

UNIVERSIDADE FEDERAL DE PELOTAS
Faculdade de Odontologia
Programa de Pós-Graduação em Odontologia



Tese

**Influência e interações (epistáticas e gene-ambiente) de *SNPs* na
experiência de cárie: evidências a partir de revisões sistemáticas e estudos
prospectivos**

Luiz Alexandre Chisini

Pelotas, 2020

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**Dedico este trabalho a todas as populações marginalizadas,
excluídas e que não tiveram acesso à educação,
vítimas deste sistema excludente e opressor.
Da mesma forma, a todxs que lutam por
uma educação universal e de
qualidade, assim como pelo
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“Seria uma atitude ingênua esperar que as classes dominantes desenvolvessem uma forma de educação que proporcionasse às classes dominadas perceber as injustiças sociais de maneira crítica.”

(Paulo Freire)

Luiz Alexandre Chisini

**Influência e interações (epistáticas e gene-ambiente) de *SNPs* na
experiência de cárie: evidências a partir de revisões sistemáticas e
estudos prospectivos**

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Resumo

CHISINI, Luiz Alexandre. **Influência e interações (epistáticas e gene-ambiente) de SNPs na experiência de cárie: evidências a partir de revisões sistemáticas e estudos prospectivos**. 2020. 622f. Tese – Programa de Pós-Graduação em Odontologia. Universidade Federal de Pelotas, Pelotas, 2020.

É inquestionado – e a literatura suporta com forte evidência - que os principais fatores para o desenvolvimento e progressão da doença cárie são relacionados aos fatores biológicos, comportamentais e socioeconômicos. No entanto, alguns indivíduos expostos aos mesmos fatores de risco e/ou de proteção podem apresentar um padrão de ocorrência de cárie diferente. Desta forma, estudos recentes têm investigado a possibilidade de influência genética na ocorrência de cárie dental, objetivando explicar essa parte do efeito não explicada pelos fatores de risco já conhecidos. Desta forma, o objetivo do presente estudo foi investigar a influência e a interação (epistática e gene-ambiente) de *Single Nuclotide Polymorphism* (SNPs) na experiência de cárie dental a partir de revisões sistemáticas e estudos prospectivos na coorte de nascimento de 1982 de Pelotas. Revisões sistemáticas e meta-análises foram conduzidas para identificar os polimorfismos genéticos e seus efeitos na experiência de cárie dental em adultos e crianças. A estratégia de pesquisa foi realizada utilizando palavras-chave relevantes e entre termos MeSH considerando a estrutura de cada base de dados (PubMed/MedLine, Scopus, ISI Web of Science, BVS - Biblioteca de saúde virtual, Scielo). Somente estudos em humanos foram incluídos com desenho transversal e/ou longitudinal. Não foram consideradas quaisquer restrições de idioma ou período de publicação. Estudos com desenho de revisões de literatura, relatos de casos e séries de casos, resumos de conferências, cartas para o editor e estudos qualitativos foram excluídos. Após a identificação dos SNPs e seus efeitos estimados sobre a experiência cárie dental nas revisões, conduziu-se estudos prospectivos para avaliar o impacto e a reprodução dos efeitos destes SNPs na coorte de nascimentos. Assim, uma amostra representativa de todos os 5,914 nascidos vivos da coorte de Pelotas de 1982 foram prospectivamente

investigados e a prevalência de cárie foi acessada aos 15 anos (n=888), 24 (n=720) e 31 anos (n=539). *Group-Based trajectory modeling* foi utilizado para identificar grupos com trajetórias semelhantes do compo-dente “cariado” do CPO-D. O material genético foi coletado e SNPs relativos aos genes (*TUFT1*, *MMP20*, *MMP13*, *MMP2*, *DLX3*, *TIMP2*, *BMP7*, *TFIP11*, *TAS1R3*, *TAS1R2*, *CA6*, *MUC5B*, *AQP2*, *AQP5*, *LTF* e *MBL2*) foram genotipados. A ancestralidade genômica foi avaliada usando ADMIXTURE. Também foram investigadas renda familiar, consumo e frequência de açúcar e sangramento gengival. Investigamos interações epistáticas pelo *software Generalized Multifactor Dimensionality Reduction* (GMDR) e também modificação gene-ambiente, inserindo um termo de interação entre consumo de açúcar e genótipo / alelo. Análise paramétrica por G-fórmula foi utilizada para analisar efeitos de mediação. Resultados das revisões sistemáticas apresentaram que os principais SNPs associados com experiência de cárie foram: i) dentre os genes ligados aos tecidos minerais dentais o *TFIP11*, *AMBN*, *VRD* e *AMELX* ii) dentre os genes ligados aos genes da sensibilidade gustatória o *TAS1R2*, *TAS1R3* e *TAS2R38* ; iii) dentre os genes ligados à composição e fluxo salivar o *CA6*, *AQP5* e *AQP2*; iv) dentre os genes da resposta imune o *MBL2* e *MUC5B*. Estudos prospectivos encontraram: i) uma associação epistática envolvendo rs243847 (*MMP2*), rs2303466 (*DLX3*) e rs388286 (*BMP7*) capaz de aumentar a trajetória de cárie dental (OR=2.51, CI95%[1.54–4.09]); ii) O SNP rs307355 (*TAS1R3*) foi associado com elevada trajetória de cárie (OR=4.17, CI95%[1.21–14.42]) e apresentou uma interação epistática com rs35874116 (*TAS1R2*) (OR=1.72, CI95%[1.04-2.84]); iii) rs10875989 (*AQP2*) foi associado com elevada trajetória de cárie (OR=1.38 CI95%[1.07–1.78]) e apresentou uma interação de três locus com rs2274333 (*CA6*) e rs3759129 (*AQP5*) que elevou a chances de estar no grupo de elevada trajetória de cárie (OR=2.31, CI95%[1.53–3.47]); g-formula mostrou que o efeito entre rs10875989 (*AQP2*) e cárie foi mediada pelo sangramento gengival ($p<0.05$) e não pela consumo de açúcar ($p>0.05$). iv) rs11716497 (*LTF*) foi associada com elevada trajetória de cárie (OR=1.61, CI95% [1.03–2.52]) e uma interação epistática com rs4547741 (*LTF*) e rs11716497 (*LTF*) também foi observada. G-fórmula mostrou que a

associação entre rs11716497 (*LTF*) e trajetória de cárie teve um efeito direto e não foi mediada pelo consumo de açúcar ($p < 0.001$).

Assim, baseado nos resultados obtidos a partir das revisões sistemáticas e meta-análises somado com os achados dos estudos prospectivos, concluimos que a cárie dental apresenta um componente genético importante capaz de influenciar a experiência e a trajetória de cárie dos indivíduos. Além disso, interações epistáticas parecem desempenhar um papel importante na arquitetura genética da cárie dental e fatores ambientais podem modificar o efeito genético no fenótipo.

Palavras-chave: Estudos de coorte; Revisão sistemática; Epidemiologia; Polimorfismos genéticos; Single nucleotide polymorphism; Cárie Dental

Abstract

Chisini, Luiz Alexandre. **Influence and interactions (epistatic and gene-environment) of SNPs on caries experience: evidence from systematic reviews and prospective studies.** 2020. 622p. PhD in Dentistry. Graduate Program in Dentistry. Federal University of Pelotas, Pelotas, 2020.

It is unquestionable - and the literature strongly supports - that the main factors for the development and progression of caries disease are related to biological, behavioral and socioeconomic factors. However, some individuals exposed to the same risk and/or protective factors may have a different pattern of caries occurrence. Thus, recent studies have investigated the possibility of genetic influence on the occurrence of dental caries, aiming to explain this part of the effect not explained by known risk factors. Therefore, the aim of the present study was to investigate the influence and interaction (epistatic and gene-environment) of Single Nucleotide Polymorphism (SNPs) on dental caries experience from systematic reviews and prospective studies in the 1982 Pelotas birth cohort. Systematic reviews and meta-analyses were conducted to identify genetic polymorphisms and their effects on dental caries experience in adults and children. The search strategy was performed using relevant keywords and between MeSH terms considering the structure of each database (PubMed / MedLine, Scopus, ISI Web of Science, VHL - Virtual Health Library, Scielo). Only human studies were included with cross-sectional and/or longitudinal design. No language restrictions or period of publication were considered. Studies with literature review design, case reports and case series, conference abstracts, letters to the editor, and qualitative studies were excluded. After identifying SNPs and their estimated effects on dental caries experience in reviews, prospective studies were conducted to evaluate the impact and reproduction of the effects of these SNPs on the birth cohort. Thus, a representative sample of all 5,914 live births from the 1982 Pelotas cohort were prospectively investigated and the prevalence of caries was assessed at 15 years (n = 888), 24 (n = 720) and 31 years (n = 539). Group-Based trajectory modeling was used to identify groups with similar trajectories of CPO-D

“decayed” component. Genetic material was collected, and SNPs related to genes (*TUFT1*, *MMP20*, *MMP13*, *MMP2*, *DLX3*, *TIMP2*, *BMP7*, *TFIP11*, *TAS1R3*, *TAS1R2*, *CA6*, *MUC5B*, *AQP2*, *AQP5*, *LTF* and *MBL2*) were genotyped. Genomic ancestry was evaluated using ADMIXTURE. Family income, sugar consumption and frequency and gingival bleeding were also investigated. We investigated epistatic interactions by the Generalized Multifactor Dimensionality Reduction (GMDR) software and gene-environment modification, inserting an interaction term between sugar consumption and genotype / allele. Parametric analysis by G-formula was used to analyze mediation effects. Results of the systematic reviews showed that the main SNPs associated with caries experience were: i) among the genes linked to dental mineral tissues the *TFIP11*, *AMBN*, *VRD* and *AMELX* ii) among the genes linked to the taste sensitivity genes *TAS1R2*, *TAS1R3* and *TAS2R38*; iii) among the genes linked to salivary composition and salivary flow *CA6*, *AQP5* and *AQP2*; iv) among the immune response genes *MBL2* and *MUC5B*. Prospective studies found: i) an epistatic association involving rs243847 (*MMP2*), rs2303466 (*DLX3*) and rs388286 (*BMP7*) capable of increasing the dental caries trajectory (OR=2.51, 95%CI[1.54–4.09]); ii) SNP rs307355 (*TAS1R3*) was associated with high caries trajectory (OR=4.17, 95%CI[1.21–14.42]) and showed an epistatic interaction with rs35874116 (*TAS1R2*) (OR=1.72, 95%CI [1.04-2.84]); iii) rs10875989 (*AQP2*) was associated with an elegant caries trajectory (OR=1.38, 95%CI [1.07–1.78]) and presented a three locus interaction with rs2274333 (*CA6*) and rs3759129 (*AQP5*) which increased the chances of being in the group of high caries trajectory (OR=2.31, 95%CI [1.53–3.47]); g-formula showed that the effect between rs10875989 (*AQP2*) and caries was mediated by gingival bleeding ($p < 0.05$) rather than sugar consumption ($p > 0.05$); iv) rs11716497 (*LTF*) was associated with high caries trajectory (OR=1.61, 95%CI [1.03–2.52]) and an epistatic interaction with rs4547741 (*LTF*) and rs11716497 (*LTF*) was also observed. G-formula showed that the association between rs11716497 (*LTF*) and caries trajectory had a direct effect and was not mediated by sugar intake ($p < 0.001$). Thus, based on the results obtained from the systematic reviews and meta-analyzes added to the findings of prospective studies, we conclude that dental

caries has an important genetic component capable of influence the caries experience and trajectory of individuals. In addition, epistatic interactions seem to play an important role in the genetic architecture of dental caries and environmental factors may modify the genetic effect on the phenotype.

Key-words: Cohort studies; Systematic review; Epidemiology; Genetic polymorphism; Single nucleotide polymorphism; Dental Caries

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1. Introdução

A cárie dentária é uma doença crônica com alta prevalência global (KASSEBAUM *et al.*, 2015). Cerca de 2,4 bilhões de pessoas com dentição permanente e 621 milhões de crianças com dentes decíduos são afetadas pela cárie, levando a uma redução na qualidade de vida destes indivíduos (FERREIRA *et al.*, 2017). Embora a cárie dental possa ser prevenida quando atuamos nos seus principais fatores etiológicos - como hábitos de higiene bucal (biofilme), diminuição do consumo de carboidratos fermentáveis – e utilizando fluoretos, sejam eles nas mais variadas fontes (como em água fluoretada, cremes dentais com flúor, enxaguatório bucal, entre outros) (VAN LOVEREN E DUGGAL, 2001; MALTZ *et al.*, 2017), o seu controle a nível populacional é muito difícil; pois a cárie é fortemente influenciada por fatores contextuais, socioeconômicos e comportamentais (KASSEBAUM *et al.*, 2015; CHISINI, COLLARES *et al.*, 2018; CHISINI, NORONHA, *et al.*, 2018; DUTRA *et al.*, 2018). Portanto, ela continua sendo um problema de saúde pública mundial (Kassebaum *et al.*, 2015).

É indiscutível que fatores biológicos, socioeconômicos e comportamentais são as principais variáveis que explicam a ocorrência e a distribuição da doença cárie na população. No entanto, em alguns casos, indivíduos que possuem os mesmos fatores de proteção - como fluoretação da água - ou fatores de risco e com comportamento semelhante relacionado à saúde bucal, apresentam padrões diferentes de cárie dentária (VAN LOVEREN e DUGGAL, 2001; SLADE *et al.*, 2013). Para esses indivíduos, fatores genéticos podem ser uma influência intrínseca que fornece resistência ou suscetibilidade adicional a cárie dentária (VIEIRA *et al.*, 2014). Nesse contexto, estudos têm proposto que uma porção dessas variações na prevalência de cárie dentária possa ser explicada por fatores genéticos (DEELEY *et al.*, 2008;

VIEIRA *et al.*, 2014). De fato, uma grande variedade de genes foi identificada e associada com a cárie dental, demonstrando seu importante papel no desenvolvimento e progressão da doença (Vieira *et al.*, 2014).

Um pequeno número de estudos focados nos aspectos genéticos da cárie realizou associações genômicas (GWAS), que visam identificar genes potencialmente novos envolvidos com a cárie dentária (SHAFFER *et al.*, 2013; ZENG *et al.*, 2013; HAWORTH *et al.*, 2018), enquanto a maioria dos estudos que investigam a associação de componentes genéticos e cárie dentária utilizou metodologia de genes candidatos, examinando polimorfismos de nucleotídeo único (SNPs) (VIEIRA *et al.*, 2014). Dessa maneira, esses SNPs podem ser agrupados em quatro grupos principais: a) aqueles envolvidos com tecidos minerais dos dentes; b) resposta imune; c) composição e fluxo salivar; e d) genes gustativos (VIEIRA *et al.*, 2014).

Neste contexto, uma compreensão de quais SNPs e genes estão envolvidos na suscetibilidade de indivíduos à doença cárie poderia apoiar o desenvolvimento de uma abordagem viável para melhor compreender esses mecanismos complexos.

2. Projeto de Pesquisa

A Cárie dental é uma doença multifatorial altamente prevalente na população mundial (MARCENES *et al.*, 2013; KASSEBAUM *et al.*, 2015) apresentando um grande impacto econômico aos serviços de saúde (MEIER *et al.*, 2017). Estima-se que cerca de 2,4 bilhões de dentes permanentes e 621 milhões de dentes decíduos sejam afetados pela cárie dental, necessitando de tratamento tanto para a doença quanto para as manifestações clínicas da mesma (KASSEBAUM *et al.*, 2015). Além disso, a cárie é considerada como uma das principais causas de falhas de restaurações tanto em dentes decíduos (CHISINI *et al.*, 2018) quanto em dentes permanentes (DEMARCO *et al.*, 2012). Quando a doença não é tratada, a cárie pode causar inúmeras complicações que iniciam com dor e abscesso e podem evoluir para inchaço e celulite orofacial, a qual pode apresentar risco de vida ao indivíduo (KASSEBAUM *et al.*, 2015).

Embora afete grande parte da população, a cárie está distribuída de forma desproporcional nos indivíduos, apresentando, assim, uma polarização naqueles que apresentam alguma vulnerabilidade social (MARCENES *et al.*, 2013). Este fato é devido principalmente à sua etiologia multifatorial, a qual exibe uma complexa rede de fatores determinantes e mediadores (com intensidade variável de acordo com o indivíduo) (KIDD E FEJERSKOV, 2004; SPLIETH *et al.*, 2016). Estes fatores envolvem desde a estrutura e contexto social aos fatores comportamentais e nutricionais, os hábitos de higiene bucal, a exposição à fluoretos, o fluxo salivar, assim como outros fatores que ainda estão sendo definidos (KIDD E FEJERSKOV, 2004; SPLIETH *et al.*, 2016).

Dentre estes fatores, os componentes genéticos têm sido o foco de recentes estudos (VIEIRA *et al.*, 2014; CHAPPLE *et al.*, 2017; LIPS *et al.*, 2017),

embora contribuições genéticas para a ocorrência de cárie dental tenham sido propostas desde o final dos anos 80 em estudos de gêmeos (BORAAS *et al.*, 1988; CONRY *et al.*, 1993; BRETZ, CORBY, SCHORK, *et al.*, 2005; WRIGHT, 2010). Estes estudos demonstraram que cerca de 40 a 60% da susceptibilidade à cárie poderia ser geneticamente determinada (WANG *et al.*, 2010). Neste contexto, uma ampla gama de genes tem sido identificados mostrando papel importante no desenvolvimento e na progressão da cárie (VIEIRA *et al.*, 2014). Este crescente interesse em abordagens investigando componentes genéticos que poderiam explicar uma parcela da susceptibilidade do indivíduo à doença cárie pode ser explicada devido ao fato de grupos de indivíduos com, por exemplo, a mesma exposição à fluoretos e aos demais fatores de proteção/risco conhecidos apresentarem prevalências diferentes da doença (SLADE *et al.*, 2013). Neste contexto, principalmente impulsionada a partir do projeto genoma (www.genome.gov), genes específicos têm sido citados como possíveis fatores que podem influenciar a prevalência e a severidade da doença cárie (VIEIRA *et al.*, 2014). Além disso, é importante ressaltar que o genótipo humano pode influenciar não só a cárie dental, mas também pode desempenhar um papel importante na microbiota oral influenciando a patogênese de diversas doenças bucais (VIEIRA *et al.*, 2014; CHAPPLE *et al.*, 2017).

Estas abordagens genéticas têm sido investigadas através da utilização de metodologias específicas (VIEIRA *et al.*, 2008; VIEIRA, 2012; SHAFFER, FEINGOLD, WANG, LEE, *et al.*, 2013; SHAFFER, FEINGOLD, WANG, WEEKS, *et al.*, 2013; VIEIRA *et al.*, 2014; DAI E LONG, 2015; CHAPPLE *et al.*, 2017). Brevemente, existem basicamente três tipos principais de abordagens metodológicas para a condução destes estudos: estudos de gêmeos, estudos de genes candidatos e estudos de genoma (do inglês, *genome wide association study* - GWAS).

A metodologia empregada nos estudos de gêmeos (BORAAS *et al.*, 1988; CONRY *et al.*, 1993) é realizada através da comparação de um desfecho/fenótipo (neste caso, a cárie dental) entre gêmeos monozigóticos e dizigóticos. Os primeiros estudos utilizando esta abordagem (BORAAS *et al.*, 1988; CONRY *et al.*, 1993) observaram uma maior semelhança na porcentagem de dentes e superfícies cariadas e restauradas em gêmeos monozigóticos comparados aos gêmeos dizigóticos. Estes estudos, relataram que fatores

genéticos poderiam apresentar uma contribuição de aproximadamente 40% na cárie dental (BORAAS *et al.*, 1988; CONRY *et al.*, 1993). Estudos mais recentes têm corroborado na direção dos resultados (BRETZ *et al.*, 2003; BRETZ, CORBY, HART, *et al.*, 2005); no entanto, mostram que a contribuição genética poderia ser maior – 45 a 64%. Além disso, a hereditabilidade na dentição decídua tem mostrado ser mais pronunciada que na dentição permanente (WANG *et al.*, 2010).

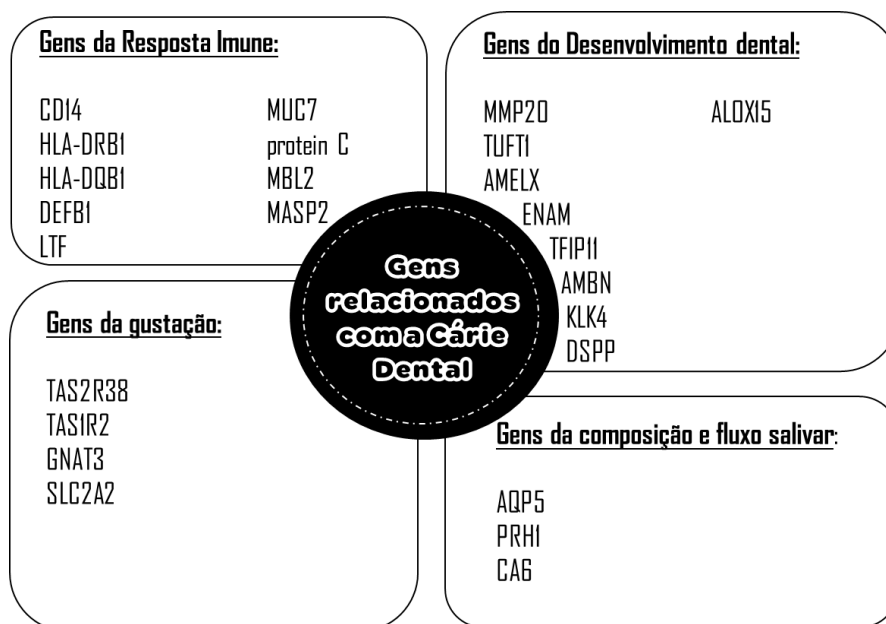
Embora estudos de gêmeos tenham sido a metodologia pioneira na investigação da associação entre componentes genéticos e cárie dental, a grande maioria dos estudos realizados é conduzido utilizando a metodologia de genes candidatos. Esta abordagem objetiva testar uma associação entre um gene específico (variantes específicas) e o fenótipo (cárie dental) (ZHU E ZHAO, 2007; PATNALA *et al.*, 2013). Marjoritariamente, os estudos de genes candidatos investigando sua influência na cárie dental têm se aprofundado principalmente na investigação de polimorfismos de nucleotídeos únicos (do inglês, *Single Nucleotide Polymorphisms* - SNPs) (VIEIRA *et al.*, 2014). É importante ressaltar que, diferentemente do GWAS, esta metodologia é realizada com uma hipótese prévia. Desta forma, o pesquisador deve previamente definir a variação genética que pretende testar (ZHU E ZHAO, 2007; PATNALA *et al.*, 2013). Assim, para utilização desta metodologia é importante que o fator genético já tenha sido previamente relatado como possível candidato ou que exista uma hipótese teórica prévia envolvida. Diferentes resultados têm sido observados entre os estudos de genes candidatos (utilizando os mesmos genes e os mesmos polimorfismos). Esta variabilidade pode ser explicada devido à grande heterogeneidade das populações e, principalmente, a questões metodológicas e de poder estatístico (VIEIRA *et al.*, 2014). Desta forma, existe um campo ainda a ser explorado, seja em relação a confirmação ou não de genes já identificados, seja na investigação de genótipos envolvidos em novas rotas que poderiam favorecer ou proteger os indivíduos à cárie dental.

Além dos estudos de gêmeos e genes candidatos, os estudos de GWAS têm sido utilizados principalmente como estudo exploratório objetivando identificar novos genes com potencial associação com a cárie (BALL, 2013; HAYES, 2013). Desta forma, estes estudos não apresentam (necessariamente)

uma hipótese prévia. Estudos de GWAS investigam todo o genoma (frequentemente milhões de SNPs) testando associações entre variantes do DNA e o fenótipo de maneira independente. Desta forma, representam um procedimento gerador de hipóteses, que posteriormente necessitam de confirmação. Como se trata de uma questão meramente estatística que envolve múltiplas comparações, o resultado do GWAS deve ser interpretado com cautela. Assim, um limiar típico para a significância estatística em estudos de GWAS é um valor de $p \leq 5 \times 10^{-8}$, e um resultado sugestivo de associação quando observamos um p de 5×10^{-6} (BALL, 2013; HAYES, 2013).

Utilizando estas metodologias, diversos estudos têm identificado diferentes genótipos associados com a cárie dental (VIEIRA *et al.*, 2014; CHAPPLE *et al.*, 2017). Em relação a estes genes investigados, podemos agrupá-los de acordo com as características as quais eles estão ligados (Figura 1): Genes ligados ao desenvolvimento dental, a resposta imune do hospedeiro, a composição e fluxo salivar e a sensibilidade gustativa (VIEIRA *et al.*, 2014). Uma sumarização dos principais genes e SNPs reportados na literatura e suas principais características está disponível na Figura 1. Abaixo, será discutido, brevemente, cada grupo de genes relacionados com a cárie dental:

Figura 1 – Sumarização dos principais genes candidatos com possível influência na cárie dental em humanos reportados na literatura provenientes de abordagem gene-candidato.



2.1.1. Genes da gustação

A sensibilidade gustativa, principalmente a doce, e sua relação com a cárie já foi amplamente investigada (WRIGHT, 2010; KARMAKAR *et al.*, 2016; ASHI, CAMPUS, *et al.*, 2017; ASHI, LARA-CAPI, *et al.*, 2017; SONBUL *et al.*, 2017) mostrando um importante papel da influência da percepção do paladar na opção dietética dos indivíduos. Nesta visão, talvez a opção da composição dietética não seja apenas um determinante do estilo de vida do indivíduo, mas sim influenciada, também, pela percepção gustativa que pode variar de acordo com os indivíduos. Em outras palavras, a percepção gustativa pode influenciar a escolha dietética individual (WRIGHT, 2010). De uma perspectiva evolutiva, a percepção do paladar apresenta um importante papel biológico adquirida pelos nossos ancestrais para potencializar a aquisição de nutrientes e prevenir a ingestão de substâncias tóxicas. Alimentos doces na natureza estão frequentemente relacionados com grandes quantidades de calorias provenientes de carboidratos, enquanto o amargo está relacionado com alimentos estragados e a toxinas. Desta forma, a literatura tem apontado alguns genes específicos que foram associados com influências na percepção gustativas aos alimentos doces (WRIGHT, 2010).

Um estudo conduzido em uma grande coorte de famílias (496 indivíduos em dentição decídua, 562 em dentição mista e 1391 com dentição permanente) representativas da população de West Virginia e da Pennsylvania demonstrou uma importante associação entre genes que influenciam a percepção gustativa (*TAS2R38* e *TAS1R2*) e cárie dental na dentição decídua (WENDELL *et al.*, 2010). Embora uma relação tenha sido estabelecida com a dentição decídua, o mesmo não ocorreu quando investigado nos indivíduos em dentição mista ou permanente (WENDELL *et al.*, 2010). É importante ressaltar a pequena variabilidade étnica desta população. Desta forma, a confirmação ou não destes resultados em populações com maior diversidade étnica se torna necessária para maiores extrapolações dos dados para demais populações. Os alelos individuais “G”, “G” e “C” dos polimorfismos rs713598, rs1726866 e rs10246939 (os quais representam a substituição dos amino ácidos Prolina, Alanina e Valina) do gene *taste 2 receptor member 38* - *TAS2R38* – com localização 7q34 mostraram um efeito protetor, enquanto os haplótipos “CAT” e “CAX” (sendo X nenhum nucleotídeo) foram fatores de risco para a cárie dental (WENDELL *et al.*, 2010). A combinação da Prolina, Alanina e Valina (PAV) representada pelos nucleotídeos “GGC” foi associada com maior sensibilidade ao amargo (*Supertasters*). No entanto, Alanina, Valina e Isoleucina (AVI) representada pelos nucleotídeos (CAT) foi associada com dessensibilidade ao amargo. Além disso, *supertasters* têm sido associados com uma maior sensibilidade a uma ampla gama de gostos, dentre eles o doce (KELLER *et al.*, 2002; KELLER E TEPPER, 2004; BELL E TEPPER, 2006; TEPPER, 2008). Estes resultados têm demonstrado um importante papel dos fatores genéticos nos mecanismos que levam os indivíduos a adotarem hábitos nutricionais cariogênicos.

2.1.2. Genes do Desenvolvimento dental

Durante a morfogênese dos tecidos dentais, diversas moléculas desempenham papéis indispensáveis de sinalização regendo o comportamento dos componentes celulares, dando forma, mediando principalmente as interações entre as camadas do tecido epitelial e mesenquimal, os quais

formam as principais estruturas dentais (SIMMER E HU, 2001; CHISINI *et al.*, 2016). Desta forma, proteínas sinalizadoras ou aquelas constituintes do elemento dental são de extrema importância para o correto desenvolvimento das estruturas dentais. Em contrapartida, alterações nestes componentes podem acarretar modificações no tecido dental. Do ponto de vista genético, variações nos genes envolvidos na formação do esmalte e dentina podem ocasionar alterações na constituição tecidual dos respectivos tecidos contribuindo, assim, na susceptibilidade do indivíduo à carie dental (SLAYTON *et al.*, 2005).

Diversos estudos investigando genes relacionados com o desenvolvimento dental (em populações dos Estados Unidos da América, Guatemala, Turquia, Argentina, Brasil e Filipinas) têm apontado alguns genes candidatos com potencial influência na experiência de cárie (SLAYTON *et al.*, 2005; DEELEY *et al.*, 2008; PATIR *et al.*, 2008; SHIMIZU *et al.*, 2012). A presença do alelo C no gene da *amelogenin* (*AMELX*) (SNP: rs17878486) foi associado com escores de dentes cariados, perdidos e obturados (CPO-D) superiores a oito. Além disso, o alelo T do marcador de *ameloblastin* (*AMBN*) (SNP: rs34538475) foi associado aos casos com escores de CPO-D superiores a dez (PATIR *et al.*, 2008).

Um estudo conduzido com diversas populações (Brasileira, Turca, Argentina e Filipina) reuniu dados de 1.831 indivíduos e investigou SNPs específicos para diversos genes que influenciam a formação do esmalte dental (*ameloblastin* -*AMBN*-, *amelogenin* -*AMELX*-, *enamelin* -*ENAM*-, *tuftelin* -*TUFT*-, and *tuftelin interacting protein 11* -*TUFT11*) (Shimizu 2012). Embora associações tenham sido observadas principalmente para o gene da *Amelogenin*, as associações com experiência de cárie não foram necessariamente replicadas em todos os grupos populacionais (SHIMIZU *et al.*, 2012). Estes dados reforçam a necessidade de replicação dos resultados prévios em diferentes populações para que o real papel destes genes possa ser confirmados ou descartado.

Além disso, um recente estudo (KUCHLER *et al.*, 2017) avaliou 266 crianças de ambos os sexos de escolas públicas e privadas da cidade de Curitiba e observou que algumas variações genéticas nos genes do

desenvolvimento dental (*AMELX*, *AMNB* and *ESRRB*) foram associados com uma maior quantidade de cálcio na saliva dental, assim como o gene *ENAM* foi associado com maior quantidade de fosfato. De forma semelhante, observou-se em outro estudo que a variação genética de genes de formação de esmalte (*ENAM* e *AMBN*) também influenciou as concentrações de cálcio e magnésio dos dentes (KUCHLER *et al.*, 2017), desta forma, sugerindo possível influência de polimorfismos nestes genes e a composição dental e salivar.

2.1.3. Genes da composição e fluxo salivar

A saliva apresenta componentes que podem inibir bactérias cariogênicas além de conter cálcio e fosfato que estão envolvidos ativamente no processo de desmineralização e remineralização do esmalte dental (KIDD E FEJERSKOV, 2004; SPLIETH *et al.*, 2016). Por exemplo, pacientes com glândulas salivares irradiadas podem apresentar uma maior experiência de cárie devido à diminuição do fluxo salivar. Ademais, o fluxo salivar tem o papel de diluir os microorganismos e os carboidratos ingeridos pelos indivíduos evitando que se acumulem nos tecidos dentais (KIDD E FEJERSKOV, 2004; SPLIETH *et al.*, 2016), apresentando assim, um importante papel protetor para o desenvolvimento e progressão da doença cárie.

Alguns estudos têm demonstrado que alguns SNPs em genes que regulam a produção salivar poderia ter um papel protetor à carie dental (CULP *et al.*, 2005; WANG *et al.*, 2012). A deleção direta do gene que codifica a proteína Aquaporina-5 (*Aqp5*), por exemplo, foi responsável por aumentar a susceptibilidade de camundongos à carie dental (CULP *et al.*, 2005). Aquaporinas são responsáveis por codificar uma série de proteínas de membrana que funcionam como canais de água altamente seletivos. Em especial, a proteína AQP5 está presente quase exclusivamente nas glândulas salivares e lacrimais. Ela é responsável pela geração de saliva além de ser importante na produção de lágrimas e de secreções pulmonares. Nesse contexto, alterações no gene codificante desta proteína poderia alterar a produção de saliva nos indivíduos. Apartir desta hipótese, um estudo conduzido na *Iowa Fluoride Study cohort* (333 crianças caucasianas) observou uma

importante associação entre um SNP - rs1996315 - (C/T) do *AQP5* e a experiência de cárie (WANG *et al.*, 2012). Esse SNP foi um importante fator protetor à carie dental. Além disso, embora outro SNP - rs923911 - (A/C) não tenha mostrado associação quando analisado de forma isolada, quando estudado em combinação com o primeiro SNP (análise de haplótipos), observou-se uma associação protetora para os haplótipos CA e CG (WANG *et al.*, 2012).

Outro estudo realizado na província de Gansu (355 indivíduos), no noroeste da China, observou também uma influência de SNPs no gene da *Anidrase Carbonica-6* (ACA) na susceptibilidade a cárie (LI *et al.*, 2015). O haplótipo “A” “C” “A” (referentes aos SNPs rs2274328, rs17032907 e rs11576766) foi associado com um baixo índice de dentes cariados/perdidos/obturados (CPO-D) nesta população. Isso pode ser explicado pois a proteína codificada por este gene é uma das várias isozimas de anidrase carbônica, porém a única com capacidade secretora. Esta proteína é encontrada apenas nas glândulas salivares e na saliva. Embora sua função não seja completamente conhecida, especula-se que desempenha um papel primordial na manutenção do pH bucal, o que, por sua vez, influencia a microbiota oral (LI *et al.*, 2015). Neste contexto, PERES *et al.* (2010) observou em 245 escolares brasileiros que o polimorfismo (rs2274327 (C/T)) apresentou uma associação com a capacidade tampão da saliva. O genótipo TT foi significativamente menos frequente em indivíduos com maior capacidade de tamponamento salivar, embora não tenha sido associada propriamente com a prevalência de cárie dental nesta população de crianças (7-9 anos) brasileiras (PERES *et al.*, 2010). Desta forma, SNPs nesse gene podem alterar a função da ACA, o que pode influenciar (em um segundo momento) a cárie dental (LI *et al.*, 2015).

2.1.4. Genes da Resposta Imune

Enquanto o fluxo e os componentes salivares podem afetar/modificar a doença cárie, algumas proteínas presentes na saliva podem apresentar propriedades antimicrobianas, antivirais, antifúngicas e/ou anti-inflamatórias

(FARNAUD E EVANS, 2003). Uma dessas proteínas é a Lactotransferina (LTF), a qual pode atuar como proteína de defesa do hospedeiro influenciando o sistema imunológico não específico, assim como a imunidade adaptativa.

O gene codificante da proteína LTF (o qual apresenta o mesmo nome Lactotransferina - *LTF*) é um gene membro da família de genes da transferrina e seu produto proteico é encontrado nos grânulos secundários de neutrófilos. Em relação à cárie dental, ela parece atuar apresentando um efeito na formação do biofilme bacteriano (FINE, 2015). Este efeito é devido a capacidade de sequestrar ou quelar o ferro necessário para o desenvolvimento do biofilme, influenciando assim, tanto a cárie dental como a doença periodontal (FINE, 2015). Recente investigação tem observado que o alelo “A” do SNP rs6441989 foi significativamente menos frequente no grupo com alta experiência de cárie, mostrando um efeito protetor para a cárie dental (DOETZER *et al.*, 2015); Por outro lado, nenhuma diferença na experiência de cárie foi encontrada entre a frequência dos alelos genotipados para o SNP rs1126478 (A/G) e a experiência de cárie (WANG *et al.*, 2017).

Além do gene que codifica LTF, outros genes têm sido propostos com atuação na resposta imune. Enquanto que *Mannan-binding lectin serine peptidase 2* (*MASP2*), fator bactericida que se liga a polissacarídeos expressos por certas enterobactérias, não tenha sido associada com a cárie dental (OLSZOWSKI *et al.*, 2012), a *Mannose Binding Lectin 2* (*MBL2*) tem apresentado associação na maioria dos estudos (OLSZOWSKI *et al.*, 2012; YANG *et al.*, 2013; ALYOUSEF *et al.*, 2017; SHIMOMURA-KUROKI *et al.*, 2018).

Neste contexto, o gene codificante da *Beta Defensin 1* (*DEFB1*) tem sido amplamente investigado no que diz respeito à resposta imune do hospedeiro e cáries. Este gene é responsável por formar uma família de peptídios com propriedades antimicrobianas com influência na resistência das superfícies epiteliais à colonização microbiana. Desta forma, uma possível relação entre o gene *DEFB1* e a cárie dental poderia ser esperada. De fato, alguns estudos (NAVARRA *et al.*, 2016; YILDIZ *et al.*, 2016) têm encontrado uma associação positiva. Além disso, alguns SNPs desse gene, como por exemplo o SNP rs179946, foi correlacionado com um baixo CPO-D (OZTURK *et al.*, 2010). Da mesma forma, o SNP rs11362 influenciou a susceptibilidade à cárie (KRASONE *et*

al., 2014); Por outro lado, outras investigações em populações distintas não observaram associação de polimorfismos no *DEFB1* com cárie dental em crianças (LIPS *et al.*, 2017), necessitando de estudos adicionais para a confirmação (ou rejeição) desta possível relação.

2.1.5. Justificativa

A descoberta de novos genes ou a confirmação dos já identificados e que foram associados com uma maior susceptibilidade à cárie (Tabela 1) são de extrema importância para ampliar a identificação de indivíduos com risco aumentado, os quais poderiam ser incluídos e direcionados para estratégias preventivas precocemente. Além disso, o entendimento dos caminhos complementares que podem influenciar o risco à doença cárie pode ser evidenciados com tais abordagens, principalmente quando ajustados pelos fatores de risco já conhecidos. A compreensão dos mecanismos genéticos e das vias genéticas podem prover uma interessante abordagem para discriminar de forma mais detalhada as diferenças observadas entre indivíduos com os mesmos determinantes sociais, ambientais e comportamentais, porém, com experiências de cárie diferentes.

Desta forma, a investigação da influência e interações (gene-gene) de polimorfismos genéticos relacionados à cárie dental observadas em uma população que foi acompanhada ao longo da vida somada às evidências observadas em revisões sistemáticas da literatura pode prover uma contribuição importante no entendimento do papel genético na experiência de cárie dental.

Tabela 1. Resumo dos principais genes candidatos relatados na literatura provenientes de abordagem gene-candidato com possível associação com a cárie dental.

Gene	Summary	Localização	Referência	Resultados
Genes Gustativos				
<i>Taste 2 receptor member 38 (TAS2R38)</i>	Codifica a proteína G envolvida na sensibilidade gustativa.	7q34	(WENDELL <i>et al.</i> , 2010; YILDIZ <i>et al.</i> , 2016)	Substituição do alelos G, G e C que representam as substituições de aminoácidos P, A e V no gene <i>TAS2R38</i> mostraram proteção para cáries em dentes decíduos (WENDELL <i>et al.</i> , 2010). A frequência do genótipo GG do polimorfismo rs713598 GG foi de 78,8% no grupo de baixo risco de cárie e 21,2% no grupo de alto risco de cárie (YILDIZ <i>et al.</i> , 2016) . Nenhum resultado foi observado na dentição permanente (WENDELL <i>et al.</i> , 2010)
SNPs: rs713598 (C/G); rs1726866 (G/A); rs10246939 (C/T)(WENDELL <i>et al.</i> , 2010); rs713598 (C/G) (YILDIZ <i>et al.</i> , 2016)				
<i>Taste 1 receptor member 2</i>	Codifica a proteína G envolvida na sensibilidade gustativa.	1p36.13	(WENDELL <i>et al.</i> , 2010; KULKARNI <i>et</i>	Mudança do alelo G para C (rs10246939) mostrou associação com proteção para cárie e mambas dentições (WENDELL <i>et al.</i> , 2010);

(TAS1R2)			al., 2013; HAZNEDAROGLU U <i>et al.</i> , 2015; IZAKOVICOVA HOLLA <i>et al.</i> , 2015; ROBINO <i>et al.</i> , 2015); Holla 2015; Haznedaroğlu U 2015	Homozigose GG no SNP rs3935570 foi associado com maior CPOD (ROBINO <i>et al.</i> , 2015); Genótipo TT (rs35874116) é mais frequente em indivíduos com cárie (IZAKOVICOVA HOLLA <i>et al.</i> , 2015); Associação entre número total de cáries e polimorfismo rs35874116 (HAZNEDAROGLU <i>et al.</i> , 2015) Associado tanto com alta e baixa experiência de cárie (WENDELL <i>et al.</i> , 2010; KULKARNI <i>et al.</i> , 2013)
		SNPs: rs4920566 (G/A); rs9701796 (G/C) (WENDELL <i>et al.</i> , 2010); rs3935570 (G/T) (ROBINO <i>et al.</i> , 2015); rs35874116 (C/T) (IZAKOVICOVA HOLLA <i>et al.</i> , 2015); rs35874116 (C/T), rs9701796 (C/G) (HAZNEDAROGLU <i>et al.</i> , 2015)		
Guanine nucleotide binding protein, alpha transducing 3 (GNAT3)	Acredita-se que esteja envolvida com a preferência dietética	7q21.11	(WENDELL <i>et al.</i> , 2010)	Polimorfismos não foram associados ao desfecho (WENDELL <i>et al.</i> , 2010)
		SNPs: rs2074674 (G/A); rs6962693 (T/G) (WENDELL <i>et al.</i> , 2010)		
Solute carrier family 2	Codifica uma glicoproteína integral facilitando o transporte	3q26.2	(ENY <i>et al.</i> , 2008)	Associado (rs5400) com alta ingestão de açúcares (ENY <i>et al.</i> , 2008)

(SLC2A2)		(bdirecional) da glucose		
SNPs: rs5400 (C/T) (ENY et al., 2008)				
Genes do Desenvolvimento dental				
Matrix metallopeptidas e 20 (MMP20)	Envolvida na degradação da matriz extracelular A proteína codificada por este gene degrada a amelogenina, o principal componente proteico da matriz do esmalte dental e, portanto, acredita-se que possa desempenhar um papel na formação do esmalte dentário.	11q22.3	(TANNURE et al., 2012; WANG et al., 2012; ABBASOGLU et al., 2015; ANTUNES et al., 2016; FILHO et al., 2017; GERRETH et al., 2017);	A mudança de C/T no SNP rs1784418 resultou em uma proteção para cárie (FILHO et al., 2017). Associado com cárie dental (TANNURE et al., 2012; ANTUNES et al., 2016; FILHO et al., 2017). Associação não observada (WANG et al., 2012; ABBASOGLU et al., 2015; GERRETH et al., 2017). Sugestão de associação com experiência de cárie, principalmente entre indivíduos caucasianos e com pobre higiene bucal. Alelo C apresentou frequência de 58% entre os afetados por cárie e 56% entre os livre de cárie e o alelo T, 42% entre os afetados e 44% entre os livres de cárie (TANNURE et al., 2012)
	SNPs: rs1784418 (C/T), rs2245803 (G/T), rs7109663 (C/G) (WANG et al., 2012); rs1784418 (C/T) (TANNURE et al., 2012); rs1784418 (C/T), rs1711437 (A/G) (FILHO et al., 2017), rs1784418 (A/G) (ABBASOGLU et al., 2015); rs1784418 (A/G), rs1711437 (A/G) (ANTUNES et al., 2016); rs1784418 (C/T) (GERRETH et al., 2017);			
Tuftelin (TUFT1)	Tuftelin é uma proteína ácida que parece desempenhar um	1q21.3	(SLAYTON et al., 2005;	Mudança de C/T no SNP rs3790506 foi um fator de proteção para cárie precoce em crianças (Abbasoğlu

*papel na mineralização do
esmalte dental implicando na
susceptibilidade do indivíduo à
cárie dental*

DEELEY *et al.*, 2015).
2008; PATIR SNP rs4970957 apresenou proporção A:G 85:33 no
et al., 2008; grupo de alta experiência e A:G 91:61 no grupo de
WANG *et al.*, baixa experiência de cárie (SHIMIZU *et al.*, 2012). Teste
2010; SHIMIZU de microdureza do esmalte após desafio cariogênico
et al., 2012; foi associado com SNP da TUFT1 (SHIMIZU *et al.*,
WANG *et al.*, 2012); Interação entre TUFT1 e *Streptococcus Mutans*
2012; também foi observada, sendo que 26.8% da variação
JEREMIAS *et* no CPOD foi explicada por essa interação (SLAYTON *et*
al., 2013; *al.*, 2005); No entanto, não mostrou associação em
ERGOZ *et al.*, outros estudos (WANG *et al.*, 2012; JEREMIAS *et al.*,
2014; 2013; ERGOZ *et al.*, 2014)
ABBASOGLU *et*
al., 2015;
GERRETH *et*
al., 2017)

SNPs: rs3748609 (A-G), rs11204846 (A-G), rs3748608 (A-G), rs7526319 (C-T), rs3828054 (A-G), rs6587597 (A-G),
rs7554707 (G-T), rs2337360 (A-G) (WANG *et al.*, 2010); rs4970957 (A/G) (SHIMIZU *et al.*, 2012); rs3790506 (A/G),
rs2337360 (G/A) (DEELEY *et al.*, 2008); rs3790506 (T/C), rs2337360 (G/A) (PATIR *et al.*, 2008); rs2337360 (A/G)
(GERRETH *et al.*, 2017); rs3748609 (A/G), rs11204846 (A/G), rs3748608 (A/G), rs7526319 (C/T), rs3828054 (A/G),

rs6587597 (A/G), rs7554707 (G/T), rs2337360 (A/G) (WANG et al., 2012); rs3790506 (A/G), rs233736 (A/G), rs4970957 (A/G) (ERGOZ et al., 2014); rs3790506 (A/G), rs233736 (A/G), rs4970957 (A/G, C/T) (JEREMIAS et al., 2013); rs7526319 (C/T), rs4970957 (A/G), rs3828054 (C/T), rs3790506 (C/T), rs2337360 (A/G) (ABBASOGLU et al., 2015)

Amelogenin (AMELX)	As amelogeninas estão envolvidas na biomineralização durante o desenvolvimento do esmalte dental. Mutações neste gene causam amelogênese imperfeita. O splicing alternativo resulta em múltiplas variáveis de transcrição que codificam diferentes isoformas.	Xp22.2	(DEELEY et al., 2008; PATIR et al., 2008; KANG et al., 2011; OLSZOWSKI et al., 2012; SHIMIZU et al., 2012; GASSE et al., 2013; JEREMIAS et al., 2013; ERGOZ et al., 2014; ABBASOGLU et al., 2015;	Foi associada com alta experiência de cárie (DEELEY et al., 2008; PATIR et al., 2008; KANG et al., 2011; SHIMIZU et al., 2012; JEREMIAS et al., 2013). A frequência do alelo T foi maior em indivíduos com experiência de cárie (C:T 354:241 no grupo de alta experiência de cárie) e C:T 242:114 no grupo de baixa experiência de cárie. Sobrerrepresentação do Alelo C foi associado com CPOD maior que 8 (SHIMIZU et al., 2012). Outros estudos não observaram associação com cárie (SLAYTON et al., 2005; OLSZOWSKI et al., 2012; GASSE et al., 2013; JEREMIAS et al., 2013; ERGOZ et al., 2014; ABBASOGLU et al., 2015; YILDIZ et al., 2016)

		YILDIZ <i>et al.</i> , 2016; GERRETH <i>et al.</i> , 2017)	
		SNPs: rs946252 (C/T) (SHIMIZU <i>et al.</i> , 2012); rs17878486 (T/C) (PATIR <i>et al.</i> , 2008); rs17878486 (T/C) (GERRETH <i>et al.</i> , 2017); hCV2190967 (C/T) (DEELEY <i>et al.</i> , 2008); rs17878486 (C/T), rs5933871 (C/T), rs5934997 (C/T) (KANG <i>et al.</i> , 2011); rs17878486 (C/T), rs946252 (C/T) (JEREMIAS <i>et al.</i> , 2013); rs2106416 (A/C/T) (OLSZOWSKI <i>et al.</i> , 2012); rs17878486 (C/T), rs946252 (C/T) (ERGOZ <i>et al.</i> , 2014); rs17878486 (C/T), rs946252 (C/T) (JEREMIAS <i>et al.</i> , 2013); rs17878486 (C/T), rs946252 (C/T) (ABBASOGLU <i>et al.</i> , 2015); rs6639060 (C/T) (YILDIZ <i>et al.</i> , 2016); rs184371797 (A/C), rs946252 (A/G), rs200163085 (A/G), rs2106416 (A/C/T), rs138249749 (G/T), rs7052450 (C/T) (GASSE <i>et al.</i> , 2013)	
Enamelin (ENAM)	Este gene codifica a maior proteína na matriz de esmalte do desenvolvimento de dentes. Esta proteína está envolvida na mineralização e organização estrutural do esmalte. Defeitos neste gene resultam em amelogenese imperfeita (tipo 1C)	4q13.3	(SLAYTON <i>et al.</i> , 2005; DEELEY <i>et al.</i> , 2008; PATIR <i>et al.</i> , 2008; OLSZOWSKI <i>et al.</i> , 2012; SHIMIZU <i>et al.</i> , 2012; WANG <i>et al.</i> , 2012; <i>et al.</i> , 2012; Associado com alta experiencia de cáie (PATIR <i>et al.</i> , 2008; SHIMIZU <i>et al.</i> , 2012; JEREMIAS <i>et al.</i> , 2013; CHAUSSAIN <i>et al.</i> , 2014; WANG <i>et al.</i> , 2017) Nenhuma evidência de associação (SLAYTON <i>et al.</i> , 2005; DEELEY <i>et al.</i> , 2008; OLSZOWSKI <i>et al.</i> , 2012; WANG <i>et al.</i> , 2012; ERGOZ <i>et al.</i> , 2014; BORILOVA LINHARTOVA <i>et al.</i> , 2017). Genótipo GG em ENAM (rs1264848) foi um fator protetor para cárie em crianças (ABBASOGLU <i>et al.</i> , 2015; GERRETH <i>et al.</i> , 2017).

JEREMIAS <i>et al.</i> , 2013; CHAUSSAIN <i>et al.</i> , 2014; ERGOZ <i>et al.</i> , 2014; ABBASOGLU <i>et al.</i> , 2015; BORILOVA LINHARTOVA <i>et al.</i> , 2017; GERRETH <i>et al.</i> , 2017; WANG <i>et al.</i> , 2017);	Significante associação entre ENAM e experiência de cárie. (A:G = 101:157 no grupo de indivíduos com alta experiência de cárie. A:G=266:554 no grupo de indivíduos com baixa experiência de cárie (SHIMIZU <i>et al.</i> , 2012)

SNPs: rs12640848 (A/G); rs3796704 (A/G); rs7671281 (C/T) (WANG 2012); rs12640848 (A/G) (SHIMIZU *et al.*, 2012); rs3796704 (G/A) (PATIR *et al.*, 2008); rs3796704 (A/G), rs12640848 (A/G) (JEREMIAS *et al.*, 2013); rs3796704 (A/G) (DEELEY *et al.*, 2008); rs3796704 (A/G), rs12640848 (A/G) (ERGOZ *et al.*, 2014); rs12640848 (A/G), rs3796704 (A/G) (ABBASOGLU *et al.*, 2015); rs12640848 (A/G) (BORILOVA LINHARTOVA *et al.*, 2017); rs3796703 (C/T) (WANG *et al.*, 2017); rs2609428 (C/T), rs7671281 (C/T), rs36064169 (A/C/T), rs3796704 (A/G), rs12640848 (A/G), rs144929717 (A/G),

rs139228330 (A/G) (GERRETH et al., 2017); rs182835987 (A/T), rs147876348 (A/G), rs144929717 (A/G), rs2609429 (A/C), rs202231676 (C/T), rs34251790 (C/T), rs149086531 (A/G), rs147177510 (A/G), rs139228330 (A/G), rs74511578 (A/G), rs2609428 (C/T), rs6813313 (C/T), rs7671281 (C/T), rs36064169 (A/C/T), rs3796704 (A/G), rs138729240 (C/T), rs71599965 (A/G) (CHAUSSAIN et al., 2014)

<i>Tuftelin Interacting Protein 11 (TFIP11)</i>	Os polimorfismos neste gene estão associados a carie dental sugerindo um papel na amelogênese	22q12.1	(SLAYTON et al., 2005; DEELEY et al., 2008; PATIR et al., 2008; SHIMIZU et al., 2012; JEREMIAS et al., 2013; ERGOZ et al., 2014; ABBASOGLU et al., 2015; GERRETH et al., 2017)	Está associado com lesões cariosas e maior experiência em carie (SHIMIZU et al., 2012; JEREMIAS et al., 2013). Além disso está associada com hipomineralização molar incisivo (JEREMIAS et al., 2013). Não foi associada com cárie (SLAYTON et al., 2005; DEELEY et al., 2008; SHIMIZU et al., 2012; ERGOZ et al., 2014; ABBASOGLU et al., 2015; GERRETH et al., 2017)

SNPs: rs5997096 (C/T) (SHIMIZU et al., 2012); rs134136 (C/T) (PATIR et al., 2008); rs5997096 (C/T), rs134136 (C/T)

(ABBASOGLU <i>et al.</i> , 2015), rs134136 (C/T) (DEELEY <i>et al.</i> , 2008); rs134136 (C/T), rs5997096 (C/T) (ERGOZ <i>et al.</i> , 2014); rs134136 (C/T), rs5997096 (C/T) (GERRETH <i>et al.</i> , 2017),			
<i>Ameloblastin</i> (AMBN)	Codifica a proteína da matriz de esmalte não amelogenina e ameloblastina. A proteína codificada pode ser importante na formação e mineralização da matriz de esmalte. Este gene está localizado no agrupamento de genes de fosfoproteína de ligação ao cálcio no cromossomo 4. As mutações neste gene podem estar associadas a dentinogênese imperfeita e amelogênese imperfeita autossômica dominante.	4q13.3	(SLAYTON <i>et al.</i> , 2005; DEELEY <i>et al.</i> , 2008; PATIR <i>et al.</i> , 2008; SHIMIZU <i>et al.</i> , 2012; JEREMIAS <i>et al.</i> , 2013; ERGOZ <i>et al.</i> , 2014; ABBASOGLU <i>et al.</i> , 2015; GERRETH <i>et al.</i> , 2017; WEBER <i>et al.</i> , 2018)
	Associação com alta experiência de cárie (PATIR <i>et al.</i> , 2008; SHIMIZU <i>et al.</i> , 2012; ERGOZ <i>et al.</i> , 2014; WEBER <i>et al.</i> , 2018). Forte associação ente SNP (rs4694075) e crianças com cárie (GERRETH <i>et al.</i> , 2017). A frequência do alelo C em indivíduos com alta experiência de cárie foi maior do que no grupo com baixa experiência de cárie (C:T 210:388 no grupo de alta experiência) e C:T 105:285 no grupo de baixa experiência. A sobreexpressão do alelo T foi associada com CPOD maior que 10 (SHIMIZU <i>et al.</i> , 2012). Nenhuma associação observada (SLAYTON <i>et al.</i> , 2005; DEELEY <i>et al.</i> , 2008; JEREMIAS <i>et al.</i> , 2013; ABBASOGLU <i>et al.</i> , 2015)		
	SNPs: rs4694075 (C/T) (SHIMIZU <i>et al.</i> , 2012); rs34538475 (G/T) (PATIR <i>et al.</i> , 2008); rs34538475 (G/T), rs4694075		

(C/T) (GERRETH <i>et al.</i> , 2017); rs4694075 (C/T), rs34538475 (G/T) (ABBASOGLU <i>et al.</i> , 2015); hCV496502 (G/T) (DEELEY <i>et al.</i> , 2008); rs496502 (G/T), rs4694075 (C/T) (JEREMIAS <i>et al.</i> , 2013); rs34538475 (G/T), rs4694075 (C/T) (ERGOZ <i>et al.</i> , 2014); rs4694075 (C/T) (WEBER <i>et al.</i> , 2018)			
<i>Kallikrein-related peptidase 4 (KLK4)</i>	Degrada proteínas do esmalte	19q13.41	(WANG <i>et al.</i>, 2012; ABBASOGLU <i>et al.</i>, 2015; CAVALLARI <i>et al.</i>, 2017; GERRETH <i>et al.</i>, 2017; WEBER <i>et al.</i>, 2018) Associado com baixa experiência de cárie; No entanto, o alelo G (rs2235091) foi associado a um aumento do risco de cárie (WANG <i>et al.</i>, 2012); No rs198969, foi observado uma associação do genótipo GG em crianças com cáries comparadas com crianças sem cárie (GERRETH <i>et al.</i>, 2017); No SNP rs2235091 não foi observada nenhuma diferença entre a distribuição do genótipo e a frequência de cárie dental (GERRETH <i>et al.</i>, 2017); rs2235091 (A/G) mostrou estar associado com baixa experiência de cárie em comparação a crianças com alta experiência assim como Cáries Free em comparação com alta experiência de cárie (WEBER <i>et al.</i>, 2018); Genótipos AG e GG no KLK4 (rs198968) foram fatores de risco para cárie precoce em crianças (ABBASOGLU <i>et al.</i>, 2015); Alelo A do SNP rs2242670 foi associado com u aumento da susceptibilidade à cárie dental (CAVALLARI <i>et al.</i>, 2017).

SNPs: rs2235091 (A/G); rs198969 (C/G) (WANG <i>et al.</i> , 2012); rs198969 (C/G), rs2235091 (A/G) (GERRETH <i>et al.</i> , 2017); rs198969 (C/G), rs2235091 (A/G) (WEBER <i>et al.</i> , 2018); rs2235091 (C/T), rs198968 (A/G) (ABBASOGLU <i>et al.</i> , 2015); rs2242670 (A/G), rs2242670 (A/G), rs2235091 (A/G), rs2978642 (A/T), rs2978642 (A/T), rs2978643 (C/G) (CAVALLARI <i>et al.</i> , 2017)				
<i>Dentin sialophosphoprotein (DSPP)</i>	Envolvida no processo de mineralização da dentina	4q22.1	(WANG <i>et al.</i> , 2012)	Associada com uma baixa experiência de cárie (WANG <i>et al.</i> , 2012).
SNPs: rs2615487 (C/T) (WANG <i>et al.</i> , 2012)				
<i>Arachidonate 15-lipoxygenase (ALOX15)</i>	Embora este gene tenha sido relacionado à resposta inflamatória, ele também foi associado com a mineralização óssea. Desta forma, especula-se que ele poderia estar envolvido na formação das estruturas minerais do dente	17p13.2	(ABBASOGLU <i>et al.</i> , 2015)	O genótipo TT em rs7217186 foi um fator de risco para cárie precoce em crianças (ABBASOGLU <i>et al.</i> , 2015).
SNP: rs2619112 (A/G), rs7217186 (C/T) (ABBASOGLU <i>et al.</i> , 2015)				
Genes da resposta imune				
<i>Beta defensin 1 (DEFB1)</i>	As defensinas formam uma família de peptídios microbicidas e citotóxicos produzidos por	8p23.1	(OZTURK <i>et al.</i> , 2010; KRASONE <i>et al.</i>	Associada com cárie dental (NAVARRA <i>et al.</i> , 2016; YILDIZ <i>et al.</i> , 2016); rs179946 foi correlacionado com baixo CPO-D (OZTURK <i>et al.</i> , 2010); O SNP rs11362

	neutrófilos. Os membros da família da defensina são altamente similares na sequência de proteínas. Este gene codifica a defensina, beta 1, um peptídeo antimicrobiano implicado na resistência das superfícies epiteliais à colonização microbiana		<i>al.</i> , 2014; NAVARRA <i>et al.</i> , 2016; YILDIZ <i>et al.</i> , 2016; LIPS <i>et al.</i> , 2017)	influenciou a susceptibilidade à cárie (KRASONE <i>et al.</i> , 2014); Polimorfismos não foram associados com cárie em crianças (LIPS <i>et al.</i> , 2017)
	rs11362 (G/A) (YILDIZ <i>et al.</i> , 2016); rs11362 (A/G), rs1800972 (C/G), rs179946 (A/T) (OZTURK <i>et al.</i> , 2010), rs11362 (A/G) (KRASONE <i>et al.</i> , 2014); rs11362 (C/T), rs1799946 (C/T) (LIPS <i>et al.</i> , 2017); rs11362 (A/G), rs1799946 (T/C) (NAVARRA <i>et al.</i> , 2016)			
<i>Lactotransferrin (LTF)</i>	Este gene é um membro da família de genes da transferrina e seu produto proteico é encontrado nos grânulos secundários de neutrófilos. A proteína é uma importante proteína de ligação de ferro no leite e secreções corporais com	3p21.31	(AZEVEDO <i>et al.</i> , 2010; FINE <i>et al.</i> , 2013; VOLCKOVA <i>et al.</i> , 2014; ABBASOGLU <i>et al.</i> , 2015;	Alelo A do SNP rs6441989 foi significativamente menos frequente no grupo com alta experiência de cárie, mostrando um efeito protetor para a cárie dental (DOETZER <i>et al.</i> , 2015); De forma semelhante, rs1126478 (A/G) foi protetor para cárie (AZEVEDO <i>et al.</i> , 2010; FINE <i>et al.</i> , 2013). Genótipo CT no SNP rs4547741 foi um fator de risco para cárie em crianças (ABBASOGLU <i>et al.</i> , 2015)

uma atividade antimicrobiana, tornando-se um componente importante do sistema imune não específico. A proteína demonstra um amplo espectro de propriedades, incluindo regulação da homeostase de ferro, defesa do hospedeiro contra uma ampla gama de infecções microbianas, atividade antiinflamatória, regulação do crescimento celular e diferenciação e proteção contra o desenvolvimento do câncer e metástases. Foi encontrada atividade antimicrobiana, antiviral, antifúngica e antiparasitária para esta proteína e seus péptidos.	DOETZER <i>et al.</i>, 2015; WANG <i>et al.</i>, 2017)	Nenhuma diferença na experiência de cárie foi encontrada entre o SNP rs1126478 e a frequência dos alelos genotipados (WANG <i>et al.</i>, 2017). Nenhuma associação com SNP rs1126478 (A/G) (VOLCKOVA <i>et al.</i>, 2014)
rs6441989 (A/G), rs2073495 (C/G), rs11716497 (A/G) (DOETZER <i>et al.</i>, 2015); rs1126478 (A/G) (WANG <i>et al.</i>, 2017);		

	rs2269436 (A/G), rs743658 (A/G), rs4547741 (C/T), rs17078878 (A/C) (ABBASOGLU <i>et al.</i> , 2015); rs1126478 (A/G) (VOLCKOVA <i>et al.</i> , 2014); rs1126478 (A/G) (FINE <i>et al.</i> , 2013); rs1126478 (A/G) (AZEVEDO <i>et al.</i> , 2010)			
<i>Mannose binding lectin 2 (MBL2)</i>	Codifica a lectina solúvel em ligação ao manose ou a proteína de ligação à manose encontrada no soro. A proteína codificada pertence à família coletora e é um elemento importante no sistema imune inato. A proteína reconhece manose e N-acetilglucosamina em muitos microorganismos e é capaz de ativar a via de complemento clássica. As deficiências deste gene têm sido associadas à susceptibilidade a doenças auto-imunes e infecciosas	10q21.1	(OLSZOWSKI <i>et al.</i> , 2012; YANG <i>et al.</i> , 2013; ALYOUSEF <i>et al.</i> , 2017; SHIMOMURA-KUROKI <i>et al.</i> , 2018)	Foi observada uma maior percentagem de indivíduos portadores do alelo G no polimorfismo rs7096206 em indivíduos do grupo com alta experiência de cárie comparado com baixa experiência (OLSZOWSKI <i>et al.</i> , 2012); Polimorfismo rs11003125 no gene <i>MBL2</i> foi associado com alta prevalência de cárie (ALYOUSEF <i>et al.</i> , 2017) CPO-D foi associado com <i>MBL2</i> (SHIMOMURA-KUROKI <i>et al.</i> , 2018); Nenhuma associação foi observada (YANG <i>et al.</i> , 2013)
	rs7096206 (C/G) (OLSZOWSKI <i>et al.</i> , 2012); rs7096206 (C/G), rs11003125 (C/G) (ALYOUSEF <i>et al.</i> , 2017)			
<i>Mannan-binding</i>	Este gene codifica um membro	1p36.22	(OLSZOWSKI	Nenhuma associação observada (OLSZOWSKI <i>et al.</i> ,

<i>lectin serine peptidase 2 (MASP2)</i>	da família peptidase S1 de serina proteases. A pré- proteína codificada é processada proteoliticamente para gerar cadeias A e B que se heterodimerizam para formar a protease madura. Esta protease cliva os componentes do complemento C2 e C4 para gerar C3 convertase na via lectina do sistema do complemento. Fator bactericida que se liga aos polissacarídeos Ra e R2 expressos por certas enterobactérias		<i>et al.</i> , 2012) Olszowski 2012	2012)
rs72550870 (A/G) (OLSZOWSKI <i>et al.</i> , 2012)				
Genes da Composição e Fluxo Salivar				
<i>Aquaporin 5 (AQP5)</i>	Desempenha um papel na produção de saliva	12q13.12	(WANG <i>et al.</i> , 2012; ANJOMSHOAA	Associações consistente como proteção contra cárie dental (WANG <i>et al.</i> , 2012) Associação entre AQP5 e experiencia de cárie

			<i>et al.</i> , 2015)	(ANJOMSHOAA <i>et al.</i> , 2015)
SNPs: rs923911 (A-C); rs1996315 (A-G) (WANG <i>et al.</i> , 2012); rs3759129 (A/C) (ANJOMSHOAA <i>et al.</i> , 2015)				
<i>Protein-rich Protein HaeIII subfamily 1 (PRH1)</i>	Codifica glicoproteínas salivares ricas em prolina secretadas nas glândulas parótida e submandibular/ sublingual. Certos alelos deste gene estão associados à susceptibilidade a cárie dental.	12p13.2	(ZAKHARY <i>et al.</i> , 2007)	Associada com uma alta experiência de cárie e colonização por <i>Streptococcus Mutans</i> (ZAKHARY <i>et al.</i> , 2007)
<i>Carbonic Anhydrase 6 (CA6)</i>	A proteína codificada por este gene é uma das várias isozimas de anidrase carbônica. Esta proteína é encontrada apenas nas glândulas salivares e na saliva. Ela pode desempenhar um papel na hidratação reversível do dióxido de carbono, embora sua função na saliva seja desconhecida.	1p36.23	(PERES <i>et al.</i> , 2010; LI <i>et al.</i> , 2015; SENGUL <i>et al.</i> , 2016; YILDIZ <i>et al.</i> , 2016)	Halótipo ACA (rs2274328, rs17032907 e rs11576766) foi associado com um baixo CPOD (LI <i>et al.</i> , 2015); Não foi observada associação (SENGUL <i>et al.</i> , 2016; YILDIZ <i>et al.</i> , 2016); A distribuição do genótipo CA6 e das frequências de alelos no grupo de baixo risco de cárie não diferiu do grupo de alto risco de cárie (YILDIZ <i>et al.</i> , 2016)

SNP: rs2274328 (A/C); rs17032907 (C/T); rs11576766 (A/C); rs2274333 (G/A); rs10864376 (T/C); rs3765964 (T/C);
rs6680186 (A/G) (Li *et al.*, 2015); rs2274327 (C/T); rs2274328 (A/C), rs2274333 (A/G) (PERES *et al.*, 2010);
rs2274327 (C/T CA6) (YILDIZ *et al.*, 2016); rs2274327 (A/G) (SENGUL *et al.*, 2016)

2.2.1 Objetivo geral

Investigar a associação e a interação (gene-gene) dos polimorfismos genéticos (SNPs ligados ao desenvolvimento dental, a resposta imune do hospedeiro, a composição e fluxo salivar e a sensibilidade gustativa) com a experiência de cárie dental em revisões sistemáticas e entre os indivíduos da coorte de nascimento de 1982 de Pelotas, no sul do Brasil.

2.2.2 Objetivos específicos

- Revisar sistematicamente a literatura científica identificando e discutindo os principais polimorfismos relacionados com os genes da sensibilidade gustativa e a cárie dental.
- Revisar sistematicamente a literatura científica identificando e discutindo os principais polimorfismos relacionados com os genes do desenvolvimento dental e a cárie dental.
- Revisar sistematicamente a literatura científica identificando e discutindo os principais polimorfismos relacionados com os genes da resposta imune do hospedeiro e a cárie dental.
- Revisar sistematicamente a literatura científica identificando e discutindo os principais polimorfismos relacionados com os genes da composição/função e fluxo salivar e a cárie dental.
- Investigar a associação e a interação dos genes gustativos (*TAS2R38*, *TAS1R2*, *GNAT3* e *SLC2A2*) com os hábitos alimentares e com a experiência de cárie dental dos indivíduos da coorte de 1982 de Pelotas
- Investigar a associação e a interação dos genes do desenvolvimento dental (*MMP20*, *ALOX15*, *TUFT1*, *AMELX*, *ENAM*, *TFIP11*, *AMBN*, *KLK4* e *DSPP*) com a experiência de cárie dental dos indivíduos da coorte de 1982 de Pelotas
- Investigar a associação e a interação dos genes da resposta imune (*MBL2*, *DEFB1*, *MASP2* e *LTF*) com a experiência cárie dental dos indivíduos da coorte de 1982 de Pelotas

- Investigar a associação e a interação dos genes da composição/função salivar (*AQP5*, *PRH1* e *CA6*) com a experiência de cárie dental dos indivíduos da coorte de 1982 de Pelotas

2.2.3 Hipóteses

As hipóteses a serem testadas serão que polimorfismos genéticos podem influenciar a experiência de cárie dental ao longo da vida, ainda, que a interação gene-gene entre grupos de polimorfismos (relacionados ao desenvolvimento dental, a resposta imune do hospedeiro, a composição e fluxo salivar e a sensibilidade gustatória) podem aumentar o risco destes indivíduos no estabelecimento e/ou progressão da cárie dental.

2.3. Materiais e métodos

Os estudos observacionais serão reportados seguindo o STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) (VON ELM *et al.*, 2007), enquanto as revisões sistemáticas serão reportadas pelo PRISMA (MOHER *et al.*, 2009)

2.3.1. Revisões Sistemáticas

Inicialmente, será realizado o registro das revisões no PROSPERO (*International Prospective Register of Systematic Reviews*).

2.3.1.1. Perguntas de pesquisa: as perguntas de pesquisa serão estruturadas seguindo o modelo P.I.C.O.

- **Participantes/população:** Indivíduos (adultos e crianças)
- **Intervenção/exposição:** Polimorfismos genéticos
- **Comparação/controle:** Ausência do polimorfismo
- **Desfecho:** Experiência de cárie

Quais são os polimorfismos genéticos relacionados com a (1- composição e formação do esmalte dental; 2- sensibilidade gustativa; 3- resposta imune do hospedeiro; e 4- composição/função e fluxo salivar) que influenciam a experiência de cárie dental em adultos e/ou crianças? Desta forma, serão realizadas 4 revisões sistemáticas agrupando os quatro principais grupos de polimorfismos relacionados com a cárie dental reportados acima.

2.3.1.2. Estratégia de Busca

A estratégia de pesquisa será realizada utilizando palavras-chave relevantes e entre termos MeSH considerando a estrutura de cada base de dados. A estratégia de pesquisa completa é detalhada na tabela 2.

Tabela 2 - Estratégia de busca estruturada seguindo a sintaxe da base de dados MEDLINE/PubMed. A pesquisa seguirá a estrutura de cada base de dados.

Sintaxe da busca	
PubMed	#1 (“Dental Decay” OR “Caries, Dental” OR “Decay, Dental” OR “Cariou Dentin” OR “Cariou Dentins” OR “Dentin, Cariou” OR “Dentins, Cariou” OR “Dental White Spot” OR “White Spots, Dental” OR “White Spots” OR “Spot, White” OR “Spots, White” OR “White Spot” OR “Dental White Spots” OR “White Spot, Dental” OR “Susceptibility, Dental Caries” OR “Caries Susceptibility, Dental” OR “Caries Resistance, Dental” OR “Resistance, Dental Caries” OR “Dental Caries Resistance”)
	#2 (“Polymorphisms, Genetic” OR “Genetic Polymorphisms” OR “Genetic Polymorphism” OR “Polymorphism” OR “Polymorphisms” OR “Nucleotide Polymorphism, Single” OR “Nucleotide Polymorphisms, Single” OR “Polymorphisms, Single Nucleotide” OR “Single Nucleotide Polymorphisms” OR “SNPs” OR “Single Nucleotide Polymorphism”)

*** Combinação das buscas: #1 AND #2**

Serão pesquisados cinco bancos de dados (PubMed/MedLine, Scopus, ISI Web of Science, BVS - Biblioteca de saúde virtual, Scielo) utilizando as sintaxes de buscas descritas acima. Os artigos que serão encontrados irão ser carregados no software EndNote™ (Thomson Reuters, Rochester, Nova York, NY, EUA), criando assim uma biblioteca virtual. Os estudos duplicados serão identificados e excluídos. Dois revisores independentes irão ler os títulos e resumos de todos os artigos. Serão incluídos artigos que objetivem avaliar a associação entre polimorfismos genéticos (em crianças ou adultos) e fatores genético relacionados com 1) composição e formação do esmalte dental; 2) sensibilidade gustativa; 3) resposta imune do hospedeiro; e 4) composição/função e fluxo salivar. Além disso, somente estudos

em humanos serão incluídos com desenho transversal e/ ou longitudinal. Não serão consideradas quaisquer restrições de idioma ou período de publicação. Estudos com design de revisões de literatura, relatos de casos e séries de casos, resumos de conferências, cartas para o editor e estudos qualitativos serão excluídos.

Os mesmos revisores lerão o texto completo e avaliarão os artigos. Em caso de desentendimento, os mesmos revisores discutirão até obter um consenso. Em caso de desacordo, a decisão será determinada por um terceiro revisor.

2.3.1.3. Coleta de Dados

A extração de dados será realizada de forma independente por dois revisores em uma planilha eletrônica predefinida. Os seguintes dados serão extraídos: Autor, ano, país, desenho do estudo, amostra, idade, etnia da amostra (% para cada etnia), percentagem entre os sexos da amostra, cálculo de poder estatístico, avaliação de categorização da cárie dental, polimorfismos avaliados, frequência do menor alelo do polimorfismo, abordagem analítica, análise de dados (valores de análise bruta e ajustada e seus respectivos intervalos de confiança), co-variáveis e principais resultados.

2.3.1.4. Qualidade dos estudos

A qualidade dos estudos incluídos será verificada através da escala *Appraisal Checklist for observational studies* (Joanna Briggs Institute) (T.J.B., 2014). Esta ferramenta apresenta 10 questões avaliando diferentes pontos no estudo, que devem ser respondidas com "Não", "não claro" ou "Sim". Cada resposta "Sim" corresponde a um ponto, portanto, o escore da ferramenta varia de 0 a 10. Estudos sinalizados com 0 a 3 serão considerados de baixa qualidade; 4 a 6 serão de qualidade média qualidade; e 7 a 10 serão considerados de alta qualidade. Para classificar os estudos, dois revisores realizarão a classificação de forma independente. Os desentendimentos serão sanados através da discussão até que o consenso seja alcançado. Da mesma forma, quando não for encontrado um

consenso entre os avaliadores, um terceiro autor será responsável por tomar a decisão final.

2.3.1.5. Estratégia para síntese de dados:

Uma meta-análise poderá ser realizada para cada uma das revisões. Quando o mesmo polimorfismo for identificado em pelo menos 3 estudos distintos, o mesmo será considerado para análise estatística. Em estudos que apresentarem mais de uma categoria para o desfecho ou para a variável de exposição, será considerada a categoria mais severa da doença.

Para a meta-análise, serão incluídos, de preferência, os resultados ajustados. Nos casos em que os resultados ajustados não foram relatados, as estimativas brutas serão consideradas ou calculadas. Quando os dados não estiverem disponíveis, os autores serão contatados. Razão de ODDS (OR) será usada para medir o tamanho do efeito com 95% de Intervalo de Confiança (IC). As medidas de razão de prevalência serão convertidas para OR utilizando a fórmula proposta por Zhang e Yu: $PR = odds\ ratio / 1 - risk_0 + risk_0 \times odds\ ratio$, onde $risk_0$ é a prevalência de doença entre indivíduos não expostos (ZHANG E YU, 1998). Modelos fixos e randômicos serão utilizados para avaliar a associação entre os polimorfismos e a cárie dental.

A heterogeneidade será avaliada com a estatística I^2 e considerada alta quando I^2 for superior a 50%. Para se observar o efeito de cada estudo sobre a estimativa agrupada, será utilizada a análise de sensibilidade. Todas as análises serão realizadas usando o software Stata 12.0 (StataCorp, College Station, TX, EUA)

2.3.2. Estudos observacionais

2.3.2.1. Desenho do estudo

O presente estudo será conduzido de forma observacional e prospectiva a partir de uma coorte de nascimentos. No ano de 1982, todos os nascidos vivos nas 3 maternidades da cidade de Pelotas (localizada no estado do Rio Grande do Sul) foram identificados e submetidos a um inquérito de saúde perinatal. Estas crianças foram pesadas e as mães foram submetidas a uma entrevista sobre condições socioeconômicas, demográficas e de saúde geral. No total, 5.914 crianças foram identificadas, isso representou 99,2% dos nascimentos daquele ano na cidade de Pelotas. Esta população segue sendo acompanhada ao longo da vida (BARROS *et al.*, 2008).

2.3.2.2. Estudos de saúde geral

Após a realização do primeiro levantamento nas maternidades, estes mesmos indivíduos foram novamente procurados nos anos de 1983, 1984, 1986, 1995, 1997, 1998, 2000, 2001, 2004, 2006 e 2013. No entanto, em alguns anos apenas algumas subamostras foram acessadas. No ano de 2004, toda a amostra da coorte de 1982 foi entrevistada e, além de perguntas relativas à saúde destes indivíduos, foi aplicado um questionário de frequência alimentar. Além deste questionário, foi realizada uma coleta de material genético (com a realização posterior de genotipagem) destes indivíduos (maiores detalhes na seção de variáveis independentes).

2.3.2.3. Estudos de saúde bucal (ESB)

Quando os indivíduos tinham 15 anos de idade (1997), um estudo de saúde bucal (ESB-97) foi realizado em uma amostra desta mesma coorte. Tal amostra representativa foi obtida através da busca dos indivíduos da coorte em 70 setores censitários (27% do total) da zona urbana de Pelotas. Foram localizados 1076 indivíduos pertencentes a coorte, dos quais, 900 foram aleatoriamente selecionados, compondo a amostra do ESB-97. Neste acompanhamento, foi realizado uma entrevista contendo questões sobre os hábitos de higiene bucal, utilização de serviços odontológicos e dor de origem dental. Além disso, exames odontológicos foram realizados avaliando a presença de cárie e problemas oclusais.

Os 888 jovens participantes (98,7%) do ESB-97 foram contatados em 2006 (ESB-06) para uma nova entrevista, composta também pela realização de exames odontológicos. Desta forma, foi possível coletar informações sobre cárie dentária, qualidade das restaurações em dentes posteriores e lesões bucais, entre outros. Além disto, na entrevista os indivíduos foram questionados sobre uso de serviços odontológicos, episódios de dor de origem dental e hábitos de higiene oral. Um total de 720 indivíduos compuseram o ESB-06, representando 80% da amostra inicial (PERES *et al.*, 2011).

Após isso, no ano de 2013, os 900 indivíduos da amostra inicial, foram novamente contatadas com finalidade de aumentar a amostra perdida durante o período. Todos os indivíduos localizados e que aceitaram continuar do estudo (agora com 31 anos de idade) compuseram o Estudo de Saúde Bucal de 2013 (ESB-13). De forma semelhante aos estudos anteriores, no ESB-13 os indivíduos também foram entrevistados (sendo aplicado um questionário) e exames clínicos foram realizados. Diversas condições de saúde bucal foram avaliadas neste estudo, dentre as quais a presença de lesões cariosas e de restaurações nos dentes destes indivíduos.

2.3.2.4. Variáveis dependentes (fenótipo)

A variável desfecho do presente estudo será a cárie dental dos participantes que será avaliada em 3 pontos da vida dos indivíduos (15, 24 e 31 anos). Foram colhidas os CPO-D aos 15 e 24 anos. Aos 31 anos de idade, a cárie dental foi avaliada através do índice CPO-S (WHO, 1997), proporcionando assim, um maior detalhamento em relação às exatas superfícies acometidas pela cárie.

Desta forma serão obtidas diferentes categorizações de cárie dental:

- (1) CPOD-total:** número total de superfícies dentárias com experiência de cárie (componentes cariado, perdido e obturado) aos 31 anos (variável contínua);
- (2) CPOS-Oclusal:** número total de superfícies dentárias com experiência de cárie (componentes cariado, perdido e obturado) aos 31 anos nas superfícies oclusais (variável contínua);

(3) CPOS-Livres: número total de superfícies dentárias com experiência de cárie (componente cariado, perdido e obturado) aos 31 anos nas superfícies livres (variável contínua);

(4) CPOD-alto/médio/baixo: Indivíduos serão categorizados em baixo CPOD ($CPOD \leq 5$), médio (CPOD entre 5 e 14) e alto ($CPOD \geq 14$) (variável categórica) (Yildiz 2015).

(5) CPOD-cárie não-tratada: indivíduos que apresentaram nas três avaliações clínicas (15, 24 e 31 anos) componente C do CPOD ≥ 1 (variável categórica).

(6) Traj-CPOD: será criada a trajetória do CPO-D dos indivíduos através do *Group-Based trajectory modelling*. (Maiores detalhes são descritos abaixo) (variável categórica)

(7) Traj-cárie: de forma semelhante a Traj-CPOD, será criada a trajetória apenas do componente C do CPO-D, através do *Group-Based trajectory modelling* (variável categórica).

(8) Cárie-Livre: Serão categorizados os indivíduos cárie livre Vs. Com experiência de cárie (variável dicotômica).

(Variáveis categóricas: CPOD-alto/médio/baixo, CPOD-cárie não tratada, Traj-CPOD, Traj-cárie; variável dicotômica e variáveis lineares: CPOD-total, CPOS-Oclusal, CPOS-Livres).

O *Group-Based trajectory modelling* será utilizado para identificar grupos com trajetórias semelhantes do componente “C” (Traj-cárie) e do CPO-D (Traj-CPOD) no percurso da vida (ESB-97, ESB-06 e ESB-13). Assim, os modelos serão estimados com o comando “traj” no programa Stata 12.0 (JONES *et al.*, 2001) Identificando a similaridade da trajetória entre os indivíduos avaliados. Os parâmetros para a trajetória de modelos serão determinada baseada na máxima verossemelhança pelo método de *quasi-Newton* (DENNIS *et al.*, 1981; JONES E NAGIN, 2007). A seleção dos modelos será considerada e estimada pelo número latentes de categorias e pela ordem polinomial de cada trajetória latente. O número de trajetórias será determinado quando através das comparações sequenciais do *Bayesian information criterion* (BIC) e com seus critérios de ajustes entre o modelo com K e K+1 trajetórias não produzirá diferença substancial no escore BIC do modelo k + 1. Assim, será definido o número de grupos de trajetórias para as variáveis desfecho Traj-Cárie e Traj-CPOD.

2.3.2.5. Coleta de material genético e genotipagem

A coleta de material genético dos participantes da coorte de nascimentos de 1982 de Pelotas foi coletada durante o período de outubro de 2004 a agosto de 2005. Todos os participantes localizados na área urbana da cidade foram visitados. Assim, os participantes (de 22 a 23 anos) foram entrevistados e examinados em casa e convidados a visitar o laboratório de pesquisa para doar uma amostra de sangue, coletada por punção venosa. O DNA e o soro foram extraídos e congelados a -70° C. As amostras de DNA foram genotipadas usando *Illumina HumanOmni2.5-8v1 array* (VICTORA E BARROS, 2006; HORTA *et al.*, 2015).

Além disso, a ancestralidade genômica será avaliada usando ADMIXTURE (ALEXANDER *et al.*, 2009) baseado em aproximadamente 370 000 SNPs disponíveis na coorte de nascimentos de 1982 de Pelotas compatíveis com os projetos HapMap e Human Genome Diversity para a população brasileira (LIMA-COSTA *et al.*, 2015).

2.3.2.6. Variáveis independentes principais

2.3.2.6.1. *Single nucleotide polymorphism* avaliados

As variáveis independentes principais a serem relacionadas com o fenótipo serão os SNPs. Alguns polimorfismos já foram identificados previamente na introdução deste documento (Tabela 1):

- Genes gustativos: *TAS2R38*, *TAS1R2*, *GNAT3* e *SLC2A2*
- Genes do desenvolvimento dental: *MMP20*, *ALOX15*, *TUFT1*, *AMELX*, *ENAM*, *TFIP11*, *AMBN*, *KLK4* e *DSPP*
- Genes da resposta imune *MBL2*, *DEFB1*, *MASP2* e *LTF*
- Genes da composição/função salivar: *AQP5*, *PRH1* e *CA6*

Além dos genes já identificados, serão conduzidas revisões sistemáticas da literatura com finalidade de identificar genes adicionais com possível implicação na cárie dental (maiores detalhes na seção 3.1.). Desta forma, cada SNP identificado (Tabela 1 + revisões sistemáticas) em cada grupo de genes reportado acima será

investigado de forma independente. Serão calculadas as frequências dos respectivos alelos e genótipos de cada um dos SNPs. Será testado o equilíbrio de Hardy-Weinberg (maiores detalhes na seção de análise de dados). Serão utilizados dois modelos de efeito genético:

- Aditivo: Cada genótipo é codificado como uma categoria distinta de acordo com a quantidade de alelos de risco que apresenta. Desta forma, em um SNP hipotético onde temos a mudança das bases nitrogenadas de “A” para “G” (A/G) podemos ter os seguintes genótipos: AA (categoria 0), AG (categoria 1) e GG (categoria 2).

- Dominante: Serão codificadas apenas duas categorias. Desta forma, para o mesmo SNP hipotético, teremos: AA (categoria 0), AG (categoria 0) e GG (Categoria 1).

2.3.2.7. Variáveis independentes (covariáveis)

As variáveis independentes de ajuste que serão utilizadas no estudo foram obtidas dos levantamentos realizados no nascimento, aos 22, 24 e 31 anos de idade. Uma lista das variáveis independentes (controle) e suas respectivas categorizações pode ser observada na tabela 3.

2.3.2.7.1. Variáveis individuais demográficas e socioeconômicas

O sexo dos indivíduos foi coletado no primeiro levantamento em 1982, logo após o nascimento dos indivíduos. A ancestralidade genômica será utilizada sendo definida por dez componentes na análise de componentes principais. Para escolaridade do indivíduo, foram coletados o número de anos de estudo. Estes serão categorizados em três grupos (>12 ; de 9 a 11; ≤ 8 anos).

A renda familiar aos 31 anos de idade será coleta de forma contínua e os indivíduos serão categorizados em tercís para realização das análises. Assim, os participantes serão agrupados em uma categoria denominada “tercil mais pobre” (1º tercís) e em outra denominada de “tercis menos pobres” (2º e 3º tercís de renda).

2.3.2.7.2. Variáveis comportamentais

A utilização de serviços odontológicos foi medida aos 24 e será novamente avaliada aos 31 anos de idade através da pergunta: “Alguma vez na vida foste ao consultório do dentista?” com possíveis respostas Sim e Não. Além disto, através da pergunta “Onde você foi atendido na última consulta?” será possível obter a informação referente ao tipo de serviço que a pessoa utilizou: a) dentista particular; b) dentista de convênio; c) faculdade de odontologia, d) posto de saúde; e) no local onde trabalho; F) outro. Desta forma, as variáveis serão categorizadas de acordo com a forma de pagamento dos serviços odontológicos prestados. As opções “faculdade de odontologia” e “posto de saúde” serão agrupadas em serviço público; “dentista particular” será considerado serviço privado; e “dentista de convênio” e “local de trabalho” serão agrupados em convênio.

Um questionário de frequência alimentar foi aplicado no ano de 2004 para todos os indivíduos da coorte. Neste questionário, perguntas referentes ao consumo de alimentos doces (sorvete, balas, chocolate, pudins doces, refrigerantes) e de açúcar foram realizadas. Além do consumo propriamente dito, a frequência (variando de 0 a 10) diária, semanal, mensal ou anual. Desta forma, será realizada a soma bruta da quantidade de açúcar diária consumida para cada indivíduo em um ano. Esta, por sua vez, será categorizada em tercís.

2.3.2.7.3. Variáveis clínicas

A qualidade da higiene bucal dos pacientes será realizada através da presença de sangramento gengival de cada indivíduo. O Sangramento do tecido gengival foi examinado clinicamente em seis sítios para cada elemento dental (sítio mesiovestibular, vestibular, distovestibular, mesiolingual, lingual e distolingual) (SUSIN *et al.*, 2005). Os indivíduos serão classificados com presença de sangramento gengival quando possuírem $\geq 10\%$ dos sítios com sangramento gengival (GARLET *et al.*, 2012; AAP, 2015). Em contrapartida, indivíduos que apresentarem $< 10\%$ dos sítios com sangramento gengival serão considerados sem sangramento (periodontalmente saudáveis).

Tabela 3 – Lista de variáveis independentes (controle) e suas respectivas categorizações.

Variáveis	Categorias
1. Individuais	
Sexo	Masculino e feminino
Ancestralidade genômica	Europeus, Africanos e Nativos Americanos
2. Sociodemográficas	
Escolaridade aos 31 anos	≥12, de 9 a 11, ≤ 8 anos
Renda familiar 31 anos de idade	“Menor tercil” (1º tercil) e “Maiores Tercis” (2º e 3º tercis de renda)
3. Comportamentais	
Utilização de serviços odontológicos no último ano aos 31 anos	Sim e não
Tipo de serviço utilizado	Serviço público, privado e convênio
Consumo de açúcar (22 anos)	Alto / Médio / Baixo
Escovação dental	Pouco Frequente (Nunca e Sim às vezes), Frequente (1 vez ao dia todos os dias, 2 vezes ao dia todos os dias, 3 vezes ao dia ou + todos os dias)
Uso de fio dental	Não (Nunca e às vezes) e Sim (Sempre)
4. Clínicas	
Sangramento gengival	Sim (≥ 10% dos sítios) e Não (< 10% dos sítios)

2.3.2.8. Trabalho de campo

Os exames odontológicos foram realizados por 06 alunos do Programa de Pós-Graduação em Odontologia da Universidade Federal de Pelotas (UFPeL). Além

destes, oito anotadores também participaram da equipe. Todos os examinadores e entrevistadores foram treinados e calibrados seguindo metodologia previamente descrita (PERES *et al.*, 2001). A reprodutibilidade diagnóstica foi aferida pela estatística Kappa (variáveis categóricas). O menor valor de kappa aceitável neste estudo foi de 0,65. Além disso, 10% das entrevistas foram repetidas com uma versão resumida do questionário.

Para realização do exame foram utilizados avental, máscara, gorro e luvas descartáveis, luz artificial adaptada à cabeça do examinador, espelho bucal e sonda periodontal NIDR, sendo os dois últimos previamente autoclavados.

2.3.2.9. Análise dos dados

O software STATA versão 12.0. (StataCorp, College Station, TX, EUA) será utilizado para organização do banco de dados. Posteriormente o Programa PILNK será utilizado para realização da análise dos dados. Será realizada uma análise descritiva para determinar a frequência relativa e absoluta das variáveis independentes e dependentes em relação aos genótipos avaliados. SNPs que não estiverem em equilíbrio de Hardy-Weinberg serão excluídos das análises de associação (maiores detalhes na sessão 3.2.9.1.).

Para controle dos possíveis fatores confundidores na associação entre os genótipos e fenótipos serão utilizados modelos de regressão logística para variáveis categóricas (CPOD-alto/médio/baixo, CPOD-cárie, Traj-CPOD, Traj-cárie) e modelos de regressão linear para variáveis contínuas (CPOD-total, CPOS-Oclusal, CPOS-Livres). Correções de Bonferroni serão utilizadas para correções por múltiplos testes. Além disso, dois modelos de efeitos genéticos serão utilizados para cada um dos SNPs: aditivo e dominante. Posteriormente, serão realizadas análises complementares de regressão para cada grupo de genes (gustativos, desenvolvimento dental, resposta imune, composição/função salivar) para testar as interações gene-gene (*Gene-Gene Interaction Analysis*) (ANJOMSHOAA *et al.*, 2015).

Somente as variáveis que na análise bivariada apresentarem valor $p < 0,25$ serão incluídas nos modelos finais. Serão obtidas as razões de ODDS para as variáveis de interesse e seus respectivos intervalos de confiança de 95%.

2.3.2.9.1. *Hardy-Weinberg equilibrium*

A equação de Hardy-Weinberg será utilizada para medir se as frequências de genótipos observadas na presente população diferem das frequências previstas pela equação. As frequências (Hardy-Weinberg equilibrium) em ambos os grupos (observada Vs. esperada) serão determinadas usando o teste exato de Fisher e o teste de X^2 para determinar se a frequência observada difere ou não estatisticamente da frequência esperada.

O equilíbrio de Hardy-Weinberg se baseia no princípio de que a quantidade de variação genética em uma população permanecerá constante de uma geração para outra na ausência de fatores perturbadores. Os principais pressupostos: grande tamanho da população, não ocorrência de mutação, não ocorrência de imigração ou emigração, ocorrência de acasalamento aleatório e o sucesso reprodutivo aleatório. Quando estes pressupostos são mantidos, uma distribuição randômica dos alelos é esperada.

Assim, iremos examinar, por exemplo, um locus genético simples no qual existem dois alelos A e a através da equação Hardy-Weinberg, a qual é expressa como:

$$p^2 + 2pq + q^2 = 1$$

, onde p é a frequência do alelo "A" e q é a frequência do alelo "a" na população. p^2 representa a frequência do genótipo homozigoto AA, q^2 representa a frequência do genótipo homozigoto "aa" e 2pq representa a frequência do genótipo heterozigótico Aa. Além disso, a soma das frequências de alelos para todos os alelos no locus deve ser 1, então $p + q = 1$. Se as frequências de alelos p e q são conhecidas, então as frequências dos três genótipos podem ser calculadas usando a equação de Hardy Weinberg (RYCKMAN E WILLIAMS, 2008). Para a realização do cálculo, será utilizada a *Hardy-Weinberg Equilibrium Calculator* disponível online (<http://scienceprimer.com/hardy-weinberg-equilibrium-calculator>).

2.3.2.9.2. Análise de interação gene-gene

A análise de interação gene-gene será testada para investigar se existe interação entre os genes investigados e a experiência de cárie dos indivíduos. As combinações de pares de SNPs dentro de cada um dos grupos descritos previamente (gustativos, desenvolvimento dental, resposta imune, composição/função salivar) serão testados de forma a se investigar possíveis interações alélicas utilizando o programa PLINK. O teste padrão usa regressão linear ou logística, dependendo dos diferentes fenótipos utilizados. O programa PLINK faz um modelo baseado na dosagem de alelo para cada SNP, A e B, e se encaixa no modelo na forma de:

$$Y \sim b_0 + b_1.A + b_2.B + b_3.AB + e$$

O teste para interação é baseado no coeficiente b_3 . Este teste, portanto, considera apenas epistasia alélica (epistasia é a interação gênica em que a expressão de um gene "mascara" a presença do outro). Atualmente, as covariáveis não podem ser incluídas ao usar este comando do programa. Desta forma, serão testadas apenas as interações independentemente das demais covariáveis.

2.3.2.9.3. Análise de ancestralidade

Para evitar o efeito de estratificação populacional, as regressões serão ajustadas pelos dez primeiros componentes principais da análise de componentes principais. Além disso, uma estratificação populacional poderá também ser realizada utilizando indicadores quantitativos de ascendência genômica (europeus, africanos e nativos americanos).

2.4. Questões éticas

Este projeto foi submetido ao Comitê de Ética em Pesquisa da Faculdade de Medicina da UFPel. Todas as entrevistas e exames serão realizados após assinatura de termo de consentimento livre e esclarecido. Os indivíduos que apresentarem necessidade de tratamento serão encaminhados à clínica do Programa de Pós-graduação em Odontologia para atendimento.

2.5. Orçamento

Tabela 4 – Orçamento do estudo

Item	Quantidade	Valor (Unidade)	Valor (Reais)
Material - Exame Clínico			
Espátulas de madeira	6 pacotes	4,80	28,80
Gaze	1 pacote	-	15,00
Embalagem autoclave	1 rolo	-	48,00
Espelho bucal	40 unidades	9,50	380,00
Sonda Milimetrada	40 unidades	12,00	480,00
Lanternas portáteis para exame	8 unidades	10,00	80,00
Luvas	8 caixas	14,00	112,00
Toucas	2 pacotes	12,00	24,00
Mascaras	8 caixas	10,00	80,00
Toalhas de papel	10 rolos	1,00	10,00
Jalecos	8 unidades	30,00	240,00
Sacos de lixo	2 pacotes	9,00	18,00
Subtotal			1.515,80
Material Permanente			
Máquina fotográfica digital Nikon Coolpix 4300	1 unidade	-	3.000,00
Conjunto de pilhas recarregáveis, carregador, jogo de espelhos intra oral, afastadores, cartão de memória, câmera, cabo USB, cabo para tomadas macro	1 conjunto	-	2.100,00
Flash macro cool light SL-1	1 unidade	-	613,00
Computador notebook	1 unidade	-	3.200,00
Impressora Laser HP 3300	1 unidade	-	1.999,00
Subtotal			10.912,00
Pessoa Física			
Pagamento por exame aos examinadores/entrevistadores de	720	40,00	28.000,00

campo			
Secretaria	1	-	3.000,00
Subtotal			31.000,00
Pessoa Jurídica			
Gráfica (Impressão questionários)	900	2,50	2.250,00
Revisão de inglês	1	-	100,00
Subtotal			2.350,00
TOTAL			45.777,80

2.7. Referências

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3.0. Relatório do Trabalho de campo

3.1. Introdução

O Programa de Pós-graduação em Epidemiologia (PPGE) da Universidade Federal de Pelotas (UFPel) realiza estudos de coorte de nascimentos desde o ano de 1982. A partir do ano de 1997, iniciou-se uma parceria com o Programa de Pós-Graduação em Odontologia (PPGO) da UFPel. Assim, uma subamostra da coorte de nascimento de 1982 foi investigada para a realização dos estudos de saúde bucal. Os estudos de saúde bucal iniciaram quando os membros da coorte estavam com 15 anos, que se repetiu em 2006 e em 2013.

O primeiro acompanhamento de saúde bucal foi realizado no ano de 1997, onde elegeram-se sistematicamente 70 (dos 265 setores censitários de Pelotas), o que corresponde a 27% dos domicílios. Desta forma, foi realizada uma busca dos indivíduos nascidos no ano de 1982 nestes locais, onde 1076 participantes da coorte foram localizados. Destes, aleatoriamente, obteve-se uma amostra probabilística de 900 indivíduos. Neste acompanhamento de saúde bucal, foram realizados exames de saúde bucal, o qual foi composto de aplicação de um questionário e de exames odontológicos ($n = 888$). Todo o trabalho de campo foi realizado por oito estudantes do curso de odontologia, os quais foram treinados e calibrados previamente aos exames. Os demais levantamentos (24 anos e 31 anos) seguiram a mesma metodologia, sendo que em 2006 720 membros foram examinados e em 2013, aos 31 anos, 539 indivíduos.

Tabela 6. Descrição dos acompanhamentos da coorte de 1982.

Ano	Acompanhamento
1982	Todas as crianças (estudo perinatal)
1983	1/3 da coorte (nascidos entre os meses de janeiro e abril)
1984	Todas as crianças
1986	Todas as crianças
1997	27% dos setores censitários da cidade
2000	Todos os homens (alistamento militar)
2001	27% dos setores censitários da cidade (os mesmos de 1997)
2004-2005	Todas as crianças – Coleta de Material Genético
2006	27% dos setores censitários da cidade (os mesmos de 1997)

Este levantamento, após ter seu projeto aprovado pelo comitê de ética da Faculdade de Medicina da Universidade Federal de Pelotas, iniciou-se com treinamento prévio dos alunos de pós-graduação que foram os examinadores de campo e com os acadêmicos de Odontologia e/ou pós-graduandos que atuaram como anotadores.

3.2. Questionário e ficha clínica:

Para levantamento dos dados, uma planilha eletrônica foi criada, na qual foram anotadas as condições de saúde bucal de cada pessoa examinada, assim como um questionário contendo perguntas relacionadas aos hábitos de saúde bucal, uso de serviço e qualidade de vida dos indivíduos entrevistados. Cada campo da planilha estava previamente codificado; evitando assim, possíveis erros de anotação.

Esta planilha continha uma parte geral, onde estariam contidos os dados gerais do indivíduo, como número da coorte, endereço, telefones, um questionário e uma ficha clínica. Como já mencionado, neste questionário estavam contidas perguntas previamente validadas sobre qualidade de vida, uso de serviço odontológico, dor dentária, qualidade de vida, hábitos parafuncionais além de informações relacionadas a tratamentos odontológicos previamente realizados.

Os anotadores, durante a realização do campo, levavam um *netbook* e preencheram a planilha durante os exames.

Esta planilha foi testada previamente pelos pesquisadores durante o processo de treinando e calibração. Algumas adaptações foram necessárias, e, ao final, estava pronta para ser inserida nos computadores disponibilizados pela equipe de campo.

3.3. Manual de Instruções

Os mestrandos e doutorando envolvidos com o presente projeto elaboraram um manual de instruções objetivando auxiliar no treinamento e no trabalho de campo. Assim, o manual serviu como material de consulta em caso de dúvidas. Cada dupla (isto é, um entrevistador e um anotador) possuía uma versão digital documento no *desktop* do *netbook*.

O manual continha, assim, orientações sobre o preenchimento de cada uma das abas da planilha (folha de rosto, questionário e exame bucal), incluindo detalhes sobre o que se pretendia coletar com a questão, as opções de resposta e se estas deveriam ser lidas ou não. No que diz respeito ao exame de saúde bucal, estavam definidas todas as patologias, acompanhadas de fotos para facilitar o exame.

3.4. Amostra

Para o levantamento de 2013, buscou-se todos os 888 indivíduos participantes da subamostra do levantamento da coorte de 1997, o que permitiria as análises de todas as temáticas envolvidas na pesquisa.

Os supervisores, auxiliados pela secretária e um doutorando, providenciaram o contato telefônico de cada um dos 888 indivíduos previamente examinados. Neste contexto, o primeiro contato se deu via chamada telefônica, com uma explanação sobre o objetivo deste novo levantamento e com o convite para participar do mesmo. Em caso de concordância, preferências de dia e horário para realização das entrevistas eram obtidas.

Em caso de os sujeitos não terem número telefônico disponível, os mapas dos endereços de todos os indivíduos, conforme os dados dos levantamentos anteriores, foram acessados. Estes domicílios foram visitados pelo examinador responsável, que entregou carta de apresentação da pesquisa aos indivíduos, convidando-os para participar do estudo.

3.5. Capacitação e Calibração

A capacitação teórico/prática teve duração de uma semana e realizou-se na segunda semana de setembro do ano de 2013. Assim, foram apresentados todos os temas que iriam compor o questionário de avaliação da saúde bucal, e também os pontos do exame clínico bucal propriamente dito. Foram incluídos nestes exames de lesões de tecidos moles, índice periodontal, índice CPO-S, índice de estética dental (DAI), e qualidade de restaurações previamente realizadas.

Assim, os pesquisadores foram orientados de todos os procedimentos de busca dos examinados, os quais seriam iniciados pelos supervisores e de como deveriam estar identificados e portarem-se durante as entrevistas, as quais, preferencialmente se realizariam no domicílio dos entrevistados.

Após esta capacitação, os prováveis examinadores passaram por processo de calibração, também com duração de uma semana, realizado na

semana seguinte à capacitação teórica. O examinador padrão-ouro foi determinado pelo coordenador da pesquisa. Os examinados no processo de calibração eram pessoas da população de Pelotas, buscados na triagem da faculdade de Odontologia da UFPel, além de pessoas da comunidade, por convite dos participantes da pesquisa. Para serem elegíveis, deveriam ter entre 25 e 40 anos e não pertencerem à coorte de nascimentos de 1982.

Cada examinador foi orientado a realizar o exame completo em oito indivíduos, os quais eram anotados pelo candidato ao posto de anotador. Depois, aleatoriamente, escolheram-se algumas variáveis para serem testados os índices Kappa e de concordância entre os examinadores. Os resultados obtidos foram: uso e necessidade de prótese (Kappa 0.84); DAI (Kappa 0.65); CPO-S (Kappa 0.89); condições periodontais (coeficiente de correlação intra-classe 0.85). Como resultado deste processo, seis examinadores estavam calibrados e prontos para ir a campo assim como dez anotadores.

Para a parte específica de patologia bucal a calibração foi feita *in lux* e, após os examinadores realizaram um teste com base nas fotos apresentadas (Kappa 0.91).

3.6. Supervisão e acompanhamento do trabalho de campo

Para melhor organizar o andamento da pesquisa, foi definido que dois doutorandos, um do PPGE (Lenise Menezes Seerig) e um do PPGO (Gustavo Giacomelli Nascimento), seriam os responsáveis pela supervisão do trabalho de campo, auxiliados por uma secretária.

Toda o preparo das entrevistas, incluindo divisão dos bairros e direcionamento das datas e horários ocorreu sob a responsabilidade destes estudantes de doutorado.

A inclusão dos dados provenientes das entrevistas em um banco de dados foi de responsabilidade da doutoranda Lenise Menezes Seerig, o que era feito em tempo real. Assim que terminada a entrevista, a planilha era enviada para um endereço eletrônico específico para este fim, e era tabulada e

salva pela supervisora no programa Excel e, após, transferida para o programa *Stata 12.0*, que seria o utilizado para as análises.

3.7. Logística do Trabalho de Campo

O trabalho de campo foi realizado por seis mestrandos e doutorandos do PPGO da UFPel que atuara, como examinadores de campo, dez acadêmicos e mestrandos do PPGO que atuaram como anotadores, além de uma secretária contratada especificamente para esta finalidade com jornada de trabalho de oito horas diárias. Todo o trabalho foi supervisionado por dois doutorandos, além dos coordenadores da pesquisa.

Para a busca dos entrevistados, utilizou-se dados obtidos no levantamento de saúde geral aos 30 anos desta coorte, dados do último levantamento de saúde bucal e, se necessário, dados de outros levantamentos e da secretaria de saúde municipal. Todos os 888 indivíduos da amostra de saúde bucal do ano de 1997 foram procurados.

Cada examinador disponibilizou uma agenda semanal, incluindo finais de semana, apresentando os horários disponíveis para a realização das entrevistas. De acordo com esta agenda, a secretária agendava as visitas ao domicílio do entrevistado e organizava a dupla entrevistador/anotador que faria a entrevista. Após, o supervisor de campo, fazia a marcação da entrevista por e-mail para a dupla elegível para ir a campo. Sempre que possível, as entrevistas eram agendadas em endereços próximos, reduzindo assim, custos de combustível e o tempo dedicado. Um dos integrantes da dupla disponibilizava carro próprio para ir ao domicílio do entrevistado.

Devido à peculiaridade da coorte de nascimentos de 1982, a alguns membros que haviam sido entrevistados na sede do centro de pesquisas no ano anterior, também foi oferecida a possibilidade do exame ocorrer neste local. Para os membros da amostra que haviam mudado de cidade, foi oferecido pagamento de passagem para o comparecimento à entrevista. Em alguns casos de cidades, onde havia número significativo de entrevistados,

uma dupla de examinador e anotador deslocou-se para realizar as entrevistas (casos das cidades de Rio Grande, Caxias do Sul e região metropolitana de Porto Alegre)

Os examinadores e anotadores foram orientados a enviar a planilha eletrônica para o e-mail da pesquisa, salva com arquivo nomeado com o número da coorte do integrante, assim que retornasse da entrevista, evitando assim, perda de dados. A supervisora responsável pelo armazenamento dos dados, diariamente, fazia a checagem e *download* das entrevistas.

Semanalmente, durante os quatro meses de realização do campo (outubro de 2013 a janeiro de 2014) realizou-se reuniões no PPGO da UFPel, realizadas às terças-feiras, com duração de duas horas, sob a coordenação dos supervisores de campo. Nestas reuniões eram debatidos o andamento das entrevistas, as dificuldades encontradas, sugeridas possíveis mudanças para evitar recusas e perdas, além de serem esclarecidas alguma inconsistência encontrada na digitação dos dados.

A secretária tinha a responsabilidade de comunicar decisões da coordenação e supervisão aos examinadores e anotadores, fazer as ligações tentando agendar as consultas, participar das reuniões semanais e auxiliar nos demais afazeres solicitados pelos supervisores.

A realização de entrevistas iniciou no dia 27 de setembro de 2013, sendo finalizada no dia 30 de janeiro do ano de 2014.

As duplas de entrevistadores e anotadores iam a campo identificadas por camiseta com o logo do Centro de Pesquisas em Epidemiologia e crachá. Levavam consigo todo o material necessário para a execução das entrevistas (*netbook*, instrumentais esterilizados, luvas, gaze, máscara e gorro). Ainda, levavam consigo os termos de consentimento livre e esclarecido (TCLE), para ser assinado pelo entrevistado. Antes de iniciar a entrevista, este termo era lido e assinado, ficando uma cópia arquivada no CPE e outra cópia com o entrevistado. Inicialmente era preenchida a folha de rosto, a seguir o questionário e, por último, era realizado o exame bucal. A duração de cada visita teve tempo médio de 25 minutos, desde a chegada do examinador até a finalização do exame clínico bucal.

O controle do andamento do número de entrevistas e das inconsistências era feito semanalmente pela supervisora do campo e reportado nas reuniões semanais. Estes números eram discutidos em reuniões semanais com a participação dos coordenadores da pesquisa.

Ao final do trabalho de campo, foram realizadas 539 entrevistas, obtendo-se um percentual de 5% de recusas e de 34% de perdas, e taxa de participação de 61%, quando comparado com a amostra obtida em 1997. Foram consideradas perdas quando não foi possível contato via telefone ou e-mail, assim como contato pessoal no endereço prévio de referência de cada indivíduo após três visitas em horários e dias diferentes.

3.8. Controle de Qualidade

Para assegurar a qualidade dos dados obtidos, foram adotadas várias estratégias, como: capacitação dos examinadores e anotadores, calibração dos examinadores, elaboração de manual de instruções, testagem da planilha dos questionários e exames bucais, verificação semanal de inconsistências no banco de dados e reforço das questões que frequentemente apresentavam problemas.

Após a realização das entrevistas e exames, 10% dos indivíduos foram randomicamente escolhidos para a realização de um questionário por telefone, contendo dez questões previamente escolhidas pelos supervisores. Os supervisores de campo ficaram responsáveis pela aplicação deste questionário e pela tabulação dos resultados, verificando as consistências das respostas. Adicionalmente, foi perguntado sobre a satisfação do participante quanto ao trabalho da dupla que foi ao seu domicílio.

Os resultados mostraram boa concordância das respostas, com índices Kappa superiores a 8 em todas as perguntas. Quanto à satisfação, a nota média das visitas foi de 9,3, variando de 7,0 a 10,0.

3.9. Cronograma

Tabela 7. Cronograma do Estudo de Saúde Bucal de 2013

	2013						2014
Atividades/Período	Julho	Agosto	Setembro	Outubro	Novembro	Dezembro	Janeiro
Entrega do projeto	X						
Treinamento dos examinadores/ anotadores		X	X				
Mapeamento dos entrevistados	X	X	X				
Elaboração dos questionários	X	X	X				
Elaboração manual de instruções	X	X	X				
Realização do trabalho de campo				X	X	X	X

3.10. Orçamento

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Processo: 402357/2012-3

Condições de saúde geral, socioeconômicas, comportamentais, clínicas e de acesso a serviços ao longo do ciclo vital: associação com saúde bucal em uma coorte de nascidos vivos no Sul do Brasil.

Proponente: Flávio Fernando Demarco

Co-proponentes: Bernardo Horta; Denise Gigante; Marco Peres; Karen Peres; Sandra Tarquínio; Marcos Britto Corrêa

Valor Aprovado: R\$ 59.021,30

4.0 Revisões Sistemáticas

Neste capítulo, serão apresentadas as revisões sistemáticas produzidas com o objetivo de investigar os possíveis genes e *Single Nucleotide Polimorphisms* já reportados na literatura com possíveis influências na experiência de cárie dental em crianças e adultos. Além disso, meta-análises foram realizadas para sumarizar quantitativamente os achados.

4.1 Artigo 01

Artigo formatado seguindo as normas da revista *Clinical Oral Investigations*

Genes in the pathway of tooth mineral tissues and dental caries risk: A systematic review and Meta-Analysis

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Key words: Polymorphisms. Dental caries. Mineral tissues. Genetic. Gene.

Declarations of conflict of interest: none

Running tile: Tooth mineral tissues genes and caries

Clinical Significance: Several Single Nucleotide Polymorphisms related to tooth mineral formation genes are linked with the occurrence of dental caries and these genes have been shown to be important to explain differences in dental caries risk.

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Carta de Sumbissão

To: Professor Dr. Matthias Hannig
Editor-in-Chief,

Dear Editor:

Based on the importance of *Clinical Oral Investigations*, we are sending the manuscript entitled “**Genes in the pathway of tooth mineral tissues and dental caries risk: A systematic review and Meta-Analysis**” to be appraised by the Journal’s Editorial Board.

This is the first systematic review with meta-analysis investigating the association between single nucleotide polymorphisms (SNPs) of tooth mineral tissues genes and dental caries experience. The present findings showed that some genes are linked with dental caries occurrence. The meta-analysis suggests that the genes TFIP11, AMBN and AMELX have an important role on dental caries, confirming therefore, the positive influence of SNPs of tooth mineral tissues genes on dental caries experience.

A great number of studies were included in this review and meta-analysis making wide review of current available literature. Also, we performed the analysis considering different analysis (allelic and genotype) providing a robustness to our findings. We did quality control filters in order to minimize the bias in our estimates, such as to investigate and exclude SNPs in linkage disequilibrium for the gene-pooled approach, as well as excluded palindromic ones. Besides, we have not identified publication bias across included studies.

This is a review manuscript and has not been considered for publication elsewhere. The paper was read and approved by all authors. All authors have made substantive contribution to this study, and all have reviewed the final paper prior to its submission. The authors declare that there are no potential competing interests. Furthermore, I attest the validity and legitimacy of data and its interpretation. There are no conflicts of interest for authors listed above. We sign for and accept responsibility for releasing this material.

Thank you very much for your consideration.

Yours sincerely,

Prof. Marcos Britto Corrêa, PhD. (**Corresponding Author**)
Graduate Program in Dentistry, Federal University of Pelotas

Carta de Aceite do Manuscrito

Ref.: Ms. No. CLOI-D-19-00967R1

Genes in the pathway of tooth mineral tissues and dental caries risk: A
systematic review and Meta-Analysis
Clinical Oral Investigations

Dear Mr Correa,

It is a pleasure to accept your manuscript entitled "Genes in the pathway of
tooth mineral tissues and dental caries risk: A systematic review and Meta-
Analysis" in its current form for publication in the 'Clinical Oral Investigations'.

Thank you for your fine contribution. On behalf of the Editors of the 'Clinical Oral
Investigations', we look forward to your continued contributions to the Journal.

With kind regards

Matthias Hannig, Univ.-Prof. Dr.
Editor-in-Chief
Clinical Oral Investigations

**Genes in the pathway of tooth mineral tissue and dental caries risk: A systematic review
and Meta-Analysis**

Running title: Tooth mineral tissue genes, and caries

Genes in the pathway of tooth mineral tissue and dental caries risk: A systematic review and Meta-Analysis

Running title: Tooth mineral tissue genes, and caries

Abstract:

Objectives: to perform a systematic review of the literature, investigating the influence of tooth mineral tissues genes on dental caries.

Materials and methods: Five databases were searched. Only human studies with cross-sectional, longitudinal and case-control design were included. Meta-analysis was performed for each polymorphism, providing allele and genotype estimates. A meta-analysis was performed, pooling several polymorphisms for each gene. A Funnel Plot and Egger test were also performed.

Results: A total of 1,124 records were found. Of these, 25 papers were included in the systematic review and 18 in the meta-analysis. Most of the studies (52%) were of medium quality. With regard to the allele analysis, the T allele of rs134136 (*TFIP11*) (OR 1.51; 95%CI 1.02–2.22) showed an association with high experience of caries and the summarization of polymorphisms investigated in the *TFIP11* gene, after exclusion of SNP linkage disequilibrium, showed an association with caries experience (OR 1.64; 95%CI 1.08–2.50). An analysis of the homozygous genotype did not show any significant association. The pooled SNPs of *AMBN* showed associations with caries (OR 0.45; 95%CI 0.29 – 0.72). The pooled polymorphisms of *AMELX* were associated with caries experience (OR 1.78; 95%CI 1.23–2.56). In the analysis of the homozygous genotype, no SNP showed a significant association. Egger's test showed no significant publication bias for all models ($p > 0.05$).

Conclusion: The present findings showed that the genes *TFIP11*, *AMBN* and *AMELX* play an important role in dental caries.

Clinical Relevance: Several Single Nucleotide Polymorphisms related to the genes in the formation of tooth mineral are linked to the occurrence of dental caries and these genes have proved to be important for an explanation of differences in the risk of dental caries.

Key words: Polymorphisms. Dental caries. Mineral tissues. Genetic. Gene.

Introduction

Dental caries is a chronic disease with high global prevalence [1]. About 2.4 billion people with permanent dentition and 621 million children with primary teeth are affected by caries, leading to a reduction in the quality of life [2]. Although dental caries can be prevented by addressing the etiological factors, such as oral hygiene habits (biofilm), decrease in the consumption of fermentable carbohydrates and the use of fluorides as in fluoridated water, fluoride toothpastes, mouthwash, among others [3-4], its control at the population level is very difficult as caries is strongly influenced by contextual, socioeconomic and behavioral factors [1, 5-7]. Therefore, it remains a worldwide public health problem [1].

It is undisputable that biological, socioeconomic and behavioral factors are the main variables explaining the occurrence and distribution of dental caries in the population. However, in some cases, individuals possessing the same protective factors – such as water fluoridation – or risk factors, and with similar oral health-related behavior, present with different patterns of dental caries [4, 8]. For these individuals, genetic factors could be an intrinsic influence providing additional resistance or susceptibility to dental caries [9]. In this context, studies have proposed that a proportion of these variations in the prevalence of dental caries may be explained by genetic factors [9-10]. In fact, a wide range of genes have been identified, demonstrating their important role in the development and progression of caries [9].

A small number of studies focusing on the genetic aspects of caries have performed Genome-Wide Associations (GWAS), which aim to identify potentially new genes involved with dental caries [11-13], while most studies investigating the association of genetic components and dental caries have used candidate gene methodology, examining Single Nucleotide Polymorphisms (SNPs) [9]. In this way, these SNPs can be pooled into four main groups: a) those involved with tooth mineral tissues, b) immune response, c) salivary composition/flow and d) gustatory genes [9]. Among these groups of genes, the SNPs involved with tooth mineral tissues are currently responsible for the majority of the available literature [9].

Thus, an understanding of which SNPs and genes are involved in individuals' susceptibility to caries disease, could support the development of a viable approach to better comprehend these complex mechanisms. Accordingly, the aim of the present study was to perform a systematic review of the literature, investigating the influence of Single Nucleotide Polymorphisms related to tooth mineral tissues genes on the experience of dental caries, as well as to perform a meta-analysis using the data.

Methods

The present systematic review was registered in PROSPERO (International Prospective Register of Systematic Reviews) under protocol number CRD42018098809. This review was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline [14].

Review question and Searches: The research question was structured obeying the PICO model: “Are gene polymorphisms in tooth mineral formation a risk factor for dental caries in children and adults?”

- Participants/population: individuals, adults and children
- Intervention/exposure: Single nucleotide polymorphisms in the formation of tooth mineral. The effect allele in this study was standardized as the least frequent allele reported in the studies. Whenever the minor allele frequency varied among the studies, the effect allele was referred to as the minor allele in the majority of the studies. Similarly, to do the estimates stratifying by genotype, we opted for the minor homozygote and heterozygotes as the effect genotypes.
- Comparator/control: Single nucleotide polymorphisms in the formation of tooth mineral. Thus, the effect allele was compared to the reference allele, defined as that which is most frequent in the population. To perform genotype analysis, the major homozygote was chosen as the reference.
- Outcome: Dental caries experience.

The search strategy was achieved using appropriate keywords and entry terms related to MeSH Terms, taking into consideration the particularity and structure of each database (**Table 1**). Five databases were researched (PubMed, Scopus, ISI Web of Science, BVS virtual health library, Scielo), through November 2018. All retrieved records were uploaded to EndNote software (Thomson Reuters, Rochester, New York, NY, USA). Thus a virtual library was assembled. Identified duplicate studies were excluded. Two independent reviewers (LAC and MCMC) read the titles and abstracts of all the papers. Inclusion criteria comprised articles that aim to evaluate the association between genetic tooth mineral tissues in children or adults. Only human studies with cross-sectional, longitudinal and case-control design were included. No restrictions were placed on language or date of publication. Studies with *design of literature* reviews, case reports and case series, conference abstracts, letters to the editor, and qualitative studies were all excluded. The same reviewers read the full-text and adjudged the articles. In the event of disagreement, the same reviewers discussed the issue until a consensus was reached.

Data collection: Data extraction was performed independently by two reviewers in a predefined database. The following data were extracted: Author, year, country, study design, sample, age, ethnicity of the sample (% for each ethnic group (skin color or origin of population)), proportion of males and females for each sample, calculation of statistical power, categorization of dental caries, minor allele frequency calculation for each polymorphism, analytical approach, Hardy-Weinberg equilibrium, effect estimate (crude and adjusted analysis values and their respective confidence intervals) covariables and the main results.

Quality of studies: The quality of the included studies was verified in accordance with the Appraisal Checklist for Observational Studies scale (Joanna Briggs Institute) (T.J.B., 2014). This tool presents 10 questions evaluating different points in the study, which should be answered with "No", "not clear" or "Yes". Each "Yes" response corresponds to one point, so the tool score ranges from 0 to 10. Studies totaling between 0 and 3 points were considered low quality; 4 to 6 were of medium quality; and 7 to 10 were considered high quality. To classify the studies, two reviewers will perform the classification independently. Disagreements will be remedied through discussion until consensus is reached.

Strategy for data synthesis: A meta-analysis was planned to be performed when the same polymorphisms were identified in at least three different studies, when the effects were shown or where it was possible to calculate the effect measures. However, due to the fact that the studies analyzed different polymorphisms in the same gene, it was decided to perform a global meta-analysis pooling the same polymorphisms across the studies, as well as pooling different polymorphisms in the same gene across the studies. Thus, for the meta-analysis, only SNPs present in at least two different studies were considered in the pooled polymorphism and gene results. In addition, meta-analysis was performed pooled by gene, including results of individual studies. Moreover, in the analysis, a division was made between allele and genotype models, calculating the estimates for the effect allele and effect heterozygote and homozygote genotypes, pooling by both polymorphism and gene. The effect allele and genotypes were compared to the reference allele and genotype, respectively, in different analyses. In studies that present more than one categorization for dental caries, DMF/dmf=0 vs. DMF/dmf $\geq$ 1 was chosen.

For the meta-analysis, the results of the adjusted models (adjusting for ethnicity) were preferably included. In cases where the adjusted results have not been reported, the unadjusted estimates were considered or calculated, to be included in the analysis. In cases where results

were only shown by stratified analysis, we included the group with the highest number of individuals. The odds ratio (OR) was used to measure effect size with a 95% Confidence Interval (CI). The prevalence ratio measures were converted to OR using the formula proposed by Zhang and Yu: $PR = \text{odds ratio} / 1 - \text{risk}_0 + \text{risk}_0 \times \text{odds ratio}$, where “risk₀” is the prevalence of disease among non-exposed individuals [15-16]. It is important to emphasize that, in genetic studies, non-genetic factors known to be associated with risk of disease can exist in the intermediate pathways between the genetic risk marker and disease development and, therefore, should be included in the adjusted analysis to avoid over-adjustment [17]. To address the absence of the reporting of ethnicity, an investigation was reported of allele frequencies stratified by populations based on the human genome (GRCh37.p13).

To avoid inconsistencies with the data analysis, data harmonization for palindromic SNPs was performed. When the palindromic SNP was present in two different studies, we only kept the SNP in the analysis if the study reported the DNA strand. If this information was missing in the papers, the SNP was excluded from further analysis. In order to avoid biased estimates due to linkage disequilibrium (LD) in the gene pool analysis, a pruning was performed, by LD, for those studies that analyzed more than one polymorphism in the same gene. To this end, a pairwise comparison was carried out including only SNPs which were independent ($r^2 < 0.3$) from the others. For the SNPs in $LD \geq 0.3$, the analysis included the one with the lowest P-value for the association. When the studies did not provide estimates of linkage disequilibrium, those retrieved from the 1000 Genomes global population as a reference panel, were considered. Thus, when the SNPs included in the meta-analysis (in gene stratification) were extracted from the same study, they were only maintained in the analysis when r^2 of equilibrium linkage was ≤ 0.30 , according to the investigated population. Due to the high degree of heterogeneity (I^2 statistic) observed across the studies, random models were carried out. All analyses were performed using Stata 12.0 software (StataCorp, College Station, TX, USA)

To investigate possible publication bias, the Egger test and contour-enhanced funnel-plot were used. This test details statistical significance on a funnel-plot, demonstrating the level of significance of each estimate (allele, homozygote and heterozygote analysis), and graph pooling by gene was also plotted [18]. Contour-enhanced funnel plots were performed to examine the context of the statistical significance of the results [18].

Results

Study selection

A total of 1,124 records were found in the initial searches (Figure 1). Excluding duplicates, 719 manuscripts remained for title and abstract screening. Twenty-eight full-text articles were assessed for eligibility, of which three were excluded. The studies and reasons for exclusion are shown in **table 2**. Thus, 25 papers were included in the systematic review and 18 in the meta-analysis (**Figure 1**). Studies evaluating the same population (French children) [19-20] and (Polish children) [21-22] were included because different genes and SNPs of tooth mineral tissues were investigated in each study.

Study characteristics

Most of the 25 studies included were in the form of a case-control design. The studies were performed most frequently in populations from Brazil (n=6; 24%), followed by Turkey (n=4; 16%). Most of the studies were published after 2011 (**Figure 2**). Single Nucleotide Polymorphisms of the Enamelin (*ENAM*) gene were investigated by 56% of the studies, followed by the Amelogenin (*AMELX*) gene, which was investigated in 48% of the studies (**Figure 3**). **Table 4** and **table 5** show the main characteristics of the studies included and the effects of polymorphism on dental caries. With regard to the evaluation of dental caries, a significant variation was noted across the studies. The main categorization was DFM/dfm=0 vs. DFM/dfm $\geq$ 1. Moreover, few studies reported power analysis, nor did they report the ethnicity of the studied population. Effect estimates of some studies were not displayed, making it impossible to calculate the odds ratio.

Risk of bias within the studies

Regarding the quality assessment, **table 3** displays the Critical Appraisal Checklist for observational studies (Joanna Briggs Institute). Most of the studies (52%) presented medium quality of assessment while 44% presented low quality.

Overview of Single Nucleotide Polymorphisms

Forty-five single nucleotide polymorphisms were found by investigating possible associations with dental caries experience. These SNPs were present in eighteen genes. Most of the SNPs were situated in intron region (57.5%), 2.3% in missense variants and 1.8% were synonymous. Furthermore, 80.9% of SNPs are related to a possible functional impact on protein

coding according to the 1000 Genomes global population. More details of SNPs and their functional impact on protein are available in **table 4**. No palindromic SNPs needed to be removed. SNP rs496502 (G/T) was excluded due to inconsistencies between the chromosome region described in the manuscript [23] and the database available at http://grch37.ensembl.org/Homo_sapiens. Linkage disequilibrium was observed between the single nucleotide polymorphism present in *DLX3*, *MMP20* and *TFIP11*; accordingly, SNPs in disequilibrium were excluded from the final analysis. *Only the SNP with the strongest association was included in the analyses.*

Results of individual studies

The main characteristics of the studies included in this systematic review are available in **supplementary material S1**. Overall, a high methodological variability (inclusion criteria, caries diagnosis method and classification, age of population, study design and analytic approach) was observed across the studies included, which evaluated a large number of genes and polymorphisms. Moreover, most of the polymorphisms were investigated by only one study. These polymorphisms will also be described in this section. In additions, some observations concerning individual studies will be described here.

With regard to the allelic analysis, the allele C of polymorphisms rs2609428 (*ENAM*) (OR 3.89 [1.47 – 10.31]) in the French population [19], allele T of rs3796703 (*ENAM*) (OR 1.65 [1.11 – 2.45]) in the Chinese population [24], allele G of rs198969 (*KLK4*) (OR 2.38 [1.30 – 4.35]) in the Polish population [22] and allele G of rs2235091 (*KLK4*) (OR 2.30 [1.15 – 4.62]) also in the Polish population [22], showed an association with high experience of dental caries. On the other hand, allele T of rs34538475 (*AMBN*) (OR 0.15 [0.08 – 0.30]) in the Polish population [22], allele T of rs2278163 (*DLX3*) (OR 0.30 [0.15 – 0.64]) in the Japanese population, evaluating individuals with a high level of *Mutans streptococci* [25], and allele G of rs2252070 (matrix metalloproteinase 13 - *MMP13*) (OR 0.67 [0.51 – 0.89]) in the Brazilian population [26], showed a protective effect for dental caries.

Considering the genotype analysis, the genotype GG of rs198969 (*KLK4*) (OR 18.07 [2.10 – 155.49]) in the Polish population [22] was associated with high dental caries experience in this population [22]. However, the genotype CC of rs2278163 (*DLX3*) (OR 0.07 [0.01 – 0.46]) in Japanese individuals with high levels of *Mutans streptococci* [25], GG of rs198968 (*KLK4*) (OR 0.17 [0.03 – 0.94]) in the Turkish population [27], and GG of rs2252070 (*MMP13*) (OR 0.54 [0.31 – 0.93]) in the Brazilian population [26], showed a protective effect against dental caries.

Some associations were also observed in respect of heterozygote genotypes. The genotype CT of rs3796703 (*ENAM*) (OR 1.61 [1.03 – 2.52]) in the Chinese population [24] and

the AG of rs198968 (*KLK4*) (OR 0.15 [0.03 – 0.82]) in the Turkish population [27] showed an association with high dental caries experience, while the genotype TC of rs5933871 (*AMELX*) (OR 0.05 [0.01 – 0.53]) in the Korean population [28] and TC of rs5934997 (*AMELX*) (OR 0.05 [0.01 – 0.52]), also in the Korean population [28], were associated with low caries experience.

Although few studies performed an analysis that considered the participants' ethnicity, differences in genotype and ethnicity were observed in the polymorphism rs1784418 C/T (matrix metalloproteinase 20 - *MMP20*); differences in genotype distribution and caries experience were observed in Caucasian children, but not in afro-descendants [29]. Cavallari et al. [30] performed an analysis of dominant and additive models and observed different results. The allele A of rs2978642 A/T (kallikrein-related peptidase 4 - *KLK4*) in the dominant model (AA+AT Vs. TT) was associated with dental caries (OR 3.48 [1.00-13.07]), allele T in the additive model (AA/TT/AT) ($p=0.15$) and dominant model (OR 0.87 (0.50-1.52)), were not. Moreover an influence of some genotypes (tuftelin 1 - *TUFT1*) was also identified interacting with levels of *S. mutans* infection in children, leading to higher levels of caries [31]. Similarly, when the sample was stratified by water fluoridation, different results were observed: the *AMELX* gene was associated with experience of dental caries in non-fluoridated water. The genotype TT in rs5933871 and TT in rs5934997 led to a higher risk for dental caries. Taking all subjects into consideration, no associations were observed [28].

Synthesis of results (meta-analysis)

Eighteen studies were included in the meta-analysis. The summarization of individual and meta-analysis results (at least two studies evaluating the same SNP) according to allele and genotype models, are displayed in **table 5**. To perform the analysis pooled by gene, the results of single articles were considered. Overall, 45 polymorphisms were included.

In the allele analysis, 38 polymorphisms were investigated. Only the polymorphism rs134136 (*TFIP11*) situated in an intron region with a potential impact on protein coding, showed an association with dental caries. The allele T of this SNP was associated with a high experience of caries (OR 1.51 [1.02 – 2.22]).

For the analysis concerning genotypes, 43 polymorphisms were included. No polymorphisms included in this analysis showed significant association with caries in the meta-analysis, considering the minor homozygote or heterozygote genotypes.

When several SNPs were pooled in order to test the association for the whole gene, the summarization of polymorphisms investigated in the *DLX3* gene revealed an association with dental caries experience (OR 0.67 [0.47 – 0.94]) in an initial analysis that considered alleles, although this association was lost after the exclusion of SNPs in linkage disequilibrium (OR

0.53 [0.26 – 1.07]). In addition to the intron region, SNPs in the *DLX3* gene were found in the promoter region, 5' UTR and 3' UTR. After correction for linkage disequilibrium, the gene *TFIP11* was associated with caries experience after pooling all the polymorphisms (OR 1.64 [1.08 – 2.50]). The *AMBN* and *AMELX* genes were also associated with dental caries after pooling estimates for minor homozygote genotypes (OR 0.37 [0.17 – 0.82] and OR 1.78 [1.23 – 2.56], respectively). All the SNPs for both genes are situated intronically.

Funnel plot results showed no significant publication bias across the studies. Egger's test confirmed these observations (Allele [$p=0.558$] and genotype - Homozygote [$p=0.330$] and Heterozygote [$p=0.093$]- analysis) (**Figure 4**).

Discussion

To the best of our knowledge, this is the first systematic review with meta-analysis investigating the association between single nucleotide polymorphisms of tooth mineral tissues genes and dental caries experience. The present findings showed that some genes are linked to the occurrence of dental caries. The meta-analysis suggests that the genes *TFIP11*, *AMBN* and *AMELX* play an important role in dental caries, thus confirming the positive influence of the SNPs of tooth mineral tissues genes on dental caries experience.

The main association sustained by the meta-analysis was the effect allele T of SNP rs134136 (*TFIP11*), situated in an intronic region, which showed an association with a high experience of dental caries. The gene of *TFIP11* encodes a protein component of the spliceosome that promotes the release of the lariat-intron during late-stage splicing. Therefore, polymorphisms in this gene can play a role in the amelogenesis process resulting in a change in susceptibility to caries. A recent study carried out in Turkey suggested the genetic variation in genes *TFIP11* was linked to the hypomineralization of tooth enamel and, hence, dental caries [23]. So it seems that genetic variations in this gene can alter the composition or organization of mineral tissue, such as enamel, and influence the progression of dental caries [23], which could explain the observed association of this gene with dental caries. The functional role of rs134136 has not been explored by experimental studies to date. So the possibility of this polymorphism being in the linkage disequilibrium with another functional one cannot be excluded. The *AMELX* gene was also detected as being relevant for dental caries. It is involved in biomineralization during tooth enamel development. SNPs in this gene were associated with dental caries in the genotype (homozygote) analysis, which highlights the relevance of *AMELX* for susceptibility to dental caries. This important function of *AMELX* on tooth development, together with the *ENAM* gene, which encodes the largest protein involved in the mineralization and structural organization of enamel, may explain the increasing interest in investigating SNPs related to these genes. In fact, several studies have attempted to study SNPs related to *ENAM* and *AMLX* genes.

Moreover, a substantial influence on the results was found when grouping together the SNPs belonging to the *AMBN* gene, which encodes the non-amelogenin enamel matrix protein ameloblastin, the second most abundant enamel matrix protein expressed during amelogenesis. Furthermore, *AMBN* is located in the calcium-binding phosphoprotein gene present in chromosome 4. Ameloblasts secrete mainly amelogenin and ameloblastin, which quickly form a nucleus with the calcium hydroxyapatite in enamel crystals. Subsequently, in the maturation phase, the mineral deposition is completed [32]. Thus, the protein coded by this gene seems to

be important for the formation and mineralization of the enamel matrix. SNPs in this gene can lead to dentinogenesis/amelogenesis imperfecta [33]. In fact, genotype mutations in the *AMBN* gene had influenced the complete transcription of AMBN protein in ameloblasts, being associated, therefore, with amelogenesis imperfecta in human deciduous teeth [34]. This previous finding corroborates the results observed in the present systematic review, showing that alterations in *AMBN* could change the normal mineral process of enamel and, therefore, also have an influence on individuals' caries experience.

DLX3 has also been reported as being involved in tooth mineralization in addition to having a relationship with imperfect amelogenesis. It is important to stress that a large number of SNPs in *DLX3* are present in promoter, 3 and 5 prime UTR as well as the TF binding site, which might suggest an influence on the regulatory and coding protein process. However, the present results have found a large number of SNPs in linkage disequilibrium in the *DLX3* gene, which could lead to a biased outcome. Therefore, any SNP in linkage disequilibrium reported by the authors was excluded from the final analysis and a supplementary investigation was carried out based on Human genome (GRCh37.p13) in cases not reported/investigated by authors. Therefore, a loss of association in the grouped *DLX3* results. This outcome may be explained by the proximity observed between investigated SNPs of this gene, which do not appear to be independently segregated. This highlights, for future studies, the compelling need to investigate the linkage disequilibrium and report of the findings.

Although the present findings have shown that some SNPs related to the genes of tooth mineral formation are linked to the etiology of dental caries, these results should be interpreted with caution. It is important to highlight the significant methodological differences observed amongst the studies. The first point is related to the ethnicity of samples investigated and population stratification. The population may be a problem for genetic studies, leading to bias in the estimates of association. A very small portion of the studies conducted are adjusted for any type of ancestry information. Important differences between allele frequencies and population ethnicity have been identified when reported SNPs were investigated in a supplementary database. This emphasizes the need to perform checks on this variable to decrease possible bias in the studies. The other limitation relating to different ethnicities relates to the analysis grouped together by gene, in which the estimates were combined regardless of the ancestry background of the population. It is already known that genetic effect sizes may differ among populations, at least for some traits, and allele heterogeneity could have an important impact on the potential for generalizing about association results across populations. Failures with transferability findings have been clearly demonstrated for polygenic risk scores [35]. So the estimates for polymorphisms and genes should be carefully considered.

Despite the important limitations observed, no publication bias was identified through the funnel plot and was complemented by the Egger's test. This result can be explained by the fact that this topic is extremely new and negative results (no associations) are frequently published. In addition, many articles have carried out investigations on different SNPs, hence, some SNPs being published with association and some without, decreasing possible publication bias. Furthermore, a lack of information was observed in some studies, in which only the *p*-values were reported, precluding the inclusion of these studies in the meta-analysis. The use and the reporting of appropriate descriptions and estimates are essential for making comparisons between studies. Similarly, studies have used different alleles as a reference in the analysis. To circumvent this situation, the reference SNP was standardized as the allele most commonly found in most of the studies included.

Moreover, most of the papers were of medium quality and had lower scores in the "sample representativeness of the target population" and "participants recruited in an appropriate way", which reflects studies with samples that are not representative of the population. Several cut-off points on caries categorizations were performed, despite DMF/dmf=0 vs. DMF/dmf $\geq$ 1 being chosen (when available) for inclusion in the analyses. However, some studies used dmfs $\geq$ 4 compared to dmfs=0, taking into consideration the severity of dental caries [31]. This wide variation of cut-off points may lead to a significant bias in the results. As a counterpoint to this observation, Shimizu et al. [36] reported variations in cut-off definitions and demonstrated that these alterations did not affect the findings.

One important limitation is that only candidate gene studies were included in the analyses. Other genes from the same pathway may also be important, but have not been studied to date and, consequently, were not included in this meta-analysis. Moreover, some genes are poorly studied while others are better studied, which reinforces the need for conducting further studies. Studies on a genomic scale are more robust for identification of genetic components because they are not based on a prior knowledge of the pathophysiology and should be used to identify new routes to direct future studies, since there is little overlap in terms of existing studies. The available literature on genomic studies and dental caries is still in its infancy [9]. Initial studies have suggested an association in some loci (1q42-q43, 11p13, and 17q23.1) [37-38]. Similarly, a consortium genome-wide association study was carried out with a large number of individuals (n=19,003) between 2.5 and 18 years old and found an association of rs1594318 with dental caries [13]. This SNP is an Allantoicase gene and participates in the uric acid degradation pathway, reinforcing the need for further statistically well-powered genome-wide studies in order to understand the genetic architecture of dental caries etiology.

Another factor to be considered was the inclusion of deciduous and permanent teeth in the same analysis, performed in some studies [19-20, 23, 26, 29, 36, 39-42]. A Polish cohort including children with both dentitions showed that Mannose-binding lectin 2 (*MBL2*) - an important gene for innate immunity – was associated with dental caries experience, although the direction of effects in the analysis was the opposite in the permanent and deciduous dentitions [43] highlighting the importance of stratification by this factor. Primary teeth are less mineralized and present higher susceptibility to dental caries, which can also result in the fast progression of dental caries [6]. This same study included SNPs of *AMELX* and *ENAM* and did not observe an association with dental caries in either dentition [43]. To avoid possible bias, some papers stratified the sample between children with deciduous and permanent teeth. [43-44]. However, in the two studies, differences between the SNP evaluated and permanent and primary teeth were not observed.

Another important issue refers to the *AMELX* gene, which is situated in the X-chromosome. It was not possible to stratify the analyses according to gender since the studies reported the estimates only for mixed-sex models. This approach, however, may lead to important limitations in interpretation, as pointed out by Clayton [45]. Firstly, the associations can be confused by differences in the sex ratio between cases and controls in population-based case-control studies involving both male and female subjects. Moreover, the phenomenon of X inactivation, which randomly affects most loci on the X chromosome in females, leads to differences between females and males in terms of the risk attributable to a single allele. It reinforces the need for further studies that take this into consideration.

On the other hand, the present study has positive attributes that should be emphasized. A large number of studies were included in this review and meta-analysis, providing a broad review of the literature currently available. In addition, the analysis was performed taking into account different analyses (allele and genotype) providing a robustness in the present findings. Moreover, quality control filters were used in order to minimize the bias in present estimates, such as investigating and excluding SNPs in linkage disequilibrium for the gene-pooled approach, as well as excluding palindromic SNPs. No publication bias was identified across the included studies. Accordingly, it was observed that studies focusing on this topic started in 2005 but only in 2012 was there a significant increase in the number of published investigations, highlighting that studies investigating the relationship between dental caries and genetic polymorphism are a relatively new topic. To support the presented results with a high degree of evidence, further studies are needed, preferably including representative samples of the target population, with populations of different ethnic groups. In addition, the combining of databases to increase the sample sizes and to perform replication of studies is highly recommended and

should be encouraged. In addition, the studies should carry out and present sample calculations to ensure that non-associations are not due to lack of statistical power, since a small proportion of the studies investigated contained calculations of this kind. This can lead to false-negative type inferential errors. Moreover, further studies can also focus on epigenetic issues, interactions between genetic and environmental factors as well as performing control of variables by dental and individual/contextual variables. Genome-wide-association studies can also be an interesting alternative to help to identify new SNPs related to dental caries experience as well as being extremely necessary as a basis for understanding the polygenic trait and genetic architecture of this phenotype.

Conclusion

The present findings showed that several Single Nucleotide Polymorphisms related to the genes of tooth mineral formation are linked to the occurrence of dental caries, mainly those in the genes *TFIP11*, *AMBN* and *AMELX*. These genes have shown themselves to be important to explain differences in dental caries risk. Studies with high methodological and reporting quality must be performed to support and confirm the present findings. The evidence in the literature is recent and encouraging. Further studies can also consider epigenetic issues, interactions between genetic and environmental factors as well as performing control of variables by dental and individual/contextual variables.

Compliance with Ethical Standards:

Conflict of Interest: Luiz Alexandre Chisini declares that he has no conflict of interest. Mariana Gonzales Cademartori declares that she has no conflict of interest. Marcus Cristian Muniz Conde declares that he has no conflict of interest. Luciana Tovo-Rodrigues declares that she has no conflict of interest. Marcos Britto Correa declares that he has no conflict of interest

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Ethical approval: not required

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Legends:

Table 1. Search strategy

Table 2. Excluded studies and reasons for exclusion

Table 3. Critical Appraisal Checklist for observational studies (Joanna Briggs Institute) in the systematic review according to the 10-items

Table 4. Description of single nucleotide polymorphism investigated in the present systematic review according genes*

Table 5. Summarization of results (meta-analysis and individuals) according by allelic and genotype (homozygote and heterozygote) analysis pooled by gene.

S1. Main characteristics of studies included in this systematic review

Figure 1: Prisma flow diagram

Figure 2. Studies grouped by year of publication.

Figure 3. Number of studies investigates by gene

Figure 4. Funnel plot of meta-analysis included studies

Table 1. Search strategy

Search syntax	
PubMed	#1 (“Dental Decay” OR “Caries, Dental” Or “Decay, Dental” OR “Cariou s Dentin” OR “Cariou s Dentins” OR “Dentin, Cariou s” OR “Dentins, Cariou s” OR “Dental White Spot” OR “White Spots, Dental” OR “White Spots” OR “Spot, White” OR “Spots, White” OR “White Spot” OR “Dental White Spots” OR “White Spot, Dental” OR “Susceptibility, Dental Caries” OR “Caries Susceptibility, Dental” OR “Caries Resistance, Dental” OR “Resistance, Dental Caries” OR “Dental Caries Resistance”)
	#2 (“Polymorphisms, Genetic” OR “Genetic Polymorphisms” OR “Genetic Polymorphism” OR “Polymorphism” OR “Polymorphisms” OR “Nucleotide Polymorphism, Single” OR “Nucleotide Polymorphisms, Single” OR “Polymorphisms, Single Nucleotide” OR “Single Nucleotide Polymorphisms” OR “SNPs” OR “Single Nucleotide Polymorphism”)

* Search combination: #1 AND #2

Table 2. Excluded studies and reasons for exclusion

Studies	Reason
Kuchler, et al. [51]	Not investigated the association between genetic polymorphisms and dental caries
Saha, et al. [52]	Not report the specific polymorphism
Lu, et al. [53]	Review

Table 3. Critical Appraisal Checklist for observational studies (Joanna Briggs Institute) in the systematic review according to the 10-items

Study, year	NIH Criteria										Final score
	1	2	3	4	5	6	7	8	9	10	
Slayton, et al. [31]	-	-	+	-	-	+	+	+	-	-	Medium Quality (4)
Deeley, et al. [10]	-	-	-	+	-	+	+	+	-	-	Medium Quality (4)
Patir, et al. [46]	-	-	+	-	-	+	+	+	-	-	Low quality (3)
Kang, et al. [28]	-	-	-	-	-	+	-	+	+	-	Low quality (3)
Olszowski, et al. [43]	-	-	+	-	-	+	+	+	-	-	Low quality (3)
Shimizu, et al. [36]	-	-	-	-	-	-	+	-	-	-	Low quality (1)
Tannure, et al. [29]	-	-	-	+	-	+	+	+	+	+	Medium Quality (6)
Tannure, et al. [26]	-	-	-	-	-	+	+	+	+	-	Medium Quality (4)
Wang, et al. [39]	+	+	/	+	-	+	+	+	+	+	High Quality (8)
Gasse, et al. [20]	-	-	+	+	-	+	+	+	-	-	Medium Quality (5)
Jeremias, et al. [23]	-	-	-	-	-	+	+	+	-	-	Medium Quality (3)
Ergoz, et al. [40]	-	-	-	-	-	+	+	+	-	-	Low Quality (3)
Chaussain, et al. [19]	-	-	+	+	-	+	+	+	-	-	Medium Quality (5)
Ohta, et al. [25]	/	/	-	-	-	+	+	-	-	+	Low Quality (2)
Abbasoglu, et al. [27]	-	-	-	+	-	+	+	+	-	-	Medium Quality (4)
Romanos, et al. [47]	-	-	-	/	+	+	+	-	+	+	Medium Quality (5)
Shaffer, et al. [41]	/	/	+	-	/	+	+	+	+	/	Medium Quality (5)

Antunes, et al. [48]	-	-	-	-	-	+	+	+	+	-	Medium quality (4)
Yildiz, et al. [49]	-	-	+	-	-	+	+	+	-	-	Medium Quality (4)
Gerreth, et al. [21]	-	-	-	-	-	-	+	+	-	-	Low Quality (2)
Cavallari, et al. [30]	-	-	+	-	-	+	+	-	-	-	Low Quality (3)
Filho, et al. [50]	-	-	+	-	-	+	+	-	-	-	Low quality (3)
Gerreth, et al. [22]	-	-	-	-	-	-	+	+	-	-	Low Quality (2)
Borilova Linhartova, et al. [44]	-	-	-	-	-	+	+	+	-	-	Low Quality (3)
Wang, et al. [24]	-	-	+	-	-	+	+	+	-	-	Medium Quality (4)
Weber, et al. [42]	+	+	+	+	+	+	+	+	-	-	High Quality (8)

+ Yes; - No; /: Unclear

Table 4. Description of single nucleotide polymorphism investigated in the present systematic review according genes*

Gene	Polymorphism	Chromosomal position	Variation	Allele Frequencies by populations (%) *					Allele Referenc e/ allele Effect used	Ancestral allele
				African	American	East Asian	Europe	South Asia		
AMBN	rs34538475 (G/T)	4:71471176	Intron	G:72% T:28%	G:82% T:18%	G:99% T:1%	G:76% T:24%	G:91% T:9%	G / T	T
	rs4694075 (C/T)	4:71466914	Intron	C:33% T:67%	C:58% T:42%	C:46% T:54%	C:53% T:47%	C:57% T:43%	C / T	T
AMELX	rs17878486 (C/T)	X:11313948	Intron	T:99% C:1%	T:91% C:9%	T:100% C:0%	T:75% C:25%	T:93% C: 7%	C / T	C
	rs2106416 (C/T)	X:11316742	Synonymous	C:72% T:28%	C:89% T:11%	C:98% T:2%	C:78% T:22%	C:87% T:13%	C / T	C

	rs5933871 (T/C)	X:11313657	Intron	T:40% C:60%	T:85% C:15%	T:98% C:2%	T:75% C:25%	T:79% C:21%	T / C	T
	rs5934997 (T/C)	X:11313733	Intron	T:41% C:59%	T:85% C:15%	T:98% C:2%	T:75% C:25%	T:79% C:21%	T / C	C
	rs6639060 (C/T)	X:11316977	Synonymous	NA	NA	NA	NA	NA	C / T	C
	rs946252 (C/T)	X:11313027	Intron	T:14% C:86%	T:22% C:78%	T:44% C:56%	T:33% C:67%	T:47% C:53%	C / T	C
	rs7052450 (T/C)	X:11318948	Intron	T:14% C:86%	T:22% C:78%	T:44% C:56%	T:33% C:67%	T:47% C:53%	T / C	T
<i>BMP2</i>	rs1884302 (T/C)	20:7106289	Intron	T:34% C:66%	T:61% C:39%	T:61% C:39%	T:67% C:33%	T:75% C:25%	T / C	C
<i>BMP4</i>	rs2761887 (A/C)	14:54425052	Intron	C:40% A:60%	C:47% A:53%	C:47% A:53%	C:41% A:59%	C:34% A:66%	A / C	C
<i>BMP7</i>	rs388286 (T/C)	20:55465424	TF binding site	C:63% T:37%	C:55% T:45%	C:51% T:49%	C:52% T:48%	C:45% T:55%	T / C	C
<i>DLX3</i>	rs10459948 (T/G)	17:48072496	5 prime UTR	G:93% T:7%	G:94% T:6%	G:61% T:39%	G:92% T:8%	G:90% T:10%	T / G	G
	rs11656951 (T/C)	17:48072865	Promoter	C:77% T:23%	C:86% T:14%	C:52% T:48%	C:80% T:20%	C:82% T:18%	T / C	C

	rs12452477 (T/C)	17:48067953	3 prime UTR	T:61% C:39%	T:21% C:79%	T:30% C:70%	T:16% C:84%	T:34% C:66%	T / C	T
	rs16948563 (A/G)	17:48065141	TF binding site	G:91% A:9%	G:86% A:14%	G:82% A:18%	G:94% A:6%	G:87% A:13%	A / G	G
	rs2278163 (T/C)	17:48072426	5 prime UTR	G:67% A:33%	G:77% A:23%	G:32% A:68%	G:76% A:24%	G:60% A:40%	T / C	A
	rs2303466 (A/G)	17:48070878	Synonymous	C:85% T:15%	C:87% T:13%	C:53% T:47%	C:81% T:19%	C:82% T:18%	A / G	C
	rs3891034 (A/G)	17:48070225	Intron	C:85% T:15%	C:87% T:13%	C:53% T:47%	C:81% T:19%	C:82% T:18%	A / G	T
ENAM	rs12640848 (A/G)	4:71506412	Intron	A:97% G:3%	A:66% G:34%	A:77% G:23%	A:33% G:67%	A:53% G:47%	A / G	A
	rs2609428 (T/C)	4:71508869	Missense	T:87% C:13%	T:99% C:1%	T:100%	T:99% C:1%	T:100%	T / C	T
	rs3796703 (C/T)	4:71509314	Missense	C:100% T:0%	C:100% T:0%	C:96% T:4%	C:99% T:1%	C:99% T:1%	C / T	C
	rs3796704 (A/G)	4:71509431	Missense	G:62% A:38%	G:90% A:10%	G:98% A:2%	G:95% A:5%	G:95% A:5%	A / G	G
KLK4	rs198968 (A/G)	19:51413328	Intron	A:15%	A:22%	A:71%	A:17%	A:37%	A / G	G

				G:85%	G:78%	G:29%	G:83%	G:63%		
	rs198969 (C/G)	19:51413802	Intron	G:47%	G:35%	G:84%	G:49%	G:59%	C / G	G
				C:53%	C:65%	C:16%	C:51%	C:41%		
	rs2235091 (A/G)	19:51410471	Intron	A:56%	A:64%	A:84%	A:61%	A:60%	A / G	A
				G:44%	G:36%	G:16%	G:39%	G:40%		
	rs2242670 (A/G)	19:51412315	5 prime UTR	A:51%	A:51%	A:80%	A:45%	A:64%	A / G	NA
				G:49%	G:49%	G:20%	G:55%	G:36%		
	rs2978642 (A/T)	19:51413906	Intron	A:36%	A:72%	A:78%	A:75%	A:69%	A / T	A
				T:64%	T:28%	T:22%	T:25%	T:31%		
	rs2978643 (C/G)	19:51412326	5 prime UTR	C:59%	C:74%	C:91%	C:75%	C:71%		
				G:41%	G:26%	G:9%	G:25%	G:29%	C / G	NA
<i>MMP13</i>	rs2252070 (A/G)	11:102826539	Open chromatin region	C:28%	C:35%	C:50%	C:30%	C:41%	A / G	T
				T:72%	T:65%	T:50%	T:70%	T:59%		
	rs243847 (T/C)	16:55523998	Intron	T:81%	T:71%	T:59%	T:59%	T:52%	T / C	T
<i>MMP2</i>				C:19%	C:29%	C:41%	C:41%	C:48%		
	rs243865 (C/T)	16:55511806	Intron	C:97%	C:76%	C:90%	C:74%	C:88%	C / T	C
				T:3%	T:24%	T:10%	T:26%	T:12%		
<i>MMP20</i>	rs1711437 (G/A)	11:102465226	Intron	C:75%	C:59%	C:64%	C:57%	C:41%	G / A	C

				T:25%	T:41%	T:36%	T:43%	T:59%		
	rs1784418 (C/T)	11:102484396	Intron	C:74%	C:56%	C:56%	C:55%	C:41%	C / T	C
				T:26%	T:44%	T:44%	T:45%	T:59%		
	rs1784418 (G/A)	11:102484396	Intron	C:74%	C:56%	C:56%	C:55%	C:41%	G / A	C
				T:26%	T:44%	T:44%	T:45%	T:59%		
<i>MMP3</i>	rs522616 (A/G)	11:102715048	Intergenic Region	T:80%	T:56%	T:65%	T:77%	T:56%	A / G	T
				C:20%	C:44%	C:35%	C:23%	C:44%		
<i>MMP9</i>	rs17576 (A/G)	20:44640225	Missense	A:66%	A:77%	A:26%	A:62%	A:45%	A / G	A
				G:34%	G:23%	G:74%	G:38%	G:55%		
	rs3790506 (A/G)	1:151538366	Intron	G:91%	G:64%	G:75%	G:74%	G:63%	A / G	A
				A:9%	A:36%	A:25%	A:26%	A:37%		
<i>TFIP1</i>	rs3828054 (A/G)	1:151512895	Missense	A:84%	A:90%	A:95%	A:89%	A:89%	A / G	A
				G:16%	G:10%	G:5%	G:11%	G:11%		
	rs7526319 (C/T)	1:151524558	Intron	T:63%	T:28%	T:12%	T:36%	T:33%	C / T	T
				C:37%	C:72%	C:88%	C:64%	C:67%		
<i>TIMP1</i>	rs4898 (T/C)	X:47444985	Synonymous	T:52%	T:58%	T:54%	T:54%	T:50%	T / C	C
				C:48%	C:42%	C:46%	C:46%	C:50%		
<i>TIMP2</i>	rs7501477 (G/T)	17:76926276	TF binding site	G:70%	G:89%	G:87%	G:89%	G:94%	G / T	G
				T:30%	T:11%	T:13%	T:11%	T:6%		

<i>TUFT1</i>	rs2337360 (A/G)	1:151542127	Intron	G:75%	G:75%	G:90%	G:64%	G:67%	A / G	NA
				A:25%	A:25%	A:10%	A:36%	A:33%		
<i>TUFT1</i>	rs4970957 (A/G)	1:151517388	Intron	A:98%	A:70%	A:53%	A:81%	A:81%	A / G	A
				G:2%	G:30%	G:47%	G:19%	G:19%		
<i>TFIP11</i>	rs5997096 (C/T)	22:26895957	Intron	T:66%	T:52%	T:50%	T:44%	T:63%	C / T	T
				C:34%	C:48%	C:50%	C:56%	C:37%		
<i>TFIP11</i>	rs134136 (C/T)	22:26899474	Intron	T:25%	T:35%	T:33%	T:35%	T:49%	C / T	T
				C:75%	C:65%	C:67%	C:65%	C:51%		

* Based on Human (GRCh37.p13), available on: http://grch37.ensembl.org/Homo_sapiens. NA: not available

Table 5. Summarization of results (meta-analysis and individuals) according by allelic and genotype (homozygote and heterozygote) analysis pooled by gene.

Gene	Polymorphism	Allelic		Genotype			
				Homozygote		Heterozygote	
		N	Pooled Odds Ratio (95%CI)	N	Pooled Odds Ratio (95%CI)	N	Pooled Odds Ratio (95%CI)
AMBN	rs34538475 (G/T)	1	0.15 (0.08 – 0.30) #	2	0.15 (0.02 – 1.14)	2	0.66 (0.36 – 1.23)
	rs4694075 (C/T)	2	0.77 (0.54 – 1.09)	3	0.59 (0.34 – 1.03)	3	0.87 (0.44 – 1.74)
	Overall AMBN		0.45 (0.17 – 1.18)		0.37 (0.17 – 0.82) #		0.76 (0.51 – 1.14)
AMELX	rs17878486 (C/T)	2	4.33 (0.82 – 22.91)	4	4.83 (0.91 – 25.69)	4	2.21 (0.98 – 4.99)
	rs2106416 (C/T)	3	1.17 (0.76 – 1.82)	1	1.50 (0.17 – 13.55)	1	1.21 (0.66 – 2.21)
	rs5933871 (T/C)	-	-	1	1.13 (0.04 – 30.61)	1	0.05 (0.01 – 0.53) #
	rs5934997 (T/C)	-	-	1	1.10 (0.04 – 29.41)	1	0.05 (0.01 – 0.52) #
	rs6639060 (C/T)	1	1.08 (0.63 – 1.85)	1	1.04 (0.43 – 2.51)	1	1.27 (0.49 – 3.30)
	rs946252 (C/T)	2	0.95 (0.56 – 1.63)	2	1.28 (0.69 – 2.39)	2	1.29 (0.75 – 2.22)
	rs7052450 (T/C)	1	0.46 (0.08 – 2.64)	-	-	-	-
	Overall AMELX		1.39 (0.79 – 2.45)		1.78 (1.23 – 2.56) #		1.16 (0.68 – 1.93)

<i>BMP2</i>	rs1884302 (T/C)	-	-	1	1.08 (0.72 – 1.62)	1	1.36 (0.99 – 1.86)
<i>BMP4</i>	rs2761887 (A/C)	1	1.17 (0.96 – 1.42)	1	0.71 (0.42 – 1.18)	1	1.07 (0.74 – 1.53)
<i>BMP7</i>	rs388286 (T/C)	1	0.97 (0.74 – 1.26)	1	0.90 (0.63 – 1.29)	1	0.72 (0.51 – 1.04)
<i>DLX3</i>	rs10459948 (T/G)	1	0.48 (0.22 – 1.05)	1	0.26 (0.05 – 1.52)	1	1.00 (0.18 – 5.60)
	rs11656951 (T/C) ^a	1	0.94 (0.46 – 1.93)	1	1.06 (0.23 – 4.90)	1	1.89 (0.46 – 7.77)
	rs12452477 (T/C) ^a	1	0.84 (0.37 – 1.89)	1	0.73 (0.07 – 7.71)	1	0.93 (0.01 – 9.85)
	rs16948563 (A/G)	1	1.00 (0.49 – 2.05)	1	1.07 (0.34 – 3.36)	1	3.20 (0.72 – 14.24)
	rs2278163 (T/C)	1	0.30 (0.15 – 0.64) #	1	0.07 (0.01 – 0.46) #	1	0.45 (0.14 – 1.46)
	rs2303466 (A/G) ^a	1	0.94 (0.46 – 1.93)	1	1.06 (0.23 – 4.90)	1	1.89 (0.46 – 7.77)
	rs3891034 (A/G) ^a	1	0.50 (0.23 – 1.10)	1	0.21 (0.02 – 2.07)	1	0.46 (0.05 – 4.25)
	Overall <i>DLX3</i>	0.67 (0.47 – 0.94) #		0.58 (0.31 – 1.07)		1.15 (0.64 – 2.08)	
	Overall <i>DLX3</i> ^{LD}	0.53 (0.26 – 1.07)		0.31 (0.06 – 1.56)		1.06 (0.32 – 3.50)	
	rs12640848 (A/G)	3	0.85 (0.64 – 1.14)	4	0.59 (0.26 – 1.33)	4	0.91 (0.52 – 1.61)
<i>ENAM</i>	rs2609428 (T/C)	1	3.89 (1.47 – 10.31) #	-	-	-	-
	rs3796703 (C/T)	1	1.65 (1.11 – 2.45) #	1	5.52 (0.26 – 116.29)	1	1.61 (1.03 – 2.52) #
	rs3796704 (A/G)	1	1.11 (0.57 – 2.16)	1	0.27 (0.02 – 3.07)	2	0.63 (0.28 – 1.41)
	Overall <i>ENAM</i>	1.08 (0.78 – 1.51)		0.77 (0.53 – 1.13)		0.99 (0.65 – 1.50)	
<i>KLK4</i>	rs198968 (A/G)	-	-	1	0.17 (0.03 – 0.94) #	1	0.15 (0.03 – 0.82) #

	rs198969 (C/G)	1	2.38 (1.30 – 4.35) #	1	18.07 (2.10 – 155.49) #	1	1.57 (0.66 – 3.58)
	rs2235091 (A/G)	1	2.30 (1.15 – 4.62) #	3	2.15 (0.60 – 7.18)	3	1.15 (0.73 – 1.83)
	rs2242670 (A/G)	1	0.87 (0.58 – 1.30)	1	0.73 (0.29 – 1.84)	1	2.07 (0.97 – 4.40)
	rs2978642 (A/T)	1	0.77 (0.49 – 1.21)	1	0.29 (0.078 – 1.08)	1	1.01 (0.56 – 1.81)
	rs2978643 (C/G)	-	-	1	0.79 (0.26 – 2.43)	1	1.12 (0.62 – 2.01)
	Overall KL4		1.32 (0.75 – 2.33)		0.91 (0.57 – 1.45)		1.17 (0.85 – 1.60)
<i>MMP13</i>	rs2252070 (A/G)	1	0.67 (0.51 – 0.89) #	1	0.54 (0.31 – 0.93) #	1	0.71 (0.47 – 1.08)
	rs243847 (T/C)	1	0.97 (0.75 – 1.25)	1	0.97 (0.55 – 1.71)	1	0.93 (0.65 – 1.34)
<i>MMP2</i>	rs243865 (C/T)	1	1.31 (0.98 – 1.76)	1	1.50 (0.79 – 2.85)	1	1.23 (0.80 – 1.89)
	Overall MMP2		1.12 (0.83 – 1.50)		1.17 (0.77 – 1.79)		1.04 (0.79 – 1.38)
	rs1711437 (G/A)	1	0.98 (0.76 – 1.26)	1	0.85 (0.50 – 1.45)	1	1.15 (0.79 – 1.68)
	rs1784418 (C/T)	3	0.83 (0.67 – 1.04)	4	0.97 (0.63 – 1.51)	4	0.82 (0.52 – 1.28)
<i>MMP20</i>	rs1784418 (G/A) ^a	1	1.16 (0.91 – 1.47)	1	1.26 (0.78 – 2.03)	1	1.33 (0.92 – 1.93)
	Overall MMP20		0.95 (0.80 – 1.13)		1.02 (0.77 – 1.35)		1.03 (0.77 – 1.38)
	Overall MMP20 ^{LD}		0.90 (0.76 – 1.06)		0.92 (0.66 – 1.29)		0.93 (0.66 – 1.32)
<i>MMP3</i>	rs522616 (A/G)	1	1.05 (0.80 – 1.38)	1	1.01 (0.59 – 1.74)	1	1.14 (0.78 – 1.69)
<i>MMP9</i>	rs17576 (A/G)	2	1.09 (0.88 – 1.34)	2	1.01 (0.49 – 2.09)	2	1.17 (0.48 – 2.86)

<i>TFIP1</i>	rs3790506 (A/G)	2	1.30 (0.88 – 1.92)	3	0.85 (0.20 – 3.59)	3	0.92 (0.44 – 1.90)
	rs3828054 (A/G)	-	-	1	0.44 (0.04 – 4.95)	1	1.06 (0.52 – 2.17)
	rs7526319 (C/T)	-	-	1	1.36 (0.60 – 3.09)	1	1.34 (0.73 – 2.45)
	Overall <i>TFIP1</i>		1.30 (0.88 – 1.92)		0.68 (0.40 – 1.16)		1.08 (0.77 – 1.50)
<i>TIMP1</i>	rs4898 (T/C)	1	1.05 (0.79 – 1.39)	1	1.26 (0.59 – 2.68)	1	1.47 (0.68 – 3.18)
<i>TIMP2</i>	rs7501477 (G/T)	2	1.13 (0.76 – 1.69)	2	0.69 (0.30 – 1.58)	2	1.27 (0.88 – 1.84)
<i>TUFT1</i>	rs2337360 (A/G)	2	0.47 (0.11 – 1.93)	2	0.71 (0.24 – 2.14)	2	0.20 (0.02 – 2.06)
	rs4970957 (A/G)	2	1.16 (0.78 – 1.73)	3	0.62 (0.31 – 1.27)	3	1.33 (0.84 – 2.10)
	Overall <i>TUFT1</i>		0.96 (0.64 – 1.45)		0.83 (0.52 – 1.34)		0.71 (0.23 -2.20)
<i>TFIP11</i>	rs134136 (C/T)	2	1.51 (1.02 – 2.22) #	3	1.04 (0.65 – 1.64)	3	1.01 (0.46 – 2.06)
	rs5997096 (C/T) ^a	2	0.76 (0.57 – 1.03)	2	0.68 (0.28 – 1.63)	3	0.65 (0.33 – 1.28)
	Overall <i>TFIP11</i>		1.01 (0.62 - 1.63)		0.87 (0.59 – 1.28)		0.76 (0.44 – 1.31)
	Overall <i>TFIP11</i> ^{LD}		1.64 (1.08 – 2.50) #		0.99 (0.60 – 1.62)		0.99 (0.48 – 2.06)

statistical significance (p<0.05); ^{LD} Results after exclusion of SNPs in linkage disequilibrium; ^a Polymorphisms in linkage disequilibrium excluded of analysis; Overall ^{LD} result excluding SNPs in linkage disequilibrium

S1. Main characteristics of studies included in this systematic review

Author , year	-Country	-Age	-Evaluation	dental	caries	Analytical	Adjustment
	-Study design	(permanent/	-categorization			Approach	variables
	-Sample (% Males)	deciduous)				SNPs in Hardy–Weinberg	
		-Ethnicity				equilibrium	
		-Statistic power					
		(yes/no)					
	-EUA	-3 to 5 y	-dmf			Chi-square and regression models	NR
	-Case and control	(deciduous)	-Cases dmfs ≥4 and control			NR	
	- 184 (55%)	- White (63%)	dmfs=0; children whit dmfs > 0 but				
		Non-white (37%)	< 4 were excluded of sample				
Slayton, et al. [31]		-Yes					
		AMBN Rs:NR G/A data not show. Not associated; AMELX Rs:NR C/T 1.1 (0.7-1.7); AMELX Rs:NR C/G1.1 (0.7-1.7)					
	CA (95% CI)	AMELX Rs:NR C/T 0.9 (0.5-1.4); ENAM Rs:NR G/A 1.6 (0.8-2.9); KLK4 Rs:NR A/C data not show. Not associated;					
		TFIP11 Rs:NR T/C data not show. Not associated; TFIP11 Rs:NR C/T 0.2 (0.0-4.7); TUFT1 Rs:NR T/C 5.8 (0.7-50.6)					
	AA (95% CI)	TFIP11 Rs:NR G/A 1.4 (0.7-2.3);					
		None association was observed. Data not show in paper					
Deeley, et al.	-Guatemala	-14 to 60y	-DMFT			Linear regression	NR

between 5 and 8 inclusive or dmfs
scores between 6 and 10 inclusive.
Severe: Cases with dmft scores
higher than 8 or dmfs scores
higher than 10.

CA (95% CI) **# AMBN rs34538475 G/T** effect NR p (Moderate Vs. Caries-free, p = 0.04); **# AMELX rs17878486 T/C** effect NR p
(Severe Vs. Caries-free, p = 0.01; Moderate Vs. Caries-free, p = 0.04); **ENAM rs3796704 G/A** effect NR. Not
associated; **# TUFT1 rs3790506 T/C** effect NR p to genotype CT (Moderate Vs. Caries-free, p = 0.05; and dmfs > 6, p
= 0.05); **TUFT1 rs2337360 G/A** effect NR. Not associated; **TFIP11 rs134136 C/T** effect NR. Not associated
AA (95% CI) Parameter estimates beta indicate that dmfs is increased when the T allele of ds3790506 (tuftelin) was involved and
when the C allele of rs17878486 (amelogenin) was involved.

Kang, et al. [28]	-Korea	-mean	23y	-DMFT and DMFS	Logistic regression.	Stratification	by
	-Cohort	(permanent)		- very low experience (DFMT and	Fisher's exact test	fluorite water	
	-120 (72%)	-European,		DMFS ≤ 2) Vs. higher experience	Yes		
		chinese,		(DMFT AND DMFS ≥ 3);			
		Japanese, sub-					
		saharan African					
		and Korean					
		-No					
	CA (95% CI)	Considering DMFT scores: AMELX rs17878486 T/C Genotype TC compared to TT OR 1.32 (0.05 – 34.13); Genotype					

CC compared to TT OR 0.44 (0.01 – 23.14); **AMELX rs5933871 T/C** Genotype TC compared to TT OR 0.05 (0.01 – 1.11); Genotype CC compared to TT OR 1.13 (0.04 – 29.35); **AMELX rs5934997 T/C** Genotype TC compared to TT OR 0.05 (0.00 – 1.08); Genotype CC compared to TT OR 1.10 (0.04 – 28.59);

Considering all subjects, no associations were observed with codominant and dominant models. Associations were only observed when the sample was stratified. Not associations observed in haplotypes analyses; Stratified analysis is displayed as follow: **AMELX rs17878486 T/C** Not associated; **# AMELX rs5933871 C/T** p (Genotype TT associated with higher experience in non-fluoridation water, p = 0.003); **# AMELX rs5934997 C/T** p (Genotype TT associated with higher experience in non-fluoridation water, p < 0.001);

AA (95% CI)									
-Poland	-5	and	13	-DMFT	and	dmft	Fisher's exact test		NP
-Cohort	(deciduous	and	-higher	experience	DMFT/dmft	≥3	Stratified by age (5y and 13y)		
-199 (41%)	permanent)		and lower	experience	DMFT/dmft	Yes			
	-NR		<3						
	-Yes								

Olszowski, et
al. [43]

Results of 5 years old children:

AMELX rs2106416 C/T Genotype CT compared to TT OR 0.95 (0.36 – 2.52); Genotype TT compared to CC OR 3.29 (0.01 – 7.56); Allele C compared to T OR 0.82 (0.36 – 1.90);

CA (95% CI)

Results of 13 years old children:

AMELX rs2106416 C/T Genotype CT compared to TT OR 1.40 (0.64 – 3.01); Genotype TT compared to CC OR 3.23

(0.60 – 17.46); Allele C compared to T OR 1.54 (0.85 – 2.81);

AA (95% CI)		-	
Shimizu, et al. [36]	-Multicentric (Brazil, Philippines, Turkey)	- <u>Brazil</u> : 2 cohorts: 10 to 14y; 2 to 21y (permanent and deciduous)	-DMFT/dmft; categorizations were performed in each country.
	-Multicentric cohort -1,831 (51%)	<u>Philippines</u> : up to 12y (permanent and deciduous)	<u>Philippines</u> : Low caries (DMFT/dmft ≤ 2) Vs. high (DMFT/dmft ≥ 3)
		<u>Argentina</u> : 1 to 72y	<u>Argentina</u> : NR
		<u>Turkey</u> : 3 to 6y (deciduous)	<u>Turkey</u> : Caries =-free Vs. dmft ≥ 4
		-NR	
		-No	
		# AMELX rs946252 C/T p (Philippines: Allele T associated with high caries, p = 0.01) (Turkey: Allele T associated with caries group, p = 0.004); # TUFT1 rs4970957 A/G p (Argentina: Allele A associated with high caries, p = 0.03) (Brasil: Allele A associated with high caries, p = 0.04); # AMBN rs4694075 C/T p (Philippines: Allele T associated with high caries, p = 0.007); # ENAM rs12640848 A/G rs12640848 (Brazil: Allele G associated with high caries, p = 0.04) TFIP11 rs4970957 A/G rs5997096 Not associated; Associations were performed separately for each country.	
	CA (95% CI)		

	AA (95% CI)		-		
Tannure, et al. [29]	-Brazil	-5 to 14y	-DMFT/dmft	chi-square tests and binary logistic regression	Stratification by
	-Cohort	(permanent and deciduous)	-Caries-free (dmft/DMFT = 0) Vs. Caries affected (dmft/DMFT ≥ 1);	Yes	Genotype, ethnicity and
	-388 (52%)	-Caucasian (58%) and Afro-descendants (42%)	Caries-free (dmft/DMFT = 0), low caries (dmft/DMFT = 1), Moderate caries (dmft/DMFT >1 and ≤ 3) and High caries experience (dmft/DMFT ≥ 4)		variables related to oral health habits (Visible plaque, Tooth-brushing, Use of dental floss daily, Use of fluoride mouthwash daily, Dietary factors)
		-No			
	CA (95% CI)	MMP20 rs1784418 C/T Genotype CT compared to CC OR 0.71 (0.45 – 1.12); Genotype TT compared to CC OR 0.93 (0.52 – 1.66); Allele C compared to T OR 0.92 (0.69 – 1.23)			
	AA (95% CI)	# MMP20 rs1784418 C/T Genotype CT compared to CC OR 0.53 (0.29 – 0.98); Genotype TT compared to CC OR 1.20 (0.55 – 2.63); An Interaction genotype and ethnicity was observed; Significance was only observed when adjusted by ethnicity.			
Tannure, et al. [26]	-Brazil	-mean 8y	-DMFT/dmft	Binary logistic regression	Candidate genes,
	-Cross-sectional	(permanent and deciduous)	-Caries-free (dmft/DMFT = 0) Vs. caries experience (dmft/	Yes	type of dentition and dietary factors
	-505 (53% Males)				

-Caucasian (57%) DMFT ≥ 1)

and Afro-
descendants

(43%)

-No

CA (95% CI)

MMP2 rs243865 C/T Genotype CT compared to CC OR 1.27 (0.86–1.89); Genotype TT compared to CC OR 1.54 (0.86–2.74); Allele T compared to C OR 1.31 (0.98 – 1.76); **MMP9 rs17576 A/G** Genotype AG compared to AA OR 0.75 (0.51 – 1.11); Genotype GG compared to AA OR 1.13 (0.77 – 2.31); Allele A compared to G OR 1.04 (0.79 – 1.37); **# MMP13 rs2252070 A/G** Genotype AG compared to AA 0.70 (0.48-1.04); Genotype GG compared to AA OR 0.54 (0.31–0.93); Allele A compared to G OR 0.67 (0.51 – 0.89); **TIMP2 G/T rs7501477** Genotype GT compared to GG 1.413 (0.95 – 2.10). Genotype TT compared to GG OR 0.88 (0.36 – 2.13); Allele T compared to G OR 1.38 (0.97 – 1.95)

AA (95% CI)

MMP2 rs243865 C/T Genotype CT compared to CC OR 1.23 (0.80–1.89). Genotype TT compared to CC OR 1.50 (0.79–2.86); **MMP9 rs17576 A/G** Genotype AG compared to AA OR 0.74 (0.49–1.13); Genotype GG compared to AA OR 1.35 (0.75 – 2.46); **# MMP13 rs2252070 A/G** Genotype AG compared to AA 0.71 (0.46-1.07); Genotype GG compared to AA OR 0.54 (0.31–0.92); **# TIMP2 G/T rs7501477** Genotype GT compared to GG OR 1.53 (1.01-2.34); Genotype TT compared to GG OR 0.87 (0.33 – 2.93)

Wang, et al.	-EUA	-4	to	7y	-dmfs	and	WS	-Linear	and	logistic	regression	-Age, sex, race,
[39]	-Cohort	Study	(permanent/	- 1)	total number of tooth surfaces	model						tooth-brushing
	(longitudinal)		deciduous)		with frank cavitated or filled caries	Yes						frequencies and

-575 (48%)	-	Caucasian experience (d2fs-total); 2) pit and fissure surfaces with caries experience (d2fs-pit/fissure); and 3) caries experience of all other tooth surfaces (d2fs-smooth surface). These scores were dichotomized in the downstream analyses as cases (children with scores ≥ 61) and controls (scores = 0).	fluoride intake from water, tooth-brushing frequency.
CA (95% CI)		# DSPP rs2615487 C/T p (Allele T show protective effect against caries in d2fs-total, $p < 0.001$; d2fs-pit/fissure, $p < 0.001$; and d2fs-smooth surface, $p = 0.002$); TUFT1 rs3748609 A/G effect NR. Not associated; TUFT1 rs11204846 A/G effect NR. Not associated; TUFT1 rs3748608 A/G effect NR. Not associated; TUFT1 rs7526319 C/T effect NR. Not associated; TUFT1 rs3828054 A/G effect NR. Not associated; TUFT1 rs6587597 A/G effect NR. Not associated; TUFT1 rs7554707 G/T effect NR. Not associated; TUFT1 rs2337360 A/G effect NR. Not associated; SPP1 rs10516800 C/G effect NR. Not associated; SPP1 rs6840362 C/T effect NR. Not associated; SPP1 rs10516799 C/G effect NR. Not associated; SPP1 rs11728697 C/T effect NR. Not associated; ENAM rs12640848 A/G effect NR. Not associated; ENAM rs3796704 A/G effect NR. Not associated; ENAM rs7671281 C/T effect NR. Not associated; MMP20 rs1784418 C/T effect NR. Not associated; MMP20 rs2245803 G/T effect NR. Not associated; MMP20 rs7109663 C/G effect NR. Not associated; # KLK4 rs2235091 A/G p (Allele A show protective effect against caries in d2fs-total, $p = 0.02$; d2fs-pit/fissure, $p = 0.03$; and d2fs-smooth surface, $p = 0.02$); KLK4 rs198969 C/G effect NR. Not associated	

AMELX rs2106416 C/T Allele T compared to C OR 0.90 (0.32-2.46); **Male:** Allele T Compared to C OR 2.72 (0.48-19.51); **AMELX rs138249749 G/T** effect NR. Not associated; **AMELX s7052450 T/C** Allele C compared to T OR 0.46 (0.08-2.63); **Male:** Allele T Compared to C OR 1.10 (0.12-infinite)

-Brazil and Turkey	- NC (permanent	-DMFT/dmft	and	WS	Fisher's exact test and odds ratio	-Streptococcus
-Cohort Study	and deciduous)	-NC			calculation	mutans
-405 (48%)	-NR			Yes		colonization status
	-No					and molar-incisor
						hypomineralization
						status

Jeremias, et
al. [23]

CA (95% CI)

AMELX rs946252 (C/T) Genotype CT compared to CC OR 0.95 (0.40 – 2.25); Genotype TT compared to CC OR 1.03 (0.29 – 3.75); Allele T compared to C OR 1.01 (0.54 – 1.89); **# AMELX rs17878486 (C/T)** Genotype CT compared to CC OR 5.11 (1.33 – 19.72); Genotype TT compare to CC OR 3.82 (1.19 – 12.21); Allele T compared to C OR 1.87 (1.06 – 3.30); **# TFIP11 rs5997096 (C/T)** Genotype CT compared to CC OR 0.35 (0.14 – 0.88); Genotype TT compared to CC OR 0.42 (0.16 – 1.10); Allele T compared to C OR 0.72 (0.46 – 1.13); **# TFIP11 rs134136 (C/T)** Genotype CT compared to CC OR 0.71 (0.34 – 1.45); Genotype TT compared to CC OR 1.15 (0.47 – 2.80); Allele T compared to C OR 1.78 (1.11 – 2.86); **AMBN rs4694075 (C/T)** Genotype CT compared to CC OR 0.53 (0.25 – 1.15); Genotype TT compared to CC OR 0.70 (0.28 – 1.71); Allele T compared to C OR 0.84 (0.54 – 1.30); **AMBN rs496502 G/T** Genotype GT compared to GG OR 1.00 (0.51 – 1.98); Genotype TT compared to GG OR 0.50 (0.12 – 2.12); Allele T compared to G OR 1.19 (0.70 – 2.05); **ENAM rs12640848 (A/G)** Genotype AG compared to AA OR 1.73 (0.77 – 3.91); Genotype GG compared to AA OR 1.62 (0.64 – 4.09); Allele G compared to A OR 0.94 (0.59 – 1.48); **ENAM rs3796704 (A/G)**

Genotype AG compared to AA OR 0.15 (0.01 – 1.46); Genotype GG compared to AA OR 0.27 (0.03 – 2.47); Allele G compared to A OR 1.11 (0.57 – 2.15); **TUFT1 rs3790506 (A/G)** Genotype AG compared to AA OR 0.95 (0.31 – 2.92); Genotype GG compared to AA OR 1.23 (0.41 – 3.71); Allele G compared to A OR 1.19 (0.73 – 1.92); **TUFT1 rs2337360 (A/G)** Genotype AG compared to AA OR 0.67 (0.21 – 2.13); Genotype GG compared to AA OR 0.73 (0.23 – 2.32); Allele G compared to A OR 0.94 (0.58 – 1.53); **TUFT rs4970957 (A/G)** Genotype AG compared to AA OR 1.29 (0.66 – 2.55); Genotype GG compared to AA OR 0.69 (0.21 – 2.28); Allele G compared to A OR 0.99 (0.60 – 1.66)

AMELX rs946252 (C/T) Genotype CT compared to CC OR 0.93 (0.39 – 2.22); Genotype TT compared to CC OR 1.02 (0.28 – 3.73); **# AMELX rs17878486 (C/T)** Genotype CT compared to CC OR 5.38 (1.38 – 20.98); Genotype TT compared to CC OR 3.91 (1.21 – 12.55); **# TFIP11 rs5997096 (C/T)** Genotype CT compared to CC OR 0.34 (0.13 – 0.88); Genotype TT compared to CC OR 0.41 (0.41 – 1.09); **TFIP11 rs134136 (C/T)** Genotype CT compared to CC OR 0.52 (0.26 – 1.05); Genotype TT compared to CC OR 0.84 (0.35 – 2.1); **AMBN rs4694075 (C/T)** Genotype CT compared to CC OR 0.53 (0.25 – 1.16); Genotype TT compared to CC OR 0.69 (0.28 – 1.70); **AMBN rs496502 G/T** Genotype GT compared to GG OR 0.60 (0.29 – 1.2); Genotype TT compared to GG OR 0.86 (0.19 – 3.88); **ENAM rs12640848 (A/G)** Genotype AG compared to AA OR 1.73 (0.76 – 3.96); Genotype GG compared to AA OR 1.60 (0.62 – 4.11); **ENAM rs3796704 (A/G)** Genotype AG compared to AA OR 1.15 (0.01 – 1.50); Genotype GG compared to AA OR 0.27 (0.02 – 2.58); **TUFT1 rs3790506 (A/G)** Genotype AG compared to AA OR 0.91 (0.29 – 2.84). Genotype GG compared to AA OR 1.17 (0.38 – 3.59); **TUFT1 rs2337360 (A/G)** Genotype AG compared to AA OR 0.66 (0.20 – 2.12); Genotype GG compared to AA OR 0.71 (0.22 – 2.32); **TUFT rs4970957 (A/G)** Genotype AG compared to AA OR 1.02 (0.53 – 1.95); Genotype GG compared to AA OR 0.27 (0.66 – 2.57);

AA (95% CI)

		Saharan Africa	frequency
		(4%); Asia (5%);	
		others (9%)	
		-Yes	
		ENAM rs182835987 T/A effect NR. Not associated; ENAM rs147876348 A/G effect NR. Not associated; ENAM rs144929717 G/A effect NR. Not associated; ENAM rs2609429 G/T effect NR. Not associated; ENAM rs202231676 /T effect NR. Not associated; ENAM rs34251790 T/C effect NR. Not associated; ENAM rs149086531 G/A effect NR. Not associated; ENAM rs147177510 G/A effect NR. Not associated; ENAM rs139228330 A/G effect NR. Not associated. ENAM rs74511578 G/A effect NR. Not associated; # ENAM rs2609428 T/C Allele C compared to T was associated with caries OR 3.89(1.47–10.33); ENAM rs6813313 C/T effect NR. Not associated; ENAM rs7671281 T/C effect NR. Not associated; ENAM rs36064169 C/T effect NR. Not associated; ENAM rs3796704 G/A effect NR. Not associated; EMAM rs138729240 T/C effect NR. Not associated; EMAM rs71599965 G/A effect NR. Not associated.	
	CA (95% CI)		
	AA (95% CI)	# Haplotype analysis show that association of ENAM rs7671281 T/C and ENAM rs3796704 G/A were associated with caries susceptibility even after adjustment for environmental factors OR 2.66 (0.99–7.20)	
		-5 to 6y -	dmft
		(deciduous)	Welch's t test
		-Japanese	Stratification by
		-NR	or Student t-test; correlation
			between the high level Mutans streptococci was
			streptococci and caries experience performed
			was assessed by Spearman's
Ohta, et al. [25]	-Japan -cohort -201 (52.7%)	- low caries experience dmft ≤ 2 and high caries experience dmft > 3	

correlation test

NR

In Low level Mutans streptococci:

DLX3 rs11656951 T/C Genotype TC compared to TT OR 0.57 (0.18-1.80); Genotype CC compared to TT OR 0.50 (0.11-2.22); Allele C compared to T OR 0.7 (0.34-1.46); **DLX3 rs10459948 T/G** Genotype TG compared to TT OR 0.45 (0.11-1.74); Genotype GG compared to TT OR 0.53 (0.13-2.14); Allele G compared to T OR 0.84 (0.41-1.75)

DLX3 rs2278163 T/C Genotype TC compared to TT OR 0.59 (0.18-1.89); Genotype CC compared to TT OR 1.89 (0.43-8.35); Allele T compared to C OR 1.08 (0.50-2.35); **DLX3 rs2303466 A/G** Genotype AG compared to AA OR 0.39 (0.12-1.24); Genotype GG compared to AA OR 0.45 (0.10-1.94); Allele G compared to A OR 0.63 (0.30-1.32);

DLX3 rs3891034 A/G Genotype AG compared to AA OR 1.26 (0.24-6.75); Genotype GG compared to AA OR 2.49 (0.49-12.06); Allele G compared to A OR 1.75 (0.79-3.84); **DLX3 rs12452477 T/C** Genotype TC compared to TT OR 1.53 (0.17-13.96); Genotype CC compared to TT OR 2.4 (0.28-20.61); Allele C compared to T OR 1.57 (0.68-3.64)

DLX3 rs16948563 A/G Genotype AG compared to AA OR 1.41 (0.40-5.01); Genotype GG compared to AA OR 1.37 (0.39-4.89); Allele G compared to A OR 1.13 (0.54-2.33)

CA (95% CI)

In High level Mutans streptococci:

DLX3 rs11656951 T/C Genotype TC compared to TT OR 1.89 (0.46-7.78); Genotype CC compared to TT OR 1.06 (0.23-4.92); Allele C compared to T OR 0.94 (0.46-1.94); **DLX3 rs10459948 T/G** Genotype TG compared to TT OR 1.00 (0.18-5.65); Genotype GG compared to TT OR 0.26 (0.05-1.70); Allele G compared to T OR 0.48 (0.22-1.05)

DLX3 rs2278163 T/C Genotype TC compared to TT OR 0.45 (0.14-1.47); Genotype CC compared to TT OR 0.07 (0.01-0.04); Allele C compared to T OR 0.30 (0.14-0.64); **DLX3 rs2303466 A/G** Genotype AG compared to AA OR 1.89

(0.46-7.78); Genotype GG compared to AA OR 1.06 (0.23-4.92); Allele G compared to A OR 1.06 (0.52-2.19)

DLX3 rs3891034 A/G Genotype AG compared to AA OR 0.46 (0.05-4.26); Genotype GG compared to AA OR 0.21 (0.02-1.94); Allele G compared to A OR 0.50 (0.23-1.11); **DLX3 rs12452477 T/C** Genotype TC compared to TT OR 0.93 (0.09-10.09); Genotype CC compared to TT OR 0.73 (0.07-7.80); Allele C compared to T OR 0.84 (0.37-1.87)
DLX3 rs16948563 A/G Genotype AG compared to AA OR 3.20 (0.72-14.25); Genotype GG compared to AA OR 1.07 (0.34-3.35); Allele G compared to A OR 1.00 (0.49-2.05)

AA (95% CI)		-	
-Turkey	-2 to 5y -dmst and WS	-Fisher's exact tests and logistic regression analysis	-Frequency, sugar and/or acid drink consumption and time of first toothbrushing
-Cross-sectional	(deciduous) -Caries-free (dmft = 0) Vs. Caries experience (dmft ≥ 1)	rs17878486, rs946252, rs3796704, rs2337360 were not in Hardy-Weinberg equilibrium	
-259 (50%)	-NR		
	-No		
CA (95% CI)		AMBN rs4694075 C/T Genotype CT compared to CC OR 1.99 (0.78-5.08); Genotype TT compared to CC OR 0.51 (0.21-2.28); AMBN rs34538475 G/T Genotype GT compared to GG OR 0.84 (0.43-1.64); Genotype TT compared to GG OR 0.42 (0.12-1.45); AMELX rs17878486 C/T Genotype CT compared to CC OR 1.10 (0.46-2.65); Genotype TT compared to CC OR 1.28 (0.46-2.65); AMELX rs946252 C/T Genotype CT compared to CC OR 1.54 (0.80-2.96); Genotype TT compared to CC OR 1.59 (0.81-3.13); ENAM rs12640848 A/G Genotype AG compared to AA OR 0.65 (0.32-1.30); Genotype GG compared to AA OR 0.53 (0.25-1.13); ENAM rs3796704 A/G Genotype GG compared to AG OR 0.63 (0.29-1.37); KLK4 rs2235091 A/G Genotype AG compared to AA OR 1.58 (0.38-6.55); Genotype GG compared to AA OR 1.78 (0.46-6.88); KLK4 rs198968 A/G Genotype AG compared to AA OR 0.43 (0.09-1.90);	

Abbasoglu,
et al. [27]

Genotype GG compared to AA OR 0.45 (0.11-1.87); **MMP20 rs1784418 C/T** Genotype CT compared to CC OR 1.25 (0.66-2.37); Genotype TT compared to CC OR 1.04 (0.52-2.05); **TFIP11 rs5997096 C/T** Genotype CT compared to CC OR 0.75 (0.37-1.51); Genotype TT compared to CC OR 1.19 (0.52-2.68); **TFIP11 rs134136 C/T** Genotype CT compared to CC OR 1.18 (0.67-2.06); Genotype TT compared to CC OR 1.39 (0.66-2.90); **TFIP1 rs7526319 C/T** Genotype CT compared to CC OR 1.38 (0.79-2.40); Genotype TT compared to CC OR 1.32 (0.62-2.85); **TFIP1 rs4970957 A/G** Genotype AG compared to AA OR 0.85 (0.48-1.50); Genotype GG compared to AA OR 0.64 (0.25-1.63); **TFIP1 rs3828054 A/G** Genotype AG compared to AA OR 1.17 (0.59-2.29); Genotype GG compared to AA OR 0.51 (0.05-5.66); **# TFIP1 rs3790506 A/G** Genotype AG compared to AA OR 0.93 (0.34-2.53); Genotype GG compared to AA OR 0.34 (0.12-0.94)

AA (95% CI)

AMBN rs4694075 C/T Genotype CT compared to CC OR 1.74 (0.66-4.61); Genotype TT compared to CC OR 0.58 (0.22-1.49); **AMBN rs34538475 G/C** Genotype GT compared to GG OR 0.75 (0.36-1.57); Genotype TT compared to GG OR 0.41 (0.11-1.54); **AMELX rs17878486 C/T** Genotype CT compared to CC OR 1.04 (0.41-2.64); Genotype TT compared to CC OR 1.28 (0.59-2.77); **AMELX rs946252 C/T** Genotype CT compared to CC OR 1.59 (0.79-3.18); Genotype TT compared to CC OR 1.37 (0.67-2.78); **# ENAM rs12640848 A/G** Genotype AG compared to AA OR 0.61 (0.29-1.29); Genotype GG compared to AA OR 0.41 (0.18-0.92); **ENAM rs3796704 A/G** Genotype GG compared to AG OR 0.59 (0.25-1.37); **KLK4 rs2235091 A/G** Genotype AG compared to AA OR 1.65 (0.36-7.57); Genotype GG compared to AA OR 1.70 (0.40-7.18); **# KLK4 rs198968 A/G** Genotype AG compared to AA OR 0.15 (0.03-0.89); Genotype GG compared to AA OR 0.17 (0.03-0.92); **MMP20 rs1784418 C/T** Genotype CT compared to CC OR 1.20 (0.61-2.39); Genotype TT compared to CC OR 1.02 (0.49-2.12); **TFIP11 rs5997096 C/T** Genotype CT compared to CC OR 0.64 (0.31-1.35); Genotype TT compared to CC OR 1.03 (0.44-2.39); **TFIP11 rs134136 C/T** Genotype CT

compared to CC OR 1.58 (0.80-3.11); Genotype TT compared to CC OR 1.06 (0.58-1.91); **TFIP1 rs7526319 C/T** Genotype CT compared to CC OR 1.34 (0.73-2.43); Genotype TT compared to CC OR 1.36 (0.60-3.09); **TFIP1 rs4970957 A/G** Genotype AG compared to AA OR 0.98 (0.53-1.79); Genotype GG compared to AA OR 0.57 (0.22-1.48); **TFIP1 rs3828054 A/G** Genotype AG compared to AA OR 1.06 (0.52-2.17); Genotype GG compared to AA OR 0.44 (0.04-5.06); **# TFIP1 rs3790506 A/G** Genotype AG compared to AA OR 0.64 (0.21-1.98); Genotype GG compared to AA OR 0.23 (0.07-0.74)

-Brazil	- 1 to 6y -dmft	Logistic regression	age, gender,
- cohorts	(deciduous) - low caries dmft ≤ 2 and higher	rs388286 in BMP7 in the Nova	ethnicity,
-850 (53.2%) including	-Caucasian and caries experience dmft > 3	Friburgo cohort was not in Hardy-	toothbrushing,
two cohorts.	afro-descendants	Weinberg equilibrium	daily use of dental
	-NR		floss and ingesting
			sweets between
			meals

Romanos, et
al. [47]

Results are displayed separately of each cohort. To perform meta-analysis, results were included separately;

Results from Nova Friburgo Cohort:

BMP2 rs1884302 T/C Genotype CT compared to TT OR 1.58 (0.98 – 2.57); Genotype CC compared to TT OR 0.88 (0.46 – 1.69); Allele C compared to T OR 1.01 (0.75 – 1.38); **BMP4 rs2761887 A/C** Genotype AC compared to AA OR 0.98 (0.62 – 1.57); Genotype CC compared to AA OR 0.71 (0.38 – 1.33); Allele C compared to A OR 1.16 (0.86 – 1.57); **BMP7 rs388286 T/C** Genotype CT compared to TT OR 0.86 (0.48 – 1.55); Genotype CC compared to TT OR 0.99 (0.58 – 1.71); Allele C compared to T OR 0.99 (0.71 – 1.37)

CA (95% CI)

	-EUA	-3 to 12 and ≥18	-	DMFT	and	dmft	Logistic regression		Fluoridated
	-Multicentric	(permanent and deciduous)	-	caries experience	DMFT>1	Vs no	NR		Water; Daily tooth
	-3,600 (54% Males)			caries; dmft >1	Vs no caries				Brushing; Tooth
Shaffer, et		-black and white							brushing
al. [41]		-NR							per day
	CA (95% CI)						-		
	AA (95% CI)								
				AMBN rs17149026 G/T	No association, effect NR;	AMBN rs17733915 C/T	effect NR p (dmft≥1 p < 0.05);		

Antunes, et al. [48]

(0.68 – 1.39); Genotype CC compared to TT OR 1.26 (0.59-2.66); Allele T compared to C OR 1.05 (0.79 – 1.39);

TIMP2 rs7501477 G/T Genotype TG compared to GG OR 1.05 (0.68 – 1.62); Genotype GG compared to TT 0.5 (0.14 - 1.80); Allele G compared to T OR 0.92 (0.62 – 1.33); **# MMP9 rs17576 A/G** Genotype GA compared to AA OR 1.85 (1.18 – 2.90); Genotype GG compared to GA OR 0.63 (0.25 - 1.63); Allele G compared to A OR 1.16 (0.84 – 1.61)

Yildiz, et al. [49]	-Turkey	-20 to 60y	-DMFT	-Qui square and multiple linear	-Plaque
	-Case control	(permanent)	-low caries risk (DMFT ≤ 5) and	regression analyses	amount,
	-154 (% Males)	-NR	high caries risk (DMFT ≥ 14)	NR	toothbrushing
		-Yes			frequency, dietary intake between meals, saliva secretion rate, saliva buffer capacity, mutans streptococci counts, and lactobacilli counts
	CA (95% CI)	AMELX rs6639060 C/T Genotype CT compared to CC OR 1.27 (0.49 -3.30); Genotype TT compared to CC OR 1.04 (0.43-2.51); Allele C compared to T OR 1.08 (0.63 - 1.84)			
	AA (95% CI)	-			
Gerreth, et	-Poland	-1 and 2y	-WS and cavitated caries	Fisher's exact test and Odds ratio	NR

al. [21]	-Case control	(deciduous)	-Caries experience and Control	Calculation	
	-96 (49%)	-NR	without caries experience	Yes	
		-NR			
	ENAM rs2609428 C/T effect NR. Not associated; ENAM rs7671281 C/T effect NR. Not associated.				
	CA (95% CI)	ENAM rs36064169 G/T effect NR. Not associated; ENAM rs3796704 A/G effect NR. Not associated; # ENAM rs12640848 A/G Genotype AG compared to AA OR 0.71 (0.19 – 2.61). Genotype GG compared to AA OR 0.08 (0.02 – 0.46); Allele G compared to A 0.45 (0.25 – 0.80)			
	AA (95% CI)	-			
Cavallari, et al. [30]	-Brazil	-12 to 34y	-ICDAS	criteria	Fisher exact test
	-Case control	(permanent)	- zero score and scores ≥ 1	Yes	NP
	-200 (39%)	-Caucasian (92%), Afro-descendent (8%)			
		-Yes			
	CA (95% CI)	KLK4 rs2242670 A/G Genotype AG compared to AA OR 2.07 (0.97 – 4.38); Genotype GG compared to AA OR 0.73 (0.29 – 1.84); Allele G compared to A OR .87 (0.58 – 1.29); KLK4 rs2235091 A/G Genotype AG compared to AA OR 1.24 (0.68 – 2.24); Genotype GG compared to AA OR 1.0 (0.33 – 3.06); KLK4 rs2978642 A/T Genotype AT compared to AA OR 1.01 (0.56 – 1.80); Genotype TT compared to AA OR 0.29 (0.08 – 1.11); Allele T compared to A OR 0.77 (0.49 – 1.20); KLK4 rs2978643 C/G Genotype CG compared to CC OR 1.12 (0.62 – 2.00); Genotype GG compared to			

CC OR 0.79 (0.26 – 2.45)

KLK4 rs2242670 A/G Allele A in additive model ($p = 0.009$) and dominant (AA+AG Vs. GG OR 2.37 (1.16-4.84)); Model were associated with dental caries. Allele G in dominant model was not significant (GG + AG Vs. AA OR 1.58 (0.77-3.27)); Genotype AG compared to AA OR 2.07 (0.97-4.37); Genotype GG compared to AA 0.73 (0.29-1.83); **KLK4 rs2235091 A/G** Additive model (AA/GG/AG) ($p=0.77$), A dominant model (AA+AG Vs. GG) OR 1.13 (0.39-3.25) and G dominant (GG + AG Vs. AA) OR 1.20 (0.67-2.19) were not associated; Genotype AG compared to AA OR 1.13 (0.61-2.05); Genotype GG compared to AA OR 1.00 (0.33-3.06); **# KLK4 rs2978642 A/T** Allele A in dominant model (AA+AT Vs. TT) was associated with dental caries OR 3.48 (1.00-13.07) and allele T in the additive (AA/TT/AT) ($p=0.15$) and dominant (OR 0.87 (0.50-1.52), $p=6.62$) was not associated; **KLK4 rs2978643 C/G** Additive model (CC/CG/GG) ($p=0.83$), C dominant model (CC + CG Vs. GG) OR 1.32 (0.44-3.96), and G dominant model (GG + CG Vs. GG) OR 1.66 (0.67-1.85) were not associated.

AA (95% CI)

Filho, et al. [50]	-Brazil	-4 to 7 y	-Dmf	- fisher exact test and odds ratio	NP
	-Cross-sectional	Deciduous	-Caries free vs low caries (dmf=1),	Yes	
	- 184 (48.4%)	- white (65), moderate (dmf=2-3) and high black (26), mixed experience (dmf=4 or more) (93)			
		- Yes			
	CA (95% CI)	MMP20 rs1784418 C/T = Allele T compared to C OR 0.64(0.41-1.00); Genotype CT compared to CC OR 1.4 (0.38 – 5.16); TT compared to CC OR 0.71 (0.28 – 1.80)			
	AA (95% CI)			-	
Gerreth, et	-Poland	-1 and 2y	-WS and cavitated caries	Fisher's exact test	NR

al. [22]	-Case control	(deciduous)	-Caries experience and Control Yes
	-96 (48%)	-NR	without caries experience
		-NR	

CA (95% CI)

AMELX rs17878486 C/T Genotype CT compared to CC OR 2.77 (0.90-8.52); Genotype TT compared to CC OR 38.75 (9.38-160.11); Allele T compared to C was associated with caries OR 10.23 (5.25–19.94); **# AMBN rs34538475 G/T** Genotype GT compared to GG OR 0.50 (0.19-1.79); Genotype TT compared to GG OR 0.05 (0.01-0.17); Allele G compared to T was associated with caries OR 0.15 (0.07-0.27); **AMBN rs4694075 C/T** Genotype CT compared to CC OR 0.83 (0.31-2.23); Genotype TT compared to CC OR 0.50 (0.18-1.42); Allele T compared to C was associated with caries OR 0.66 (0.37–1.16); **TUFT1 rs3790506 A/G** Genotype AG compared to AA OR 2.25 (0.39-13.07); Genotype GG compared to AA OR 3.04 (0.54-17.17); Allele G compared to A was associated with caries OR 1.53 (0.80–2.91) **TUFT1 rs4970957 A/G** Genotype AG compared to AA OR 2.29 (0.99-5.26); Genotype GG compared to AA OR 0.75 (0.06-8.89); Allele G compared to A was associated with caries OR 1.50 (0.80–2.90); **# TUFT1 rs2337360 A/G** Genotype AG compared to AA OR 0.06 (0.02-0.17); Genotype GG compared to AA OR 0.70 (0.03-18.69); Allele G compared to A OR 0.22 (0.11–0.45); **TFIP11 rs134136 C/T** Genotype CT compared to CC OR 1.30 (0.54-3.17); Genotype TT compared to CC OR 1.37 (0.40-4.66); Allele T compared to C was associated with caries OR 1.19 (0.67–2.12); **TFIP11 rs5997096 C/T** Genotype CT compared to CC OR 1.27 (0.49-3.29); Genotype TT not present in sample; Allele T compared to C OR 1.23 (0.50–3.00); **MMP20 rs1784418 C/T** Genotype CT compared to CC OR 0.75 (0.32-1.77); Genotype TT compared to CC OR 0.85 (0.21-3.44); Allele T compared to C OR 0.87 (0.48–1.57); **# KLK4 rs2235091 A/G** Genotype GA compared to AA OR 0.9 (0.40-2.1); Genotype GG compared to AA OR 12.4 (1.5-101.0); Allele G compared to A OR 2.3 (1.2–4.84); **# KLK4 rs198969 C/G** Genotype CG compared to CC OR 1.57 (0.66-3.78);

Genotype GG compared to CC OR 18.07 (2.10-155.49); Allele G compared to C OR 2.38 (1.30–4.34)

AA (95% CI)		-	
Borilova Linhartova, et al. [44]	-Czech republic	-2 to 6y and 13	-dmft
	-Case Control	to	15y
	-718 (53%)	(deciduous and experience (dmft ≥ 1)	15y)
		permanent)	NR
		-NR	
		-No	
Stratified by deciduous teeth:			
ENAM rs12640848 A/G Genotype AG compared to AA OR 0.33 (0.10 – 1.10); Genotype GG compared to AA OR 0.51 (0.15 - 1.70); Allele G compared to A OR 0.99 (0.64 – 1.52);			
Stratified by permanent teeth:			
ENAM rs12640848 A/G Genotype AG compared to AA OR 1.47 (0.83 – 2.61); Genotype GG compared to AA OR 1.18 (0.67 – 2.08); Allele G compared to A OR 0.97 (0.75 – 1.24)			
AA (95% CI)		-	
Wang, et al. [24]	-China	-under 4y	-dmft
	-Case Control	(deciduous)	-Caries-free (dmft = 0) vs. caries
	-1005	-NR	experience (dmft ≥ 1)
		-Yes	
		Chi-square or Fisher's exact test and binary logistic regression test	
		-Diet, oral behavioural habits and application of topical fluoride	

		# ENAM rs3796703 C/T Genotype CT compared to CC OR 1.68 (1.12 - 2.54); Genotype TT compared to CC OR 5.52 (0.26-115.40); Allele T compared to C was associated with caries OR 1.65 (1.11 – 2.44)		
CA (95% CI)				
AA (95% CI)		# ENAM rs3796703 C/T Genotype CT compared to CC OR 1.61 (1.03-2.52)		
-Norway	-Children follow	-DMFT/dmft; approximal lesions	-Bonferroni correction	was NR
-Longitudinal study	from 5 to 18y.	recorded during the radiographic	implemented to correct for multiple	
-856 (47%)	(permanent/deciduous)	examination, only dentin lesions	Comparisons	
	-NR	were included (D/d3–5).	Yes	
	-Yes	- No caries vs. low caries; No caries vs. high caries; Low caries vs. high caries; No primary caries (dmft) vs. low primary caries (dmft); No primary caries (dmft) vs. high primary caries (dmft); Low primary caries (dmft) vs. high primary caries (dmft); No caries vs. very high caries; Very high caries vs. low caries; Very high caries vs. high caries; Acute increase in DMFT vs. no acute increase in DMFT; Acute increase in DMFT vs. no caries		

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[42]

CA (95% CI)	# KLK4 rs2235091 A/G p ($p = 0.0008$ in a recessive model for low primary caries vs. high primary caries experience, and $p = 0.0004$ in a recessive model for no primary caries vs. high primary caries experience)
AA (95% CI)	-

NR: not reported; # Statistical association; CI: Confidence Interval; OR: Odds Ratio; PR: Prevalence Ratio; dmft (decayed, missing teeth due to caries, filled teeth); WSL: white spot lesions; ICDAS: International Decay Detection and Assessment System; CA: Crude association; AA: adjusted association; All measure effects showed are ODDS Ratio. Different measures are reported; SNP: Single Nucleotide Polymorphism.

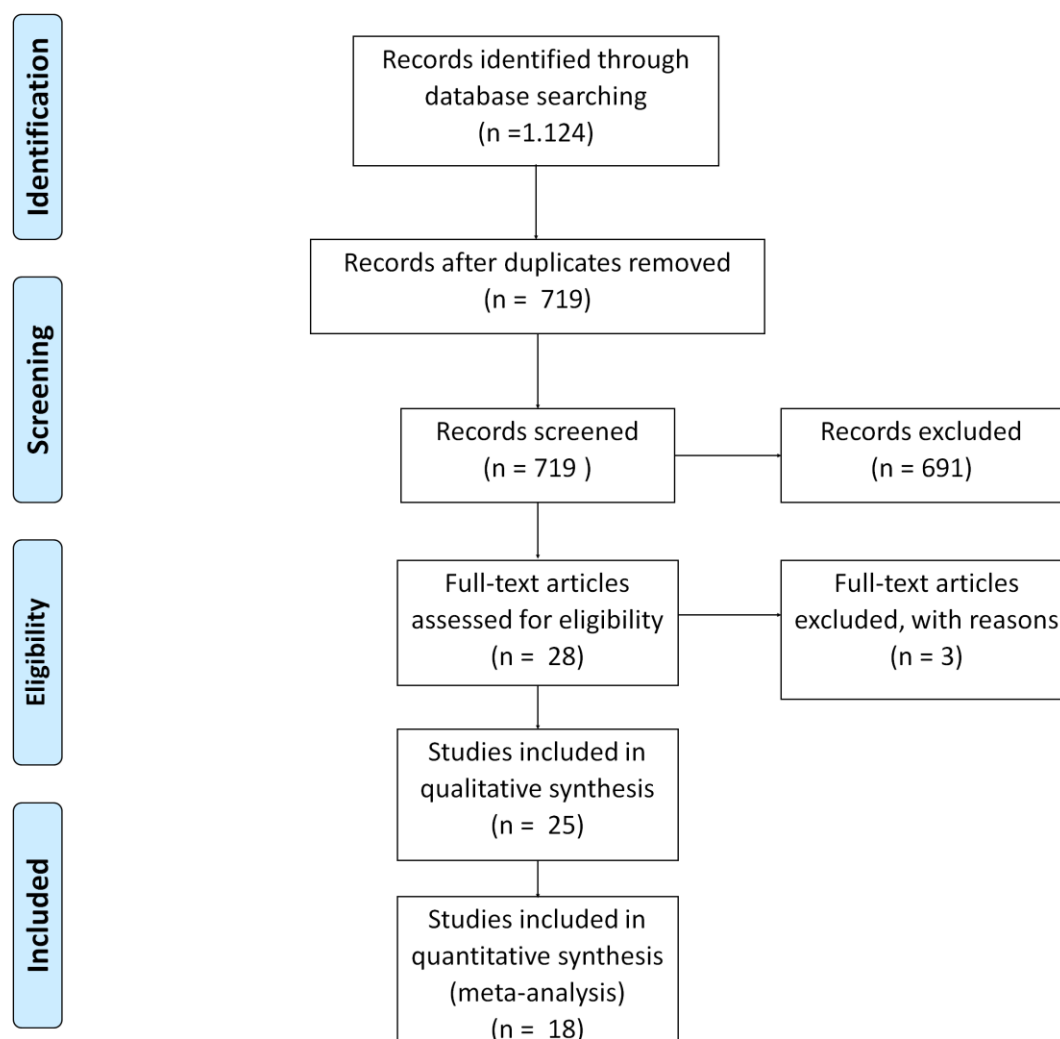
Figure 1: Prisma flow diagram

Figure 2. Studies grouped by year of publication.

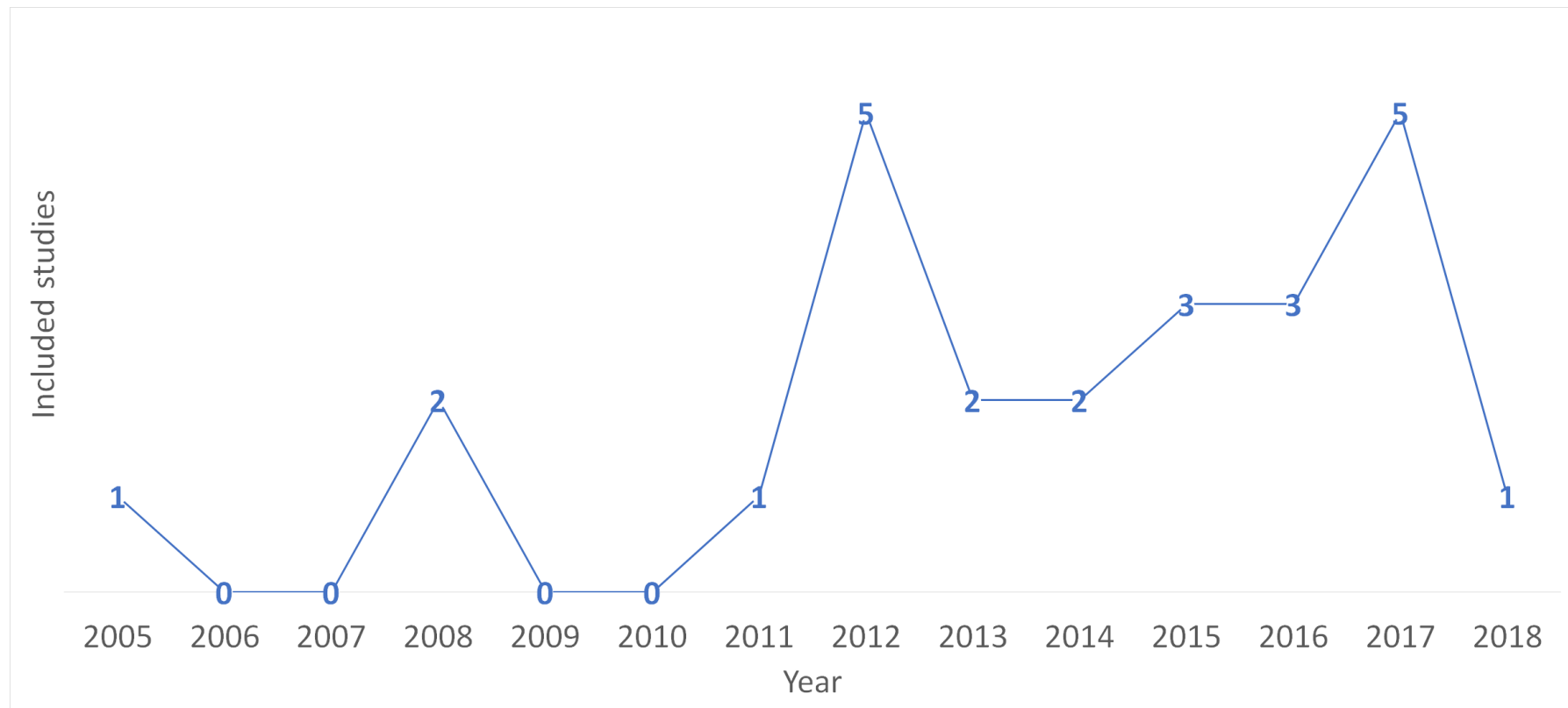


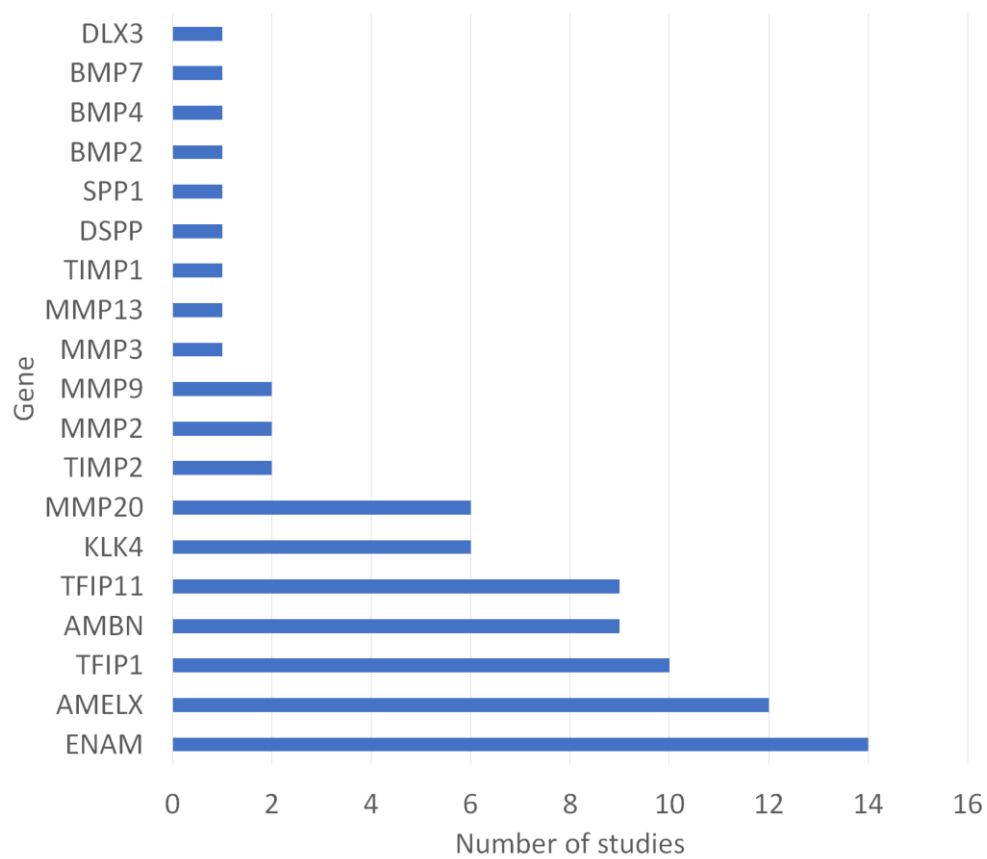
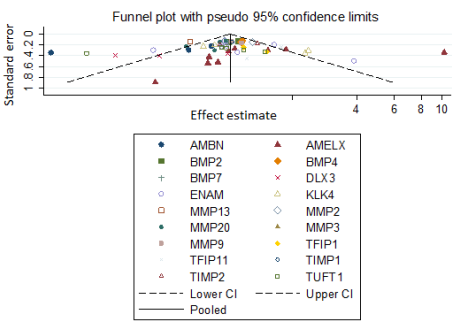
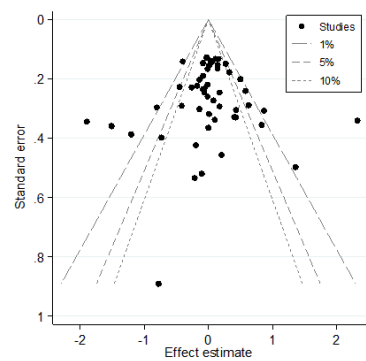
Figure 3. Number of studies investigates by gene

Figure 4. Funnel plot of meta-analysis included studies

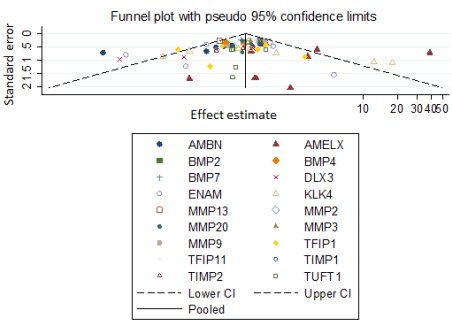
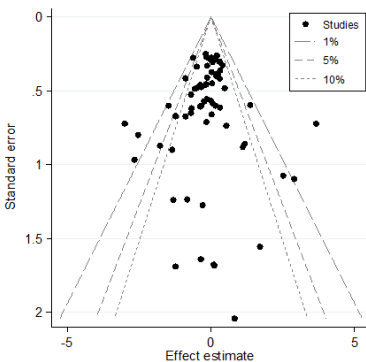
Allelic

Contour enhanced funnel plot with pseudo 95% confidence limits



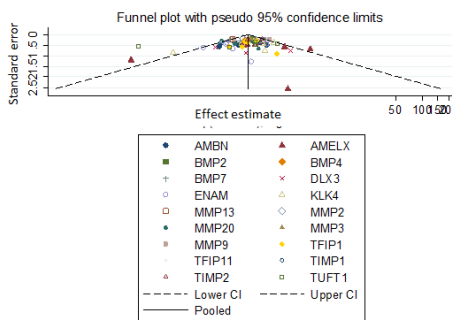
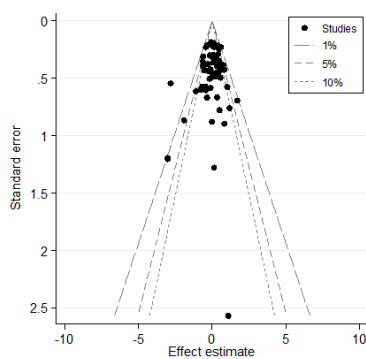
Homozygote

Contour enhanced funnel plot with pseudo 95% confidence limits



Heterozygote

Contour enhanced funnel plot with pseudo 95% confidence limits



4.2 Artigo 2

Artigo formatado seguindo as normas da Revistas *Archives of Oral Biology*

Single Nucleotide Polymorphisms of Taste Genes and Caries: A systematic Review and Meta-analysis

Running Title: Polymorphisms and Caries

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Key words: Polymorphisms, Dental caries, Taste genes, Single Nucleotide Polymorphism

Running title: Taste polymorphisms and caries

Declarations of conflict of interest: none

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Cover letter

To: Prof. Dr. G.B. Proctor

Dear Editor:

Based on the importance of *ARCHIVES OF ORAL BIOLOGY*, we are sending the manuscript entitled “**Single Nucleotide Polymorphisms of Taste Genes and Caries: A systematic Review and Meta-analysis**” to be appraised by the Journal’s Editorial Board.

This is the first systematic review with meta-analysis investigating the association between single nucleotide polymorphisms (SNPs) of taste genes and dental caries experience. Twelve Single Nucleotide Polymorphism presented in four different genes (*TAS1R2*, *TAS2R38*, *TAS1R3* and *GLUT2*) were identified suggesting an impact in eating behavior and an influence on dental caries experience. Most of these SNPs showed a protective effect for the minor allele, suggesting that these genetic variations may be involved in taste sensibility. The meta-analysis results suggested that the SNP rs713598 presents in *TAS2R38* may play an important role on dental caries susceptibility.

We did quality control filters in order to minimize the bias in our estimates, such as to investigate and exclude SNPs in linkage disequilibrium for the gene-pooled approach, as well as excluded palindromic ones. This is a review manuscript and has not been considered for publication elsewhere. The paper was read and approved by all authors. All authors have made substantive contribution to this study, and all have reviewed the final paper prior to its submission. The authors declare that there are no potential competing interests. Furthermore, I attest the validity and legitimacy of data and its interpretation.

There are no conflicts of interest for authors listed above. We sign for and accept responsibility for releasing this material.

Thank you very much for your consideration.

Yours sincerely,

Prof. Luiz Alexandre Chisini, PhD. (**Corresponding Author**)

University of Vale do Taquari

Graduate Program in Dentistry, Federal University of Pelotas

**Single Nucleotide Polymorphisms of Taste Genes and Caries: A systematic Review and
Meta-analysis**

Running Title: Polymorphisms and Caries

Single Nucleotide Polymorphisms of Taste Genes and Caries: A systematic Review and Meta-analysis

Running Title: Polymorphisms and Caries

Abstract

Objectives: to systematically review the literature investigating the Single Nucleotide Polymorphisms (SNP) related to taste genes are associated with caries experience.

Materials and methods: Search was performed in five databases to respond the question: “Are the polymorphisms of taste genes associated with dental caries?”. Studies in humans were included. Quality of studies, meta-analysis and sensibility analysis were performed.

Results: Seven studies were included in the systematic review and two in meta-analysis. Most of studies (71.4%) presented cohort design with low-level evidence. A total of 4,032 individuals were evaluated. Four different taste genes (*TAS1R2*, *TAS2R38*, *TAS1R3* and *GLUT2*) and 12 SNPs were reported. Most SNPs of taste genes show a protective effect of the minor allele against to dental caries. Meta-analysis included the SNP rs713598 placed in the *TAS2R38* gene. The results suggest an effect of the heterozygote genotype (CG), which was associate with low caries experience (OR 0.35 CI95% [0.17 – 0.75]). However, the genotype GG not was associated (OR 0.17 CI95% [0.03 – 1.04]). Sensibility analysis showed an important influence of one study in the results.

Conclusion: Single Nucleotide Polymorphisms of taste genes were associated with caries experience. Interpretations show be taken with caution and the results must be replicate in different populations.

Clinical Relevance: Single Nucleotide Polymorphisms related seems to be linked with the occurrence of dental caries and these genes have been shown to be important to explain differences in dental caries risk.

Key words: Polymorphisms, Dental caries, Taste genes, Single Nucleotide Polymorphism

Highlights:

- *TAS1R2*, *TAS2R38*, *TAS1R3* and *GLUT2* were identified suggesting an impact in eating behavior and dental caries
- *TAS2R38* may play an important role on dental caries susceptibility
- Genotype CG rs713598 presented a reduction of 75% on the odds for dental caries

Introduction

Dental caries is one of the most common chronic disease affecting population from all ages worldwide.(Dutra et al., 2018; Kassebaum et al., 2015) It is the main cause of need for dental treatments as well as the main reason for treatment failures in both primary (Chisini et al., 2018) and permanent dentition.(Demarco et al., 2017) When the disease is not treated, caries lesions can lead to numerous complications, from pain and abscess, progressing to swelling and orofacial cellulitis, which can be life-threatening to the individual.(Kassebaum et al., 2015)

Dental caries is a complex disease strongly linked to biological, socioeconomic, behavior and cultural components. In this way, treatments not addressing this multifactorial and amplified approach tend to fail.(L. A. Chisini et al., 2019; Cury, de Oliveira, dos Santos, & Tenuta, 2016; Maltz, Alves, & Zenkner, 2017) Biological components are mainly linked with low hygiene habits, low exposure to fluoride and high consumption of fermentable carbohydrates.(Cury et al., 2016; Maltz et al., 2017) In this way, some studies have directed efforts to understand possible factors that may influence dietary preferences among individuals.(Chamoun, Mutch, et al., 2018; Eny, Wolever, Fontaine-Bisson, & El-Sohemy, 2008) They have indicated a biological plausibility to predisposition of genetic and non-genetic eating behaviors, which determine a different taste perception for each individual.(Fay & German, 2008; Grimm & Steinle, 2011) Authors suggest the existence of some taste genes that influence the gustative perception as well as some factors (such as life stage, physical activity, and gut microbiota) which can co-exist for the determination of gustatory perception.

In this way, Single Nucleotide Polymorphisms (SNPs) presented in taste genes seems to modify the taste sensitivity influencing the preference and choice for sweet foods, leading the individual to present a higher sweet food intake.(Chamoun, Mutch, et al., 2018; Eny et al., 2008) Consequently, these individuals could present and increased risk for several diseases, including obesity and dental caries. Changes in the perception to sugar gustatory sensibility has been associated to genetic variations influencing dental caries susceptibility.(Keskitalo et al., 2007; Kim, Wooding, Riaz, Jorde, & Drayna, 2006; Kulkarni et al., 2013)

Therefore, the better comprehension of the genetic contributions in gustatory perception may be valuable information to help to personalize and amplify the strategies to prevent dental caries and other sugar related-diseases. In this way, the aim of present study was systematically reviewed the literature investigating the Single Nucleotide Polymorphisms related to taste genes and their influence on dental caries experience.

Materials & Methods

The study was registered in PROSPERO (International Prospective Register of Systematic Reviews) under protocol number CRD42019121484. This systematic review was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline.(Moher, Liberati, Tetzlaff, Altman, & Group, 2009)

Review question and Searches:

A structured search was performed in five databases (Pubmed/Medline, Scopus, Web of Science, BIREME – BVS Virtual health library and Scielo) up to January of 2019. Keywords were selected based on the research questions, which was build and structured following PICO model: “Are the polymorphism of taste genes associated with dental caries?”

- Participants/ population: adults and children
- Intervention/exposure: Mutants Single Nucleotide Polymorphisms; The effect allele in this study was standardized as the minor allele reported in the studies. When the minor allele frequency varied across the studies, the effect allele was referred as the minor alleles in most of studies. Likewise, to do the estimates stratifying by genotypes, we opted for choosing the minor homozygote and heterozygotes as effect genotypes.
- Comparator/control: Wildtype Single Nucleotide Polymorphisms; The effect allele was compared to reference allele, defined as that most frequent in the population. To perform genotype analysis, the major homozygote was chose as the reference.
- Outcome: Dental caries experience. Dental caries was the main outcome of this review, which was considered by the follow criteria: International Caries Detection and Assessment System (ICDAS) and DMF/dmf (Decayed, Missing, Filled) teeth/surface. It was preferentially considered groups caries-free vs caries experience. When more than one criteria to investigate dental caries was displayed, DMFT/dmft=0 (caries-free) vs. DMFT/dmft $\geq$ 1 was chose.

Relevant MeSH terms were considered, even as the relevant entry terms. The complete structure of search strategy is descripted in the **Table S1**. All the retrieved records were uploaded into the EndNoteTM software (Thomson Reuters, Rochester, New York, NY, USA). Thus, a virtual library was built. Duplicated records were excluded by software. Two independent reviewers (LAC and MCMC) read all reports titles and abstracts, under the following criteria:

- a) Inclusion criteria: comprised articles that aim to evaluate the association between genetic taste genes and dental caries in children or adults. Only human studies with cross-sectional, longitudinal and case control design were included. No restrictions on language or publication period were considered.

- b) Exclusion criteria: comprised the studies with design of literature reviews, case reports and case series, abstracts of conference, letters to the editor as well as qualitative studies were excluded of the present revision.

The same reviewers (LAC and MCMC) read the full-text and judged the papers. If any disagreement was found, the reviewers attempted to reach a consensus through discussions. Persistent disagreements were resolved by a third reviewer (MBC), which take the final decision. Grey literature was manually investigated using (Dental caries AND polymorphism) as key words in the annals of International Association for Dental Research (IADR) and Researchgate (<http://researchgate.net/>).

Data collection:

Full data extraction was independently executed by both reviewers (LAC and MCMC) in a previously tested and predefined database. The following data were extracted: Author, year, country, study design, sample, age, ethnicity of the sample (% for each ethnic group), percentage of the sexes of the sample, calculation of statistical power, evaluation and categorization of dental caries, analytical approach, data analysis (crude and adjusted analysis values and their respective 95% confidence intervals (CI)), covariables and main results. Disagreements between the collected data were checked.

Quality of studies:

Two instruments were used to perform the quality of studies: First, was used the Appraisal Checklist for Observational Studies (Joanna Briggs Institute) (Institute, 2014). This tool presents 10 questions assessing different arguments in the study, which must be answered with three possibilities as follow: "No", "not clear" or "Yes". Each "Yes" answer corresponds to one point, therefore the tool score can range from 0 to 10. Studies scored between 0 to 3 were considered of low quality; 4 to 6 were of medium quality; and 7 to 10 were considered of high quality. To score the studies, two reviewers (LAC and MCMC) performed the evaluation independently. Disagreements were remedied through discussion until consensus was reached. The second instrument was adapted to a 10-point scoring (control group, Hardy–Weinberg equilibrium, case group, primer, reproducibility, blinding, power calculation, Statistics, corrected statistics, independent replication) from a sheet previously used (Clark & Baudouin, 2006; Salles, Antunes, Carvalho, Kuchler, & Antunes, 2017) in genetic studies. This tool present two different criteria to evaluate such of 10 points (Yes=1) or (no/undetermined=0). The same reviewer performed independently the evaluation. Studies that obtained until four points were classified as low quality, five to seven, medium quality; eight or more, high quality.

Strategy for data synthesis:

A meta-analysis was adopted to pooling the polymorphisms. Due to low number of polymorphisms it was not possible to perform analysis pooling the polymorphisms by respective genes. Only SNPs present in at least two different studies/populations were considered in meta-analysis. Besides, meta-analysis was performed by genotypic (homozygote and heterozygote). Allelic analysis was not performed because allelic results were not presented in studies included in meta-analysis. To perform the analysis, we calculated the estimates for the effect heterozygote and homozygote genotypes pooling by polymorphism. In studies presenting more than one category for dental caries, we chose the DMF/dmf=0 vs. DMF/dmf $\geq$ 1.

To avoid inconsistencies in data analysis we performed the data harmonization for palindromic SNPs. When the palindromic SNP was present in two different studies, we only kept the SNP in the analysis if the study reported the DNA strand. If this information was missing in the papers, the SNP was excluded from further analysis.

The results of adjusted models were preferably included. In cases where the adjusted results were not reported, the unadjusted estimates were considered or calculated. In cases where results were only showed by stratified analysis, we included the group with higher number of individuals. Odds ratio (OR) was used to measure effect size with 95% Confidence Interval (CI). The prevalence ratio measures were converted to OR using the formula proposed by Zhang and Yu: $PR = odds\ ratio / (1 - risk_0 + risk_0 \times odds\ ratio)$, where risk₀ is the prevalence of disease among non-exposed individuals.(L. Chisini et al., 2019; Zhang & Yu, 1998) When high heterogeneity (I^2 statistic >50%) was observed, random models were performed while when heterogeneity was less than 50%, analysis was performed with fixed models. Moreover, to assess the effect of each study on the pooled estimate, sensitivity analysis was used. Analyzes were performed using Stata 12.0 software (StataCorp, College Station, TX, USA)

Results

Study selection

The search resulted in 1,200 initial records, which 985 remained after the removal of duplicated papers. After evaluation of abstracts, ten papers were selected to full-text assessment, from which seven were included in the systematic review (Haznedaroglu et al., 2015; Holla et al., 2015; Kulkarni et al., 2013; Robino et al., 2015; Shimomura-Kuroki, Nashida, Miyagawa, & Sekimoto, 2018; Wendell et al., 2010; Yildiz, Ermis, Calapoglu, Celik, & Turel, 2016) and two in the meta-analysis.(Shimomura-Kuroki et al., 2018; Yildiz et al., 2016) Three studies were excluded in full-text evaluation.(Ashi et al., 2017; Eny et al., 2008; Wright, 2010) The reasons to exclusion are justified in the flowchart of **Figure 1**.

Study characteristics

From the 7 included studies, 71.4% (n=5) presented cohort design and 28.6% were case control studies (n=2). These studies were carried out mainly in Turkey (28.6%) (Haznedaroglu et al., 2015; Yildiz et al., 2016) and North America (28.6%).(Kulkarni et al., 2013; Wendell et al., 2010) Other studies were performed in Czech Republic (Holla et al., 2015), Italy (Robino et al., 2015) and Japan.(Shimomura-Kuroki et al., 2018). Most of studies (57.1%) reported included only Caucasian individuals while the others have not reported the ethnicity of investigated population. All Studies used DMF/dmf to assessment dental caries investigating only permanent (57.1%) and permanent/primary teeth (42.9%). Therefore, 4,032 individuals were evaluated.

Risk of bias within studies

Regarding to the quality assessment through Critical Appraisal Checklist for observational studies (Joanna Briggs Institute), most of studies (57.1%) were considered of low quality and 28% of medium quality (**Table 1**). Similarly, considering methodological scoring protocol based on quality assessment for genetic studies, it was observed that 71.4% of the studies were classified as low level of evidence and the remained as medium level of evidence (**Table 2**).

Overview of Single Nucleotide Polymorphisms

Twelve single nucleotide polymorphisms were found investigating possible associations between SNPs of taste genes and dental caries experience. These SNPs were present in four genes. Most of SNPs were missense (58.3%), followed by intronic (33.3%). Moreover, 91.7% of SNPs are related to possible functional impact in protein. More details of SNPs and their

functional impact on protein are available on **table 3**. One SNP was included in the meta-analysis and presented a palindromic sequence. However, studies reported the evaluated sequence, therefore, it was possible to perform the meta-analysis.

Results of individual studies

Four different taste genes - taste 1 receptor member 2 (*TAS1R2*), taste 2 receptor member 38 (*TAS2R38*), taste 1 receptor member 3 (*TAS1R3*) and glucose transporter 2 (*GLUT2*) - and 12 polymorphisms were related in the literature investigating possible associations with dental caries experience. The summarized results according gene and polymorphism in the studies is displayed in the **Table 4**. In general, it was possible to observe that most of allele and genotype effects of taste genes SNPs presented a protective factor against dental caries.

In the table **S2** is showed the main characteristics of studies included. Wendell et al. (2010) observed different results between permanent/mixed and primary teeth related to taste genes (*TAS2R38* [rs713598, rs1726866, rs10246939] and *TAS1R2* [rs4920566]). While both SNPs were associated with dental caries in primary teeth, no associations were observed in permanent teeth. Moreover, the SNP rs9701796 (*TAS1R2*) was only associated in primary teeth. (Wendell et al., 2010) Holla et al. (2015) presented different approaches to categorize dental caries, observing different results. When DMFT=0 Vs DMFT $\geq$ 1 were compared, no statistical differences were observed to rs35874116 (*TAS1R2*) and rs5400 (*GLUT2*). However, when considered DMFT=0 Vs Decayed teeth $\geq$ 1, statistical difference was observed in these SNPs.

Although some SNPs have been reported in several papers, not all studies displayed the effects of measurement or presented the data in way to make it possible to calculate the Odds Ratio and be included in the meta-analysis.

Synthesis of results (meta-analysis)

Two studies were included in the meta-analysis (Shimomura-Kuroki et al., 2018; Yildiz et al., 2016) evaluating the SNP rs713598 in *TAS2R38* gene. For this analysis, the genotype heterozygote (**Figure 2**) and the homozygote (**Figure 3**) analysis were considered. Considering the heterozygote, a low heterogenicity was observed ($I^2 = 44\%$) and fixed model was used in the analysis. The pooled effect in the heterozygote analysis shown that genotype CG was associate with low caries experience (OR 0.35 [0.17 – 0.75]).

On the other hand, considering homozygote analysis, high heterogenicity was observed ($I^2 = 72.0\%$ and OR 0.15 [0.06 – 0.38]). So, a random model was performed, presenting no significative association considering the genotype GG (OR 0.17 [0.03 – 1.04]). Sensibility analysis shown an important influence of Yildiz et al. (2016) in the results (**Figure 4**).

Risk of bias across studies

Due to low number of studies included in the meta-analysis (less than 7), it was not possible to perform the Funnel Plot and Egger's Test.

Discussion

Twelve Single Nucleotide Polymorphism presented in four different genes (*TAS1R2*, *TAS2R38*, *TAS1R3* and *GLUT2*) were identified suggesting an impact in eating behavior and an influence on dental caries experience. Most of these SNPs showed a protective effect for the minor allele, suggesting that these genetic variations may be involved in taste sensibility. The meta-analysis results suggested that the SNP rs713598 presents in *TAS2R38* may play an important role on dental caries susceptibility.

While polymorphism in *CD36* suggesting possible influence in the fast taste perceptions decreasing the attraction to this foods in mice,(Sclafani, Ackroff, & Abumrad, 2007) *TAS2R38* gene - taste receptor gene cluster on chromosome 12p13 / taste receptor, type-2, member 38 – is responsible to sensitivity to the bitter compound of propylthiouracilis. They are member of the G-protein-coupled receptor superfamily. These proteins are expressed mainly in the epithelia cells of tongue and palate. In special, the SNP rs713598 lead a change of amino acid alanine to proline at position 49. Besides, it is a candidate gene to sweet taste perception.(Inoue et al., 2013; Khataa, Stewart, Brenner, Cornelis, & El-Sohemy, 2009; Mennella, Pepino, & Reed, 2005) Homozygote to Alanine (AA) individuals are referred as “nontasters”, while allele heterozygote (Alanine and Proline) are referred as “medium-tasters” and allele homozygote to Proline (PP) are referred as “supertasters” individuals to bitter.(Chamoun, Mutch, et al., 2018; Mennella et al., 2005) Thus, seems that individuals medium and supertasters can be more taste sensitive to high diversity of substances and, therefore, more prone to decrease sugar intake in detriment to different flavors when are compared with individuals considered “nontasters”.(Chamoun, Mutch, et al., 2018; Mennella et al., 2005) Therefore, a gradual intensity of phenotype change is expected, since “supertasters” homozygote to mutant allele should presented the less sugar intake and, consequently, decrease of dental caries experience. In this study, the genotype CG of SNP rs713598 showed an odds 75% lower to presented dental caries. Genotype GG of the same SNP presented a borderline result (OR 0.17 [0.03 – 1.04]), revealing a tendency of protection against dental caries, although it was not statistically significant. The lack of the significance for the CC homozygote can be explained due to elevate heterogenicity between the methodological approach observed in the included studies even as to significant weight of Shimomura-Kuroki et al. (2018) on result observed in the sensibility analysis. Despite the results, have presented similar tendencies in both studies, the study of Shimomura-Kuroki et al. (2018) was decisive in the final result.

Moreover, the *TAS1R2* - taste receptor, type-1, member 2 - was also associated with low intake of sugar consumption.(Eny, Wolever, Corey, & El-Sohemy, 2010) High number of studies have found association of *TAS1R2* and dietary behaviors, such as sucrose/carbohydrate

preference.(Chamoun, Carroll, et al., 2018; Han, Keast, & Roura, 2017) In this way, seems that *TAS1R2* can contribute to sensitivity to sweet taste and influence the sugar consumption. Based in this pathway, was proposed that hypothesis that *TAS1R2* could also influence the caries experience. Thus, while effect SNPs of rs3935570, rs4920566 and rs9701796 (presents in *TAS1R2*) minor alleles were associated with decrease of dental caries,(Robino et al., 2015; Wendell et al., 2010) rs35874116 show contrasting results across the studies.(Haznedaroglu et al., 2015; Holla et al., 2015; Kulkarni et al., 2013) *TAS1R3* have analogous mechanisms and is involved in sweet perception, which is determined via a G-protein-linked.(Haznedaroglu et al., 2015) Only one study investigated the influence of *TAS1R3* on caries, showing also a protective effect.(Haznedaroglu et al., 2015) In this context, glucose transporter type 2 (*GLUT2*) facilitates the first step in glucose induced insulin secretion, brain detection of glucose,(Barroso et al., 2003) as well as facilitative glucose transporter in the plasma membrane of the intestinal and provide metabolites stimulating the transcription of glucose sensitive genes.(Leturque, Brot-Laroche, Le Gall, Stolarczyk, & Tobin, 2005) Henceforth, this polymorphism seems to be associated with higher habitual consumption of sugar,(Eny et al., 2008) and some studies have detected possible associations with dental caries experience.(Holla et al., 2015; Kulkarni et al., 2013)

Moreover, another important point must be discussed. In the Wendell et al. (2010) study, statistically differences between (*TA1R2* and *TAS2R38*) were observed when primary/mixed dentition were considered. However, when permanent teeth were investigated, these statistical differences were not observed, although the direction of effect have been preserved.(Wendell et al., 2010) These can be explained due to other non-genetic factors, which can be more expressed in adulthood (Chamoun, Mutch, et al., 2018; Connors, Bisogni, Sobal, & Devine, 2001; Scheibehenne, Miesler, & Todd, 2007). Thus, studies have supposed that genetic taste influence would be more correlated in childhood. Over time, cultural and environmental contributions could contribute more significantly,(Chamoun, Mutch, et al., 2018; Connors et al., 2001; Scheibehenne et al., 2007) explaining the observed results.

Thus, it is important to highlight that less than half of the included studies investigated primary teeth, and this high heterogenicity can be the main limitation to be considered in the interpretation of presents results. It was one of the factors that conduct to low quality of included studies in both of tools used. Yet, in the meta-analysis, only crude estimative were included. In addition, despite 4,032 individuals included in the studies, the results were based mainly in Caucasian population distributed basically in North America and Europe. Therefore, we must emphasize ethnicity of samples investigated and population stratification. Populations diversity not controlled can introduce important bias in genetic studies, leading to problems in association estimates. A limited part of the included studies adjusted the results for any type of

ancestry information. Fundamental differences between allele frequencies and population ethnicity have been identified when investigated SNPs were analyzed in complementary database. This point highlights the necessity to perform a control for this variable to decrease possible bias of studies. Therefore, conclusion and interpretations of the results should be carried out taking into account these limitations. Besides, most of studies presented low quality of evidence in both instruments used to investigate this point as well as we only include candidate-gene studies in present study. Thus, other genes or pathways related to taste genes can be important to explain the relationship between taste gene and dental caries experience. However, the low number of studies address to investigate this topic reinforce the need to carry further studies investigating this issue with different genetic methods. Studies at genomic scales are more robust for identification of genetic component since they are not based on previous knowledge of the pathophysiology and should be used to detect new routes and identify new candidate genes. However, the available literature on caries experience and genomic is limited.(Vieira, Modesto, & Marazita, 2014)

We have also to emphasize that was performed an analysis with quality control filters aim to decrease possible biases in our estimates. We investigate SNPs in linkage disequilibrium as well as palindromic SNPs. Moreover, the perform sensibility analysis to observe the weight of studies in meta-analysis. It highlights that studies focus in the relationship between taste genetic single nucleotide polymorphisms and dental caries experience is a relatively new topic and to support with high evidence the presents observations are necessary further studies. Preferably, it has included representative and wide samples (presenting power calculation) with populations of different ethnic groups. Epigenetic issues, genome wide-associations studies and interactions between genetic and environmental factors have been encouraged, since that they are necessary to complement the founds observed in gene-candidate studies.

Therefore, Single Nucleotide Polymorphisms of taste genes seems to be associated with experience of dental caries. The genotype CG of SNP rs713598 present in *TAS2R38*-gene presented a reduction of 75% on the odds for dental caries. Presents findings were mostly based in studies with low evidence, performed in Caucasian individuals; Henceforth, interpretations show be taken with caution and the results must be replicate in different populations with high quality level of evidence. Further studies should also consider epigenetic issues, interactions between genetic and environmental factors as well as perform control of variables by dental and individual/contextual variables.

Compliance with Ethical Standards:

Conflict of Interest: Luiz Alexandre Chisini declares that he has no conflict of interest. Mariana Gonzales Cademartori declares that she has no conflict of interest. Marcus Cristian Muniz Conde declares that he has no conflict of interest. Luciana Tovo-Rodrigues declares that she has no conflict of interest. Marcos Britto Correa declares that he has no conflict of interest

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Legends:

Table S1. Search strategy

Table S2. Main characteristics of studies included in this systematic review

Table 1. Critical Appraisal Checklist for observational studies (Joanna Briggs Institute) in the systematic review according to the 10-items

Table 2. Methodological scoring protocol based on quality assessment for genetic studies.

Table 3. Description of single nucleotide polymorphism investigated in the present systematic review according genes*

Table 4. Summarization results according gene and polymorphism in the studies

Figure 1: Prisma flow diagram

Figure 2. Pooled effect of *TAS2R38* rs713598 in genotype heterozygote. Data are presented as odds ratio for each study (boxes), 95% CIs (horizontal lines) and summary as odds ratio with 95% CI (diamond). Fixed model was performed.

Figure 3. Pooled effect of *TAS2R38* rs713598 in genotype homozygote. Data are presented as odds ratio for each study (boxes), 95% CIs (horizontal lines) and summary as odds ratio with 95% CI (diamond). Randomic model was performed.

Figure 4 Sensibility analysis of included studies. In A) Heterozygote genotype and B) homozytote genotype.

Table S1. Search strategy

Search syntax	
PubMed	#1 (“Dental Decay” OR “Caries, Dental” Or “Decay, Dental” OR “Cariious Dentin” OR “Cariious Dentins” OR “Dentin, Cariious” OR “Dentins, Cariious” OR “Dental White Spot” OR “White Spots, Dental” OR “White Spots” OR “Spot, White” OR “Spots, White” OR “White Spot” OR “Dental White Spots” OR “White Spot, Dental” OR “Susceptibility, Dental Caries” OR “Caries Susceptibility, Dental” OR “Caries Resistance, Dental” OR “Resistance, Dental Caries” OR “Dental Caries Resistance”)
	#2 (“Polymorphisms, Genetic” OR “Genetic Polymorphisms” OR “Genetic Polymorphism” OR “Polymorphism” OR “Polymorphisms” OR “Nucleotide Polymorphism, Single” OR “Nucleotide Polymorphisms, Single” OR “Polymorphisms, Single Nucleotide” OR “Single Nucleotide Polymorphisms” OR “SNPs” OR “Single Nucleotide Polymorphism”)

* Search combination: #1 AND #2

Table S2. Main characteristics of studies included in this systematic review

Author , year	-Country -Study design -Sample (% Males)	-Age (permanent/ deciduous) -Ethnicity -Statistic power (yes/no)	-Evaluation dental -categorization	caries	Analytical Approach	Adjustment variables
Wendell et al. (2010)	-EUA -Cohort -2249 (NR)	- Permanent= 1391 individuals mean age 29.4y; mixed= 562 individuals mean age 9.8y; and deciduous= 496 individuals, mean age 3.4y) -Caucasian -No	- DMFS/dmfs - DMFT/dmft=0 Vs DMFS/dmfs≥1		-Family Based Association Test and Haplotype analysis	-None
	Crude Analysis		Deciduous/mixed dentition: # TAS2R38: rs713598 C/G Protective effect (p = 0.007); Effect not reported # TAS2R38: rs1726866 G/A Protective effect (p = 0.03); Effect not reported # TAS2R38: rs10246939 C/T Protective effect (p = 0.01); Effect not reported # TAS1R2: rs4920566 G/A Protective effect (p = 0.03); Effect not reported # TAS1R2: rs9701796 G/C Risk effect (p = 0.02); Effect not reported			

		Permanent dentition:	
		No statistically significant results were found in the permanent dentition group	
Adjusted Analysis		-	
Kulkarni et al. (2013)	-Canada	-21 to 32y	- DMFT, X-ray and ICDAS
	-Cohort	(permanent)	-Mean of DMFT and ICDAS were
	-80 (30%)	-Caucasian	used
		-No	
Crude Analysis		# TAS1R2 rs35874116 Ile191Val: Protective effect (p= 0.05) in DMFT, Protective effect in DMF + X-ray (P = 0.01) and Protective effect in ICDAS (P = 0.003). Effects not reported	
Adjusted Analysis		# GLUT2 rs5400 Thr110Ile: Risk effect (p = 0.04) in DMFT; Not associated in DMF + X-ray (P = 0.14) and Not associated in ICDAS (P = 0.22). Effects not reported	
		-	
Haznedaroglu et al. (2015)	-Turkey	-7 to 12	-DMFT/dmft
	-Cohort	(permanent and	-Low risk (dft+DMFT 0-3 scores);
	-184 (45.1%)	deciduous)	Moderate risk (dft+DMFT 4-7
		-NR	scores); High Risk (dft+DMFT >8
		-No	scores); Analysis compared the low vs. High caries
Crude Analysis		# TAS1R2 rs35874116 T/C: Genotype CT compared to TT OR 0.10 (0.05 – 0.24); Genotype CC compared to TT OR 0.26 (0.04 – 1.65);	
		TAS1R2 rs9701796 G/C: Genotype GC compared to GG OR 1.08 (0.45 – 2.60)	
		# TAS1R3 rs307355 C/T: Heterozygous genotype carriers were found to be the highest among the moderate-risk group (4–7 caries; p = 0.04)	

Crude Analysis

TAS1R2 rs3935570 G/T: Protector factor ($p = 0.012$, Beta -0.937); allele G showed higher DMFT compared to both heterozygous G/T and homozygous for the allele T

Adjusted Analysis

GLUT2 rs1499821: Protector factor ($p = 0.027$, Beta -1.047); allele G showed higher DMFT compared to both heterozygous G/A and homozygous A/A

GLUT2 rs5398: Protector factor ($p = 0.204$, Beta -0.508)

GLUT2 rs5400: Risk factor ($p = 0.453$, Beta $+0.422$)

GLUT2 rs11924032: Protector factor ($p = 0.360$, Beta -0.378)

-Turkey	-20 to 60y	-DMFT	-Qui square and Mann-Whitney U test.	-Plaque
-Case Control	(permanent)	-Low caries risk ($DMFT \leq 5$) and high	Multiple linear regression analyses to	amount,
-154 (NR)	-NR	caries risk ($DMFT \geq 14$)	gene-environment associations	toothbrushing
	-Yes			frequency, dietary
				intake between
				meals, saliva
				secretion rate, saliva
				buffer capacity,
				mutans streptococci
				counts,
				and lactobacilli
				counts

Yildiz et al.
(2016)

Crude Analysis

TAS2R38 rs713598 C/G: Genotype CG compared to CC OR 0.25 (0.10 – 0.60); Genotype GG compared to CC OR 0.07 (0.02 – 0.23); Allele G compared to C OR 0.34 (0.21 – 0.53)

Adjusted Analysis				-
Shimomura-Kuroki et al. (2018)	-Japan	-3 to 11y	-DMFT/dmft	-Regression analysis (results not shown -
	-Case Control	(permanent and deciduous)	-DMFT/dmft=0 Vs DMFT/dmft≥1	to TAS2R38 e GLUT2)
	-81 (49,4 %)	-NR		
		-Statistic power (yes/no)		
			TAS2R38 rs713598 C/G: Genotype CG compared to CC OR 0.73 (0.20 – 2.65); Genotype GG compared to CC OR 0.45 (0.10 – 1.98)	
	Crude Analysis		GLUT2 rs5400 T/C: Genotype CT compared to TT OR 0.93 (0.25 – 3.51); Genotype CC compared to TT OR 1.62 (0.06 – 41.17)	
	Adjusted Analysis			

NR: not reported; # Statistical association; CI: Confidence Interval; OR: Odds Ratio; PR: Prevalence Ratio; dmft (decayed, missing teeth due to caries, filled teeth); WSL: white spot lesions; ICDAS: International Decay Detection and Assessment System; CA: Crude association; AA: adjusted association; All measure effects show are ODDS Ratio. Different measures are reported; p: only p value reported; SNP: Single Nucleotide Polymorphism.

Table 1. Critical Appraisal Checklist for observational studies (Joanna Briggs Institute) in the systematic review according to the 10-items

Study, year	NIH Criteria										Final score
	1	2	3	4	5	6	7	8	9	10	
Wendell et al. (2010)	-	/	/	-	+	+	+	-	/	/	Low Quality (3)
Kulkarni et al. (2013)	-	-	-	-	-	+	+	-	-	/	Low Quality (2)
Haznedaroglu et al. (2015)	-	-	-	+	+	+	+	-	/	/	Low Quality (3)
Holla et al. (2015)	+	+	+	+	+	+	+	-	+	/	High Quality (8)
Robino et al. (2015)	+	+	/	+	+	+	+	-	/	/	Medium Quality (6)
Yildiz et al. (2016)	-	-	+	-	-	+	+	+	-	-	Medium Quality (4)
Shimomura-Kuroki et al. (2018)	-	-	/	-	-	+	+	-	/	/	Low Quality (2)

+ Yes; - No; /: Unclear

Table 2. Methodological scoring protocol based on quality assessment for genetic studies.

Study, year	Genetic Criteria										Score	Evidence
	Control group	Hardy–Weinberg equilibrium	Case group	Primer	Reproducibility	Blinding	Power calculation	Statistics	Corrected statistics	Independent replication		
Wendell et al. (2010)	0	1	0	1	1	0	0	0	0	0	3	Low
Kulkarni et al. (2013)	0	0	0	0	1	0	0	0	0	1	2	Low
Haznedaroglu et al. (2015)	0	0	0	0	1	0	0	0	0	1	2	Low
Holla et al. (2015)	1	1	1	1	1	0	1	0	0	1	7	Medium
Robino et al. (2015)	1	0	1	0	1	0	0	0	0	1	4	Low
Yildiz et al. (2016)	1	0	0	1	1	0	1	0	0	1	5	Medium
Shimomura-Kuroki et al. (2018)	0	0	0	1	1	0	0	0	0	1	3	Low

*For the quantification of criteria: «1» means present, and «0» absent

Table 3. Description of single nucleotide polymorphism investigated in the present systematic review according genes*

Allele Frequencies by populations (%) *												
Gene	Polymorphism	Chromosomal position	Variation	Biotype / impact functional	African	American	East Asian	Europe	South Asia	All	Allele Reference/ allele Effect used	Ancestral allele
TAS1R2	rs3935570 (G/T)	1:19167371	Intron	Protein Coding	G:66% T:34%	G:83% T:17%	G:88% T:12%	G:74% T:26%	G:69% T:31%	G:75% T:25%	G/T	T
	rs4920566 (G/A)	1:19179824	Intron	Protein Coding	A:20% G:80%	A:64% G:36%	A:40% G:60%	A:64% G:36%	A:45% G:55%	A:44% G:56%	G/A	G
	rs9701796 (G/C)	1:19186129	Missense	Protein Coding	G:18% C:82%	G:16% C:84%	G:23% C:77%	G:22% C:78%	G:22% C:78%	G:20% C:80%	G/C	C
	rs35874116 (T/C)	1:19181393	Missense	Protein Coding	T:66% C:34%	T:73% C:27%	T:90% C:10%	T:68% C:32%	T:72% C:28%	T:73% C:27%	T/C	C
TAS2R38	rs713598 (C/G)	7:141673345	Missense	Protein Coding	C:52% G:48%	C:34% G:66%	C:32% G:68%	C:58% G:42%	C:66% G:34%	C:50% G:50%	C/G	G
	rs1726866 (G/A)	7:141672705	Missense	Protein Coding	G:67%	G:71%	G:68%	G:46%	G:36%	G:57%	G/A	G

					A:33%	A:29%	A:32%	A:54%	A:64%	A:43%		
	rs10246939 (C/T)	7:141672604	Missense	Protein Coding	T:52%	T:31%	T:32%	T:54%	T:64%	T:48%	C/T	C
					C:48%	C:69%	C:68%	C:46%	C:36%	C:52%		
TAS1R3	rs307355 (C/T)	1:1265154	Regulatory	TF Binding Site C	T:52%	T:12%	T:17%	T:8%	T:17%	T:24%	C/T	C
					C:48%	C:88%	C:83%	C:92%	C:83%	C:48%		
	rs1499821 (NR)	3:170724729	Intron	Protein Coding	C:88%	C:87%	C:81%	C:85%	C:90%	C:86%	NR	C
					T:12%	T:13%	T:19%	T:15%	T:10%	T:14%		
	rs5398 (NR)	3:170715830	Missense	Protein Coding	G:36%	G:69%	G:76%	G:71%	G:72%	G:63%	NR	A
GLUT2					A:64%	A:31%	A:24%	A:29%	A:28%	A:37%		
	rs5400 (G/A)	3:170732300	Missense	Protein Coding	G:51%	G:83%	G:98%	G:86%	G:84%	G:78%	G/A	A
					A:49%	A:17%	A:2%	A:14%	A:16%	A:22%		
	rs11924032 (NR)	3:170735099	Intron	Protein Coding	G:59%	G:74%	G:79%	G:74%	G:77%	G:72%	NR	G
					A:41%	A:26%	A:21%	A:26%	A:23%	A:28%		

* Based on Human (GRCh37.p13), available on: http://grch37.ensembl.org/Homo_sapiens. NR: not reported

Table 4. Summarization results according gene and polymorphism in the studies

Gene	Polymorphism (Reference allele/ effect allele)	Study, Year						
		Wendell et al. (2010)	Kulkarni et al. (2013)	Haznedaroglu et al. (2015)	Holla et al. (2015)	Robino et al. (2015)	Yildiz et al. (2016)	Shimomura-Kuroki et al. (2018)
TAS1R2	rs3935570 (G/T)					- #		
	rs4920566 (G/A)	-# ^a						
	rs9701796 (G/C)	-# ^a		+				
	rs35874116 (T/C)		-#	-#	+# ^b			
TAS2R38	rs713598 (C/G)	-# ^a					-#	-
	rs1726866 (G/A)	-# ^a						
	rs10246939 (C/T)	-# ^a						
TAS1R3	rs307355 (C/T)			-#				
GLUT2	rs1499821 (NR)					-#		
	rs5398 (NR)					-		
	rs5400 (G/A)		+ #		+ # ^b	+		
	rs5400 (C/T)							-/+ ^c
	rs11924032 (NR)					-		

Legends: - Protector factor; + Risk factor; # Statistically associated; ^a Statistical difference only in deciduous/mixed dentition; NA: not associated, direction of effect not showed; ^b Statistical difference only in DMFT=0 Vs component D_≥1; ^c Genotype CT compared to TT protective effect and genotype CC compared to TT risk effect; NR: not clearly reported

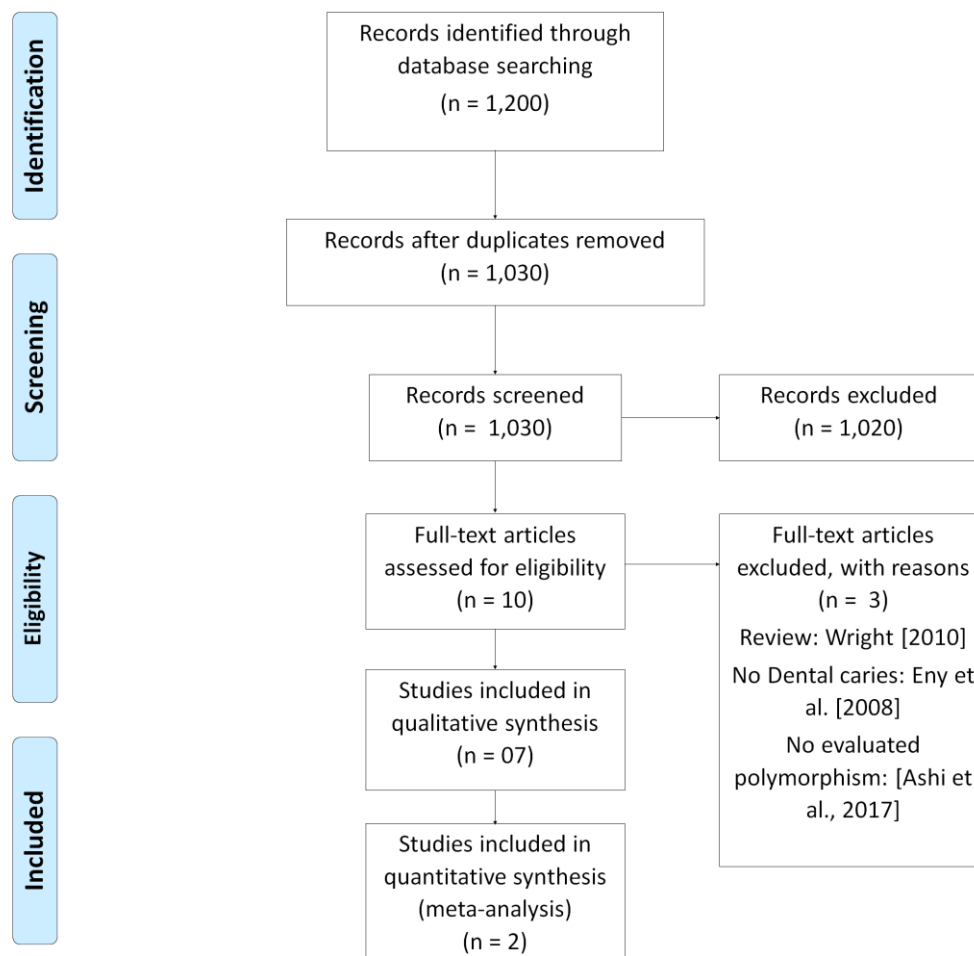
Figure 1: Prisma flow diagram

Figure 2. Pooled effect of *TAS2R38* rs713598 in genotype heterozygote. Data are presented as odds ratio for each study (boxes), 95% CIs (horizontal lines) and summary as odds ratio with 95% CI (diamond). Fixed model was performed.

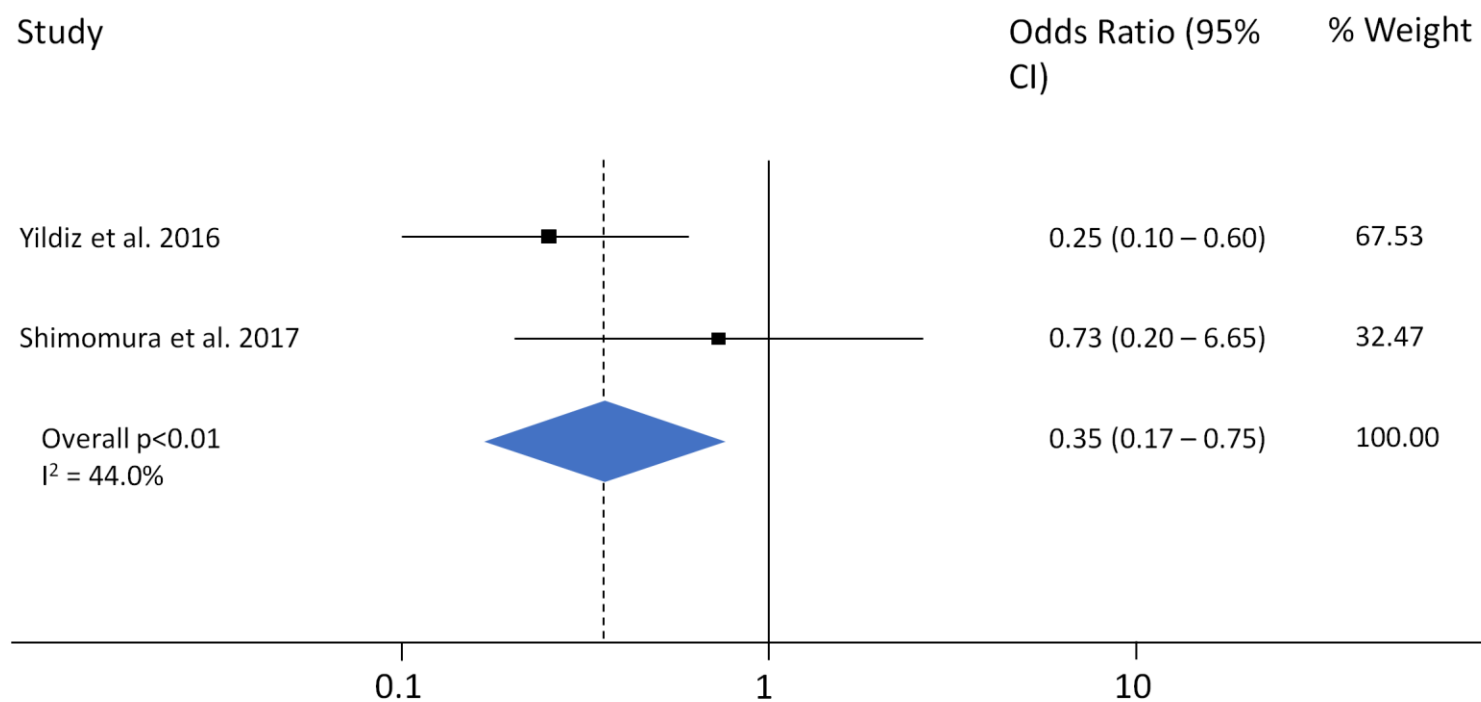


Figure 3. Pooled effect of *TAS2R38* rs713598 in genotype homozygote. Data are presented as odds ratio for each study (boxes), 95% CIs (horizontal lines) and summary as odds ratio with 95% CI (diamond). Randomic model was performed.

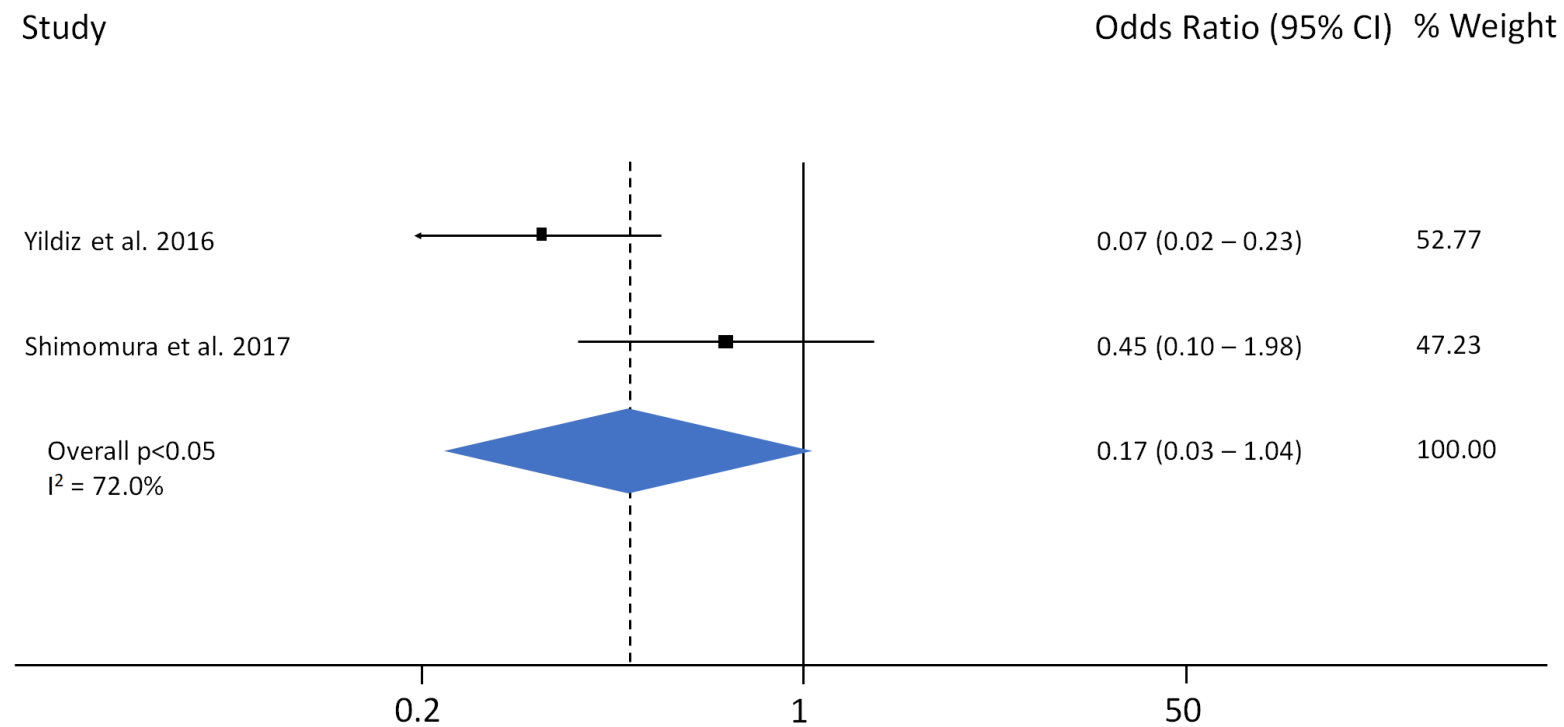
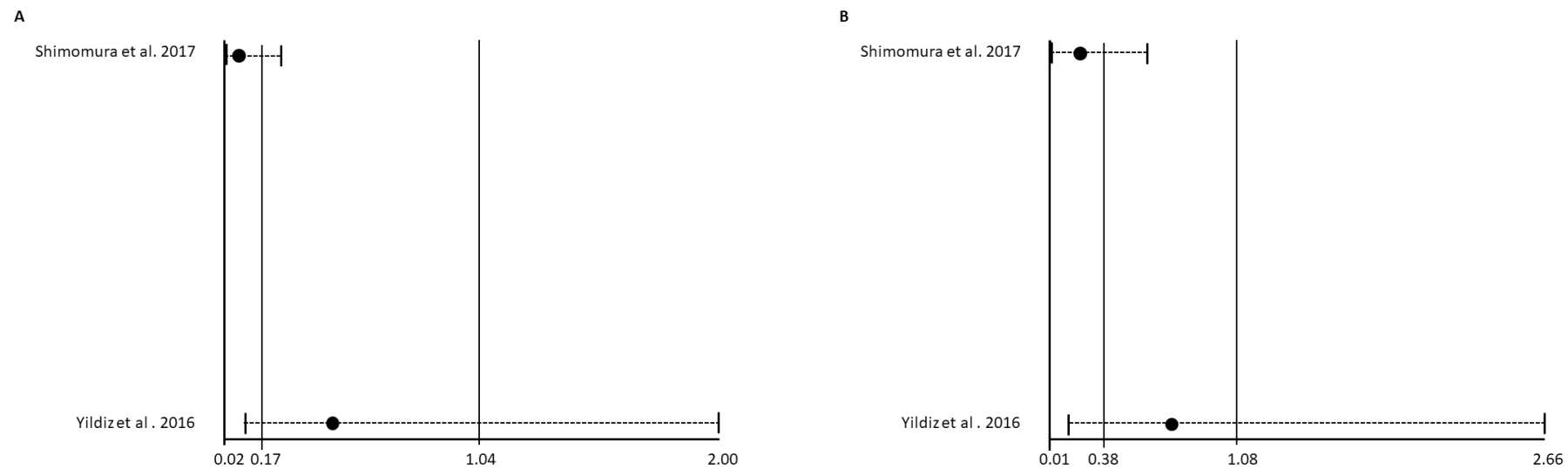


Figure 4 Sensibility analysis of included studies. In A) Heterozygote genotype and B) homozytote genotype.



Supplemental Material: Prisma Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3/4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4 and S1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4/5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5

Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

4.3 Artigo 3

Artigo formatado seguindo as normas da Revista *Brazilian Oral Research*

Cariology

Is there a role of composition and salivary flow genes in dental caries? A systematic review
and Meta-Analysis

Short tile: Salivary polymorphisms and caries

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Key words: Polymorphisms, Dental caries, Saliva

Declarations of conflict of interest: none

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Mariana Gonzalez Cademartori: Data collection and data analysis

Marcus Cristian Muniz Conde: Write the paper and perform the literature review

Luciana Tovo-Rodrigues: Revision of the paper

Marcos Britto Corrêa: Conception of the design, perform the data analysis and review of paper

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Short title: Salivary polymorphisms and caries

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Abstract

The aim of study was to review the literature to assess whether Single Nucleotide Polymorphisms (SNPs) of composition and salivary flow genes can influence the susceptibility of individuals to dental caries. Five databases (Pubmed/Medline, Scopus, Web of Science, BIREME and Scielo) were systematically searched to respond the question: “Do the composition and salivary flow genes polymorphisms influence the susceptibility to dental caries in childhood and adult life?”. Human studies with cross-sectional, longitudinal and case control design were included. No restrictions on language/publication period was considered. Quality of studies was evaluated by Appraisal Checklist for Observational Studies and for a 10-point scoring sheet used to genetic studies. Meta-analysis was performed. From 1,200 identified records, seven were included in the qualitative and two in quantitative synthesis. Most of studies (57.1%) used a cohort/cross-section design. The systematic review comprised 2,861 individuals. Most of the included studies (71.4%) presented low quality of assessment. Overall, three different genes (*CA6*, *AQP5* and *AQP2*) and 15 different polymorphisms were analyzed. Findings of systematic review show that rs1996315 and rs3759129 in *AQP5*; rs467323 and rs10875989 in *AQP2*; and rs17032907 in *CA6* were associated with dental caries. Only the SNP rs2274327, in *CA6* gene, was included in the meta-analysis. It did not show association with caries (heterozygote, OR=0.73 [0.44 – 1.25]; homozygote, OR=0.79 [0.36 – 1.75]).

Results of systematic review shown that some Single Nucleotide Polymorphisms of composition and flow salivary were associated with the caries. Results of meta-analysis in rs2274327 of *CA6*-gene did not was associated with caries.

Key words: Polymorphisms, Dental caries, Saliva

Introduction

Dental caries affect an expressive number of individual in worldwide ^{1, 2} being the main reason to failure to dental restorations ^{3, 4}. Although it affects a large part of the population, caries prevalence is disproportionately distributed among the individuals, thus, presenting a polarization in those that present some social vulnerability ⁵. This fact is mainly due to multifactorial etiology of caries, which exhibits a complex network of determinants and mediators (with varying intensity according to the individual) ^{1, 5}. Social, biological and behavioral factors have been widely discussed and reported in the literature and is unquestionable that they are the mains explanations to populational differences in dental caries prevalence ^{1, 5-8}.

Although genetic contributions to the occurrence and susceptibility of dental caries have been proposed since the late 80's in twin studies, the interest on this topic has increased in recent years being the focus of some studies ⁹⁻¹¹. These studies aim found possible explanations the fact that groups of individuals with the same risk factors and behaviors in oral health can presented differences in prevalence of caries. In this context, some Single Nucleotide Polymorphism (SNPs) of salivary-gene have been shown a potential influence on dental caries susceptibility ^{12, 13}. In fact, a systematic review suggests that alteration in salivary proteins may influencing the individual caries experience ¹⁴

Saliva has components that can inhibit cariogenic bacteria in addition to containing calcium and phosphate that are actively involved in the process of demineralization and remineralization of dental enamel (KIDD E FEJERSKOV, 2004; SPLIETH et al., 2016). For example, patients with irradiated salivary glands may have a higher caries experience due to decreased salivary flow. Moreover, salivary flow has the role of diluting the microorganisms and carbohydrates ingested by individuals, avoiding them accumulating in dental tissues ¹⁵, therefore, presenting an important protective role for development and progression of caries disease.

Although some studies have shown the possible influence of SNPs in salivary flow and composition related-genes ^{13, 16}, this not a consensus ¹⁷. Therefore, the better understanding of the possible influence of these genetic variants in the susceptibility of individuals to caries disease could support the development of feasible approach. Thus, the aim of present study was to review the literature and perform a meta-analysis to investigates if SNPs in composition and salivary flow genes can influenced the susceptibility of individuals to dental caries.

Methods

The present systematic review was registered in PROSPERO (International Prospective Register of Systematic Reviews) under protocol number CRD42019121477. Besides, we describe the study according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline ¹⁸.

Review question and Searches: To structure the research question: “Do the composition and salivary flow genes polymorphisms influence the susceptibility of adults and children to dental caries?”, the P.I.C.O model has been employed. PICO stands for:

- Participants/ population: Individuals adults and children
- Intervention/exposure: Minor allele. The effect allele in this study was standardized as the minor allele reported in the studies. When the minor allele varied across the studies, the effect allele was referred as the minor alleles in most of studies. Likewise, to do the estimates stratifying by genotypes, we opted for choosing the minor homozygote and heterozygotes as effect genotypes.
- Comparator/control: Major allele. The effect allele was compared to the reference allele, defined as that most frequent in the population. To perform genotype analysis, the major homozygote was chosen as the reference.
- Outcome: Dental caries experience (DMF/dmf). Dental caries experience was the main outcome of this review, which was considered by the follow criteria: International Caries Detection and Assessment System (ICDAS) and DMF/dmf (Decayed, Missing, Filled) teeth/surface. It was preferentially considered groups caries-free vs caries experience. When more than one criteria to investigate dental caries was displayed, DMFT/dmft=0 (caries-free) vs. DMFT/dmft≥1 was chose.

The eligibility criteria were defined as: a) inclusion criteria: articles that aim to evaluate the association between genetic single nucleotide polymorphisms of flow and composition salivary genes in children or adults. Only human studies with cross-sectional, longitudinal and case control design. No restrictions on language or publication period were considered. b) exclusion criteria: studies with design of literature reviews, case reports and case series, abstracts of conference, letters to the editor, and qualitative studies.

Regarding the information sources, studies were identified by electronic searching in five different databases, including Pubmed/Medline, Scopus, Web of Science, BIREME – BVS Virtual health library and Scielo. up to January of 2019. Keywords selected included relevant entry terms and MeSH terms, which were combined to build the structured research. The complete structure is showed in the **supplemental material S1**. All retrieved records were upload in the EndNote Basic (www.myendnoteweb.com) aim to delete the duplicated ones. Besides, two independent reviewers (LAC and MCMC) read the titles and abstracts and judged all the founded papers considering the inclusion/exclusion criteria. The articles that were selected at this stage were full-text assessed and again judged. If any disagreement was found in relation to the inclusion of some study, the reviewers discussed the matter to obtain consensus. If a consensus was not reached, a third reviewer (MBC) talked the final judgment.

Data collection: Data collection was completed independently by two reviewers in a predefined file. The subsequent information were collected: Author, year, country, study design, sample, age, ethnicity of the sample (% for each ethnic group), percentage of the sexes of the sample, calculation of statistical power, evaluation of categorization of dental caries, analytical approach, data analysis (crude and adjusted analysis values and their respective confidence intervals), covariables and main results.

Quality of studies: Quality of studies was performed by two instruments. First, it was verified through the Appraisal Checklist for Observational Studies (Joanna Briggs Institute) (T.J.B., 2014) scale. This instrument has 10 questions evaluating diverse arguments in the study, which necessity be responded with three possibilities as follow: "No", "not clear" or "Yes". Each "Yes" answer corresponds to one point. Studies scored between 0 to 3 were considered low quality; 4 to 6 were of medium quality; and 7 to 10 were considered high quality. Two reviewers (LAC and MCMC) achieved the evaluation independently. Second, we perform as complementary evaluation through of instrument to evaluated genetic studies, adapted to a 10-point scoring sheet ^{19, 20}. This instrument is composed of two different measures to estimate such one of 10 points (Yes=1) or (no/undetermined=0). Same reviewer performed independently the evaluation.

Strategy for data synthesis: to synthesize quantitative the data, a meta-analysis was carried to pool the odds of each polymorphisms. Only SNPs present in at least two different studies/populations were included in meta-analysis. The allelic and genotypic effect were tested when available in the studies. To perform the analysis, it was calculated the estimates for the effect heterozygote and homozygote genotypes pooling by polymorphism. Studies that present more than one category for the dental caries, we chose the DMF/dmf=0 vs. DMF/dmf≥1.

To prevent discrepancies in data analysis the data harmonization for palindromic SNPs was done. When the palindromic SNP was present in two different studies, we only kept the SNP in the analysis if the study reported the DNA strand it was considering for the allele call. If this information was missing in the papers, the SNP was excluded from further analysis. Odds ratio (OR) was used to measure effect size with 95% Confidence Interval (CI). The prevalence ratio measures were converted to OR using the formula: $PR = \text{odds ratio} / (1 - \text{risk}_0 + \text{risk}_0 \times \text{odds ratio})$, where risk₀ is the prevalence of disease among non-exposed individuals^{21, 22}. Due to low heterogeneity (I^2 statistic <20%) observed, fixed models were performed. Analyzes were performed using Stata 12.0 software (StataCorp, College Station, TX, USA)

Results

Study selection

The initial search resulted in 1,200 records. Additional records identified through the references of included studies. After duplicates exclusion, 1,031 manuscripts remained in the digital library, from which 13 were selected by full-text evaluation. In this stage, six studies were excluded after full-text reading ²³⁻²⁸. The reasons for exclusion and flow diagram of study are displayed in the **figure 1**. Therefore, seven studies were included in the qualitative and two in quantitative synthesis.

Study characteristics

The majority of included studies were conducted in Turkey (28.6%; n=2) ^{17, 29} and Asia (28.6%; n=2) ^{11, 13}. A multicentric study included different populations (EUA, Turkey, Argentina, Brazil) ¹⁰. Four studies (57.1%) used a cohort/cross-sectional design ^{16, 17, 29, 30} and two (28.6%) case control design ^{11, 13}. Considering all studies, 2,861 individuals were included. Most of studies perform the analysis considering both permanent and deciduous teeth ^{10, 13, 16, 17, 30}, while two investigated only permanent teeth ^{11, 29}. None study performs power calculation and the studies did not reported clearly the ethnicity of populations.

Risk of bias within studies

Regarding to the quality assessment through Critical Appraisal Checklist for observational studies (Joanna Briggs Institute), most of studies (71.4%) presented low quality of assessment (**Table 1**). Similarly, considering methodological scoring protocol based on quality assessment for genetic studies, was observed that 71.4% of the studies were classified as low evidence, 14.3% as medium and 14.3% as high-quality evidence. The complete scoring is displayed in the **Table 2**.

Overview of Single Nucleotide Polymorphisms

Fifteen single nucleotide polymorphisms, covering three genes, were tested over salivary flow and composition with dental caries experience. Most of SNPs were in introns (46.2%), followed by missense (30.8%), 3 prime UTE (15,3%) and exon (7.7%). More information of SNPs is available on **table 3**. Only one SNP was included in the meta-analysis (rs2274327 of Carbonic Anhydrase 6 - CA6 - gene) without palindromic alleles. SNPs rs142460367 and rs142460368 were not in

consonance in chromosomic location reported in study ²⁹ and reference annotation (http://grch37.ensembl.org/Homo_sapiens), thus, both polymorphisms were excluded from further analysis

Results of individual studies

Were found three different genes which were related with possible influences with dental caries experience in adults and children: carbonic anhydrase 6 (*CA6*), aquaporin 5 (*AQP5*) and aquaporin 2 (*AQP2*). The summarization of results according gene and polymorphism in the studies is displayed in the **Table 4**. In general, effect allele of *CA6* did not were associated with dental caries susceptibility. Only the rs17032907 show association among the *CA6* investigated SNPs. While the heterozygote model did not was associated (OR=0.81 [CI95% 0.51 – 1.28]), the genotype TT in homozygote model was associated to increase dental caries experience (OR=2.14 [CI95% 1.10 – 4.20]) in this Chinese population.

From three SNPs investigated in *AQP5*, two presented association with dental caries. In a cohort study carried in a children (4 to 7 years) EUA population, the rs1996315 exhibited an association with dental caries ¹⁶. This study was carried with Caucasian (95%), Afro-descendents (2%) and other racial/ethnic groups (3%). Similarly, rs3759129 (*AQP5*) was associated with dental caries experience in a multicentric study carried in a EUA, Turkey, Argentina and Brazil. This study included a large sample size (n=1,383) with different age populations. In the EUA population, the mean of age was to 45.6, while the Turkey was 5.4, the Argentina was to 21.7 and the Brazil 55.8 years. Consequently, primary and permanent teeth were included in the analysis and different categorizations of dental caries were perform: a) individuals with age between 23 to 39: low caries was considered as DMFT=0-8 and compared to high caries experience (dmft >8); b) Individuals with age from 40 to 59 years: Low caries was considered as DMFT 0-19 and compared to high caries experience (DMFT>20); and c) Individuals with 60 years or more: low caries was considered as DMFT 0-21 and compared to high caries experience (DMFT>21).

Regarding the gene *AQP2*, both SNPs investigated were associated with dental caries experience. Anjomshoaa et al. ¹⁰ found that rs467323 and rs10875989 were associated with dental caries in a multicentric study (EUA, Turkey, Argentina, Brazil). Considering the rs10875989 (*AQP2*) associations with caries were observed only in recessive model in the same populations.

Wang et al. ¹⁶ performed haplotype analysis conducting associations between dental caries experience and haplotypes of 2 SNPs within the same gene. Thus, when the C of rs923911

(AQP5) was combined with the allele A or G of rs1996315 (AQP5) both haplotypes (CA and CG) exhibited a protective effect against caries for all caries categories (EUA population) ¹⁶ (**Table S2**).

Besides, an association between buffer capacity and the SNP rs2274327 (CA6) was observed. Allele T and genotype TT were less frequent in individuals with the highest buffer capacity in a Brazilian population ³⁰, Similarly, in a Turkey population, allele T and genotype TT of same SNP were less frequent in individuals with the highest buffer capacity ²⁹.

Synthesis of results (meta-analysis)

Considering the quantitative analysis, two studies were included ^{13, 30} in the analysis. The heterozygote (CT) (**Figure 2**) and homozygote (TT) (**Figure 3**) genotypes were tested for the SNP rs2274327 of CA6 gene. It was not possible to run the allelic association analysis. Both models presented low heterogeneity (I^2 statistic <20%) and fixed models were performed. The pooled effect in the heterozygote genotype show a tendency to protection of genotype CT to caries without statistical significance (OR=0.73 CI95% [0.44 – 1.25]). Considering homozygote genotype not significant associations were observed considering the genotype TT (OR=0.79 CI95% [0.36 – 1.75]).

Risk of bias across studies

Due to low number of studies included in the meta-analysis (less than 7), did not possible to perform the Funnel Plot and Egger's Test.

Discussion

To the best of our knowledge, this is the first systematic review and meta-analysis aim to evaluate the influence of Single Nucleotide Polymorphisms of salivary composition and flow related-gene in the caries experience of children and adults. Therefore, we identify 15 different polymorphisms understood in three different genes (*CA6*, *AQP5* and *AQP2*). Considering results of systematic review, rs1996315 and rs3759129 were associated with susceptibility of dental caries in *AQP5*; while in *AQP2*, rs467323 and rs10875989 were associated with dental caries in a multicentric study. Similarly, in *CA6*, the s17032907 show important role in dental caries experience. Thus, the results observed in the qualitative analysis suggesting that some SNPs can influence dental caries occurrence, although the meta-analysis of rs2274327 of *CA6*-gene did not show association with dental caries in (heterozygote and homozygote) genotype analysis.

The aquaporin 5 (*AQP5*) is a protein that is responsible to water channel being highly selective, codified by the respective gene (*AQP5*) and is localized into 12q13. Particularly, the protein *AQP5* plays a role in the generation of tears, pulmonary secretions and saliva being expressed in the apical membranes of serous acinar cells both in salivary as in lacrimal glands ³¹. In this context, the hypothesis is that some influence caused by SNPs of this gene can change the natural salivary flow or composition and unbalance the homeostasis. In fact, initial observations in mice models, which were targeted deletion of the gene encoding *AQP5*, exhibited a reduction on salivary flow and, hence, increase of dental caries ³². Yet, similar studies observed that water permeability to determines the flow and ionic composition of mice saliva are controlled mainly through the *AQP5* gene ³³. Therefore, the association between higher caries experience and rs3759129 (*AQP5*) was observed in the individuals of Argentine sample ¹⁰. This fact can be explained because rs3759129 is a non-coding transcript variant. In this way, rs1996315 from the *AQP5* related-gene was also associated with dental caries in a EUA population ¹⁶, and this SNP can presented as consequence a transcript variant of a non-coding RNA gene.

Similarly, the aquaporin 2 (*AQP2*) is also a gene that encodes water channels protein being localized into 12q13, very close to *AQP5*. Both SNPs (rs467323 and rs10875989) from *AQP2* related-genes were associated with dental caries experience ¹⁰. rs467323 and rs10875989 are an alteration in a three prime untranslated region (3'-UTR), which contains regulatory regions that post-transcriptionally influence gene expression, therefore, explain the presents results.

Most of SNPs investigated in the present review were gene-related to Carcarbonic Anhydrase 6 (CA6). The protein encoded by this gene is an isozyme of carbonic anhydrase. It is found only in salivary glands and in saliva. In addition, it seems to play an important role in the reversible hydration of carbon dioxide, although its function in saliva is already unknown ¹¹. Decrease in salivary buffering capacity in healthy children was found related to SNP rs2274327, which is a missense variant can change the protein coding. Therefore, the findings of study show that the allele T and genotype TT of this SNP were less frequent in individuals with the highest buffer capacity in a Brazilian sample ³⁰. However, significant alterations in caries experiences were not observed ³⁰. In a Turkey students' sample, salivary pH and buffering capacity were evaluated. Thus, also was observed an association between buffer capacity and the rs2274327 (CA6). Similarly, allele T and genotype TT were less frequent in individuals with the highest buffer capacity ²⁹. On the other hand, a study carried with Chinese population with similar behaviors investigated seven SNPs related to CA6, from which, one (rs17032907) was associated with dental caries experience. The genotype TT was linked to an increase of caries in this population. On the other hand, the haplotype analysis (ACA) considering the SNPs rs2274328, rs17032907 and rs11576766 from CA6 was associated with decrease of caries experience in this Chinese population. Corroborating, SNPs of CA6 displayed a significative association with the activity of the protein in the saliva ³⁴.

Although specific SNPs related to salivary flow and composition have shown potential association with dental caries experience in children and adults, a wide variation in the methodologies used and the SNPs investigated was observed. Most SNPs were evaluated only in one study, and some studies did not show the effects measure of the results. Thus, only two studies could be included in the meta-analysis, which did not show association. Moreover, other points have been highlighted. Besides, the limited report of power calculation in included study should be carefully interpreted; sample of studies ranged from 44 to 1,383 individuals and the lack of report in the sample size calculation can lead to decrease of power, since that non-significant results could be just a sample problem and not a lack of association. This can lead us to false negative type inferential errors. Similarly, few studies reported the ethnicity/ancestry information of populations. Considering all SNPs investigated in present systematic review, significant variances between allele frequencies and sample ethnicity have been detect when reported SNPs were explored in complementary database. It is finding highlight the need to carried adjustment of ancestry

information because genetic effects sizes can also change among populations, at least for some traits.

Moreover, it is important highlight that only gene candidate studies were included in present systematic review and in analysis and this should be also considered in the results interpretations. Other pathways related to salivary flow and composition might be influence dental caries experience, although not yet known and studied so far. In this way, we reinforce the importance of further conduction of gene candidate studies with thorough methodological rigor and quality as well as genomic scales studies. Genomic studies and caries experience is an topic still in initial phase ⁹ discouraging, even, a revision considering this subject. However, genomic scale investigations do not presuppose the prior knowledge of pathways as well as the etiopathogenesis of diseases, which facilitates the discovery of new routes of genes and polymorphisms promoting robustness on identification of these pathways.

Furthermore, these points were reflecting in the quality of studies evaluated by to instruments, which in both show a low quality. We chose to perform the analysis of quality through two tools because the first (T.J.B., 2014) is more linked to evaluate the observational studies and their report. We observed through this tool a low representativity of included sample of the real population. Most of studies chose convenience samples. As alternative to complement this instrument we perform a second evaluation through of instrument to evaluated genetic studies ¹⁹, ²⁰. This instrument measures mainly methodological points to genetic factors. In this way, both tools evidenced the low quality of evidence in which the results of the present study are based. Therefore, all considerations and interpretations of presented results need to be performed with caution taken this in account.

Despite the limitations observed, our study has strengths points that must be emphasized. A wide review was performed, with several control filters aim to reduce possible bias in or estimates, investigating possible palindromic SNPs as well as standardized the reference genotype. Therefore, providing robustness to our results. We highlight that this topic is extremely new and further studies with different populations are needed to provide more robust estimate. Further studies should include representative samples of the target populations with sample calculations and addressed to different ethnic samples. Collaborations between research groups can be an interesting strategy aim to combinate databases to increase sample sizes and/or replicate the results could be conducted and are recommended. Genome wide association studies are important

strategies and alternative approach to help the identification of new SNPs and pathways associated to caries experience being also essential as a basis for understanding the polygenic trait and genetic architecture of this phenotype.

Conclusion

Results of systematic review shown that some Single Nucleotide Polymorphisms of composition and flow salivary were associated with the caries experience - in genes related to *CA6*, *AQP5* and *AQP2*. High heterogenicity between the results were observed. Results of meta-analysis in rs2274327 of *CA6*-gene did not was associated with caries experience. Henceforth, interpretations show be taken with caution and further studies must be performed to support the presents findings, which must be replicate in studies with high methodological and report quality including different populations. Further studies can investigate presented genes and SNPs even as perform controls adjustments and presented power calculation of estimates.

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Legends:

Table 1. Critical Appraisal Checklist for observational studies (Joanna Briggs Institute) in the systematic review according to the 10-items

Table 2. Methodological scoring protocol based on quality assessment for genetic studies.

Table 3. Description of single nucleotide polymorphism investigated in the present systematic review according genes*

Table 4. Summarization results according gene and polymorphism in the studies

Table S1. Search strategy

Table S2. Main characteristics of studies included in this systematic review

Figure 1: Prisma flow diagram

Figure 2. Pooled effect of CA6 rs2274327 (C/T) to teste the effect of heterozygote genotype. Data are presented as odds ratio for each study (boxes), 95% CIs (horizontal lines) and summary as odds ratio with 95% CI (diamond). Fixed model was performed.

Figure 3. Pooled effect of CA6 rs2274327 (C/T) in homozygote model. Data are presented as odds ratio for each study (boxes), 95% CIs (horizontal lines) and summary as odds ratio with 95% CI (diamond). Fixed model was performed.

Table 1. Critical Appraisal Checklist for observational studies (Joanna Briggs Institute) in the systematic review according to the 10-items

Study, year	NIH Criteria										Final score
	1	2	3	4	5	6	7	8	9	10	
Peres et al. ³⁰	-	-	/	-	-	+	+	-	/	/	Low Quality (2)
Yarat et al. ²⁹	-	-	-	-	-	+	+	-	/	/	Low Quality (2)
Wang et al. ¹⁶	+	+	-	+	+	+	+	-	/	/	Medium Quality (6)
Anjomshoaa et al. ¹⁰	+	+	/	+	+	+	+	+	+	/	High Quality (8)
Li et al. ¹¹	-	-	-	-	-	+	+	-	/	-	Low Quality (2)
Sengul et al. ¹⁷	-	-	/	-	-	+	+	-	-	/	Low Quality (2)
¹³	-	-	/	-	-	+	+	-	/	/	Low Quality (2)

+ Yes; - No; /: Unclear

Table 2. Methodological scoring protocol based on quality assessment for genetic studies.

Study, year	Genetic Criteria										Score	Evidence
	Control group	Hardy–Weinberg equilibrium	Case group	Primer	Reproducibility	Blinding	Power calculation	Statistics	Corrected statistics	Independent replication		
Peres et al. ³⁰	1	0	1	1	1	0	0	0	0	0	4	Low
Yarat et al. ²⁹	0	0	0	1	0	0	0	0	0	1	2	Low
Wang et al. ¹⁶	1	1	1	1	1	0	0	0	0	0	5	Medium
Anjomshoaa et al. ¹⁰	1	1	1	1	1	0	0	1	1	1	8	High
Li et al. ¹¹	0	1	0	1	1	0	0	1	0	0	4	Low
Sengul et al. ¹⁷	0	1	0	1	0	0	0	0	0	0	2	Low
Shimomura-Kuroki et al. ¹³	0	0	0	1	1	0	0	0	0	1	3	Low

*For the quantification of criteria: «1» means present, and «0» absent

Table 3. Description of single nucleotide polymorphism investigated in the present systematic review according genes*

Gene	Polymorphism	Chromosomal position	Variation	Allele Frequencies by populations (%) *					Reference Allele / Effect allele used	Anc estr al allel e
				African	American	East Asian	Europe	South Asia		
CA6	rs2274327 C/T	1:9009406	Missense	C:92%	C:62%	C:76%	C:58%	C:67%	C/T	C
				T:8%	T:38%	T:24%	T:42%	T:33%		
	rs2274328 A/C	1:9009444	Missense	A:44%	A:53%	A:59%	A:46%	A:50%	A/C	C
				C:56%	C:47%	C:41%	C:54%	C:50%		
rs2274333 A/G	1:9017204	Missense	A:89%	A:61%	A:44%	A:71%	A:54%	A/G	A	
			G:11%	G:39%	G:56%	G:29%	G:46%			
rs17032907 C/T	1:9010405	Intron	C:82%	C:85%	C:60%	C:85%	C:81%	C/T	C	
			T:18%	T:15%	T:40%	T:15%	T:19%			

	rs11576766 A/C	1:9010984	Intron	A:79%	A:59%	A:70%	A:51%	A:59%	A/C	A
				C:21%	C:41%	C:30%	C:49%	C:41%		
	rs10864376 T/C	1:9030372	Intron	C:27%	C:60%	C:35%	C:72%	C:50%	T/C	T
				T:73%	T:40%	T:65%	T:28%	T:50%		
	rs3765964 T/C	1:9034421	Missense	G:68%	G:57%	G:32%	G:61%	G:48%	T/C	G
				A:32%	A:43%	A:68%	A:39%	A:52%		
	rs6680186 A/G	1:9039704	Exon	G:26%	G:51%	G:32%	G:51%	G:48%	A/G	G
				A:74%	A:49%	A:68%	A:49%	A:52%		
AQP5	rs923911 A/C	12:50358174	Intron	C:52%	C:86%	C:84%	C:86%	C:91%	A/C	A
				A:48%	A:14%	A:16%	A:14%	A:9%		
	rs1996315 A/G	12:50364707	Intron	G:83%	G:54%	G:58%	G:42%	G:38%	A/G	G
				A:17%	A:46%	A:42%	A:58%	A:62%		
	rs3759129 A/C	12:50354437	Intron	A:95%	A:88%	A:98%	A:82%	A:94%	A/C	A
				C:5%	C:12%	C:2%	C:18%	C:6%		
	rs467323 A/C	12:50349765	3 prime UTR	C:88%	C:50%	C:74%	C:28%	C:73%	A/C	T
				T:12%	T:50T	T:26%	T:72%	T:27%		
AQP2	rs10875989 C/T	12:50351075	3 prime UTR	T:52%	T:63%	T:42%	T:73%	T:32%	C/T	T
				C:48%	C:37%	C:58%	C:27%	C:68%		

* Based on Human (GRCh37.p13), available on: http://grch37.ensembl.org/Homo_sapiens. NA: not available; rs142460367 and rs142460368 were observed in chromosome position 6, not coincident with *CA6* gene location, thus, this polymorphism did not was reported in this table

Table 4. Summarization results according gene and polymorphism in the studies

Gene	Polymorphism	Study, Year						
		Peres et al. ³⁰	Yarat et al. ²⁹	Wang et al. ²²	Anjomshoaa et al. ¹³	Li et al. ¹⁴	Sengul et al. ²³	Shimomura-Kuroki et al. ¹⁶
CA6	rs2274327 C/T	-NA						NA
	rs2274327 A/G						+	
	rs2274328 A/C	NA				NA		
	rs2274333 A/G	-NA				NA		
	rs142460367 A/G		NA					
	rs142460368 A/C		NA					
	rs17032907 C/T					+ # ^b		
	rs11576766 A/C					NA		
	rs10864376 T/C					NA		
	rs3765964 T/C					-		
	rs6680186 A/G					NA		
AQP5	rs923911 A/C			NA				
	rs1996315 A/G			- #				
	rs3759129 A/C				+ #			
AQP2	rs467323 A/C				+ #			
	rs10875989 C/T				+ # ^a			

Legends: - Protector factor; + Risk factor; NA not associated; # Statistically associated; ^a Only in recessive model; ^b Association with homozygote genotype

Table S1. Search strategy

Search syntax	
PubMed	#1 (“Dental Decay” OR “Caries, Dental” OR “Decay, Dental” OR “Cariou Dentin” OR “Cariou Dentins” OR “Dentin, Cariou” OR “Dentins, Cariou” OR “Dental White Spot” OR “White Spots, Dental” OR “White Spots” OR “Spot, White” OR “Spots, White” OR “White Spot” OR “Dental White Spots” OR “White Spot, Dental” OR “Susceptibility, Dental Caries” OR “Caries Susceptibility, Dental” OR “Caries Resistance, Dental” OR “Resistance, Dental Caries” OR “Dental Caries Resistance”)
	#2 (“Polymorphisms, Genetic” OR “Genetic Polymorphisms” OR “Genetic Polymorphism” OR “Polymorphism” OR “Polymorphisms” OR “Nucleotide Polymorphism, Single” OR “Nucleotide Polymorphisms, Single” OR “Polymorphisms, Single Nucleotide” OR “Single Nucleotide Polymorphisms” OR “SNPs” OR “Single Nucleotide Polymorphism”)

* Search combination: #1 AND #2

Table S2. Main characteristics of studies included in this systematic review

Author , year	-Country -Study design -Sample (% Males)	-Age (permanent/ deciduous) -Ethnicity -Statistic power (yes/no)	-Evaluation dental -categorization	caries	Analytical Approach	Adjustment variables
	-Brazil -cohort (convenience sample) -245 (51.4 %)	-7 to 9 (permanent/ deciduous) -NR -No	-dmft/DMFT -dmft/DMFT=0 Vs dmft/DMFT≥1		x ² , Student's, and Mann–Whitney tests	-
Peres et al. ³⁰						Crude Analysis CA6 rs2274327 (C/T): Genotype CT compared to TT OR=0.83 (0.46 – 1.48); Genotype TT compared to CC OR 0.70 (0.31 – 1.60); CA6 rs2274328 (A/C): Genotype AC compared to AA OR 0.80 (0.48 – 1.34); Genotype CC compared to AA OR 1.16 (0.38 – 3.55) CA6 rs2274333 (A/G): Genotype AG compared to AA OR 0.96 (0.57 – 1.62); Genotype GG compared to AA OR 0.72 (0.25 – 2.05) Was observed an association between buffer capacity and the rs2274327 C/T (CA6). Allele T and genotype TT were less frequent in individuals with the highest buffer capacity. Adjusted Analysis -
Yarat et al. ²⁹	-Turkey -cohort	-19 to 26y (permanent)	-DMFT - DMFT=0 Vs. DMFT>0≤6		Student's t-test and chi-square test between groups and Pearson	-

-44 (54.5 %)	-NR	correlation analysis
	-No	

CA6 rs142460367 A/G: Effect direction not report; Effect not reported, Not associated

CA6 rs142460368 A/C: Effect direction not report; Effect not reported, Not associated

Crude Analysis

No associations between the evaluated SNPs and caries distribution as well as no correlation between these SNPs and the salivary parameters.

Adjusted Analysis

-

-EUA	-4 to 7y	-dmfs	and	WS	-Linear and logistic regression	-Age, sex, race,
-Cohort	Study (permanent/deciduous)	- 1) total number of tooth surfaces with frank cavitated or filled caries			model	tooth-brushing
(longitudinal)						frequencies and
-575 (48%)	- Caucasian	experience (d2fs-total); 2) pit and fissure surfaces with caries				fluoride intake
	(95%), Afro-descendents (2%)	experience (d2fs-pit/fissure); and				from water, tooth-brushing
	and other racial/ethnic groups (3%)	3) caries experience of all other tooth surfaces (d2fs-smooth surface). These scores were				frequency.
	-No	dichotomized in the downstream analyses as cases (children with scores ≥ 1) and controls (scores = 0).				

Wang et al.

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Crude Analysis

AQP5 rs923911 A/C: Not associated; Effect not reported; # **AQP5 rs1996315 A/G:** Protective effect ($p = 0.02$); Effect not reported

When rs923911 A/C was combined in haplotype analysis with rs1996315 (CA and CG) exhibited a protective effect against caries for all caries categories

Adjusted Analysis

-EUA, Turkey, Argentina, Brazil	- EUA 45.6y, Turkey 5.4y / 4.6y, Argentina 21.7y, Brazil 55.8y (permanent and deciduous)	-DMFT/dmft; white spots in enamel were scored as decayed	Logistic regression analysis	Age and Sex; the data from EUA was also adjusted by salivary flow and the use of medications that cause dry mouth.
-Multicentric study		- individuals with age between 23 to 39: low caries DMFT= 0-8 Vs. High caries dmft >8; Individuals with age from 40 to 59 years: Low caries DMFT 0-19 Vs High caries DMFT>20; Individuals with 60 years or more: low caries DMFT 0-21 Vs high caries DMFT>21		
-1383 (40.1 %)				

Anjomshoaa
et al. ¹⁰

Crude Analysis

AQP5 rs3759129 A/C: Risk factor (p = 0.03); Effect not reported; # AQP2 rs467323 A/C: Risk factor (p = 0.03); Effect not reported

Recessive model:

AQP2 rs10875989 C/T: Risk factor (p = 0.01); Effect not reported

Adjusted Analysis

Haplotypes analysis, in dominant Model:

AQP5 rs461872-rs1996315: Risk factor (p = 0.03); Effect not reported; AQP5 rs461872-rs1996315: Risk factor (p = 0.05); Effect not reported; # AQP5 rs3759129-rs1996315: Risk factor (p = 0.01); Effect not reported;

The best model analysis exhibited that higher caries was influenced by older age, use of medications and genetic

variation in **AQP5 SNPs rs3759129** ($p = 0.03$) and **rs10875989** ($p = 0.04$).

-China	- 51y high caries	-DMFT	chi-square test, and dominant and	Gender and age
-Case control	and 47 low caries	-Low caries DMFT $\leq$ 2 Vs High caries	co-dominant genetic models;	
-355 (51.5 %)	(permanent)	DMFT $\geq$ 3	logistic regression	
	-NR			
	-No			

Li et al. ¹¹

Crude Analysis

CA6 rs2274328 A/C: Genotype AC compared to AA OR 0.80 (0.50 – 1.27); Genotype CC compared to AA OR 1.47 (0.79 – 2.75); **# CA6 rs17032907 C/T**: Genotype CT compared to CC OR 0.81 (0.51 – 1.28); Genotype TT compared to CC OR 2.14 (1.10 – 4.20); **CA6 rs11576766 A/C**: Genotype AC compared to AA OR 0.74 (0.47 – 1.19); Genotype CC compared to AA OR 1.09 (0.55 – 2.14) **CA6 rs2274333 A/G**: Genotype AG compared to AA OR 0.83 (0.47 – 1.47); Genotype GG compared to AA OR 1.01 (0.60 – 2.00); **CA6 rs10864376 T/C**: Genotype TC compared to TT OR 0.88 (0.56 – 1.40); Genotype CC compared to TT OR 1.00 (0.51 – 1.98); **CA6 rs3765964 T/C**: Genotype TC compared to TT OR 0.76 (0.49 – 1.19); Genotype CC compared to TT OR 0.88 (0.40 – 1.98); **CA6 rs6680186 A/G**: Genotype AG compared to AA OR 0.76 (0.50 – 1.19); Genotype GG compared to AA OR 1.32 (0.57 – 2.96)

Dominant Model

CA6 rs2274328 A/C: Genotype AC/CC compared to AA OR 0.93 (0.60 – 1.44); **CA6 rs17032907 C/T**: Genotype CT/TT compared to CC OR 0.99 (0.64 – 1.53); **CA6 rs11576766 A/C**: Genotype AC/CC compared to AA OR 0.82 (0.54 – 1.25); **CA6 rs2274333 A/G**: Genotype AG/GG compared to AA OR 0.94 (0.55 – 1.60); **CA6 rs10864376 T/C**: Genotype TC/CC compared to TT OR 0.92 (0.60 – 1.39); **CA6 rs3765964 T/C**: Genotype TC/CC compared to TT OR 0.78 (0.52 – 1.19); **CA6 rs6680186 A/G**: Genotype AG/GG compared to AA OR 0.82 (0.54 – 1.26)
Haplotype (ACA) (rs2274328, rs17032907, and rs11576766) was associated with a lower caries index.

Adjusted Analysis

-

Sengul et al. 17	-Turkey	-6 to 16y	-dmft/DMFT	Two way ANOVA, and an	-
	-Cohort	(permanent and	-not clear	independent samples t test	
	-178 (45.5%)	deciduous)			
		-NR			
		-No			
	Crude Analysis	CA6 rs2274327 A/G: Genotype AG compared to AA OR 1.64 (0.82 – 3.25); Genotype GG compared to AA OR 1.07 (0.47 – 2.45); Allele G compared to A OR 1.09 (0.72 – 1.69)			
	Adjusted Analysis	-			
Shimomura-Kuroki et al. 13	-Japan	-3 to 11y	-DMFT/dmft	-Regression analysis	-
	-Case Control	(permanent and	-DMFT/dmft=0 Vs DMFT/dmft≥1		
	-81 (49,4 %)	deciduous)			
		-NR			
		-No			
	Crude Analysis	CA6 rs2274327 C/T: Genotype CT compared to CC OR 0.51 (0.19 – 1.32); Genotype TT compared to CC OR 4.04 (0.21 – 79.82)			
	Adjusted Analysis	-			

NR: not reported; # Statistical association; CI: Confidence Interval; OR: Odds Ratio; PR: Prevalence Ratio; dmft (decayed, missing teeth due to caries, filled teeth); WSL: white spot lesions; ICDAS: International Decay Detection and Assessment System; CA: Crude association; AA:

adjusted association; All measure effects show are ODDS Ratio. Different measures are reported; p: only p value reported; SNP: Single Nucleotide Polymorphism.

Figure 1: Prisma flow diagram

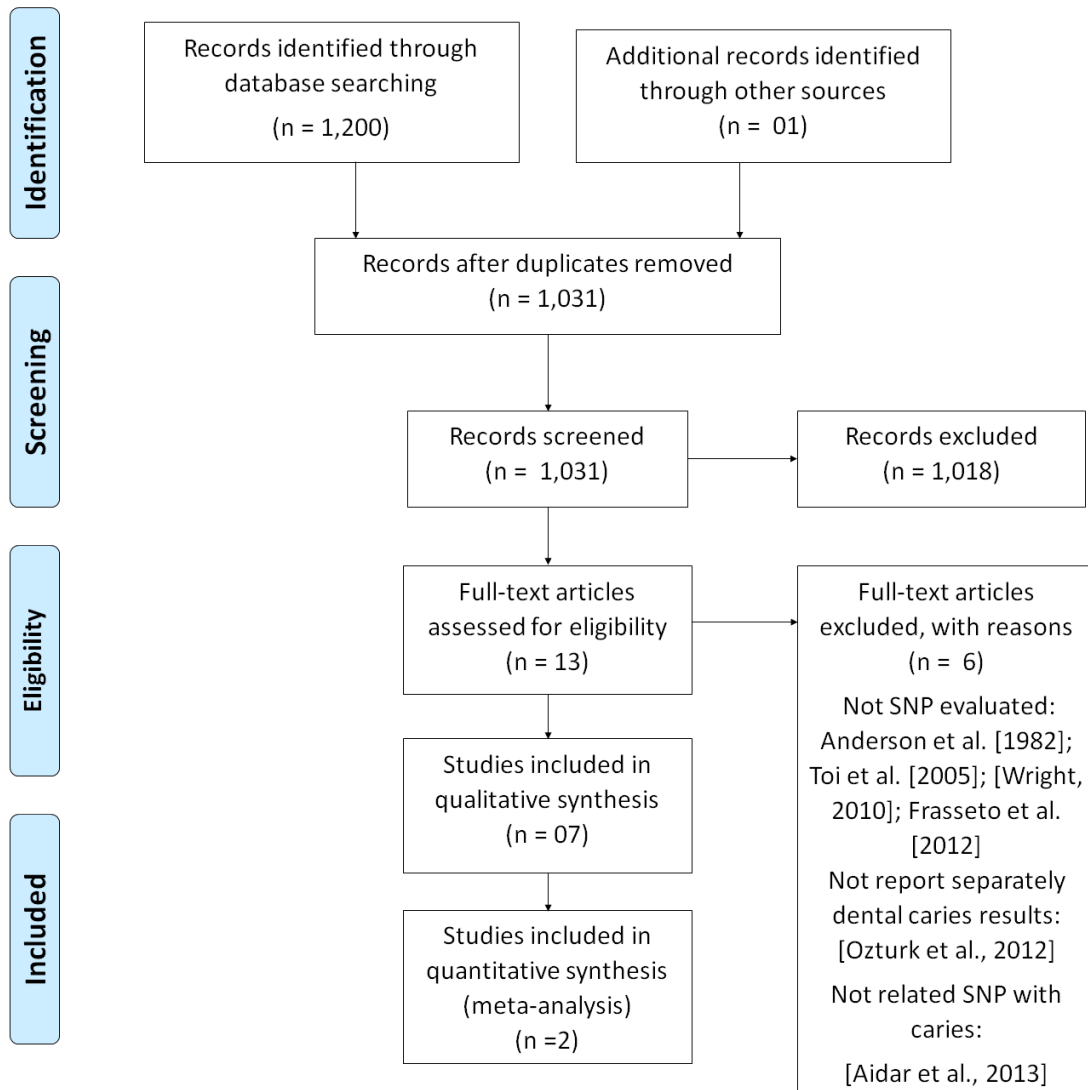


Figure 2. Pooled effect of CA6 rs2274327 (C/T) to test the effect of heterozygote genotype. Data are presented as odds ratio for each study (boxes), 95% CIs (horizontal lines) and summary as odds ratio with 95% CI (diamond). Fixed model was performed.

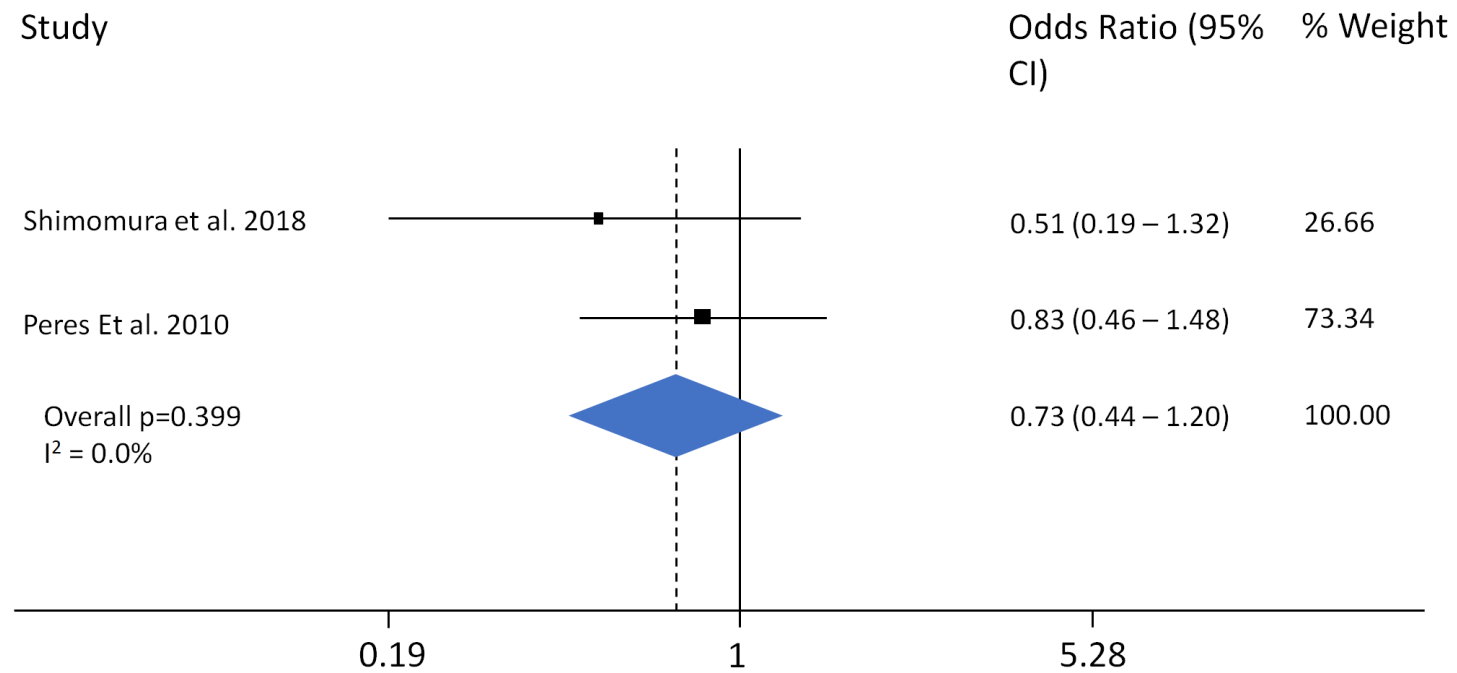
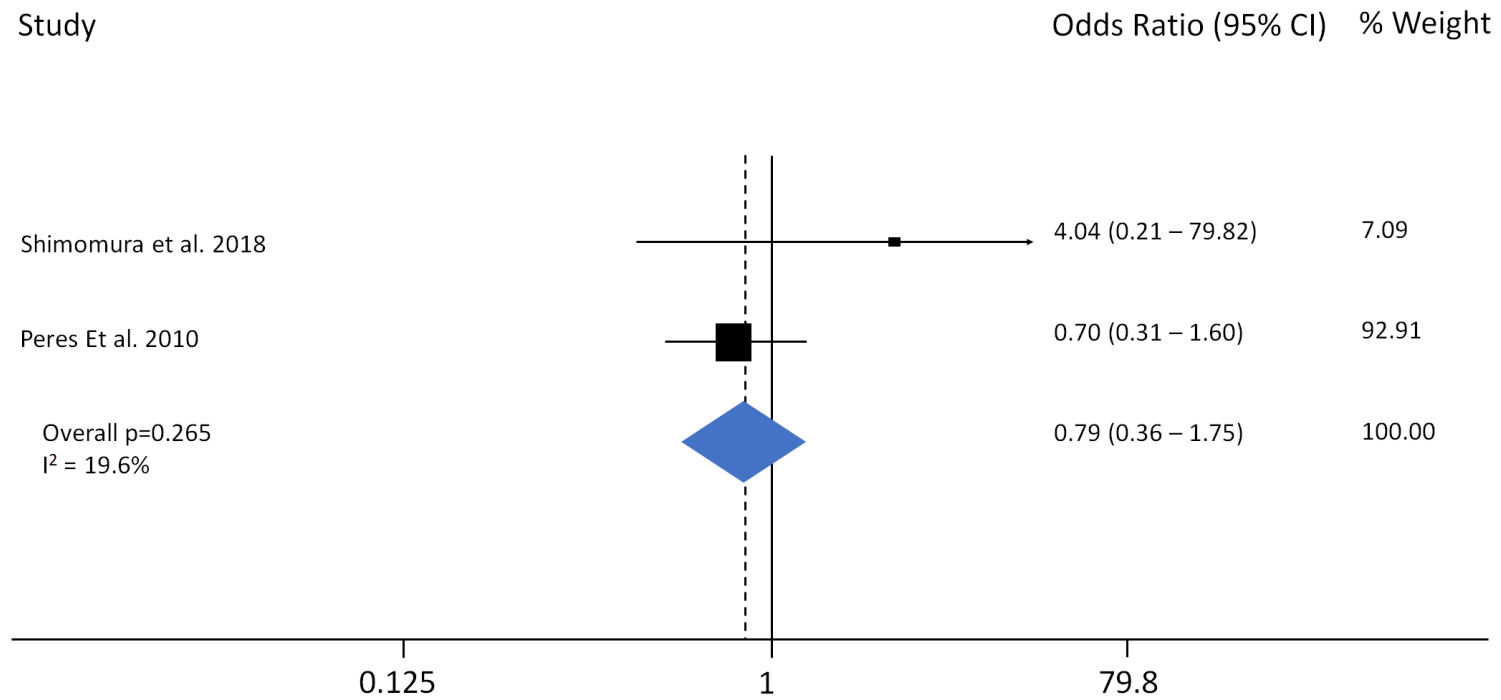


Figure 3. Pooled effect of CA6 rs2274327 (C/T) in homozygote model. Data are presented as odds ratio for each study (boxes), 95% CIs (horizontal lines) and summary as odds ratio with 95% CI (diamond). Fixed model was performed.



4.4 Artigo 4

Artigo formatado seguindo as normas da Revista *Clinical Oral Investigations*

Genes and SNPs in the pathway of immune response and dental caries risk: A systematic Review and Meta-analysis

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Key words: Polymorphisms. Dental caries. Immune response. Genetic. Gene.

Declarations of conflict of interest: none

Running title: Immune response polymorphisms and caries

Clinical Relevance: Several Single Nucleotide Polymorphisms related to immune response genes were linked with dental caries experience. Therefore, these genes have been shown to be important to explain differences in dental caries risk.

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Cover Letter

To: Professor Dr. Matthias Hannig
Editor-in-Chief,

Dear Editor:

Based on the importance of *Clinical Oral Investigations*, we are sending the manuscript entitled “**Genes and SNPs in the pathway of immune response and dental caries risk: A systematic Review and Meta-analysis**” to be appraised by the Journal’s Editorial Board.

This is the first systematic review with meta-analysis investigating the association between single nucleotide polymorphisms (SNPs) of immune response genes and dental caries experience.

The present systematic review included 6,947 individuals founding twenty-two SNPs linked to six different immune response genes (*MBL2*, *LFT*, *MASP2*, *DEFB1*, *FCN2* and *MUC5B*). The present findings showed that some genes are linked with dental caries occurrence. The meta-analysis suggests that the genes *MBL2* and *MUC5B* have an important role on dental caries, confirming the positive influence of response immune-genes on dental caries occurrence. Moreover, the pooled of all genes related to immune response in genotype (homozygote) analysis displayed association with dental caries experience.

MBL2-gene was associated with dental caries experience after control to linkage disequilibrium in genotype homozygote (OR=2.12 CI95% [1.12 – 3.99]) and heterozygote (OR=2.22 CI95% [1.44 – 3.44]) analysis. *MUC5B* was also associated in genotype heterozygote analysis (OR=1.83 CI95% [1.08–3.09]).

A great number of studies were included in this review and meta-analysis making wide review of current available literature. Also, we performed the analysis considering different analysis (allelic and genotype) providing a robustness to our findings. We did quality control filters in order to minimize the bias in our estimates, such as to investigate and exclude SNPs in linkage disequilibrium for the gene-pooled approach, as well as excluded palindromic ones. Besides, we have not identified publication bias across included studies.

This is a review manuscript and has not been considered for publication elsewhere. The paper was read and approved by all authors. All authors have made substantive contribution to this study, and all have reviewed the final paper prior to its submission. The authors declare that there are no potential competing interests. Furthermore, I attest the validity and legitimacy of data and its

interpretation. There are no conflicts of interest for authors listed above. We sign for and accept responsibility for releasing this material.

Thank you very much for your consideration.

Yours sincerely,

Prof. Marcos Britto Corrêa, PhD. (**Corresponding Author**)

Graduate Program in Dentistry, Federal University of Pelotas

**Genes and SNPs in the pathway of immune response and dental caries risk: A systematic
Review and Meta-analysis**

Running tile: Immune response polymorphisms and caries

Genes and SNPs in the pathway of immune response and dental caries risk: A systematic Review and Meta-analysis

Running title: Immune response polymorphisms and caries

Abstract

Objectives: to systematically review the literature pooling the Single Nucleotide Polymorphisms (SNPs) related to immune response-genes and their influence on dental caries experience.

Materials and methods: Five databases (Pubmed/Medline, Scopus, Web of Science, BIREME and Scielo) were searched. Two reviewers independently judged the papers. Were included only human studies with cross-sectional, longitudinal and case control design without restrictions on language or publication period. Quality of studies was evaluated by Appraisal Checklist for Observational Studies (ACOS) and for a 10-point scoring sheet used to genetic studies (GS). Meta-analysis was performed.

Results: From 1,200 records, 19 studies were included in the review and 18 in the meta-analysis. Overall, 6,947 individuals were evaluated most in cohort studies (57.9%). Quality of studies was considered as low (42.1%) in ACOS and medium (63.2%) in GS. Twenty-two SNPs were evaluated, which are linked to six different immune response genes (*MBL2*, *LFT*, *MASP2*, *DEFB1*, *FCN2* and *MUC5B*). Most of SNPs are in intron region (36.4%) and 81.8% are related to possible functional impact in protein coding. *MBL2*-gene was associated with dental caries experience after control to linkage disequilibrium in genotype homozygote (OR=2.12 CI95%[1.12 – 3.99]) and heterozygote (OR=2.22 CI95%[1.44 – 3.44]) analysis. *MUC5B* was also associated in genotype heterozygote analysis (OR=1.83 CI95%[1.08–3.09]).

Conclusion: Single Nucleotide Polymorphisms related to immune response genes are linked to phenotype caries experience. The meta-analysis showed that the genes *MBL2* and *MUC5B* have an important role on dental caries. Results should be interpreted with caution due to the quality of the evidence.

Clinical Relevance: Several Single Nucleotide Polymorphisms related to immune response genes were linked with dental caries experience. Therefore, these genes have been shown to be important to explain differences in dental caries risk.

Introduction

Dental caries is a multifactorial and complex pathology being considered one of most prevalent chronic disease that affect children and adults [1-4]. Although affects high part of global population [4], it is disproportionately distributed among the individuals, thus, presenting a polarization in those that present some social vulnerability [3]. This fact is mainly due to its multifactorial etiology, which exhibits a complex network of determinants and mediators (with varying intensity according to the individual) [3]. Besides of socioeconomic components that perform important role in caries, a wide range of components, such as fluoride exposition, dietary habits, salivary flow and bacteria colonization's, are extensively reported in the literature [5].

It is mostly accepted that cariogenic bacteria influenced by dietary habits play a key role in the individual microenvironment being mediate by contextual factors [4-5]. Despite this multifactorial network explain the most part of caries prevalence in worldwide, there is still a lack of evidence to full-understanding of all mechanisms of this complex disease. Hence, complementarily to consolidated knowledge, genetic factors have been one of the subject of studies demonstrating important genetic role in determination of caries susceptibility [6]. Besides of evidence found in animal models [7-8], twins studies suggest that 40 to 60% of caries susceptibility can be determinate by genetic (hereditary) factors [9-12]. Moreover, with the development of the biological and molecular methodologies, such as DNA sequence analysis, which were increased with human genome project, was possible investigated the direct relationships between the genes and the Single Nucleotide Polymorphisms (SNPs) with dental caries disease [6].

In this way, recent reviews [6, 13] have shown a wide range of gene that are implicated in caries etiology. Besides that, using candidate gene methodologies SNPs have been associated influencing these results can increase the caries prevalence [14] as well as decrease then [15]. Some proteins codified by specific genes and present in saliva have been related to individual immune response have an antimicrobial, antiviral, antifungal and/or anti-inflammatory properties [16]. The lactotransferin (*LTF*), defensin beta 1 (*DEFB1*) and mannose binding lectin 2 (*MBL2*) are some of these genes related to response immune, which studies have suggested act as a host defense protein by influencing the nonspecific immune system, as well as adaptive immunity affecting, hence, dental caries experience [6, 13].

Although some reviews have been published [6, 13], they investigating the influence of SNPs in dental caries susceptibility only present an overview of individual studies and did not pooled the same genes and polymorphism in analytic approach, which can be an interesting

strategy to better understanding the real role of SNPs in dental caries experience. Therefore, the aim of present study was to systematically review the literature pooling the SNPs related to immune response-genes and their influence on dental caries experience.

Methods

The present review was registered with protocol of CRD42019121486 in International Prospective Register of Systematic Reviews (PROSPERO). Besides, we reported the study according to PRISMA guideline (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) [17].

Review question and Searches:

Five databases (Pubmed/Medline, Scopus, Web of Science, BIREME – BVS Virtual health library and Scielo) were searched through structured search syntaxis up to January of 2019. Chose keywords were selected to answer the study question (Are the polymorphisms of immune response genes associated with dental caries?) using the PICO model:

- Participants/ population: individuals of all age groups.
- Intervention/exposure: Mutants Single Nucleotide Polymorphisms in immune response gene. The effect allele in this study was standardized as the less frequent allele reported in the studies. When the minor allele frequency varied among the studies, the effect allele was referred as the minor alleles in the majority of the studies. Similarly, to do the estimates stratifying by genotypes, we opted for choosing the minor homozygote and heterozygotes as effect genotypes.
- Comparator/control: Wildtype Single Nucleotide Polymorphisms in immune response gene. Thus, the effect allele was compared to the reference allele, defined as the most frequent in the population. To perform genotype analysis, the major homozygote was choosing as the reference.
- Outcome: Dental caries experience.

All relevant MeSh and entry terms were also included in the syntaxis. **Supplemental material S1** display the complete structure of search strategy. After the search, a virtual library was build uploading the retrieved papers into the EndNote ^M software (Thomson Reuters, Rochester, New York, NY, USA). Duplicated records were excluded after the duplicate identification by software. Thus, two reviewers (LAC and MCMC) read independently all founded title and abstracts reports performed the judgment of the papers through the following inclusion/exclusion criteria:

- a) Inclusion criteria: comprised articles that aim to evaluate the association between genetic immune response genes and dental caries in children or adults. Only human studies with cross-sectional, longitudinal and case control design were included. No restrictions on language or publication period were considered.

- b) Exclusion criteria: comprised the studies with design of literature reviews, case reports and case series, abstracts of conference, letters to the editor as well as qualitative studies were excluded of the present revision.

At this stage, the reviewers (LAC and MCMC) read the full-text and judged the remained papers. When disagreements were observed, a consensus between the reviewers through discussion was reached.

Data collection:

The Full data extraction in a tested database was performed independently by the same reviewers (LAC and MCMC). The following data were extracted: Author, year, country, study design, sample, age, ethnicity of the sample (% for each ethnic group), percentage of the sexes of the sample, calculation of statistical power, evaluation and categorization of dental caries, analytical approach, data analysis - crude and adjusted analysis values and their respective 95% confidence intervals (CI95%) -, covariables and main results. Disagreements between the collected data were checked.

Quality of studies:

To investigate the quality of studies, two tools were used. The first is indicated to observational studies: Appraisal Checklist for Observational Studies (Joanna Briggs Institute) [18]. This tool presents 10 questions assessing different arguments in the study. Studies that reach a scored up to three were considered low quality; four to six were of medium quality; and seven to ten were considered high quality. The second instrument was adapted to a 10-point scoring sheet previously used [19-20] to genetic studies. This tool present two different criteria to evaluate such one of 10 points (Yes=1) or (no/undetermined=0). Studies that obtained until four points were classified as low quality, five to seven, medium quality; eight or more, high quality. Two reviewers (LAC and MCMC) performed the evaluations independently. Disagreements were solved through discussion until consensus.

Strategy for data synthesis:

Was adopted a meta-analysis to pooling the SNPs and another stratifying by gene. We divide the analysis between allelic and genotype (homozygote and heterozygote) analysis, pooling by both polymorphism and gene. The effect allele and genotypes were compared to the reference allele and genotype, respectively, into the different analysis. Studies that present more than one category for the dental caries, we chose the DMF/dmf=0 vs. DMF/dmf $\geq$ 1.

Results of adjusted models were – when possible – included. Unadjusted estimates were considered (or calculated) when adjusted results have not been displayed. Odds ratio (OR) was used to measure effect size with 95% Confidence Interval (CI95%). Prevalence ratio measures were transformed to OR by the formula: $PR = odds\ ratio / 1 - risk_0 + risk_0 \times odds\ ratio$, where $risk_0$ is the prevalence of disease among non-exposed individuals [21-22]. In cases that results were only showed by stratified analysis, we included the group with higher number of individuals.

To avoid inconsistencies in data analysis we did data harmonization for palindromic SNPs. When the palindromic SNP was present in two different studies, we only kept the SNP in the analysis if the study reported the DNA strand. If this information were missing in the papers, the SNP was excluded from further analysis. Aiming to avoid biased estimates due to linkage disequilibrium (LD) in the gene pooled analysis, we performed a pruning by LD for those studies that analyzed more than one polymorphism in the same gene. For that, we made a pairwise comparison and included only SNPs which were independent ($r^2 < 0.3$) from the others. For the SNPs in $LD \geq 0.3$, we have included in the analysis the one with lowest P-value in the association. When the studies have not showed linkage disequilibrium estimates, the once retrieved from the 1000Genomes global population as reference pannel was considered. Therefore, when the SNPs included in the meta-analysis (in gene stratification) was extract from the same study we only maintained in the analysis when r^2 of equilibrium linkage was ≤ 0.30 , according investigated population.

Because we observed a high heterogeneity (I^2 statistic $> 50\%$) across the studies, random models were used. Analyzes were performed using Stata 12.0 software (StataCorp, College Station, TX, USA).

Published bias was also investigated by Egger test and funnel-plot. This plot details statistical significance on a funnel-plot, indicating the level of significance of each analysis (allelic and genotype - homozygote and heterozygote). Moreover, we plot the graphs pooling by gene.

Results

Study selection

The search found 1,200 initial records. After duplicated removal, 1,029 records remained in the virtual library. In the initial screening, 1,000 records were excluded and 29 followed to full-text reading. From these, 10 papers [23-32] were excluded in this stage. Reasons of exclusion are show in the PRISMA flow chart (**Figure 1**). Therefore, 19 studies were included in the systematic review [14-15, 33-49] and 18 in the meta-analysis [14, 33-49].

Study characteristics

The most frequent study design was the cross-sectional, followed by cohort design (n=11; 57.9%) and case-control design (n=8; 42.1%). Studies were conducted mostly in Brazil (n=5; 26.3%) [34, 41-42, 45-46] and China (n=3; 15.8%) [37, 44, 49]. Only three studies reported the ethnicity of investigated populations: Caucasian [39] and Caucasian/Afro-American [35, 45]. Most of studies used DMF/dmf (Decayed, Missing, Filled) and only one [45] used the International Caries Detection and Assessment System (ICDAS) to assessment dental caries. Permanent (n=7; 36.8%), primary (n=7; 36.8%) and permanent/primary teeth (n=5; 26.3%) were investigated. Moreover, 6,947 individuals were evaluated.

Risk of bias within studies

Concerning Critical Appraisal Checklist for observational studies (Joanna Briggs Institute), was observed a high number of studies classified as low (n=8; 42.1%) and medium (n=7; 36.8%) quality of assessment (**Table 1**). Likewise, regarding the tool to evaluate the quality of assessment in genetic studies, most of them were classified as medium (n=12; 63.2%) evidence (**Table 2**).

Overview of Single Nucleotide Polymorphisms

Twenty-two single nucleotide polymorphisms were found investigating possible associations with dental caries experience. These SNPs were present in six genes. Most of SNPs were placed in intron region (36.4%), 27.2% in missense variants and 18.2% were 5 prime UTR. Furthermore, 81.8% of SNPs are related to possible functional impact in protein coding, according to 1000Genomes global population. Details of SNPs and their functional impact on protein are available on **table 3**. Palindromic SNPs were founded and needed to be removed: rs11003125 of *MBL2* of Alyousef, et al. [14] and SNP rs1800972 of gene *DEFB1* investigated in the study fulfilled by Ozturk, et al. [35]. Abbasoglu, et al. [40] did not reported primer

sequence and was excluded of analysis. The SNP *rs7096206* of Shimomura-Kuroki, et al. [48] presented different nucleotide (G/T to C/G) and was excluded of gene analysis.

Linkage disequilibrium was observed among several single nucleotide polymorphism presents in *MBL2*, *MUC5B*, *LTF*, *DEFB1* and *FCN2*; therefore, SNPs in disequilibrium were excluded of final analysis. Only the SNP with the strongest association was included in the analyses. **Table 4** display complete description of excluded SNPs and respective D value.

Results of individual studies

Twenty-two Single Nucleotide Polymorphisms were evaluated, which are linked to six different immune response genes (*MBL2*, *LFT*, *MASP2*, *DEFB1*, *FCN2* and *MUC5B*) suggesting possible association with dental caries experience. Full characteristics of studies included in this systematic review are available in **supplementary material S2**. A high number of SNPs were evaluated in only one study; thus, these polymorphisms will be described in this section. Besides, some observations of individuals studies will be described here. The summarization of founded SNPs according gene in the studies are described in the **table 5**.

Considering the *MBL2* gene, was observed that genotype CG of SNP *rs11003125*, present in a regulatory region with potential TF binding, was associated with increase of dental caries experience in Iranian population [47]. When the genotype CG was compared to CC - in genotype (heterozygote) analysis – was observed an OR 2.54 CI95%(1.36 – 4.17); Besides, the genotype GG – in genotype (homozygote) analysis – shown an OR 2.05 CI95%(1.01 – 4.14); similar association was observed by authors when genotype CG+GG was compared was compared to CC (OR 2.40 CI95% [1.3 – 4.40]); On the other hand, allelic model did not show association (OR 1.19 CI95% [0.89 – 1.57]) [47].

Any of four studies that investigated SNPs related to *LTF* (*rs1126478*, *rs1126477*, *rs2269436*, *rs743658*, *rs4547741*, *rs6441989*, *rs2073495*, *rs11716497*) show associations with caries. Similarly, the SNP *rs72550870*, with possible impact in protein regulation, present in gene *MASP2* had been investigated in only two population (13 and 5 year old children) of one study, no associations with this SNPs were observed in a Polish children [36]. In this way, SNPs related to *FCN2* (*rs17514136*, *rs3124953*, *rs3124952*) also did not show associations with caries experience in Poland individuals [43].

Considering the SNPs related to *DEFB1*, was observed that genotype AA in SNP *rs11362* was associated with higher dental caries experience (OR 5.76 CI95%[1.83 – 18.14]) as well as the genotype AG (OR 2.04 CI95%[1.04 – 4.01]) in EUA individuals [35]. This SNP is present in a 5 prime UTR region with possible impact in the protein coding. On the other hand, the genotype GA of *rs1799946*, also in 5 prime region with potential to change protein coding,

was associated with decrease of dental caries (OR 0.34 CI95%[0.16 – 0.71]), although, genotype AA was not associated (OR 0.39 CI95%[0.15 – 1.06]) in the same population [35].

Regarding SNPs presents in the MUC5B gene, only one study investigated this gene and found association with three of five SNPs investigated. Genotype CT (OR 2.14 CI95%[1.11 – 4.12]) and TT (OR 6.69 CI95%[2.79 – 16.03]) in SNP rs2735733 were associated with higher number of teeth with dental caries in Brazilian population [45]. This SNP presented in an intron region can be influence the protein coding. Similarly, genotype TT of rs2249073 (OR 31.56 CI95%[10.52 – 94.66]) and genotype CT (OR 2.76 CI95%[1.32 – 5.78]) were associated with higher occurrence of caries. In the SNP rs2857476 (present in intro region and potential influence in protein coding) genotype CT (OR 2.77 CI95%[1.39 – 5.51]) and genotype TT (OR 21.43 CI95%[6.59 – 69.72]) were associated with dental caries.

Synthesis of results (meta-analysis)

Eighteen studies were included in the meta-analysis. The summarization of the meta-analysis results according by allelic and genotype (homozygote and heterozygote) analysis are displayed in **table 6**. Overall, 21 SNPs were included.

Considering the allelic analysis, 14 SNPs were included. No SNPs included in this meta-analysis showed significant association when were considered individually. In genotype analysis, 22 SNPs were included. Considering genotype (homozygotes or heterozygote) analysis, no SNPs were associated with dental caries in the meta-analysis.

When several SNPs were pooled in order to test the association for the whole gene, some genes shown associations. In allelic analysis, before exclusion of Linkage disequilibrium, *MBL* was associated (OR 1.24 CI95%[1.00 – 1.56]) with dental caries. After exclusion of SNPs in Linkage Disequilibrium the association was lost (OR 1.24 CI95%[0.96 – 1.59]). The pooled of all genes of immune response in allelic model also did not show association (OR 1.01 CI95%[0.95 – 1.08]).

Considering genotype analysis, pooled of gene *MBL2* in homozygote was associated with dental caries experience after control to linkage disequilibrium (OR 2.12 CI95%[1.12 – 3.99]). Moreover, the pooled of all genes related to immune response-gene in genotype (homozygote) analysis show an increase of 42% in odds of dental caries experience (OR 1.42 CI95%[1.01 – 1.99]), highlight that pooled of these genes these SNPs in general presents risk to dental caries. Considering the heterozygote, *MBL2* gene was also associated with occurrence of dental caries after exclusion of SNPs in linkage disequilibrium (OR 2.22 CI95%[1.44 – 3.44]). Likewise, *MUC5B* gene were associated with dental caries (OR 1.83 CI95%[1.08 – 3.09]).

Risk of bias across studies

Funnel plot results presented no significant publication bias across studies. Egger's test confirmed these observations (Allelic [p=0.554], Homozygote [p=0.299] and Heterozygote models [p=0.200]) (**Figure 2**).

Discussion

The present systematic review included 6,947 individuals founding twenty-two SNPs linked to six different immune response genes (*MBL2*, *LFT*, *MASP2*, *DEFB1*, *FCN2* and *MUC5B*). The present findings showed that some genes are linked with dental caries occurrence. The meta-analysis suggests that the genes *MBL2* and *MUC5B* have an important role on dental caries, confirming the positive influence of response immune-genes on dental caries occurrence. Moreover, the pooled of all genes related to immune response in genotype (homozygote) analysis displayed association with dental caries experience.

Mannose binding lectin 2 (*MBL2*) is a gene that encodes the soluble mannose-binding lectin protein found in serum. This protein is linked to innate immune system, identifies mannose and N-acetylglucosamine on several microorganisms, being capable to identify a elevate range of pathogenic microorganisms activating complement cascade via the antibody-independent pathway [50]. Therefore, has been proposed that could influenced the microorganism's colonization and consequently dental caries. Low-producers haplotypes of this protein had been suggest a link to increase of severe septic shock and sepsis in sick patients as well as increase of MBL production might exacerbate an proinflammatory response [51]. In fact, the genotype CG and GG of SNP rs11003125 shown an increase of dental caries experience [14]. Likewise, pool of *MBL2*-gene was associated with caries in genotype (both homozygote as heterozygote) analysis, even after exclusion of linkage disequilibrium SNPs.

In this way, SNPs related to defensin beta 1 (*DFB1*) showed also associations with phenotype. The protein encoded through this gene is an antimicrobial peptide that present influence in the resistance to microbiological colonization of epithelial surfaces. Thus, SNP rs1799946 is in 5 prime UTR region with potential to influence the protein coding. The genotype AG of this SNP was associated with a reduction of phenotype in a cohort of individuals in EUA (Caucasian and Afro-Americans) with age between 17 and 84 years [35], while in a cohort of Italian individuals (18 to 65 years) was observed an association with increase of dental caries [15]. Some factors can explain this difference observed in the studies. The first is linked to difference between the ethnicity of populations, which could influence the distribution of alleles and, hence, the findings. Another possibility is the difference in the categorization of dental caries. Although both authors used DMFT index, Italian study complemented the clinical diagnostic with use of panoramic radiographic [15]. Besides, the analysis of the data was taken with linear model regression while EUA study choice multiple logistic regression models with the follow categorization: Low caries (DMFT<14) Vs High

Caries (DMFT $\geq$ 14) in individuals below 30 years and Low caries (DMFT<9) Vs High Caries (DMFT $\geq$ 9) in individuals equal or above 30 years.

The gene encoding the LTF protein (which has the same name Lactotransferrin - *LTF*) is a member gene of the transferrin gene family and its protein product is found in the secondary neutrophil granules. Indeed, it can be considered an important mediator of immune response, protecting the organism to pathogenic injuries through regulation of enzyme activities [52]. In relation to phenotype dental caries, it seems to act with an effect on the formation of bacterial biofilm [6, 39]. This effect is due to the ability to sequester or chelate the iron necessary for the development of the biofilm, thus influencing both the dental caries such as periodontal disease [6, 39]. LFT was the gene with more SNPs investigated among the included, however, no difference in caries experience was found between the frequency of genotypes/allele and phenotype in the me-analysis (WANG et al., 2017).

Similarly, SNPs related to mannan binding lectin serine peptidase 2 (*MASP2*) - rs72550870 - and ficolin 2 (*FCN2*) - rs17514136, rs3124953, rs3124952 - did not show also associations with dental caries experience [36, 43]. This SNPs were investigated in few studies [36, 43] and further investigations must be performing to confirm these initial observations. *MASP2* encoded pre-proprotein that heterodimerize to form the complete protease. It cleaves complements converting in the lectin pathway of the complement system, besides to participate to proteolytically processed [53]. On the other hand, *FCN2* has shown to have carbohydrate binding and opsonic activities, and low levels of FCN2 in serum seems increase the predisposition to infectious diseases [54]

MUC5B is a gene that encoding specific glycoprotein, which can play such an important role in maintaining oral health, hence, have been associated with an protection of surfaces from colonization by cariogenic bacteria [54]. Indeed, the mucins (among them MUC5B) presents a protective effect against airway infection shown a prevention of the progress of inflammatory lung disease [55-56]. In fact, it can decrease the biofilm formation through the reduction of *Streptococcus mutans* adhesion [54]. Despite only one study evaluate the association of (five) SNPs linked to this gene, three of them (rs2735733, rs2249073 and rs2857476) were associated with the phenotype. Moreover, the pooled of gene in genotype (heterozygote) analysis displayed in meta-analysis shown an increase of 83% the odds of having dental caries. These observations suggest that refereed SNPs might change the normal expression of encoded proteins in the buccal environment favoring the microbiological adhesion and, hence, the increase of dental caries in these individuals. In fact, all SNPs investigated present potential influence in protein coding, being majority presents in introgenic regions. However, further studies are needed to

confirm this findings should compare also the SNPs even as the expression of this proteins in the saliva.

Yet, some limitations and strong points of present systematic review should be highlight. Results were based mainly in studies with moderate and low quality of evidence and a limited number of studies presents the ethnicity of target population, which could introduce a considered bias in the results. The population can be an important bias font in genetic studies, leading to erroneous association estimates. Significant variances between allele frequencies and population ethnicity have been identify when reported SNPs were investigated in complementary database. This highlight that ancestry information should be adjusted in further studies with focus in this topic to decrease possible biases. The additional limitation connected to unlike ethnicities refers to the pooled analysis, in which the estimates were joined regardless of the population ancestry background. It is previously known that genetic effect sizes can be fluctuate between different populations, at least for some traits, and allelic heterogeneity could have a vital influence on the generalizability potential of association results across populations. Problems in transferability of results have been evidently verified for polygenic risk scores [57]. Therefore, the estimates for both polymorphisms and genes must be prudently considered. Besides, only gene candidates were included in the analysis. It is can be considered a limitation because other genes from the same pathway could also to be influencing the phenotype. To identify these possible genes, it is necessary additional literature focused in genomic analysis, such as Genome Wide Association Studies (GWAS), which presents a scarce literature with the phenotype of interest of the present revision [6]. Investigations at genomic scales are more robust for identification of genetic component. It is explained due to genomic studies are not performed on previous knowledge but are designed to be used to identify new genes or routes to, in a second stage, easily direct further studies.

Moreover, case and controls groups not always were well described and presented matched, which can conduct a different sample being comparable in the studies. Moreover, considering the low number of SNPs replicate/investigate in several studies, its important interpret the data with caution, since that could potentially be false positive. Besides that, considering that few numbers of studies perform power calculation, this could implicate also in false negative findings, which could be an important limitation to be considered. It is important that studies present sample calculations to ensure that non-associations are not due to lack of statistical power, since a small part of the studies investigated presented such calculations. This can lead us to false negative type inferential errors. Different caries categorizations were observed, perhaps caries-free (DMFT/dmft=0) vs caries-affected (DMFT/dmft $\geq$ 1) were performed. Thus, we recommend the preferable use of this categorization to standardize the

further studies. The use of correct analytic approach control by environment and contextual factors even as with post hoc corrections should be encouraged.

Some strong points should be also emphasized. We did not identify publication bias through the funnel plot and egger's test in all analysis performed, perhaps due to this topic is recent and negative and positive results (without associations) were frequently published. Even as most of studies performed analysis of several SNPs, where some presented associations and another not. Included studies have used different allele as reference in the analysis. To include in the meta-analysis, we performed a standardization of reference allele as the major frequent allele present in most of the included studies. Yet, some genes are poorly investigated while others are better studied, which reinforces the need for conducting further studies. Moreover, was performed all analysis considering allelic and genotype (heterozygote and homozygote), which provide robustness to ours results. Several control quality filters were performed in the analysis. For the gene-pooled approach, we excluded SNPs in linkage disequilibrium to provide better quality of results. An elevate number of SNPs were excluded, which could be a source of bias for our results. Moreover, palindromic SNPs were also excluded in meta-analysis.

However, a high amount of studies was included and evaluated in the meta-analysis shown important observations concern this recent topic although it is important that, to support presents results, further studies are need with ethnic groups control, including representative samples, with power calculations, in different populations, with longitudinal designs, wide populations and use of combinate datasets, stratifying by dentition types. The relation of gene candidate/SNPs and dental caries is only one of diverse approach possible to genetic research. Epigenetic, interactions of genetic and environmental factors as well as genome wide association studies should be explored to complement the suggestive evidences.

Conclusion

The results showed that Single Nucleotide Polymorphisms related to immune response genes are linked to phenotype caries experience. The meta-analysis showed that the genes *MBL2* and *MUC5B* have an important role on dental caries, confirming the positive influence of this genes in caries and help us to explain the differences in dental caries risk among the individuals. Studies with elevate report quality and high methodological approach should be performed to support and confirm the presents results.

Compliance with Ethical Standards:

Conflict of Interest: Luiz Alexandre Chisini declares that he has no conflict of interest. Mariana Gonzales Cademartori declares that she has no conflict of interest. Marcus Cristian Muniz Conde declares that he has no conflict of interest. Luciana Tovo-Rodrigues declares that she has no conflict of interest. Marcos Britto Correa declares that he has no conflict of interest

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Ethical approval: not required

Informed consent: not required

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Legends:

Figure 1: Prisma flow diagram

Figure 2. Funnel plot of meta-analysis included studies

Table 1. Critical Appraisal Checklist for observational studies (Joanna Briggs Institute) in the systematic review according to the 10-items

Table 2. Methodological scoring protocol based on quality assessment for genetic studies.

Table 3. Description of single nucleotide polymorphism investigated in the present systematic review according genes*

Table 4. Description of studies with SNPs in Linkage Disequilibrium and respective D' value.

Table 5. Summarization results according gene and polymorphism in the studies by included study

Table 6. Summarization of SNP meta-analysis results according by allelic, homozygote and heterozygote analysis; Analysis were also stratified according gene.

Table S1. Search strategy

Table S2 Supplementary material S2. Main characteristics of studies included in this systematic review

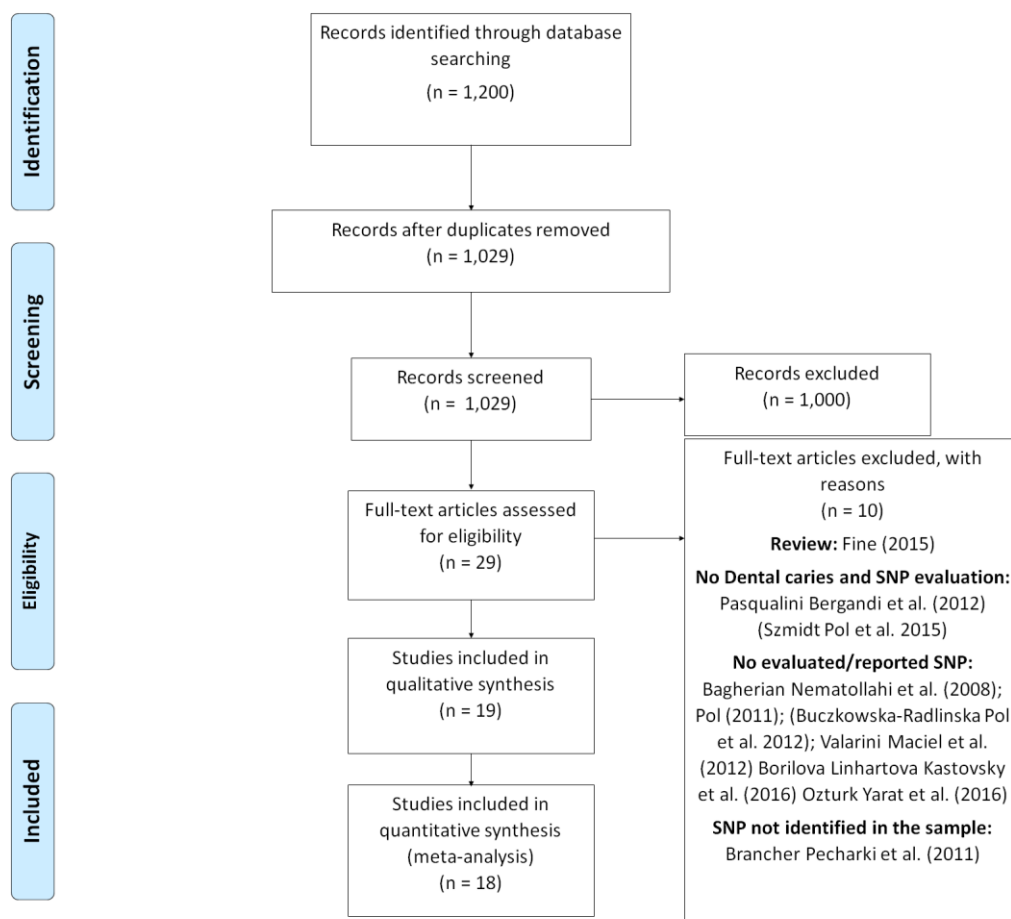
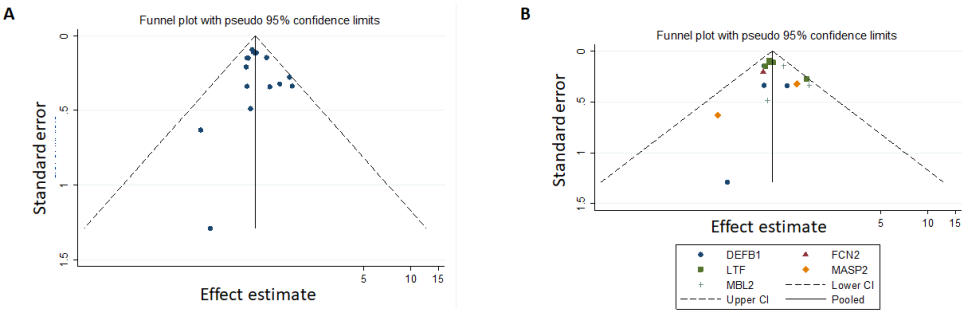
Figure 1: Prisma flow diagram

Figure 2. Funnel plot of meta-analysis included studies

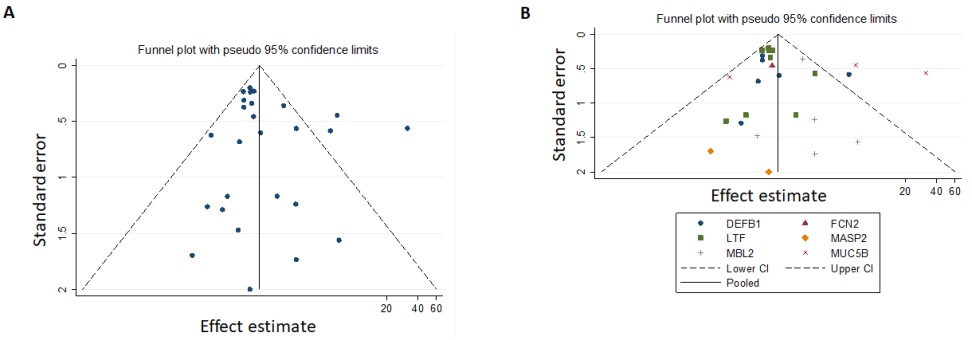
Allelic:

Egger's test: $p=0.554$



Homozygote:

Egger's test: $p=0.299$



Heterozygote:

Egger's test: $p=0.200$

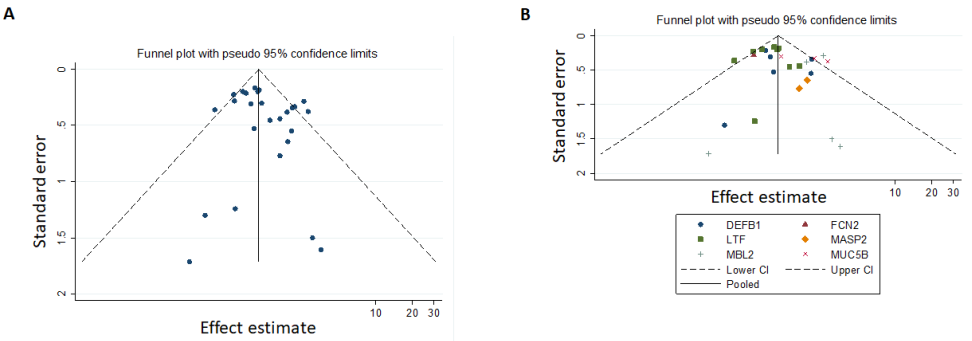


Table 1. Critical Appraisal Checklist for observational studies (Joanna Briggs Institute) in the systematic review according to the 10-ite

Study, year	NIH Criteria										Final score
	1	2	3	4	5	6	7	8	9	10	
Pehlivan, et al. [33]	-	-	-	-	-	/	/	-	-	-	Low (0)
Azevedo, et al. [34]	-	-	-	-	-	+	+	-	-	-	Low (2)
Ozturk, et al. [35]	-	-	-	-	-	+	+	+	/	-	Low (3)
Olszowski, et al. [36]	-	-	+	-	-	+	+	+	-	-	Low (3)
Yang, et al. [37]	-	-	-	-	-	+	+	-	-	-	Low (2)
Krasone, et al. [38]	-	+	-	+	-	+	+	+	-	-	Medium (5)
Volckova, et al. [39]	+	+	+	+	+	+	+	+	/	-	High (8)
Abbasoglu, et al. [40]	-	-	-	+	-	+	+	+	-	-	Medium (4)
Doetzer, et al. [41]	+	+	+	+	+	+	+	+	-	-	High (8)
Navarra, et al. [15]	+	+	/	+	+	+	+	+	+	-	High (8)
Alyousef, et al. [14]	-	-	+	-	/	+	+	-	-	-	Low (3)
Lips, et al. [42]	+	+	/	+	/	+	+	-	-	-	Medium (5)
Olszowski, et al. [43]	/	/	-	+	+	+	+	-	-	-	Medium (4)
Wang, et al. [44]	-	-	+	-	-	+	+	+	-	-	Medium (4)
Cavallari, et al. [45]	+	+	+	+	/	+	+	+	/	-	High (7)
de Oliveira, et al. [46]	-	+	-	+	+	+	+	-	-	-	Medium (5)
Mokhtari, et al. [47]	-	/	/	-	/	+	+	-	/	/	Low (2)
Shimomura-Kuroki, et al. [48]	-	-	/	-	-	+	+	-	/	/	Low (2)

Table 2. Methodological scoring protocol based on quality assessment for genetic studies.

[illegible]

Wang, et al. [44]	1	1	1	1	1	0	1	1	0	0	7	Medium
Cavallari, et al. [45]	1	1	1	1	1	0	1	1	0	0	7	Medium
de Oliveira, et al. [46]	1	1	1	1	1	0	0	1	0	1	7	Medium
Mokhtari, et al. [47]	0	0	0	1	1	0	0	0	0	0	2	Low
Shimomura-Kuroki, et al. [48]	0	0	0	1	1	0	0	0	0	1	3	Low
Wang, et al. [49]	0	1	0	1	1	0	1	0	0	0	4	Low

*For the quantification of criteria: «1» means present, and «0» absent

Table 3. Description of single nucleotide polymorphism investigated in the present systematic review according genes*

Allele Frequencies by populations (%) *												
<i>Gene</i>	<i>Polymorphism</i>	Chromosom ic position	Variation	Biotype / impact functional	African	American	East Asian	Europe	South Asia	All	allele Effect used	Ancestral allele
MBL2	rs1800450 G/A	10:54531235	Missense	Protein Coding	C:99% T:1%	C:78% T:22%	C:85% T:15%	C:86% T:14%	C:85% T:15%	C:88% T:12%	G/A	C
	rs7096206 G/C	10:54531685	Regulatory region	Regulatory	G:15% C:85%	G:13% C:87%	G:19% C:81%	G:22% C:78%	G:28% C:72%	G:20% C:80%	G/C	A
	rs11003125 C/G	10:54532014	Regulatory region	TF binding site	G:91% C:9%	G:60% C:40%	G:55% C:45%	G:61% C:39%	G:69% C:31%	G:69% C:31%	C/G	G
LTF	rs1126478 A/G	3:46501213	Missense	Protein Coding	T:4% C:96%	T:51% C:49%	T:35% C:65%	T:65% C:35%	T:47% C:53%	T:37% C:63%	A/G	C
	rs1126477 G/A	3:46501268	Missense	Protein Coding	C:23% T:77%	C:58% T:42%	C:58% T:42%	C:74% C:26%	C:63% T:26%	C:63% T:37%	G/A	T
	rs2269436 A/G	3:46487253	Intron	Protein Coding	A:82% G:18%	A:93% G:7%	A:87% G:13%	A:97% G:3%	A:89% G:11%	A:89% G:11%	A/G	A

	rs743658 A/G	3:46488488	Intron	Protein Coding	G:82% A:18%	G:93% A:7%	G:87% A:13%	G:97% A:3%	G:89% A:11%	G:89% A:11%	A/G	G
	rs4547741 C/T	3:46500458	Intron	Protein Coding	C:99% T:1%	C:96% T:4%	C:94% T:6%	C:91% T:9%	C:84% T:16%	C:93% T:7%	C/T	C
	rs6441989 A/G	3:46474899	TF binding site	Regulatory region	A:44% G:56%	A:35% G:65%	A:29% G:71%	A:52% G:48%	A:54% G:46%	A:43% G:57%	A/G	G
	rs2073495 C/G	3:46480958	Missense	Protein Coding	C:94% G:6%	C:65% G:35%	C:63% G:37%	C:68% G:32%	C:61% A:1% G:38%	C:72% A:0% G:28%	C/G	C
	rs11716497 A/G	3:46503498	Intron	Protein Coding	A:21% G:79%	A:54% G:46%	A:52% G:48%	A:65% G:35%	A:47% G:53%	A:46% G:54%	A/G	G
MASP2	rs72550870 A/G	1:11106666	Missense	Protein Coding	T:100% C:0%	T:98% C:2%	T:100%	T:96% C:4%	T:100% % C:0%	T:99% C:1%	A/G	T
DEFB1	rs11362 C/T	8:6735399	5 prime UTR	Protein Coding	C: 70% T:30%	C:56% T:44%	C:57% T:43%	C:56% T:44%	C:58% T:42%	C:60% T:40%	G/A	C
	rs1800972 C/G	8:6735423	5 prime UTR	Protein Coding	C:5% G:95%	C:26% G:74%	C:10% G:90%	C:20% G:80%	C:15% G:85%	C:14% G:86%	C/G	G
	rs1799946 C/T	8:6735431	5 prime UTR	Protein Coding	C:41% T:59%	C:70% T:30%	C:53% T:47%	C:64% T:36%	C:57% T:43%	C:55% T:45%	C/T	C
FCN2	rs17514136 A/G	9:137772664	5 prime UTR	Protein Coding	A:79% G:21%	A:77% G:23%	A:93% G:7%	A:74% G:26%	A:81% G:19%	A:81% G:19%	A/G	A
	rs3124953 G/A	9:137772066	Intergenic variant	NA	A:2% G:98%	A:22% G:78%	A:2% G:98%	A:21% G:79%	A:11% G:89%	A:10% G:90%	G/A	G

<i>MUC5B</i>	rs2735733 C/T	11:1261640	Intron	Protein Coding	C:69% T:31%	C:45% T:55%	C:34% T:66%	C:57% T:43%	C:57% T:43%	C:54% T:46%	C/T	C
	rs2249073 C/T	11:1273833	Intron	Protein Coding	T:51% C:49%	T:40% C:60%	T:34% C:66%	T:50% C:50%	T:48% C:52%	T:45% C:55%	C/T	C
	rs2672812 A/G	11:1249372	Intron	Protein Coding	G:56% A:44%	G:40% A:60%	G:36% A:64%	G:50% A:50%	G:48% A:52%	G:47% A:53%	A/G	A
	rs2672785 A/G	11:1246941	Missense	Protein Coding	A:66% G:34%	A:72% G:28%	A:56% G:44%	A:76% G:24%	A:68% G:32%	A:67% G:33%	A/G	G
	rs2857476 C/T	11:1281134	Intron	Protein Coding	T:42% C:58%	T:39% C:61%	T:33% C:67%	T:50% C:50%	T:47% C:53%	T:42% C:58%	C/T	T

* Based on Human (GRCh37.p13), available on: http://grch37.ensembl.org/Homo_sapiens. NA: not available

Table 4. Description of studies with SNPs in Linkage Disequilibrium and respective D' value.

Study	Gene	SNPs		D'
Alyousef et al. [2017]	BML2	rs7096206 C/G *	rs11003125 C/G	0.90
Cavallari et al. [2018]	MUC5B	rs2249073 C/T	rs2857476 C/T *	0.37
Cavallari et al. [2018]	MUC5B	rs2249073 C/T	rs2672812 A/G *	0.30
Doetzer et al. [2015]	LTF	rs6441989 A/G	rs2073495 C/G *	0.32
Krasone et al. [2014]	DEFB1	rs11362 C/T *	rs1800972 C/G	0.99
Lips et al. [2017]	DEFB1	rs11362 C/T	rs1799946 C/T *	0.99
Olszowski et al. [2012]	MBL2	rs1800450 G/A *	rs7096206 G/C	0.65
Olszowski et al. [2017]	FCN2	rs3124953 G/A	rs17514136 A/G	1.00
Ozturk et al. [2010]	DEFB1	rs11362 G/A	rs1799946 G/A *	0.99
Ozturk et al. [2010]	DEFB1	rs11362 G/A	rs1800972 C/G *	0.99
Wang and Qin [2018]	LTF	rs1126477 G/A	rs1126478 A/G *	1.00
de Oliveira et al. [2018]	DEFB1	rs1799946 C/T	rs11362 C/T *	0.99

* Excluded SNP in gene analysis due to Linkage Disequilibrium

	rs4547741 C/T rs6441989 A/G rs2073495 C/G rs11716497 A/G						NA	NA NA NA NA							
MASP2	rs72550870 A/G			NA											
DEFB1	rs11362 G/A rs11362 C/T rs1800972 C/G rs1799946 G/A rs1799946 C/T rs1047031 G/A rs1800971 A/G		+# + ^{#a} -#		-# NA		NA NA	-# NA + NA NA		NA NA			+ ^{#b} NA		
FCN2	rs17514136 A/G rs3124953 G/A rs3124952 A/G									NA NA NA					
MUC5B	rs2735733 C/T rs2249073 C/T rs2672812 A/G rs2672785 A/G rs2857476 C/T											+ + NA NA +			

Legends: - Protector factor; + Risk factor; # Statistically associated; NA: not associated, direction of effect not showed; ^a only considering logistic regression not

stratified between heterozygote and homozygote; ^b associated only in Ribeirão Preto cohort;

Table 6. Summarization of SNP meta-analysis results according by allelic, homozygote and heterozygote analysis; Analysis were also stratified according gene.

Gene	Polymorphism	Allelic		Genotype			
		N	Pooled Odds Ratio (95%CI)	Homozygote		Heterozygote	
				N	Pooled Odds Ratio (95%CI)	N	Pooled Odds Ratio (95%CI)
MBL2	rs1800450 G/A ^b	2	1.46(0.80 – 2.50)	4	0.90 (0.22 – 3.64)	4	1.48 (0.89 – 2.47)
	rs7096206 G/C		-	2	2.15 (0.24 – 19.04)	2	3.23 (0.38 – 27.75)
	rs7096206 C/G ^{a b}	1	1.25 (0.73- 2.15)	1	2.48 (0.11 – 54.73)	1	1.10 (0.31 – 3.92)
	rs11003125 C/G	1	1.19 (0.90 – 1.58)	1	2.05 (1.01 – 4.15) #	1	2.54 (1.45 – 4.45) #
	Overall MBL2		1.24 (1.00 – 1.56) #		1.76 (0.97 – 3.23)		1.92 (1.33 – 2.79) #
	Overall MBL2 ^{LD}		1.24 (0.96 – 1.59)		2.12 (1.12 – 3.99) #		2.22 (1.44 – 3.44) #
LTF	rs1126478 A/G ^b	4	0.97 (0.82 – 1.14)	4	1.02 (0.77 – 1.37)	4	1.00 (0.80 – 1.26)
	rs1126477 G/A	1	1.00 (0.80 – 1.26)	1	0.98 (0.61 – 1.57)	1	1.09 (0.76 – 1.57)
	rs2269436 A/G		-	1	1.77 (0.18 – 17.45)	1	1.34 (0.55 – 3.26)
	rs743658 A/G		-	1	0.59 (0.06 – 5.85)	1	0.69 (0.06 – 7.87)
	rs4547741 C/T		-	1	0.38 (0.03 – 4.50)	1	0.47 (0.23 – 0.96)
	rs6441989 A/G	1	0.98 (0.79 – 1.21)	1	0.84 (0.53 – 1.33)	1	0.67 (0.43 – 1.04)
	rs2073495 C/G ^b	1	0.92 (0.74 – 1.15)	1	0.89 (0.56 – 1.41)	1	0.83 (0.60 – 1.15)
	rs11716497 A/G	1	1.02 (0.82- 1.27)	1	1.06 (0.68 – 1.66)	1	1.00 (0.72 – 1.38)
	Overall LFT		0.97 (0.89 -1.05)		0.97 (0.81 – 1.15)		0.93 (0.80 – 1.07)

	Overall LFT ^{LD}		0.99 (0.90 – 1.10)		0.99 (0.81 – 1.22)		0.92 (0.77 – 1.11)
MASP2	rs72550870 A/G	2	0.92 (0.30 – 2.81)	2	0.46 (0.04 – 5.82)	2	1.77 (0.67 – 4.66)
	Overall MASP2		0.92 (0.30 – 2.81)		0.46 (0.04 – 5.82)		1.77 (0.67 – 4.66)
DEFB1	rs11362 G/A ^b	4	0.88 (0.70 – 1.11)	1	5.76 (1.83 – 18.14) #	1	2.04 (1.04 – 4.01) #
	rs11362 C/T ^b		-	5	0.92 (0.615 – 1.38)	5	0.92 (0.50 – 1.69)
	rs1800972 C/G ^b	1	0.52 (0.04 – 6.5)	1	0.53 (0.04 – 6.62)	1	0.39 (0.03 – 4.99)
	rs1799946 G/A ^b		-	1	0.39 (0.15 – 1.04)	1	0.34 (0.16 – 0.72) #
	rs1799946 C/T ^b	3	1.15 (0.9 – 1.45)	3	1.31 (0.83 – 2.07)	3	1.11(0.75 – 1.64)
	Overall DEFB1		1.00 (0.85 – 1.18)		1.08 (0.72 – 1.61)		0.96 (0.65 – 1.42)
	Overall DEFB1 ^b		0.93 (0.73 – 1.19)		1.17 (0.64 – 2.14)		1.13 (0.78 – 1.62)
FCN2	rs17514136 A/G	1	0.88 (0.57 – 1.32)	1	1.05 (0.43 – 2.57)	1	0.68 (0.39 – 1.18)
	rs3124953 G/A ^b	2	1.07 (0.81 – 1.42)	2	1.26 (0.68 – 2.33)	2	0.91 (0.60 – 1.37)
	Overall FCN2		-		1.19 (0.71 – 1.97)		0.82 (0.59 – 1.14)
	Overall FCN2 ^{LD}		0.88 (0.59 – 1.32)		1.05 (0.43 – 2.57)		0.68 (0.39 – 1.18)
MUC5B	rs2735733 C/T		-	1	6.69 (2.79 – 16.03) #	1	2.14 (1.11 -4.12) #
	rs2249073 C/T		-	1	31.56 (10.52 – 94.66) #	1	2.76 (1.32 – 5.78) #
	rs2672812 A/G ^b		-	1	1.02 (0.38 – 2.77)	1	0.82 (0.42 – 1.61)
	rs2672785 A/G		-	1	0.41 (0.12 – 1.39)	1	1.14 (0.62 – 2.07)
	rs2857476 C/T ^b		-	1	21.43 (6.59 – 69.70) #	1	2.77 (1.39 – 5.52) #
	Overall MUC5B		-		4.54 (0.96 – 21.46)		1.70 (1.05 – 2.74) #
	Overall MUC5B ^{LD}		-		4.51 (0.47 – 42.92)		1.83 (1.08 – 3.09) #
	Overall		1.00 (0.94 – 1.07)		1.32 (1.02 – 1.73) #		1.10 (0.93 – 1.29)
	Overall ^{LD}		1.01 (0.93 – 1.09)		1.42 (1.01 – 1.99) #		1.16 (0.96 – 1.39)

N: represents the populations investigated; **#:** statistic difference; ^a Excluded of gene analysis; ^b Polymorphisms in linkage disequilibrium excluded of analysis; Overall LD result excluding SNPs in linkage disequilibrium

Table S1. Search strategy

Search syntax	
PubMed	#1 ("Dental Decay" OR "Caries, Dental" Or "Decay, Dental" OR "Cariou Dentin" OR "Cariou Dentins" OR "Dentin, Cariou" OR "Dentins, Cariou" OR "Dental White Spot" OR "White Spots, Dental" OR "White Spots" OR "Spot, White" OR "Spots, White" OR "White Spot" OR "Dental White Spots" OR "White Spot, Dental" OR "Susceptibility, Dental Caries" OR "Caries Susceptibility, Dental" OR "Caries Resistance, Dental" OR "Resistance, Dental Caries" OR "Dental Caries Resistance")
	#2 ("Polymorphisms, Genetic" OR "Genetic Polymorphisms" OR "Genetic Polymorphism" OR "Polymorphism" OR "Polymorphisms" OR "Nucleotide Polymorphism, Single" OR "Nucleotide Polymorphisms, Single" OR "Polymorphisms, Single Nucleotide" OR "Single Nucleotide Polymorphisms" OR "SNPs" OR "Single Nucleotide Polymorphism")

* Search combination: #1 AND #2

Table S2 Supplementary material S2. Main characteristics of studies included in this systematic review

Author , year	-Country -Study design -Sample (% Males)	-Age (permanent/ deciduous) -Ethnicity -Statistic power (yes/no)	-Evaluation -categorization	dental caries	Analytical Approach	Adjustment variables
Pehlivan Koturoglu et al. (2005)	-Turkey	-9.78y	mean	-NR	Chi-square test	-
	-Case control	(deciduous)	-NR			
	-82 (NR %)	-NR				
		-No				
	Crude Analysis	MBL rs1800450 A/G: Genotype AG compared to AA OR 0.84 (0.29 – 2.44); Genotype GG compared to AA OR 2.82 (0.11 – 71.84); Allele G compared to A OR 1.07 (0.41 – 2.78) MBL rs1800450 G/A: Genotype AG compared to GG OR 0.29 (0.01 – 8.3); Genotype AA compared to GG OR 2.71 (0.01 – 9.00); Allele A compared to G OR 0.94 (0.36 – 2.44)				
	Adjusted Analysis	-				
Azevedo Pecharki et al. (2010)	-Brazil	-NR (permanent)	-DMFT	(white lesions were	chi-square test	-
	-Cohort	-NR	considered)			
	-110 (NR %)	-No	-DMFT=0 Vs DMFT≥1			
	Crude Analysis	LTF rs1126478 A/G: Genotype AG compared to AA OR 1.62 (0.68 – 3.84); Genotype GG compared to AA OR 2.71 (0.90 – 8.23); Allele G compared to A OR 1.67 (0.97 – 2.87)				
	Adjusted Analysis	-				

-EUA	-17 to 84y	-DMFT and DMFS	Multiple logistic regression models	Age, sex, race,
-Cohort	(permanent)	-Low caries (DMFT<14) Vs High		smoking status, and
-296 (NR %)	-Caucasian 68%, African Americans 27%	Caries (DMFT≥14) in individuals below 30y. Low caries (DMFT<9) Vs High Caries (DMFT≥9) in individuals		the presence of periodontal disease
	-No	equal or above 30y		

Considering DMFT

DEFB1 rs11362 G/A: Genotype AG compared to GG OR 2.04 (1.04 – 4.01); Genotype AA compared to GG OR 5.76 (1.83 – 18.14);

DEFB1 rs1800972 C/G: Genotype CG compared to CC OR 0.75 (0.39 – 1.45); Genotype GG compared to CC OR 2.43 (0.29 – 20.50);

DEFB1 rs1799946 G/A: Genotype AG compared to GG OR 0.34 (0.16 – 0.71); Genotype AA compared to GG OR 0.39 (0.15 – 1.06);

Considering DMFS

Crude Analysis # **DEFB1 rs11362 G/A:** Genotype AG compared to GG OR 1.23 (0.65 – 2.33); Genotype AA compared to GG OR 3.89 (1.58 – 9.53);

DEFB1 rs1800972 C/G: Genotype CG compared to CC OR 0.58 (0.32 – 1.07); Genotype GG compared to CC OR 0.47 (0.11 – 2.04);

DEFB1 rs1799946 G/A: Genotype AG compared to GG OR 0.69 (0.38 – 1.28); Genotype AA compared to GG OR 0.84 (0.36 – 1.99);

The authors reported that performed some variations in cut-off definitions found a suggestion that variations do not affect the findings

**Ozturk
Famili et al.
(2010)**

Logistic regression not stratified between heterozygote and homozygote

Considering DMFT

DEFB1 rs11362 G/A: Genotype OR 5.40 (1.58 – 18.44)

DEFB1 rs1800972 C/G: Genotype OR 3.44 (0.35 – 33.90)

DEFB1 rs1799946 G/A: Genotype OR 0.32 (0.14 – 0.72)

Considering DMFS

DEFB1 rs11362 G/A: Genotype OR 5.28 (1.99 – 14.05);

DEFB1 rs1800972 C/G: Genotype OR 2.00 (1.04 – 3.94);

DEFB1 rs1799946 G/A: Genotype AG compared to GG OR 0.73 (0.38 – 1.39);

Haplotype Analysis: confirm the observations of SNPs; haplotype rs11362 (G), rs1800972 (C) and rs1799946 (A) show a increase of dental caries experience OR 2.19 (1.10 – 4.35)

Adjusted Analysis

-Poland	-5	and	13	-DMFT	and	dmft	Fisher's exact test	NP
-Cohort	(deciduous	and	-higher	experience	DMFT/dmft	≥3	Stratified by age (5y and 13y)	
-199 (41%)	permanent)		and lower	experience	DMFT/dmft	<3		
	-NR							
	-Yes							

(Olszowski

Adler et al.

2012)

Results of 5-year-old children

MBL2 rs7096206 G/C: Genotype GC compared to GG OR 3.00 (0.16 – 57.37); Genotype CC compared to GG OR 0.75 (0.04 – 12.70);

Crude Analysis

MBL2 rs1800450 A/G: Genotype AG compared to AA OR 2.11 (0.04 – 124.53); Genotype GG compared to AA OR 0.93 (0.02 – 48.68);

MBL2 rs1800450 G/A: Genotype AG compared to GG OR 2.41 (0.67 – 8.72); Genotype AA compared to GG OR 1.07 (0.02 – 55.75);

MASP2 rs72550870 A/G: Genotype AG compared to AA OR 1.62 (0.36 – 7.34); Genotype GG compared to AA OR 0.97 (0.02 – 50.36); Allele G compared to A OR 0.45 (0.13 – 1.54)

Results of 13-year-old children

MBL2 rs7096206 G/C: Genotype GC compared to GG OR 3.52 (0.15 – 81.93); Genotype CC compared to GG OR 6.97 (0.33 – 149.71);

MBL2 rs1800450 A/G: Genotype AG compared to AA OR 4.92 (0.49 – 49.61); Genotype GG compared to AA OR 4.97 (0.53 – 46.62);

MBL2 rs1800450 G/A: Genotype AG compared to GG OR 0.99 (0.42 – 2.35); Genotype AA compared to GG OR 0.20 (0.02 – 1.89);

MASP2 rs72550870 A/G: Genotype AG compared to AA OR 1.88 (0.53 – 6.66); Genotype GG compared to AA OR 0.27 (0.01 – 7.72); Allele G compared to A OR 1.45 (0.82 – 2.90)

Adjusted Analysis		-				
Yang Wang et al. (2013)	-China	-1	to	5y	-dmft (including white-spot lesions)	chi-square tests
	-Case control	(deciduous)			-dmft=0 Vs dmft≥1	
	-130 (50 %)	-NR				
		-No				
	Crude Analysis	MBL2 rs1800450 G/A: Genotype AG compared to GG OR 1.85 (0.89 – 3.98); Genotype AA compared to GG OR 2.68 (0.24 – 30.70); Allele G compared to A OR 1.74 (0.90 – 3.36)				
Adjusted Analysis						
Krasone Lace et al. (2014)	- Latvia	-2	to	12y	-DMFT/dmft and DMFS/dmfs	Chi-square and odds ratio
	-Cohort	(permanent	and		-categorization was performed	
	-69 (66.7 %)	deciduous).			according to age. 2 to 3 years: dmft≤2	

	-No	time of first toothbrushing
Crude Analysis	DEFB1 rs11362 C/T: Genotype CT compared to CC OR 0.98 (0.56 – 1.74); Genotype TT compared to CC OR 0.97 (0.49 – 1.92);	
	DEFB1 rs1800972 C/G: Genotype CG compared to CC OR 1.89 (0.39 – 9.22); Genotype GG compared to CC OR 1.35 (0.29 – 6.18);	
	LTF rs2269436 A/G: Genotype AG compared to AA OR 1.12 (0.50 – 2.50); Genotype GG compared to AA OR 2.68 (0.27 – 26.20);	
	LTF rs743658 A/G: Genotype AG compared to AA OR 0.38 (0.03 – 4.24); Genotype GG compared to AA OR 0.37 (0.03 – 3.69);	
	LTF rs4547741 C/T: Genotype CT compared to CC OR 0.47 (0.23 – 0.95); Genotype TT compared to CC OR 0.38 (0.03 – 4.21);	
Adjusted Analysis	DEFB1 rs11362 C/T: Genotype CT compared to CC OR 0.93 (0.51 – 1.71); Genotype TT compared to CC OR 0.85 (0.41 – 1.77);	
	DEFB1 rs1800972 C/G: Genotype CG compared to CC OR 2.57 (0.45 – 14.70); Genotype GG compared to CC OR 1.39 (0.26 – 7.34);	
	LTF rs2269436 A/G: Genotype AG compared to AA OR 1.34 (0.55 – 3.26); Genotype GG compared to AA OR 1.77 (0.18 – 17.50);	
	LTF rs743658 A/G: Genotype AG compared to AA OR 0.69 (0.06 – 7.80); Genotype GG compared to AA OR 0.59 (0.06 – 5.89);	
	LTF rs4547741 C/T: Genotype CT compared to CC OR 0.44 (0.21 – 0.96); Genotype TT compared to CC OR 0.24 (0.02 – 2.79);	

	-Brazil	-12y (permanent)	-DMFT	Chi-square teste and Odds Ratio	-
	-Cohort	-NR	-DMFT=0 Vs DMFT≥1; was also		
	-677 (44.8 %)	-yes	tested different categorizations		
Doetzer Brancher et al. (2015)	Crude Analysis	LTF rs6441989 A/G: Genotype AG compared to AA OR 0.67 (0.43 – 1.04); Genotype GG compared to AA OR 0.84 (0.53 – 1.33); Allele G compared to A OR 0.98 (0.79 – 1.21); LTF rs2073495 C/G: Genotype CG compared to CC OR 0.83 (0.60 – 1.16); Genotype GG compared to CC OR 0.89 (0.57 – 1.42); Allele G compared to C OR 0.92 (0.74 – 1.15); LTF rs11716497 A/G: Genotype AG compared to AA OR 1.00 (0.72 – 1.38); Genotype GG compared to AA OR 1.06 (0.68 – 1.67); Allele G compared to A OR 1.02 (0.82 – 1.28);			
	Adjusted Analysis	-			
	-Italy	-18 to 65y	-DMFT; panoramic radiographic was	Linear mixed model regression analysis	Sex and age
	-Cohort	(permanent)	also used		
	-536 (44.4 %)	-NR	-No categorization was performed		
		-No			
Navarra Robino et al. (2016)	Crude Analysis	-			
		# DEFB1 rs1799946 G/A: Risk factor (β +0.820; p = 0.030); DEFB1 rs1800972 C/G: Effect not reported; Not associated;			
	Adjusted Analysis	# DEFB1 rs11362 G/A: Protective effect (β –1.014; p = 0.008); G/G homozygous individuals showed a higher DMFT index compared to both G/A heterozygous and A/A homozygous individuals. DEFB1 rs1047031 G/A: Effect not reported; Not associated; DEFB1 rs1800971 A/G: Effect not reported; Not associated;			
Alyousef	-Saudi Arabia	-5 to 13y	-DMFT/dmft	Chi-square test and T test	-

Borgio et al. (2017)	-Case-control -102 (NR %)	(permanent deciduous) -NR -Yes	and -NR			
	Crude Analysis	MBL2: rs7096206 C/G: Allele G compared to C OR 1.25 (0.73 – 2.15) # MBL2: rs11003125 C/G: Allele G compared to C OR 1.83(1.11 – 3.02)				
	Adjusted Analysis					
	-Brazil -Cohort -678 (48.8 %)	-2 to 6y (deciduous) -NR -No	-dmft -dmft=0 Vs dmft≥1	Chi-square test and logistic regression	Sweet ingestion, Presence of biofilm and age	
Lips Antunes et al. (2017)		DEFB1 rs11362 C/T: Genotype CT compared to CC OR 0.85 (0.56 – 1.30); Genotype TT compared to CC OR 0.85 (0.46 – 1.56); Allele T compared to C OR 0.89 (0.67 – 1.20); DEFB1 rs1799946 C/T: Genotype CT compared to CC OR 1.02 (0.65 – 1.62); Genotype TT compared to CC OR 1.44 (0.84 – 2.46); Allele T compared to C OR 1.19 (0.91 – 1.57);				
	Crude Analysis					
	Adjusted Analysis	-				
Olszowski Milona et al. (2017)	-Poland -Cohort -260 (55.4 %)	-15y (permanent) -NR -No	-DMFT -DMFT≤5 Vs DMFT>5	Chi-square teste	-	
	Crude Analysis	FCN2 rs17514136 A/G: Genotype AG compared to AA OR 0.68 (0.39 – 1.18); Genotype GG compared to AA OR 1.05 (0.43 – 2.57); Allele G compared to A OR 0.88 (0.58 – 1.31);				

FCN2 rs3124953 G/A: Genotype AG compared to GG OR 0.99 (0.57 – 1.73); Genotype AA compared to GG OR 1.22 (0.39 – 3.82); Allele A compared to G OR 1.04 (0.67 – 1.61);

FCN2 rs3124952 A/G: Genotype AG compared to AA OR 0.82 (0.45 – 1.50); Genotype GG compared to AA OR 1.27 (0.61 – 2.65); Allele G compared to A OR 1.09 (0.75 – 1.58);

Adjusted Analysis		-	
-China	-under 4y -dmft	Chi-square or Fisher's exact test and	-Diet, oral
-Case-Control	(deciduous)	binary logistic regression test	behavioral habits
-1005 (52.7 %)	-NR experience (dmft ≥ 1)		and application of
	-Yes		topical fluoride
Wang Qin et al. (2017)			
Crude Analysis		LTF rs1126478 A/G: Genotype AG compared to AA OR 1.06 (0.72 – 1.58); Genotype GG compared to AA OR 0.97 (0.65 – 1.43); Allele G compared to A OR 0.96 (0.80 – 1.15); LTF rs1126478 G/A: Genotype AG compared to GG OR 1.10 (0.85 – 1.44); Genotype AA compared to GG OR 1.04 (0.70 – 1.54); Allele A compared to G OR 1.04 (0.87 – 1.26);	
Adjusted Analysis		LTF rs1126478 G/A: Genotype AG compared to GG OR 1.11 (0.72 – 1.71); Genotype AA compared to GG OR 1.60 (0.79 – 1.43);	
-Brazil	-12 to 34 -ICDAS	Multivariate analysis (logistic	socioeconomic,
-Case-control	(permanent)	regression) and chi-square test	dietary and buccal
-200 (39 %)	-Caucasian 90.5%;		hygiene
	African-american		
	9.5%		
	-Yes		
Cavallari Salomao et al. (2018)			
Crude Analysis		# MUC5B rs2735733 C/T: Genotype CT compared to CC OR 2.14 (1.11 – 4.12); Genotype TT compared to CC OR 6.69	

(2.79 – 16.02); C dominance model (CC + CT vs TT) OR 0.23 (0.11 – 0.51); T dominance model (TT + CT vs CC) OR 2.98 (1.61 – 5.52);

MUC5B rs2249073 C/T: Genotype CT compared to CC OR 2.76 (1.32 – 5.78); Genotype TT compared to CC OR 31.56 (10.52 – 94.64); C dominance model (CC + CT vs TT) OR 0.09 (0.03 – 0.24); T dominance model (TT + CT vs CC) OR 5.42 (2.72 – 10.91);

MUC5B rs2672812 A/G: Genotype AG compared to AA OR 0.82 (0.42 – 1.61); Genotype GG compared to AA OR 1.02 (0.45 – 3.31); A dominance model (AA+ AG vs GG) OR 0.89 (0.44 – 1.69); G dominance model (GG+ AG vs AA) OR 0.88 (0.46 – 1.66);

MUC5B rs2672785 A/G: Genotype AG compared to AA OR 1.14 (0.63 – 2.07); Genotype GG compared to AA OR 0.41 (0.12 – 1.38); A dominance model (AA + AG vs GG) OR 2.58 (0.78 – 8.54). G dominance model (GG + AG vs AA) OR 0.97 (0.55 – 1.71);

MUC5B rs2857476 C/T: Genotype CT compared to CC OR 2.77 (1.39 – 5.51); Genotype TT compared to CC OR 21.43 (6.59 – 69.72); C dominance model (CC+ Ct vs TT) OR 0.09 (0.03 – 0.26); T dominance model (TT + CT vs CC) OR 4.26 (2.20 – 8.23);

Adjusted Analysis

-

de Oliveira Segato et al. (2018)	-Brazil	-10 to 12 and 6 to	-DMFT/dmft	Chi-square or Fisher's exact tests and	-
	-Cohort (2 different populations: Manaus Cohort and Ribeirão Preto Cohort)	12y (permanent and deciduous)	-DMFT/dmft=0 Vs DMFT/dmft≥1	odds ratio	
	-312 (48.7 %)	-NR			
		-No			

Manaus Cohort:

Crude Analysis

DEFB1 rs11362 C/T: Genotype CT compared to CC OR 0.87 (0.30 – 2.51); Genotype TT compared to CC OR 1.30 (0.32 – 5.24); Allele T compared to C OR 1.10 (0.55 – 2.19);

DEFB1 rs1799946 C/T: Genotype CT compared to CC OR 2.02 (0.69 – 5.94); Genotype TT compared to CC OR 1.23 (0.38 – 4.00); Allele T compared to C OR 1.25 (0.64 – 2.44);

Ribeirão Preto Cohort:

DEFB1 rs11362 C/T: Genotype CT compared to CC OR 3.57 (1.29 – 9.86); Genotype TT compared to CC OR 2.23 (0.58 – 8.55); Allele T compared to C OR 0.89 (0.46 – 1.73);

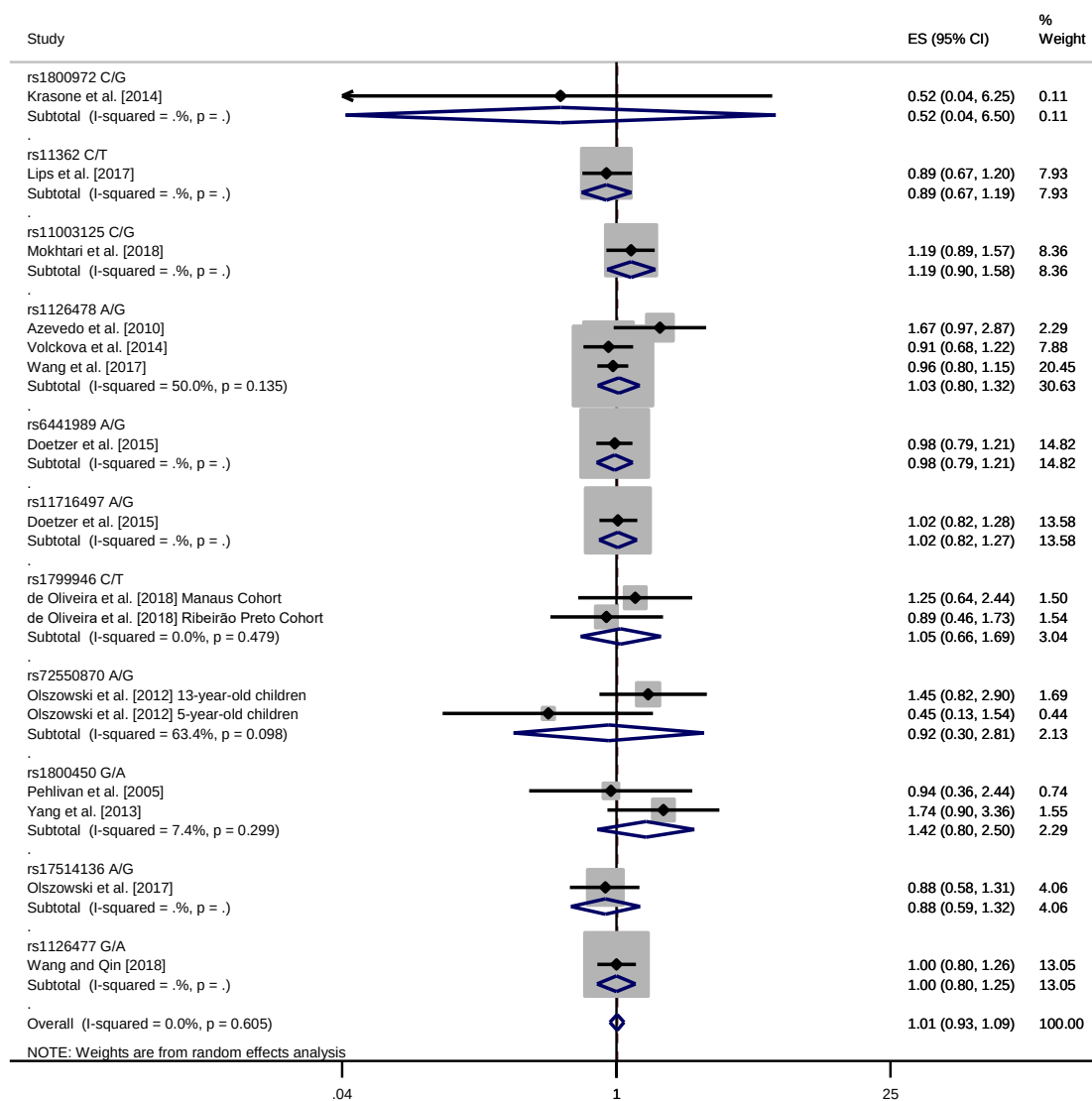
DEFB1 rs1799946 C/T: Genotype CT compared to CC OR 0.99 (0.35 – 2.79); Genotype TT compared to CC OR 0.77 (0.20 – 2.90); Allele T compared to C OR 0.89 (0.46 – 1.73);

Adjusted Analysis			-		
Mokhtari Koohpeima et al. (2018)	-Iran	-20 to 34y	-DMFT	Odds ratio and logistic regression	-
	-Case-control	(permanent)	-DMFT $\geq$ 6 Vs DMFT $>$ 6		
	-404 (37.8 %)	-NR			
		-No			
Crude Analysis			# MBL2 rs11003125 C/G: Genotype CG compared to CC OR 2.54 (1.36 – 4.17); Genotype GG compared to CC OR 2.05 (1.01 – 4.14); Genotype CG+GG compared to CC OR 2.40 (1.3 – 4.40); Allele G compared to C OR 1.19 (0.89 – 1.57)		
Adjusted Analysis					
Shimomura- Kuroki Nashida et al. (2018)	-Japan	-3 to 11y	-DMFT/dmft	-Regression analysis	-
	-Case Control	(permanent and	-DMFT/dmft=0 Vs DMFT/dmft $\geq$ 1		
	-81 (49,4 %)	deciduous)			
		-NR			
			-No		

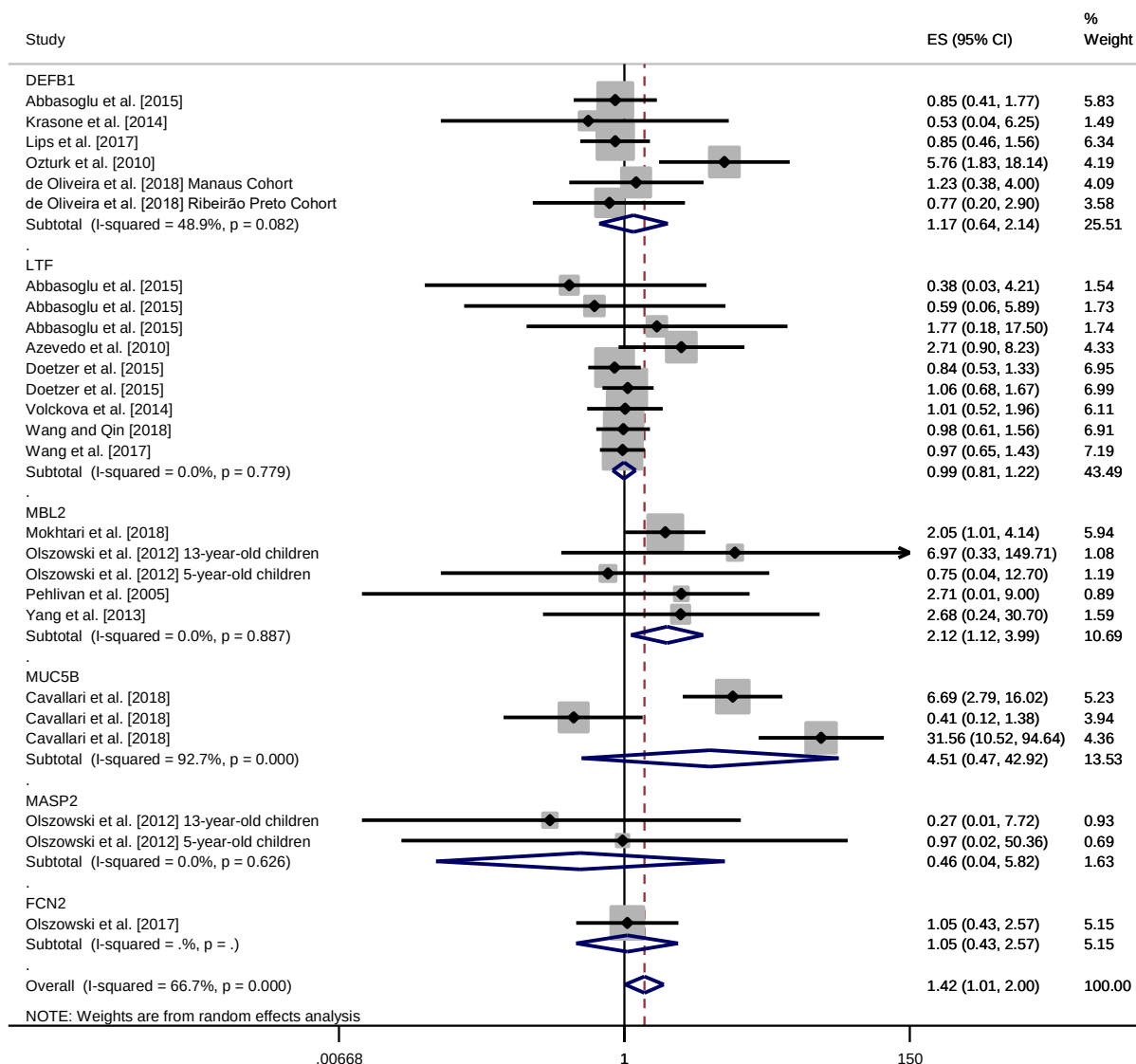
	Crude Analysis	MBL2 rs7096206 C/G: Genotype CG compared to CC OR 1.10 (0.31 – 3.94); Genotype GG compared to CC OR 2.48 (0.11 – 53.57); In the multivariate analysis, allele C was associated with decrease of caries (β -0.173; p =0.042)					
	Adjusted Analysis						
Wang and Qin (2018)	-China	-2 to 4y	-dmft	and	white-spot	Chi-square test	-
	-Cohort	(deciduous)	-No caries (dmft=0 and no white-				
	-910 (48.9 %)	-NR	spot), moderate caries ($8 \leq \text{dmft} \leq 12$)				
		-Yes	and severe caries ($13 \leq \text{dmft} \leq 20$)				
	<u>Caries Free vs Moderate caries:</u>						
	LTF rs1126477 G/A: Genotype AG compared to GG OR 1.09 (0.76 – 1.57); Genotype AA compared to GG OR 0.98 (0.61 – 1.56); Allele A compared to G OR 1.00 (0.80 – 1.26);						
	LTF rs1126478 A/G: Genotype AG compared to AA OR 1.03 (0.61 – 1.74); Genotype GG compared to AA OR 1.06 (0.63 – 1.78); Allele G compared to A OR 1.03 (0.81 – 1.32);						
	<u>Moderate caries vs Severe caries:</u>						
	LTF rs1126477 G/A: Genotype AG compared to GG OR 0.89 (0.60 – 1.32); Genotype AA compared to GG OR 0.88 (0.53 – 1.46); Allele A compared to G OR 0.93 (0.72 – 1.19);						
	LTF rs1126478 A/G: Genotype AG compared to AA OR 1.09 (0.62 – 1.91); Genotype GG compared to AA OR 0.85 (0.48 – 1.48); Allele G compared to A OR 0.82 (0.63 – 1.07);						
	Crude Analysis	<u>Caries Free vs Severe caries:</u>					
		LTF rs1126477 G/A: Genotype AG compared to GG OR 0.97 (0.69 – 1.36); Genotype AA compared to GG OR 0.86 (0.55 – 1.34); Allele A compared to G OR 0.93 (0.75 – 1.16);					
		LTF rs1126478 A/G: Genotype AG compared to AA OR 1.12 (0.69 – 1.82); Genotype GG compared to AA OR 0.90 (0.55 – 1.46); Allele G compared to A OR 0.89 (0.72 – 1.13);					
	Adjusted Analysis	-					

NR: not reported; # Statistical association; CI: Confidence Interval; OR: Odds Ratio; PR: Prevalence Ratio; dmft (decayed, missing teeth due to caries, filled teeth); WSL: white spot lesions; ICDAS: International Decay Detection and Assessment System; CA: Crude association; AA: adjusted association; All measure effects show are ODDS Ratio. Different measures are reported; SNP: Single Nucleotide Polymorphism.

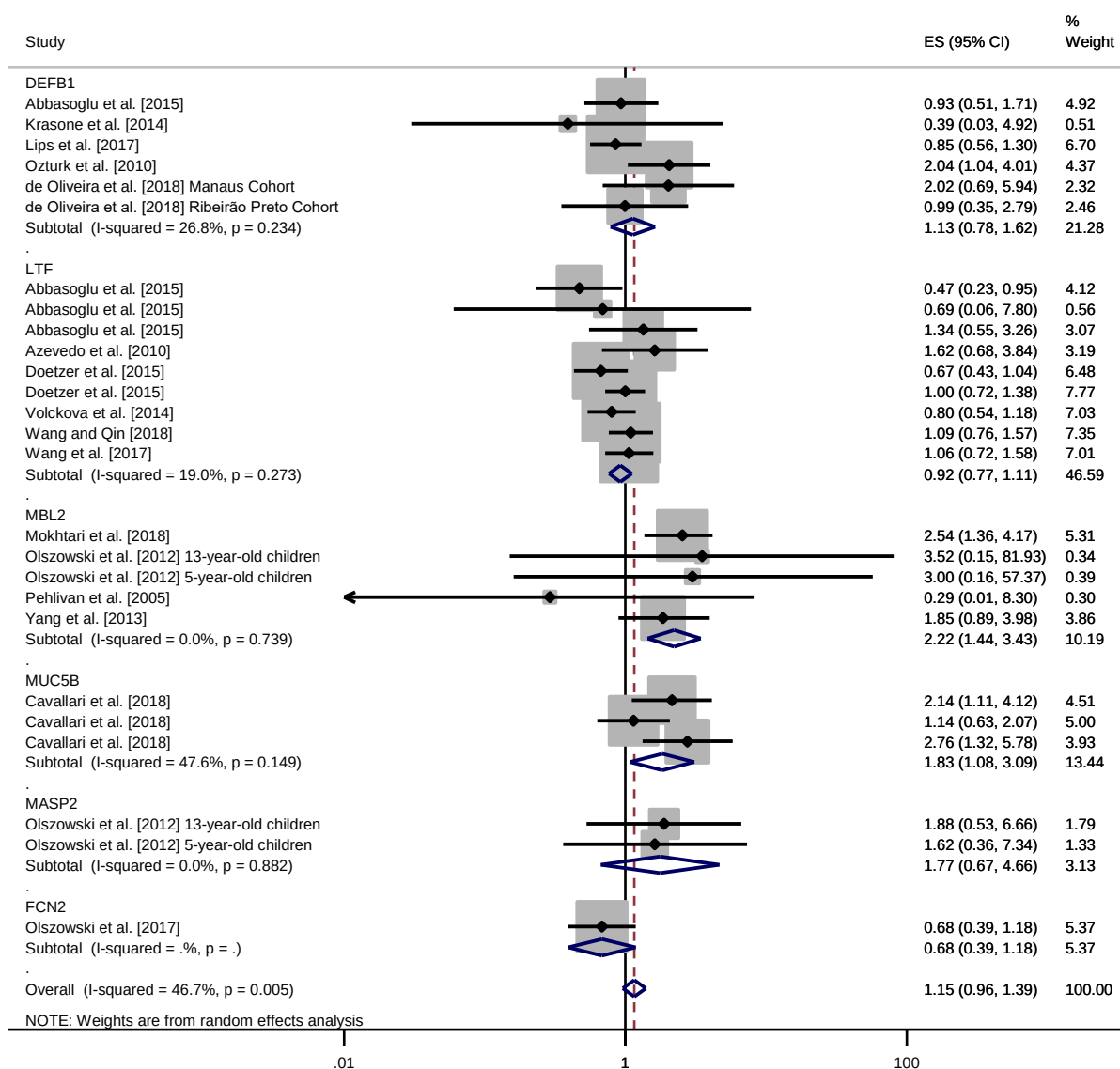
Supplementary material S3. Pooled effect of imate response SNPs in allelic model. Data are presented as odds ratio for each study (boxes), 95% CIs (horizontal lines) and summary as odds ratio with 95% CI (diamond). Randomic model was performed. In a) full model and b) SNPs stratified by gene.



Supplementary material S4. Pooled effect of imate response SNPs in homozygote model. Data are presented as odds ratio for each study (boxes), 95% CIs (horizontal lines) and summary as odds ratio with 95% CI (diamond). Randomic model was performed. In a) full model and b) SNPs stratified by gene.



Supplementary material S5. Pooled effect of imate response SNPs in heterozygote model. Data are presented as odds ratio for each study (boxes), 95% CIs (horizontal lines) and summary as odds ratio with 95% CI (diamond). Randomic model was performed. In a) full model and b) SNPs stratified by gene.



4.5 Artigo 5

Artigo formatado seguindo as normas da Revista *Caries Research*

Are the Single Nucleotide Polymorphisms in Vitamin D Receptor Gene associated with dental caries experience: A systematic Review and Meta-Analysis

Short title: Vitamin D Receptor TaqI and caries

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Key words: Polymorphisms, Dental caries, Vitamin D

Declarations of conflict of interest: none

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Cover Letter

Dear Editor, Beighton, D.

Based on the importance of *Caries Research*, we are sending the manuscript entitled “**Are the Single Nucleotide Polymorphisms in Vitamin D Receptor Gene associated with dental caries experience: A systematic Review and Meta-Analysis**” to be appraised by the Journal’s Editorial Board.

This is the first systematic review and meta-analysis to summarize the results of literature about the influence of Single Nucleotide Polymorphisms related to Vitamin D Receptor gene. Therefore, the meta-analysis findings showed that Foq I (rs10735810) was associated with dental caries in heterozygous genotype analysis. Moreover, allelic and genotype homozygous genotype analysis have been in borderline, but not significantly associated.

A considered number of studies were included in this review and meta-analysis making wide review of current available literature. Also, we performed the analysis considering different analysis (allelic and genotype) providing a robustness to our findings. We did quality control filters in order to minimize the bias in our estimates, such as to investigate and exclude SNPs in linkage disequilibrium, as well as excluded palindromic ones.

This is a review manuscript and has not been considered for publication elsewhere. The paper was read and approved by all authors. All authors have made substantive contribution to this study, and all have reviewed the final paper prior to its submission. The authors declare that there are no potential competing interests. Furthermore, I attest the validity and legitimacy of data and its interpretation. There are no conflicts of interest for authors listed above. We sign for and accept responsibility for releasing this material.

Thank you very much for your consideration.

Yours sincerely,

Prof. Luiz Alexandre Chisini. (Corresponding Author)

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Are the Single Nucleotide Polymorphisms in Vitamin D Receptor Gene associated with dental caries experience: A systematic Review and Meta-Analysis

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Abstract

Aim: the aim of this study was to evaluate whether single nucleotide polymorphisms (SNPs) in the vitamin D receptor's gene are associated with dental caries experience.

Methods: five databases were searched up to December 2019 following a structured search syntax comprising terms and entry terms. Pubmed/Medline, Scopus, Web of Science, BIREME – BVS Virtual health library and Scielo were the databases searched. We included cross-sectional or longitudinal, even as case control designs. We did not imposed limitations regarding language as well as publication time or population (children/adults).

Results: A total of 1,029 papers were founded, six were included in the present systematic review and five in meta-analysis. Four SNP were found to be related to *VDR* gene (rs731236, rs1544410, rs7975232 and rs10735810) and a total of 2,092 individuals were included in the analyses. Meta-analysis showed that rs10735810 was associated with dental caries in genotype heterozygote (OR=2.09 95%CI [1.03–4.22]) although borderline results were observed in in allelic (OR=1.43 95%CI [0.98–2.08] and genotype homozygote (OR=2.43 95%CI [0.99–4.38]). rs731236 was not associated with caries in allelic (OR=1.49 95%CI [0.85–2.62]), genotype homozygote (OR=1.53 95%CI [0.51–4.59]) and heterozygote (OR=1.28 95%CI [0.92–1.80]). No associations were observed with rs1544410 in allelic (OR=0.87 95%CI [0.51–1.49]) and the genotype heterozygote (OR= 0.79 CI95% [0.36–1.73]).

Conclusion: The results of our systematic review and meta-analysis suggest that *VDR* (rs10735810) is associated with dental caries. Further well-design studies in large samples sizes will be necessary to validate the presents associations.

Introduction

Dental caries is a chronic disease of multifactorial etiology, which can lead to the destruction of the dental structure [Fejerskov, 2004]. In the absence of disease control, mainly caused by mechanical disorganization of this biofilm associated with the presence of fluoride in the oral cavity, the lesions evolve to completely destroy the tooth structure; thus, culminating in the loss of the dental element [Chisini et al., 2018; Dutra et al., 2018; Kassebaum et al., 2015]. In order to better understand the existing criteria for the control of caries disease, it is essential to understand its etiological factors [Fejerskov, 2004].

Vitamin D (VD) is an important controller of osteomineral physiology by regulating calcium and phosphorus levels playing a key role in craniofacial development, including teeth [Uwitonze et al., 2018]. Higher serological levels of this molecule are associated with better oral health conditions [Uwitonze et al., 2018]. VD deficiency, during odontogenesis, is associated with developmental defects of dental mineralized tissues, such as enamel hypoplasia [Reed et al., 2017], being considered a risk factor periodontal disease [Jagelaviciene et al., 2018] and dental caries development [Seminario and Velan, 2016]. Such hypomineralization condition are not exclusively associated with vitamin D deficiencies.

Vitamin D receptor (VDR) plays a mediating role in this biomineralization process during tissue formation. The *VDR* gene locus corresponds to the 12q13.1 region of the human genome [Wang et al., 2012b]. This receptor possess 10 kb having the ability to generate numerous tissue specific transcripts. More than 200 *VDR* gene polymorphisms are currently recognized, including four single nucleotide polymorphisms (SNPs) such as Apa I (rs7975232), Fok I (rs10735810), Bsm I (rs1544410) and Taq I (rs731236). Literature reports that *VDR* polymorphisms may be responsible for increased susceptibility to periodontitis and early loss of dental implants [Guido Mangano et al., 2018; Jagelaviciene et al., 2018]. In fact, Fok I polymorphism was associated with susceptibility to tooth decay in the permanent teeth of Chinese adolescents [Yu et al., 2017]. Besides, another study showed that the presence of Taq I (tt) genotypes was highest among children with active caries in Turkish children [Cogulu et al., 2016].

In such a context, the effect of behavioral and socioeconomic factors over the establishment and progression of dental caries has been extensively studied and, therefore, is relatively known [Kassebaum et al., 2015]. However, such factors are not enough to explain caries

susceptibility and there is still slight evidence to clarify the influence of genetic components on this outcome [Chisini et al., 2020; Vieira et al., 2014].

Thus, the aim of this study is to evaluate whether single nucleotide polymorphisms (SNPs) in the vitamin D receptor's gene are associated with dental caries experience.

Methods

The present systematic review is awaiting registration confirmation in the International Prospective Register of Systematic Reviews (PROSPERO). This study has been reported following the PRISMA guideline [Moher et al., 2009].

Review question and Searches:

To perform this review, five databases were searched up to December 2019 following a structured search syntaxis with terms and entry terms (**Supplemental material S1** presents the complete structure of search strategy). PubMed/Medline, Scopus, Web of Science, BIREME – BVS Virtual health library and Scielo were the databases searched. Specific keywords and MeSH terms were used, based on PICO-structured questions, viz:

- Population: adults and children
- Exposure: The allele frequency and genotype. We standardized as effect allele/genotype the less frequent allele/genotype considering most of the included studies.
- Comparison: The more frequent allele/genotype
- Outcome: Caries experience. We considered different measures to investigate dental caries such as DMF-T/S or International Caries Detection and Assessment System (ICDAS). Preferable, we selected the DMFT/ICDAS =0 vs. DMF/ICDAS $\geq$ 1.

The review question was: Are the Single Nucleotide Polymorphisms in Vitamin D Receptor's Gene associated with dental caries experience?

Retrieved records were uploaded into a reference manager (EndNote) to delete the duplicated ones and to create a virtual library (VL). Two independent reviewers (LAC and MCMC) read all the titles and abstracts contained in the VL considering the given predefined criteria as follows:

- Inclusion criteria: Every paper evaluating possible association between dental caries susceptibility and VDR Single Nucleotide Polymorphisms in both, children or adults/older population. Studies corresponding to a cross-sectional or longitudinal design, even as case control design. We did not have impose limitation regarding language as well as publication time.

- Exclusion criteria: literature reviews, case reports or case series or studies that not fit the inclusion criteria.

In order to confirm if the included studies, in fact, satisfied the inclusion criteria, the same reviewers judged, independently, each selected study's full text. Eventual disagreements were discussed until a consensus.

Data collection:

Data extraction has been performed independently by each of the reviewers, based on the studies selected based on the inclusion criteria, recording the follow information: Author, year, country, study design, sample size, age, sample ethnicity (% for each ethnic group), percentage of the sexes of the sample, calculation of statistical power, Hardy-Weinberg equilibrium, evaluation and categorization of dental caries, analytical approach, data analysis and covariables.

Quality of studies:

Two guides aiming to assess the methodological quality of included studies were used. Appraisal Checklist for Observational Studies (Joanna Briggs Institute) [Institute, 2014] was used to investigate the quality of observational design and a second tool was modified to a 10-point scoring sheet previously used [Clark and Baudouin, 2006; Salles et al., 2017] in observational studies investigating genetic influence on disease.

Strategy for data synthesis:

Data synthesis was performed using a meta-analysis investigation the odds ratio (OR) of each SNPs on dental caries experience. Moreover, we pooled all minor frequency allele/genotype of SNPs related to *VDR* Gene to estimate the pooled effect of different SNPs. So, we stratified the analysis between allelic and genotype (i.e. homozygote and heterozygote). We preferable included adjusted results in meta-analysis. When studies did not reported OR, and 95% Confidence Interval (CI), we used the online software MedCalc (https://www.medcalc.org/calc/odds_ratio.php). Prevalence ratio were converted in OR, as previously reported [Chisini et al., 2019; Zhang and Yu, 1998].

Data harmonization for palindromic SNPs was performed, i.e. when more than one palindromic SNP was observed in different studies, we only kept the SNP in the analysis if was reported the DNA strand. Furthermore, in the gene pooled analysis the data were pruning by Linkage Disequilibrium (LD). We only maintained in the gene pooled analysis SNPs not LD. i.e $LD < 0.3$. In cases of SNPs in LD, we maintained in the analysis the one with lowest P-value in the association. In cases where studies have not performed LD evaluation, we used data from the 1000Genomes to estimate LD, considering the global population as reference panel.

Due to the observed high heterogeneity (I^2 statistic) across the studies, random models were used. All analyzes were carried using Stata 12.0 software (StataCorp, College Station, TX, USA).

Results

Study selection

The initial search resulted in 1,417 records, corresponding to 1,029 papers after duplicates exclusion. Eight full text were considered as eligible following the inclusion criteria. Two studies were excluded [Cavallari et al., 2019; Fine, 2015]. The **Figure 1** displays the Prisma diagram flow and the reasons for paper full-text exclusions. Therefore, six papers were included in systematic review [Cogulu et al., 2016; Holla et al., 2017; Hu et al., 2015; Kong et al., 2017; Raivisto et al., 2018; Yu et al., 2017] and five in meta-analysis [Cogulu et al., 2016; Holla et al., 2017; Hu et al., 2015; Kong et al., 2017; Yu et al., 2017].

Study characteristics

The selected studies were performed mainly in China [Hu et al., 2015; Kong et al., 2017; Yu et al., 2017] (n=3, 50%). Three studies were case control design [Holla et al., 2017; Hu et al., 2015; Yu et al., 2017] and the other three cohort design [Izakovicova Holla et al., 2017; Kong et al., 2017; Raivisto et al., 2018]. All studies evaluated the phenotype as DMF-T/dmf-f index and only Raivisto et al. [2018] complemented the clinical examination with bite-wing x-ray. Permanent dentition was evaluated by 66.6% of studies [Holla et al., 2017; Hu et al., 2015; Raivisto et al., 2018; Yu et al., 2017] followed by deciduous [Kong et al., 2017] and mixed [Cogulu et al., 2016] with 16.7% each. A total of 2,092 individuals were included in the selected studies. **Table 1** displays the main characteristics of studies included in this systematic review. Four SNP were found to be related to *VDR* gene [Taq I (rs731236), Bsm I (rs1544410), Apa I (rs7975232) and Fok I (rs10735810)]. Bsm I and Apa I were in an intron region while Taq I is a synonymous and Fok I star lost variation. Complete data of SNPs are available in **Table 2**.

Risk of bias within studies

The risk of bias investigated to Critical Appraisal Checklist for observational studies (Joanna Briggs Institute) showed that 50% of studies presented a high quality and the other 50% a low quality (**Table 3**). Methodological scoring protocol based on quality assessment for genetic studies showed that 50% of studies were considered as medium and 50% low quality of evidence.

Independent replication, reproducibility and corrected statistics were the criteria with the worst scores (**Table 4**).

Results of individual studies

The SNP *VDR* Apa I (rs7975232) was not included in meta-analysis and have not showed association with dental caries experience. In allelic effect, the allele risk T display an Odds ratio of 1.26 (95% CI 0.91 – 1.73); similarly, genotype homozygote (TT) presented a Odds ratio of 1.45 (95% CI 0.78 – 2.70) and genotype heterozygote (TG) an Odds of 1.20 (0.75 – 1.92). Furthermore, Yu et al. [2017] perform an haplotype analysis and observed that combination of “TCGT” ($p = 0.001$) and “TCGT” ($p = 0.011$) [respectively, Bsm I (rs1544410), Taq I (rs731236), Apa I (rs7975232), and Fok I (rs10735810)] were associated with dental caries.

Synthesis of results (meta-analysis)

No palindromic SNPs needed to be removed for analysis. Linkage disequilibrium (LD) was observed between all the SNPs related to *VDR* gene; thus, it was not possible to pool the SNPs in the *VDR* gene. Therefore, meta-analysis was only performed separately considering each SNPs. Therefore, three SNPs were included in meta-analysis. **Table 5** shown the LD and r^2 considered in present study.

Overall, we observed some differences in methodology of included studies, i.e. analytic approach, age of population; thus, considering also that most of models presented I-square statistics $> 50\%$, we chose to perform all the analysis under random effects. However, the studies presented a consonance among SNPs investigated, making possible to include almost all studies in meta-analysis, according allelic and genotype (homozygote and heterozygote). Raivisto et al. [2018] was not included in the meta-analysis because the allele change observed in the population (T/A) was different to that one observed by Kong et al. [2017] and Yu et al. [2017], i.e. (T/C).

With regard the allelic analysis of Taq I (rs731236) (**Figure 2**), we observed that risk allele C was not associated with caries (OR = 1.49 95% CI [0.85 – 2.62]); Similarly, genotype homozygote (CC) presented an Odds ratio of 1.53 (95% CI 0.51 – 4.59) and the genotype heterozygote (CT) an Odds ratio of 1.28 (95% CI 0.92 – 1.80).

Considering the SNP Fok I (rs10735810), we considered the risk allele as being the C; risk genotype homozygote as being CC; and risk genotype heterozygote as CT (**Figure 3**). Therefore, the

allele C was in borderline, but not associated with dental caries (OR = 1.43 95% CI [0.98 – 2.08]); Similarly, the genotype homozygote CC was not associated in presents findings (OR = 2.43 95% Ci [0.99 – 4.38]). On the other hand, genotype heterozygote CT showed an increase in 2.09 fold on odds of presenting caries (OR = 2.09 95% CI [1.03 – 4.22]).

No associations were observed with Bsm I (rs1544410). In allelic model, the risk allele A an Odds of 0.87 (95% CI 0.51 – 1.49) and the genotype heterozygote AG an Odds of 0.79 (95% CI 0.36 – 1.73) (**Figure 4**).

Discussion

To the best of our knowledge, this is the first systematic review and meta-analysis to summarize the results of literature about the influence of Single Nucleotide Polymorphisms related to *VDR* gene. Therefore, the meta-analysis findings showed that *Foq I* (rs10735810) was associated with dental caries in heterozygous genotype analysis. Moreover, allelic and genotype homozygous genotype analysis have been in borderline, but not significantly associated.

The main etiological factors of dental caries are well known as a disease being mediated by social determinants in health, which could lead to poor oral health habits and diet. However, people exposed to the same socio-cultural condition can present different susceptibility to caries [Yildiz et al., 2016]; Aiming to explain these knowledge differences, and stimulated by breakthroughs in genome project, studies have been focused on the use of several tools and strategies (i.e. twin studies, genome wide associations studies [GWAS] and candidate gene) to investigate possible genetic contributions in these associations. Despite GWAS are considered the more robust strategy to identify associations between diseases and genes or variants, *a priori* knowledge of etiology is not necessary, i.e. it is not necessary a previous hypothesis, few studies using this approach are available in literature investigating dental caries and did not point to 12q13.1 locus [Vieira et al., 2014]. In fact, genome consortium's have identified some loci (1q42-q43, 11p13 and 17q23.1) [Shaffer et al., 2011; Wang et al., 2012a] (*RPS6KA2*, *PTK2B*, *RHOA*, *FZD1*, *ADMTS3* and *ISL1*) as associated with dental caries. Thus, few specific genetic loci have been identified by genomic association and needed to further studies to confirm these results. On the other hand, gene candidate analysis have presented a wide literature on dental caries [Chisini et al., 2020; Vieira et al., 2014].

Therefore, taking into account mainly the gene candidate studies, it has been possible to identify some groups of genes with impact in dental caries [Chisini et al., 2020; Vieira et al., 2014] and *VDR* is one of them [Izakovicova Holla et al., 2017]. In fact, mutations in *VDR* gene can change the biological activity of the protein *VDR* leading to alterations on Vitamin D. Thus, *VDR* plays a role in the calcium absorption and, therefore, influencing the quality of enamel formation [Houari et al., 2016; Zhang et al., 2009]. Our results found four SNPs potentially related to these changes, affecting dental caries susceptibility. *Taq I* (rs731236) has been the more investigated SNP and

displayed different frequencies of allele and genotypes between the group's - caries free and caries experience - concerning the included studies.

In fact, it was possible to observed that all studies evaluating Chinese populations found a potential increase on dental caries in the risk allele [Hu et al., 2015; Kong et al., 2017; Yu et al., 2017], despite only Hu et al. [2015] found association in allelic model. On the other hand, considering Czech children, the same allele presented opposed direction of estimates, highlighting possible ethnic background, which can influence phenotype expression. In the study of Hu et al. [2015] it was observed that “t” allele and “Tt” genotype increased the Odds for caries in Chinese adults ($\cong$ 50 years old). A non-significant increase in Odds for caries was observed in “tt” genotype of Turkish children (6/12) [Cogulu et al., 2016]. It is important to highlight that different dentitions were included in the analysis. While Cogulu et al. [2016] included mixed dentition, and [Kong et al., 2017] deciduous, others only included permanent [Holla et al., 2017; Hu et al., 2015; Yu et al., 2017].

While no associations were observed in meta-analysis regarding Taq I (rs731236), we found association about Foq I (rs10735810) in genomic heterozygote model; despite other models (allelic and genotype homozygote) presented the same directions and a borderline result. In fact, individuals with risk genotype CT (Ff) showed an Odds twice as large of having experienced caries compared with individuals with genotype TT (ff) . Foq I (rs10735810) is a start lost variant, i.e. a codon variant changing at least one nitrogen base of the canonical start codon and have been associated with a range of diseases, including periodontal chronic diseases [Murthykumar et al., 2019; Nazemismalman et al., 2019; Smolders et al., 2009]. It is important to highlight that in our study only Chinese population were evaluated considering Foq I (rs10735810). In this SNP a low ethnic background influence could be observed. So, extrapolation of this data can be performed with caution to other populations.

On the other hand, Bsm I (rs1544410) presented results more discrepant in the included studies. The risk allele A (b) and the genotype AG (bB) were associated with decrease in caries experience in Chinese deciduous dentition [Kong et al., 2017]. No associations were observed in Chinese children considering permanent teeth [Yu et al., 2017]. Bsm I (rs1544410) has been associated with bone mineral disease such as osteoporosis [Marozik et al., 2018] and chronic osteomyelitis [Jiang et al., 2016].

The differences found at the VDR-linked SNPs in the association of dental caries can be

explained due to ethnic differences presented in the different studies, as well as the differences in methodological approaches, which lead to different sample size. Therefore, we proposed assess the quality of studies through two different tools. The first one consisted of investigating the assessment quality considering observational studies. Therefore, we observed that half of studies presented high methodological quality. In addition, we use a modified sheet to a 10-point scoring for genetic observational studies. Such assessment showed that all studies were classified in low/medium quality of evidence. Points related to statistical analysis were the criteria with minors scores. In general, reproducibility of study and independent replications were poor scored. Likewise, studies perform only chi-square tests and odds calculation with respective 95% CI, without adjustment – except by Raivisto et al. [2018]. In fact, studies did not perform corrections by multiple comparisons. It is well known that gene candidate studies present an elevate potential for false positive associations, i.e. type I error. To avoid false positive results, it is recommended that tests to multiple corrections are carried out. However, recent studies contested this recommendation highlighting some true positive results losing significance due to corrections [Vieira et al., 2017; Vieira et al., 2008]. Type II error was also poorly investigated through power calculations. Besides, few studies perform test to Hardy-Weinberg equilibrium, i.e. next generation alleles for any individual are chosen independently. So, it is possible that SNPs that are not in balance include bias in results. Moreover, linkage disequilibrium was evaluated in only one study [Yu et al., 2017] and we complemented the evaluation using 1000Genomes to estimate LD. So, we identify an elevate LD among the *VRD* SNPs and, thus, we did not perform the analysis pooling all the SNPs. After evaluating the presence of heterogeneity, we detected considered ($I^2 > 50\%$) heterogeneity. This finding cannot be ignored, since may influenced in our results and must be considered in data interpretation. To avoid influence of heterogeneity, we perform the analysis with random effects model.

In addition, despite we did not include a large number of studies in the analysis, it is important to highlight that our inclusion criteria are embracing; so, we included every studies investigating the SNPs related to *VDR* and dental caries in an elevate number of data bases. Yet, the analysis has been performed considering different strategies (allelic and genotype [homozygote and heterozygote]) providing strength to our findings. A elevate number of control filters were provided to avoid inclusion of additional biases in our estimates. Between them we investigate palindromic SNPs and linkage disequilibrium. Taking into account the limitation of our study, our results must be

interpreted with caution considering the intrinsic limitations of a meta-analysis and the gene candidate studies included in the review. To support presents results, it is necessary to conduct well-design longitudinal studies with large sample size performing studies' replication. Power calculation must be carried out in further studies to ensure that non-associations are not due to lack of statistical power. So, further studies address in the current search topic must be encouraged even as studies in genomic scale. Gene-environment interactions and epistatic, i.e. gene-gene interactions can also provide more contribution of current literature to understand the polygenic trait.

Conclusion

The results of our systematic review and meta-analysis suggest that *VDR* Foq I (rs10735810) is associated with dental caries. Further well-design studies in large samples sizes will be necessary to validate the presents associations. Epistatic and gene-environments interactions can contribute in the understanding of influence of *VDR* in dental caries.

Compliance with Ethical Standards:

Conflict of Interest: Luiz Alexandre Chisini declares that he has no conflict of interest. Marcus Cristian Muniz Conde declares that he has no conflict of interest. Marcos Britto Correa declares that he has no conflict of interest

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Ethical approval: no necessary

Informed consent: no necessary

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Legends:

Table S1. Search strategy

Table 1. Main characteristics of studies included in this systematic review

Table 2. Description of single nucleotide polymorphism investigated in the present systematic review

Table 3. Critical Appraisal Checklist for observational studies (Joanna Briggs Institute) in the systematic review according to the 10-items

Table 4. Methodological scoring protocol based on quality assessment for genetic studies.

Table 5. Linkage Disequilibrium of single nucleotide polymorphisms

Figure 1: Prisma flow diagram

Figure 2. Meta-analysis of results of Taq I (rs731236) according allelic (a) and genotype [homozygote (b) and heterozygote (c)] analysis. Effect allele C (t); Genotype Homozygote effect CC (tt); Genotype Heterozygote effect CT (Tt)

Figure 3. Meta-analysis of results of Fok I (rs10735810) according allelic (a) and genotype [homozygote (b) and heterozygote (c)] analysis. Risk allele: C (F); Risk genotype homozygote: CC (FF); Risk genotype heterozygote: CT (Ff)

Figure 4. Meta-analysis of results of Bsm I (rs1544410) according allelic (a) and genotype [heterozygote (b)] analysis. Risk allele: A (b); Risk genotype homozygote: AA (bb); Risk genotype heterozygote: AG (bB)

Table S1. Search strategy

Search syntax	
PubMed	#1 ("Dental Decay" OR "Caries, Dental" OR "Decay, Dental" OR "Cariou Dentin" OR "Cariou Dentins" OR "Dentin, Cariou" OR "Dentins, Cariou" OR "Dental White Spot" OR "White Spots, Dental" OR "White Spots" OR "Spot, White" OR "Spots, White" OR "White Spot" OR "Dental White Spots" OR "White Spot, Dental" OR "Susceptibility, Dental Caries" OR "Caries Susceptibility, Dental" OR "Caries Resistance, Dental" OR "Resistance, Dental Caries" OR "Dental Caries Resistance")
	#2 ("Polymorphisms, Genetic" OR "Genetic Polymorphisms" OR "Genetic Polymorphism" OR "Polymorphism" OR "Polymorphisms" OR "Nucleotide Polymorphism, Single" OR "Nucleotide Polymorphisms, Single" OR "Polymorphisms, Single Nucleotide" OR "Single Nucleotide Polymorphisms" OR "SNPs" OR "Single Nucleotide Polymorphism")

* Search combination: #1 AND #2

Table 1. Main characteristics of studies included in this systematic review

Author , year	-Country	-Age	-Evaluation	dental	caries	Analytical	Adjustment
	-Study design	(permanent/ deciduous)	-categorization			Approach	variables
	-Sample (% Males)	-Ethnicity				Hardy-Weinberg equilibrium	
		-Statistic power (yes/no)					
Hu et al. [2015]	-China	-Mean $\cong$ 50	-DMF-T			-Odds ratio calculation and chi- square test	-
	-Case Control	years	-Caries experience (DMFT $\geq$ 1) vs				
	-480 (50.7%)	(permanent)	caries free (DMF-T=0)			-Hardy-Weinberg equilibrium	
		-NR					
		-NR					
	CA					<i>VDRTaqI</i> (rs731236) t MAF	
						Homozygote: OR = 3.80 (1.79 – 8.07); Allele: t vs T = 3.59 (1.79 – 7.21)	
	AA					-	
Cogulu et al. [2016]	-Turkey	-6/12	-Evaluation	dental	caries	-Fisher's exact test	-
	-cohort	(permanent and	-1)high caries (dmft/DMFT>4), 2)			-Hardy-Weinberg equilibrium	
	-350 (50%)	deciduous)	moderate caries (dmft/DMFT=1-4)				
		-Ethnicity	and 3): caries-free				
		-Statistic power (yes/no)	(dmft/DMFT=0)				

VDR TaqI (rs731236)

t MAF

Heterozygote: OR = 1.11 (0.51 – 2.41); Allele: OR = 1.11 (0.52 – 2.35);

VDR Apal (rs7975232)

A MAF

Homozygote: OR = 1.45 (0.78 – 2.70); Heterozygote: OR = 1.20 (0.75 – 1.92); Allele: OR = 1.26 (0.91 – 1.73);

VDR FokI (rs10735810)

F MAF

Homozygote: OR = 1.43 (0.79 – 2.62); Heterozygote: OR = 1.48 (0.87 – 2.53); Allele: OR = 1.18 (0.88 – 1.60);

-China	-12	years	-DMF-T	-chi-square test	-
-Case control	(permanent)		- Caries experience (DMFT≥1) vs	-Hardy-Weinberg equilibrium	
-400 (49%)	-NR		caries free (DMF-T=0)		
	-Yes				

Yu et al.
[2017]

CA

VDR BsmI (rs1544410)

b MAF

Heterozygote: OR = 1.20 (0.71 – 2.03); Allele: OR = 1.18 (0.72 – 1.94);

VDR Taq I (rs731236)

t MAF

Heterozygote: OR = 1.57 (0.93 – 2.63; Allele: OR = 1.50 (0.92 – 2.46);

VDR FokI (rs10735810)

F MAF

Homozygote: OR = 3.06 (1.92 – 6.76); **Heterozygote:** OR = 3.04 (1.65 – 5.61); **Allele:** OR = 1.73 (1.30 – 2.30);

	AA	-	
Raivisto et al. [2018]	-Finland	-15 to 17 -DMF-T and x-ray bite-wing	Logistic regresion
	-Study design	(permanent) -Caries experience (at least one	NR
	-94 (50%)	-Ethnicity decayed tooth) vs caries free (no	
		-Statistic power decayed)	
		(yes/no)	
	CA	VDR FokI (rs10735810 / rs2228570)	
		Allele T: OR = 2.49 (1.18 – 5.25)	
	AA	VDR FokI (rs10735810 / rs2228570):	
		Allele T: OR = 2.68 (1.20 – 5.98)	

NR: not reported; OR: Odds Ratio; dmft (decayed, missing teeth due to caries, filled teeth); ICDAS: International Decay Detection and Assessment System; CA: Crude association; AA: adjusted association; All measure effects show comprises ODDS Ratio. Different measures are reported; SNP: Single Nucleotide Polymorphism. MAF minor allele frequency

Table 2. Description of single nucleotide polymorphism investigated in the present systematic review

Gene	Polymorphism	Chromosomic position	Variation	Allele Frequencies by populations (%)						Allele change	Ancestral allele
				African	American	East Asian	Europe	South Asia	All		
VDR	VDR TaqI (rs731236)	12:48238757	synonymous	A:71% G: 29%	A: 74% G: 26%	A: 93% G: 7%	A: 60% G: 40%	A: 60% G:37%	A: 72% G: 28%	A/G	A
	VDR BsmI (rs1544410)	12:48239835	Intron	C: 73% T: 27%	C: 74% T: 26%	C: 94% T:6%	C: 60% T: 40%	C: 52% T: 48%	C: 70% T: 30%	C/A/G /T	C
	VDR ApaI (rs7975232)	12:48238837	Intron	C: 36% A: 64%	C: 56% A: 44%	C: 71% A: 29%	C: 45% A: 55%	C: 41% A: 59%	C: 48% A: 52%	C/A	C
	VDR FokI (rs10735810)	12:48272895	start lost	A: 19% G: 81%	A: 48% G:52%	A: 42% G: 58%	A: 38% G: 62%	A: 26% G:74%	A: 33% G: 67%	A/C/G /T	A

Table 3. Critical Appraisal Checklist for observational studies (Joanna Briggs Institute) in the systematic review according to the 10-items

Study, year	NIH Criteria										Final score
	1	2	3	4	5	6	7	8	9	10	
Hu et al. [2015]	-	-	/	-	/	+	+	-	-	/	Low
Cogulu et al. [2016]	/	/	/	-	-	+	+	-	-	+	Low
Holla et al. [2017]	1	1	1	1	1	1	1	-	-	-	High
Kong et al. [2017]	1	1	1	1	1	1	1	-	-	-	High
Yu et al. [2017]	1	1	1	1	1	1	1	-	-	-	High
Raivisto et al. [2018]	-	-	/	-	/	1	1	-	/	/	Low

+ Yes; - No; /: Unclear

Table 4. Methodological scoring protocol based on quality assessment for genetic studies.

Study, year	Genetic Criteria										Score	Evidence
	Control group	Hardy–Weinberg equilibrium	Case group	Primer	Reproducibility	Blinding	Power calculation	Statistics	Corrected statistics	Independent replication		
Hu et al. [2015]	1	1	1	0	0	0	0	0	0	0	3	Low
Cogulu et al. [2016]	1	1	1	1	0	0	0	0	0	0	4	Low
Holla et al. [2017]	1	0	1	1	0	0	1	0	0	0	4	Medium
Kong et al. [2017]	1	1	1	1	0	0	1	0	0	0	5	Medium
Yu et al. [2017]	1	1	1	1	0	0	1	0	0	0	5	Medium
Raivisto et al. [2018]	0	0	0	0	0	0	0	1	1	0	2	Low

*For the quantification of criteria: «1» means present, and «0» absent

Table 5. Linkage Disequilibrium of single nucleotide polymorphisms

Linkage Disequilibrium		
SNP	D'	r ²
rs731236 and rs1544410	1.0	0.77
rs731236 and rs7975232	0.9	0.60
rs731236 and rs10735810	NA	NA

NA: not available

Figure 1: Prisma flow diagram

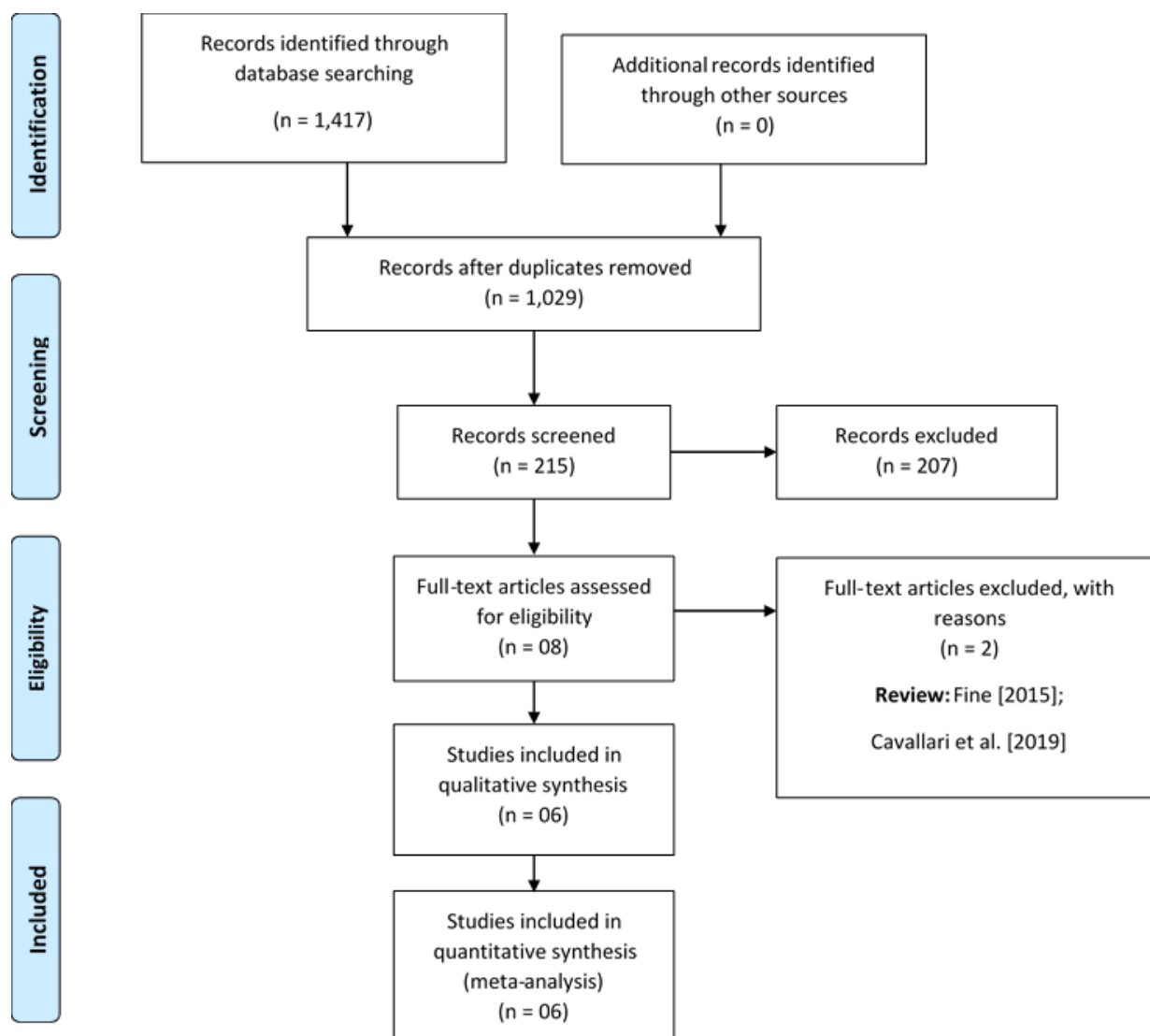
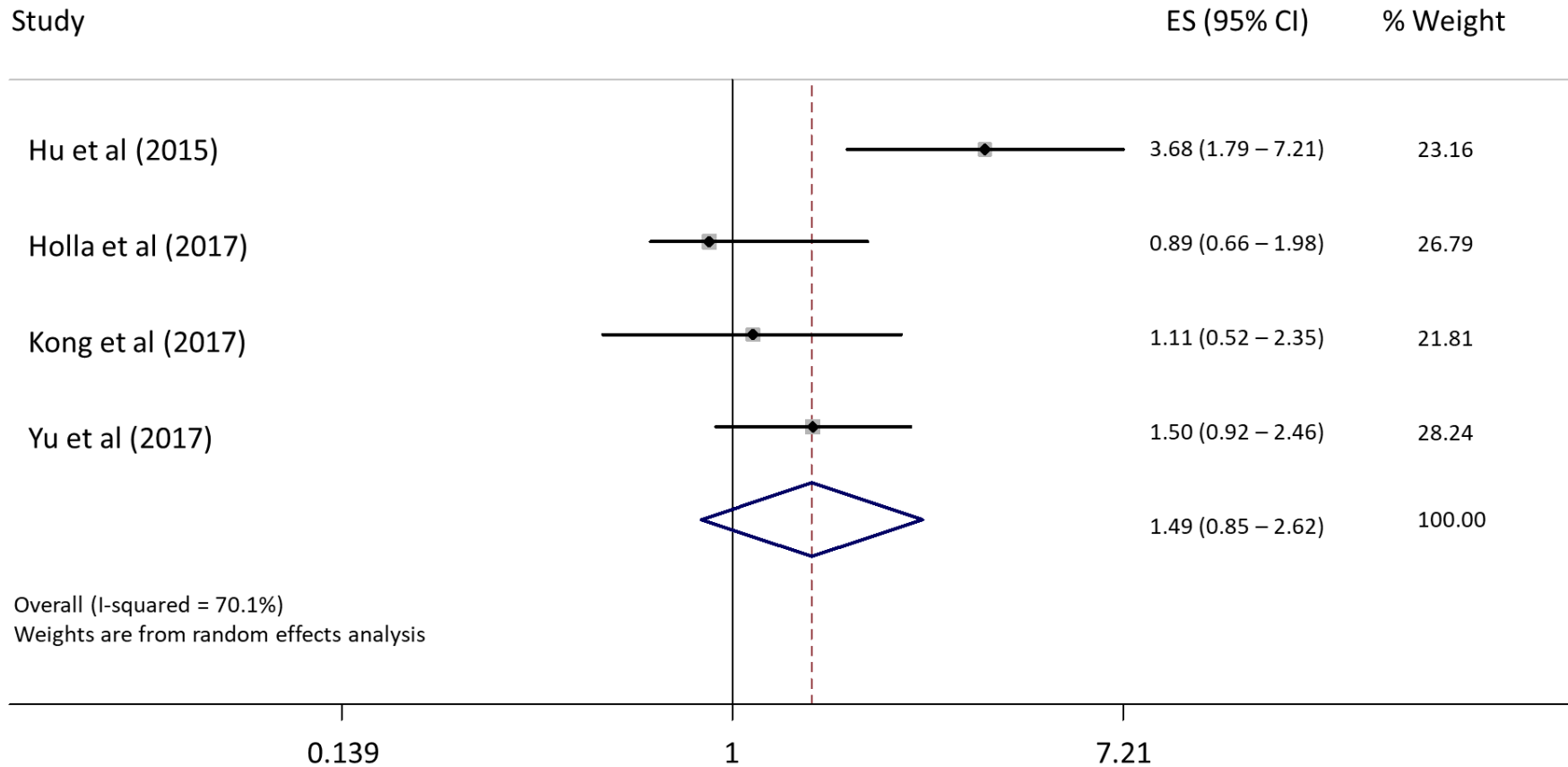
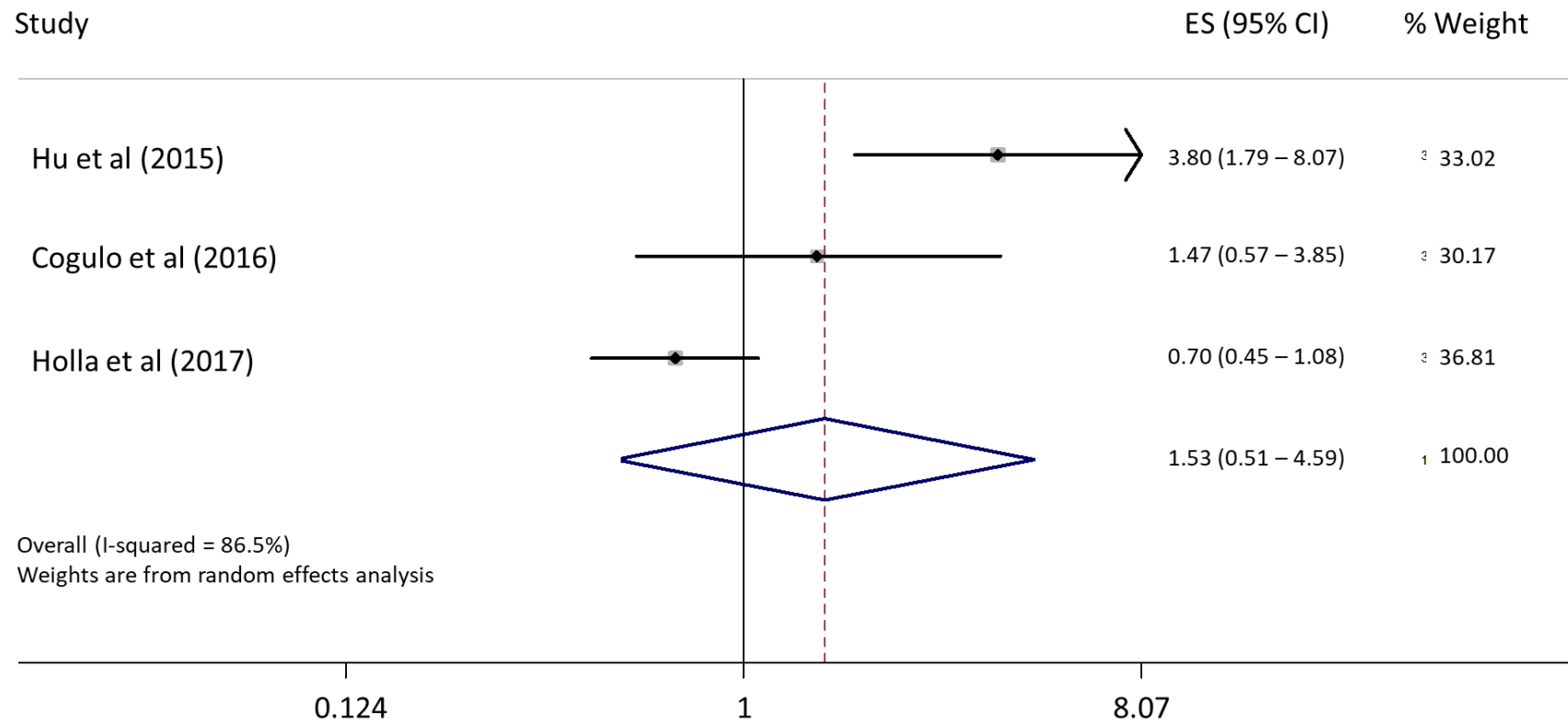


Figure 2. Meta-analysis of results of Taq I (rs731236) according allelic (a) and genotype [homozygote (b) and heterozygote (c)] analysis. Effect allele C (t); Genotype Homozygote effect CC (tt); Genotype Heterozygote effect CT (Tt)

a)



b)



c)

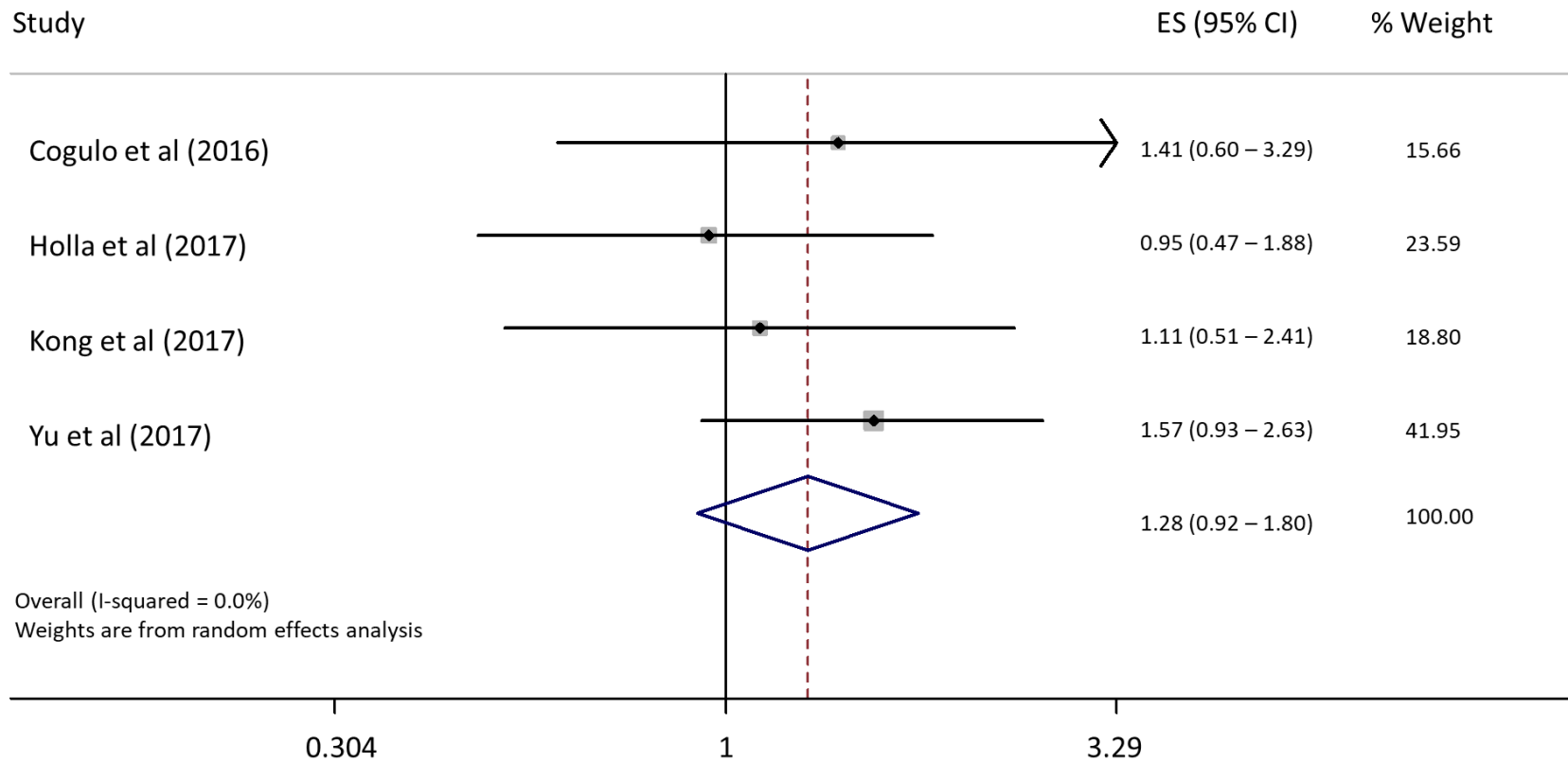
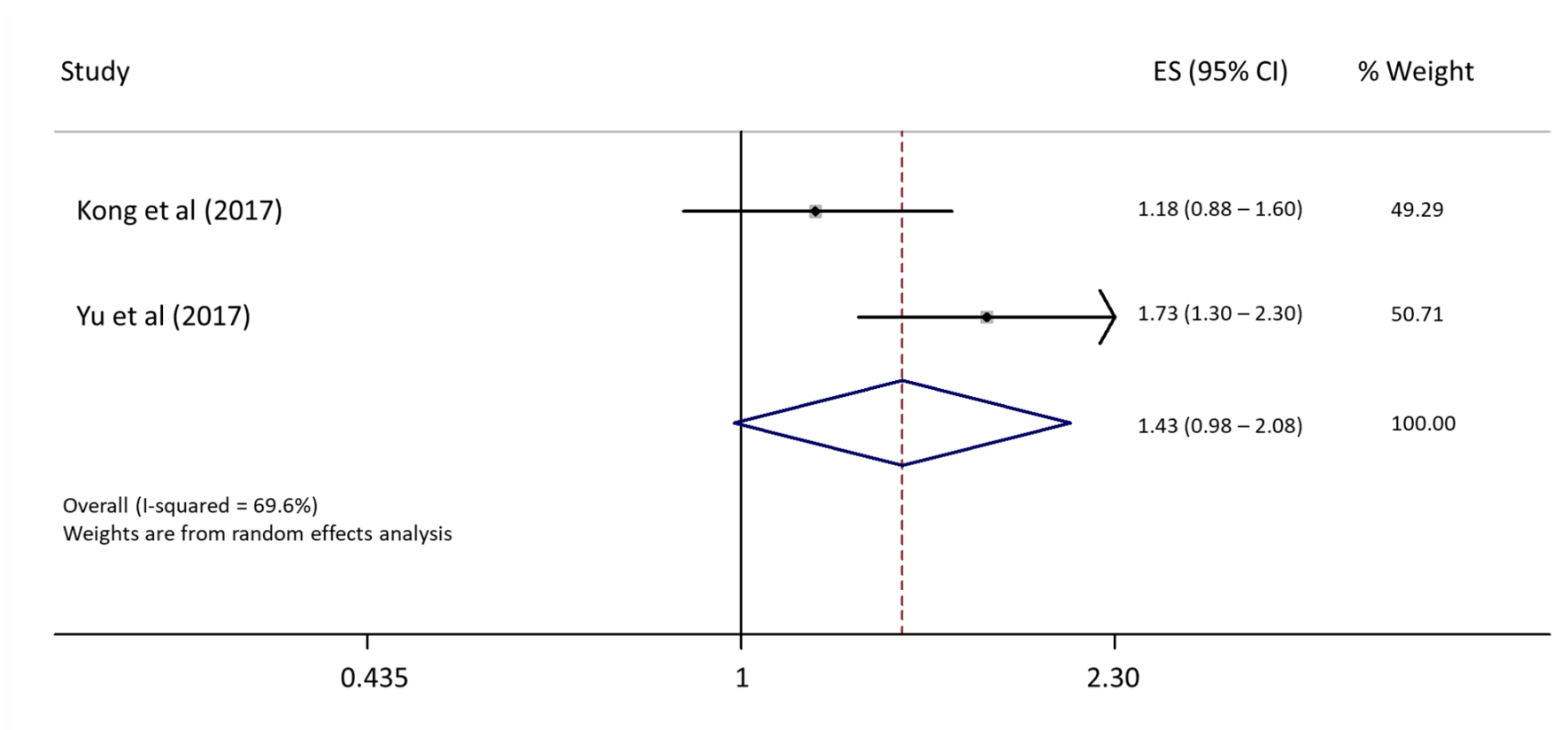
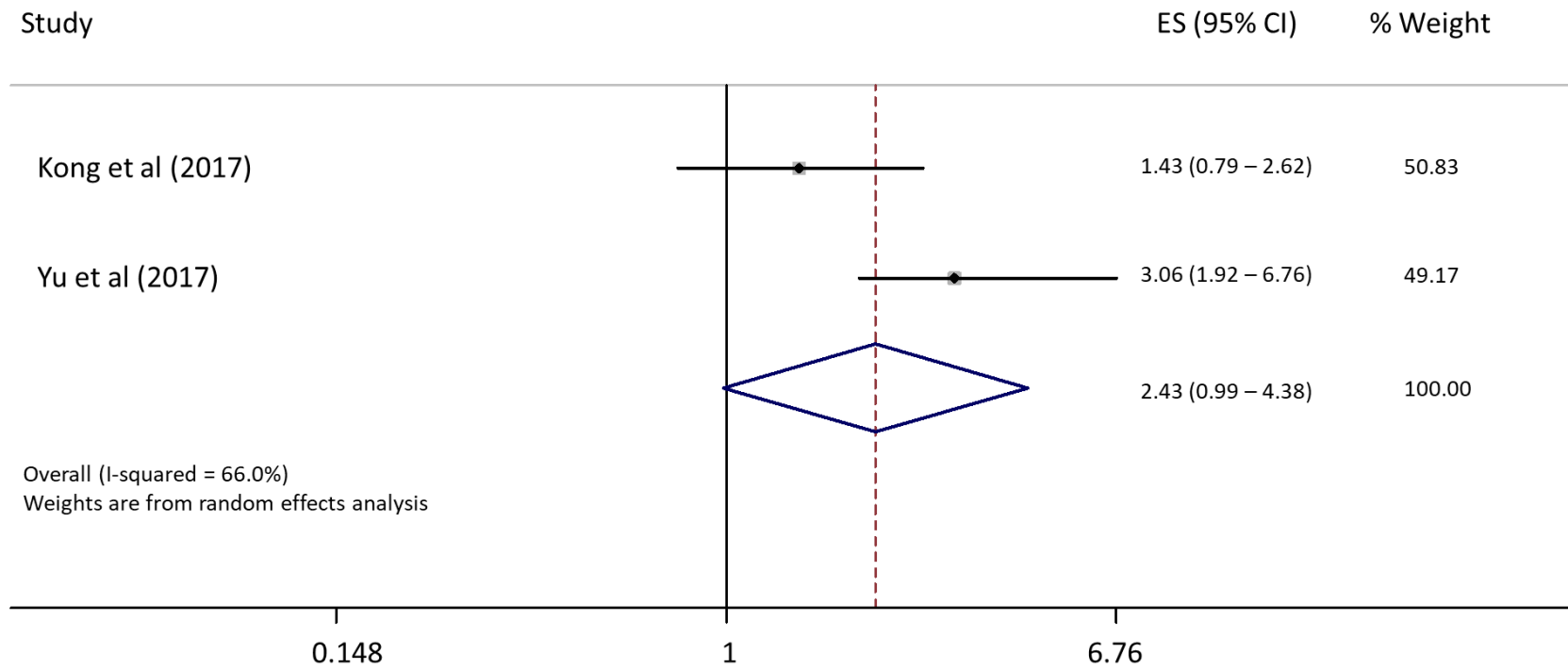


Figure 3. Meta-analysis of results of Foq I (rs10735810) according allelic (a) and genotype [homozygote (b) and heterozygote (c) analysis. Risk allele: C (F); Risk genotype homozygote: CC (FF); Risk genotype heterozygote: CT (Ff)

a)



b)



c)

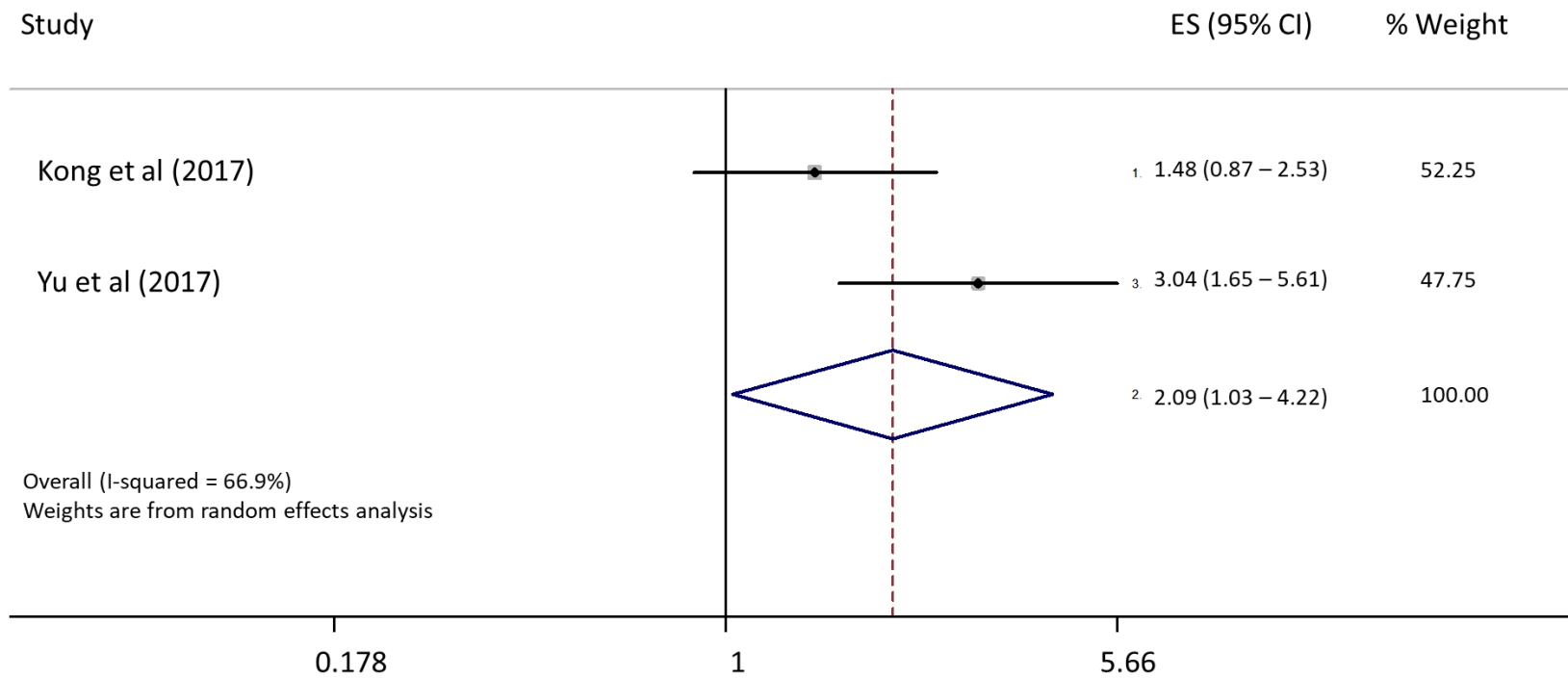
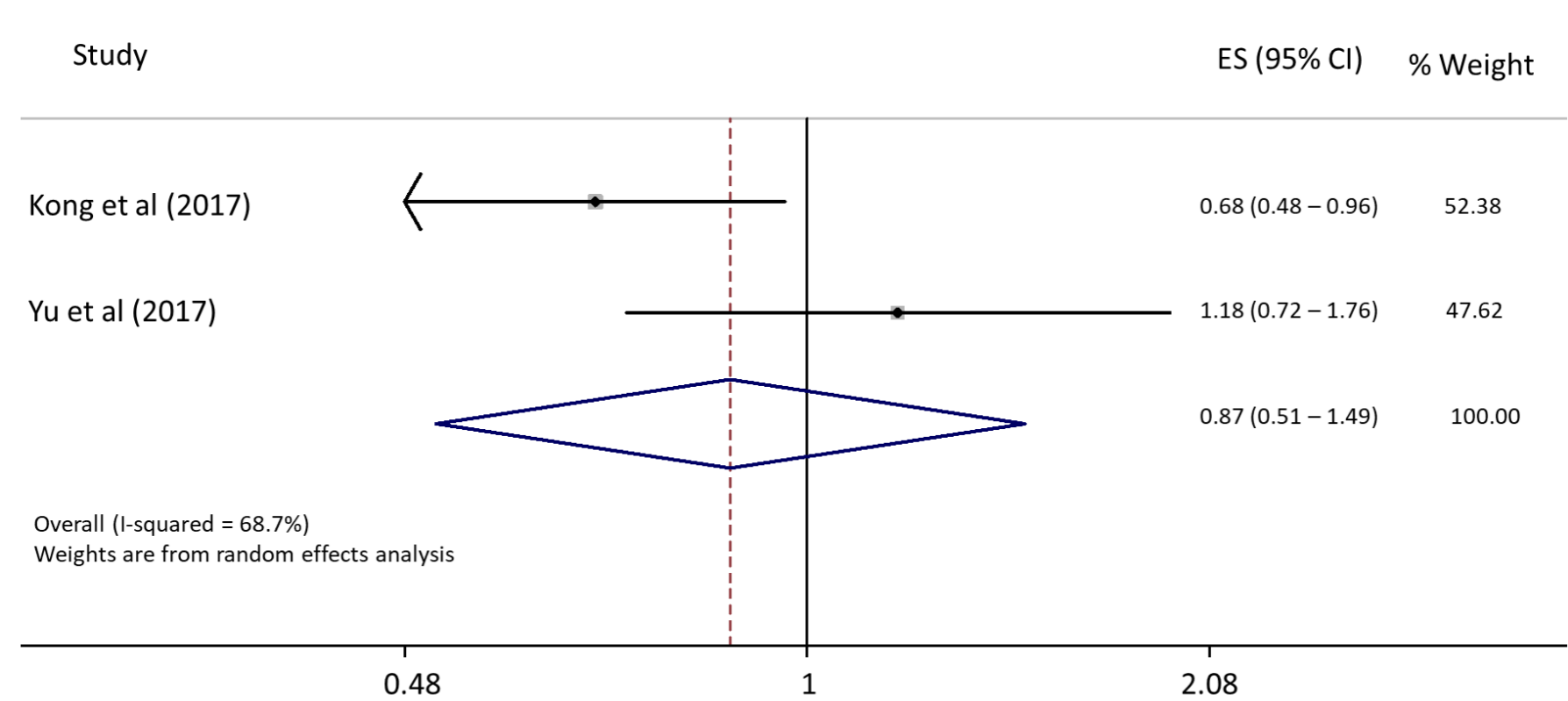
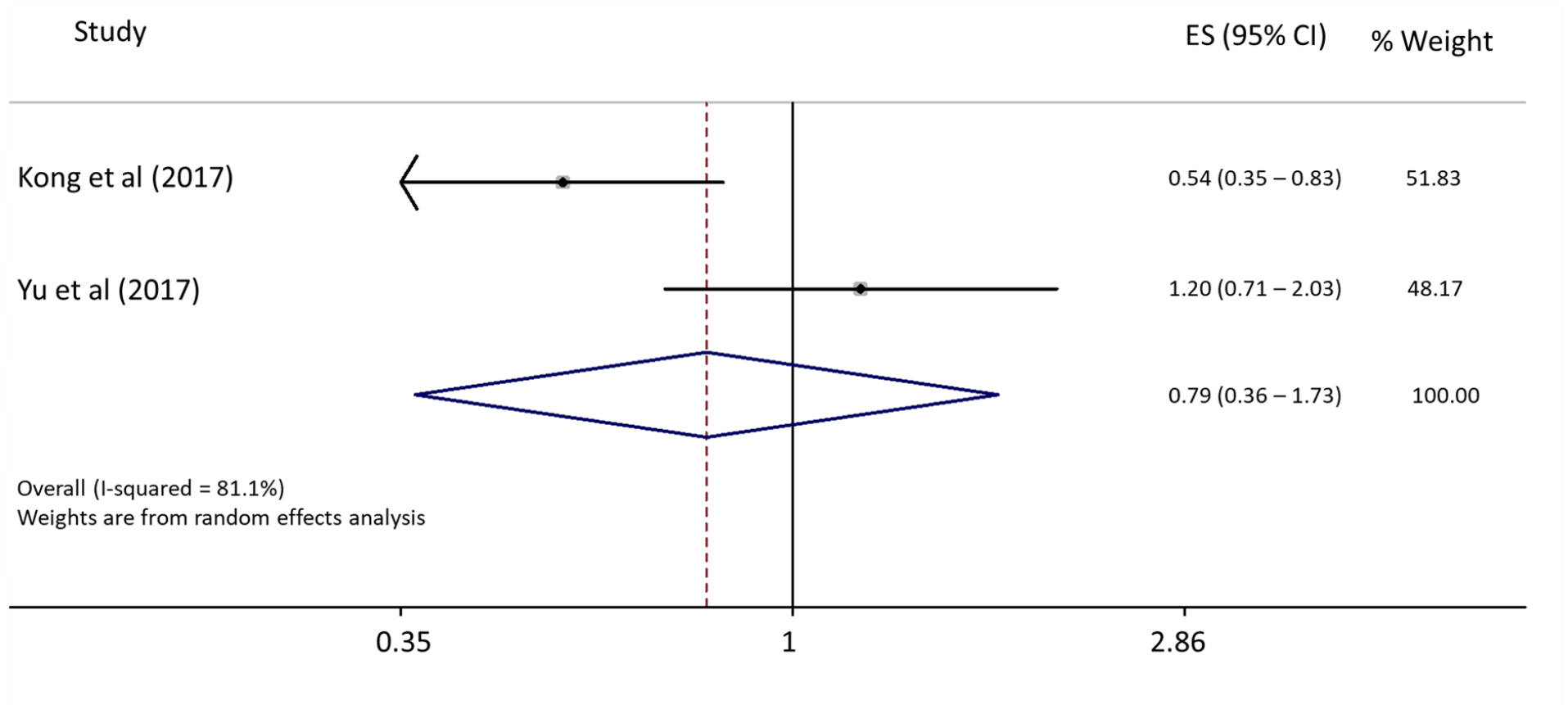


Figure 4. Meta-analysis of results of Bsm I (rs1544410) according allelic (a) and genotype [heterozygote (b)] analysis. Risk allele: A (b); Risk genotype homozygote: AA (bb); Risk genotype heterozygote: AG (bB)

a)



b)



5.0 Estudos Prospectivos

Neste capítulo, serão apresentados os resultados obtidos a partir dos estudos prospectivos com objetivo de testar e replicar os resultados obtidos nas revisões sistemáticas em uma coorte de nascimentos. Desta forma, iremos investigar a influência de todos os SNPs disponíveis na coorte de nascimentos de 1982 de Pelotas (identificados previamente nas revisões sistemáticas) com desfecho longitudinal de cárie. Análises de mediação foram realizadas para testar e confirmar as possíveis explicações teóricas para cada grupo de polimorfismo assim como interações epistáticas (gene-gene) e interações gene-ambiente.

5.1 Artigo 6

Artigo formatado seguindo as normas da Revista *Journal of Dentistry*.

***TAS1R3* of rs307355 is associated with caries trajectory in the life course: A gene-environment mediation in a birth cohort**

Running title: *TAS1R3* and dental caries

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Cover Letter

To: Dr. Christopher D. Lynch
Editor-in-Chief, *Journal of Dentistry*

Dear Editor:

Based on the importance of *Journal of Dentistry*, we are sending the manuscript entitled **“TAS1R3 of rs307355 is associated with caries trajectory in the life course: A gene-environment mediation in a birth cohort”** to be appraised by the Journal’s Editorial Board.

In this study, we assessed the hypothesis that trajectory of dental caries in the life course can be influenced by the rs307355 (*TAS1R3*) and rs35874116 (*TAS1R2*) genotypes and allele; moreover, we investigated if sugar consumption modifies the association between SNPs and caries trajectory. Our analysis corroborates with current literature confirming the association between rs307355 (*TAS1R3*) and dental caries. Our results showed that genotypes TC and TT, as well as allele T of rs307355 were associated with high caries trajectory in the life course. In this way, we added an interaction term between rs307355 (*TAS1R3*) and sugar consumption to investigate possible gene-environment modification. Thus, in both (genotypic T-dominant effect and allelic) analysis strategies we found that high sugar consumption modifies the relationship between allele T and dental caries trajectory, increasing the odds of caries. This result emphasizes the previous finding that exists an important relationship between T-alleles and sugar consumption, since that T-allele increased the consumption of sweets. Although we did not find direct association of rs35874116 (*TAS1R2*) and caries trajectory – and partially discordant from previous studies – we found an important association between rs307355 (*TAS1R3*) and rs35874116 (*TAS1R2*) both in haplotype analysis even as in gene-gene interaction on dental caries trajectory. Therefore, highlight the complex architecture of genetic influence of dental caries and showing a possible epistatic interaction underlying initial observations of rs35874116 (*TAS1R2*) since dental caries seem to be a complex trait. Moreover, we also observed

in rs307355 (*TAS1R3*) that in females the allele T in TC-genotype presented a recessive effect; while in males the allele presented a dominant effect.

This is a review manuscript and has not been considered for publication elsewhere. The paper was read and approved by all authors. All authors have made substantive contribution to this study, and all have reviewed the final paper prior to its submission. The authors declare that there are no potential competing interests. Furthermore, I attest the validity and legitimacy of data and its interpretation. There are no conflicts of interest for authors listed above. We sign for and accept responsibility for releasing this material.

Thank you very much for your consideration.

Yours sincerely,

Prof. Marcos Britto Corrêa, PhD. (**Corresponding Author**)
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Abstract:

Aim: was to Investigate if the trajectory of dental caries in the life course is associated with the rs307355 (*TAS1R3*) and rs35874116 (*TAS1R2*); and if the association can differ between subgroups of sugar consumption in a Gene-Environment modification.

Methods: A representative sample of all 5,914 births from the 1982 in Pelotas birth cohort study was prospectively investigated, and the caries trajectory in the life course was assessed at 15 (n=888) 24 (n=720) and 31 years old (n=539). Group-Based trajectory modeling was used to identify groups with similar trajectories of component “decayed” in the life course. Genetic material was collected and SNP rs307355 of *TAS1R3* and rs35874116 of *TAS1R2* were genotyped. Genomic ancestry was evaluated using ADMIXTURE. Family income at 24 years, consumption and frequency of sugar were asked were also investigated. We investigated epistatic interactions and gene-environment modification inserting an interaction term between sugar consumption and genotype/allele.

Results: Considering rs307355, genotype TT of rs307355 was associated with high caries trajectory in additive (OR=4.17, CI95%[1.21–14.42]), dominant genotype analysis (OR=1.53, CI95%[1.05–2.23]) and allelic (OR=1.55, CI95%[1.11–2.15]). Sugar consumption significantly modified with the allelic and genotype rs307355 influencing dental caries trajectory. rs35874116 of *TAS1R2* was not associated with caries in regression. Positive epistatic interactions were observed involving rs307355 and rs35874116 (OR=1.72, CI95%[1.04-2.84])

Conclusions: Trajectory of dental caries in the life course was positively associated with the rs307355 (*TAS1R3*) genotypes and allele. Presence of T-allele and high sugar consumption modify the caries trajectory. Thus, seem exist a Gene-Environment modification. Epistatic interaction between rs307355 and rs35874116 increasing caries trajectory.

Introduction

Dental caries is a multifactorial and chronic oral disease that affect a high number of children [1] and adults [2] worldwide, being the most prevalent health condition globally [3]. Interaction between cariogenic biofilm, main compost by *streptococcus mutants*, and fermentable carbohydrate are indispensable factors to caries development [4]. However, socioeconomic and behavioral issues are important variables that can explain the incidence and distribution of dental caries at population level [5]. In fact, inequalities in oral health can drastically influence prevalence of dental caries: individuals with lower socioeconomic status were associated with an increase of dental caries experience in a meta-analysis that included twenty-five studies [5].

On the other hand, a significant number of individuals, even when exposed to the same degree of individual and environment risk factors, seem to be more susceptible to dental caries than others. Some authors presented the hypothesis that these differences might be due to genetic factors, which can influence the etiopathogenesis of dental caries by different pathways [6-8]. In this way, recent studies confirmed that genetics plays a substantial role on dental caries susceptibility [6]. One of these pathways could be linked with taste genes [6, 9, 10]. A recent systematic review and meta-analysis performed by our group identify four (*TAS1R2*, *TAS2R38*, *TAS1R3* and *GLUT2*) taste genes with potential to influence dental caries experience.

The *TAS1R3* and *TAS1R2* encoded two of three main proteins of sweet taste receptor, the T1R3 (taste receptor type 1, member 3) and T1R2 (taste receptor type 1, member 2) [11]. Human sweet perception is intermediated by the products of the *TAS1R2* and *TAS1R3* gene [11]. Thus, the ability of perform fine discriminations in sucrose sweetness was reduced in individuals with CT or TT genotypes in rs307355, a Single Nucleotide Polymorphism (SNP) of *TAS1R3* [12]. Besides, considering this SNP, the heterozygous CT was associated with higher consumption of Soju, a Korean alcoholic beverage that contains a variety of natural or artificial sweeteners [13]. Corroborating, individuals with genotype CC were associated with higher consumption of proteins when compared to TC or TT-individuals [14]. In fact, rs307355 seems to affect the function of the *TAS1R3* regulatory region [12], changing the sweet taste perception [12, 13]. Similarly, one study showed an important association between the genotype CT of rs307355 and dental caries experience [15]. Although the literature has presented important evidences that rs307355 can change taste perceptions as well as caries experience, the studies supporting this hypothesis have not explored the different pathways of these associations with

robust models from longitudinal approaches. Moreover, it is not known whether there are possible interactions between polymorphisms in genes of *TAS1R3* and *TAS1R2*.

In this study, we aimed to investigate the effect of rs307355 (*TAS1R3*) and rs35874116 (*TAS1R2*) on dental caries and possible Gene-Environment mediation. Precisely, we addressed three research questions: a) is trajectory of dental caries in the life course positively associated with the rs307355 (*TAS1R3*) and rs35874116 (*TAS1R2*) genotypes (homozygotes and heterozygote) and allele?; b) is the association of rs307355/rs35874116 with caries trajectory different between subgroups of sugar consumption (i.e. is there a Gene-Environment interaction/effect modification)? C) Is there an epistatic interaction between SNPs of rs307355 (*TAS1R3*) and rs35874116 (*TAS1R2*)?

Methods

The present study was reported according to the STROBE guidelines for cohort observational studies [16].

Study design, setting and participants

This birth cohort study was performed in Pelotas, a Southern Brazilian city. All live births in the maternity hospitals of Pelotas in 1982 (5,914 children [99.2% of the births]) were identified and included in a perinatal health survey. This population continues to be followed throughout life [17]. After the first survey at the maternity ward, these same individuals were again sought in the years 1983, 1984, 1986, 1995, 1997, 1998, 2000, 2001, 2004, 2006 and 2013. However, in some years only a few subsamples were accessed with specific objectives. In 2004, the entire 1982 cohort sample was interviewed and, in addition to questions regarding the health of these individuals, a food frequency questionnaire was applied. In addition to this questionnaire, genetic material (with subsequent genotyping) was collected from these individuals.

Regarding the oral health studies, when subjects were 15 years old (1997), an oral health study (ESB-97) was conducted on a sample of this same cohort. This representative sample was obtained by searching the cohort individuals in 70 census tracts (27% of the total) in the urban area of Pelotas. We found 1,076 cohort individuals, of which 900 were randomly selected, composing the ESB-97 sample. In this follow-up, an interview was conducted containing questions about oral hygiene habits, use of dental services and pain of dental origin. In addition, dental examinations were performed to assess the presence of caries and occlusal problems.

The 888 young participants (98.7%) of the ESB-97 were contacted in 2006 (ESB-06) for a new interview, which also included dental examinations. Thus, it was possible to collect information about dental caries, quality of restorations in posterior teeth and oral lesions, among others. In addition, in the interview, individuals were asked about the use of dental services, episodes of dental pain and oral hygiene habits. A total of 720 individuals composed the ESB-06, representing 80% of the initial sample.

After that, in 2013, the 900 individuals from the initial sample were contacted again in order to increase the sample lost during the period. All individuals located and who agreed to continue the study (now 31 years old) made up the 2013 Oral Health Study (ESB-13). Similar to

previous studies, at ESB-13 individuals were also interviewed and clinical examinations were performed. Several oral health conditions were evaluated in this study, including the presence of carious lesions and restorations in the teeth of these individuals.

Outcome variable (phenotype)

The outcome variable of the present study was the dental caries trajectory of the participants (15, 24 and 31 years). The DMF-T were collected at 15, 24 and 31 years [18]. The component “decayed” of each follow-up was estimated and the individuals were dichotomously categorized into two groups for each of the three follow-ups: a) with untreated dental caries b) without untreated dental caries.

Moreover, the group-Based trajectory modeling was used to identify groups with similar trajectories of component “decayed” in the life course (15, 24 and 31 years). The model was estimated with the command “traj” in the program Stata 12.0. [19, 20]. Identifying the similarity of the trajectory among the evaluated individuals. The parameters for the model trajectory was determined based on the maximum likelihood by the quasi-Newton method [21, 22]. Model selection was considered and estimated by the latent number of categories and the polynomial order of each latent trajectory. The number of trajectories was determined when through sequential comparisons of the Bayesian information criterion (BIC) and its fit criteria between the K and K + 1 trajectory model have not produced substantial difference in the k + 1 model BIC score. Therefore, two untreated caries trajectories groups (low and high) were produced including 673 individuals. (**Figure 1**).

Independent variables

Collection of genetic material and genotyping (genotype)

The collection of genetic material from the Pelotas 1982 birth cohort participants was performed from October 2004 to August 2005. All participants located in the urban area of the city were visited. Thus, participants (22 to 23 years old) were interviewed and examined at home and invited to visit the research laboratory to donate a blood sample, collected by venipuncture. DNA and serum were extracted and frozen at -70 °C. DNA samples were genotyped using Illumina *Illumina HumanOmni2.5-8v1 array* and the SNP of *TAS1R3* rs307355 and rs35874116 of *TAS1R2* were genotyped in this population [23, 24]. In addition, genomic

ancestry was evaluated using ADMIXTURE [25], based on approximately 370,000 SNPs available from the 1982 Pelotas birth cohort compatible with the HapMap and Human Genome Diversity projects for the Brazilian population [26].

Independent Variables (covariates)

The independent variables that were used in the study were obtained from previous waves of the cohort performed at birth, at 22 and 24 years old. Family income at 24 years was collected continuously and individuals were categorized into tertiles. Besides, a food frequency questionnaire was applied in 2004 for all cohort individuals. In this questionnaire, questions regarding the consumption of sweet foods (ice cream, candies, chocolate, sweet puddings, sodas) and sugar were asked. In addition to consumption itself, the frequency (ranging from 0 to 10) daily, weekly, monthly or yearly was obtained. The gross sum of the amount of daily sugar consumed for everyone in a year was obtained and categorized into tertiles and coded into: a) higher sugar consumption (highest tertile); b) low sugar consumption (intermediate and low sugar consumption)

Power calculation

Power calculation was performed in *OpenEpi* (https://www.openepi.com/Menu/OE_Menu.htm) Considering the present sample size, an alpha of 0.05, prevalence of caries in non-exposed group of 32.05% and 45% in the exposed, this study has 80% power to detect incidence rate ratios of 1.4 or greater.

Statistical methods

Stata statistical package, version 12.0, was used for all statistical analysis (Stata Corporation, College Station, USA). *Hardy–Weinberg equilibrium* and allele frequency estimation of the population regarding the SNPs were tested using the command “*genhw*” [27]. To avoid the population stratification effect, regressions were adjusted by the first ten major components of the principal component analysis (European, African and Native American). Descriptive analysis determined the absolute and relative frequency of independent variables and dental caries trajectory, genotype and allele variables were performed. Fisher exact test

was performed for exploratory analysis of these data and compare the proportions of genotype or alleles.

To analyze the association between caries trajectories and SNPs, two different analysis were conducted with a forward stepwise logistic regression. To avoid possible false positives results we calculated the p value corrections for multiple testing using Bonferroni correction. The primary analysis involved genotype of individuals. Considering genotype analysis, initially, we assuming an additive genetic effect of the T allele in rs307355 (*TAS1R3*) polymorphism (i.e. homozygotes CC individuals = 0; heterozygotes CT =1; and homozygotes TT = 2) and C allele in rs35874116 (*TAS1R2*) (i.e. homozygotes TT individuals = 0; heterozygotes CT =1; and homozygotes CC = 2). So, we also perform an analysis considering a dominant effect of allele T (i.e. homozygotes CC individuals = 0; heterozygotes CT =1; and homozygotes TT = 1) to rs307355 (*TAS1R3*) and C dominant effect (i.e. homozygotes TT individuals = 0; heterozygotes CT =1; and homozygotes CC = 1) to rs35874116 (*TAS1R2*).

Moreover, we tested an interaction term between sugar consumption (low and high) and genotypes. Three models were performed to genotype analysis: (i) unadjusted (i.e. no covariates); (ii) adjusted 1: controlling for ancestry genetic and sex; and (iii) adjusted 2: same covariates in adjusted 1 model, as well as family income and sugar consumption.

For all allele analysis, a forward stepwise multilevel logistic regression model was used, considering mixed effects and two hierarchical levels: genetic- (level 1) and personal-level (level 2). Same adjustment performed in genotype analysis were performed in the allelic analysis.

Aiming to deeply improving the current understanding of rs307355 (*TAS1R3*) and rs35874116 (*TAS1R2*) on dental caries, we also performed genotype and allelic analysis (in both models: unadjusted and adjusted 2) according the different dental caries follow-ups, i.e. a) dental caries at 15 years (no caries = 0; caries = 1), b) dental caries at 24 years (no caries = 0; caries = 1) and dental caries at 31 years (no caries = 0; caries = 1). Yet, we perform a data stratification by sex to investigate possible differences in phenotype between the sex.

Linkage disequilibrium analysis was performed aim to establish the non-random association of alleles. The estimating of D' and r^2 were performed using the SHEsis, an online software (available in <https://analysis.bio-x.cn/myAnalysis.php>) [28, 29]. Haplotype analysis were performed using the same software.

Generalized multifactor dimensionality reduction (GMDR) software was utilized to investigate epistatic interactions, i.e. gene–gene interactions. To perform this analysis, we used

the caries trajectory as main outcome. Logistic regressions models and the genotypes of all SNPs were performed being adjusted by ancestry genetic, sex, income and sugar consumption.

Ethical issues

This project was approved by the UFPel Ethics Committee. All the examinations and interviews were performed with individual authorization after participants signed informed consent forms. Individuals who had treatment needs were identified and referred for treatment.

Results

General information

A total of 539 individuals were assessed in the OHS-13, which corresponds to a response rate of 59.9%. Considering the three follow-ups with oral health data, group-Based trajectory modeling produced two caries trajectories categories (low and high) including 888 individuals (53.8% male), which were present in at least 2 accompaniments and presented genetic analysis. Thus, 68.1% of participants were included in low caries trajectory and 31.9% in high caries trajectory.

The proportion of males was 34.7% in the high caries trajectory group while females was 28.5% (χ^2 test, $p = 0.028$). Similarly, proportion of individuals from lowest income tertile (49.3%) was superior in high caries trajectory compared to higher income tertile (15.2%) (χ^2 test, $p < 0.001$). Most of individuals with European ancestrally genetic (68.2%) presented low caries trajectory, in contrast, most of individuals with African ancestrally genetic (54.6%) presented high caries trajectory (χ^2 test, $p < 0.001$). The groups of sugar consumption showed significant differences in terms of caries trajectory (χ^2 test, $p = 0.003$) where the group with high sugar consumption being more prevalent in the high caries trajectory group.

Genetic informations

The SNPs rs307355 (*TAS1R3*) and rs35874116 (*TAS1R2*) were in Hardy–Weinberg equilibrium ($p > 0.05$) and the minor allele frequency (MAF) was of 0.1475 and 0.3278 in this population, respectively (**table 1**). SNPs were not in linkage disequilibrium: D' (rs307355 rs35874116) = 0.05, r^2 (rs307355 rs35874116) = 0 (figure **Supplementary S1**). Considering the first of ten major components of the principal component analysis, 89.1% of individuals were considered as European and 10.9% African. No individuals were considered as Native American.

The genetic profile – genotypic and allelic - was compared in terms of caries trajectory groups and general characteristics in **table 2**. Considering genotype frequencies, rs307355 (*TAS1R3*) showed significant differences in terms of sex distribution (χ^2 test, $p = 0.036$), ancestry genetic (χ^2 test, $p < 0.001$) sugar consumption (χ^2 test, $p = 0.003$) and caries trajectory (χ^2 test, $p = 0.001$); while no differences in genotype frequencies were found for income (χ^2 test, $p = 0.110$). Similarly, considering allelic frequencies, rs307355 showed significant differences in terms of sex distribution (χ^2 test, $p = 0.0013$), ancestry genetic (χ^2 test, $p < 0.001$), caries

trajectory (χ^2 test, $p < 0.001$) and sugar consumption (χ^2 test, $p < 0.001$). Allelic distribution has not presented differences among income tertile (χ^2 test, $p = 0.069$).

On the other hand, both genotype as allele distribution of rs35874116 (*TAS1R2*) was not associated with any general characteristic (χ^2 test, $p > 0.05$). Complete description of variables according rs35874116 (*TAS1R2*) is available in **Table 3**.

Genetic analyses

Genotypic

Forward stepwise logistic regression model reporting ODDS Ratio (OR) was performed to investigate the genetic influence of *TAS1R3* rs307355 genotype on caries trajectory in the life course (**Table 4**). Considering additive effect, in the unadjusted model the genotype CT (OR = 1.59, CI95% [1.05 – 2.40]) and TT (OR = 5.30, CI95% [1.38 – 20.32]) showed an association with caries trajectory. In the final model (adjusted by ancestry genetic, sex, income and sugar consumption), genotype CT lost the association (OR = 1.39, CI95% [0.89 – 2.17]) while genotype TT was associated with high caries trajectory group (OR = 4.17, CI95% [1.21 – 14.42]). Similar results were observed when T allele was considered as dominant in genotype analysis (CC vs. CT/TT) in unadjusted (OR) (OR = 1.74, CI95% [1.22 – 2.46]) and adjusted model (OR = 1.53, CI95% [1.05 – 2.23]).

The genetic influence of rs35874116 (*TAS1R2*) genotype on caries trajectory in the life course is displayed in **Table 5**. Both additive as dominant models were not associated with caries trajectory in adjusted or unadjusted effects.

Allelic

Allele T of rs307355 (*TAS1R3*) was associated with an increased Odds of 75% in unadjusted model (OR = 1.75, CI95% [1.28 – 2.37]) (**Table 6**); After adjustments by ancestry genetic and sex, the same allele showed an Odds Ratio of 1.64 (CI 95% [1.20 – 2.25]) for being in high caries trajectory group. In the final model adjusted by ancestry genetic, sex, income and sugar consumption, the individuals that presented the allele T showed a 55% higher odds of being in the group with high caries trajectory (OR = 1.55, CI95% [1.11 – 2.15]). On the other hand, rs35874116 (*TAS1R2*) was no associated in allelic models.

Table **Supplementary S2** presents complementary results of genotype and allelic analysis of dental caries according different follow-ups separately (15, 24 and 31 years) for rs307355 (*TAS1R3*). Both genotype as allele were associated with all follow-ups, except the

genotype at 15 years follow-up. Table **Supplementary S3** presents complementary results for rs35874116 (*TAS1R2*). No associations were founded between this SNP and caries at 15, 24 or 31 years.

Haplotype analysis was performed aiming to test the relationship of different allele and dental caries trajectories. The combination of allele “C” of rs307355 (*TAS1R3*) and allele “T” of rs35874116 (*TAS1R2*) was associated with individuals in low caries trajectory group (OR = 0.71, CI95% [0.57 – 0.89]) and combination of allele “T” of rs307355 (*TAS1R3*) and allele “T” of rs35874116 (*TAS1R2*) was associated with individuals in high caries trajectory group (OR = 1.83, CI95% [1.28 – 2.61]).

“G” of rs6441989 (*LTF*), “A” of rs2269436 (*LTF*), “G” of rs743658 (*LTF*), “C” of rs4547741 (*LTF*), “G” of rs11716497 (*LTF*) and “C” of rs7096206 (*MBL2*) was associated with individuals in high caries trajectory group (OR = 1.43 CI95% [1.01 – 2.04], p value = 0.046). Complete haplotype analysis is available in **Table 7**.

Gene-environment Interaction

Sugar consumption significantly interacted with the allelic and genotype (dominant effect) of rs307355 (*TAS1R3*) modifying dental caries trajectory. Overall, it was possible to observe that presence of T-allele and high sugar consumption increased the risk to be in high caries trajectory group. Positive interactions were observed in T-dominant effect (CT/TT-genotype) and high consumption of caries (p = 0.002), increasing the odds to be in high caries trajectory group in 98%. Similarly, effect modification of caries trajectory was observed in allelic analysis. T-allele in high sugar consumption was associated with higher odds to be in high caries trajectory group (p = 0.008). Effect modification between genotype (considering additive effect) was not observed (p = 0.147). No effect gene-environment effect modification was observed considering rs35874116 (*TAS1R2*).

Sex influence

When stratifying analysis by sex, genotypes of rs307355 (*TAS1R3*) behaved differently according to this variable (**Figure 2**). While an additive and linear effect in caries trajectory was observed in males with TC and TT genotype, no effect was observed in females with genotype TC. However, females with TT genotype presented similar risk to be in high caries trajectory

than males. No significant difference was observed in sex stratifying analysis by sex in rs35874116 (*TAS1R2*).

Epistasis Analysis (Gene-gene Interaction)

Table 8 shows the compilation of results for gene-gene interaction and dental caries trajectory in the life course achieved from the generalized multifactor dimensionality reduction analysis. We found significant associations between rs307355 (*TAS1R3*) and rs35874116 (*TAS1R2*) ($p = 0.034$); This result indicates a potential gene-gene interaction between these SNPs. Furthermore, this model shows a high cross validation consistency of 10/10, an elevated training-balanced accuracy of 56.09% and testing-balanced accuracy of 55.77%. Therefore, combination of these SNPs presented an Odds of 1.72 (CI95% 1.04 – 2.84) of being in high in caries trajectory group (**Figure 3**).

Discussion

In this study, we assessed the hypothesis that trajectory of dental caries in the life course can be influenced by the rs307355 (*TAS1R3*) and rs35874116 (*TAS1R2*) genotypes and allele; moreover, we investigated if sugar consumption modifies the association between SNPs and caries trajectory. Our analysis corroborates with current literature [15] confirming the association between rs307355 (*TAS1R3*) and dental caries. Our results showed that genotypes TC and TT, as well as allele T of rs307355 were associated with high caries trajectory in the life course. In this way, we added an interaction term between rs307355 (*TAS1R3*) and sugar consumption to investigate possible gene-environment modification. Thus, in both (genotypic T-dominant effect and allelic) analysis strategies we found that high sugar consumption modifies the relationship between allele T and dental caries trajectory, increasing the odds of caries. This result emphasizes the previous finding that there exists an important relationship between T-alleles and sugar consumption, since that T-allele increased the consumption of sweets [12]. Although we did not find direct association of rs35874116 (*TAS1R2*) and caries trajectory – and partially discordant from previous studies [9, 15, 30] – we found an important association between rs307355 (*TAS1R3*) and rs35874116 (*TAS1R2*) both in haplotype analysis even as in gene-gene interaction on dental caries trajectory. Therefore, highlight the complex architecture of genetic influence of dental caries and showing a possible epistatic interaction underlying initial observations of rs35874116 (*TAS1R2*) since dental caries seem to be a complex trait. Moreover, we also observed in rs307355 (*TAS1R3*) that in females the allele T in TC-genotype presented a recessive effect; while in males the allele presented a dominant effect.

TAS1R3 has been described as the responsible for the saccharin preferring phenotype [31] localized in chromosome Chromosome 1 at position 1265154. In fact, sweet tastes are intermediated by G-protein-coupled receptors superfamily, being mainly expressed in the epithelial cells of tongue and palate, but not only in these tissues: gastrointestinal tract [32, 33], pancreatic islets [32] and the brain [34, 35] are another tissues with T1R expression. Cells of taste receptors of G-protein-coupled family can present three members: T1R1, T1R2 and T1R3. Initial evidence that *TAS1R3* plays an important role in sweet taste was through the mice study that isolated *Tas1r3* from taster mice and rescued non-taster mice by transfection with the taster form of the gene [36]. The receptors of sweet are composed T1R2+T1R3 [37] and although the studies present co-expression of T1R2 and T1R3 as heterodimers [36, 38] a recent review [31] have present the possibility of T1R2+T1R3 heterodimers have a function as sweet taste receptors alone [39, 40]. In fact, due to proximity between them, the rs307355 is in

disequilibrium linkage with wide number of SNPs of *TAS1R2* (rs3935570, D' 0.366; rs4920566, D' 0.599; rs9701796, D' 0.534) according to studies that found association with dental caries experience [15, 41, 42] when based on Human (GRCh37.p13 and available on: http://grch37.ensembl.org/Homo_sapiens). However, we did not observe linkage disequilibrium in our sample between rs307355 (*TAS1R3*) and rs35874116 (*TAS1R2*). In fact, presents result show that epistatic interaction between these SNPs can increase the odds ratio to dental caries trajectory and identify genetic interactions underlying the phenotypes considering genetic architecture of complex traits.

Corroborating with our results, a cross-sectional study of 184 Turkey children with age range 7 to 12 years found association between the heterozygous genotype (TC) of rs307355 (*TAS1R3*) and dental caries [15]. Between the moderate-risk group (i.e., dft+DMFT between 4-7) the genotype TC was the most prevalent. This tendency was reproduced in our study where 29.7% of individuals with high caries trajectory presented the genotype TC compared with 21.9% of individuals with low caries trajectory. However, in adjusted logistic regression considering an additive effect (i.e., CC = 0, TC = 1 and TT = 2), we have not found association of genotype TC; despite genotype TT was associated with an odds four-fold higher of being in high caries group. It is important highlight that only two individuals with genotype TT were found in the study of Haznedaroglu, et al. [15], and, thus, the authors excluded TT of analysis; In fact, we also found a low number of individuals with this genotype (n=14). However, differently of this study, we made the decision to keep this genotype. This decision impact in our findings, decreasing the power in some strata and widening the confidence intervals limit. Therefore, it must be taken into consideration when analyzing our data in additive effect. Moreover, when we consider a possible dominant effect of allele T in the genotype (i.e., CC = 0, CT = 1 and TT = 1) we observed also a higher odds of high caries trajectory in individuals CT/TT, even after adjustments.

So, we also tested the hypothesis that risk alleles might be linked with higher odds of dental caries. Therefore, we chose an analytical approach that consider two hierarchical levels as previously suggest [43]: genetic- (level 1) and personal-level (level 2). Thus, we clustered the alleles in each individual. In this way, allele T of rs307355 (*TAS1R3*) presented an Odds 55% higher to be in high caries trajectory. It is important highlight that allelic analysis was similarly associated when non-multilevel analysis was performed (data not showed), contributing to the robustness of our results. Moreover, as complementary analysis in the **Table S1** (see Supplementary Material, available as Supplementary data for detailed the analysis in all follow

ups), we repeat the genotype and allelic analysis – unadjusted and adjusted - of dental caries according different follow-ups (15, 24 and 31 years old). In this analysis it was observed that allele T was associated in all analyzes, except at 15 years in the adjusted (2) model. Besides, considering the genotype analysis, only in adjusted 15 years follow-up associations were not found. These observations reinforce the robustness of our results and highlight the need of studies with longitudinal design.

An unexpected result was observed linked to individual sex. In fact, we observed different relationship between heterozygote genotype of rs307355 (*TAS1R3*) (i.e. CT) and the phenotype according to gender. In males, a linear trend effect in caries trajectory was observed with the addition of one (TC) and two (TT) alleles in genotype; on the other hand, no effect on caries trajectory was observed in females with genotype TC, despite females with TT genotype presented similar risk to be in high caries trajectory group than males. In fact, one study investigating the influence of *TAS1R3* on sensitivity to sweet taste observed that sex of individuals was an important factor to differences among children in Sucrose detection thresholds [44]. However, the available literature on *TAS1R3* reports no other observations in this regard. One of the possible explanation for this result can be linked to epistatic interactions (i. e. Gene x Gene interactions). It would be possible that other gene or SNP present in X chromosome or unpaired region of Y chromosome interact with *TAS1R3*. Besides, interactions between SNPs related to *TASR2* can be expected, since that co-expression of T1R2 and T1R3 as heterodimers are reported. A less possible explanation can be through the hormonal effect, which can sex-limited gene expression.

Our study had limitations that need to be discussed. Some of them relate to the small numbers of individuals in some categories. Genotype analysis with additive effect presented the low number of individuals and should be evaluated carefully. Interpreting the magnitude of estimates, especially in this group, for the effect of rs307355 (*TAS1R3*) and dental caries trajectory requires caution. Considering that is a longitudinal study, some losses in the follow-ups were observed. To avoid power decrease, group-based trajectory modeling inputted missing data. Thus, individuals who were not followed in one of the accompaniments were modeled and not excluded of longitudinal analysis. This analytical approach should be emphasized. Besides, only two SNPs were investigated in the present study, while the literature presents diverse number of SNPs related with taste genes can influence dental caries experience. Thus, further analysis should include other SNP of *TAS1R3* well as *TAS1R2* *GLUT2* and *TAS2R38*. We suggest that epistatic interaction should be performed in further studies to confirm our results

and investigating possible influence with sex of individuals. It is also possible that there are others gene-gene interactions (epistatic) implicated in this pathway and this was not investigated in our study. This can imply that focusing only two variants in two genes perhaps not capture the whole complexity involved. It was highlight when we only observed positive influence of rs35874116 (*TAS1R2*) in caries when epistatic interaction was considered.

So, strengths of our study also need to be highlight. We use a well-characterized and large birth cohort. Group-Based trajectory modeling was used to identify similar groups of caries experience in the life course and this is the first study to investigate the influence of genes with longitudinal caries evaluation. Our results were reproducible in all follow-ups, highlight the robustness of our findings. Other strengths were that we the robust analytical approach carries that investigate the of gene-gene interaction and gene-environment modification, were found in rs307355 (*TAS1R3*) an increase of dental caries in T-allele individuals in high sugar consumption group.

Conclusion

In this study we observed that high trajectory of untreated dental caries in the life course was positively associated with the rs307355 (*TAS1R3*) genotypes. TT-genotype in additive model and CT/TT-genotype in dominant model were associated with high odds to be in high trajectory of untreated dental caries. Similarly, T-allele was associated with high untreated caries trajectory group in the individuals of present birth cohort. Moreover, we tested and confirmed the hypothesis that sugar consumption significantly interacted with the allelic and genotype (dominant effect) of rs307355 modifying dental caries trajectory. Thus, seem exist a Gene-Environment modification. Moreover, epistatic interactions between rs307355 (*TAS1R3*) and rs35874116 (*TAS1R2*) can increase trajectory of untreated dental caries. Presents conclusions must be interpreted taking into account all possible biases of present study.

Compliance with Ethical Standards:

Conflict of Interest: Luiz Alexandre Chisini declares that he has no conflict of interest. Francinde dos Santos Costa declares that she has no conflict of interest. Marcus Cristian Muniz Conde declares that he has no conflict of interest. Luciana Tovo-Rodrigues declares that she has no conflict of interest. Marcus Bernardo Lessa Horta declares that he has no conflict of interest. Flávio Fernando Demarco declares that he has no conflict of interest. Marcos Britto Correa declares that he has no conflict of interest

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Table 1. Description of allele frequency and results of Hardy-Weinberg equilibrium

		Hardy–Weinberg equilibrium		
rs307355 (<i>TAS1R3</i>)	Allele Frequency	Tests	p value	
	C	0.8525	Pearson chi2	0.184
			likelihood-ratio chi2	0.191
	T	0.1475	Exact significance prob	0.182
rs35874116 (<i>TAS1R2</i>)				
		Pearson chi2	0.846	
T	0.6722	likelihood-ratio chi2	0.846	
		Exact significance prob	0.879	
C	0.3278			

Table 2. Description of variables by rs307355 (*TAS1R3*) and outcome: dental Caries trajectory (low and high)

Variables	Caries Trajectory (15, 24 and 31 years old), N (%)			Genotype rs307355 (<i>TAS1R3</i>), N (%)				Allele rs307355 (<i>TAS1R3</i>), N (%)		
	Low	High	p value	CC	TC	TT	p value	C	T	p value
Sex distribution			0.028				0.036			0.013
Male	312 (65.27)	166 (34.73)		270 (77.36)	72 (20.63)	7 (2.01)		612 (87.68)	86 (12.32)	
Female	293 (71.46)	117 (28.54)		222 (68.73)	94 (29.10)	7 (2.17)		538 (83.28)	108 (16.72)	
Income tertile (at 23 y)			<0.001				0.110			0.069
Lowest tertile (1 st)	115 (50.66)	112 (49.34)		127 (66.84)	58 (30.53)	5 (2.63)		312 (82.11)	68 (17.89)	
Medium (2 nd)	187 (62.75)	111 (37.25)		196 (75.38)	57 (21.92)	7 (2.69)		449 (86.35)	71 (13.65)	
Higher tertile (3 rd)	223 (29.66)	40 (15.21)		169 (76.13)	51 (22.97)	2 (0.90)		389 (87.61)	55 (12.39)	
Ancestry-informative genetic			<0.001				<0.001			<0.001
European	428 (68.15)	200 (31.85)		463 (75.41)	141 (22.96)	10 (1.63)		1067 (86.89)	161 (13.11)	
African	35 (45.45)	42 (54.55)		29 (50.00)	25 (43.10)	4 (6.90)		83 (71.55)	33 (28.45)	
Sugar consumption (tertile)			0.003				0.001			<0.001
Low	387 (69.11)	173 (30.89)		335 (77.19)	95 (21.89)	4 (0.92)		765 (88.13)	103 (11.87)	
High	138 (58.72)	97 (41.28)		158 (66.11)	71 (29.71)	10 (4.18)		387 (80.96)	91 (19.04)	

Caries Trajectory				<i>0.001</i>		<i><0.001</i>
Low	335 (77.19)	95 (21.89)	4 (0.92)		765 (88.13)	103 (11.87)
High	158 (66.11)	71 (29.71)	10 (4.18)		387 (80.96)	91 (19.04)

p-values are presented in italics when the differences are significant ($p < 0.05$); Fischer exact test

Table 3. Description of variables according rs35874116 (*TAS1R2*)

Variables	Genotype rs35874116 (<i>TAS1R2</i>), N (%)				Allele rs35874116 (<i>TAS1R2</i>), N (%)		
	TT	CT	CC	p value	T	C	p value
Sex distribution				0.712			0.283
Male	167 (48.27)	146 (42.20)	33 (9.54)		480 (69.36)	212 (30.64)	
Female	145 (45.17)	145 (45.17)	31 (9.66)		435 (67.76)	207 (32.24)	
Income tertile (at 23 y)				0.168			0.527
Lowest tertile (1 st)	84 (44.44)	93 (49.21)	12 (6.35)		261 (69.05)	117 (30.95)	
Medium (2 nd)	117 (45.35)	111 (43.02)	30 (11.63)		345 (66.86)	171 (33.14)	
Higher tertile (3 rd)	111 (50.45)	87 (39.55)	22 (10.00)		309 (70.23)	131 (29.77)	
Ancestry-informative genetic				0.652			0.259
European	288 (47.29)	263 (43.19)	58 (9.52)		839 (68.88)	379 (31.12)	
African	24 (41.38)	28 (48.28)	6 (10.34)		76 (65.52)	40 (34.48)	
Sugar consumption (tertile)				0.328			0.393
Low	213 (45.61)	212 (45.40)	42 (8.99)		638 (68.31)	296 (31.69)	
High	99 (49.50)	79 (39.50)	22 (11.00)		277 (69.25)	123 (30.75)	
Caries Trajectory				0.625			0.189
Low	208 (47.93)	187 (43.09)	39 (8.99)		603 (69.47)	265 (30.53)	
High	104 (44.64)	104 (44.64)	25 (10.73)		312 (66.95)	154 (33.05)	

p-values are presented in italics when the differences are significant ($p < 0.05$); Fischer exact test

Table 4. Forward stepwise logistic regression reporting ODDS Ratio (OR) of the association between *TAS1R3* rs307355 genotype (additive and recessive effect) and dental caries trajectory. An interaction of rs307355 and sugar consumption (daily sugar consumption) was performed using an interaction term between the variables. (n = 673)

Additive effect	OR (95% CI)	p value	Dominant effect	OR (95% CI)	p value
Unadjusted			Unadjusted		
CC	1		CC	1	
CT	1.59 (1.05 – 2.40)	0.025	CT/TT	1.74 (1.22 – 2.46)	0.002
TT	5.30 (1.38 – 20.32)	0.011			
Adjusted (1)			Adjusted (1)		
CC	1		CC	1	
CT	1.51 (0.99 – 2.31)	0.059	CT/TT	1.64 (1.14 – 2.35)	0.007
TT	4.52 (1.15 – 17.74)	0.027			
Adjusted (2)			Adjusted (2)		
CC	1		CC	1	
CT	1.39 (0.89 – 2.17)	0.192	CT/TT	1.53 (1.05 – 2.23)	0.026
TT	4.17 (1.21 – 14.42)	0.024			
Adjusted (2) + interaction with sugar consumption			Adjusted (2) + interaction with sugar consumption		
CC # Low	1		CC # Low	1	
CC # High	1.36 (0.88 – 2.09)	0.165	CC # High	1.35 (0.88 – 2.08)	0.168
CT # Low	1.49 (0.93 – 2.40)	0.100	CT/TT # Low	1.57 (0.98 – 2.49)	0.055
CT # High	1.74 (0.95 – 3.18)	0.071	CT/TT # High	1.98 (1.10 – 3.56)	0.022
TT # Low	2.78 (0.70 – 10.94)	0.144			
TT # High	-				

p value was adjusted by multiple comparisons; Adjusted (1): Ancestry-informative genetic and sex; Adjusted (2): Ancestry-informative genetic, sex, income and sugar consumption. Interaction: rs307355 and sugar consumption

Table 5. Forward stepwise logistic regression reporting ODDS Ratio (OR) of the association between *TAS1R2* rs35874116 genotype (additive and recessive effect) and dental caries trajectory. An interaction of rs35874116 and sugar consumption (daily sugar consumption) was performed using an interaction term between the variables. (n = 673)

Additive effect	OR (95% CI)	p value	Dominant effect	OR (95% CI)	p value
Unadjusted			Unadjusted		
TT	1		TT	1	
CT	1.10 (0.75 – 1.61)	1.000	CT/CC	1.13 (0.83 – 1.56)	0.432
CC	1.29 (0.69 – 2.43)	0.700			
Adjusted (1)			Adjusted (1)		
TT	1		TT	1	
CT	1.09 (0.74 – 1.69)	1.000	CT/CC	1.13 (0.82 – 1.55)	0.473
CC	1.29 (0.68 – 2.43)	0.750			
Adjusted (2)			Adjusted (2)		
TT	1		TT	1	
CT	1.03 (0.69 – 1.54)	1.000	CT/CC	1.09 (0.78 – 1.53)	0.613
CC	1.04 (0.72 – 2.73)	0.498			
Adjusted (2) + interaction with sugar consumption			Adjusted (2) + interaction with sugar consumption		
TT # Low	1		TT # Low	1	
TT # High	1.13 (0.68 – 1.09)	0.655	TT # High	1.13 (0.69 – 1.91)	0.648
CT # Low	0.96 (0.62 – 1.47)	0.834	CT/CC # Low	0.98 (0.65 – 1.47)	0.911
CT # High	1.34 (0.76 – 2.35)	0.300	CT/CC # High	1.55 (0.92 – 2.58)	0.094
CC # Low	1.08 (0.52 – 2.27)	0.830			
CC # High	2.52 (0.99 – 6.38)	0.051			

p value was adjusted by multiple comparisons; Adjusted (1): Ancestry-informative genetic and sex; Adjusted (2): Ancestry-informative genetic, sex, income and sugar consumption. Interaction: rs307355 and sugar consumption

Table 6. Forward stepwise multilevel logistic regression reporting ODDS Ratio (OR) of the association between *TAS1R3* rs307355 / *TAS1R2* rs35874116 allele and dental caries trajectory. An interaction of rs307355 / rs35874116 and sugar consumption (daily sugar consumption) was performed using an interaction term between the variables. (n = 673)

rs307355 (<i>TAS1R3</i>)	OR (95% CI)	p value	rs35874116 (<i>TAS1R2</i>)	OR (95% CI)	p value
Unadjusted		<0.001	Unadjusted		
C	1		T	1	
T	1.75 (1.28 – 2.37)		C	0.11 (0.87 – 1.42)	0.397
Adjusted (1)		0.002	Adjusted (1)		
C	1		T	1	
T	1.64 (1.20 – 2.25)		C	1.12 (0.88 – 1.42)	0.371
Adjusted (2)		0.009	Adjusted (2)		
C	1		T	1	
T	1.55 (1.11 – 2.15)		C	1.12 (0.87 – 1.45)	0.367
Adjusted (2) + interaction with sugar consumption			Adjusted (2) + interaction with sugar consumption		
C # Low	1		T # Low	1	
C # High	1.33 (1.01 – 1.76)	0.042	T # High	1.21 (0.89- 1.64)	0.232
T # Low	1.34 (0.96 – 2.31)	0.059	C # Low	1.01 (0.74 – 1.37)	0.969
T # High	2.08 (1.21 – 3.57)	0.008	C # High	1.17 (0.95 – 2.58)	0.078

p value was adjusted by multiple comparisons; Adjusted (1): Ancestry-informative genetic and sex; Adjusted (2): Ancestry-informative genetic, sex, income and sugar consumption. Interaction: rs307355 and sugar consumption

Table 7. Haplotype analysis of loci for hap-analysis: rs307355 (*TAS1R3*) rs35874116 (*TAS1R2*).

Haplotype	High caries	Downward caries	Fisher's p	Odds Ratio (95% CI)
	Trajectory	Trajectory		
	Frequency	Frequency		
C C	0.279	0.268	0.065	1.06 (0.824 – 1.36)
C T	0.531	0.614	0.003	0.71 (0.57 – 0.89)
T C	0.052	0.038	0.220	1.40 (0.82 – 2.38)
T T	0.139	0.081	<0.001	1.83 (1.28 – 2.61)

Table 8. Summary of Generalized Multifactor Dimensionality Reduction results for gene-gene interactions.

Best Model	Tr-BA (%)	Te-BA (%)	Sign test (<i>p</i>)	CVC	P value	Odds Ratio (CI95%)
rs307355 (<i>TAS1R3</i>) / rs35874116 (<i>TAS1R2</i>)	56.09	55.77	9 (0.011)	10/10	0.034	1.72 (1.04 – 2.84)

Abbreviations: CVC, cross validation consistency; Te-BA, testing-balanced accuracy; Tr-BA, training balanced accuracy; Results were adjusted by ancestry genetic, sex, income trajectory, sugar consumption, oral health habits

Figure S1. Linkage disequilibrium of rs307355 (*TAS1R3*) rs35874116 (*TAS1R2*). The Single Nucleotides Polymorphisms were tested using SHEsis and estimated with D' and r^2 . D' (rs307355 rs35874116) = 0.05, r^2 (rs307355 rs35874116) = 0

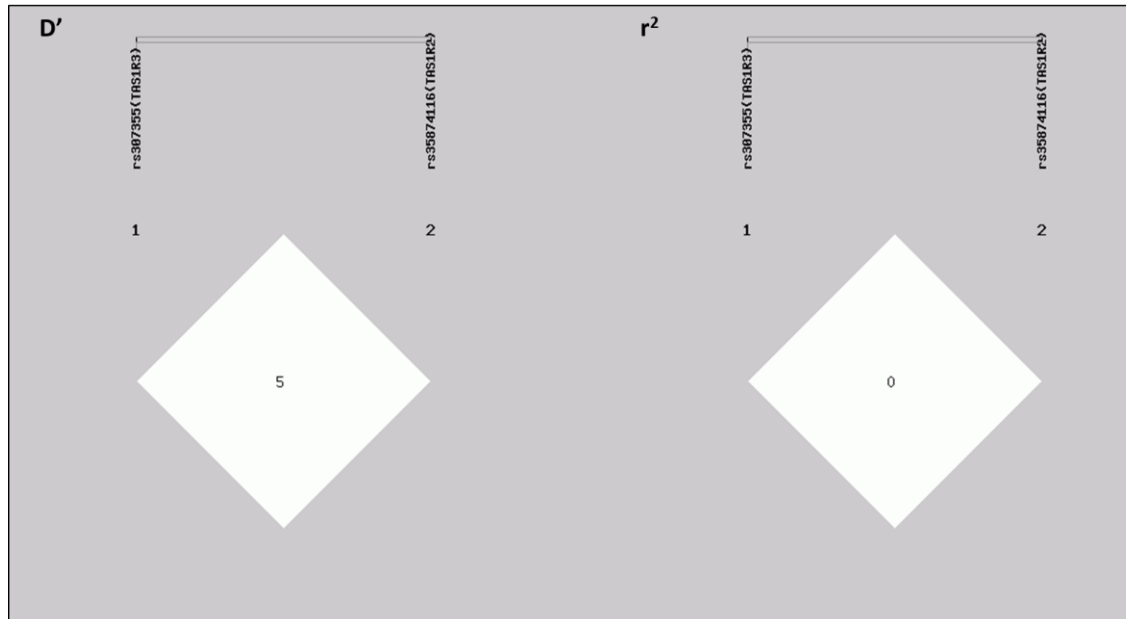


Table S2. Genotype and allelic analysis of dental caries according different follow-ups

rs307355 (TAS1R3)	Dental caries 15 years (n=667)		Dental caries 24 years (n=599)		Dental caries 31 years (n=446)	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
	Genotype Analysis Logistic regression					
Unadjusted						
CC	1		1		1	
CT	1.43 (0.88 – 2.33)	0.203	1.48 (0.97 – 2.27)	0.079	1.76 (1.07 – 2.91)	0.023
TT	4.86 (0.47 – 50.27)	0.259	12.79 (1.21 – 134.30)	0.030	4.20 (0.90 – 19.56)	0.073
Adjusted (2)						
CC	1		1		1	
CT	1.32 (0.85 – 2.05)	0.214	1.28 (0.85 – 1.19)	0.228	1.60 (1.00 – 2.55)	0.049
TT	4.30 (0.54 – 33.77)	0.165	9.12 (1.13 – 74.02)	0.038	3.18 (0.79 – 12.65)	0.101
	Allelic Analysis Multilevel logistic regression					
Unadjusted		0.027		0.001		0.001
C	1		1		1	
T	1.54 (1.04 – 2.27)		1.71 (1.23 – 2.38)		1.86 (1.28- 2.70)	
Adjusted (2)		0.076		0.031		0.014
C	1		1		1	
T	1.43 (0.96 – 2.12)		1.47 (1.04 – 2.08)		1.65 (1.10 – 2.45)	

p value was adjusted by multiple comparisons; Adjusted (1): Ancestry-informative genetic and sex; Adjusted (2): Ancestry-informative genetic, sex, income and sugar consumption. Interaction: rs307355 and sugar consumption

Table S3. Genotype and allelic analysis of dental caries according different follow-ups

rs35874116 (TAS1R2)	Dental caries 15 years (n=667)		Dental caries 24 years (n=599)		Dental caries 31 years (n=446)	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
	Genotype Analysis Logistic regression					
Unadjusted						
TT	1		1		1	
CT	0.67 (0.44 – 1.03)	0.074	0.93 (0.62 – 1.36)	1.000	1.22 (0.58 – 2.55)	1.000
CC	0.91 (0.44 – 1.88)	1.000	1.18 (0.62 – 2.23)	1.000	0.96 (0.45 – 2.01)	1.000
Adjusted (2)						
TT	1		1		1	
CT	0.65 (0.42 – 1.01)	0.054	0.91 (0.60 – 1.37)	1.000	1.20 (0.75 – 1.94)	0.764
CC	0.91 (0.43 – 1.90)	1.000	1.24 (0.64 – 2.43)	0.930	1.10 (0.50 – 2.38)	1.000
	Allelic Analysis Multilevel logistic regression					
Unadjusted						
T	1		1		1	
C	0.84 (0.65 – 1.09)	0.199	1.02 (0.80 – 1.31)	0.842	1.10 (0.83 – 1.46)	0.499
Adjusted (2)						
T	1		1		1	
C	0.83 (0.64 – 1.09)	0.178	1.04 (0.80 – 1.34)	0.779	1.09 (0.82 – 1.47)	0.542

p value was adjusted by multiple comparisons; Adjusted (1): Ancestry-informative genetic and sex; Adjusted (2): Ancestry-informative genetic, sex, income and sugar consumption. Interaction: rs307355 and sugar consumption

Figure 1. Dental Caries trajectory in the life course (group-based trajectory modelling) (n=888)

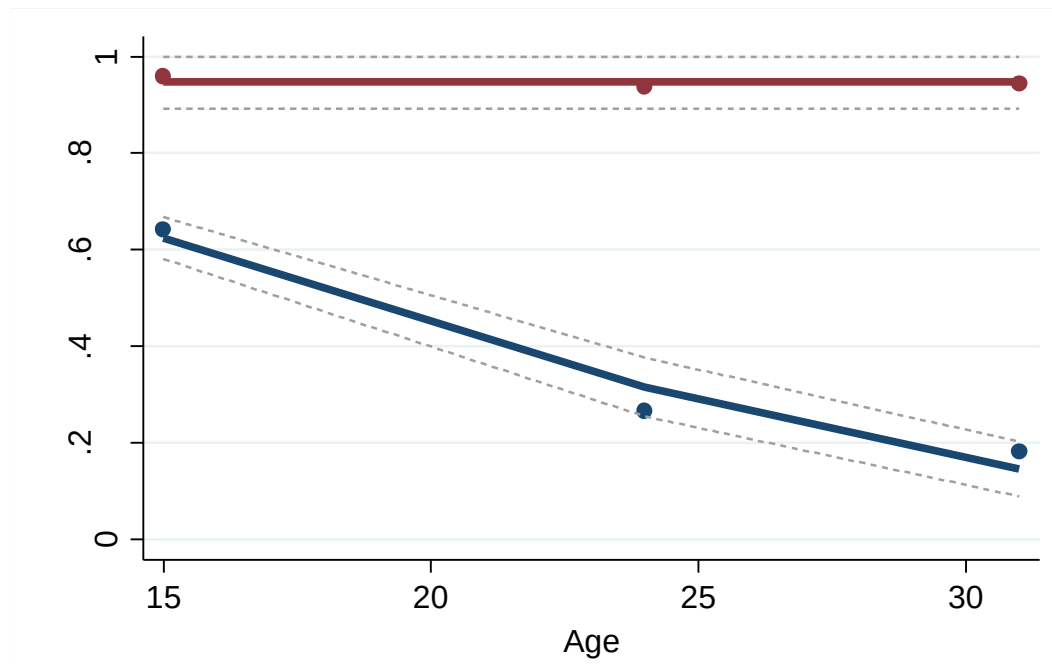
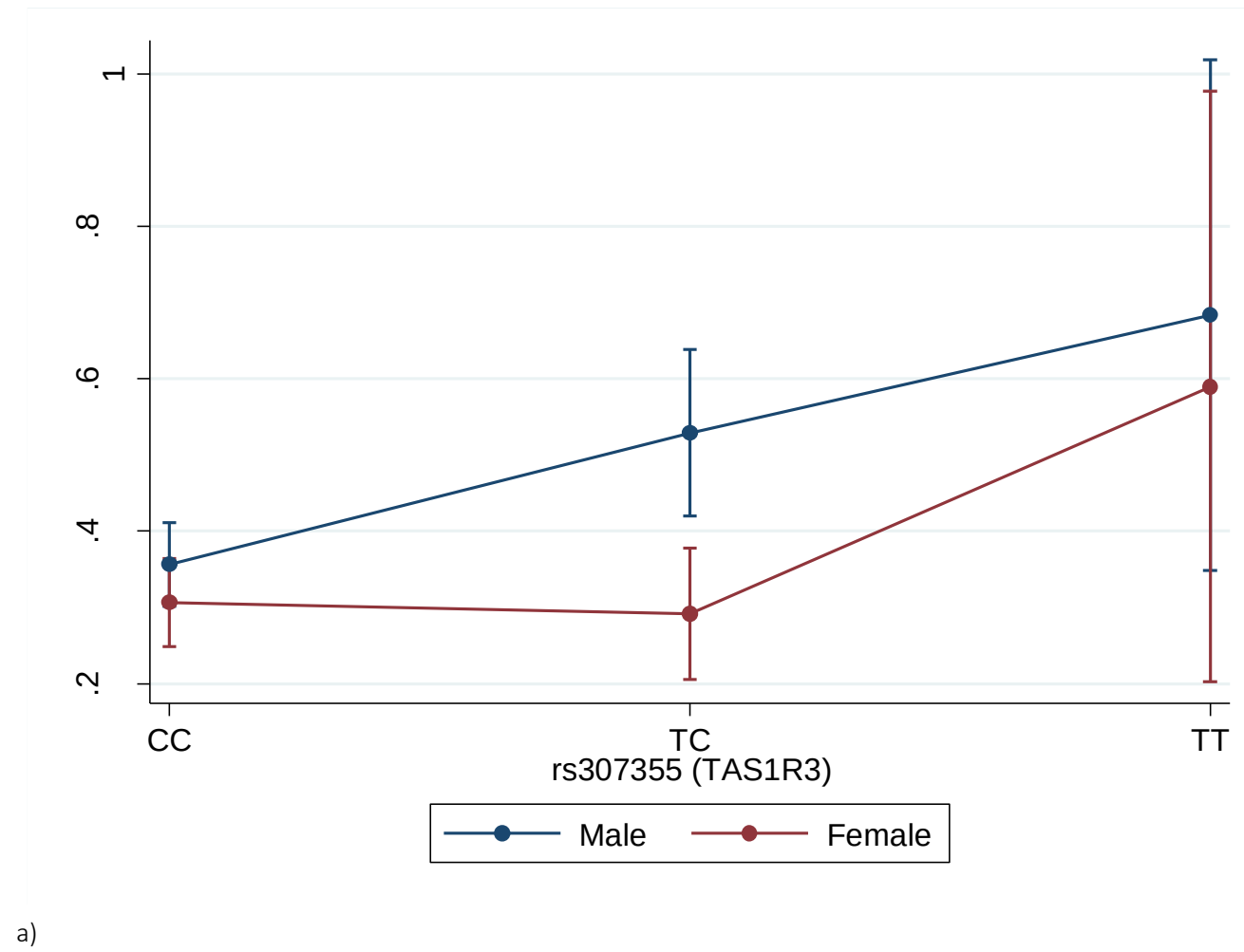


Figure 2. Caries trajectory in the life course in males and females according different genotypes of a) rs307355 (*TAS1R3*) and b) rs35874116 (*TAS1R2*).



b)

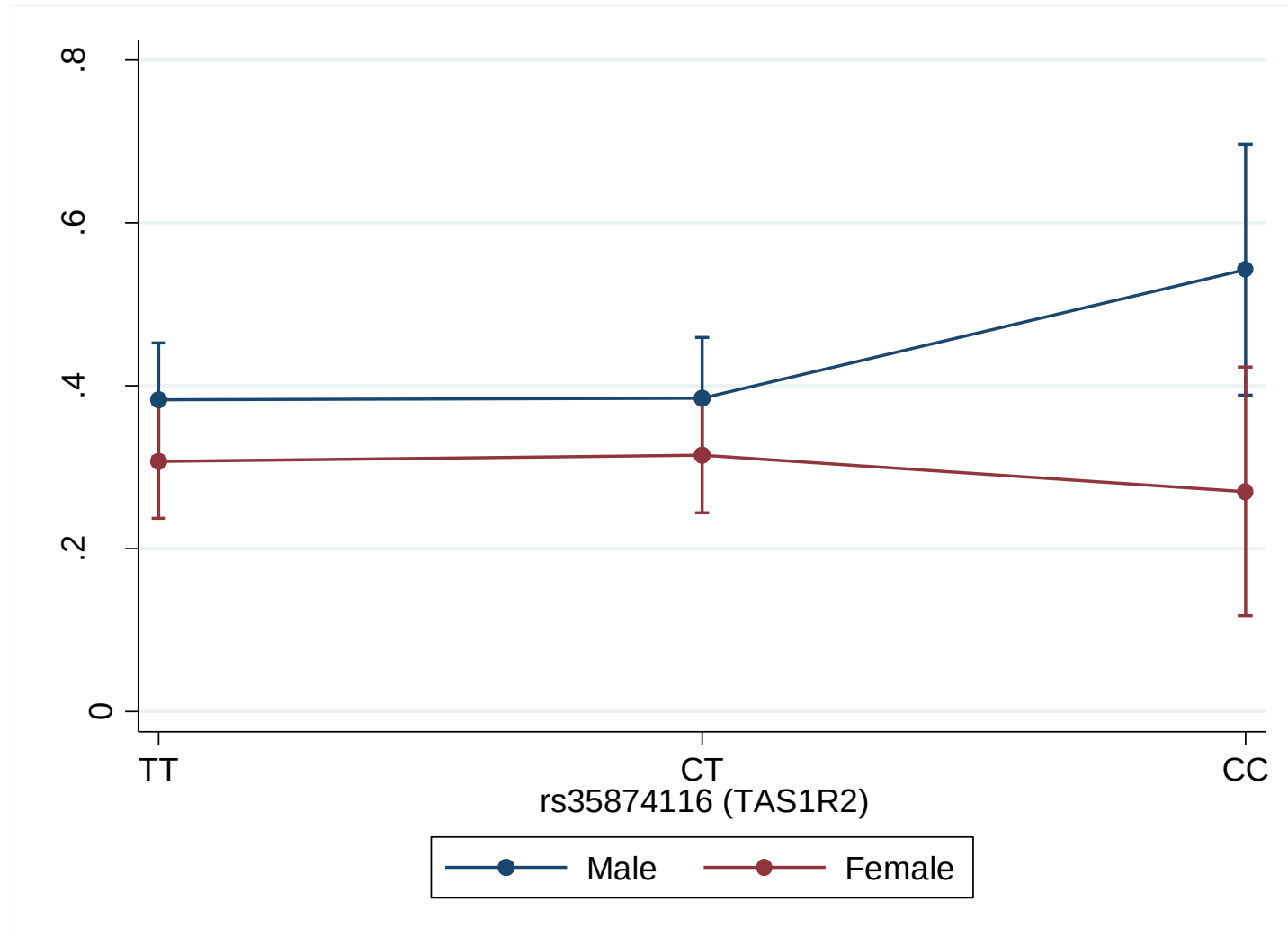
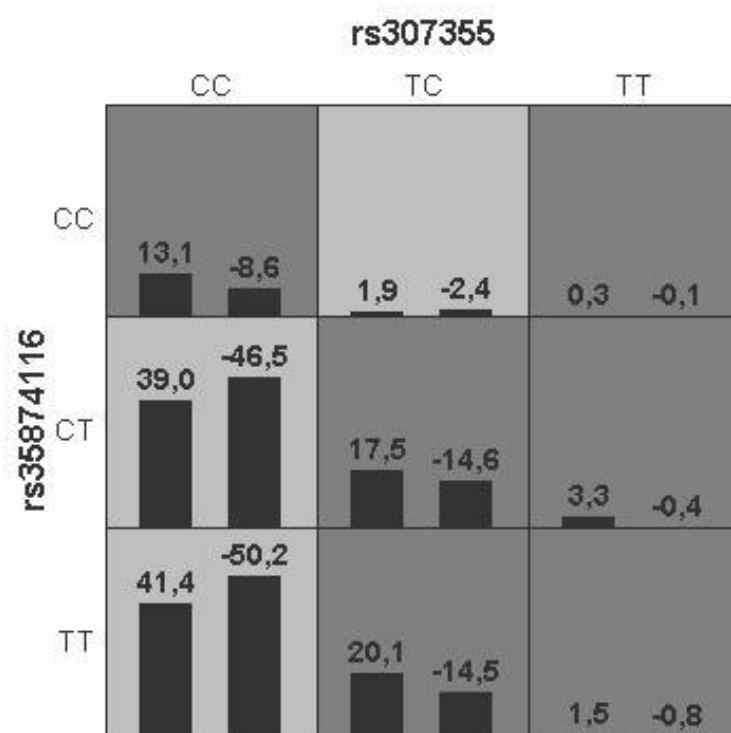


Figure 3. Graphical model of gene–gene interaction analysis



5.2 Artigo 7

Artigo formatado seguindo as normas da Revista *Clinical Oral Investigations*.

Genes in the pathway of tooth mineral tissues and trajectory of dental caries: Results of a longitudinal birth cohort study

Running title: Tooth-mineral genes *and* Dental Caries

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Key words: Polymorphisms. Dental caries. *Tooth-mineral genes*. Genetic. Gene.

Declarations of conflict of interest: none

Running title: Tooth-mineral genes *and Dental Caries*

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Cover Letter

To: Professor Dr. Matthias Hannig
Editor-in-Chief,

Dear Editor:

Based on the importance of *Clinical Oral Investigations*, we are sending the manuscript entitled “**Genes in the pathway of tooth mineral tissues and trajectory of dental caries: Results of a longitudinal birth cohort study**” to be appraised by the Journal’s Editorial Board.

In the present study we deeply investigated - with a longitudinal birth cohort design - the association between dental caries trajectory and SNPs present in genes of the pathway of tooth mineral tissues (TUFT1, MMP20, MMP13, MMP2, DLX3, TIMP2, BMP7 and TFIP11), increasing thus the knowledge of current literature. rs4970957 (TUFT1) in allelic, additive and dominant effect was associated with presence of dental caries at 31 years, while allele C of rs243847 (MMP2) was associated with caries at 15 years in unadjusted models. The lack of association of these SNPs in other follow-ups highlight the need of carefully interpretation of our results. Furthermore, we identify epistatic interaction between investigated SNPs and caries trajectory in the life course. GMDR analysis found a three-locus model significant involving rs243847 (MMP2), rs2303466 (DLX3) and rs388286 (BMP7). Individuals with the combination of these SNPs showed an Odds 2.51 (1.54 – 4.09) to be in high caries trajectory group.

This is an original manuscript and has not been considered for publication elsewhere. The paper was read and approved by all authors. All authors have made substantive contribution to this study, and all have reviewed the final paper prior to its submission. The authors declare that there are no potential competing interests. Furthermore, I attest the validity and legitimacy of data and its interpretation. There are no conflicts of interest for authors listed above. We sign for and accept responsibility for releasing this material.

Thank you very much for your consideration.

Yours sincerely,

Prof. Marcos Britto Corrêa, PhD. (**Corresponding Author**)
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Genes in the pathway of tooth mineral tissues and trajectory of dental caries: Results of a longitudinal birth cohort study

Running title: Tooth-mineral genes *and Dental Caries*

Genes in the pathway of tooth mineral tissues and trajectory of dental caries: Results of a longitudinal birth cohort study

Abstract:

Aim: investigate if the dental caries is associated with Single Nucleotide Polymorphisms (SNPs) presents in the genes of tooth mineral tissues.

Methods: Representative sample of all 5,914 births from the 1982 in Pelotas birth cohort study was prospectively investigated. Caries trajectory in the life course was assessed at 15 (n=888) 24 (n=720) and 31 years old (n=539). Associations were investigated using logistic regression models with Bonferroni multiple correction test considering allelic and genotype effects (additive/dominant). Models were adjusted by ancestry genetic, sex, family income, sugar consumption and trajectory of gingival bleeding. Generalized multifactor dimensionality reduction software was used to analyze gene–gene interactions.

Results: rs4970957 (*TUFT1*) presented weak association with dental caries at 31 years. This SNP was not associated with caries in any other follow-ups or caries trajectory. rs243847 (*MMP2*) was associated with dental caries only at 15 years in allelic analysis. Epistatic interaction was found significant in a three-locus models ($p < 0.001$) involving rs243847 (*MMP2*), rs2303466 (*DLX3*) and rs388286 (*BMP7*). The analysis of combination of these genotypes showed and odds of 2.51 (1.54 – 4.09) to be in high caries trajectory group.

Conclusions: Although rs4970957 (*TUFT1*) has been associated with dental caries at 31 years old in adjusted model and rs243847 (*MMP2*) at 15 years old in unadjusted model, presents results have not supported with strength that this SNPS are associated with dental caries in the life course in the present sample. However, we found an epistatic interaction which seems to be an important pathway to explain susceptibility for dental caries.

Introduction

Although knowledge about etiopathogenesis of dental caries have evolved from initial Keyes model to robustness and complex multifactorial models that included the influence of contextual and behavioral of individuals, caries prevalence is still a disease with elevate prevalence at global level [Kassebaum et al., 2017] affecting the quality of life of the individuals [Jaggi et al., 2019].

Using the tools developed by the Human Genome Project together with the increase of interest on understanding the biological and molecular mechanisms underlying individual predisposition to caries, a large number of studies have begun to be drawn from this perspective [Chisini et al., 2020; Vieira et al., 2014]. Initial studies investigating genetic influence on dental caries with consistent results started with simple methods in the 80' years from studies of twins reared apart, where the estimated genetic contribution to dental caries was up to 40% [Boraas et al., 1988]. On the other hand, recent studies using genome wide association tools have identified a large number of Single Nucleotide Polymorphisms (SNPs) with potential influence on dental caries [Shaffer et al., 2013; Shungin et al., 2019]. SNP is a DNA sequence variation occurring when a single nucleotide in the genome differs between members of species or paired chromosomes in an individual.

A recent meta-analysis that included 18 studies identified several SNPs related to genes in the formation of tooth mineral linked to the occurrence of dental caries [Chisini et al., 2020]. In fact, it was observed an elevate heterogenicity among the studies with contrasting results in some SNP from different pathways. In this way, a Polish cohort with children with both dentitions found that Mannose binding lectin 2 (*MBL2*) was linked with dental caries and the directions of effects in the analysis was the opposite in the permanent and deciduous dentitions [Olszowski et al., 2012]. These observations highlight the need of conduction of longitudinal studies to assess the effect of SNPs in dental caries susceptibility across the life course of individuals, and not only in one moment in the life, which resting the potential inferences. In fact, no longitudinal studies were found evaluating dental caries gene candidates in recent systematic review [Chisini et al., 2020], corroborating with the demand of carrying out population-based longitudinal studies.

In the present study, it was aimed to increase the current understanding of SNPs present in genes in the pathway of tooth mineral tissues (such as *TUFT1*, *MMP20*, *MMP13*, *MMP2*, *DLX3*, *TIMP2*, *BMP7* and *TFIP11*) on dental caries in the life course and investigate possible epistatic interaction (i.e. gene-gene interaction). Specifically, it was addressed two research questions: a) is dental caries at different ages and caries trajectory associated with SNPs present in the genes of tooth mineral tissues considering genotypes (additive and dominant effect) and

allele distribution?; (b) Is there an epistatic interaction between investigated SNPs and caries trajectory in the life course?

Methods

Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) was used to guide the report of present study. [von Elm et al., 2007].

Study design, setting and participants

The present study has a birth cohort design, being carried out in Pelotas, Brazil. The live births of 1982 in Pelotas were identified and followed in the life course. In 1982, 5,914 children (99.2% of the births) were included in a perinatal health survey [Barros et al., 2008]. Several follow-ups of this population were performed. In 2004, all cohort was interviewed, and a food frequency questionnaire was applied as well as genotyping of genetic material was performed in this wave.

Oral health studies were carried out in a representative sample of this cohort. In 1997, at 15 years old, the first follow-up was performed with a representative sample of this population. Thus, 70 census tracts, corresponding about 27% of the total, of the urban area of Pelotas were screened and 1,076 cohort individuals were founded. Of these individuals, 900 were randomly selected and 888 were assessed in first oral health study with an interview and dental examinations. In 2006, a new oral health study was conducted in this cohort, in which 720 individuals were included from the originals ample (80%). The third oral health follow-up was carried out in 2013, where the 888 individuals from the initial sample were searched. In this study, 539 individuals (59.9% of initial sample) were included. Similarly, individuals were interviewed, and clinical oral examinations were performed.

Genotyping

Genetic material (blood sample) of participants was collected in the research laboratory by venipuncture. Materials were freezing at -70 °C. The genotyping of DNA samples was performed using Illumina (*Illumina HumanOmni2.5-8v1 array*). More details were previously reported [Horta et al., 2015; Victora and Barros, 2006]. So, in this study we use ten SNPs, which were genotyped: rs4970957 (*TUFT1*); rs1711437 (*MMP20*); rs1784418 (*MMP20*); rs2252070 (*MMP13*); rs243847 (*MMP2*); rs2303466 (*DLX3*); rs11656951 (*DLX3*); rs7501477 (*TIMP2*); rs388286 (*BMP7*); and, rs5997096 (*TFIP11*).

We also performed genomic ancestry evaluation using ADMIXTURE.[Alexander et al., 2009]. This analysis was based on around 370,000 SNPs accessible from the 1982 Pelotas birth cohort, which is compatible with the HapMap and Human Genome Diversity projects for the Brazilian population [Lima-Costa et al., 2015].

Outcome variable (phenotype)

The presence of dental caries was the phenotype/outcome of present study. DMF-T of participants was collected in clinical examination at 15, 24 and 31 years. Decayed teeth in each wave were estimated and individuals dichotomized into: (i) presence of untreated dental caries (one or more decayed tooth); or (ii) absence of dental caries (none decayed tooth). We investigate the phenotype in four ways: (i) presence of untreated dental caries at 15 years; (ii) presence of untreated dental caries at 24 years; (iii) presence of untreated dental caries at 31 years; and, (iv) untreated dental caries trajectory from 15 to 31 years of age.

Thus, dental caries trajectory was modeled by group-based trajectory using the component “decayed” of three follow-ups [Dennis et al., 1981; Jones and Nagin, 2007]. Briefly, the model was estimated with the command “traj” in the program Stata 12.0 [Jones et al., 2001; Silva et al., 2018]. Identifying the similarity of the trajectory among the evaluated individuals. The parameters for the model trajectory was determined based on the maximum likelihood by the quasi-Newton method [Dennis et al., 1981; Jones and Nagin, 2007]. Model selection was considered and estimated by the latent number of categories and the polynomial order of each latent trajectory. The number of trajectories was determined when through sequential comparisons of the Bayesian information criterion (BIC) and its fit criteria between the K and $K + 1$ trajectory model have not produced substantial difference in the $k + 1$ model BIC score. So, we identify two categories of caries trajectories: low and high.

Independent variables

Independent variables were used aim to adjust the logistic regressions models and were obtained from waves performed at birth, 22 and 24 years. Income was continuously collected at 24 years and divided in tertiles. In 2004, food frequency questionnaire was also applied. Questions about the sweet intake and sugar were asked. Moreover, frequency (varying from zero to ten) daily in the last year was questioned. A sum of the quantity of daily sugar consumed in a year was estimated. Posteriorly, it was categorized into tertiles. The quality of oral hygiene of participants was achieved through presence of gingival bleeding at 24 and 31 years. Gingival tissue bleeding was clinically examined at six sites for each dental element. Individuals were classified as having gingival bleeding when they have $\geq 10\%$ of sites with gingival bleeding. Thus, considering the two follow-ups, we classified individuals into three groups: i) absence of gingival bleeding; ii) at least one follow-up with gingival bleeding and iii) gingival bleeding in both assessment.

Power calculation

Power calculation was estimated using the present sample size and prevalence of caries in exposed and non-exposed from caries trajectory of each SNP, considering an α of 0.05; thus,

we have 80% power to detect incidence rate ratios of 1.6 or greater in rs4970957 (*TUFT1*); 1.9 or greater in rs1711437 (*MMP20*); 1.8 or greater in rs1784418 (*MMP20*); 1.9 or greater in rs2252070 (*MMP13*); 1.8 or greater in rs243847 (*MMP2*); 1.8 or greater in rs2303466 (*DLX3*); 1.8 or greater in rs11656951 (*DLX3*); 1.2 or greater in rs7501477 (*TIMP2*); 1.6 or greater in rs388286 (*BMP7*); and, 1.9 or greater in rs5997096 (*TFIP11*).

Statistical methods

The *Hardy–Weinberg equilibrium test* and allele estimation frequency were performed to each investigated SNP using the “*genhw*” command [Newton] in the Stata 12.0 (Stata Corporation, College Station, USA). All subsequent analyses were performed using this software. Descriptive analysis was performed calculating the absolute and relative frequency of each SNP according outcomes using Fisher Exact Test.

Associations analysis were investigated by forward stepwise logistic regression models between SNPs and four caries outcomes following two different strategy of analysis. It is important to highlight that estimations were performed using Bonferroni multiple correction test. Regression were also adjusted by ancestry genomic, which was estimated by the first ten major components of the principal component analysis of genetic. This strategy was adopted to prevent population stratification effect. First, we performed the analysis considering the genotype of participants. In this way, we consider two possible genetic effects: i) additive, i.e. when the heterozygote and homozygote containing the minor frequent allele are coded differently; in a representative SNP A/B (being A the more frequent allele and B the minor frequent allele) the homozygote AA are coded = 0; the heterozygote AB are coded = 1; and homozygote BB are coded = 2; and ii) dominant effects, i.e. when the heterozygote and homozygote contain the minor frequent allele are coded in the same category. Considering the same example, homozygote AA are coded = 0; the heterozygote AB are coded = 1; and homozygote BB are coded = 1. Recessive effects were not performed due to low number the individuals in the homozygote minor frequent genotype.

Considering the second strategy analysis, we also investigated the allele association with dental caries outcomes. In this way, forward stepwise multilevel logistic regression model was used, considering mixed effects and two hierarchical levels: genetic and individual. Two models were used in the analysis: (i) unadjusted (i.e. no covariates), and (ii) adjusted (i.e. controlling for ancestry genetic, sex, family income, sugar consumption and trajectory of gingival bleeding)

Linkage Disequilibrium and haplotype analysis

Linkage disequilibrium analysis was carried out to determine the non-random association of alleles in the same chromosome estimated with D' (i.e. D' equal to 1 represent total *linkage disequilibrium* while $D' = 0$ correspond to no *linkage disequilibrium*). So, we perform the analysis using SHEsis, online software (<https://analysis.bio-x.cn/myAnalysis.php>) [Shi and He, 2005; Wang and Qin, 2018] Moreover, we carried out associations between dental caries trajectory and haplotype of 2 or more SNPs in the same gene in the same online software.

Epistasis Analysis - Generalized Multifactor Dimensionality Reduction (GMDR)

Generalized multifactor dimensionality reduction software was used to analyze gene–gene interactions. To perform this analysis, it was used the caries trajectory as outcome considering logistic models and the genotypes in additive effect of all SNPs.

Ethical issues

Federal University of Pelotas Ethics committee approved this project. Authorization of all participants were done individually even as all participants signed informed consent terms.

Results

Sample of follow-ups:

A total of 888 individuals were assessed at 15 years, 720 at 24 years and 539 at 31 years. The group-Based trajectory modeling created two caries trajectories: i) low caries trajectory (68.1%) and ii) high caries trajectory (31.9%). Individuals not followed in one wave were inputted by group-based trajectory modeling; therefore, 888 individuals were included in this outcome, being 53.8% male. Concern the ancestry, 89.1% of participants presented main similarity as European ancestrally genetic and 10.9% with African. Proportion of female in low caries trajectory group was 71.5% while proportion of males was 65.3%. Individuals into higher income tertile were more frequent in low caries (29.7%). **Table S1** display the proportion of genotypes in additive effect and co-variables.

General genetic information

All evaluated SNPs were in Hardy–Weinberg equilibrium ($p > 0.05$). Description of allele frequency and results of Hardy-Weinberg equilibrium are available in **Table S2**. Minor allele frequency ranges from 19.7% in rs4970957 (*TUFT1*) to 45.5% rs388286 (*BMP7*). *Linkage Disequilibrium* analysis was measured by D' and r^2 to determine possible differences between observed and expected frequencies. The result found two genes with nonrandom association of alleles between loci. The SNPs of *DLX3* rs2303466 and rs11656951 were in

linkage disequilibrium ($D' = 0.98$, $r^2 0.85$). Similar, SNPs of *MMP20* rs1711437 and rs1784418 were in disequilibrium ($D' = 0.97$, $r^2 0.85$). **Figure 1** display complete linkage disequilibrium analysis to SNPs in the same chromosome (i.e. *DLX3*, *TIMP2* and *BMP7*; *MMP20* and *MMP13*).

Haplotype analysis of the low caries trajectory and high caries trajectory was carried out in association test between haplotypes of two or more SNPs in the same chromosome. Frequencies <0.01 were ignored in this analysis. No significant associations were observed in the haplotype and caries trajectory. **Table S3 and S4** presents complete haplotype analysis of loci (see Supplementary Material, available as Supplementary data for detailed haplotype analysis).

Genetic distribution and dental caries

Description of Genotype analysis in additive effect according dental caries trajectory, Dental caries at 15, 24 and 31 years is displayed in **Table S5**. No associations ($P > 0.05$) were observed with Fisher exact test for any SNP evaluated. However, weak associations were observed in dominant effect (**Table S6**). rs4970957 (*TUFT1*) was associated with dental caries at 31 years ($p = 0.042$) and rs243847 (*MMP2*) was associated with dental caries at 15 years ($p = 0.046$). The other SNPs were not associated with caries trajectory or with the follow-ups. Considering the allelic distribution, was observed that C allele of rs243847 (*MMP2*) was less frequent in individuals without dental caries at 15 years ($p = 0.041$) (**Table S7**).

Genetic Analysis

Complete models of logistic regression investigating the associations between SNPs and dental caries are available in **Table S8** to genotype additive effect, in **Table S9** to genotype recessive effect and **Table S10** to allelic. (see Supplementary Material, available as Supplementary data for detailed analysis). **Table 1** presents the summary of associated SNPs. Untreated caries trajectory in the life course was not associated with investigated SNPs in any model. rs4970957 of *TUFT1* presented weak association with dental caries at 31 years. Genotype GA in additive effect presented an Odds of 1.71 (1.04 – 2.81) for caries in adjusted model. Considering dominant effect, GA/GG showed an Odds 1.74 (1.14 – 2.65) for caries when compared to AA. Allele analysis of rs4970957 (*TUFT1*) also was associated with dental caries at 31 years. Allele G present an Odds of 1.56 (1.09 – 2.20). However, this SNP did not was associated in any other follow-ups or caries trajectory. Finally, rs243847 of *MMP2* was associated with dental caries at 15 years in allelic analysis. Allele C was associated with increase of 30% in odds for dental caries in unadjusted model. However, the associations were lost in the adjusted model.

Epistasis Analysis (Gene-gene Interaction)

Table 2 presents the summarization of results for gene-gene interaction on the odds of high dental caries trajectory obtained from the GMDR analysis. It was found three-locus models significant ($p < 0.001$) involving rs243847 (*MMP2*), rs2303466 (*DLX3*) and rs388286 (*BMP7*). This result indicates a potential gene-gene interaction between these SNPs. However, this model shows a cross validation consistency of 7/10, training-balanced accuracy of 60.49% and testing-balanced accuracy of 52.57%. The analysis of combination of these genotypes showed an odds of 2.51 (1.54 – 4.09) to be high caries trajectory group, being this the best gene-gene combination associated with caries trajectory. Besides, GMDR found a two-locus interaction model between the rs243847 (*MMP2*) and rs388286 (*BMP7*) ($p = 0.013$). This model also revealed an intermediate cross validation consistency (6/10), training-balanced accuracy (57.08%) and testing-balanced accuracy (53.46%). The analysis of combination of genotypes showed an odds of 1.81 (1.13 – 2.91) to high caries trajectory group. Interactions of gene-gene combination is represented in **Figure 2**; **Figure 3** illustrate the interaction graph summarizing the measures of information gain.

Discussion

In the present study we deeply investigated - with a longitudinal birth cohort design - the association between dental caries trajectory and SNPs present in genes of the pathway of tooth mineral tissues (*TUFT1*, *MMP20*, *MMP13*, *MMP2*, *DLX3*, *TIMP2*, *BMP7* and *TFIP11*), increasing thus the knowledge of current literature. rs4970957 (*TUFT1*) in allelic, additive and dominant effect was associated with presence of dental caries at 31 years, while allele C of rs243847 (*MMP2*) was associated with caries at 15 years in unadjusted models. The lack of association of these SNPs in other follow-ups highlight the need of carefully interpretation of our results. Furthermore, we identify epistatic interaction between investigated SNPs and caries trajectory in the life course. GMDR analysis found a three-locus model significant involving rs243847 (*MMP2*), rs2303466 (*DLX3*) and rs388286 (*BMP7*). Individuals with the combination of these SNPs showed an Odds 2.51 (1.54 – 4.09) to be in high caries trajectory group.

GMDR analysis have been described as a power tool to evaluated epistatic interactions [Hou et al., 2019]. In our study, the best gene–gene interaction model was selected across all multi-locus models that maximized testing accuracy and CVC for prediction of caries trajectory. Therefore, the combination of rs243847 (*MMP2*), rs2303466 (*DLX3*) and rs388286 (*BMP7*) was considered as the best gene–gene interaction model due to higher cross validation consistency (7/10) testing-balanced accuracy (60.49%) and training balanced accuracy (62.30%). Thus, the combination of variant genotypes can increase the effect, leading to an increase in the chance of having caries in the life-course. Despite this SNPs alone did not exhibit association with dental caries, the interaction of then seems be important pathways to explain the dental caries susceptibility in our sample. Considering these genes, the matrix metalloproteinase 2 (*MMP2*) encoded the gelatinase A, type IV collagenase; variations in the gene have been associated with tooth agenesis [Kuchler et al., 2011] and dental caries [Tannure et al., 2012]. Bone morphogenetic protein 7 (*BMP7*) encodes a secreted ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins. Variations in this gene were associated with molar-incisor hypomineralization [Jeremias et al., 2016] while distal-less homeobox 3 (*DLX3*) has also been reported as being involved in tooth mineralization and dental caries [Ohta et al., 2015].

Although the direct association analyzes did not shown consistent effects of SNPs on untreated caries trajectory, when epistatic interaction analysis was performed, we found a 2.5-fold odds to increase untreated caries. In fact, the epistatic interaction of these genes seemed to be an important way to explain the genetic effect of dental caries that would not be identified in direct association analyzes. Often the direct association between a polymorphism and the disease can be not adequate enough to explain a complex gene structure that presents several possibilities of interaction, especially when working with multifactorial phenotypes, such as

dental caries. These findings underline the importance of conducting robust analyzes that consider gene-environment and epistatic interactions so that we can understand the polygenic trait of dental caries.

Tuftelin 1 (*TUFT1*) is a protein coding gene present in the location 1q21.3. The acid protein tufletin plays a role in dental enamel mineralization and recent studies found that it is implicated in caries susceptibility [Deeley et al., 2008; Ergoz et al., 2014; Gerreth et al., 2017; Patir et al., 2008; Shimizu et al., 2012]. Moreover, influence of some genotypes of *TUFT1* was also identified interacting with levels of *Streptococcus. mutans* in children, which lead to increase of levels of caries [Slayton et al., 2005]. In our study, we only found association between dental caries and *TUFT1* at 31 years, as well as we have not identified association with caries trajectory in the life course. Regarding the rs4970957 (*TUFT1*), which is an intron variation, previously studies showed contradictory results. Shimizu et al. [2012] found association in an Argentinian cohort with individuals ranging from 1 to 72 years of age and replicate the results in a Brazilian cohort with individuals with age under 21 years. On the other hand, a study carried with turkey children (6 to 12y) did not found association of this SNP with dental caries [Ergoz et al., 2014]; Similarly, rs4970957 (*TUFT1*) in children from Poland (1 to 2y) also was not associated with experience of caries [Gerreth et al., 2017]. These inconsistent results presented in the literature can be explained due to different ancestry, lack of statistical power and possible epistatic interactions, which can influence the results.

In fact, it is important consider that genetic association studies are more complex than genetic analysis based only in occurrence of recombination during the meiosis. Thus, can occur three main justifications for an association of between allele/genotype and phenotype, i.e. caries risk. First, an indirect association due to linkage disequilibrium can be present, where the alleles are in linkage disequilibrium with disease, rather than the casual allele itself [Slatkin, 2008]. In this way, in our study we investigated the possible linkage disequilibrium in studied SNPs. However, investigations concern disequilibrium linkage are poor investigated in the literature, since some studies present this investigation [Deeley et al., 2008; Gerreth et al., 2017; Shimizu et al., 2012] while others do not report them [Ergoz et al., 2014; Patir et al., 2008]. Therefore, lacking of investigation of linkage disequilibrium can introduce important bias in the results of studies [Slatkin, 2008].

Second, type I error (false positive associations) are common due to multiple comparisons. Type I error is when the study concludes that there is an association when it is not present. The main source of false positive results is the absence of control for multiple comparisons, as Bonferroni corrections in logistic regressions [Gao et al., 2008]. Bonferroni corrections remains the standard approach for avoid false positive owing to multiple comparisons, since that in the significant level of Bonferroni adjustments is determined dividing

the α by the number of tests [Gao et al., 2008]. Furthermore, SNPs in Hardy-Weinberg disequilibrium can be a source of false positive association. False-positive associations are inflated if homozygotes are less frequent than expected and, therefore, all associated studies must perform control for Hardy-Weinberg equilibrium. In fact, recent systematic review found a low number of studies investigating dental caries in gene-associations studies with correction by multiple comparisons [Chisini et al., 2020].

And third, can exist a direct and casual relationship, being necessary to investigate the strength and consistency of the association, the temporal sequence, possible dose-response and biological plausibility. In this way, our study was the first to investigate the influence of SNPs linked to mineral tissues and dental caries in a longitudinal approach. We have not observed an association of rs4970957 (*TUFT1*) and rs243847 (*MMP2*) in different follow-ups. In fact, rs4970957 (*TUFT1*) was only associated in one of waves, putting in check the results and hypothesis of a causal association. In this way, we must be careful to infer a real causality between this association, since it was not observed when considering the trajectory of caries or other follow-ups. Moreover, the findings available in literature about this SNP showed contradictory results. Thus, it is necessary further confirmations to infer real causality between dental caries and rs4970957 (*TUFT1*).

Besides, we cannot rule out the possibility that the lack of association of the other SNPs may be due to type II error, i.e. lack of statistical power due to sample size. In fact, our study has a large sample size compared to previous studies [Deeley et al., 2008; Filho et al., 2017; Gerreth et al., 2017; Kang et al., 2011; Olszowski et al., 2012; Patir et al., 2008; Slayton et al., 2005; Yildiz et al., 2016]. Taking in account that present study is longitudinal, losses are expect in long-time follow-ups. Group-based trajectory modeling used in present study is an interesting strategy to compensate losses inputting missing data for individuals that were not assessed in all follow-ups.

One important source of bias in genetic studies is the ancestry of population. Population stratification can confound the results of genetic association studies and cannot be ignored because can lead to misleading results or false-positives [Hellwege et al., 2017]. In this way, we controlled all the logistic regressions models to ancestry. Thus, aiming to reduce possible confounding in our results, ancestry was estimated based on approximately 370,000 SNPs available from the 1982 Pelotas birth cohort compatible with the HapMap and Human Genome Diversity projects for the Brazilian population [Lima-Costa et al., 2015]. In fact, a large number of studies evaluated in a wide systematic review have not controlled the results for ancestry and only one performed control for skin color [Chisini et al., 2020].

Conclusion

Although rs4970957 (*TUFT1*) has been associated with dental caries at 31 years old in adjusted model and rs243847 (*MMP2*) at 15 years old in unadjusted model, presents results did not support with strengths that this SNPS are associated with dental caries in the life course in the present sample. However, we found an epistatic interaction which seem be important pathways to explain the dental caries susceptibility. Results suggest that interaction between three-locus model involving rs243847 (*MMP2*), rs2303466 (*DLX3*) and rs388286 (*BMP7*) are associated with high caries trajectory in the life course.

Compliance with Ethical Standards:

Conflict of Interest: Luiz Alexandre Chisini declares that he has no conflict of interest. Marcus Cristian Muniz Conde declares that he has no conflict of interest. Bernardo Lessa Horta declares that he has no conflict of interest. Luciana Tovo-Rodrigues declares that she has no conflict of interest. Marcus Flávio Fernando Demarco declares that he has no conflict of interest. Marcos Britto Correa declares that he has no conflict of interest

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Ethical approval: Federal University of Pelotas Ethics committee approved this project.

Informed consent: Authorization of all participants were done individually even as all participants signed informed consent terms.

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Table S1. Proportion of genotypes in additive effect and co-variables.

Variables	Sex distribution			Income tertile				Ancestry			Sugar Consumption			Gingival bleeding		
	Male	Female	p value	Lowest tertile (1st)	Medium (2nd) N (%)	Higher tertile (3rd)	p	European	African	p	Low	High	p	Never	1 Folow- up	2 Follow-up
	N (%)	N (%)		N (%)		N (%)	value	N (%)	N (%)	value	N (%)	N (%)	value	N (%)	N (%)	N (%)
rs4970957 (TUFT1)			0.147				0.172			0.031			0.925			0.372
AA	225 (52.20)	206 (47.80)		130 (30.16)	165 (38.28)	136 (31.55)		385 (89.33)	46 (10.67)		304 (70.53)	127 (19.47)		353 (87.59)	29 (7.20)	21 (5.21)
GA	103 (49.05)	107 (50.95)		55 (26.19)	78 (37.14)	77 (36.67)		198 (94.29)	12 (5.71)		145 (69.05)	65 (30.95)		176 (88.44)	12 (6.03)	11 (5.53)
GG	18 (69.23)	8 (30.77)		4 (15.38)	15 (57.69)	7 (26.92)		26 (100)	0 (00)		18 (69.23)	8 (30.77)		22 (84.62)	4 (15.38)	0 (00)
rs1711437 (MMP20)			0.620				0.499			0.125			0.819			0.459
CC	116 (51.56)	109 (48.44)		62 (27.56)	80 (35.56)	83 (36.89)		200 (88.89)	25 (11.11)		156 (69.33)	69 (30.67)		194 (88.58)	12 (5.48)	13 (5.94)
TC	167 (53.3)	145 (46.47)		86 (27.56)	126 (40.38)	100 (32.05)		292 (93.59)	20 (6.41)		222 (71.15)	90 (28.85)		246 (86.62)	26 (9.15)	12 (4.23)
TT	63 (48.46)	67 (51.54)		41 (31.54)	52 (40.00)	37 (28.46)		117 (90.00)	13 (10.00)		89 (68.46)	41 (31.54)		111 (88.80)	7 (5.60)	7 (5.60)

0.7120.4670.0860.3270.488

rs1784418
(MMP20)

CC	110 (52.13)	101 (47.87)	59 (27.96)	74 (35.07)	78 (36.97)	188 (89.10)	23 (10.90)	148 (70.14)	63 (29.86)	180 (88.24)	11 (5.39)	13 (6.37)
TC	164 (53.07)	145 (46.93)	84 (27.18)	125 (40.45)	100 (32.36)	290 (93.85)	19 (6.15)	223 (72.17)	86 (27.83)	246 (87.23)	25 (8.87)	11 (3.90)
TT	72 (48.87)	75 (51.02)	46 (31.29)	59 (40.14)	42 (28.57)	131 (89.12)	16 (10.88)	96 (65.31)	51 (34.69)	125 (88.03)	9 (6.34)	8 (5.63)

0.3620.9560.9610.0350.016

rs2252070
(MMP13)

TT	157 (52.86)	140 (47.14)	86 (28.96)	113 (38.05)	98 (33.00)	272 (91.58)	25 (8.42)	203 (67.89)	96 (32.11)	239 (87.23)	27 (9.85)	8 (2.92)
TC	146 (49.32)	150 (50.68)	83 (28.04)	118 (39.86)	95 (32.09)	269 (90.88)	27 (9.12)	223 (74.83)	75 (25.17)	246 (86.93)	17 (6.01)	20 (7.07)
CC	43 (58.11)	31 (41.89)	20 (27.03)	27 (36.49)	27 (36.49)	68 (91.89)	6 (8.11)	46 (61.33)	29 (38.67)	66 (92.96)	1 (1.41)	4 (5.63)

0.4770.6880.6440.4210.802

rs243847
(MMP2)

TT	135 (49.45)	138 (50.55)	80 (29.30)	110 (40.29)	83 (30.40)	246 (90.11)	27 (9.89)	184 (67.40)	89 (32.60)	224 (86.82)	18 (6.98)	16 (6.20)
TC	157 (52.68)	141 (47.32)	86 (28.86)	110 (36.91)	102 (34.23)	275 (92.28)	23 (7.72)	212 (71.14)	86 (28.86)	247 (88.53)	19 (6.81)	13 (4.66)
CC	54 (56.25)	42 (43.75)	23 (23.96)	38 (39.58)	35 (36.46)	88 (91.67)	8 (8.33)	71 (73.96)	25 (26.04)	80 (87.91)	8 (8.79)	3 (3.30)

0.5990.5160.0910.4050.239

rs2303466

(DLX3)

CC	249 (52.20)	228 (47.80)	128 (26.83)	193 (40.46)	156 (32.70)	435 (91.19)	42 (8.81)	328 (68.76)	149 (31.24)	402 (89.33)	28 (6.22)	20 (4.44)
TC	82 (49.70)	83 (50.30)	54 (32.73)	55 (33.33)	56 (33.94)	154 (93.33)	11 (6.67)	119 (72.12)	46 (27.88)	128 (82.58)	16 (10.32)	11 (7.10)
TT	15 (60.00)	10 (40.00)	7 (28.00)	10 (40.00)	8 (32.00)	20 (80.00)	5 (20.00)	20 (80.00)	5 (20.00)	21 (91.30)	1 (4.35)	1 (4.35)

0.5790.6290.0870.3940.388

rs11656951

(DLX3)

CC	249 (52.31)	227 (47.69)	129 (27.10)	192 (40.34)	155 (32.56)	434 (91.18)	42 (8.82)	327 (68.70)	149 (31.30)	400 (89.09)	29 (6.46)	20 (4.45)
TC	82 (49.40)	84 (50.60)	53 (31.93)	56 (33.73)	57 (34.34)	155 (93.37)	11 (6.63)	120 (72.29)	46 (27.71)	130 (83.33)	15 (9.62)	11 (7.05)
TT	15 (60.00)	10 (40.00)	7 (28.00)	10 (40.00)	8 (32.00)	20 (80.00)	5 (20.00)	20 (80.00)	5 (20.00)	21 (91.30)	1 (4.35)	1 (4.35)

0.4020.3800.1840.0870.039

rs7501477

(TIMP2)

GG	255 (51.72)	238 (48.28)	133 (26.98)	191 (38.74)	169 (34.28)	455 (92.29)	38 (7.71)	434 (91.18)	42 (8.82)	414 (89.42)	32 (6.91)	17 (3.67)
TG	87 (53.70)	75 (46.30)	53 (32.72)	60 (37.04)	49 (30.28)	144 (88.89)	18 (11.11)	155 (93.37)	11 (6.63)	127 (83.01)	13 (8.50)	13 (8.50)
TT	4 (33.33)	8 (66.67)	3 (25.00)	7 (58.33)	2 (16.67)	10 (83.33)	2 (16.67)	20 (80.00)	5 (20.00)	10 (83.33)	0 (0.00)	2 (16.67)

													0.212	0.457	0.474	0.322	0.674
rs388286																	
(BMP7)																	
CC	105 (56.45)	81 (43.55)	49 (26.34)	76 (40.86)	61 (32.80)	169 (90.86)	17 (9.14)	126 (67.74)	60 (32.26)	150 (86.71)	16 (9.25)	7 (4.05)					
TC	167 (48.69)	176 (51.31)	93 (27.11)	130 (37.90)	120 (34.99)	317 (92.42)	26 (7.58)	249 (72.59)	94 (27.41)	285 (87.42)	22 (6.75)	19 (5.83)					
TT	74 (53.62)	64 (46.38)	47 (34.06)	52 (37.68)	39 (28.26)	123 (89.13)	15 (10.87)	92 (66.67)	46 (33.33)	116 (89.92)	7 (5.43)	6 (4.65)					
													0.443	0.803	0.022	0.657	0.370
rs5997096																	
(TFIP11)																	
CC	93 (55.69)	74 (44.31)	42 (25.15)	67 (40.12)	58 (34.73)	157 (94.01)	10 (5.99)	114 (68.26)	53 (31.74)	138 (87.90)	13 (8.28)	6 (3.82)					
TC	164 (51.57)	154 (48.43)	94 (29.56)	118 (37.11)	106 (33.33)	295 (92.77)	23 (7.23)	228 (71.70)	90 (28.30)	257 (85.95)	25 (8.36)	17 (5.69)					
TT	89 (48.90)	93 (51.10)	53 (29.34)	73 (40.11)	56 (30.77)	157 (86.26)	25 (13.74)	125 (68.68)	57 (31.32)	156 (90.70)	7 (4.07)	9 (5.23)					

p-values are presented in bold when the differences are significant (p < 0.05); Fischer exact test

Table 1. Summary of logistic regression results for SNPs that presented association.

Variables	Dental caries 15 years		Dental caries 31 years	
	(n=667)		(n=446)	
	OR (95% CI)	p value	OR (95% CI)	p value
TUFT1				
rs4970957 (Adj)				
AA	-		1	
GA			1.71 (1.04 – 2.81)	0.030
GG			2.07 (0.59 – 7.28)	0.390
rs4970957 (Adj)				
AA	-		1	
GA/ GG			1.74 (1.14 – 2.65)	0.010
rs4970957 (Adj)				
A	-		1	
G			1.56 (1.09 – 2.20)	0.010
MMP2				
rs243847 (Unad)				
T	1		-	-
C	1.30 (1.01 – 1.68)	0.042		

p value was adjusted by multiple comparisons (Bonferroni); Unad: Unadjusted; Adj: Ancestry-informative genetic, sex, income, sugar consumption, gingival bleeding

Table S2. Description of allele frequency and results of Hardy-Weinberg equilibrium

		Hardy-Weinberg equilibrium	
<i>Gene / Chromosome: location</i>			
SNP	Allele Frequency	Tests	p value
<i>TUFT1 / 1: 151517388</i>			
rs4970957		Pearson chi2	0.348
A	0.8025	likelihood-ratio chi2	0.351
G	0.1975	Exact significance prob	0.340
<i>MMP20 / 11: 102465226</i>			
rs1711437		Pearson chi2	0.748
C	0.5748	likelihood-ratio chi2	0.748
T	0.4252	Exact significance prob	0.757
<i>MMP20 / 11:02484396</i>			
rs1784418		Pearson chi2	0.956
C	0.5466	likelihood-ratio chi2	0.956
T	0.4534	Exact significance prob	0.973
<i>MMP13 / 11:02826539</i>			
rs2252070		Pearson chi2	0.644
T	0.6669	likelihood-ratio chi2	0.644
C	0.3331	Exact significance prob	0.678
<i>MMP2 / 16:55523998</i>			
rs243847		Pearson chi2	0.296
T	0.6340	likelihood-ratio chi2	0.297
C	0.3660	Exact significance prob	0.294
<i>DLX3 /17:48070878</i>			
rs2303466		Pearson chi2	0.161
C	0.8341	likelihood-ratio chi2	0.166
T	0.1659	Exact significance prob	0.163
<i>DLX3 /17:48072865</i>			
rs11656951	0.8336	Pearson chi2	0.282

C	0.1664	likelihood-ratio chi2	0.286
T		Exact significance prob	0.276
<i>TIMP2 / 17:76926276</i>			
rs7501477		Pearson chi2	0.580
G	0.8594	likelihood-ratio chi2	0.582
T	0.1406	Exact significance prob	0.578
<i>BMP7 / 20:55465424</i>			
rs388286		Pearson chi2	0.273
C	0.5450	likelihood-ratio chi2	0.273
T	0.4550	Exact significance prob	0.279
<i>TFIP11 / 22:26895957</i>			
rs5997096		Pearson chi2	0.949
C	0.5124	likelihood-ratio chi2	0.949
T	0.4876	Exact significance prob	0.946

Table S3. Haplotype analysis of loci for hap-analysis: rs1711437, rs1784418, rs2252070

Haplotype	High caries	Downward caries	Fisher's p	Odds Ratio (95% CI)
	Trajectory	Trajectory		
	Frequency	Frequency		
C C C	0.169	0.143	0.214	1.21 (0.89 – 1.64)
C C T	0.375	0.396	0.399	0.91 (0.71 – 1.14)
C T C	0.011	0.005	0.817	1.09 (0.528 – 2.25)
T T C	0.004	0.004	0.605	0.92 (0.69- 1.25)
T T T	0.252	0.250	0.96	1.01 (0.78 – 1.30)

All those frequency<0.01 were ignored in analysis

Table S4. Haplotype analysis of loci for hap-analysis: rs2303466, rs11656951, rs7501477.

Haplotype	High caries	Downward caries	Fisher's p	Odds Ratio (95% CI)
	Trajectory	Trajectory		
	Frequency	Frequency		
C C G	0.717	0.727	0.717	0.95 (0.73 – 1.24)
C C T	0.123	0.109	0.448	1.52 (0.799- 1.66)
T C G	0.003	0.002	0.949	1.08 (0.09 – 11.93)
T T G	0.126	0.129	0.854	0.96 (0.678 – 1.38)
T T T	0.032	0.028	0.740	1.123 (0.56 -2.34)

All those frequency<0.01 were ignored in analysis

Table S5. Description of genotypes in additive effect by outcomes: dental Caries trajectory (downward and high), dental caries at 15, 24 and 31 years.

Variables	Caries Trajectory			Dental caries 15 years			Dental caries 24 years			Dental caries 31 years		
	(15, 24 and 31 years old) (n=888)			(n=667)			(n=599)			(n=446)		
	Downward N (%)	High N (%)	p value	No caries N (%)	Caries N (%)	p value	No caries N (%)	Caries N (%)	p value	No caries N (%)	Caries N (%)	p value
rs4970957 (TUFT1)			0.472			0.880			0.516			0.177
AA	287 (66.13)	147 (33.87)		106 (24.59)	325 (75.41)		190 (49.61)	193 (50.39)		167 (60.29)	110 (39.71)	
GA	131 (61.50)	82 (38.50)		55 (26.19)	155 (73.81)		96 (50.53)	94 (49.47)		80 (51.95)	74 (48.05)	
GG	16 (61.54)	10 (38.51)		7 (26.92)	19 (73.08)		16 (61.51)	10 (38.46)		7 (46.67)	8 (53.33)	
rs1711437 (MMP20)			0.916			0.698			0.571			0.823
CC	145 (63.60)	83 (63.40)		60 (26.67)	165 (73.33)		109 (51.66)	102 (48.34)		84 (54.90)	69 (45.10)	
TC	202 (64.54)	111 (35.46)		74 (23.72)	238 (76.28)		129 (48.13)	139 (51.87)		117 (58.21)	84 (41.79)	
TT	87 (65.91)	45 (34.09)		34 (26.15)	96 (73.85)		64 (53.33)	56 (46.67)		53 (57.61)	39 (42.39)	
rs1784418 (MMP20)			0.961			0.845			0.825			0.847
CC	139 (64.95)	75 (35.05)		56 (26.54)	155 (73.46)		100 (51.02)	96 (48.98)		80 (55.17)	65 (44.83)	
TC	198 (63.87)	112 (36.13)		75 (24.27)	234 (75.73)		131 (49.06)	136 (50.94)		115 (58.38)	82 (41.62)	
TT	97 (65.10)	52 (34.90)		37 (25.17)	110 (74.83)		71(52.21)	65 (47.79)		59 (56.73)	45 (43.27)	

rs2252070 (MMP13)			0.262		0.344		0.086		0.238
TT	201 (67.00)	99 (33.00)		71 (23.91)	226 (76.09)			108 (57.45)	80 (42.55)
TC	182 (61.07)	116 (38.93)		82 (27.70)	214 (72.30)	140 (53.64)	121 (46.36)	107 (53.77)	92 (46.23)
CC	51 (68.00)	24 (32.00)		15 (20.27)	59 (79.73)	123 (45.56)	147 (54.44)	39 (66.10)	20 (33.90)
						39 (57.35)	29 (42.65)		
rs243847 (MMP2)			0.662		0.133		0.656		0.914
TT	174 (63.04)	102 (36.96)		59 (21.61)	214 (78.39)	123 (50.00)	123 (50.00)	106 (58.24)	76 (41.76)
TC	199 (66.33)	101 (33.67)		79 (26.51)	219 (73.49)	140 (52.04)	129 (47.96)	113 (56.22)	88 (43.78)
CC	61 (62.89)	36 (37.11)		30 (31.25)	66 (68.75)	39 (46.43)	45 (53.57)	35 (55.56)	28 (44.44)
rs2303466 (DLX3)			0.999		0.858		0.652		0.934
CC	311 (64.52)	171 (35.48)		122 (25.58)	355 (74.42)	216 (50.47)	212 (49.53)	182 (57.05)	137 (42.95)
TC	107 (64.46)	59 (35.54)		41 (24.85)	124 (75.15)	77 (51.68)	72 (48.32)	61 (55.96)	48 (44.04)
TT	16 (64.00)	9 (36.00)		5 (20.00)	20 (80.00)	9 (40.91)	13 (59.09)	11 (61.11)	7 (38.89)
rs11656951 (DLX3)			0.975		0.806		0.514		0.967
CC	309 (64.24)	172 (35.76)		123 (25.84)	353 (74.16)	213 (49.88)	214 (50.12)	180 (56.78)	137 (43.22)
TC	109 (65.27)	58 (34.73)		40 (24.10)	126 (75.90)	80 (53.33)	70 (46.67)	63 (56.76)	48 (43.24)
TT	16 (64.00)	9 (36.00)		5 (20.00)	20 (80.00)	9 (40.91)	13 (59.09)	11 (61.11)	7 (38.89)
rs7501477 (TIMP2)			0.734		0.936		0.798		0.693
GG	322 (64.79)	175 (35.21)		126 (25.56)	367 (74.44)	226 (50.67)	220 (49.33)	180 (56.78)	137 (43.22)

TG	105 (64.42)	58 (35.58)	39 (24.07)	123 (75.93)	70 (48.95)	73 (51.05)	68 (58.62)	48 (41.22)
TT	7 (53.85)	6 (46.15)	3 (25.00)	9 (75.00)	6 (60.00)	4 (40.00)	6 (46.15)	7 (53.85)
rs388286 (BMP7)			0.528		0.196		0.535	0.484
CC	118 (62.77)	70 (37.23)	39 (20.97)	147 (79.03)	82 (47.67)	90 (52.33)	70 (60.87)	45 (39.13)
TC	230 (66.47)	116 (33.53)	96 (27.99)	247 (72.01)	162 (52.60)	146 (47.40)	133 (56.84)	101 (43.16)
TT	86 (61.87)	53 (38.13)	33 (23.91)	105 (76.09)	58 (48.74)	61 (51.26)	51 (52.58)	46 (47.42)
rs5997096 (TFIP11)			0.511		0.329		0.321	0.496
CC	104 (60.82)	67 (39.18)	35 (20.96)	132 (79.04)	73 (48.99)	76 (51.01)	67 (56.78)	51 (43.22)
TC	211 (65.94)	109 (34.06)	86 (27.04)	232 (72.96)	152 (53.52)	132 (46.48)	110 (54.46)	92 (45.54)
TT	119 (65.38)	63 (34.62)	47 (25.82)	135 (74.18)	77 (46.39)	89 (53.61)	77 (61.11)	49 (38.89)

p-values are presented in bold when the differences are significant ($p < 0.05$); Fischer exact test

Table S6. Description of genotypes in dominant effect by outcomes: dental Caries trajectory (downward and high), dental caries at 15, 24 and 31 years.

Variables	Caries Trajectory (15, 24 and 31 years old) (n=888)			Dental caries 15 years (n=667)			Dental caries 24 years (n=599)			Dental caries 31 years (n=446)		
	Downward	High	p value	No caries	Caries	p value	No caries	Caries	p value	No caries	Caries	p value
	N (%)	N (%)		N (%)	N (%)		N (%)	N (%)		N (%)	N (%)	
rs4970957 (<i>TUFT1</i>)			0.133			0.349			0.329			0.042
AA	287 (66.13)	147 (33.87)		106 (24.59)	325 (75.41)		190 (49.61)	193 (50.39)		167 (60.29)	110 (39.71)	
GA/ GG	147 (61.51)	92 (38.49)		62 (26.27)	174 (73.73)		112 (51.85)	104 (48.15)		87 (51.48)	82 (48.52)	
rs1711437 (<i>MMP20</i>)			0.396			0.296			0.571			0.297
CC	154 (63.60)	83 (36.40)		60 (26.67)	165 (73.33)		109 (51.66)	102 (48.34)		84 (54.90)	69 (45.10)	
TC/TT	289 (64.94)	156 (35.06)		108 (24.43)	334 (75.57)		193 (49.74)	195 (50.26)		170 (58.02)	123 (41.98)	
rs1784418 (<i>MMP20</i>)			0.467			0.324			0.453			0.335
CC	139 (64.95)	75 (35.05)		56 (26.54)	155 (73.46)		100 (51.02)	96 (48.98)		80 (55.17)	65 (44.83)	
TC/TT	295 (64.27)	164 (35.73)		112 (24.56)	344 (75.44)		202 (50.12)	201 (49.88)		174 (57.81)	127 (42.19)	
rs2252070 (<i>MMP13</i>)			0.127			0.277			0.096			
TT	201 (67.00)	99 (33.00)		71 (23.91)	226 (76.09)		140 (53.64)	121 (46.36)		108 (57.45)	80 (42.55)	0.467
TC/CC	233 (62.47)	140 (37.53)		97 (26.22)	273 (73.78)		162 (47.93)	176 (52.07)		146 (56.59)	112 (43.41)	

rs243847 (MMP2)			0.284		0.046		0.465		0.360
TT	174 (63.04)	102 (36.96)		59 (21.61)	214 (78.39)		123 (50.00)	132 (50.00)	106 (58.24) 76 (41.76)
TC/CC	260 (65.49)	137 (34.51)		109 (27.66)	285 (72.34)		179 (50.71)	174 (49.29)	148 (56.06) 116 (43.94)
rs2303466 (DLX3)			0.522		0.397		0.521		0.514
CC	311 (64.52)	171 (35.48)		122 (25.58)	355 (74.42)		216 (50.47)	212 (49.53)	182 (57.05) 137 (42.95)
TC/TT	123 (64.40)	68 (35.60)		46 (24.21)	144 (75.79)		86 (50.29)	85 (49.71)	72 (56.69) 55 (43.31)
rs11656951 (DLX3)			0.453		0.305		0.374		0.498
CC	309 (64.24)	172 (35.76)		123 (25.84)	353 (74.16)		213 (49.88)	214 (50.12)	180 (56.78) 137 (43.22)
TC/TT	125 (65.10)	67 (34.90)		45 (23.56)	146 (76.44)		89 (51.74)	83 (48.26)	74 (57.36) 55 (42.64)
rs7501477 (TIMP2)			0.426		0.397		0.452		0.498
GG	322 (64.79)	175 (35.21)		126 (25.56)	367 (74.44)		226 (50.67)	220 (49.33)	180 (56.78) 137 (43.22)
TG/TT	112 (63.64)	64 (36.36)		42 (24.14)	132 (75.86)		76 (49.67)	77 (50.33)	74 (57.36) 55 (42.64)
rs388286 (BMP7)			0.311		0.071		0.223		0.191
CC	118 (62.77)	70 (37.23)		39 (20.97)	147 (79.03)		82 (47.67)	90 (52.33)	70 (60.87) 45 (39.13)
TC/TT	316 (65.15)	169 (34.85)		129 (26.82)	352 (73.18)		220 (51.52)	207 (48.48)	184 (55.59) 147 (44.41)
rs5997096 (TFIP11)			0.143		0.087		0.380		0.525
CC	104 (60.82)	67 (39.18)		35 (20.96)	132 (79.04)		73 (48.99)	76 (51.01)	67 (56.78) 51 (43.22)
TC/TT	330 (65.74)	172 (34.26)		133 (26.60)	367 (73.40)		229 (50.89)	221 (49.11)	187 (57.01) 141 (42.99)

p-values are presented in bold when the differences are significant ($p < 0.05$); Fischer exact test

Table S7. Description of allele by outcomes: dental Caries trajectory (downward and high), dental caries at 15, 24 and 31 years.

Variables	Caries Trajectory (15, 24 and 31 years old) (n=888)			Dental caries 15 years (n=667)			Dental caries 24 years (n=599)			Dental caries 31 years (n=446)		
	Downward	High	p value	No caries	Caries	p value	No caries	Caries	p value	No caries	Caries	p value
	N (%)	N (%)		N (%)	N (%)		N (%)	N (%)		N (%)	N (%)	
rs4970957 (TUFT1)			0.258			0.633			0.389			0.071
A	705 (65.22)	376 (34.78)		267 (24.91)	805 (75.09)		476 (49.79)	480 (50.21)		414 (58.47)	294 (41.53)	
G	163 (61.51)	102 (38.49)		69 (26.34)	193 (73.66)		128 (52.79)	114 (47.11)		94 (51.09)	90 (48.91)	
rs1711437 (MMP20)			0.653			0.792			0.918			0.610
C	492 (63.98)	277 (36.02)		194 (25.46)	568 (74.54)		347 (50.29)	343 (49.71)		285 (56.21)	222 (43.79)	
T	376 (65.16)	301 (34.84)		142 (24.83)	430 (75.17)		257 (50.59)	251 (49.41)		223 (57.92)	162 (42.08)	
rs1784418 (MMP20)			0.992			0.715			0.885			0.750
C	476 (64.50)	262 (35.50)		187 (25.58)	544 (74.42)		331 (50.23)	328 (49.77)		275 (56.47)	212 (43.53)	
T	392 (64.47)	216 (35.53)		149 (24.71)	454 (75.29)		273 (50.65)	266 (49.35)		233 (57.53)	172 (42.47)	
rs2252070 (MMP13)			0.553			0.982			0.652			0.528
T	584 (65.03)	314 (34.97)		224 (25.17)	666 (74.83)		403 (50.88)	389 (49.12)		323 (56.17)	252 (43.83)	
C	284 (63.39)	164 (36.61)		112 (25.23)	332 (74.77)		201 (49.51)	205 (50.49)		185 (58.36)	132 (41.64)	

rs243847 (MMP2)			0.774		0.041		0.780		0.650
T	547 (64.20)	305 (35.80)		197 (23.34)	647 (76.66)		386 (50.72)	375 (49.28)	325 (57.52) 240 (42.48)
C	321 (64.98)	173 (35.02)		139 (28.37)	351 (71.63)		218 (49.89)	219 (50.11)	183 (55.96) 144 (44.04)
rs2303466 (DLX3)			0.964		0.589		0.717		0.938
C	729 (64.51)	401 (35.49)		285 (25.47)	834 (74.53)		509 (50.65)	496 (49.35)	425 (56.89) 322 (43.11)
T	139 (64.35)	77 (35.65)		51 (23.72)	164 (76.28)		95 (49.22)	98 (50.78)	83 (57.24) 62 (42.76)
rs11656951 (DLX3)			0.869		0.451		0.976		0.815
C	727 (64.39)	402 (35.61)		286 (25.58)	832 (74.42)		506 (50.40)	498 (49.60)	423 (56.78) 322 (43.22)
T	141 (64.98)	76 (35.02)		50 (23.15)	166 (76.85)		98 (50.52)	96 (49.48)	85 (57.82) 62 (42.18)
rs7501477 (TIMP2)			0.637		0.736		0.976		0.872
G	749 (64.74)	408 (35.26)		291 (23.35)	857 (74.65)		522 (50.43)	513 (49.57)	428 (57.07) 322 (42.93)
T	119 (62.96)	70 (37.04)		45 (14.19)	141 (75.81)		82 (50.31)	81 (49.69)	80 (56.34) 62 (43.66)
rs388286 (BMP7)			0.963		0.441		0.752		0.236
C	466 (64.54)	246 (35.46)		174 (24.34)	541 (75.66)		326 (50.00)	326 (50.00)	273 (58.84) 191 (41.16)
T	402 (64.42)	222 (35.58)		162 (26.17)	457 (73.83)		278 (50.92)	268 (49.08)	235 (54.91) 193 (45.09)
rs5997096 (TFIP11)			0.368		0.300		0.597		0.462
C	419 (63.29)	243 (36.71)		156 (23.93)	496 (76.07)		298 (51.20)	284 (48.80)	244 (55.71) 194 (44.29)
T	449 (65.64)	235 (34.36)		180 (26.39)	502 (73.61)		306 (49.68)	310 (50.32)	264 (58.15) 190 (41.85)

p-values are presented in bold when the differences are significant ($p < 0.05$); Fischer exact test

Table S8. Forward stepwise logistic regression reporting ODDS Ratio (OR) of the association between SNPs (genotype additive effect) and different outcomes: dental Caries trajectory (downward and high), dental caries at 15, 24 and 31 years.

Variables	Caries Trajectory (15, 24 and 31 years old) (n=888)		Dental caries 15 years (n=667)		Dental caries 24 years (n=599)		Dental caries 31 years (n=446)	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
<i>TUFT1</i>								
rs4970957 (Unad)	1		1		1		1	
AA	1.22 (0.83 – 1.80)	0.49	0.91 (0.60 – 1.42)	1.00	0.96 (0.65 – 1.43)	1.00	1.40 (0.89 – 2.21)	0.18
GA	1.22 (0.48-3.09)	1.00	0.89 (0.31 – 2.46)	1.00	0.61 (0.24 – 1.56)	0.49	1.74 (0.53 – 5.71)	0.60
GG								
rs4970957 (Adj)								
AA	1		1		1		1	
GA	1.43 (0.93 -2.21)	0.13	0.90 (0.57 – 1.43)	1.00	1.04 (0.68 – 1.61)	1.00	1.71 (1.04 – 2.81)	0.03
GG	1.36 (0.50 – 3.68)	0.98	0.99 (0.35 – 2.86)	1.00	0.66 (0.24 – 1.80)	0.71	2.07 (0.59 – 7.28)	0.39
<i>MMP20</i>								
rs1711437 (Unad)								
CC	1		1		1		1	

TC	0.95 (1.17 -1.44)	1.00	1.16 (0.75 – 1.84)	0.87	1.15 (0.76 – 1.74)	0.88	0.87 (0.54 – 1.42)	1.00
TT	0.90 (0.51 – 1.51)	1.00	1.02 (0.56 – 1.80)	1.00	0.94 (0.56 – 1.56)	1.00	0.89 (0.49 -1.63)	1.00
rs1711437 (Adj)								
CC	1		1		1		1	
TC	1.09 (0.69 – 1.71)	1.00	1.13 (0.70 – 1.83)	1.00	1.19 (0.76 – 1.86)	0.78	0.95 (0.57 – 1.60)	1.00
TT	0.84 (0.48 – 1.48)	0.99	0.93 (0.52 – 1.67)	1.00	0.88 (0.51 – 1.54)	1.00	0.88 (0.46 – 1.66)	1.00
rs1784418 (Unad)								
CC	1		1		1		1	
TC	1.04 (0.69 – 1.59)	1.00	1.13 (0.71 – 1.78)	1.00	1.08 (0.71 – 1.65)	1.00	0.87 (0.53 – 1.44)	1.00
TT	0.99 (0.60 – 1.64)	1.00	1.07 (0.62 – 1.86)	1.00	0.95 (0.58 – 1.57)	1.00	0.94 (0.53 – 1.68)	1.00
rs1784418 (Adj)								
CC	1		1		1		1	
TC	1.23 (0.78 – 1.97)	0.59	1.06 (0.65 – 1.73)	1.00	1.15 (0.73 – 1.81)	0.99	1.02 (0.60 – 1.74)	1.00
TT	0.91 (0.52 – 1.57)	1.00	0.94 (0.53 – 1.67)	1.00	0.88 (0.51 – 1.50)	1.00	0.97 (0.52 – 1.80)	1.00
MMP13								
rs2252070 (Unad)								
TT	1		1		1		1	
TC	1.29 (0.88 – 1.89)	0.26	0.82 (0.54 – 1.25)	0.58	1.38 (0.94 – 2.04)	0.13	1.16 (0.73 – 1.84)	0.93
CC	0.95 (0.51 -1.77)	1.00	1.23 (0.60 – 2.53)	1.00	0.86 (0.46 – 1.59)	1.00	0.69 (0.34 – 1.40)	0.48

rs2252070 (Adj)

TT	1		1		1		1	
TC	1.26 (0.82 – 1.91)	0.45	0.82 (0.52 – 1.27)	0.61	1.45 (0.95 – 2.21)	0.09	1.09 (0.67 – 1.79)	1.00
CC	0.89 (0.45 – 1.77)	1.00	1.35 (0.63 – 2.89)	0.74	0.94 (0.49 – 1.85)	1.00	0.67 (0.32 – 1.42)	0.46

MMP2

rs243847 (Unad)

TT	1		1		1		1	
TC	0.86 (0.58 – 1.28)	0.81	0.76 (0.49 – 1.18)	0.35	0.92 (0.62- 1.37)	1.00	1.09 (0.68 -1.73)	1.00
CC	1.01 (0.58 – 1.74)	1.00	0.61 (0.34 – 1.10)	0.12	1.15 (0.65 – 2.04)	1.00	1.12 (0.58 – 2.16)	1.00

rs243847 (Adj)

TT	1		1		1		1	
TC	0.92 (0.60 – 1.42)	1.00	0.79 (0.49 – 1.25)	0.48	1.02 (0.68 – 1.56)	1.00	1.12 (0.68 – 1.84)	1.00
CC	1.11 (0.61 – 2.03)	1.00	0.67 (0.36 – 1.27)	0.32	1.36 (0.74 – 2.52)	0.51	1.20 (0.59 – 2.41)	1.00

DLX3

rs2303466 (Unad)

CC	1		1		1		1	

TC	1.00 (0.66 – 1.53)	1.00	1.04 (0.65 – 1.66)	1.00	0.95 (1.46)	1.00	1.05 (0.63 – 1.73)	1.00
TT	1.02 (0.39 – 2.66)	1.00	1.37 (0.44 – 4.32)	1.00	0.54 (3.98)	0.77	0.85 (0.28 – 2.57)	1.00
rs2303466 (Adj)								
CC	1		1		1		1	
TC	0.96 (0.60 – 1.53)	1.00	1.00 (0.61 – 1.63)	1.00	0.94 (0.59 – 1.49)	1.00	1.10 (0.64 – 1.88)	1.00
TT	1.07 (0.36 – 3.18)	1.00	2.75 (0.65 – 11.60)	0.23	1.62 (0.55 – 4.81)	0.64	0.84 (0.24 – 2.85)	1.00
rs11656951 (Unad)								
CC	1		1		1		1	
TC	0.95 (0.62 – 1.46)	1.00	1.10 (0.69 – 1.76)	1.00	0.87 (0.57 – 1.33)	0.93	1.00 (0.61 – 1.65)	1.00
TT	1.01 (0.39 – 2.64)	1.00	1.39 (0.44 – 4.38)	1.00	1.44 (0.53 – 3.89)	0.83	0.84 (0.27 – 2.54)	1.00
rs11656951 (Adj)								
CC	1		1		1		1	
TC	0.92 (0.58 – 1.48)	1.00	1.07 (0.65 – 1.75)	1.00	0.86 (0.54 – 1.37)	0.93	1.07 (0.63 – 1.84)	1.00
TT	1.06 (0.36 – 3.15)	1.00	2.80 (0.67 – 11.80)	0.21	1.58 (0.53 – 4.71)	0.68	0.84 (0.25 – 2.84)	1.00
TIMP2								
rs7501477 (Unad)								
GG	1		1		1		1	
TG	1.01 (0.66 -1.55)	1.00	1.08 (0.67 – 1.74)	1.00	1.07 (0.69 – 1.65)	1.00	0.93 (0.57 – 1.52)	1.00

TT	1.58 (0.45 – 5.59)	0.84	1.03 (0.23 – 4.67)	1.00	0.68 (0.16 – 2.96)	1.00	1.53 (0.42 – 5.47)	0.90

rs7501477 (Adj)

GG	1		1		1		1	
TG	0.80 (0.49 – 1.29)	0.60	0.96 (0.59 – 1.59)	1.00	0.89 (0.56 – 1.43)	1.00	0.81 (0.47 – 1.38)	0.74
TT	1.25 (0.31 – 4.97)	1.00	0.99 (0.21 – 4.66)	1.00	0.70 (0.15 – 3.30)	1.00	1.37 (0.35 – 5.36)	1.00

BMP7

rs388286 (Unad)

CC	1		1		1		1	
TC	0.85 (0.56 – 1.29)	0.78	0.68 (0.42 – 1.11)	0.16	0.82 (0.54 – 1.26)	0.60	1.18 (0.70 – 1.99)	0.95
TT	1.04 (0.61 – 1.74)	1.00	0.84 (0.46 – 1.54)	1.00	0.96 (0.56 – 1.64)	1.00	1.40 (0.75 – 2.62)	0.45

rs388286 (Adj)

CC	1		1		1		1	
TC	0.85 (0.53 – 1.36)	0.87	0.69 (0.41 – 1.15)	0.21	0.83 (0.52 – 1.32)	0.73	1.25 (0.72 – 2.18)	0.74
TT	0.89 (0.50 – 1.58)	1.00	0.76 (0.40 – 1.44)	0.67	0.83 (0.47 – 1.48)	0.94	1.26 (0.65 – 2.48)	0.85

TFIP11

rs5997096 (Unad)

CC	1		1		1		1	

TC	0.80 (0.51 – 1.25)	0.52	0.72 (0.43 – 1.19)	0.28	0.83 (0.53 – 1.31)	0.74	1.09 (0.65 – 1.85)	1.00
TT	0.82 (0.50 – 1.35)	0.74	0.76 (0.43 - 1.35)	0.57	1.11 (0.66 – 1.84)	1.00	0.84 (0.47 – 1.50)	0.98
rs5997096 (Adj)								
CC	1		1		1		1	
TC	0.74 (0.45 – 1.20)	0.33	0.70 (0.41 – 1.20)	0.27	0.82 (0.51 – 1.34)	0.74	0.99 (0.57 – 1.75)	1.00
TT	0.71 (0.41 – 1.24)	0.34	0.75 (0.41 – 1.37)	0.57	1.09 (0.63 – 1.88)	1.00	0.72 (0.39 – 1.35)	0.49

p value was adjusted by multiple comparisons (Bonferroni); Unad: Unadjusted; Adj: Ancestry-informative genetic, sex, income, sugar consumption, gingival bleeding

Table S9. Forward stepwise logistic regression reporting ODDS Ratio (OR) of the association between SNPs (genotype dominant effect) and different outcomes: dental Caries trajectory (downward and high), dental caries at 15, 24 and 31 years.

Variables	Caries Trajectory (15, 24 and 31 years old) (n=888)		Dental caries 15 years (n=667)		Dental caries 24 years (n=599)		Dental caries 31 years (n=446)	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
<i>TUFT1</i>								
rs4970957 (Unad)								
AA	1		1		1		1	
GA/ GG	1.22 (0.88 – 1.69)	0.23	0.91 (0.63 – 1.31)	0.63	0.91 (0.65 – 1.27)	0.59	1.43 (0.98 – 2.10)	0.06
rs4970957 (Adj)								
AA	1		1		1		1	
GA/ GG	1.42 (0.99 -2.05)	0.05	0.91 (0.62 – 1.34)	0.64	0.98 (0.68 – 1.42)	0.95	1.74 (1.14 – 2.65)	0.01
<i>MMP20</i>								
rs1711437 (Unad)								
CC	1		1		1		1	
TC/TT	0.94 (0.67 – 1.31)	0.73	1.13 (0.78 – 1.62)	0.53	1.07 (0.77 – 1.51)	0.65	0.88 (0.59 – 1.31)	0.53

rs1711437 (Adj)

CC	1		1		1		1	
TC/TT	1.00 (0.70 – 1.45)	0.97	1.06 (0.72 – 1.56)	0.75	1.08 (0.75 – 1.56)	0.663	0.93 (0.61 – 1.42)	0.73

rs1784418 (Unad)

CC	1		1		1		1	
TC/TT	1.03 (0.73 – 1.44)	0.86	1.11 (0.76 – 1.61)	0.58	1.03 (0.74 – 1.46)	0.84	0.90 (0.60 – 1.34)	0.60

rs1784418 (Adj)

CC	1		1		1		1	
TC/TT	1.15 (0.76 – 1.62)	0.57	1.02 (0.69 – 1.51)	0.92	1.05 (0.72 – 1.51)	0.81	1.00 (0.65 – 1.54)	0.99

MMP13**rs2252070 (Unad)**

TT	1		1		1		1	
TC/CC	1.21 (0.89 -1.68)	0.22	0.88 (0.62 – 1.26)	0.49	1.26 (0.91 – 1.74)	0.17	1.04 (0.71 – 1.51)	0.86

rs2252070 (Adj)

TT	1		1		1		1	
TC/CC	1.17 (0.83 – 1.67)	0.36	0.89 (0.61 – 1.30)	0.56	1.33 (0.94 – 1.89)	0.11	0.98 (0.65 – 1.47)	0.93

MMP2**rs243847 (Unad)**

TT	1		1	0.07	1		1	
TC/CC	0.89 (0.65 – 1.23)	0.51	0.72 (0.50 – 1.03)		0.97 (0.70 – 1.35)	0.87	1.09 (0.75 – 1.60)	0.45
rs243847 (Adj)								
TT	1		1		1		1	
TC/ CC	0.97 (0.68 – 1.38)	0.86	0.76 (0.52 – 1.11)	0.151	1.09 (0.77 – 1.55)	0.62	1.14 (0.76 – 1.71)	0.53
DLX3								
rs2303466 (Unad)								
CC	1		1		1		1	
TC/TT	1.00 (0.71 – 1.43)	0.976	1.08 (0.73 – 1.59)	0.71	1.00 (0.71 – 1.44)	0.97	1.01 (0.67 – 1.54)	0.95
rs2303466 (Adj)								
CC	1		1		1		1	
TC/TT	0.97 (0.66 – 1.44)		1.11 (0.74 – 1.69)	0.61	1.00 (0.69 – 1.48)	0.98	1.06 (0.68 – 1.66)	0.80
rs11656951 (Unad)								
CC	1		1		1		1	
TC/TT	0.96 (0.67 – 1.37)	0.83	1.13 (0.76 – 1.67)	0.54	0.93 (0.65 – 1.32)	0.68	0.97 (0.65 – 1.48)	0.91
rs11656951 (Adj)								
CC	1		1		1		1	

CC	1		1		1		1	
TC/TT	0.81 (0.57 – 1.16)	0.25	0.73 (0.48 – 1.12)	0.15	0.93 (0.64 – 1.34)	0.69	0.99 (0.65 – 1.51)	0.97
rs5997096 (Adj)								
CC	1		1		1		1	
TC/TT	0.73 (0.49 – 1.08)	0.123	0.72 (0.46 – 1.12)	0.14	0.91 (0.61 – 1.36)	0.64	0.88 (0.56 – 1.40)	0.60

p value was adjusted by multiple comparisons (Bonferroni); Unad: Unadjusted; Adj: Ancestry-informative genetic, sex, income, sugar consumption, gingival bleeding

Table S10. Forward stepwise multilevel logistic regression reporting ODDS Ratio (OR) of the association between SNPs (Allelic) and different outcomes: dental Caries trajectory (downward and high), dental caries at 15, 24 and 31 years.

Variables	Caries Trajectory (15, 24 and 31 years old) (n=888)		Dental caries 15 years (n=667)		Dental caries 24 years (n=599)		Dental caries 31 years (n=446)	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
<i>TUFT1</i>								
rs4970957 (Unad)								
AA	1		1		1		1	
GA	1.22 (0.83 – 1.80)	0.49	0.91 (0.60 – 1.42)	1.00	0.96 (0.65 – 1.43)	1.00	1.40 (0.89 – 2.21)	0.18
GG	1.22 (0.48-3.09)	1.00	0.89 (0.31 – 2.46)	1.00	0.61 (0.24 – 1.56)	0.49	1.74 (0.53 – 5.71)	0.60
rs4970957 (Adj)								
AA	1		1		1		1	
GA	1.43 (0.93 -2.21)	0.13	0.90 (0.57 – 1.43)	1.00	1.04 (0.68 – 1.61)	1.00	1.71 (1.04 – 2.81)	0.03
GG	1.36 (0.50 – 3.68)	0.98	0.99 (0.35 – 2.86)	1.00	0.66 (0.24 – 1.80)	0.71	2.07 (0.59 – 7.28)	0.39
<i>MMP20</i>								
rs1711437 (Unad)								
CC	1		1		1		1	

TC	0.95 (1.17 -1.44)	1.00	1.16 (0.75 – 1.84)	0.87	1.15 (0.76 – 1.74)	0.88	0.87 (0.54 – 1.42)	1.00
TT	0.90 (0.51 – 1.51)	1.00	1.02 (0.56 – 1.80)	1.00	0.94 (0.56 – 1.56)	1.00	0.89 (0.49 -1.63)	1.00
rs1711437 (Adj)								
CC	1		1		1		1	
TC	1.09 (0.69 – 1.71)	1.00	1.13 (0.70 – 1.83)	1.00	1.19 (0.76 – 1.86)	0.78	0.95 (0.57 – 1.60)	1.00
TT	0.84 (0.48 – 1.48)	0.99	0.93 (0.52 – 1.67)	1.00	0.88 (0.51 – 1.54)	1.00	0.88 (0.46 – 1.66)	1.00
rs1784418 (Unad)								
CC	1		1		1		1	
TC	1.04 (0.69 – 1.59)	1.00	1.13 (0.71 – 1.78)	1.00	1.08 (0.71 – 1.65)	1.00	0.87 (0.53 – 1.44)	1.00
TT	0.99 (0.60 – 1.64)	1.00	1.07 (0.62 – 1.86)	1.00	0.95 (0.58 – 1.57)	1.00	0.94 (0.53 – 1.68)	1.00
rs1784418 (Adj)								
CC	1		1		1		1	
TC	1.23 (0.78 – 1.97)	0.59	1.06 (0.65 – 1.73)	1.00	1.15 (0.73 – 1.81)	0.99	1.02 (0.60 – 1.74)	1.00
TT	0.91 (0.52 – 1.57)	1.00	0.94 (0.53 – 1.67)	1.00	0.88 (0.51 – 1.50)	1.00	0.97 (0.52 – 1.80)	1.00
MMP13								
rs2252070 (Unad)								
TT	1		1		1		1	
TC	1.29 (0.88 – 1.89)	0.26	0.82 (0.54 – 1.25)	0.58	1.38 (0.94 – 2.04)	0.13	1.16 (0.73 – 1.84)	0.93
CC	0.95 (0.51 -1.77)	1.00	1.23 (0.60 – 2.53)	1.00	0.86 (0.46 – 1.59)	1.00	0.69 (0.34 – 1.40)	0.48

rs2252070 (Adj)

TT	1		1		1		1	
TC	1.26 (0.82 – 1.91)	0.45	0.82 (0.52 – 1.27)	0.61	1.45 (0.95 – 2.21)	0.09	1.09 (0.67 – 1.79)	1.00
CC	0.89 (0.45 – 1.77)	1.00	1.35 (0.63 – 2.89)	0.74	0.94 (0.49 – 1.85)	1.00	0.67 (0.32 – 1.42)	0.46

MMP2

rs243847 (Unad)

TT	1		1		1		1	
TC	0.86 (0.58 – 1.28)	0.81	0.76 (0.49 – 1.18)	0.35	0.92 (0.62- 1.37)	1.00	1.09 (0.68 -1.73)	1.00
CC	1.01 (0.58 – 1.74)	1.00	0.61 (0.34 – 1.10)	0.12	1.15 (0.65 – 2.04)	1.00	1.12 (0.58 – 2.16)	1.00

rs243847 (Adj)

TT	1		1		1		1	
TC	0.92 (0.60 – 1.42)	1.00	0.79 (0.49 – 1.25)	0.48	1.02 (0.68 – 1.56)	1.00	1.12 (0.68 – 1.84)	1.00
CC	1.11 (0.61 – 2.03)	1.00	0.67 (0.36 – 1.27)	0.32	1.36 (0.74 – 2.52)	0.51	1.20 (0.59 – 2.41)	1.00

DLX3

rs2303466 (Unad)

CC	1		1		1		1	

TC	1.00 (0.66 – 1.53)	1.00	1.04 (0.65 – 1.66)	1.00	0.95 (1.46)	1.00	1.05 (0.63 – 1.73)	1.00
TT	1.02 (0.39 – 2.66)	1.00	1.37 (0.44 – 4.32)	1.00	0.54 (3.98)	0.77	0.85 (0.28 – 2.57)	1.00
rs2303466 (Adj)								
CC	1		1		1		1	
TC	0.96 (0.60 – 1.53)	1.00	1.00 (0.61 – 1.63)	1.00	0.94 (0.59 – 1.49)	1.00	1.10 (0.64 – 1.88)	1.00
TT	1.07 (0.36 – 3.18)	1.00	2.75 (0.65 – 11.60)	0.23	1.62 (0.55 – 4.81)	0.64	0.84 (0.24 – 2.85)	1.00
rs11656951 (Unad)								
CC	1		1		1		1	
TC	0.95 (0.62 – 1.46)	1.00	1.10 (0.69 – 1.76)	1.00	0.87 (0.57 – 1.33)	0.93	1.00 (0.61 – 1.65)	1.00
TT	1.01 (0.39 – 2.64)	1.00	1.39 (0.44 – 4.38)	1.00	1.44 (0.53 – 3.89)	0.83	0.84 (0.27 – 2.54)	1.00
rs11656951 (Adj)								
CC	1		1		1		1	
TC	0.92 (0.58 – 1.48)	1.00	1.07 (0.65 – 1.75)	1.00	0.86 (0.54 – 1.37)	0.93	1.07 (0.63 – 1.84)	1.00
TT	1.06 (0.36 – 3.15)	1.00	2.80 (0.67 – 11.80)	0.21	1.58 (0.53 – 4.71)	0.68	0.84 (0.25 – 2.84)	1.00
TIMP2								
rs7501477 (Unad)								
GG	1		1		1		1	
TG	1.01 (0.66 -1.55)	1.00	1.08 (0.67 – 1.74)	1.00	1.07 (0.69 – 1.65)	1.00	0.93 (0.57 – 1.52)	1.00

TT	1.58 (0.45 – 5.59)	0.84	1.03 (0.23 – 4.67)	1.00	0.68 (0.16 – 2.96)	1.00	1.53 (0.42 – 5.47)	0.90
rs7501477 (Adj)								
GG	1		1		1		1	
TG	0.80 (0.49 – 1.29)	0.60	0.96 (0.59 – 1.59)	1.00	0.89 (0.56 – 1.43)	1.00	0.81 (0.47 – 1.38)	0.74
TT	1.25 (0.31 – 4.97)	1.00	0.99 (0.21 – 4.66)	1.00	0.70 (0.15 – 3.30)	1.00	1.37 (0.35 – 5.36)	1.00
BMP7								
rs388286 (Unad)								
CC	1		1		1		1	
TC	0.85 (0.56 – 1.29)	0.78	0.68 (0.42 – 1.11)	0.16	0.82 (0.54 – 1.26)	0.60	1.18 (0.70 – 1.99)	0.95
TT	1.04 (0.61 – 1.74)	1.00	0.84 (0.46 – 1.54)	1.00	0.96 (0.56 – 1.64)	1.00	1.40 (0.75 – 2.62)	0.45
rs388286 (Adj)								
CC	1		1		1		1	
TC	0.85 (0.53 – 1.36)	0.87	0.69 (0.41 – 1.15)	0.21	0.83 (0.52 – 1.32)	0.73	1.25 (0.72 – 2.18)	0.74
TT	0.89 (0.50 – 1.58)	1.00	0.76 (0.40 – 1.44)	0.67	0.83 (0.47 – 1.48)	0.94	1.26 (0.65 – 2.48)	0.85
TFIP11								
rs5997096 (Unad)								
CC	1		1		1		1	

TC	0.80 (0.51 – 1.25)	0.52	0.72 (0.43 – 1.19)	0.28	0.83 (0.53 – 1.31)	0.74	1.09 (0.65 – 1.85)	1.00
TT	0.82 (0.50 – 1.35)	0.74	0.76 (0.43 - 1.35)	0.57	1.11 (0.66 – 1.84)	1.00	0.84 (0.47 – 1.50)	0.98
rs5997096 (Adj)								
CC	1		1		1		1	
TC	0.74 (0.45 – 1.20)	0.33	0.70 (0.41 – 1.20)	0.27	0.82 (0.51 – 1.34)	0.74	0.99 (0.57 – 1.75)	1.00
TT	0.71 (0.41 – 1.24)	0.34	0.75 (0.41 – 1.37)	0.57	1.09 (0.63 – 1.88)	1.00	0.72 (0.39 – 1.35)	0.49

p value was adjusted by multiple comparisons (Bonferroni); Unad: Unadjusted; Adj: Ancestry-informative genetic, sex, income, sugar consumption, gingival bleeding

Figure 1. Linkage disequilibrium of a) *DLX3*, *TIMP2* and *BMP7* and b) *MMP20* and *MMP13* genes. The Single Nucleotides Polymorphisms were tested using SHEsis and estimated with D'. D' (rs2303466-rs11656951) = 0.983; D' (rs2303466-rs7501477) = 0.053;; D' (rs11656951-rs7501477) = 0.051; D' (rs1711437-rs1784418) = 0.967; D' (rs1711437-rs2252070) = 0.167; D' (rs1784418-rs2252070) = 0.147.

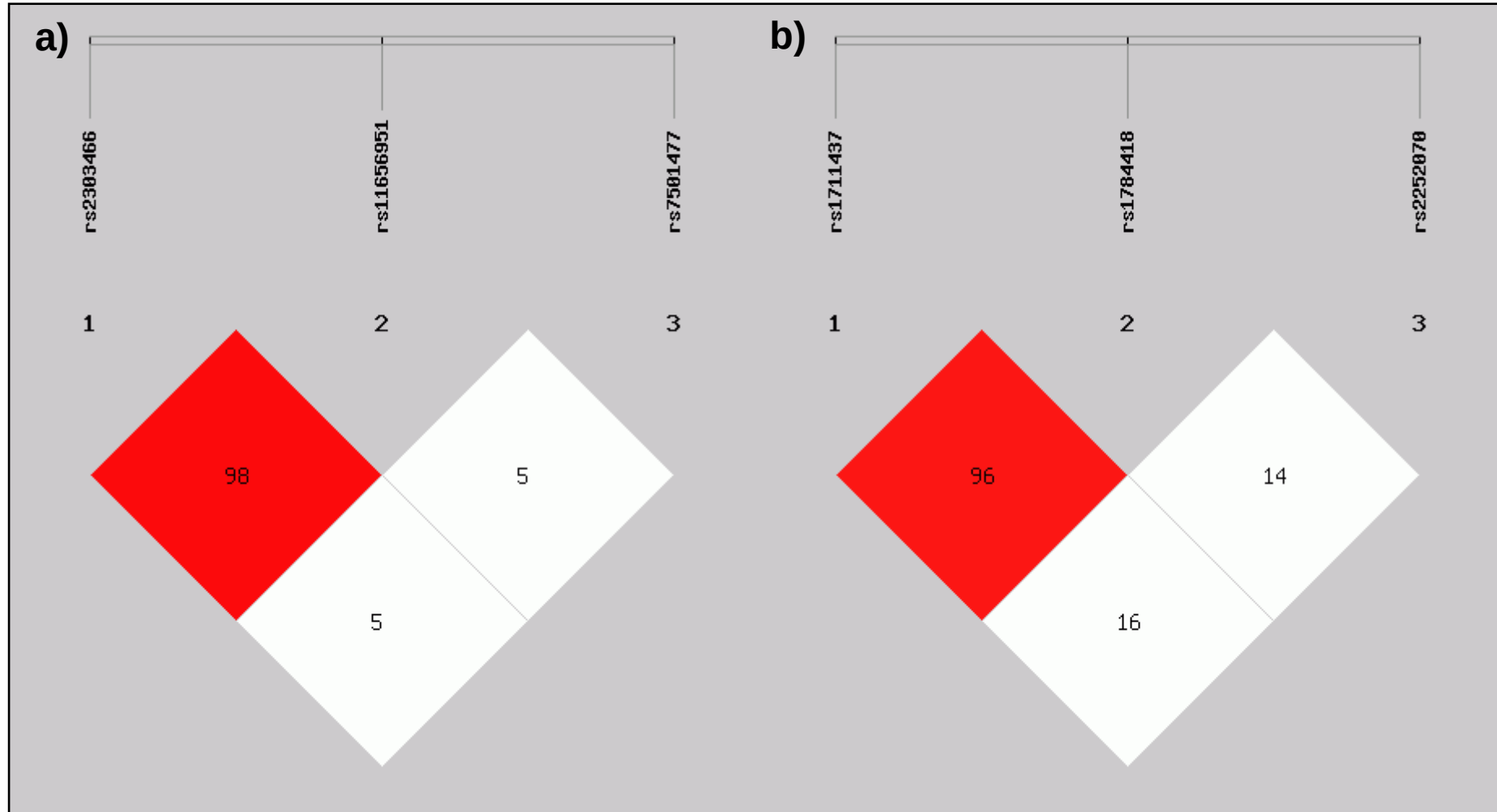


Figure 2. Graphical model of gene–gene interaction analysis

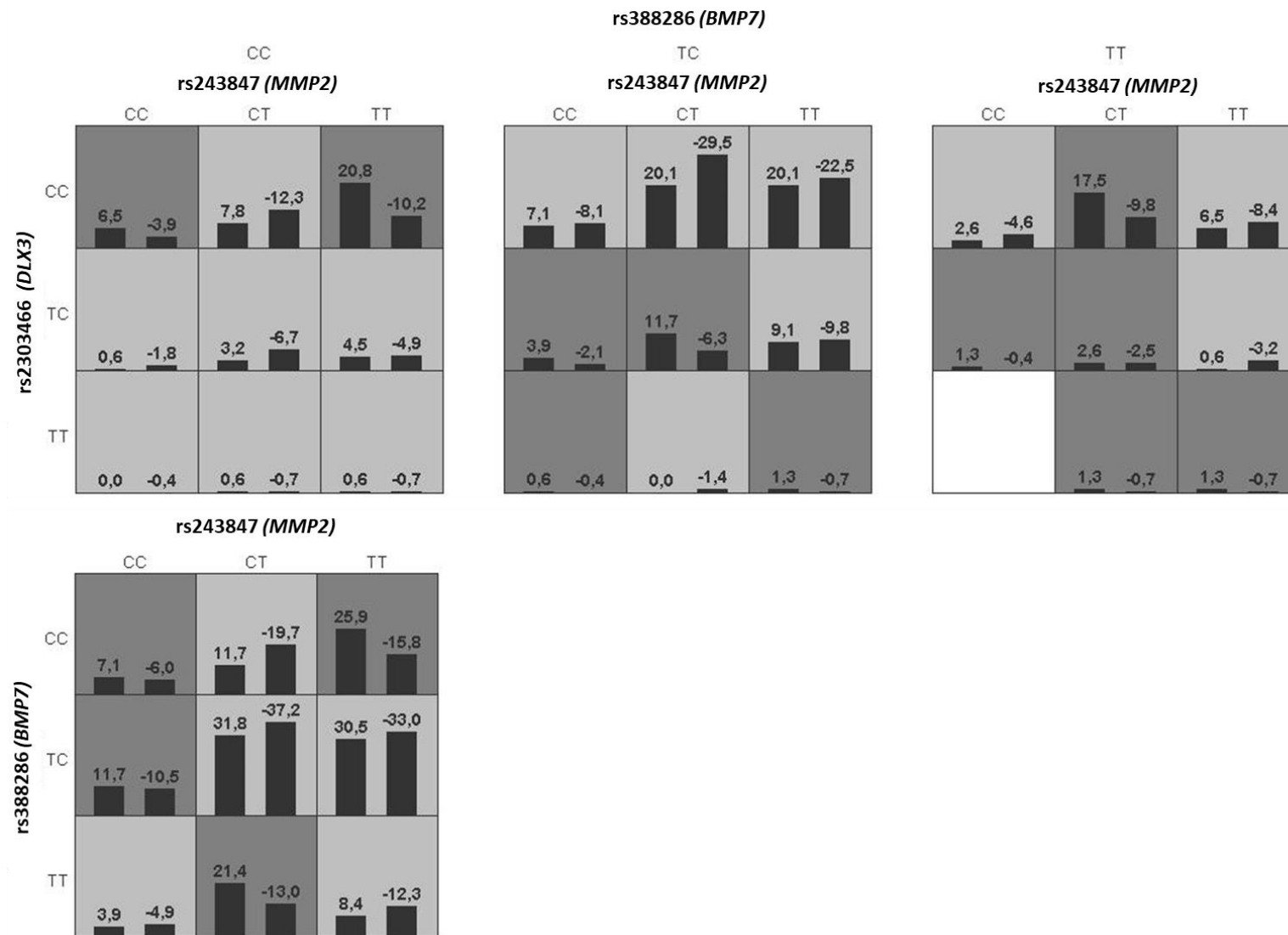
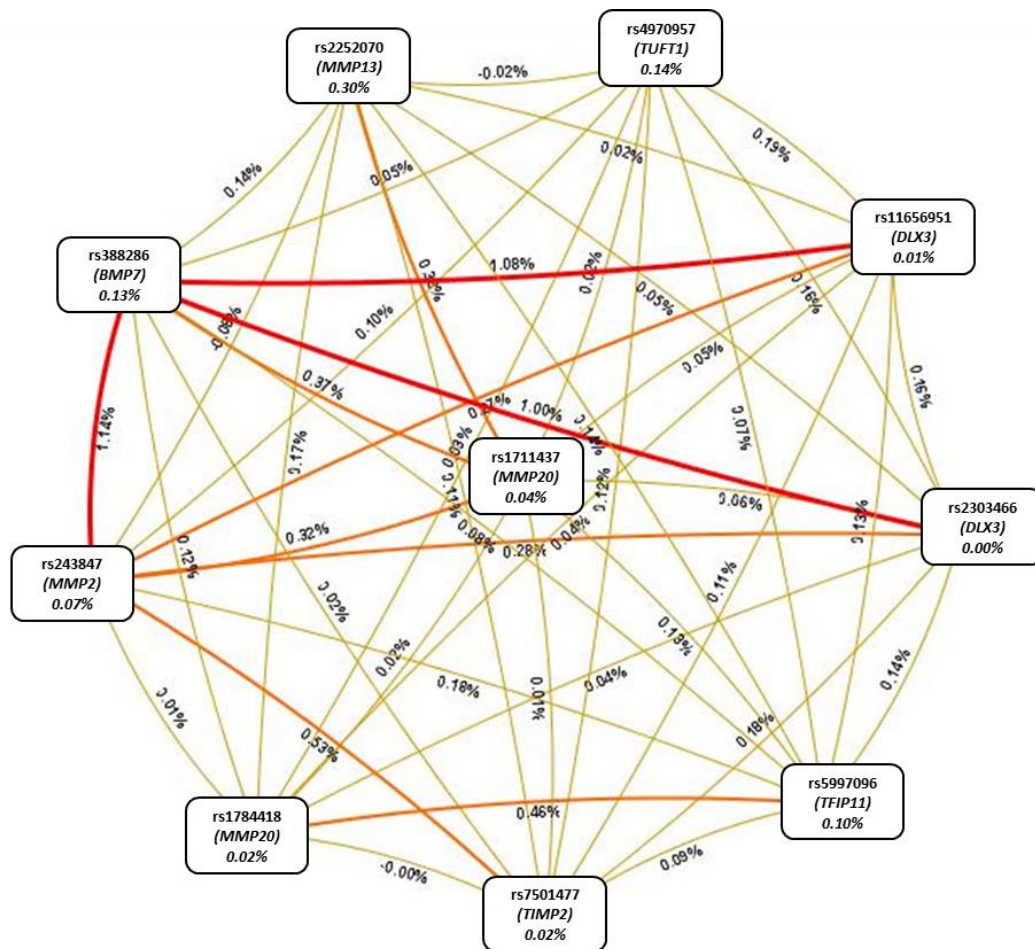


Figure 3. Illustration of interaction graph summarizing the measures of information gain. Attributes connected by red lines have stronger synergistic interactions than those connected by yellow lines



5.3 Artigo 8

Artigo formatado seguindo as normas da Revista *Clinical Oral Investigations*.

rs11716497 of Lactoferrin present a direct effect on dental caries trajectory in the life course

Running title: Lactoferrin and Caries

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Key words: Polymorphisms. Dental caries. *Lactoferrin*. Genetic. Gene.

Declarations of conflict of interest: none

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Cover Letter

To: Professor Dr. Matthias Hannig
Editor-in-Chief,

Dear Editor:

Based on the importance of *Clinical Oral Investigations*, we are sending the manuscript entitled “**rs11716497 of Lactoferrin present a direct effect on dental caries trajectory in the life course**” to be appraised by the Journal’s Editorial Board.

this is the first study investigating the association of SNPs linked to immune response genes (*LTF* and *MBL2*) and dental caries with longitudinal design in a birth cohort sample. The present results were estimated using several quality control filters aiming to reduce the bias in presented estimates linked to gene association studies; therefore, we found significant associations of rs11716497 (*LTF*) in all analysis performed – genotype additive and dominant effect and allelic – evidencing the robustness of present results. Allele G of this SNP was linked to an increase on odds of being in high caries trajectory group as well as the GG genotype. Moreover, we investigate if its association was mediated by sugar consumption through g-formula. Thus, it was found that association between rs11716497 (*LTF*) and caries trajectory is not mediated by sugar consumption but presents a direct effect. These results highlight the hypothesis that the main effect of this association is linked to immune response and not to increase on sugar consumption, which not provide a significant part of the explanation in this pathway analysis. We also performed an epistatic analysis using the Generalized Multifactor Dimensionality Reduction and our findings showed that the best model was a two-locus involving rs4547741 (*LTF*) and rs11716497 (*LTF*), which were responsible to an increase of two folds in odds of being in high caries trajectory group. Therefore, indicates a potential gene-gene interaction between these SNPs.

The presence of *LTF* in saliva can reduce *Streptococcus Mutans* in a dose-dependent effect, being consequently capable to reduce dental caries. These results corroborate with our

observations through g-formula analysis that demonstrate that the main effect of rs11716497 (*LTF*) on caries trajectory is not mediated by sugar consumption. So, we can infer that effect of its SNP seems to be direct in dental caries trajectory; possibly due to host immune response, since only 0.5% of effect was mediated by sugar consumption in our findings.

It is important highlight that we performed a wide quality control filters aiming to minimize bias in our results in the present population-based sample. In this way, we exclude SNPs in Hardy-Weinberg disequilibrium, performed analysis adjusting our final models by important factors implicated in caries occurrence – like as income and sugar consumption – even as adjusted the estimates by genomic ancestry using about 370,000 SNPs accessible from the 1982 Pelotas birth cohort, which is compatible with the HapMap and Human Genome Diversity projects for the Brazilian population . Besides, Bonferroni multiple corrections test was used in our analysis to avoid false positive. An important point is that our results are based in the trajectory of caries from 15 to 31 years of age, not limited to a specific one moment in the life course, which represents better the risk for caries in each individual.

This is an original manuscript and has not been considered for publication elsewhere. The paper was read and approved by all authors. All authors have made substantive contribution to this study, and all have reviewed the final paper prior to its submission. The authors declare that there are no potential competing interests. Furthermore, I attest the validity and legitimacy of data and its interpretation. There are no conflicts of interest for authors listed above. We sign for and accept responsibility for releasing this material.

Thank you very much for your consideration.

Yours sincerely,

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rs11716497 of Lactoferrin present a direct effect on dental caries trajectory in the life course

Running title: Lactoferrin and Caries

***rs11716497* of Lactotransferrin present a direct effect on dental caries trajectory in the life course**

Running title: Lactotransferrin and Caries

Abstract:

Aim: was to investigate if the trajectory of dental caries is associated with the SNPs related to *LTF* and *MBL2* genotypes and allele.

Methods: Representative sample of 5,914 births from the 1982 in Pelotas birth cohort were prospectively investigated. Trajectory of untreated dental caries was obtained from oral health assessments at 15, 24 and 31 yrs. (15 [n=888], 24 [n=720] and 31 years[n=539]). Single Nucleotide Polymorphisms (SNP) of Lactotransferin (*LTF*) and Mannose Binding Lectin 2 (*MBL2*) were genotyped. Logistic regressions with Bonferroni correctios were adjusted by ancestry genetic, sex, family income and sugar consumption. G-formula was used to estimate the indirect effect of sugar consumption on association and Generalized multifactor dimensionality reduction (GMDR) was utilized to investigate epistatic interactions.

Results: Six SNPS of *LTF* and two in *MBL2* were investigated. rs11716497 (*LTF*) was associated with individuals in high caries trajectory group in unadjusted ($p = 0.003$), adjusted ($p = 0.042$) and dominant ($p = 0.027$) models. Parametric g-formula analysis showed that rs11716497 (*LTF*) seem to have a direct effect on dental caries trajectory ($p < 0.001$) independently of sugar consumption. GMDR shown a significant epistatic interaction ($p < 0.004$) involving rs4547741 (*LTF*) and rs11716497 (*LTF*).

Conclusions: It was found significant associations of rs11716497 (*LTF*) and dental caries trajectory in the life course. G-formula analysis shown that association between rs11716497 (*LTF*) and caries trajectory was not mediated by sugar consumption, presenting a direct effect. Results must be interpreted taking into account all inherent limitations for genetic association studies.

Introduction

Genetic approach has increasing in the current literature as complementary strategy to improve the knowledge of multifactorial diseases [Vieira et al., 2014], clarifying in several cases part of effects observed and not yet explained by known factors [Tannure et al., 2012; Vieira et al., 2014]. Within these complex diseases, dental caries is the most prevalent in the oral cavity affecting a wide range of people around the world [Chisini et al., 2018; Dutra et al., 2018; Kassebaum et al., 2017]. The main etiological factor for dental caries is the biofilm caused by combination of elevate rate of sugar consumption and poor oral health habits [Maltz et al., 2017; von Elm et al., 2007], which can be mediated by host immune response of organism [Meng et al., 2019]. These factors are also influenced by fluoride presence (in the water, toothpaste, among others) that presents a well-known effect on caries reduction [Vieira et al., 2014]. Furthermore, the contextual factors – such as socioeconomical – are population level elements that present strongly influence in disease [Dutra et al., 2018; Kassebaum et al., 2017].

Some studies have shown the possibility that genetic factors may also explain a lower proportion of caries prevalence in several populations [Chisini et al., 2020; Slade et al., 2013; Vieira et al., 2014], since it is observed in some cases that individuals that have the same protective (such as water fluoridation) or risk factors can display different patterns of dental caries [Slade et al., 2013; von Elm et al., 2007]. Therefore, taking in account the increase of available tool from the genome project, studies have address efforts to investigate possible genetic influences on dental caries experience.

For this purpose, some strategies and study designs can be chosen. A small part of studies related with dental caries as phenotype has focus in the preformation of Genome Wide Associations (GWAS), aiming to identify new potential genes and single nucleotide polymorphisms (SNP) [Haworth et al., 2018; Meng et al., 2019; Shaffer et al., 2013; Zeng et al., 2013], while others have used the candidate gene methodological approach, which aims to examine known SNPs [Chisini et al., 2020; Vieira et al., 2014]. These studies identified several SNPs in some pooled genes with possible influence on dental caries experience [Chisini et al., 2020; Vieira et al., 2014]; among then, SNPs linked to immune response genes – such as lactotransferrin (*LTF*) and the Mannose Binding Lectin 2 (*MBL2*) - can influence the expression of some proteins present in saliva and thus presented antimicrobial, antiviral, antifungal and anti-inflammatory properties [Farnaud and Evans, 2003; Vieira et al., 2014]. Although this large increase in the number of studies investigating the influence of genetic factors on the

experience of dental caries, the literature still lacks studies presenting longitudinal phenotype evaluation designs as well as studies presenting large quality control filters, like correction for multiple comparisons avoiding false positive results. Moreover, it is necessary to confirm the real pathway of SNPs linked to immune response on dental caries.

The aim of this study was to investigate the effect of SNPs of *LTF* and *MBL2* on dental caries. Specifically, we addressed two research questions: i) is trajectory of dental caries associated with the SNPs related to *LTF* and *MBL2* genotypes and allele?; ii) is the association between response immune SNPs (*LTF* / *MBL2*) and dental caries trajectory mediated by the sugar consumption? Our hypothesis is that SNPs of immune response genes influence the caries trajectory and the associated SNPs are not mediated by sugar consumption, since that pathway (*a priori*) is due to host immune response.

Methods

Strengthening the reporting of observational studies in epidemiology (STROBE) was used to report the present study. [von Elm et al., 2007].

Study design, setting and participants

The present study was carried out in Pelotas, a Brazilian southern city. The study starts in 1982, where 99.2% of all alive births of Pelotas were identified (5,914 children) and included in a perinatal study. This population is accompanied to the present day [Barros et al., 2008]. Oral health evaluations were performed in a representative sample of the cohort (900 individuals) in 1997, at 15 years old. In 2004, the entire 1982 cohort was interviewed, and food frequency questionnaire was applied. Furthermore, individuals were invited to participate to genetic material collection. In 2006, participants included in oral health examinations were again invited to participate to second follow-up to investigate oral health. Participants were interviewed and examined by trained and calibrated dentists (Kappa > 0.65). In this stage, 720 individuals composed the second oral health follow-up, which represents 80% of the initial sample. Subsequently, in 2013 (at 31 years old), the 888 individuals from the initial sample were searched again. Participants were interviewed and clinical examinations were performed. Detailed methods of oral health studies were described by Peres et al. [2011].

Outcome variable (phenotype)

The outcome of present study was the untreated caries trajectory (15, 24 and 31 years). To calculate the caries trajectory group-Based trajectory modeling was utilized aim found groups with similar trajectories of component “decayed” of DMF-T/S index. DMF-T index was collected at 15, 24, 31 years. Component “decayed” of each follow-up was estimated and the participants were divided into: i) individuals with at least one component decayed, and ii) individuals without decayed component, in each follow-up [Dennis et al., 1981; Jones and Nagin, 2007]. The model was estimated with the command “traj” in the program Stata 12.0 [Jones et al., 2001; Silva et al., 2018]. Identifying the similarity of the trajectory among the evaluated individuals. The parameters for the model trajectory was determined based on the maximum likelihood by the quasi-Newton method [Dennis et al., 1981; Jones and Nagin, 2007]. Model selection was considered and estimated by the latent number of categories and the

polynomial order of each latent trajectory. The number of trajectories was determined when through sequential comparisons of the Bayesian information criterion (BIC) and its fit criteria between the K and $K + 1$ trajectory model have not produced substantial difference in the $k + 1$ model BIC score. [Jones et al., 2001; Silva et al., 2018]. Thus, were identified two trajectories (low and high)

Independent variables

Genetic material was collected by blood sample, collected with venipuncture. The DNA/serum was extracted and frozen at -70°C . DNA was genotyped using Illumina *Illumina HumanOmni2.5-8v1 array* [Horta et al., 2015; Victora and Barros, 2006]. Markers investigated and base pair change are displayed in **Table 1**. Genomic ancestry was also investigated by ADMIXTURE.[Alexander et al., 2009] based on approximately 370,000 SNPs available from the 1982 Pelotas birth cohort [Lima-Costa et al., 2015].

Moreover, family income at age 31 was collected in continuous (BRL) and categorized in tertiles. Posteriorly, the variable was dichotomized into higher (2nd and 3rd tertiles) and lower (1st tertile) tertiles [Chisini et al., 2019]. Food frequency questionnaire was performed with questions about the consumption of sweet foods (ice cream, candies, chocolate, sweet puddings, sodas) and sugar. The consumption was estimated using reported daily/weekly/monthly/yearly frequency, ranging from 0 to 10. The year consumption was calculated and categorized into tertiles and dichotomized in higher (3rd tertile) and lower (1st and second tertiles) tertiles of sugar consumption.

Statistical methods

The *Hardy–Weinberg equilibrium* as well as allele frequency estimation were investigated using the command “*genhw*” into Stata 12.0. [Newton]. Aiming to prevent possible population stratification effect, all the analysis was adjusted by the first ten major components of the principal component analysis considering the European, African and Native American populations. Initially, population characteristics were described by absolute and relative frequencies and dental caries trajectory using the fisher exact test.

To investigate the associations of single nucleotide polymorphism and dental caries, logistic regressions were performed calculating the Odds Ratio (OR) and respective 95% confidence intervals (CI95%). Allelic analysis was performed considering mixed effects and two hierarchical levels: i) genetic and ii) personal level. Genotype analysis were performed assuming additive and dominant effects. All final models were adjusted by ancestry genetic, sex, income and sugar consumption. Furthermore, all analyses were corrected by Bonferroni correction to multiple tests.

The parametric g-formula was used to assess the total causal effect (TCE), the natural direct effect (NDE), the natural indirect effect (NIE), even as the controlled direct effect (CDE) of SNPs on caries trajectory. Income was used as post-confounder and sugar consumption as indirect effect. Monte Carlo approach was performed to estimate the effects. The bootstrap method was used to estimate the standard errors and the confidence interval of the estimated effects. To perform this estimation, we chose to use 1000 resamples of size 10,000. Stata statistical package, version 12.0, was used for all statistical analysis (Stata Corporation, College Station, USA).

Linkage disequilibrium analysis was performed aim to establish the non-random association of alleles in the same chromosome. The estimating of D' and r^2 were performed using the SHEsis, an online software (available in <https://analysis.bio-x.cn/myAnalysis.php>) [Shi and He, 2005; Wang and Qin, 2018]. Haplotype analysis were performed using the same software; thus, associations between caries trajectory and haplotype with frequencies > 0.001 were estimated.

Generalized multifactor dimensionality reduction (GMDR) software was utilized to investigate epistatic interactions, i.e. gene–gene interactions. To perform this analysis, we used the caries trajectory as main outcome. Logistic regressions models and the genotypes of all SNPs were performed being adjusted by ancestry genetic, sex, income and sugar consumption.

Ethical issues

This study was approved by the UFPel Ethics Committee.

Results

A total of 539 participants were examined in the oral health sample at 31 years. About genetic ancestry, participants presented main (89.1%) European ancestry and African (10.9%). Individuals with main American-native ancestry were not found in this sample. Female were more prevalent in the low caries trajectory group (70.94%) than males (64.73%). Similarly, individuals from highest income tertiles were more present in low caries trajectory group (73.70%) than individuals from lowest tertile of income (50.00%). Individuals with high sugar consumption (41.28%) were more present in high caries trajectory group than individuals with low sugar consumption (30.89%). Complete population characteristics according dental caries trajectory is displayed in the **Table 2**.

General information

Genetic informations

All studied SNPs were in Hardy-Weinberg Equilibrium ($p > 0.05$), except by rs11003125 (*MBL2*); thus, it was excluded of posterior analysis. **Table S1** displays the complete description of allele frequency and results of Hardy-Weinberg equilibrium. **Table 3** presents the summary of the allele and genotype frequency comparisons related to caries trajectory.

Linkage disequilibrium was evaluated by D' and r^2 . We found a block of nonrandom associations in *LTF* SNPs evaluated. **Figure 1** presents the complete results of Linkage disequilibrium of *LTF* and *MBL2* SNPs.

Analysis of haplotype were also performed aiming to test the relationship of different allele and dental caries trajectories. The Combination of allele "G" of rs6441989 (*LTF*), "A" of rs2269436 (*LTF*), "G" of rs743658 (*LTF*), "C" of rs4547741 (*LTF*), "G" of rs11716497 (*LTF*) and "C" of rs7096206 (*MBL2*) was associated with individuals in high caries trajectory group (OR = 1.43 CI95% [1.01 – 2.04], p value = 0.046). Complete haplotype analysis is available in **Table S2**.

Genetic analyses

Allelic

Multilevel logistic regression analysis of the association between caries trajectory and genetic variation (allelic) in response immune genes (**Table 4**) found that allele G of rs11716497

(*LTF*) was associated with an increase in Odds of being in high caries trajectory group of 50% (OR = 1.50 CI95% [1.12 – 2.01]) in unadjusted model. After adjustments by ancestry genetic, sex, income and sugar consumption, allele G remained associated with high caries trajectory group (OR = 1.39 CI95% [1.06 – 1.82]). Other investigated SNPs were not associated with caries trajectory in allelic effect.

Genotypic

Considering genotype effects, the genotype AG of rs2269436 (*LTF*) was associated with an increase of odds for being in high caries trajectory group (OR = 1.61 CI95 [1.03 – 2.52]) in unadjusted model. After adjusted, the associations was lost (OR = 1.46 CI95% [0.84 – 2.53]). Similar results are observed in rs743658 (*LTF*), which presents $D' = 1.00$. On the other hand, rs11716497 (*LTF*) was associated with individuals in high caries trajectory group in unadjusted ($p = 0.003$), adjusted ($p = 0.042$) and dominant ($p = 0.027$) models. Thus, genotype GG showed an odds 89 higher of being in high caries group of 89% (OR = 1.89 CI95% [1.01 – 3.60]) in additive adjusted model. Considering the dominant adjusted model, it was observed that genotype GA/GG was associated with high odds of individuals being in high caries trajectory group (OR = 1.56 CI95% [1.05 – 2.31]). Complete summary of logistic regression analysis and genotype are displayed in **table 5**.

Epistasis Analysis (Gene-gene Interaction)

Table 6 shows the compilation of results for gene-gene interaction and dental caries trajectory in the life course achieved from the generalized multifactor dimensionality reduction analysis. We found three associated models involving three SNPs: rs6441989 (*LTF*), rs4547741 (*LTF*) and rs11716497 (*LTF*). The best model was a two-locus ($p < 0.004$) involving rs4547741 (*LTF*) and rs11716497 (*LTF*). This result indicates a potential gene-gene interaction between these SNPs. Furthermore, this model shows a high cross validation consistency of 10/10, an elevate training-balanced accuracy of 59.62% and testing-balanced accuracy of 59.12%. So, combination of these SNPs presented an Odds of 2.11 (CI95% 1.26 – 3.55) of being in high in caries trajectory group.

Likewise, a three-locus interaction model [rs6441989 (*LTF*) / rs4547741 (*LTF*) / rs11716497 (*LTF*)] was also associated with high caries trajectory group ($p = 0.001$). This model also revealed a minor cross validation consistency (8/10). Analysis of combination of genotypes found and odds 2.32 (1.36 – 3.95) of being in high caries trajectory group.

Interactions of gene–gene combination is represented in **Figure 2**; **Figure 3** illustrate the interaction graph summarizing the measures of information gain.

Parametric g-formula analysis

The parametric g-formula analysis showed that rs11716497 (*LTF*) seems to have a direct effect on dental caries trajectory (**Table 5**) independently of sugar consumption (**Table 5**; **Figure 4**). So, direct effect of rs11716497 (*LTF*) on caries trajectory was 99.5% in additive genotype model (OR 1.08; CI 95% 1.04–1.1) and only 0.5% was mediated by sugar consumption.

Discussion

To the best of our knowledge, this is the first study investigating the association of SNPs linked to immune response genes (*LTF* and *MBL2*) and dental caries with longitudinal design in a birth cohort sample. The present results were estimated using several quality control filters aiming to reduce the bias in presented estimates linked to gene association studies; therefore, we found significant associations of rs11716497 (*LTF*) in all analysis performed – genotype additive and dominant effect and allelic – evidencing the robustness of present results. Allele G of this SNP was linked to an increase on odds of being in high caries trajectory group as well as the GG genotype. Moreover, we investigate if its association was mediated by sugar consumption through g-formula. Thus, it was found that association between rs11716497 (*LTF*) and caries trajectory is not mediated by sugar consumption but presents a direct effect. These results highlight the hypothesis that the main effect of this association is linked to immune response and not to increase on sugar consumption, which not provide a significant part of the explanation in this pathway analysis. We also performed an epistatic analysis using the Generalized Multifactor Dimensionality Reduction and our findings showed that the best model was a two-locus involving rs4547741 (*LTF*) and rs11716497 (*LTF*), which were responsible to an increase of two folds in odds of being in high caries trajectory group. Therefore, indicates a potential gene-gene interaction between these SNPs.

The LTF protein is coded by the *LTF* gene located in the position 3p21.31 expressed mainly in salivary gland and bone marrow, being a gene member of the transferrin gene family [Kruzel et al., 2017]. Its protein product is found in the secondary neutrophil granules presenting high antimicrobial activity [Fine, 2015; Kruzel et al., 2017]. Besides, LTF is most important iron-binding protein in milk and body secretions and, therefore, is considered an essential element of the non-specific immune system [Fine, 2015; Kruzel et al., 2017]. The literature has displayed evidence that *LFT* can presents host defense against a broad range of microorganisms [Fine, 2015], and probably affecting the dental caries occurrence [Azevedo et al., 2010; Doetzer et al., 2015]. It seems that LTF act with an effect on the formation of bacterial biofilm [Fine, 2015] due to the ability to sequester or chelate the iron necessary for biofilm development, thus influencing both dental caries and periodontal disease [Fine, 2015]. In fact, the presence of LTF in saliva can reduce *Streptococcus Mutans* in a dose-dependent effect, being consequently capable to reduce dental caries [Fine et al., 2013]. These results corroborate with our observations through g-formula analysis that demonstrate that the main

effect of rs11716497 (*LTF*) on caries trajectory is not mediated by sugar consumption. So, we can infer that effect of its SNP seems to be direct in dental caries trajectory; possibly due to host immune response, since only 0.5% of effect was mediated by sugar consumption in our findings.

Despite rs11716497 (*LTF*) has been associated with caries trajectory in the life course, other SNPs investigates of *LTF* were not associated with tested phenotype. In a different way than that observed by Doetzer et al. [2015], we have not observed direct association of rs6441989 (*LTF*) and caries, although this loci seems to present an epistatic interaction with rs4547741 (*LTF*) and rs11716497 (*LTF*) in GMDR analysis. Combination of these three loci increased the odds of being in high dental caries trajectory group in 2.32 folds, despite the cross-validation consistency have be less than two-locus model [rs4547741 (*LTF*) / rs11716497 (*LTF*)] in GMDR. Two-locus model indicated the best gene-gene interaction displaying a 10/10 cross validation consistency.

Although this interesting result observed in our study regarding rs11716497 (*LTF*), it is important highlight that the unique studie investigating this SNP available in the literature have not found association with dental caries in Brazilian 12-year-old students [Doetzer et al., 2015]. Furthermore, genome wide associations studies investigating the phenotype of dental caries have not pointed this loci as having possible influence on caries occurrence [Haworth et al., 2018; Meng et al., 2019; Shaffer et al., 2013; Zeng et al., 2013] while other immune response genes (interleukin 32, galactokinase 2 and Elav-like family member 4) were identify in GWAS studies with children sample [Meng et al., 2019]. However, it is important to highlight the differences of ancestry between studies and that few studies performed correct adjustments by ancestry genetic, which could introduce important biases in the results. Thus, although the evidence of this loci did not corroborate with GWAS studies, rs11716497 (*LTF*) is still a likely candidate gene to pursue and must be more investigate in further studies to confirm the findings observed in present study.

Furthermore, we also investigate *MBL2* linked *SNPS*. *MBL2* is in the position 10q21.1 and encode the soluble mannose-binding lectin or mannose-binding protein found in serum, which are also critical element in the innate immune response. Its protein identifies the microorganisms the mannose even as N-acetylglucosamine initiating the classical complement pathway. Initially, we observed that rs11003125 (*MBL2*) was not in Hardy-Weinberg Equilibrium and excluded the SNP of subsequently analysis. rs7096206 (*MBL2*) have not showed association with dental caries, contrasting previous studies where this loci was associated with caries in Saudi pediatric individuals (5 to 13 years old) [Alyousef et al., 2017] and Iranian adults (20 to 34

years) [Mokhtari et al., 2019]. Possible explanations of these differences can be due to different measurement of phenotype. While we perform a group modeling trajectory to establish similar groups of individuals with presence of caries in different moments of life, Mokhtari et al. [2019] contrast individuals with high experience of caries (DMFT >6) with individuals with low caries (DMFT ≤ 6), Alyousef et al. [2017] consider DMFT in a continuous way. Moreover, the non-correction by multiple comparisons tests may have included bias on effects measurements resulting in false positive results.

Some strengths points of present study should be highlighted. We performed a wide quality control filters aiming to minimize bias in our results in the present population-based sample. In this way, we exclude SNPs in Hardy-Weinberg disequilibrium, performed analysis adjusting our final models by important factors implicated in caries occurrence – like as income and sugar consumption – even as adjusted the estimates by genomic ancestry using about 370,000 SNPs accessible from the 1982 Pelotas birth cohort, which is compatible with the HapMap and Human Genome Diversity projects for the Brazilian population. Besides, Bonferroni multiple corrections test was used in our analysis to avoid false positive. An important point is that our results are based in the trajectory of caries from 15 to 31 years of age, not limited to a specific one moment in the life course, which represents better the risk for caries in each individual.

We also must highlight and discuss some important limitations beyond the limitations inherent and well-known in genetic association studies. Thus, we need to evidence that some sample losses occurred in the follow-ups. To minimize the power decrease, group-based trajectory modeling inputting missing data when individuals were present in two follow-ups. In addition, considering that this is the first study that showed an effect of rs11716497 (*LTF*) on dental caries, it is important the conduction of further well-designs studies to confirm this association in other populations. The presents findings can contribute to a better understanding of the genetic contribution of dental caries susceptibility in humans.

Conclusion

It was found significant associations of rs11716497 (*LTF*) and dental caries trajectory in the life course. Allele G of this SNP was linked to an increased odds of being in high caries trajectory group as well as the GG genotype. g-formula analysis showed that association between rs11716497 (*LTF*) and caries trajectory was not mediated by sugar consumption but present a direct effect. Results must be interpreted taking into account all inherent limitations for genetic association studies.

Compliance with Ethical Standards:

Conflict of Interest: Luiz Alexandre Chisini declares that he has no conflict of interest. Francinde dos Santos Costa declares that she has no conflict of interest. Marcus Cristian Muniz Conde declares that he has no conflict of interest. Bernardo Lessa Horta declares that he has no conflict of interest. Marcus Flávio Fernando Demarco. declares that he has no conflict of interest. Luciana Tovo-Rodrigues declares that she has no conflict of interest. Marcos Britto Correa declares that he has no conflict of interest

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Ethical approval: Federal University of Pelotas Ethics committee approved this project.

Informed consent: Authorization of all participants were done individually even as all participants signed informed consent terms.

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Table 1 – Markers studied

Gene	Position	Marker public ID	Base pair Change
Lactotransferrin (<i>LTF</i>)	3:46474899	rs6441989	G/A
	3:46487253	rs2269436	A/G
	3:46488488	rs743658	G/A
	3:46500458	rs4547741	C/T
	3:46503498	rs11716497	A/G
Mannose binding lectin 2 (<i>MBL2</i>)	10:54531685	rs7096206	C/T
	10:54532014	rs11003125	G/C

Table S1. Description of allele frequency and results of Hardy-Weinberg equilibrium

	Allele Frequency	Hardy-Weinberg equilibrium	
		Tests	p value
rs6441989 (<i>LTF</i>)	G = 0.579 A = 0.420	Pearson chi2	0.450
		likelihood-ratio chi2	0.450
		Exact significance prob	0.469
rs2269436 (<i>LTF</i>)	A = 0.932 G = 0.068	Pearson chi2	0.066
		likelihood-ratio chi2	0.081
		Exact significance prob	0.080
rs743658 (<i>LTF</i>)	G = 0.933 A = 0.067	Pearson chi2	0.066
		likelihood-ratio chi2	0.082
		Exact significance prob	0.080
rs4547741 (<i>LTF</i>)	C = 0.957 T = 0.043	Pearson chi2	0.792
		likelihood-ratio chi2	0.788
		Exact significance prob	1.000
rs11716497 (<i>LTF</i>)	A = 0.592 G = 0.408	Pearson chi2	0.755
		likelihood-ratio chi2	0.755
		Exact significance prob	0.754
rs7096206 (<i>MBL2</i>)	C = 0.816 T = 0.184	Pearson chi2	0.173
		likelihood-ratio chi2	0.177
		Exact significance prob	0.179
rs11003125 (<i>MBL2</i>)	G = 0.677 C = 0.323	Pearson chi2	0.022
		likelihood-ratio chi2	0.022
		Exact significance prob	0.023

Table 2. Population characteristics

	Caries trajectory		p value
	N (%)		
	Low	High	
Ancestry			<0.001
European	428 (64.51)	206 (32.49)	
African	35 (44.87)	43 (55.13)	
Sex			0.028
Male	312 (64.73)	170 (35.27)	
Female	293 (70.94)	120 (29.06)	
Family income at 31 yrs. (tertiles)			<0.001
Lowest tertile (1st)	101 (50.00)	101 (50.00)	
Highest tertiles (2nd and 3rd)	325 (73.70)	116 (26.30)	
Sugar Consumption			0.003
Low	387 (69.11)	173 (30.89)	
High	138 (58.72)	97 (41.28)	

Table 3. Summary of the allele and genotype frequency comparisons related to caries trajectory

Subjects	Alleles (%)		p-value	Genotypes (%)			p-value
rs6441989 (<i>LTF</i>)	G	A	0.372	GG	AG	AA	0.878
Low	525 (60.48)	343 (39.52)		157 (36.18)	211 (48.62)	66 (15.21)	
High	284 (59.41)	194 (40.59)		85 (35.56)	114 (47.70)	40 (16.74)	
rs2269436 (<i>LTF</i>)	A	G	0.107	AA	GA	GG	0.042
Low	811 (93.43)	57 (6.57)		381 (87.79)	49 (11.29)	4 (0.92)	
High	437 (91.42)	41 (8.58)		198 (82.85)	41 (17.15)	0 (00)	
rs743658 (<i>LTF</i>)	G	A	0.107	GG	AG	AA	0.042
Low	811 (93.43)	57 (6.57)		381 (87.79)	49 (11.29)	4 (0.92)	
High	437 (91.42)	41 (8.58)		198 (82.85)	41 (17.15)	0 (00)	
rs4547741 (<i>LTF</i>)	C	T	0.108	CC	TC	TT	0.062
Low	823 (94.82)	45 (5.18)		389 (89.63)	45 (10.37)	0 (0.00)	
High	461 (96.44)	17 (3.56)		223 (93.31)	15 (6.28)	1 (0.42)	
rs11716497 (<i>LTF</i>)	A	G	0.001	AA	GA	GG	0.003
Low	555 (63.94)	313 (36.06)		182 (41.94)	191 (44.01)	61 (14.06)	
High	262 (54.81)	216 (45.19)		70 (29.29)	122 (51.05)	47 (19.67)	
rs7096206 (<i>MBL2</i>)	C	G	0.103	CC	GC	GG	0.407
Low	699 (80.53)	169 (19.47)		279 (64.29)	141 (32.49)	14 (3.23)	
High	399 (83.47)	79 (16.53)		165 (69.04)	69 (28.87)	5 (2.82)	

Bold font indicates p-values lower than 0.05; p-value: Fisher exact test

Table 4. Summary of the logistic regression analysis of the association between caries trajectory and genetic variation (allelic) in response immune genes adjusted by ancestry genetic, sex, income and sugar consumption

Subjects	Multivariate analysis Unadjusted		Multivariate analysis Adjusted	
	OR (95%CI)	p-value	OR (95%CI)	p-value
rs6441989 (<i>LTF</i>)				
G	Reference	0.701	Reference	0.609
A	0.95 (0.76 – 1.20)		0.93 (0.72 – 1.22)	
rs2269436 (<i>LTF</i>)				0.433
A	Reference	0.175	Reference	
G	1.33 (0.88 – 2.03)		1.23 (0.73 – 2.07)	
rs743658 (<i>LTF</i>)				
G	Reference	0.175	Reference	0.433
A	0.75 (0.49 – 1.13)		0.81 (0.48 – 1.37)	
rs4547741 (<i>LTF</i>)				
C	Reference	0.175	Reference	0.119
T	0.67 (0.38 – 1.19)		0.57 (0.27 – 1.15)	
rs11716497 (<i>LTF</i>)				
A	Reference	0.007	Reference	0.016
G	1.50 (1.12 – 2.01)		1.39 (1.06 – 1.82)	
rs7096206 (<i>MBL2</i>)				
C	Reference	0.183	Reference	0.699
G	0.81 (0.61 – 1.09)		0.93 (0.65 – 1.32)	

Table 5. Summary of the logistic regression analysis of the association between caries trajectory and genetic variation (genotype) in response immune genes adjusted by ancestry genetic, sex, income and sugar consumption

Gene/Marker	Genotypes	Multivariate analysis Unadjusted		Multivariate analysis adjusted			Dominant effect	
		p value	OR (95%CI)	p value	OR (95%CI)		p value	OR (95%CI)
rs6441989 (<i>LTF</i>)	GG	0.873	Reference	0.667	Reference	GG	0.955	Reference
	AG		0.99 (0.67 – 1.48)		0.95 (0.59 – 1.52)	AG/AA		1.01 (0.68 – 1.49)
	AA		1.11 (0.65 – 1.92)		1.19 (0.65 – 2.20)			
rs2269436 (<i>LTF</i>)	AA	0.038	Reference	0.184	Reference	AA	0.260	Reference
	GA		1.61 (1.03 – 2.52)		1.46 (0.84 – 2.53)	GA/GG		1.37 (0.79 – 2.37)
	GG		-					
rs743658 (<i>LTF</i>)	GG	0.038	Reference	0.184	Reference	GG	0.260	Reference
	AG		1.61 (1.03 -2.52)		1.46 (0.84 – 2.53)	AG/AA		1.37 (0.79 – 2.37)
	AA		-					
rs4547741 (<i>LTF</i>)	CC	0.080	Reference	0.033	Reference	CC	0.060	Reference
	TC		0.58 (0.31- 1.07)		0.42 (0.19 – 0.93)	TC/TT		0.48 (0.23 – 1.03)
	TT		-					
rs11716497 (<i>LTF</i>)	AA	0.003	Reference	0.042	Reference	AA	0.027	Reference
	GA		1.66 (1.10 – 2.49)		1.46 (0.92 – 2.36)	GA/GG		1.56 (1.05 – 2.31)
	GG		2.00 (1.17 – 3.43)		1.89 (1.01 – 3.60)			
rs7096206 (<i>MBL2</i>)	CC	0.394	Reference	0.753	Reference	CC	0.847	Reference

GC	0.83 (0.55 – 1.22)	0.99 (0.62 – 1.60)	GC/GG	0.96 (0.64 – 1.43)
GG	0.60 (0.18 – 1.98)	0.61 (0.14 – 2.65)		

Bold font indicates p-values lower than 0.05

Table S2. Haplotype analysis of loci for hap-analysis: rs6441989 (*LTF*), rs2269436 (*LTF*), rs743658 (*LTF*), rs4547741 (*LTF*), rs11716497 (*LTF*), rs7096206 (*MBL2*).

Haplotype	High caries	Downward caries	Fisher's p	Odds Ratio (95% CI)
	Trajectory	Trajectory		
	Frequency	Frequency		
A A G C A C	0.165	0.179	0.479	0.89 (0.66 – 1.21)
A A G C A G	0.029	0.043	0.192	0.65 (0.35 – 1.23)
A A G C G C	0.153	0.117	0.065	1.35 (0.98 – 1.87)
A A G C G G	0.028	0.022	0.527	1.25 (0.61 – 2.56)
A A G T G C	0.024	0.021	0.809	1.09 (0.52 – 2.32)
A A G T G G	0.004	0.011	0.168	0.33 (0.06 – 1.71)
G A G C A C	0.282	0.331	0.052	0.79 (0.61 – 1.00)
G A G C A G	0.073	0.087	0.344	0.81 (0.54 – 1.24)
G A G C G C	0.128	0.092	0.046	1.43 (1.01 – 2.04)
G A G C G G	0.021	0.012	0.169	1.83 (0.76 – 4.38)
G A G T G C	0.008	0.011	0.596	0.73 (0.26 – 2.36)
G G A C G C	0.071	0.051	0.139	1.42 (0.89 – 2.25)
G G A C G G	0.011	0.013	0.815	0.88 (0.31 – 2.49)
Global Result			0.099	

All those frequency <0.01 were ignored in analysis

Table 6. Summary of Generalized Multifactor Dimensionality Reduction results for gene-gene interactions

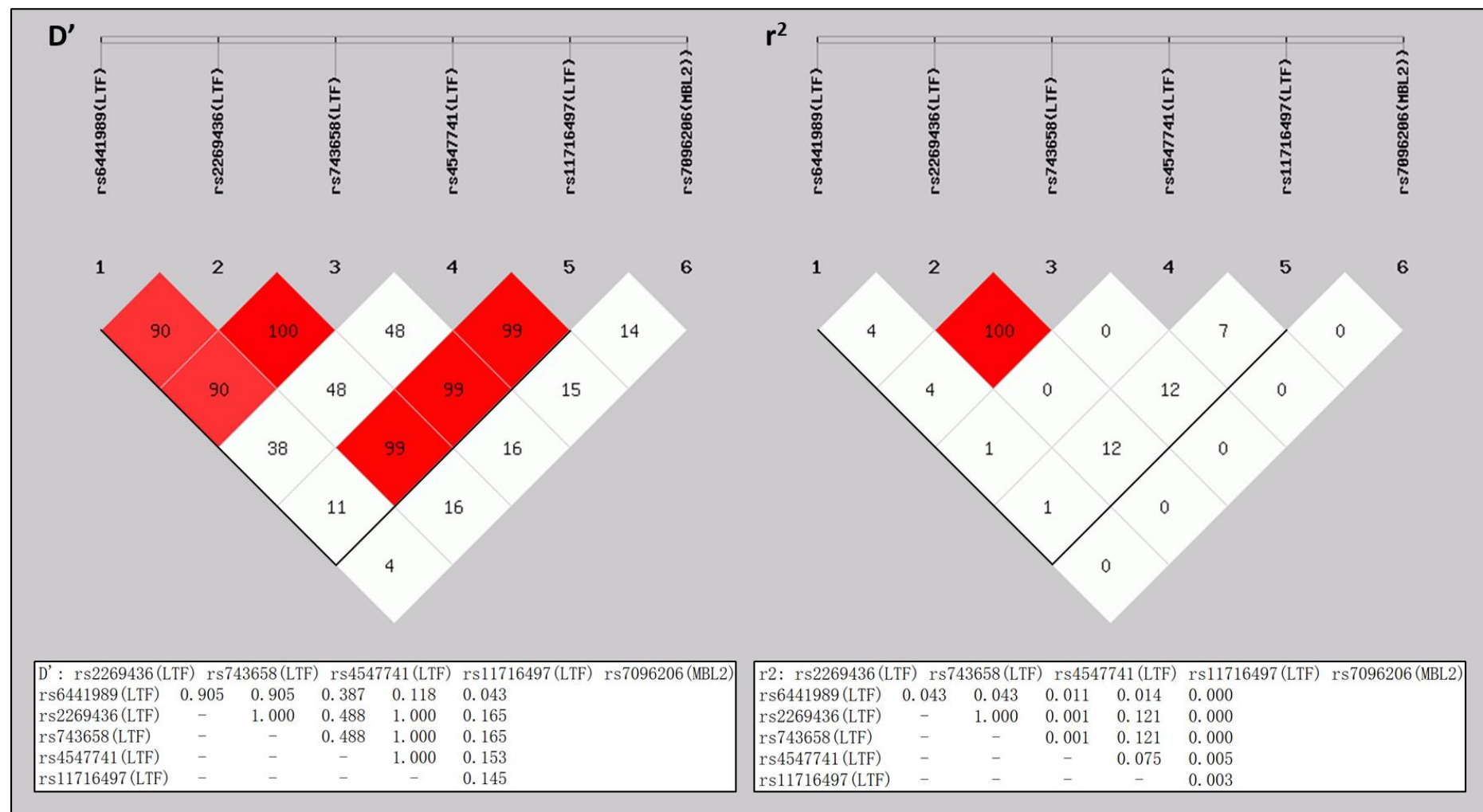
No.	Best Model	Tr-BA (%)	Te-BA (%)	Sign test (<i>p</i>)	CVC	P value	Odds Ratio (CI95%)
1	rs11716497 (<i>LTF</i>)	56.69	57.26	8 (0.0547)	10/10	0.031	1.78 (1.05 – 3.04)
2	rs4547741 (<i>LTF</i>) / rs11716497 (<i>LTF</i>)	59.12	59.62	9 (0.0107)	10/10	0.004	2.11 (1.26 – 3.55)
3	rs6441989 (<i>LTF</i>) / rs4547741 (<i>LTF</i>) / rs11716497 (<i>LTF</i>)	60.05	54.29	8 (0.0547)	8/10	0.001	2.32 (1.36 – 3.95)

Abbreviations: CVC, cross validation consistency; Te-BA, testing-balanced accuracy; Tr-BA, training balanced accuracy; Results were adjusted by ancestry genetic, sex, income and sugar consumption

Table 5. G-computation analysis of sugar consumption as mediator in the association between rs11716497 (*LTF*) and dental caries trajectory.

	G-computation estimate (OR)	Bootstrap std. err.	p value	95% CI (OR)
Genotype Additive effect				
TCE	1.08	0.0223	0.001	1.03 – 1.13
NDE	1.08	0.0161	<0.001	1.04 – 1.11
NIE	1.00	0.1107	0.974	0.98 – 1.02
CDE	1.07	0.02903	0.013	1.02 – 1.14
Genotype Dominant effect				
TCE	1.09	0.0229	<0.001	1.55 – 1.14
NDE	1.08	0.0251	0.003	1.03 – 1.13
NIE	1.15	0.0097	0.148	0.99 - 1.39
CDE	1.10	0.0212	<0.001	1.06 – 1.16
Control value(s): Sex= female. <i>TCE</i> total causal effect, <i>NDE</i> natural direct effect, <i>CDE</i> controlled direct effect, <i>NIE</i> natural direct effect				

Figure 1. Linkage disequilibrium of *LTF* and *MBL2* genes. The Single Nucleotides Polymorphisms were tested using SHEsis and estimated with D' and r^2 .



Model 1

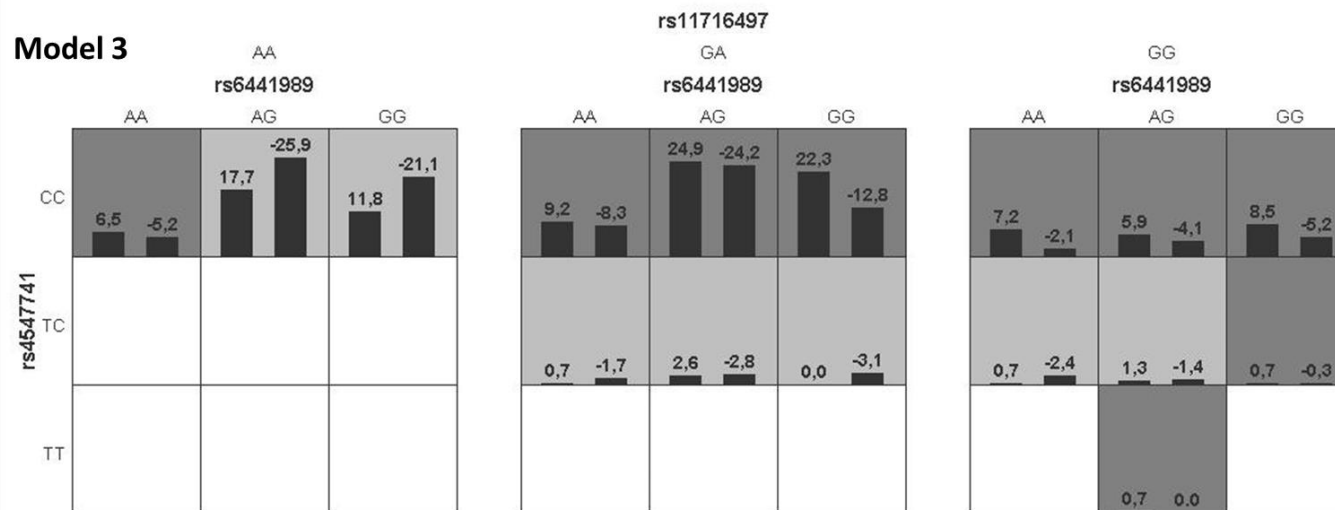


Figure 3. Illustration of interaction graph summarizing the measures of information gain. Attributes connected by red lines have stronger synergistic interactions than those connected by yellow lines

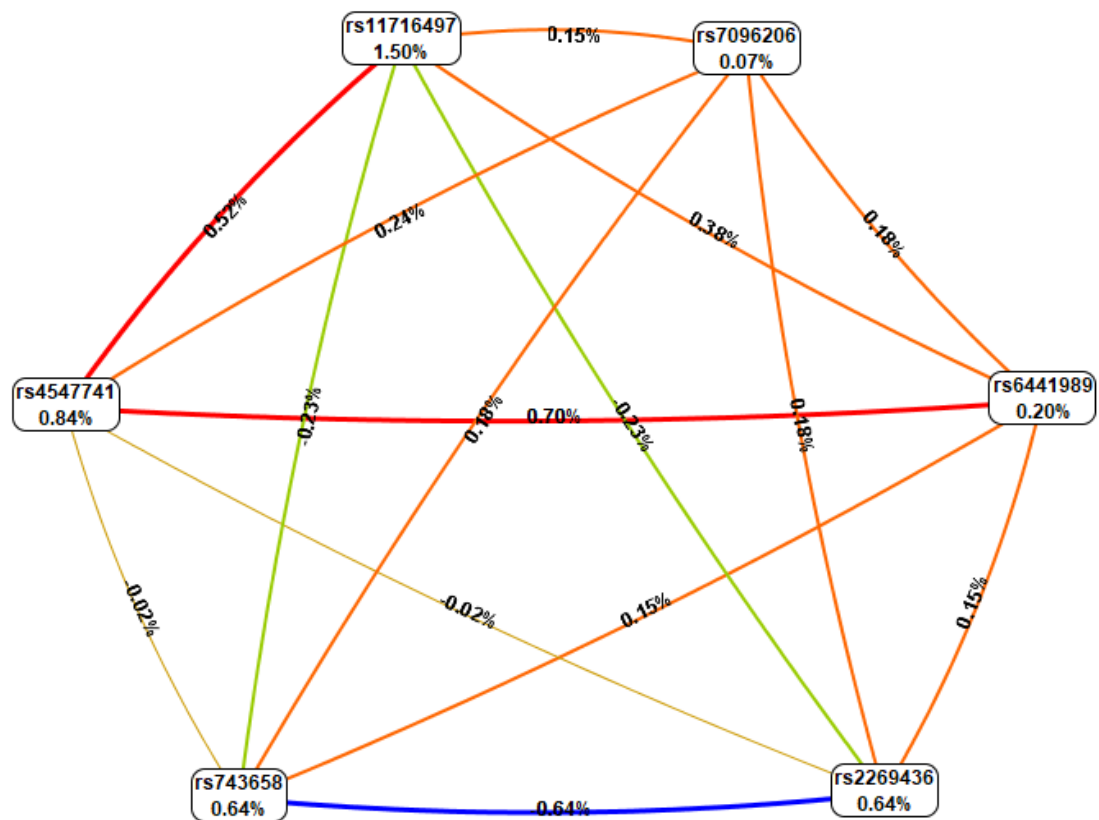
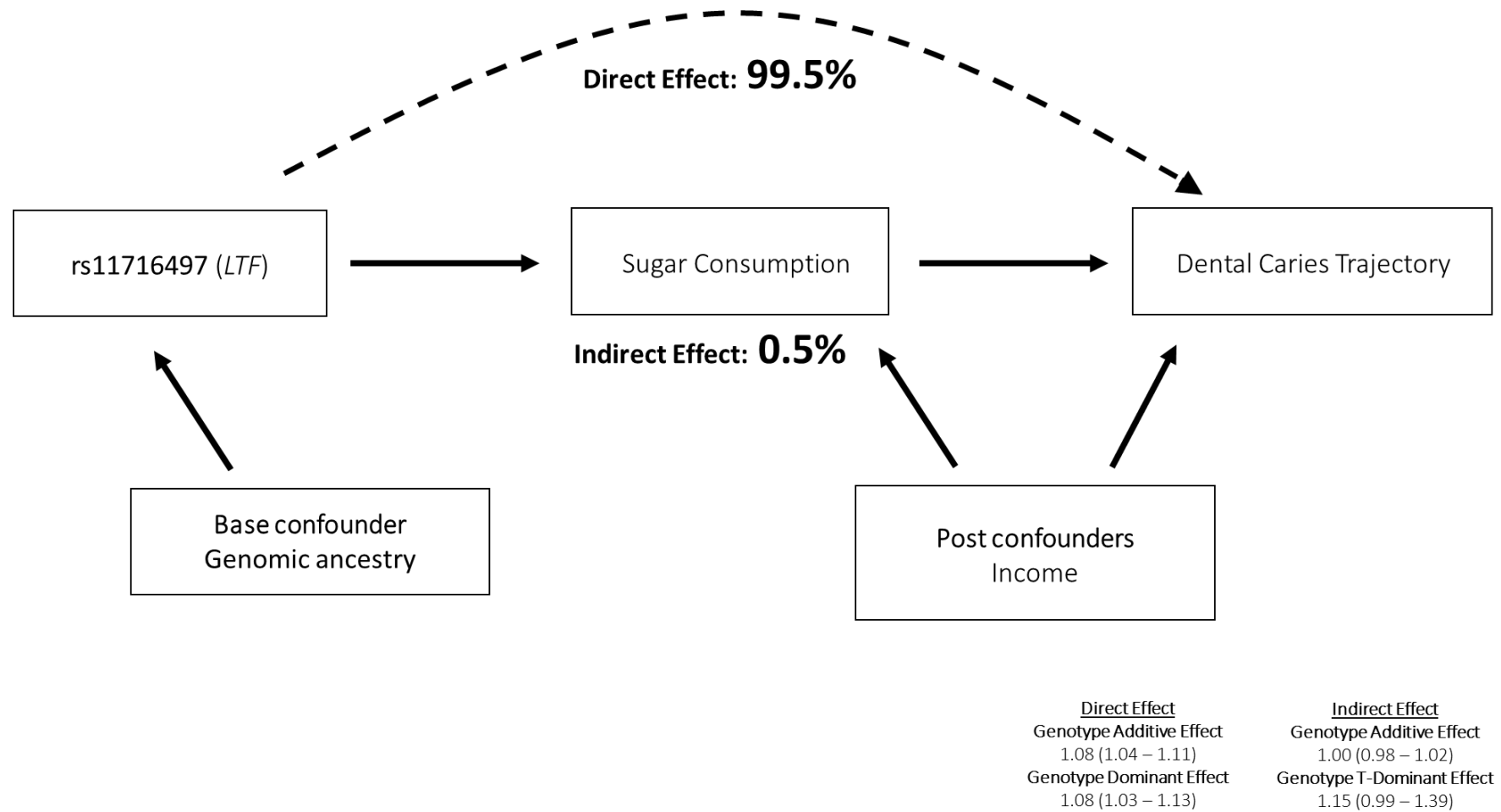


Figure 4. Model of mediation analysis with coefficients and respective confidence interval of total causal effect and natural indirect effect



5.4 Artigo 9

Artigo formatado seguindo as normas da Revista *Clinical Oral Investigations*

Genes in the pathway of salivary flow and composition and caries trajectory: A prospective birth cohort study

Running title: Salivary genes and Caries

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Declarations of conflict of interest: none

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Cover Letter

To: Professor Dr. Matthias Hannig
Editor-in-Chief,

Dear Editor:

Based on the importance of *Clinical Oral Investigations*, we are sending the manuscript entitled **“Genes in the pathway of salivary flow and composition and caries trajectory: A prospective birth cohort study”** to be appraised by the Journal’s Editorial Board.

In the present population-based birth cohort study, we assessed the hypothesis that caries trajectory assessed in the life course might be influenced by SNPs linked to pathway of salivary flow and composition genes. In fact, we investigated SNPs previously identified with potential a priori association with dental caries aiming to replicate results in the cohort. Therefore, our findings are in part in consonance with literature [Anjomshoaa et al., 2015] – in the first time with a longitudinal design – showing that rs10875989 was associated with caries in all models investigated. Allele C was associated with increase of 38% in odds to be in high caries trajectory group. Similarly, genotype CC in additive model was associated with an increase of two-fold to be in high caries group and genotype dominant model was also maintained in adjusted model. Furthermore, it was observed by parametric g-formula that the effect of rs10875989 on caries is mediated by gingival bleeding – used as a proxy to presence of the biofilm – and not by sugar consumption, which may reinforce that main effect is due to decrease in salivary flow and consequently increase of biofilm presence. We also complemented the analysis investigating possible epistatic interactions, i.e. gene-gene interaction. In this way, we found that combination of rs2274333, rs10875989 and rs3759129 was associated to increase of more than twice in odds to be in high caries trajectory group, showing a possible interaction between these genes on evaluated population underlying initial observations since dental caries seem to be a complex trait.

This is an original manuscript and has not been considered for publication elsewhere. The paper was read and approved by all authors. All authors have made substantive contribution to this study, and all have reviewed the final paper prior to its submission. The authors declare that there are no potential competing interests. Furthermore, I attest the validity and legitimacy of data and its interpretation. There are no conflicts of interest for authors listed above. We sign for and accept responsibility for releasing this material.

Thank you very much for your consideration.

Yours sincerely,

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Genes in the pathway of salivary flow and composition and caries trajectory: A prospective birth cohort study

Running title: Salivary genes and Caries

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Abstract:

Aim: to investigate if SNPs related to salivary composition and flow can influence dental caries trajectory in the life course.

Methods: Group-based trajectory modeling was used to create similar groups to dental caries trajectory in the life course (n=888 at 15 years, 720 at 24 and 539 at 31 years old) of Pelotas birth cohort. Logistic regression models adjusted by ancestry genetic, sex, income trajectory, sugar consumption and gingival bleeding (as *proxy* of oral hygiene) were used to test association. Parametric g-formula analysis was used to test mediation effect of associated polymorphisms. Epistatic interaction was investigated using Generalized multifactor dimensionality reduction approaches (GMDR).

Results: Allelic analysis found that allele C of rs10875989 was associated with dental caries trajectory (OR=1.38 CI95%[1.07–1.78]) while genotype analysis observed that in both investigated effects (additive and dominant) genotype CC was associated with high caries trajectory group (OR=2.01 CI95%[1.05–3.86]). GMDR results found epistatic interactions between rs2274333 and rs3759129 ($p = 0.004$) and between rs2274333, rs10875989 and rs3759129 ($p < 0.001$). Parametric g-formula analysis found that the association between rs10875989 and dental caries trajectory was not mediated by sugar consumption (OR=0.98 CI95%[0.95–1.02], but by gingival bleeding (OR=1.09 CI95%[1.02–1.15]).

Conclusions: rs10875989 was associated with caries trajectory in the life course considering allelic and genotype (dominant and additive) models. Parametric g-formula demonstrate that effect of rs10875989 on caries is mediated by gingival bleeding and not by sugar consumption. Epistatic interactions of rs2274333, rs10875989 and rs3759129 were observed influencing dental caries trajectory.

Introduction

Genetic influence on dental caries has been the focus of recent studies [Chisini et al., 2020; Vieira et al., 2014] aiming to explain part of the effect of this complex and multifactorial disease [Fejerskov, 2004; Frencken, 2018]. It is unquestionable that consumption of fermentable carbohydrates associated to poor habits of oral hygiene are the main factors to explain the development and progression of caries [Dutra et al., 2018; Fejerskov, 2004; Frencken, 2018], which can be strongly mediated by exposure to fluorides [Magalhaes, 2017; Parnell and O'Mullane, 2013]. Moreover, it is important consider that dental caries is behavioral and life-style disease strongly determined by socioeconomic status [Knoblauch et al., 2019]. Therefore, new genetic approach have identify that a minor part of estimate effects – not explain to knowledge associated factors – may be due influences of determinate loci in our genome [Haworth et al., 2018; Vieira et al., 2014].

Twin studies were the first approach to investigate the hypothesis that genetic factors can influence the experience of dental caries. [Boraas et al., 1988; Conry et al., 1993; Wright, 2010] These studies demonstrated a ranging between 40 to 60% of caries susceptibility could be genetically determined [Boraas et al., 1988; Conry et al., 1993; Wang et al., 2010; Wright, 2010]. In fact, a wide range of genes have been identified as having an important role in caries development and progression [Vieira et al., 2014]. In addition to twin studies, Genome Wide Association Studies (GWAS) have been the most recent tool to explore the entire genome and identify new genes and loci with potential association with caries experience [Ball, 2013; Hayes, 2013]. Although twin studies have been the pioneer methodology and GWAS the most recent strategy to identify potential candidate loci, well-design gene candidate studies with robustness methods are still important tools to confirm these findings [Chisini et al., 2020; Vieira et al., 2014]. Association studies approach aims to test an association between a specific gene (or variants) and the phenotype with knowledge relationship with disease [Patnala et al., 2013]. Thus, the use of this methodology it is important in cases that genetic factor has been previously reported as a possible candidate or that there is *a priori* theoretical hypothesis involved.

In this way, the current literature have presented interesting observations in genes related to pathway of saliva flow and composition [Lips et al., 2017; Piekoszewska-Zietek et al., 2017; Vieira et al., 2014]. Aquaporins (AQP) are a family of small integral membrane proteins which seems to plays a role in the generation of saliva and the genes coding for *AQP2*, *AQP5*,

and *AQP6* are clustered in the region 12q13 [Krane et al., 2001]. Yet, some studies have confirmed that aquaporin locus 12q13 [Anjomshoaa et al., 2015; Vieira et al., 2017] present elevate linkage disequilibrium and some variation (rs10875989 [*AQP2*] and rs3759129 [*AQP5*]) have presented associations with dental caries [Anjomshoaa et al., 2015]. Similarly, Mucin 5B (*MUC5B*) encoded proteins which are highly glycosylated macromolecular components of mucus secretions. This family member is the major gel-forming mucin in mucus. It is a major contributor to the lubricating and viscoelastic properties of whole saliva and was associated with dental caries in a Brazilian population [Cavallari et al., 2018]. Likewise, Carbonic Anhydrase 6 (*CA6*) play a role in the reversible hydratation of carbon dioxide and is present in the saliva, where seems to influence colonization by *streptococcus mutans* and occurrence of dental caries in Swedish adolescents [Esberg et al., 2019].

Thus, the aim of present study was investigated if SNPs related to salivary composition and flow can influence dental caries trajectory in the life course. Furthermore, we aim to investigate possible epistatic interaction between them using a Generalized multifactor dimensionality reduction approaches (GMDR) for detection of multifactor interactions underlying phenotypes.

Methods

Present cohort study follow the STROBE (Strengthening the reporting of observational studies in epidemiology) guideline considering longitudinal cohort design. [von Elm et al., 2007]. Ethics Committee approved the present study.

Study design, setting and participants

In 1982, 99.2% of all birth of Pelotas (a city located in southern of Brazil) were included in a perinatal study. This population is followed until nowadays [Barros et al., 2008]. In 1997, with 15 years old, a randomized and representative sample (n= 888) of entire longitudinal birth cohort (n= 5,914) participate in the first oral health survey; so, the individuals were interviewed an oral health examination (i.e. DMF-T was collected) were performed by dentists. In 2004, in a new follow-up, the individuals asked a food frequency questionnaire (consumption of sweet foods and sugar) and blood sample was collected to perform genotyping. The second oral health survey was performed in 2006, where the individuals were asked about oral health questions and again examined by dentists (i.e. DMF-T was collected, and periodontal examination was also performed). All 888 individuals were searched and invited to participate. Thus, 720 individuals were included of this second follow-up. The third oral health study was performed in 2013, where 539 individuals, now with 31 years old, participate of this follow-up; so, participants were interviewed and clinically examined by trained dentists to epidemiological studies. In this follow-up, DMF-S index was collected.

Outcome variable (phenotype)

The phenotype of present study was the dental caries trajectory, to 15 to 31 years old. Group-based trajectory modeling (G-BTM) was estimated using the command “*traj*” in Stata 12.0. [Jones et al., 2001; Silva et al., 2018]. The parameters for the model trajectory were selected based on the maximum likelihood by the quasi-Newton method. [Dennis et al., 1981; Jones and Nagin, 2007]. G-BTM was calculate using the component “decayed” previously dichotomized (i.e, 0 = without component decayed; and 1 = with component decayed) of DMF-T/S in each of follow-ups. Therefore, were identified two trajectories (low and high)

Independent variables

Blood sample were collected with venipuncture. The genotyping was performed using Illumina *Illumina HumanOmni2.5-8v1 array*. Further details of this step were previously published [Horta et al., 2015; Victora and Barros, 2006]. The markers examined in present study, respective base pair change and chromosome positions are detailed in **Table 1**. Furthermore, genomic ancestry was also established by ADMIXTURE [Alexander et al., 2009], being created considering the approximately 370,000 SNPs available in present cohort [Lima-Costa et al., 2015].

Income trajectory was measured in three time points (at birth, 23 and 31 years); participants were asked about their income collected in continuous and categorized in tertiles. In addition, the trajectories were estimated with the command “*traj*” by G-BTM being obtained three groups (low, downward and high income). Food frequency questionnaire estimated the sugar consumption reported, which range from 0 to 10 daily, weekly, monthly and yearly. Year consumption of sugar was calculated and categorized into tertiles and dichotomized in higher (3rd tertile) and lower (1st and second tertiles) tertiles. Gingival bleeding (gingivitis) was used as a *proxy* of presence of biofilm and was in two-time points (24 and 31 years). Individuals with more than 10% of gingival bleeding were considered with poor hygiene habits in each of surveys. Thus, individuals were considered with gingivitis: i) one time; ii) always; or iii) never.

Statistical methods

Stata statistical package, version 12.0, was used for statistical analysis (Stata Corporation, College Station, USA). Initially, we test the *Hardy–Weinberg equilibrium* and estimate the allele frequency [Newton]. SNPs not in *Hardy–Weinberg equilibrium* were excluded of further analysis. To avoid the effects lead to population stratification, we adjusted all analysis by the first ten major components of the principal component analysis. We considered the European, African and Native American populations in this analysis.

Fisher exact test was used to descript the populations characteristics. Associations were tested with logistic regression with Bonferroni multiple test corrections estimating Odds Ratio (OR) and respective 95% confidence intervals (CI95%). Allele analysis were performed with multilevel (mixed effects) hierarchical levels considering the genetic and personal level [Yi, 2010], so, we clustered the alleles in each individual. Genotype analysis were investigated

assuming two possible effects, i.e. i) additive; and ii) dominant. We presented unadjusted models and adjusted by ancestry, sex, income trajectory, sugar consumption, oral health habits.

The parametric g-formula approach was performed aiming to estimate the total causal effect, the natural direct effect, the natural indirect effect and the controlled direct effect. Income trajectory was applied as post-confounder and ancestry was used as base confounder. So, two mediators were used: i) sugar consumption, and ii) gingival bleeding (gingivitis). In this way, Monte Carlo method was completed to estimate the effects. The bootstrap method was used to estimate the standard errors and the confidence interval of the estimated effects. To perform this assessment, we chose to use 1000 resamples of size 10,000.

Linkage disequilibrium analysis was performed estimating the parameters D' and r^2 using SHEsis (<https://analysis.bio-x.cn/myAnalysis.php>) [Shi and He, 2005; Wang and Qin, 2018]. Moreover, SHEsis was used to investigate possible haplotype associations as complementary analysis. Aim to investigate epistatic interactions, i.e. gene-gene interactions, we used Generalized Multifactor Dimensionality Reduction (GMDR) adjusted by same independent variables [Hou et al., 2019]. Multifactor Dimensionality Reduction (MDR) was also used to plot the graphics Illustration of interaction summarizing the measures of information gain by: i) MDR combined attribute network; and ii) Cartesian product network.

Results

A total of 888 individuals were included in first oral health survey (15 years), 720 were included in second (24 years) and 539 were included in the third survey (31 years). Most of individuals presented European genomic ancestry (89.1%) while 10.9% were considered African. Population characteristics according dental caries trajectory is presented in **Table 2**. Individuals with high income trajectory were linked to a low caries trajectory ($p < 0.001$) as those that never presented gingival bleeding ($p < 0.001$).

Genetic informations

The SNP rs467323 was not in Hardy-Weinberg Equilibrium and was excluded from further analysis of association. All another SNPs were in equilibrium. Entire details of Hardy-Weinberg Equilibrium are available in supplemental material (**Table S1**).

D' and r^2 were estimated by SHEsis software to investigate linkage disequilibrium (LD) in presents SNPs according groups of caries trajectory. Full LD analysis is displayed in **Figure 1**. It is possible to observe that all SNPs of *MUC5B* (rs2672812, rs2735733, rs2249073 and rs2857476) are in LD ($D' > 0.94$; $r^2 > 0.70$). Similarly, SNPs of *CA6* (rs2274333 and rs10864376) are in LD ($D' = 0.66$; $r^2 = 0.36$) and SNPs of *AQP2* (rs467323 and rs10875989) ($D' = 0.97$; $r^2 > 0.66$); SNPs of *AQP2* (rs467323 and rs10875989) are also in LD with *AQP5* (rs3759129) ($D' > 0.80$; $r^2 = 0.06$).

We perform a complementary haplotype analysis to stablish possible association considering the loci combination of SNPs (rs2274333, rs10864376, rs10875989, rs2672812, rs2735733, rs2249073, rs2857476 and rs3759129). Combination of “A C T G C T T C” allele in respective SNPs reduce the odds of being in high caries trajectory group (OR = 0.37 CI95% [0;019 – 0.71]); In addition, haplotype “G T C G C T T A” ($p = 0.001$) and “G T T A T C C A” ($p = 0.001$) were associated with increased odds of being in high caries trajectory group. Full haplotype analysis is available in our supplemental material S2 (**Table S2**).

Genetic analyses

Allelic

Considering the allelic analysis (**Table 4**), it was found that allele T of rs10864376 was associated with dental caries trajectory in unadjusted model (OR = 1.31 CI 95% [1.04 – 1.65]). After adjustments, the associations were not maintained (OR = 1.16 CI 95% [0.90 – 1.49]). On the other hand, rs10875989 was associated in both models (adjusted and unadjusted). In unadjusted, was observed that allele C was linked to an increase in of 34% (OR = 1.34 CI 95% [1.05 – 1.69]) of being on high caries trajectory group. Similar effect estimate was observed after adjustments (OR = 1.38 CI 95% [1.07 – 1.78]). No other SNP investigated showed additional associations.

Genotypic

Considering the genotype analysis, only the SNP rs10875989 was associated with caries (**Table 5**). However, a consonance of results was observed in both investigated effects (additive and dominant). In multivariate adjusted analysis the genotype CC was associated to increase of two-folds on Odds (OR = 2.01 CI95% [1.05 – 3.86]) to be on high caries trajectory group. Considering the dominant adjusted model, similar associations were observed (OR = 1.42 CI95% [1.00 – 2.01]). Interactions of gene–gene combination is represented in **Figure 2**; **Figure 3** illustrate the interaction graph summarizing the measures of information gain considering MDR combined attribute network and cartesian product network.

Epistasis Analysis (Gene-gene Interaction)

Summary of GMDR results for gene-gene interactions is available in **Table 6**. Two associated models were found: i) model 2 [rs2274333 and rs3759129 ($p = 0.004$)] and ii) model 3 [rs2274333, rs10875989 and rs3759129 ($p < 0.001$)]. Combination of SNPs in Model 2 presented an Odds of 1.97 (CI 95% [1.20 – 2.69]) and in model 3 an Odds of 2.31 (CI 9% [1.53 – 3.47]) to be in high caries trajectory group.

Parametric g-formula analysis

Parametric g-formula analysis found that the association between rs10875989 and

dental caries trajectory was not mediated by sugar consumption (OR = 0.98 CI95% [0.95 – 1.02]) (Table 7, Figure 4). On the other hand, when we used gingival bleeding as a *proxy* to presence of biofilm it was observed an indirect effect between the association of rs10875989 and dental caries trajectory, mediated by presence of biofilm (OR = 1.09 CI 95% [1.02 – 1.15]).

Discussion

In the present population-based birth cohort study, we assessed the hypothesis that caries trajectory assessed in the life course might be influenced by SNPs linked to pathway of salivary flow and composition genes. In fact, we investigated SNPs previously identified with potential *a priori* association with dental caries aiming to replicate results in the cohort. Therefore, our findings are in part in consonance with literature [Anjomshoaa et al., 2015] – in the first time with a longitudinal design – showing that rs10875989 was associated with caries in all models investigated. Allele C was associated with increase of 38% in odds to be in high caries trajectory group. Similarly, genotype CC in additive model was associated with an increase of two-fold to be in high caries group and genotype dominant model was also maintained in adjusted model. Furthermore, it was observed by parametric g-formula that the effect of rs10875989 on caries is mediated by gingival bleeding – used as a proxy to presence the biofilm – and not by sugar consumption, which may reinforce that main effect is due decrease on salivary flow and consequently increase of biofilm presence. We also complement the analysis investigating possible epistatic interactions, i.e. gene-gene interaction. In this way, we found that combination of rs2274333, rs10875989 and rs3759129 was associated to increase of more than twice in odds to be in high caries trajectory group, showing a possible interaction between these genes on evaluated population underlying initial observations since dental caries seem be a complex traits.

Indeed, we use the parametric g-formula to investigate possible mediation of effects in the relationship between rs10875989 and caries trajectory. So, it was not observed mediation by sugar consumption. However, when gingival bleeding was used as a proxy to elevate level of biofilm, we observed that occur an important mediation on this association. This result can be interpreted considering the hypothesis that rs10875989 can influenced AQP and, consequently, salivary flow. Therefore, decrease on salivary flow lead an increase on biofilm and dental caries. In fact, genetic changes in *AQP5* lead to decrease of 60 to 65% in salivary flow of mice model, resulting in increased also in dental caries [Culp et al., 2005].

Genetic variations in locus 12q13, which are responsible by aquaporins, have presented elevate linkage disequilibrium in previous report [Anjomshoaa et al., 2015] corroborating with observed in our study. Thus, rs10875989 seems presented the higher linkage disequilibrium structure of this locality (D' and r^2), could be choose as tagSNP due to maximal representation of region [Carlson et al., 2004]. In fact, the rs10875989 is located in uncharacterized LOC101927318 in a non-coding RNA (ncRNA) close to *AQP2* and *AQP5*. Although ncRNA non

coded protein, overall, they are functionally important to include transfer RNAs, ribosomal RNAs, even as microRNA (mRNA). In this way, Anjomshoaa et al. [2015] observed an important association between rs10875989 and dental caries in Brazilian and American populations. Moreover, mRNA expression analysis of *AQP5* was performed and associated with caries experience; being also influenced by presence of fluoride in water locations.

On the other hand, our results non totally corroborate regarding rs3759129, which was associated with caries in an American population [Anjomshoaa et al., 2015] and was only associated in in our study in GMDR analysis. Thus, when GMDR software was performed to investigated epistatic influence, it was observed two models (two and three-locus) with positive interactions resulting in increase of odds of having high dental caries trajectory. The first was a three-locus involving rs2274333, rs10875989, rs3759129. We also found epistatic interaction with the combination of rs2274333 and rs3759129 genotypes resulting in an increase on odds of having a high caries trajectory. The three-locus model presented the best prediction ability due to highest accuracy of 60.52% when compared to best two-locus model (57.57%) and suggesting that caries trajectory might be best explained by the causal or indirect action of these three marker loci. However, it is necessary to take in account that cross validation consistency decrease in both models to 8/10 when compared to best one locus model.

These results can be explained because GMDR can identify genetic interactions underlying the phenotypes considering genetic architecture of complex traits. In gene-gene analysis, logistic regression presents limitations due an overfitting problem in high-order interactions while GMDR approach allows to adjust for covariates and is based in statistical scores obtained from generalized linear model on the predictor-variable and covariates. Moreover, GMDR may decrease Type I and II errors, and increase the robustness of models in a multifactorial model. Thus, these findings highlight that combination of presents SNPs presents a genetic interaction underlying conventional regression analysis, which should be carefully interpreted. We recommend that further studies investigate and confirm this epistatic interaction in other samples to confirm our observations.

Considering both two and three-locus models, rs2274333 was included in both best fitted models presenting a possible epistatic interaction. rs2274333 is a SNP of Carbonic Anhydrase 6 that play a not well known role on saliva [Esberg et al., 2019]. However, MUC5B was not associated in any model although some variations influenced caries experience in a Brazilian population [Cavallari et al., 2018]. Possible explanation to this variation of results in the same genetic variations can be linked to differences in allele frequency, demographic

characteristics or statistical power and approach, since that our study – unlike to other – perform corrections to multiple comparisons and control all the analysis by genomic ancestry.

Limitations of present study should be highlighted, some of them linked to the gene candidate design. Gene candidate studies producing a high rate of false positive, which can include biases on results. To avoid this, we perform Bonferroni multiple tests corrections in analysis. It is one of the main explanations that candidate-gene studies have not been easily replicated. Another is related to allele differences across the populations [Hutchison et al., 2004]; Therefore, we perform all analysis controlling by ancestry, avoiding to include stratification bias in our data [Hutchison et al., 2004]. Moreover, gene candidate approach can be still considered an interesting and effective tool for explain genetic makeup of complex traits like dental caries. Besides, it is important highlight losses occurred in the follow-ups and it is necessary the conduction of further well-designs studies to confirm present association, mainly regarding epistatic interactions.

Strengths of this study include a elevate number of quality control filters avoiding bias in our findings: we exclude SNP in Hardy-Weinberg disequilibrium, our models were controlled by contextual and biological factors, with multiple test correction as well as by genomic ancestry using about 370,000 SNPs accessible from the 1982 Pelotas birth cohort. Yet, the present study was the first to investigate the influence of SNPs linked to salivary flow and composition and dental caries in a longitudinal design. Moreover, the phenotype was investigate using grouped trajectory modeling, which create two groups with similar caries trajectories.

Conclusion

Present results demonstrate with robustness that rs10875989 was associated with caries trajectory in the life course considering allelic and genotype (dominant and additive) models. Allele C and the genotype CC were associated with high caries trajectory in the life-course. Parametric g-formula demonstrate that effect of rs10875989 on caries is mediated by gingival bleeding – used as a proxy to presence the biofilm – and not by sugar consumption. Results demonstrate that combination of rs2274333, rs10875989 and rs3759129 was associated with an increase on odds of being in high caries group, showing a possible interaction between these genes on evaluated population.

Compliance with Ethical Standards:

Conflict of Interest: Luiz Alexandre Chisini declares that he has no conflict of interest. Francine dos Santos Costa declares that she has no conflict of interest. Marcus Cristian Muniz Conde declares that he has no conflict of interest. Bernardo Lessa Horta declares that he has no conflict of interest. Luciana Tovo-Rodrigues declares that she has no conflict of interest. Marcus Flávio Fernando Demarco declares that he has no conflict of interest. Marcos Britto Correa declares that he has no conflict of interest

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Ethical approval: Federal University of Pelotas Ethics committee approved this project.

Informed consent: Authorization of all participants were done individually even as all participants signed informed consent terms.

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Table 1 – Markers studied

Gene	Position	Marker public ID	Base pair Change
Carbonic Anhydrase 6 (<i>CA6</i>)	1:9017204	rs2274333	A/G
	1:9030372	rs10864376	C/T
Aquaporin 2 (<i>AQP2</i>)	12:50354437	rs467323	T/C
	12:50351075	rs10875989	T/C
Aquaporin 5 (<i>AQP5</i>)	12:50354437	rs3759129	A/C
	11:1249372	rs2672812	A/G
Mucin 5B (<i>MUC5B</i>)	11:1261640	rs2735733	C/T
	11:1273833	rs2249073	C/T
	11:1281134	rs2857476	C/T

Table S1. Description of allele frequency and results of Hardy-Weinberg equilibrium

	Allele Frequency	Hardy-Weinberg equilibrium	
		Tests	p value
rs2274333 (<i>CA6</i>)	A = 0.701 G = 0.299	Pearson chi2	0.896
		likelihood-ratio chi2	0.896
		Exact significance prob	0.936
rs10864376 (<i>CA6</i>)	C = 0.627 T = 0.373	Pearson chi2	0.075
		likelihood-ratio chi2	0.075
		Exact significance prob	0.078
rs467323 (<i>AQP2</i>)	T = 0.601 C = 0.399	Pearson chi2	0.005
		likelihood-ratio chi2	0.006
		Exact significance prob	0.006
rs10875989 (<i>AQP2</i>)	T = 0.682 C = 0.317	Pearson chi2	0.572
		likelihood-ratio chi2	0.574
		Exact significance prob	0.593
rs2672812 (<i>MUC5B</i>)	A = 0.501 G = 0.499	Pearson chi2	0.893
		likelihood-ratio chi2	0.893
		Exact significance prob	0.919
rs2735733 (<i>MUC5B</i>)	C = 0.547 T = 0.433	Pearson chi2	0.503
		likelihood-ratio chi2	0.503
		Exact significance prob	0.516
rs2249073 (<i>MUC5B</i>)	C = 0.501 T = 0.499	Pearson chi2	0.866
		likelihood-ratio chi2	0.866
		Exact significance prob	0.893
rs2857476 (<i>MUC5B</i>)	C = 0.519 T = 0.481	Pearson chi2	0.983
		likelihood-ratio chi2	0.983
		Exact significance prob	1.000
rs3759129 (<i>AQP5</i>)	A = 0.857 C = 0.143	Pearson chi2	0.147
		likelihood-ratio chi2	0.154
		Exact significance prob	0.149

Table 2. Population characteristics

	Caries trajectory		p value
	N (%)		
	Low	High	
Ancestry			<0.001
European	428 (64.51)	206 (32.49)	
African	35 (44.87)	43 (55.13)	
Sex			0.028
Male	312 (64.73)	170 (35.27)	
Female	293 (70.94)	120 (29.06)	
Income trajectory			<0.001
Low	131 (49.43)	134 (50.57)	
Downward	387 (79.47)	100 (20.53)	
High	87 (63.97)	49 (36.03)	
Gingival bleeding			<0.001
Never	444 (66.17)	227 (33.83)	
One time	28 (47.46)	31 (52.54)	
Always	14 (35.90)	25 (64.10)	
Sugar Consumption			0.003
Low	387 (69.11)	173 (30.89)	
High	138 (58.72)	97 (41.28)	

Table 3. Summary of the allele and genotype frequency comparisons related to caries trajectory

Subjects	Alleles (%)		p-value	Genotypes (%)			p-value
rs2274333	A	G	0.263	AA	GA	GG	0.819
Low	593 (68.32)	275 (31.68)		196 (45.16)	201 (46.31)	37 (8.53)	
High	327 (70.17)	139 (29.83)		114 (47.70)	105 (43.93)	20 (8.37)	
rs10864376	C	T	0.014	CC	TC	TT	0.076
Low	579 (66.71)	289 (33.29)		196 (45.16)	187 (43.09)	51 (11.75)	
High	282 (60.52)	184 (39.48)		88 (36.82)	113 (47.28)	38 (15.90)	
rs10875989	T	C	0.012	TT	CT	CC	0.042
Low	611 (70.39)	257 (29.61)		214 (49.31)	183 (42.17)	37 (8.53)	
High	299 (64.16)	167 (35.84)		101 (42.26)	104 (43.51)	34 (14.23)	
rs3759129	A	C	0.081	AA	CA	CC	0.256
Low	710 (81.80)	158 (18.20)		293 (67.51)	124 (28.57)	17 (3.92)	
High	396 (84.98)	70 (17.09)		176 (73.69)	56 (23.43)	7 (2.93)	
rs2672812	A	G	0.414	AA	GA	GG	0.657
Low	439 (50.58)	429 (49.42)		108 (24.88)	223 (51.38)	103 (23.73)	
High	323 (49.79)	234 (50.21)		59 (24.69)	116 (48.54)	64 (26.78)	
rs2735733	C	T	0.190	CC	TC	TT	0.469
Low	476 (54.84)	392 (45.16)		125 (28.80)	226 (51.07)	83 (19.12)	
High	268 (57.51)	198 (42.49)		79 (33.05)	120 (50.21)	40 (16.74)	
rs2249073	C	T	0.372	CC	TC	TT	0.701
Low	338 (50.46)	430 (49.54)		109 (25.12)	220 (50.69)	105 (24.19)	
High	230 (49.36)	236 (50.64)		58 (24.27)	116 (48.54)	65 (27.20)	
rs2857476	C	T	0.430	CC	TC	TT	0.605
Low	451 (51.96)	417 (48.04)		114 (26.27)	223 (51.38)	97 (22.35)	
High	239 (51.29)	227 (48.71)		63 (26.36)	115 (48.12)	61 (25.52)	

Bold font indicates p-values lower than 0.05; p-value: Fisher exact test

Table 4. Summary of the logistic regression analysis of the association between caries trajectory and genetic variation (allelic) in response immune genes adjusted by ancestry genetic, sex, income trajectory, sugar consumption, oral health habits. (n= 669)

Subjects	Multivariate analysis Unadjusted		Multivariate analysis Adjusted	
	OR (95%CI)	p-value	OR (95%CI)	p-value
rs2274333				
A	1		1	
G	0.94 (0.74 – 1.20)	0.610	1.00 (0.77 – 1.30)	0.987
rs10864376				
C	1		1	
T	1.31 (1.04 – 1.65)	0.022	1.16 (0.90 -1.49)	0.245
rs10875989				
T	1		1	
C	1.34 (1.05 – 1.69)	0.016	1.38 (1.07 – 1.78)	0.013
rs3759129				
A	1		1	
C	0.77 (0.57 – 1.04)	0.096	0.73 (0.52 – 1.01)	0.061
rs2672812				
A	1		1	
G	1.07 (0.85 – 1.33)	0.569	1.03 (0.81 – 1.30)	0.834
rs2735733				
C	1		1	
T	0.87 (0.70 – 1.09)	0.240	0.91 (0.71 – 1.16)	0.437
rs2249073				
C	1		1	
T	1.08 (0.86 – 1.35)	0.499	1.05 (0.83 – 1.34)	0.687
rs2857476				
C	1		1	
T	1.06 (0.85 – 1.33)	0.589	1.06 (0.84 – 1.35)	0.609

Table 5. Summary of the logistic regression analysis of the association between caries trajectory and genetic variation (genotype) in response immune genes adjusted by ancestry genetic, sex, income trajectory, sugar consumption, oral health habits. (n= 669)

Gene/Marker	Genotypes	Multivariate analysis		Multivariate analysis adjusted		Dominant effect Adjusted		
		Unadjusted						
		OR (95%CI)	p-value	OR (95%CI)	p-value		OR (95%CI)	p-value
rs2274333	AA	1		1		AA	1	
	GA	0.89 (0.62 – 1.31)	1.000	0.99 (0.65 – 1.49)	1.000	GA/GG	0.99 (0.71 – 1.40)	0.974
	GG	0.93 (0.74 – 1.82)	1.000	1.03 (0.50 – 2.09)	1.000			
rs10864376	CC	1		1		GG	1	
	TC	1.34 (0.91 – 1.99)	0.179	1.32 (0.87 – 2.01)	0.279	CC/TT	1.29 (0.91 – 1.84)	0.144
	TT	1.65 (0.94 – 2.90)	0.085	1.22 (0.66 – 2.26)	0.935			
rs10875989	TT	1		1		TT	1	
	CT	1.20 (0.82 – 1.77)	0.561	1.29 (0.85 – 1.97)	0.326	CT/CC	1.42 (1.00 – 2.01)	0.047
	CC	1.94 (1.07 – 3.54)	0.024	2.01 (1.05 – 3.86)	0.031			
rs3759129	AA	1		1		AA	1	
	CA	0.75 (0.49 – 1.14)	0.255	0.70 (0.45 – 1.10)	0.164	CA/CC	0.69 (0.48 – 1.02)	0.061
	CC	0.69 (0.25 – 1.91)	0.821	0.65 (0.22 – 1.90)	0.729			
rs2672812	AA	1		1		AA	1	
	GA	0.95 (0.61 – 1.49)	1.000	0.97 (0.60 – 1.57)	1.000	GA/GG	0.99 (0.67 – 1.48)	0.997
	GG	1.13 (0.68 -1.89)	1.000	1.05 (0.61 – 1.81)	1.000			

rs2735733	CC	1		1		CC	1	
	TC	0.84 (0.56 – 1.27)	0.681	0.97 (0.63 – 1.52)	1.000	TC/TT	0.93 (0.64 – 1.34)	0.685
	TT	0.76 (0.45 – 1.30)	0.518	0.80 (0.45 – 1.42)	0.780			
rs2249073	CC	1		1		CC	1	
	TC	0.99 (0.63 – 1.55)	1.000	0.99 (0.61 – 1.60)	1.000	TC/TT	1.02 (0.69 – 1.52)	0.885
	TT	1.16 (0.70 – 1.93)	1.000	1.10 (0.64 – 1.89)	1.000			
rs2857476	CC	1		1		CC	1	
	TC	0.93 (0.60 – 1.44)	1.000	0.93 (0.58 – 1.49)	1.000	TC/TT	0.99 (0.68 – 1.47)	0.979
	TT	1.14 (0.68 -1.89)	1.000	1.14 (0.66 – 1.96)	1.000			

Bold font indicates p-values lower than 0.05

Table S2. Haplotype analysis of loci for hap-analysis: rs2274333, rs10864376, rs10875989, rs2672812, rs2735733, rs2249073, rs2857476 and rs3759129.

Haplotype	High caries	Downward caries	Fisher's p	Odds Ratio (95% CI)
	Trajectory	Trajectory		
	Frequency	Frequency		
ACCACCCA	0.017	0.007	0.111	2.28 (0.80 – 6.49)
ACCATCCA	0.094	0.089	0.638	1.09 (0.75 – 1.62)
ACCGCTTA	0.059	0.074	0.333	0.798 (0.50 – 1.26)
ACCGCTTC	0.013	0.002	0.016	5.84 (1.15 – 29.79)
ACTACCCA	0.020	0.026	0.514	0.77 (0.36 – 1.67)
ACTATCCA	0.110	0.135	0.239	0.81 (0.57 – 1.15)
ACTATCCC	0.035	0.045	0.426	0.78 (0.44 – 1.12)
ACTGCTTA	0.124	0.151	0.758	0.95 (0.69 – 1.31)
ACTGCTTC	0.023	0.061	0.002	0.37 (0.19 – 0.71)
ATCGCTTA	0.030	0.021	0.283	1.46 (0.73 – 2.95)
ATTATCCA	0.038	0.018	0.019	2.25 (1.13 – 4.50)
ATTGCTTA	0.043	0.018	0.006	2.47 (1.27 – 4.81)
GCCATCCA	0.017	0.009	0.151	2.04 (0.75 – 5.54)
GCTATCCA	0.011	0.017	0.355	0.62 (0.23 – 1.72)
GCTATCCC	0.010	0.006	0.419	1.66 (0.48 – 5.70)
GCTGCTTA	0.029	0.007	0.001	4.28 (1.66 – 11.07)
GTCATCCA	0.019	0.035	0.129	0.56 (0.26 – 1.18)
GTCGCTTA	0.058	0.025	0.001	2.47 (1.39 – 4.39)
GTTATCCA	0.020	0.059	0.001	0.33 (0.16 – 0.66)
GTTATCCC	0.021	0.020	0.904	1.05 (0.48 – 2.31)
GTTGCTTA	0.062	0.072	0.524	0.86 (0.55 – 1.36)
GTTGCTTC	0.018	0.025	0.458	0.74 (0.34 – 1.64)

Frequency <0.01 in both control and case has been ignored in analysis

Table 6. Summary of Generalized Multifactor Dimensionality Reduction results for gene-gene interactions. (n= 669)

No.	Best Model	Tr-BA (%)	Te-BA (%)	Sign test (<i>p</i>)	CVC	P value	Odds Ratio (CI95%)
1	rs3759129	54.17	53.82	8 (0.0547)	10/10	0.074	1.49 (0.96 – 2.34)
2	rs2274333 / rs3759129	57.57	52.61	8 (0.0547)	8/10	0.004	1.97 (1.20 – 2.69)
3	rs2274333 / rs10875989 / rs3759129	60.52	55.29	9 (0.0107)	8/10	<0.001	2.31 (1.53 – 3.47)

Abbreviations: CVC, cross validation consistency; Te-BA, testing-balanced accuracy; Tr-BA, training balanced accuracy; Results were adjusted by ancestry genetic, sex, income trajectory, sugar consumption, oral health habits

Table 7. G-computation analysis of sugar consumption as mediator in the association between rs10875989 and dental caries trajectory. (n= 669)

	G-computation estimate (OR)	Bootstrap std. err.	p value	95% CI (OR)
Sugar Consumption				
TCE	1.05	0.0245	0.036	1.00 – 1.10
NDE	1.07	0.0315	0.034	1.01 – 1.14
NIE	0.98	0.0158	0.328	0.95 – 1.02
CDE	1.07	0.0307	0.040	1.00 – 1.13
Gingival bleeding				
TCE	1.01	0.0621	0.880	0.89 – 1.14
NDE	0.93	0.0554	0.184	0.83 – 1.04
NIE	1.09	0.0318	0.009	1.02 – 1.15
CDE	0.95	0.0289	0.066	0.89 – 1.00
Control value(s): Sex= female. <i>TCE</i> total causal effect, <i>NDE</i> natural direct effect, <i>CDE</i> controlled direct effect, <i>NIE</i> natural direct effect				

Figure 1. Linkage disequilibrium of *CA6*, *AQP2*, *MUC5B* and *AQP5* genes. The Single Nucleotides Polymorphisms were tested using SHEsis and estimated with D' and r^2 .

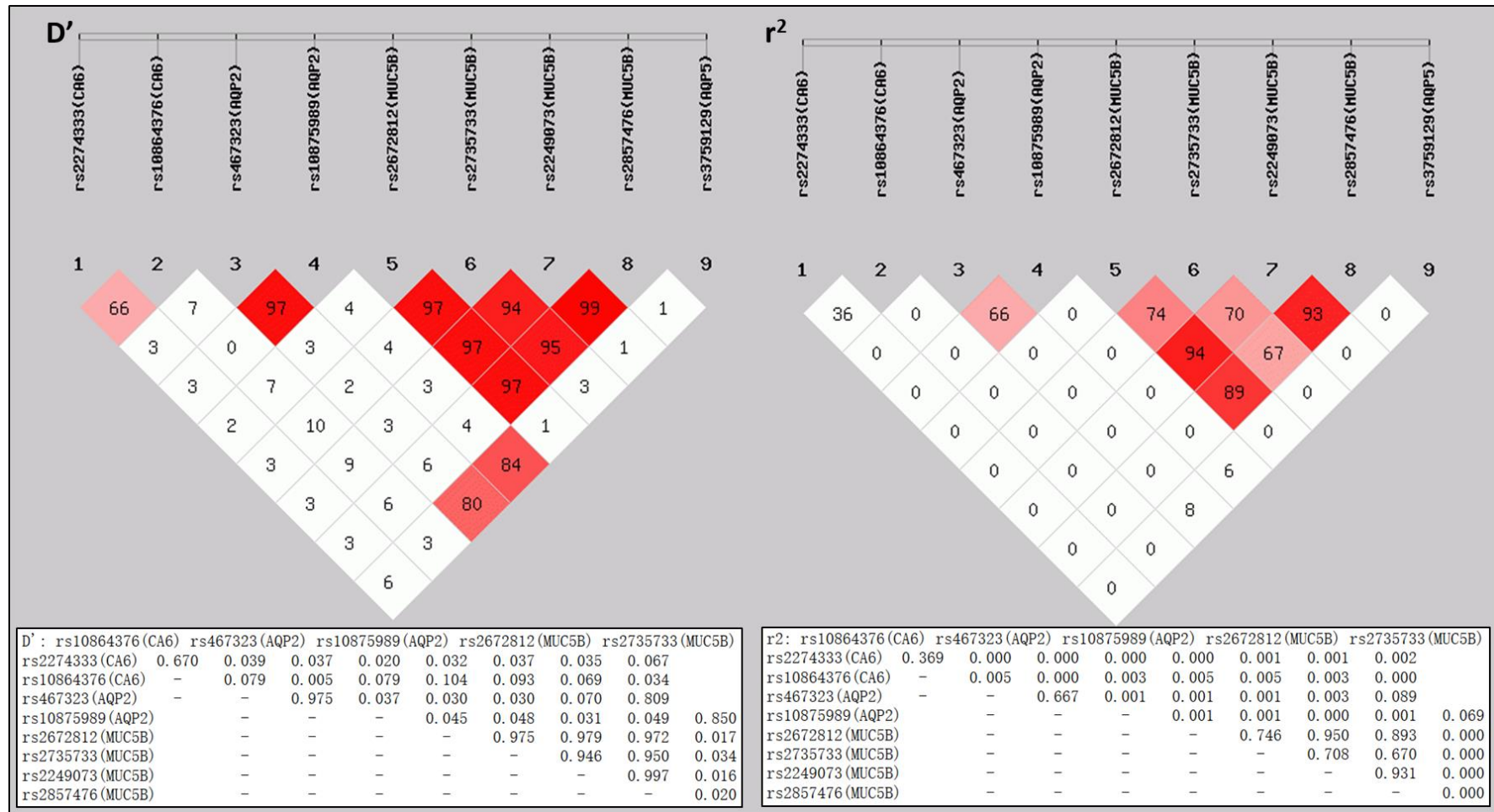


Figure 2. Graphical model of gene–gene interaction analysis

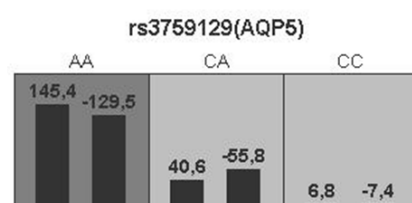
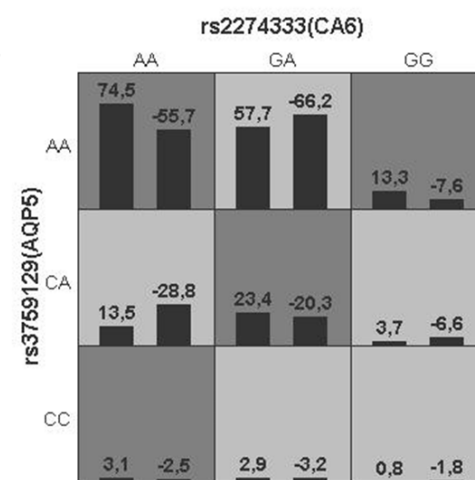
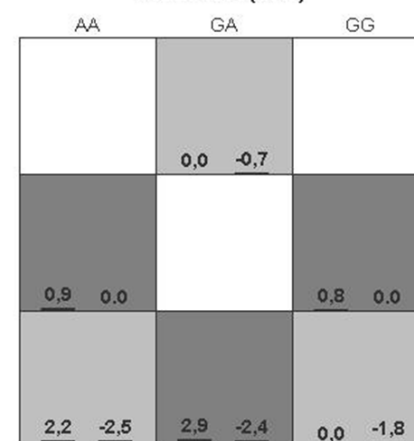
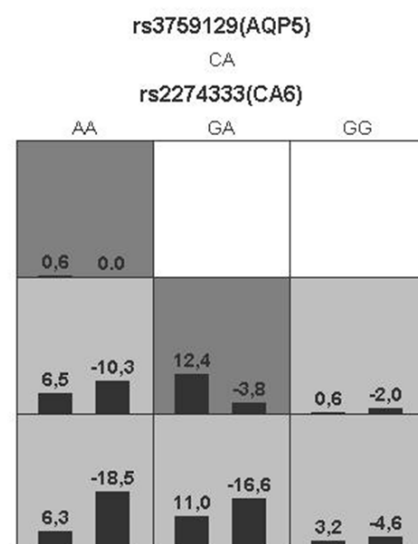
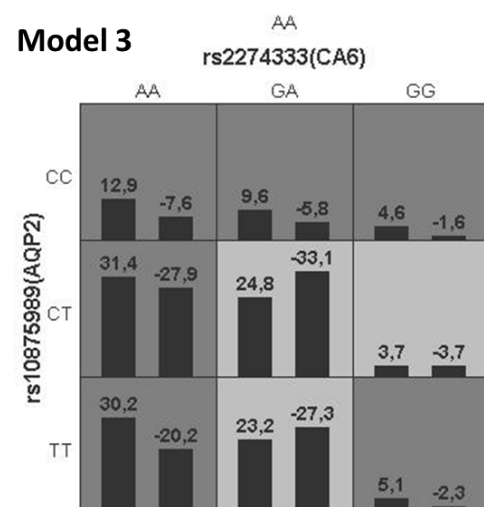
Model 1**Model 2****Model 3**

Figure 3. Illustration of interaction graph summarizing the measures of information gain. Attributes connected by red lines have stronger synergistic interactions than those connected by yellow lines

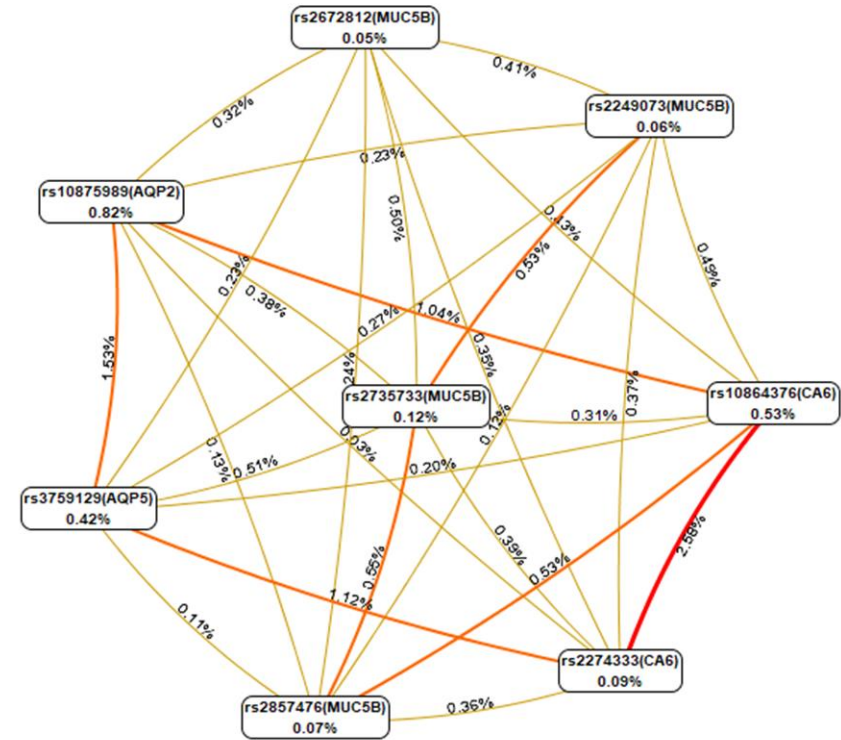
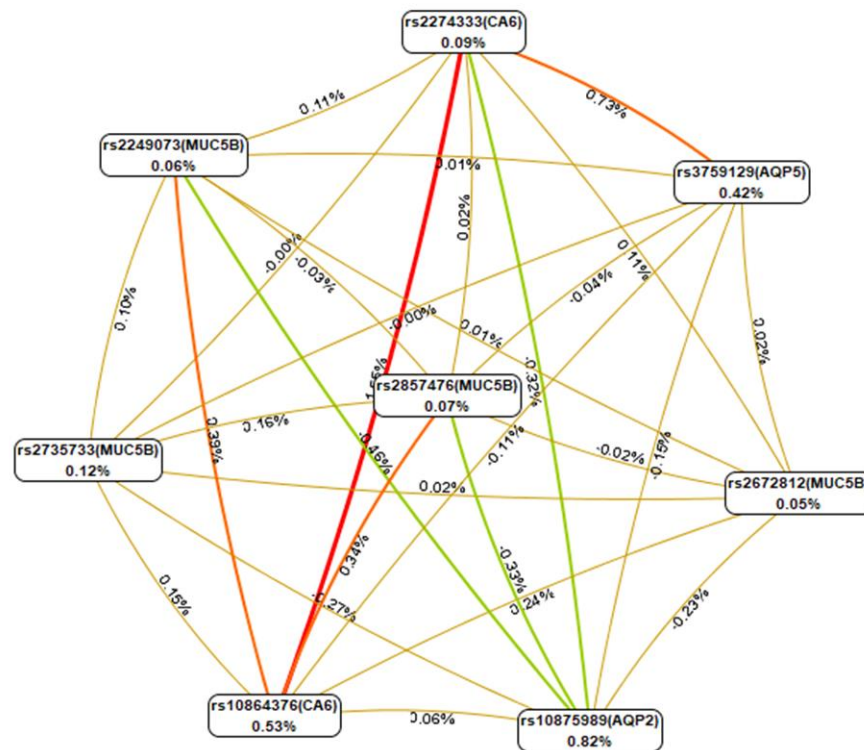
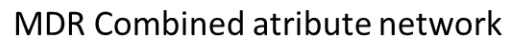
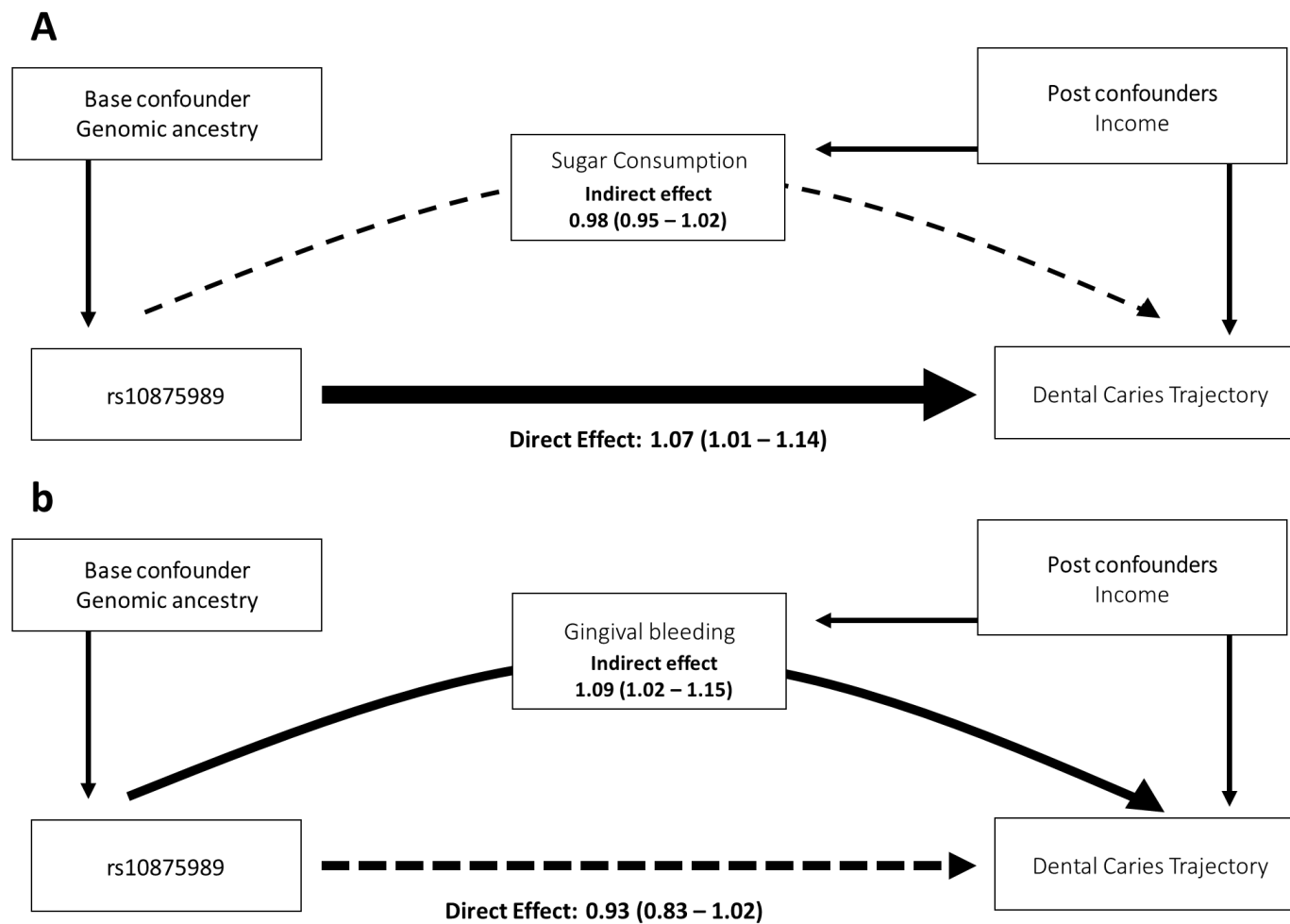


Figure 4. Model of mediation analysis with coefficients and respective confidence interval of total causal effect and natural indirect effect.



6. Sumarização dos resultados

Neste capítulo, serão apresentados os resultados obtidos na presente tese de forma sumarizada e discutidos com literatura científica disponível. Estes resultados foram redigidos em português visando a publicação em uma revista nacional e de acesso aberto para difusão dos conhecimentos obtidos com o desenvolvimento da presente tese na área de epidemiologia genética. Espera-se que tal manuscrito possa servir de base teórica para Professores utilizarem com seus respectivos Educandos ou para Cirurgiões-Dentistas que objetivem aprofundar os conhecimentos em relação aos aspectos genéticos da cárie dental.

6.1 Artigo 10

Artigo formatado seguindo as normas da *Revista da Faculdade de Odontologia da UPF*

Cariologia

Aspectos genéticos da Cárie Dental

Genetic aspects of Dental Caries

Título Abreviado: Genética e Cárie

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Aspectos genéticos da Cárie Dental**Short tile:** Genética e Cárie

Aspectos genéticos da Cárie Dental

Short tile: Genética e Cárie

Resumo

A cárie dental é uma doença crônica e multifatorial que apresenta uma elevada prevalência em termos globais. É inquestionado que os principais fatores para o desenvolvimento e progressão da doença cárie são relacionados aos fatores biológicos, comportamentais e socioeconômicos. No entanto, alguns indivíduos na presença dos mesmos fatores de risco e/ou proteção podem apresentar um padrão de ocorrência de cárie diferente. O estudo da epidemiologia genética tem apresentado evidências de que este pode ser um dos caminhos a explicar tais diferenças. Assim, o objetivo do presente estudo foi realizar uma revisão da literatura e discutir os principais aspectos genéticos da cárie dental de forma acessível à dentistas e estudantes de odontologia. A maioria dos estudos genéticos focados no fenótipo cárie tem objetivado detectar a associação de variantes genéticas (principalmente SNPs) a partir de hipóteses prévias elaboradas no conhecimento da etiopatogenia da doença. Estes estudos têm apresentado um padrão de seleção e tem sido proposto que eles poderiam ser agrupados de acordo com os mecanismos e características das rotas genéticas nas quais eles estão ligados: i) desenvolvimento dos tecidos minerais dentais; ii) resposta imune do hospedeiro; iii) composição e fluxo salivar IV) sensibilidade gustativa. É possível observar uma ampla gama de SNPs/genes que têm sido estudados em diferentes populações sugerindo que as associações com a doença cárie não são aleatórias.

As principais inconsistências parecem ser devido a fatores metodológicos dos estudos e a diferenças étnicas das diferentes populações.

Aspectos genéticos da Cárie Dental

Abstract

Dental caries is a chronic and multifactorial disease that has a high prevalence in worldwide. It is unquestionable that the main factors for the development and progression of caries disease are related to biological, behavioral and socioeconomic factors. However, some individuals in the presence of the same risk and / or protection factors may have a different pattern of caries occurrence. The study of genetic epidemiology has provided evidence that this may be one of the ways to explain such differences. Thus, the aim of the present study was to conduct a literature review and discuss the main genetic aspects of dental caries in a way accessible to dentists and dental students. Most genetic studies focused on caries phenotype have aimed to detect the association of genetic variants (mainly SNPs) based on previous hypotheses elaborated in the knowledge of the etiopathogenesis of the disease. These studies have presented a selection pattern and it has been proposed that they could be grouped according to the mechanisms and characteristics of the genetic routes to which they are linked: i) development of dental mineral tissues; ii) host immune response; iii) salivary composition and salivary flow IV) taste sensitivity. It is possible to observe a wide range of SNPs / genes that have been studied in different populations suggesting that associations with caries disease are not random. The main inconsistencies seem to be due to methodological factors of the studies and the ethnic differences of the different populations.

Introdução

A cárie dental é uma doença crônica e multifatorial que apresenta uma elevada prevalência em termos globais ¹; impactando na qualidade de vida dos indivíduos afetados ². É importante frisar que a cárie dental pode ser prevenida quando focamos nossos esforços no controle dos seus principais fatores etiológicos: hábitos de higiene oral – presença de biofilme e consumo de açúcares. ^{3, 4}. Estas estratégias podem ser mais facilmente alcançadas a níveis individuais quando trabalhamos diretamente com os usuários dos sistemas de saúde; porém, quando pensamos em termos populacionais estas estratégias encontram barreiras de difíceis transposições, uma vez que a cárie dental, por ser mediada por hábitos comportamentais, é fortemente influenciada por fatores contextuais, tais como o nível socioeconômico e educacional ^{1, 5-7}. Por isso, a cárie dental continua sendo considerada o principal problema de saúde bucal da população mundial ¹.

É inquestionado – e a literatura suporta com forte evidência - que os principais fatores para o desenvolvimento e progressão da doença cárie são relacionados aos fatores biológicos, comportamentais e socioeconômicos ^{1, 8}. No entanto, alguns indivíduos expostos aos mesmos fatores de risco e/ou de proteção podem apresentar um padrão de ocorrência de cárie diferente ^{4, 9}. Desta forma, estudos recentes têm investigado a possibilidade de influência genética na ocorrência de cárie dental, objetivando explicar essa parte do efeito não explicada pelos fatores de risco já conhecidos ¹⁰⁻¹². Embora a maior parte dos estudos sejam recentes (e uma busca rápida no pubmed nos mostra isso, **Figura 1**) estudos investigando as contribuições genéticas para a ocorrência de cárie dental têm sido propostos com certa consistência desde o final dos anos 80, a partir de estudos de

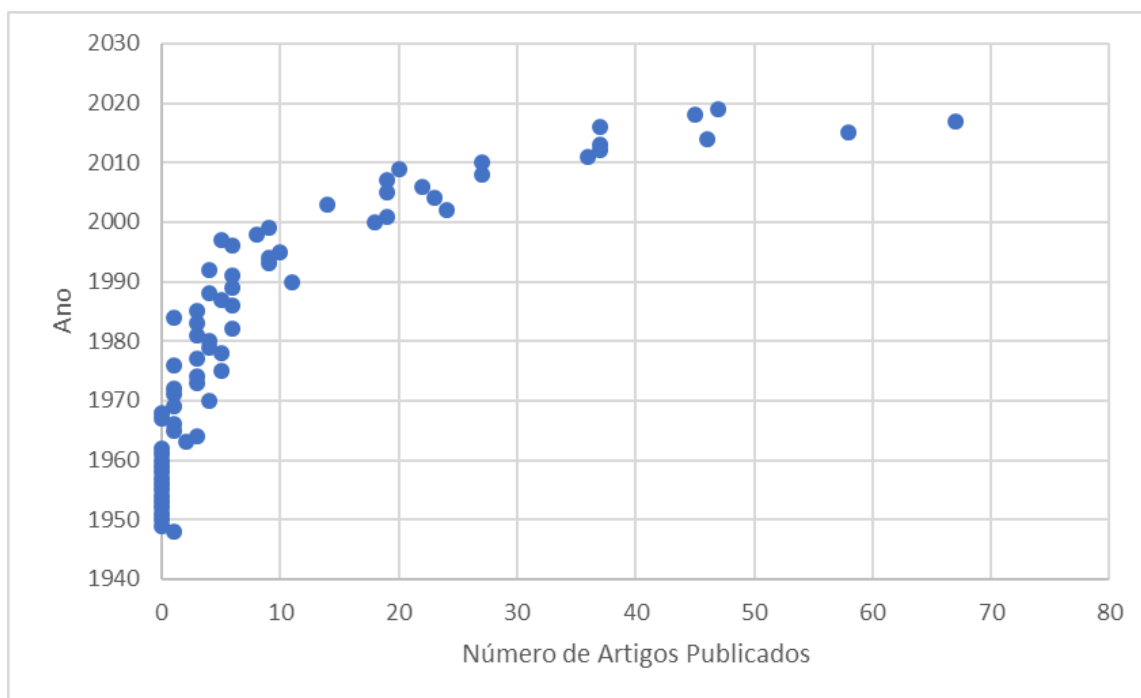
gêmeos (*twin studies*)¹³⁻¹⁶, evoluindo para estudos de associação de genes candidatos e posteriormente para estudos de associação genômica (*Genome Wide Associations Studies* [GWAS]).

Utilizando-se estas metodologias, diversos estudos têm identificado diversos genes em alguns *loci* potencialmente associados com a cárie dental, contribuindo para o entendimento mais aprofundado das bases biológicas e genéticas desta doença^{11, 17}. Brevemente, locus (ou *loci*, no plural) é a posição em que um gene (segmento de DNA que contém as informações genéticas, por exemplo, para a formação de uma proteína como a Amelogenina) ocupa em um dado cromossomo. Estes conjuntos de porções de DNA herdados, i.e. os genes, são formadas por uma sequência de nucleotídeos, os quais carregam as informações para a construção de todos os tecidos humanos^{18, 19}. Cada nucleotídeo é composto por um ácido fosfórico, um açúcar e uma base nitrogenada. Os nucleotídeos constituintes do nosso DNA são: A (adenina), C (citosina), G(guanina) e T(timina). Assim, a modificação (por mutação ou recombinação genética) destes nucleotídeos pode acarretar em diversas alterações estruturais, como, por exemplo, perda da função em uma proteína¹². As alterações podem ocorrer também em regiões não codificantes do nosso genoma, i.e. introns, que correspondem a aproximadamente 25% do genoma²⁰. Embora estas regiões não codifiquem proteínas elas apresentam funções diretas como regulação de *splicing* alternativos e controle do transporte de micro RNAs²¹. Assim, a modificação de um único nucleotídeo (*Single Nucleotide Polymorphism* [SNP]) é uma das mais frequentes variações na sequência do DNA, a qual afeta somente uma base nitrogenada; Essas modificações, ou

SNPs, são considerados as principais responsáveis pelas diferenças no genoma humano ¹⁸,
¹⁹.

Desta forma, considerando que literatura recente venha apontando para uma real contribuição de fatores genéticos na experiência de cárie ¹⁰⁻¹² e que o entendimento dos caminhos complementares (além daqueles já conhecidos) pode ser de fundamental importância para o controle da cárie em um futuro próximo, a difusão destes achados em uma linguagem acessível ao estudante de odontologia e ao clínico são de extrema importância. Além disso, considerando que estudantes e profissionais se atualizam principalmente em periódicos de língua portuguesa e de acesso livre ²², o objetivo do presente estudo foi realizar uma revisão da literatura que discute os principais aspectos genéticos da cárie dental de forma acessível à dentistas e estudantes de odontologia.

Figura 1. Frequência de estudos investigando contribuições genéticas na cárie dental por ano na base de dados PubMed.*



*A busca foi realizada com a combinação das palavras chaves “Dental Caries” AND “Genetic”

1. Tipos de estudos:

Existem três principais estratégias metodológicas para a investigação de aspectos genéticos na cárie dental. Como mencionamos anteriormente, os estudos iniciaram com a comparação dos fenótipos (isto é, características observáveis resultantes da expressão dos genes, no presente caso a cárie dental) entre gêmeos monozigóticos e dizigóticos; chamados por isso de “estudos de gêmeos”. Embora esta metodologia não possa ser considerada uma real análise genética uma vez que não realiza sequenciamento genético, as bases e as hipóteses iniciais das demais estratégias iniciaram com esta abordagem e foram

historicamente importantes. Posteriormente, com o desenvolvimento de estratégias de sequenciamento genético, floresceram os estudos de associação onde sequências genéticas específicas - principalmente SNPs – foram testados investigando sua influência no fenótipo cárie; e, atualmente, com o desenvolvimento de alternativas economicamente viáveis, o sequenciamento de todo o genoma pode ser realizado de forma mais rápida e relativamente mais econômica. Desta forma, foi possível construir metodologias estatísticas de análise de todo o genoma humano – GWAS. Devido a diferença entre estas metodologias, iremos caracterizá-las e descrevê-las de forma mais detalhada abaixo.

1.1. Estudos em gêmeos

Os primeiros estudos pesquisando a influência genética na cárie dental investigaram padrões familiares entre gêmeos e familiares e apresentaram uma forte consistência em relação aos componentes genéticos ^{11, 13, 14}. A metodologia empregada nos estudos de gêmeos ^{13, 14} é realizada através da comparação de um desfecho/fenótipo entre gêmeos monozigóticos e dizigóticos. Inicialmente, a evidência mais convincente foi apresentada a partir de estudos com gêmeos criados separadamente: gêmeos monozigóticos apresentavam semelhanças na experiência de cárie enquanto gêmeos dizigóticos não apresentavam esse padrão. Estes estudos, relataram que fatores genéticos poderiam representar uma contribuição de aproximadamente 40% na ocorrência de cárie dental ^{13, 14}. Estudos mais recentes têm corroborado na direção destes resultados ^{23, 24}; No entanto, mostram que a contribuição genética poderia ser maior – 45 a 64%. Além disso, a hereditariedade na dentição decídua tem mostrado ser mais pronunciada que na dentição permanente ²⁵.

1.2. Gene candidatos

Embora estudos de gêmeos tenham sido a metodologia pioneira, a grande maioria dos estudos realizados (até o presente momento) foi conduzida utilizando a metodologia de genes candidatos. Esta abordagem objetiva testar uma associação entre um gene específico (variantes específicas e conhecidas) e o fenótipo ^{26, 27}. Majoritariamente, os estudos de genes candidatos investigando sua influência na cárie dental têm se aprofundado principalmente na investigação de SNPs ¹¹. É importante ressaltar que, diferentemente do GWAS, esta metodologia é realizada com uma hipótese prévia. Desta forma, o pesquisador deve previamente definir a variação genética que pretende testar ^{26, 27}. Assim, para utilização desta metodologia é importante que o fator genético já tenha sido previamente relatado como possível candidato ou que exista uma hipótese teórica prévia envolvida. Diferentes resultados têm sido observados entre os estudos de genes candidatos (utilizando os mesmos genes e os mesmos polimorfismos). Esta variabilidade pode ser explicada devido à grande heterogeneidade das populações e, principalmente, a questões metodológicas e de poder estatístico ¹¹. Desta forma, existe um campo ainda a ser explorado, seja em relação a confirmação ou não de genes já identificados, seja na investigação de genótipos envolvidos em novas rotas que poderiam favorecer ou proteger os indivíduos à cárie dental.

1.3. *Genome Wide Association Studies (GWAS)*

Além dos estudos de gêmeos e genes candidatos, os estudos de GWAS têm sido utilizados principalmente como estudo exploratório objetivando identificar novas regiões

(*loci*) do genoma que provavelmente abrigam genes com potencial associação com a cárie^{28, 29}. Desta forma, estes estudos não apresentam (necessariamente) uma hipótese prévia. Estudos de GWAS investigam todo o genoma (frequentemente milhões de SNPs) testando associações entre variantes do DNA e o fenótipo de maneira independente. Desta forma, representam um procedimento gerador de hipóteses, que posteriormente necessitam de confirmação. Como se trata de uma questão meramente estatística que envolve múltiplas comparações, o resultado do GWAS deve ser interpretado com cautela. Assim, um limiar típico para a significância estatística em estudos de GWAS é um valor de $p \leq 5 \times 10^{-8}$, e um resultado sugestivo de associação quando observamos um p de 5×10^{-6} ^{28, 29}

O primeiro GWAS investigando a cárie dental foi conduzido em 2008 e identificou que os *loci* 5q13.3, 14q11.2, and Xq27.1 apresentaram baixa susceptibilidade para a cárie dental e que os *loci* 13q31.1 and 14q24.3 apresentaram elevada susceptibilidade³⁰. Além disso, este estudo mostrou que genes relacionados com o fluxo salivar e com a preferência dietética poderiam ser genes candidatos para futuras investigações³⁰. Posteriormente, foram identificados 13 possíveis *loci* significativamente associados e 17 possivelmente associados³¹. Os conjuntos de genes identificados nesse estudo abrangem amplas funções que potencialmente interagem e contribuem na resposta imune dos indivíduos, os tornando mais susceptíveis à cárie dental³¹.

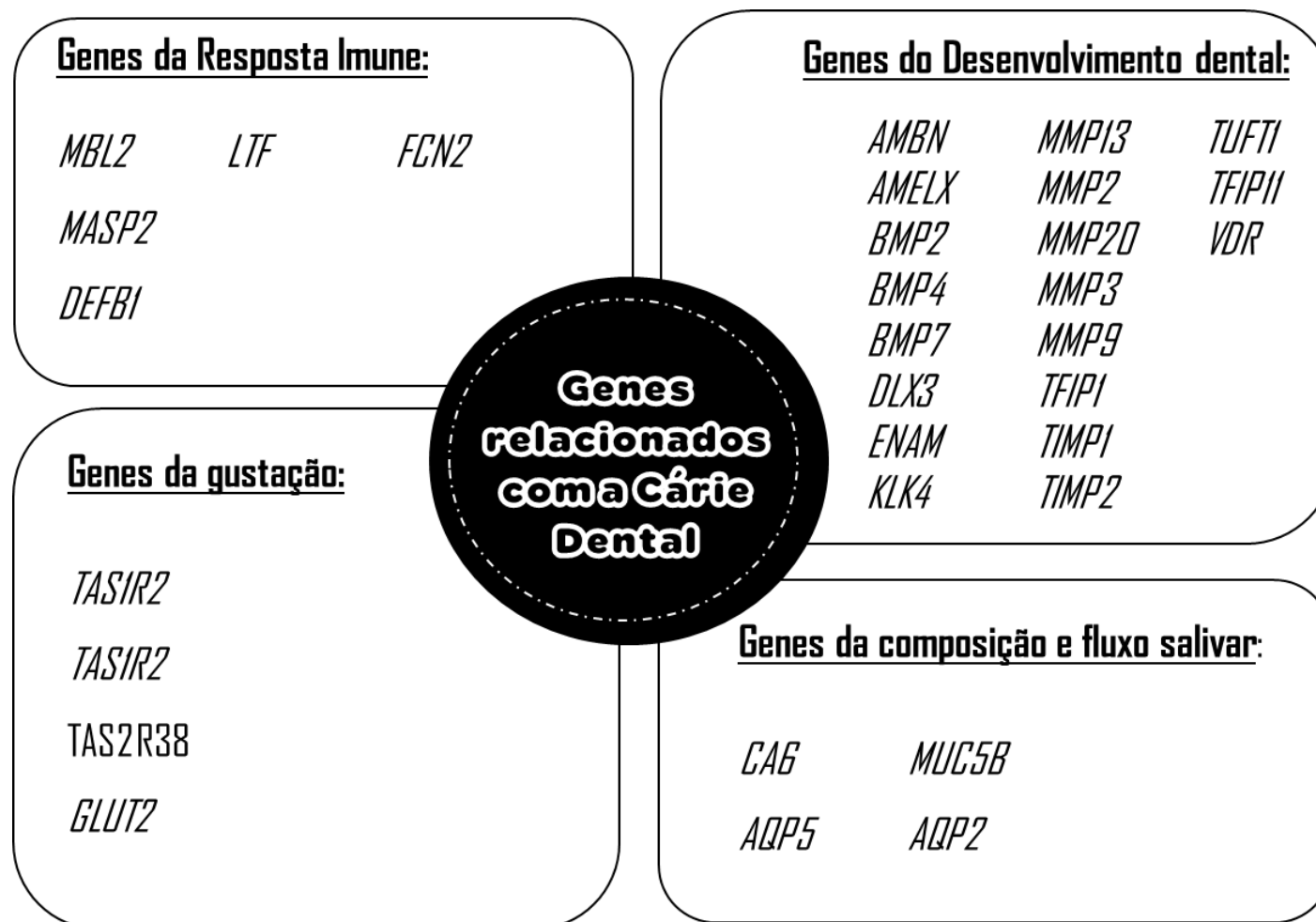
De forma geral, sucessivas abordagens genômicas foram conduzidas muitas vezes apresentando resultados que não corroboravam¹¹. De fato, os estudos de GWAS tem apresentado uma pequena sobreposição nos resultados observados. No entanto, alguns *loci*

(1p36.32 e 10p11.23) apresentaram resultados semelhantes^{32, 33}, com potencial impacto na experiência de cárie.

2. Grupos genéticos possivelmente associados com a cárie dental

A maioria dos estudos genéticos focados no fenótipo cárie tem objetivado detectar a associação de variantes genéticas (principalmente SNPs) a partir de hipóteses prévias elaboradas no conhecimento da etiopatogenia da doença. Estes estudos tem apresentado um padrão de seleção e tem sido proposto que eles poderiam ser agrupados de acordo com os mecanismos e características da rotas genéticas nas quais eles estão ligados (**Figura 2**): i) desenvolvimento dos tecidos minerais dentais; ii) resposta imune do hospedeiro; iii) composição e fluxo salivar IV) sensibilidade gustativa^{10, 11}.

Figura 2. Principais genes possivelmente associados com a cárie dental de acordo com as possíveis rotas genéticas.



2.1. Genes do desenvolvimento dental

Certamente a maior parte dos estudos genéticos são direcionados para a investigação de polimorfismos ligados aos genes do desenvolvimento dos tecidos minerais dentais ¹⁰⁻¹², principalmente por apresentarem a principal plausibilidade biológica. Tem-se tido com hipótese biológica a possibilidade de que variantes genéticas - em genes que estão relacionadas com a composição ou organização estrutural dos tecidos dentais - poderiam alterar algumas propriedades químicas da superfície dental e tornar os tecidos minerais mais susceptíveis a degradação de ácidos bacterianos provenientes do biofilme dental ^{12, 34}. Por exemplo, a proteína codificada pela *ENAM* é envolvida na mineralização e organização do esmalte dental ³⁵.

Uma recente meta-análise que incluiu 18 estudos identificou diversos SNPs ligados ao desenvolvimento dos tecidos minerais dentais e o fenótipo cárie ¹⁰. É interessante observar que uma elevada heterogeneidade foi encontrada entre os estudos, embora não tenha se identificado risco de viés de publicação. Resultados que não corroboram foram identificados em diferentes estudos. Um exemplo claro foi salientado quando se investigou SNPs ligados ao gene da *Mannose binding lectin 2 (MBL2)* que apresentou resultados em direções opostas nas estimativas de efeito de acordo com a dentição (permanente/decídua) ³⁵. No entanto, algumas outras estimativas apresentaram sobreposições nos resultados.

A principal associação mantida pela meta-análise foi relacionada ao SNP da *TFIP11* rs134136. Este SNP está localizado em uma região de intron, no gene que codifica proteínas importantes para a formação do esmalte. De fato, alterações no *TFIP11* têm sido associadas com hipomineralização do esmalte dental e com a experiência de cárie ³⁴. O

gene *AMELX* também tem sido associada com a cárie dental uma vez que a plausibilidade biológica para o seu estudo está no fato de que ela está envolvida na biomineralização do esmalte dental durante a sua formação. O agrupamento dos SNPs (considerando os genótipos de risco) mostrou um aumento de 78% na chance de cárie dental no genótipo de homozigose ¹⁰. *AMBN* é um gene amplamente estudado devido ao fato de codificar uma grande quantidade de proteínas envolvidas na mineralização e na organização da estrutura do esmalte. Assim, o agrupamento dos SNPs (considerando os genótipos de risco) na *AMBN* foram associadas com uma redução da experiência de cárie dental no genótipo homozigoto ¹⁰.

Uma outra revisão da literatura que, embora não tenha estimado os efeitos dos polimorfismos, realizou a investigação de interações genéticas e proteicas apresentou resultados semelhantes ¹². Embora também tenha observado a dificuldade dos estudos replicarem os achados em diferentes populações, apresentou alguns genes (*TUFT1*, *VDR*, *TFIP11*, *TUFT1*, *VDR* e *TFIP11*) como sendo os principais associados com a experiência de cárie dentre os genes ligados ao desenvolvimento dos tecidos minerais ¹². Além disso, esse estudo evidenciou duas redes de interação entre proteínas codificadas por esses genes (Rede 1: *MMP20*, *AMBN*, *ENAM*, *DSPP*, *TUFT1*, *TFIP11*, *AMELX*, *KLK4*; e Rede 2: *MMP13*, *MMP3* e *MMP2*) que estariam influenciando a cárie dental. Desta forma, fica evidente a importância de considerarmos a cárie dental com influência poligênica nos futuros estudos. Uma outra revisão confirmou os achados de Cavallari, Arima ¹² (2019) em relação ao gene *VDR* através de uma meta-análise. O gene do receptor da Vitamina D (*VDR*), o qual apresenta um papel no processo de biomineralização de tecidos minerais

como o esmalte, tem sido estudado e o único SNP que manteve a associação foi o rs10735810 (*VDR*). Ele foi associado com cárie dental no modelo em heterozigose, embora os modelos alélicos e homozigotos tenham apresentado tendência similar, no limite da significância estatística ³⁶. Diversos outros SNPs neste mesmo gene mostraram resultados contrastantes entre os estudos. Por exemplo, estudos com populações chinesas ³⁷⁻³⁹ observaram que o SNP rs731236 (*VDR*) apresentou um aumento no risco de experiência de cárie, enquanto apresentou-se como um fator protetor em um estudo conduzido na República Tcheca ⁴⁰.

Falhas na transferibilidade destes resultados obtidos na meta-análise foram observados em um estudo que avaliou o fenótipo cárie ao longo da vida em uma população no Sul do Brasil considerando polimorfismos de diversos genes (*TUFT1*, *MMP20*, *MMP13*, *MMP2*, *DLX3*, *TIMP2*, *BMP7* e *TFIP11*) ⁴¹. Neste sentido a literatura reconhece a falha de transferibilidade de resultados em escores de risco poligênico ⁴². Portanto, as estimativas de polimorfismos e genes devem ser cuidadosamente consideradas. Embora as análises de associações diretas não tenham corroborado com a meta-análise, quando uma análise de interação epistática, ou seja, interação gene-gene, foi realizada observou-se um aumento de 2,5 vezes na chances dos indivíduos estarem no grupo de elevada trajetória de cárie dental quando um modelo de três *locus* envolvendo os SNPs rs243847 (*MMP2*), rs2303466 (*DLX3*) e rs388286 (*BMP7*) foi considerado ⁴¹. Assim, a interação epistática destes genes pareceu ser um importante caminho para explicar o efeito genético da cárie dental que não seria identificada em análises mais simples de associação. Muitas vezes a simples associação entre um polimorfismo e a doença não é suficientemente adequada para explicar

uma complexa estrutura gênica que apresenta diversas possibilidades de interação, principalmente quando trabalhamos com fenótipos multifatoriais, como é o caso da cárie dental. Estes achados salientam a importância da realização de análises robustas que considerem interações gene-ambiente e epistáticas para que assim possamos entender o traço poligênico da cárie dental.

2.2. Genes gustativos

Um dos caminhos genéticos para explicar a potencial influência genética na cárie dental é por meio de genes que estão ligados a sensibilidade gustativa ¹¹. A plausibilidade biológica é relativa à estudos prévios que tem demonstrado que a opção dietética é influenciada por um certo grupo de genes ^{11, 43, 44}, fazendo assim, com que os indivíduos tenham um consumo de carboidratos fermentáveis (mais especificamente açúcares) de forma diferente de acordo com polimorfismos genéticos ⁴⁵.

Uma revisão sistemática destinada a esse tópico identificou doze SNPS em quatro grupos de genes (*TAS1R2*, *TAS2R38*, *TAS1R3* e *GLUT2*) objetivando encontrar associação entre experiência de cárie e fatores genéticos em estudos de gene candidato ⁴⁶. Embora os resultados tenham apresentado resultados contrastantes em alguns SNPs, dois estudos foram incluídos na meta-análise e mostraram que o genótipo CG do SNP rs713598 (*TAS2R38*) foi associado com uma redução na experiência de cárie. O gene *TAS2R38* está em um *cluster* no receptor do paladar (*locus* 12p13) e é responsável pela sensibilidade ao gosto amargo. Ele é membro da superfamília de receptores acoplados à proteína G. Essas proteínas são expressas principalmente nas células epiteliais da língua e palato, em especial,

o SNP rs713598 conduz à uma alteração do aminoácido alanina à prolina na posição 49. Além disso, é um gene candidato à percepção do sabor doce em diversos estudos ⁴⁷⁻⁴⁹

Ademais, o gene *TAS1R3* tem sido um forte gene candidato devido ao fato de codificar uma das três principais proteínas dos receptores gustativos: T1R3 (*taste receptor type 1, member 3*) ⁴⁵. Assim, já é conhecido que a percepção gustativa humana para o doce é mediada pelos genes *TAS1R2* e *TAS1R3* ⁴⁵. Um SNP do *TAS1R3* tem sido bastante estudado (rs307355) e os estudos têm corroborado nos resultados mostrando que ele é importante na regulação do *TAS1R3* ⁵⁰ e mudanças neste SNP poderiam mudar a percepção gustativas ao doce ^{50, 51}. De fato, um estudo encontrou forte associação entre o genótipo CT neste SNP e elevada experiência de cárie dental ⁵². Posteriormente, um estudo recente ⁵³ conseguiu replicar os achados considerando a trajetória de cárie ao longo da vida como desfecho. Além de realizar todos os controles estatísticos com correções por múltiplos testes, controlando por fatores contextuais e comportamentais, as associações se mantiveram em todos acompanhamentos (quando os indivíduos tinham 15, 24 e 31 anos de idade) e mostraram um efeito dose-dependente em relação ao número de alelos nos genótipos, apresentando uma robustez da associação ⁵³. Objetivando confirmar a hipótese biológica de que a associação tem relação com o consumo de açúcar, uma interação gene-ambiente foi realizada considerando o consumo de açúcar estimada durante um ano como fator ambiental; desta forma, o estudo observou que ocorreu uma interação com os grupos com elevado consumo de açúcar, confirmando a hipótese biológica da associação ⁵³. Desta forma, parece existir uma interação gene-ambiente entre rs307355 e consumo de cárie influenciado a trajetória de cárie ao longo da vida dos indivíduos.

2.3. Genes da composição e fluxo salivar

Tendo em vista que a saliva apresenta uma participação importante da etiopatogenia da doença cárie, estudos de epidemiologia genética têm pesquisado genes e polimorfismos que poderiam impactar no fluxo ou composição salivar ¹¹; Assim, esta tem sido a principal plausibilidade biológica para que estudos gene candidatos investiguem a associação entre genes ligados à saliva e cárie dental. Neste contexto, estudos de genes candidatos têm observado que estas hipóteses parecem se refletir em estudos populacionais, assim, genes ligados à composição e ao fluxo salivar têm sido associados com a experiência de cárie ⁵⁴, ⁵⁵. Além de estudos genes candidatos, um estudo de GWAS encontrou que *loci* relacionados com o fluxo salivar foi um dos principais locais do genoma a influenciar o ocorrência de cárie ³⁰

Uma revisão sistemática sem meta-análise sugeriu que proteínas salivares estariam influenciando a experiência de cárie ⁵⁶. De fato, a saliva possui componentes que podem inibir bactérias cariogênicas, além de conter cálcio e fosfato que estão ativamente envolvidos no processo de desmineralização e remineralização do esmalte dental. (KIDD E FEJERSKOV, 2004; SPLIETH et al., 2016) Além disso, o fluxo salivar tem o papel de diluir os microorganismos e carboidratos ingeridos pelos indivíduos, evitando que eles se acumulem nos tecidos dentários ⁵⁷, portanto, apresentando um importante papel protetor para o desenvolvimento e progressão da doença de cárie.

Assim, resultados de uma meta-análise que incluiu 2.861 indivíduos investigando três genes *CA6*, *AQP5* and *AQP2*) e 15 SNPs observou possível influência dos SNPs

rs1996315 e rs3759129 no gene da *AQP5*; os SNPs rs467323 e rs10875989 na *AQP2*; e os SNPs rs17032907 no gene da *CA6*. Resultados referentes ao SNP rs10875989 (*AQP2*) foram replicados em um recente estudo de coorte de nascimentos ⁵⁵. Este estudo demonstrou em um desenho longitudinal que o alelo C foi associado com um aumento de 38% na chance de ter uma trajetória de alto risco de cárie e que os genótipos, tanto em modelos aditivos quanto dominantes foram associados com a trajetória de alto risco de cárie ⁵⁵. De fato, o genótipo CC foi associado com uma chance duas vezes maior. Análises de interação gene-gene mostraram que os SNPs rs2274333 (*CA6*) e rs3759129 (*AQP5*) foram associados com a trajetória elevada de cárie semelhantemente ao modelo de três *locus*: rs2274333 (*CA6*), rs10875989 (*AQP2*) e rs3759129 (*AQP5*), confirmando relatos de possível hipóteses de interações epistáticas entre esse grupo de genes ¹².

Além disso, uma análise de mediação foi realizada demonstrando que a influência da associação entre rs10875989 (*AQP2*) e trajetória de cárie não foi mediada pelo consumo de açúcar dos indivíduos, mas sim pelo sangramento gengival, utilizado com um marcador de presença de biofilme dental [Chisini and Correa, 2020a]. Estes achados têm confirmado as hipóteses iniciais de que a relação está ligada com a saliva e, assim, influenciando a colonização do biofilme e a cárie dental. Aquaporinas (*AQP*) são uma família de pequenas proteínas integrais da membrana e parecem desempenhar um papel na geração de saliva por meio dos genes que codificam *AQP2*, *AQP5* e *AQP6* estão agrupados na região 12q13 ⁵⁸. Alguns estudos têm confirmado que o *locus* 12q13 apresenta um grande desequilíbrio de ligação, isto é, segregam-se de maneira não randômica durante o processo de meiose e que SNPs nesta localidade poderiam representar todo o *loci* para estudos de gene candidato ⁵⁹,

⁶⁰. Além disso, duas variantes polimórficas (rs10875989 [AQP2] e rs3759129 [AQP5]) apresentaram associação com a cárie dental em estudos de gene candidato ⁵⁹.

Além das aquaporinas, estudos têm apontado que a Mucina 5B (*MUC5B*) poderia apresentar uma associação com a cárie dentária em uma população Brasileira ⁶¹, as quais não foram reproduzidas posteriormente em outro grupo populacional de nacionalidade também Brasileira ⁵⁵; assim, necessitando de mais estudos para confirmar ou descartar a hipótese relacionada com SNPs deste gene. A plausibilidade biológica para as investigações em relação à *MUC5B* é devida ao fato deste gene codificar proteínas que são componentes macromoleculares glicosilados das secreções de muco. Este membro (5B) da família (Mucina) é a principal mucina formadora de gel no muco e é um dos principais contribuintes para as propriedades lubrificantes e viscoelásticas da saliva, e assim poderia influenciar a cárie dentária ⁶¹.

De forma semelhante, o gene da anidrase carbônica 6 (*CA6*) desempenha um papel na hidratação reversível do dióxido de carbono e está presente na saliva, onde parece influenciar a colonização por *streptococcus mutans* e, por esta via, influenciar a cárie dentária. De fato, SNPs neste gene influenciaram a experiência de cárie de adolescentes suecos ⁶² e só influenciaram a trajetória de cárie em uma população brasileira quando considerado em uma análise de interação epistática com outros genes ligados à saliva ⁵⁵.

Embora alguns resultados não corroborem, é possível claramente observar que diversos outros parecem apresentar resultados a suportar o papel de polimorfismos genéticos na cárie dental por meio de genes da composição e fluxo salivar ^{11, 12, 30, 54, 55}. Principais inconsistências parecem ser devidas à grande variação genética entre os estudos

e a questões metodológicas, uma vez que poucos estudos apresentam estratificação ou controle por ancestralidade genômica ¹¹.

2.4. Genes da resposta imune

Algumas proteínas codificadas por genes específicos presentes na saliva têm sido relacionadas à resposta imune individual devido ao fato de possuírem propriedades antimicrobianas, antivirais, antifúngicas e / ou anti-inflamatórias ⁶³. A Lactotransferina (*LTF*), a Defensina Beta 1 (*DEFB1*) e a Lectina de ligação à manose 2 (*MBL2*) são alguns desses genes relacionados à resposta imune. Estudos têm sugerido que elas atuam como proteínas de defesa do hospedeiro, influenciando o sistema imunológico inespecífico, bem como a imunidade adaptativa. Neste contexto, elas poderiam influenciar a colonização bacteriana na superfície dental e, consequentemente, a experiência de cárie dentária ^{11, 64}.

Uma revisão sistemática investigando a influência de SNPs ligados a resposta imune do hospedeiro que incluiu 6.947 indivíduos encontrou 22 possíveis SNPs vinculados a cinco genes diferentes de resposta imune (*MBL2*, *LFT*, *MASP2*, *DEFB1* e *FCN2*) ⁶⁵. Os presentes achados mostraram que alguns genes estão ligados à ocorrência de cárie dentária. A meta-análise sugere que os genes *MBL2* e *MUC5B* têm um papel importante na cárie dentária. Além disso, o conjunto de todos os genes relacionados à resposta imune na análise de genótipo (homozigoto) mostrou associação com a experiência de cárie dentária ⁶⁵.

A Mannose binding lectin 2 (*MBL2*) é um gene que codifica a proteína solúvel de ligação à manose encontrada no soro. Essa proteína está ligada ao sistema imunológico

inato, identifica a manose e a N-acetilglucosamina em vários microorganismos, sendo capaz de identificar uma gama elevada de microorganismos patogênicos que ativam a cascata de complemento por uma via independente de anticorpos ⁶⁶. Portanto, foi proposto que ela poderia influenciar a colonização do microrganismo e, consequentemente, a cárie experiência dentária ⁶⁷. Este estudo observou que o genótipo CG e GG do SNP rs11003125 foram responsáveis por aumentar a chance de cárie dentária na população ⁶⁷. Neste contexto, resultados de uma meta-análise que agrupou o efeito de diversos SNPs ligados ao *MBL2* (excluindo SNPs em desequilíbrio de ligação) encontrou uma forte associação nas estimativas para os genótipos em homozigose e heterozigose ⁶⁵.

O gene da *LTF* – localizada na posição 3p21.31- codifica a proteína com o mesmo nome (lactotransferina) e é expressa principalmente nas glândulas salivares ⁶⁸. Os produtos desta proteína são encontrados nos grânulos de neutrófilos e apresentam uma elevada atividade antimicrobiana sendo considerada, assim, um elemento importante para o sistema imune não específico ^{68, 69}. Neste sentido, a literatura tem investigado e encontrado evidência de que a *LTF* de fato apresenta importante papel na defesa do hospedeiro contra uma ampla gama de microrganismos ⁶⁹, o que poderia explicar sua associação com a experiência de cárie encontrada em alguns estudos ^{70, 71}.

Uma meta-análise tentou sumarizar os SNPs relacionados com a *LTF* (rs1126478, rs1126477, rs2269436, rs743658, rs4547741, rs6441989, rs2073495 e rs11716497) e não observou associações significativas. No entanto, o único estudo longitudinal (não incluído nesta meta-análise) apresentou dados robustos que contestam a meta-análise. O SNP rs11716497 (*LTF*) mostrou uma associação com indivíduos que apresentaram elevada

trajetória de cárie dental ao longo da vida. Estes dados permaneceram associados mesmo após correções por múltiplos testes de Bonferroni em modelos ajustado por fatores ambientais e individuais ⁷². Além disso, uma análise de mediação paramétrica pela g-fórmula demonstrou que rs11716497 (*LTF*) teria um efeito direto na cárie dental, independentemente do nível de consumo de açúcar. Assim, esta relação não seria mediada por pelo consumo de carboidratos fermentáveis ⁷². Além disso, a análise de *Generalized multifactor dimensionality reduction* foi realizada e demonstrou uma forte interação entre dois SNPs (rs4547741 [*LTF*] e rs11716497 [*LTF*]), que poderiam influenciar ainda mais a experiência de cárie do que quando analisados individualmente. Embora estes interessantes resultados nesta população de adultos brasileiros, é importante ressaltarmos que numa população – também brasileira - de crianças (12 anos) os dados não foram reproduzidos ⁷⁰. Além disso, estudos de GWAS investigando a cárie dental não tem apontado para o *locus* da *LTF* como possível via para explicar a experiência de cárie dental ^{32, 73-75}.

Não obstante, é importante enfatizarmos que a ancestralidade genômica tem sido pouco investigada e controlado nos estudos e que isso poderia ser uma das fontes de inconsistência entre os achados recentes. Ademais, estudos não tem realizado padronização nas formas de medir o fenótipo cárie dental, o que pode ser uma importante fonte para as inconsistências. Enquanto um modelo de trajetórias foi construído considerando o componente cariado do CPO-D em três pontos da vida ⁷², estudos tem comparado indivíduos com elevada experiência de cárie (CPOD > 6) com indivíduos com baixa experiência (CPOD ≤ 6) ⁷⁶ ou utilizado o CPO-D de forma contínua em regressões

Alyousef, Borgio ⁷⁷ (2017), o que poderia também explicar parte da falta de transposição dos resultados uma vez que os fenótipos são mensurados de forma diferente.

Espistasia (interação gene-gene)

A interação entre genes, também chamada de epistasia, tem sido considerada no estudo da epidemiologia genética da cárie mostrando traços poligênicos da doença. Assim, ela parece ser um importante fator a ser melhor explorado em estudos futuros ⁴¹. A complexidade da relação entre genótipo e fenótipo parece ter uma consequência da epistasia ou de relações de interação entre gene-ambiente. Brevemente, o termo epistasia surgiu para explicar os desvios da herança mendeliana ⁷⁸ onde um alelo em um *locus* poderia mascarar a expressão de um alelo em outro *locus*. Desta forma, relações diretas entre SNPs/*locus* podem ser mascaradas se a relação mais ampla da arquitetura genética não for considerada e talvez esta seja uma das possíveis fontes de inconsistências nos estudos de epidemiologia genética e cárie dental. É importante destacarmos que a maior parte dos estudos tem realizada análises diretas, desconsiderado a natureza poligênica da doença cárie e análises de associação direta podem não revelar a real influência genética da mesma. Por exemplo, um recente estudo somente observou associação de genes relacionados com a formação dos tecidos minerais com cárie quanto a interação epistática foi analisada ⁴¹. Assim, um modelo de três *locus* envolvendo os SNPs rs243847 (*MMP2*), rs2303466 (*DLX3*) e rs388286 (*BMP7*) apresentou uma chance 2,51 vezes maior para a ocorrência de trajetória de cárie de alto risco, embora nenhuma associação tenha sido observada quando os SNPs foram analisados individualmente. Uma possível conclusão é

que o efeito individual esteja mascarando o efeito do conjunto epistático de polimorfismos, uma vez que aspectos mais aprofundados da arquitetura genética da cárie dental ainda precisam ser desvendados. Além disso, uma recente revisão encontrou que os genes *TUFT1*, *VDR*, *TFIP11*, *LTF*, *HLA-DRB1*, *MMP2*, *MMP3* e *MUC5B* parecem estar conectados em uma rede genética com outros 10 genes, podendo assim influenciar a doença cárie ¹².

Ainda, deve-se destacar que aspectos da arquitetura genética foram pouco explorados na maioria dos estudos de associação genética, não somente para a cárie dentária, e estes estudos podem auxiliar a fornecer a base científica de conhecimento para a interpretação de resultados de GWAS. Devemos destacar que o conhecimento da diversidade de modelos genéticos subjacentes é escassa e é importante considerar cuidadosamente a plausibilidade biológica para cada modelo epistático, o que vai muito além da explicação analítica meramente. Assim, os papéis da genética experimental e da biologia de sistemas na construção de hipóteses deve ser bem fundamentado na etiopatogenia e na biologia da doença.

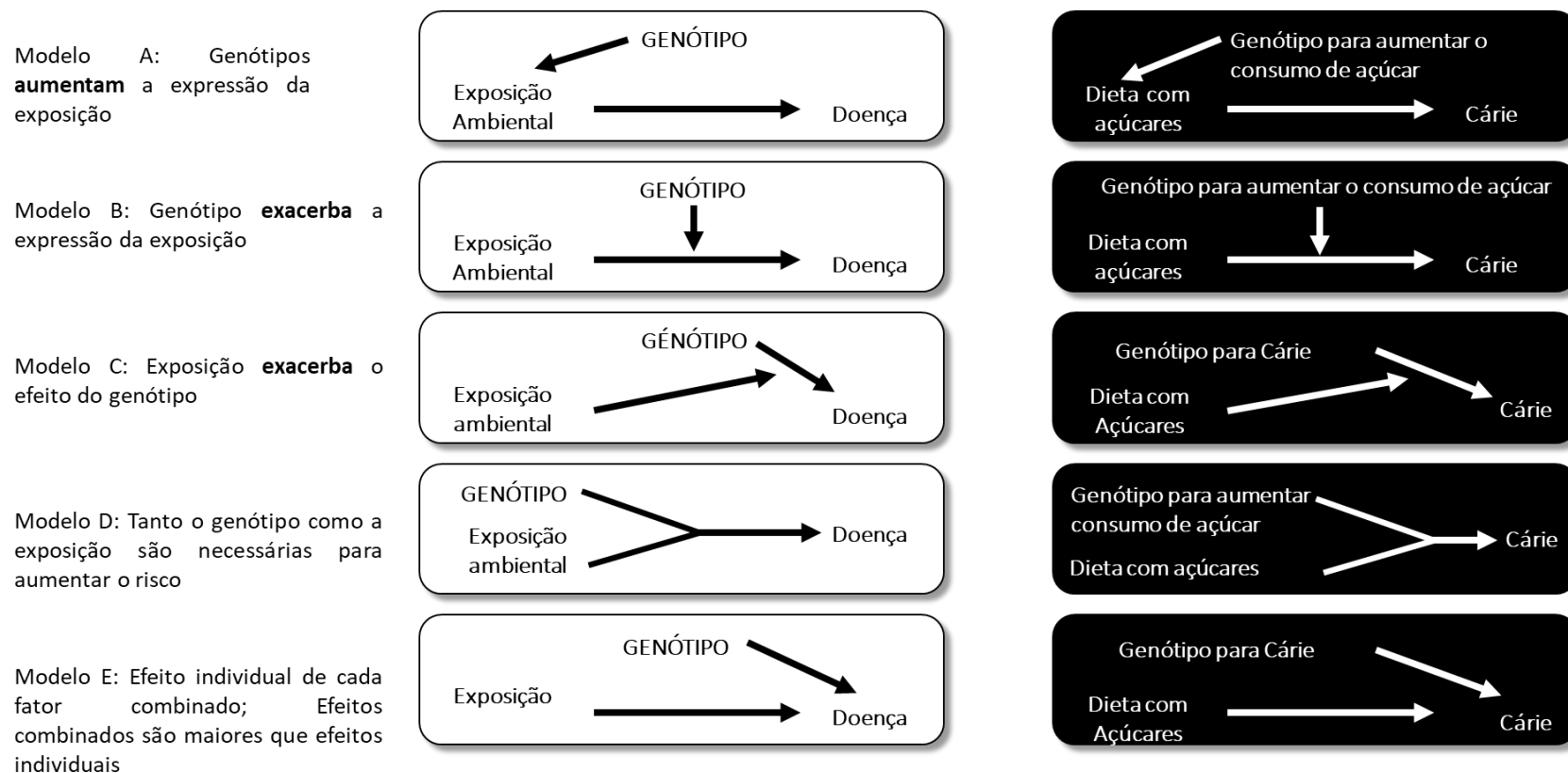
3. Interações Gene-ambiente

Modelos de interação gene-ambiente podem ser definidos como diferenças na magnitude ou na direção dos efeitos de uma exposição ambiental no risco da doença causadas por diferentes genótipos ⁷⁹. Uma ilustração dos diferentes modelos teóricos de interação gene-ambiente é apresentada na **figura 3**. É necessário considerarmos que tanto fatores ambientais quanto genéticos desempenham papel na etiopatogenia da cárie, embora

os fatores ambientais/comportamentais tenham inegavelmente a maior contribuição. No entanto, estes efeitos (ambientais e genéticos) têm sido considerados independentemente na maioria dos estudos. Essa perspectiva pode não ser a melhor abordagem se considerarmos fenótipos complexos não mendelianos que podem resultar de uma interação entre fatores genéticos e questões ambientais ⁷⁸. Assim, talvez tanto fatores genéticos como ambientais devam ser considerados em modelos teóricos mais complexos.

De fato, um estudo apresentou a possibilidade de uma modificação (interação) do efeito do SNP rs307355 (*TAS1R3*) na trajetória de cárie de acordo com o consumo de açúcar ⁵³. O estudo relatou que o consumo de açúcar interagiu significativamente com os alelos e com o genótipo modificando a trajetória da cárie dentária. Os pesquisadores observaram que um aumento na trajetória de cárie ocorreu nos indivíduos quando o alelo T esteve presente nos grupos de alto consumo de açúcar, enquanto não ocorreu modificação quando o alelo T esteve nos grupos de baixo consumo de açúcar. Assim, parece que o alelo T interage com o consumo de açúcar potencializando o efeito dos SNP na cárie dental. Desta forma, alelos e genótipos podem alterar a magnitude do efeito.

Figura 3. Principais modelos teóricos de interação gene-ambiente



Fonte: Adaptado de Austin ⁷⁹ (2012)

4. Epigenética da cárie dental

De fato, além do genótipo poder influenciar a relação entre exposição ambiental e doença é necessário considerar que o ambiente também pode influenciar a expressão gênica. Isso se deve ao que chamamos de epigenética. Brevemente, epigenética é o estudo de alterações na regulação gênica que não são causadas por alterações na sequência de DNA, ou seja, podem existir modificações na expressão de genes que sejam influenciadas por mudanças na sequência de nucleotídeos do DNA. Os mecanismos que conduzem às alterações epigenéticas são extremamente complexas e envolvem a metilação do DNA, a modificação de histonas e a regulação de genes por RNAs não codificantes ⁸⁰.

No entanto, questões envolvendo epigenética e cárie dental ainda não estão disponíveis na literatura e são um campo a ser desvendado por estudos futuros. Um recente protocolo para acessar riscos ambientais e epigenéticos da cárie dental em crianças de uma coorte de nascimentos foi publicado, enfatizando a possibilidade de crescimento de pesquisas na área nos próximos anos ⁸¹

5. Questões metodológicas dos estudos genéticos

Estudos investigando aspectos da epidemiologia genética da cárie tem apresentado resultados que não se sobrepõem com frequência considerando tanto estudos de gene candidatos como de GWAS. Essas inconsistências podem ser explicadas devido à alguns pontos estatísticos e às questões relativas a própria diversidade genética entre as populações. De fato, diferenças ancestrais parecem influenciar os resultados dos estudos ³⁶;

além disso, a falta de poder estatístico em algumas análises (principalmente em estudos de gene candidatos) pode ter favorecido a não associação de alguns polimorfismos. Ademais, é imprescindível considerar que existe a possibilidade de interações epistáticas, que podem influenciar os resultados, e que têm sido pouco exploradas pelos estudos até então.

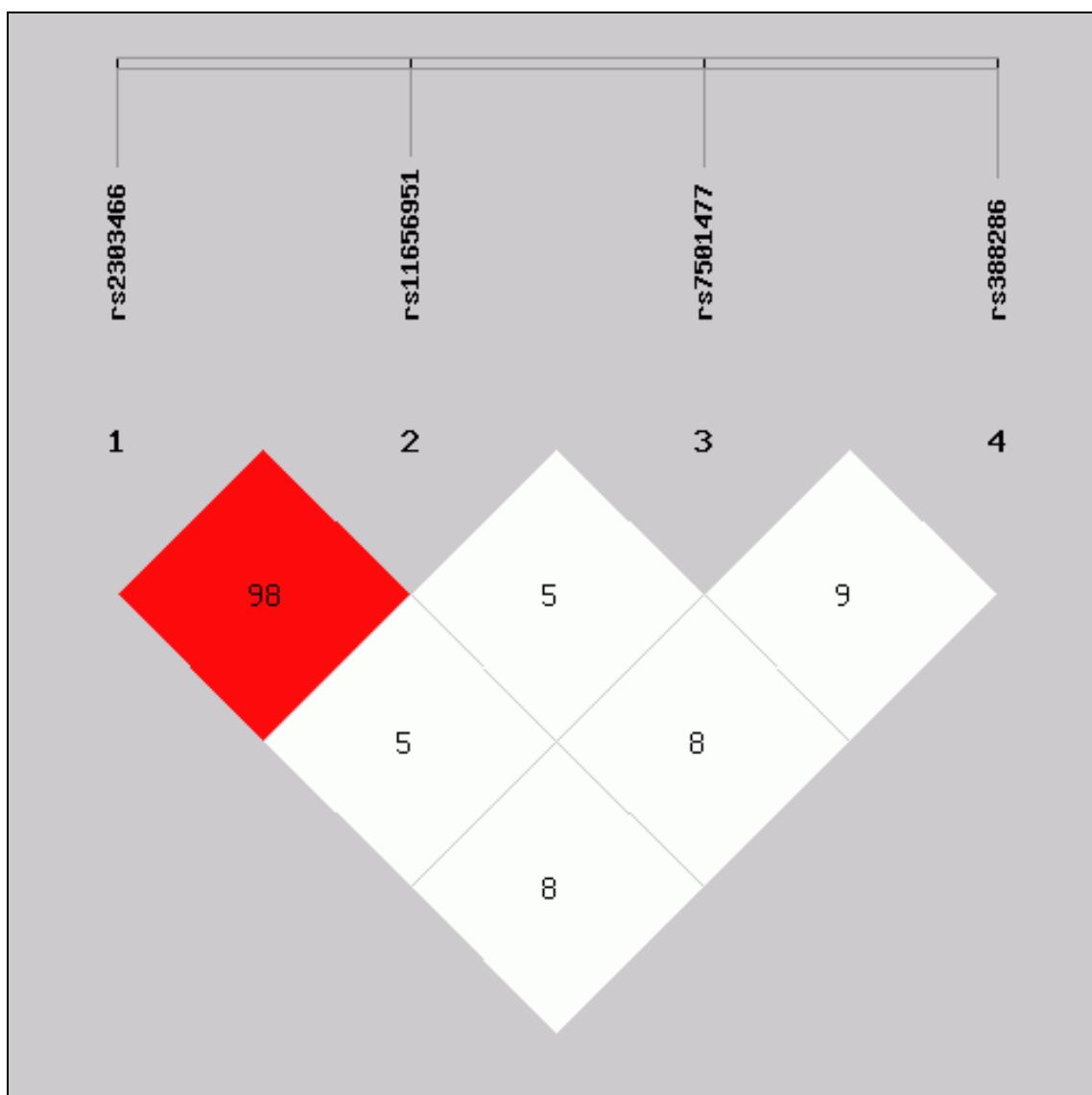
É importante considerar também que os estudos de associação genética são mais complexos que a análise genética baseada apenas na ocorrência de recombinação durante a meiose, assim apenas uma análise de qui-quadrado pode incluir importantes vieses na associação. De fato, podem ocorrer três justificativas principais para uma associação entre alelo/genótipo e o fenótipo. Primeiro, pode existir uma associação indireta devido ao desequilíbrio de ligação (*Linkage Disequilibrium* [LD]), onde os alelos associados estão em desequilíbrio de ligação com a doença ou com outro SNP ⁸². Podemos conceituar o Desequilíbrio de Ligação *Linkage* como sendo a associação não-aleatória de alelos em dois ou mais *loci*. Assim, em cruzamentos aleatórios, os alelos de qualquer gene são combinados aleatoriamente em genótipos de acordo com frequências dadas pelas proporções de Hardy-Weinberg. Desta forma, é importante que os estudos sempre avaliem se os alelos testados estão em desequilíbrio de ligação pelo teste de Hardy-Weinberg (*Hardy-Weinberg test*). Caso eles estejam em desequilíbrio, uma possível associação é espúria e não deve ser considerada uma vez que a distribuição entre os alelos não foi randômica.

Além disso, pode ocorrer LD entre um ou mais alelos investigados. Isso é importante principalmente nos casos em que se deseja agrupar os efeitos individuais de SNPs em diversos genes ¹⁰. SNPs próximos dentro de um mesmo gene apresentam uma baixa probabilidade de se recombinarem durante o processo de meiose por meio do

processo chamado de *crossing over*. De fato, existe uma proporção de quanto mais próximo os polimorfismos estão num mesmo cromossomo menor será a probabilidade de ocorrer permutação. Assim, frequentemente os SNPs próximos estão em LD e segregam-se não randomicamente.

O LD é estimado através do D' , i.e. $D' = 1$ significa um desequilíbrio de ligação total, ou seja, todos os polimorfismos segregam-se em conjunto. $D' = 0$ corresponde a nenhum equilíbrio de ligação, ou seja, os alelos segregam-se independentemente^{83, 84}. Na **Figura 4** temos o *output* de uma análise de LD realizada pelo software SHEsis. Nela foram realizadas as análises de LD em quatro SNPs. Podemos observar que há um desequilíbrio de ligação entre o SNP 1 e 2, com um $D' = 0.98$. As demais combinações apresentaram um LD baixo, ou seja, tiveram um $D' < 0.30$.

Figure 4. Exemplo de *output* de uma análise de Linkage disequilibrium usando o software online SHEsis estimando o D' .



Embora de extrema importância, as investigações sobre LD nos estudos de gene candidato que investigam a associação entre cárie e SNPs são pouco realizadas na literatura¹⁰, embora alguns estudos apresentem essa análise⁸⁵⁻⁸⁷. Portanto, a não investigação do desequilíbrio de ligação pode introduzir um viés importante nos resultados dos estudos⁸².

Segundo, erros tipo I (associações do tipo falso positivo) são comuns devido a múltiplas comparações principalmente em estudos de GWAS, embora sejam importantes de estudos de gene candidato também ⁸⁸. O erro tipo I ocorre quando o estudo conclui que existe uma associação quando de fato ela não existe. A principal fonte de falso positivo é a não realização de correções por múltiplas comparações, como, por exemplo, correções de Bonferroni [Gao et al., 2008]. Correções de Bonferroni para múltiplos testes são consideradas os padrões ouro para prevenir a incorporação de falsos positivos por múltiplos testes, e isso ocorre dividindo o valor de α pelo número de comparações [Gao et al., 2008].

Além disso, os SNPs no desequilíbrio de Hardy-Weinberg podem ser uma fonte de associação positiva falsa. Associações falso-positivas são infladas se os homozigotos forem menos frequentes do que o esperado numa distribuição normal e, portanto, todos os estudos associados devem executar o controle do equilíbrio de Hardy-Weinberg. De fato, uma revisão sistemática recente encontrou um baixo número de estudos investigando cárie dentária em estudos de associação de genes com correção por múltiplas comparações ¹⁰. No entanto, algumas publicações recentes têm salientado que correções por múltiplos testes poderiam remover também associações verdadeira e significantes ^{60, 89}. Embora exista essa discussão na literatura, o que podemos constatar é que se uma associação foi mantida mesmo após testes por múltiplas comparações essa associação era realmente forte.

Terceiro, quando existir uma relação direta e casual, é necessário investigar a força e consistência da associação, a sequência temporal, uma possível resposta dose-dependente e, principalmente, uma plausibilidade biológica para as observações. Neste sentido, alguns estudos recentes realizado por nosso grupo têm apresentado os pressupostos acima e

observado uma relação entre SNPs da gustação, resposta imune e fluxo/composição salivar e o fenótipo cárie dental ao longo da vida de indivíduos ^{41, 53, 55, 72}. Por exemplo, foi observado que a associação de rs4970957 (*TUFT1*) e rs243847 (*MMP2*) ocorreu em diferentes acompanhamentos de um estudo longitudinal e não se manteve nos demais ⁴¹. Assim, tendo em vista os pressupostos acima, os SNPs não foram considerados associados, uma vez que tal associação não se manteve ao longo dos acompanhamentos ⁴¹.

Uma outra fonte importante de viés nos estudos de associação genética é a ancestralidade da população ⁹⁰. A estratificação populacional pode confundir os resultados com estudos de associação genética e não pode ser ignorada, pois pode levar à falha por falta de resultados significativos ou falsos positivos. De fato, uma recente e ampla revisão sistemática da literatura investigando os caminhos genéticos dos genes da formação mineral dos tecidos dentais e cárie observaram que poucos estudos realizaram estratificação ou controle populacional por ancestralidade genética; apenas alguns estudos controlaram a população de acordo com a cor da pele dos indivíduos ¹⁰. No entanto, resultados têm demonstrado que deve-se ter um cuidado grande em usar a pigmentação da pele como *proxy* da ancestralidade e que a mesma pode apresentar uma correlação baixa para alguns grupos populacionais ^{91,92}.

Discussão

Os principais fatores etiológicos da doença cárie são bem conhecidos e envolvem o consumo de carboidratos fermentáveis e o biofilme dental sendo fortemente influenciado por questões comportamentais e ambientais ⁹³. No entanto, alguns indivíduos exposto aos mesmos determinantes individuais e socioculturais têm apresentado diferentes susceptibilidades para a ocorrência de cárie ⁴⁴; Objetivando explicar essas diferenças, estudos de epidemiologia genética tem apresentado resultados promissores nas últimas duas décadas, ampliando o conhecimento dos fatores etiológicas da doença cárie. A partir do projeto genoma, diversas ferramentas têm sido apresentadas e, conseqüentemente, utilizadas como estratégias de pesquisa. Estas começaram com os estudos de gêmeos e apresentaram as evidências iniciais a partir da comparação de gêmeos mono e dizigóticos que viviam separados; posteriormente, estudos de genes candidatos foram introduzidos apresentando evidências da influência de uma arquitetura genética na experiência de cárie dos indivíduos ¹¹. Estes estudos apresentaram grupos específicos de genes que parecem estar envolvidos com a formação dos tecidos minerais dentários, principalmente relacionados ao esmalte; estudos investigando genes que alteram a percepção gustativa, mais especificamente ao doce; genes que afetam a resposta imune do organismo e, conseqüentemente, a colonização do biofilme; e por fim, grupos genéticos relacionados com a composição e fluxo salivar ^{10-12, 72}.

De forma geral, diversos achados nestes SNPs têm sido replicados em diversas populações. No entanto, outros parecem não apresentar dados replicáveis em diferentes grupos étnicos. De fato, importantes diferenças metodológicas têm sido apresentadas entre

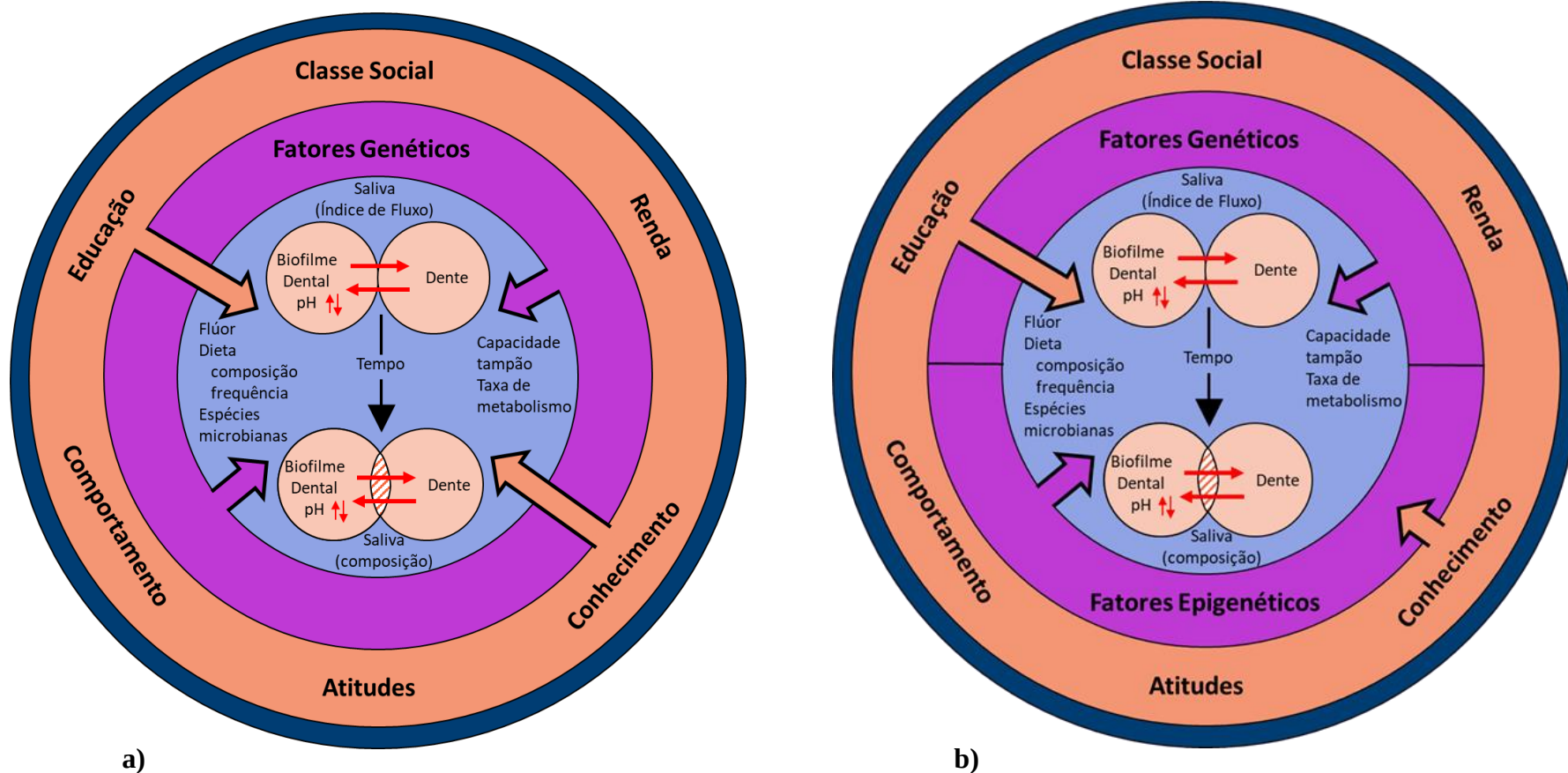
os estudos, as quais possivelmente estejam impactando na padronização dos achados ^{11, 12}. É possível observar que os estudos apresentam formas diferentes de estimar o fenótipo cárie dental, que vão da utilização do CPO-D de forma contínua, uso de grupos de alto/baixo risco, trajetórias do componente cariado, entre outras ^{10-12, 72}. Além disso, controle e/ou estratificações populacionais considerando aspectos étnicos são pontos a serem realizados nas futuras pesquisas, pois esta pode ser uma das principais fontes das inconsistências. De forma semelhante, controles estatísticos por fatores ambientais e comportamentais também devem ser considerados.

Poucos estudos investigando interações entre genes são observados na literatura e esta parece ser uma abordagem importante para que o efeito de algumas variantes genéticas não seja mascarado. Além disso, interações entre fatores ambientais (gene-ambiente interações) devem ser também consideradas para avaliarmos doenças complexas e multifatoriais como a cárie dentária ¹¹. Estudos genômicos devem ser encorajados visando a confirmação dos achados em estudos de associação e como fontes de identificação de novas rotas genéticas. Embora os GWAS sejam considerados os modelos/estratégias mais robustas para identificar associação entre doenças e variações genéticas, estudos de gene candidatos ainda são os mais frequentes e também devem continuar sendo desenvolvidos ⁹⁴. Recentes consórcios têm realizado GWAS e apresentado a possibilidade de diferentes *locus* envolvidos na etiopatogenia do doença cárie (1q42-q43, 11p13 and 17q23.1) ^{33, 95} (RPS6KA2, PTK2B, RHOU, FZD1, ADMTS3 and ISL1) os quais necessitam de replicação através de outros estudos genômicos e de genes candidatos. No entanto, diversos estudos de genes candidatos têm sido realizados e os resultados têm sido replicados considerando

diversos polimorfismos ^{10, 94}. Portanto, considerando principalmente os estudos candidatos a genes, é possível identificar alguns grupos de genes com impacto na cárie dentária ^{10, 94}. SNPs relacionados com os genes *TUFT1*, *VDR*, *TFIP11*, *LTF*, *HLA-DRB1*, *MMP2*, *MMP3*, *MUC5B*, *LTF*, *TAS1R3*, *TAS1R2*, *AQP2*, *AQP5* e *AMELX* têm sido apresentados como centrais por algumas revisões ^{11, 12} e parece ser difícil refutar a hipótese de que fatores genéticos não estejam influenciando a cárie dental.

De fato, estudos de epidemiologia genética têm apresentado sólidas evidências de que componentes genéticos fazem parte da etiopatogenia da doença cárie, embora os reais caminhos ainda não sejam totalmente compreendidos. Desta forma, a incorporação de aspectos genéticos nos modelos teóricos para a explicação da doença cárie deva ser realizado (**Figura 5, a**); embora componentes epigenéticos relacionados com a cárie dental ainda não tenham sido investigados, parece possível que eles possam também estar envolvidos no processo (**Figura 5, b**). Assim, além dos fatores contextuais influenciarem diretamente os fatores individuais eles podem estar influenciando questões genéticas que, por sua vez, influenciam os componentes individuais relacionados com a doença cárie.

Figura 5. Modelo teórico da cárie dental incluindo a) fatores genéticos e b) fatores genéticos e epigenéticos *



Fonte: Adaptado de Fejerskov ⁹⁶ (2017)

* não existem evidências de fatores epigenéticos estão envolvidas na cárie dental, embora exista plausibilidade teórica.

Conclusão

Estudos de epidemiologia genética têm crescido nas duas últimas décadas conseguindo apresentar evidências de que a doença cárie apresenta um componente genético que explica importantes diferenças no risco/proteção da doença. Novamente, destacamos a importância de se replicarem os resultados de estudos genéticos em diferentes populações para confirmar os resultados de variantes específicas; no entanto, é possível observar uma ampla gama de SNPs/genes que têm sido estudados em diferentes populações sugerindo que as associações com a doença cárie não são aleatórias. Os resultados apresentados pela presente revisão são encorajadores e novos estudos também devem considerar questões epigenéticas, interações entre fatores genéticos e ambientais, além de realizar o controle de variáveis por variáveis dentárias e individuais/contextuais.

Compliance with Ethical Standards:

Conflict of Interest: Luiz Alexandre Chisini declares that he has no conflict of interest. Marcus Cristian Muniz Conde declares that he has no conflict of interest. Marcos Britto Correa declares that he has no conflict of interest

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7. Considerações Finais

Os presentes achados confirmam a hipótese de que a doença cárie apresenta um componente genético que explica importantes diferenças no risco/proteção da doença. Destacamos a importância de se replicarem os resultados de estudos genéticos em diferentes populações para confirmar os resultados de variantes específicas; no entanto, é possível observar uma ampla gama de SNPs/genes que têm sido estudados em diferentes populações sugerindo que as associações com a doença cárie não são aleatórias.

Resultados das revisões sistemáticas e meta-análises em conjunto com os estudos prospectivos demonstram que as principais associações em SNPs e cáries foram:

- i) dentre os genes ligados aos tecidos minerais dentais: *TFIP11*, *AMBN*, *VRD* e *AMELX* nas revisões sistemáticas e *MMP2*, *DLX3* e *BMP7* nos estudos prospectivos
- ii) dentre os genes ligados aos genes da sensibilidade gustatória: *TAS1R2*, *TAS1R3* e *TAS2R38* nas revisões sistemáticas e *TAS1R3* e *TAS1R2* nos estudos prospectivos
- iii) dentre os genes ligados à composição e fluxo salivar o *CA6*, *AQP5* e *AQP2* nas revisões sistemáticas e *AQP5*, *AQP2* e *MUC5B*

iv) dentre os genes da resposta imune: *MBL2* nas revisões sistemáticas e *LTF* nos estudos prospectivos

Assim, baseado nos resultados obtidos a partir das revisões sistemáticas e meta-análises somado com os achados dos estudos prospectivos, concluímos que a cárie dental apresenta um componente genético importante capaz de influenciar a experiência e a trajetória de cárie dos indivíduos. Além disso, interações epistáticas parecem desempenhar um papel importante na arquitetura genética da cárie dental e fatores ambientais podem modificar o efeito genético no fenótipo. Novos estudos devem considerar questões epigenéticas, interações entre fatores genéticos e ambientais, além de realizar o controle de variáveis por variáveis dentárias e individuais/contextuais.

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Apêndice A

CENTRO DE PESQUISAS EPIDEMIOLÓGICAS - UFPEL



COORTE DA COORTE DE 1982 – ACOMPANHAMENTO 2013

SAÚDE BUCAL



COLAR ETIQUETA

FOLHA DE ROSTO

Nome _____ do _____ indivíduo

Número da coorte: _____ - _____

Data de nascimento: ____/____/____

Endereço: _____

Ponto de referência:

Tel 1 _____

Tel 2 _____

Tel 3 _____

Tel 1 _____

Tel 2 _____

Tel 3 _____

Tem email? Não () Sim ()

Se sim, email? _____@_____

Outra pessoa da família tem email? Não () Sim ()



Se sim, quem? _____

Email? _____@_____

COORTE 1982		
AVALIAÇÃO DE SAÚDE BUCAL AOS 31 ANOS / 2013		
IDENTIFICAÇÃO		
ENTREVISTADOR: _____	cód ____	DATA DE
ENTREVISTA: ____ / ____ / ____		
Número	do	indivíduo
qes _____ - ____		
<u>IDENTIFICAÇÃO:</u> “Sr(a) <NOME DA PESSOA > estamos trabalhando no estudo sobre saúde bucal dos adultos nascidos em 1982 em Pelotas, realizado pelo Centro de Pesquisas Epidemiológicas da UFPel. Você faz parte desse estudo desde seu nascimento e já foi visitado(a) outras vezes, e agora estamos fazendo uma pesquisa sobre a saúde bucal. Desta vez, só estamos avaliando as pessoas que já tiveram sua saúde bucal avaliada aos 15 anos (1997) e aos 24 anos (2006). Nós gostaríamos de fazer umas perguntas sobre coisas relacionadas à sua saúde bucal. Queremos também examinar seus dentes e a sua boca. Este questionário não possui respostas certas ou erradas e é muito importante para o estudo que o(a) Sr.(a). responda da maneira mais exata possível. As informações prestadas são de caráter sigiloso e seu nome não será associado com qualquer uma das respostas. Podemos conversar?” Se a resposta for afirmativa, dar o consentimento para o entrevistado assinar.		

BLOCO A – HÁBITOS

1. Tu costumavas escovar os dentes com pasta de dentes? _____	[A01]	Nunca 1 Sim às vezes 2 1 vez ao dia todos os dias 3 2 vezes ao dia todos os dias 4 3 vezes ao dia ou + todos os dias 5 IGN 9
2. Qual o tipo de água você bebe geralmente? (5) Outra. Qual? _____	[A02]	água direto da torneira 1 água da torneira filtrada/filtro 2 água l 3 água r 4 ou d 5 IGN 9
3. Você usa fio dental? <i>Ler as alternativas</i>	[A03]	Nunca 0 Às vezes 1 Sempre 2 NSA 8 IGN 9
BLOCO B – CONSULTA COM DENTISTA		
4. Alguma vez na vida foste ao consultório do dentista? <i>Se (0) → pule para a questão</i> <i>Se (9) → pule para a questão</i>	[B04]	Não 0 Sim 1 IGN 9
5. Quando você consultou o dentista pela última vez?	[B05]	Menos de 1 ano 1 1 a 2 anos 2 3 ou mais anos 3 NSA 8 IGN 9
6. Qual foi o motivo da sua última consulta com o dentista? (18) Outros _____	[B06]	Consulta de rotina/prevenção/revisão 10 Dor 11 Dente quebrado/trauma 12 Cavidades nos dentes/cárie/restauração/obturaçã 13 Ferida, caroço ou manchas na boca 14 Rosto inchado 15 Problemas na gengiva 16 Extrações/arrancar o dente (devido à cárie) 17 Outros 18 NSA 88 IGN 99
7. Onde você foi atendido? (5) Outro _____	[B07]	Posto de Saúde 0 Faculdade de odontologia 1 No local de trabalho 2 Consultório particular 3 Convênio 4 Outro 5 NSA 8 IGN 9

8. Você tem medo de ir ao dentista? <i>Ler as alternativas</i>	[B08]	Não 0 Um pouco 1 Sim 2 Sim, muito 3 IGN 9
9. Você acha que atualmente necessita ir ao dentista? <i>Se (0) → pule para a questão 11</i> <i>Se (2) → pule para a questão 12</i> <i>Se (9) → pule para a questão 12</i>	[B09]	0 1 Está em tratamento com c 2 IGN 9
10. Necessita ir a uma consulta com o dentista por qual motivo?	[B10]	Consulta de rotina/manutenção 10 Dor 11 Dente quebrado/trauma 12 Cavidades nos dentes/cárie/restauração/obturaç�o 13 Ferida, caroço ou manchas na boca 14 Rosto inchado 15 Problemas na gengiva 16 Extrações/arrancar o dente (devido à cárie) 17 Outros 18 NSA 88 IGN 99
11. Não precisa ir a uma consulta com o dentista por qual motivo? (2) Outro _____	[B11]	Por que está tudo bem com seus dentes 0 Embora ele/a tenha algum problema, isso pode esperar 1 Outro 2 IGN 8
12. Desde os últimos 6 meses, sentiste dor de dente?	[B3]	Não 0 Sim 1 NSA 8 IGN 9
13. Tu poderias apontar na linha abaixo o quanto esta dor te doeu? Tu debes pensar que 0 (zero) significa nenhuma dor e 10 (dez) uma dor muito forte (anotar o número diretamente na coluna da direita)  0123456789 [B13] NSA 88 IGN 99		

14. Qual foi a principal causa da tua dor de dente? <i>(marcar uma alternativa)</i>	[B14]	Buraco ou cavidade no dente 11 Quando comi ou bebi alimentos quentes, frios ou doces 12 Quando mastiguei alimentos duros (cenoura, maçã, etc) 13 Aparelho ortodôntico fixo ou móvel no dente 14 Quando obturei um dente 15 Quando fiz tratamento de canal 16 Quando tirei (extraí) um dente 17 Quando um dente quebrou 18 Coloquei uma prótese 19 Gengiva 20 Outra razão 21 NSA 88 IGN 99
15. Você já realizou tratamento de canal na tua vida?	[B15]	Não 0 Sim, uma vez 1 Sim, mais de uma vez 2 NSA 8 IGN 9
16. Considerando a aparência de seus dentes o senhor está <i>(ler as alternativas)?</i>	[B16]	Muito satisfeito 0 Satisfeito 1 Nem satisfeito, nem insatisfeito 2 Insatisfeito 3 Muito Insatisfeito 4
17. Considerando a cor de seus dentes o senhor está <i>(ler as alternativas)?</i>	[B17]	Muito satisfeito 0 Satisfeito 1 Nem satisfeito, nem insatisfeito 2 Insatisfeito 3 Muito Insatisfeito 4
18. Você já considerou que seus dentes estavam escuros e fez tratamento para clareá-los <i>(branqueá-los)?</i>	[B18]	Não 0 Sim, uma vez 1 Sim, mais de uma vez 2 NSA 8 IGN 9
19. Você já considerou que seus dentes estavam mal posicionados / amontoados?	[B19]	Não 0 Sim, um pouco 1 Sim, muito 2 NSA 8 IGN 9
20. Você já usou aparelho (fixo ou móvel) nos dentes?	[B20]	Não 0 Sim 1 NSA 8 IGN 9
21. Você já quebrou alguma vez algum dente da frente?	[B21]	Não 0 Sim, uma vez 1 Sim, mais de uma vez 2 NSA 8 IGN 9

22. Você deseja fazer algum destes tratamentos para melhorar a aparência dos seus dentes?		
a. Tratamento ortodôntico (aparelho dentário):	[B22]	0) Não (1) Sim (8) Não sei
b. Restaurações:		0) Não (1) Sim (8) Não sei
c. Clareamento:		0) Não (1) Sim (8) Não sei
d. Implante e/ou Prótese:		0) Não (1) Sim (8) Não sei
23. Você está satisfeito com a tua aparência?		[B23]
		Muito satisfeito 0 Satisfeito 1 Nem satisfeito, nem insatisfeito 2 Insatisfeito 3 Muito Insatisfeito 4
24. Comparado com pessoas da tua idade, você considera a saúde dos seus dentes, da boca e gengivas:		[B24]
		Muito boa 0 Boa 1 Regular 2 Ruim 3 Péssima 4
BLOCO C – SATISFAÇÃO E PROBLEMAS BUCAIS		
25. Problemas com dentes, boca e maxilares (ossos da boca) e seus tratamentos podem afetar o bem-estar e a vida diária das pessoas e suas famílias. Para cada uma das seguintes questões, por favor, escolha as opções de respostas que melhor descreve as suas experiências. Considere toda sua vida, desde o nascimento até agora, quando responder cada pergunta. Após cada pergunta ler as opções: (1) nunca, (2) quase nunca, (3) às vezes (de vez em quando), (4) com frequência, (5) com muita frequência, (9) não sei		
1. Você teve problemas para falar alguma palavra?	[OHIP1]	1 2 3 4 5 9
2. Você sentiu que o sabor dos alimentos tem piorado?	[OHIP2]	1 2 3 4 5 9
3. Você sentiu dores em sua boca ou nos seus dentes?	[OHIP3]	1 2 3 4 5 9
4. Você se sentiu incomodada ao comer algum alimento?	[OHIP4]	1 2 3 4 5 9
5. Você ficou preocupado/a?	[OHIP5]	1 2 3 4 5 9
6. Você se sentiu estressado/a?	[OHIP6]	1 2 3 4 5 9
7. Sua alimentação ficou prejudicada?	[OHIP7]	1 2 3 4 5 9
8. Você teve que parar suas refeições?	[OHIP8]	1 2 3 4 5 9
9. Você encontrou dificuldade para relaxar?	[OHIP9]	1 2 3 4 5 9
10. Você se sentiu envergonhado/a?	[OHIP10]	1 2 3 4 5 9
11. Você ficou irritado/a com outras pessoas?	[OHIP11]	1 2 3 4 5 9
12. Você teve dificuldade para realizar suas atividades diárias?	[OHIP12]	1 2 3 4 5 9
13. Você sentiu que a vida, em geral, ficou pior?	[OHIP13]	1 2 3 4 5 9
14. Você ficou totalmente incapaz de fazer suas atividades diárias?	[OHIP14]	1 2 3 4 5 9

BLOCO D – DESGASTE DENTAL / DTM		
26. Alguém já ouviu você apertando (rangendo) os dentes?	[DTM1]	Não 0 Sim 1 IGN 9
27. Você já acordou de manhã com a sua mandíbula cansada, dolorida ou com dificuldade de abrir?	[DTM2]	Não 0 Sim 1 IGN 9

28. Teus dentes ou gengiva doem ao acordar de manhã?	[DTM3]	Não 0 Sim 1 IGN 9
29. Você já teve dor do lado da cabeça ao acordar de manhã?	[DTM4]	Não 0 Sim 1 IGN 9
30. Você já percebeu estar desgastando os dentes durante o dia?	[DTM5]	Não 0 Sim 1 IGN 9
31. Você já notou estar fazendo apertamento dos seus dentes durante o dia?	[DTM6]	Não 0 Sim 1 IGN 9
32. Você já notou ruído semelhante a casca de ovo se quebrando ou estalo próximo ao ouvido?	[DTM7]	Não 0 Sim 1 IGN 9

ENCERRE A ENTREVISTA AGRADECENDO A ATENÇÃO, ENTREGANDO O BRINDE E ENTRANDO EM CONTATO COM A CENTRAL DE AGENDAMENTOS DE CONSULTAS, SE FOR O CASO.



Apêndice B – Termo de Consentimento Livre e Esclarecido

Termo de Consentimento Livre e Esclarecido

UNIVERSIDADE FEDERAL DE PELOTAS

PROGRAMAS DE PÓS-GRADUAÇÃO EM

EPIDEMIOLOGIA E ODONTOLOGIA

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO – TCLE



O Sr.(a) está sendo convidado a participar da pesquisa **“Condições de saúde geral, socioeconômicas, comportamentais e de acesso a serviços ao longo do ciclo vital: impacto na saúde bucal em uma coorte de nascidos vivos no Sul do Brasil”**. Sua colaboração neste estudo é MUITO IMPORTANTE, mas a decisão de participar é VOLUNTÁRIA, o que significa que o Sr.(a) terá o direito de decidir se quer ou não participar, bem como de desistir de fazê-lo a qualquer momento.

Esta pesquisa tem como objetivo conhecer a situação de saúde geral e de saúde bucal dos adultos que estão sendo acompanhados neste estudo de coorte e sua relação com condições socioeconômicas, demográficas, de acesso a serviços e qualidade de vida. Garantimos que será mantida a CONFIDENCIALIDADE das informações e o ANONIMATO, ou seja, o seu nome não será mencionado em qualquer hipótese ou circunstância, mesmo em publicações científicas. O benefício à sua participação será conhecer a realidade da saúde dos moradores de Pelotas, a qual poderá melhorar os serviços de saúde em sua comunidade. Além disso, se for identificada alguma necessidade de tratamento dentário, ele será realizado na Faculdade de Odontologia da UFPel, sem custo algum a você.

Será realizada uma entrevista e verificaremos algumas condições de saúde da sua boca, como por exemplo, a presença de cárie e a existência de sangramento nas gengivas. Este exame

será realizado por dentistas e não oferece nenhum risco, não causa dor alguma e todos os instrumentos utilizados estarão esterilizados ou serão descartáveis. Em caso de dúvida o(a) senhor(a) poderá entrar em contato com Professor Flávio Fernando Demarco, coordenador desta

pesquisa, nos Programas de Pós-Graduação em Odontologia e Epidemiologia da UFPel, pelo telefone (53) 3222 4162 – ramal 130 ou e-mail: ffdemarco@gmail.com.

Eu,.....

declaro estar esclarecido(a) sobre os termos apresentados e consinto por minha livre e espontânea vontade em participar desta pesquisa e assino o presente documento em duas

vias de igual teor e forma, ficando uma em minha posse.

Pelotas, ____ de _____ de 2013.

(Assinatura do participante)

Apêndice C

Formulário para solicitação de dados já coletados das coortes de nascimentos de Pelotas/RS – Brasil

Esta solicitação se refere a:

() Tese do PPGE

() Dissertação do PPGE

(X) Projeto/Artigo não vinculado à dissertação/tese do PPGE

ATENÇÃO

- A sua proposta deve incluir um plano de análise do artigo.
- Após aprovação pela Comissão de Publicações, se seu Projeto/Artigo não estiver vinculado a dissertação/tese do PPGE você terá 6 meses para submeter o artigo para publicação.
- Verifique que a seção de Agradecimentos do artigo esteja de acordo com o que consta no Anexo B deste formulário. Se for o caso, não esquecer financiamentos específicos da FAPERGS, CNPq etc.
- Com base nos acompanhamentos, cujas variáveis serão utilizadas no artigo, verifique no Anexo C o nome dos potenciais co-autores da proposta.
- Coloque na proposta o nome e a descrição breve das variáveis que você pretende utilizar.
- É obrigatório o preenchimento do Termo de Confidencialidade e Sigilo, que consta no Anexo D, e a submissão à Comissão de Publicações, junto com a proposta.
- As publicações com dados financiados pelo Wellcome Trust, UK, deverão ser depositados no PMC (open-access), com ônus para o proponente.
- Após as análises não se esqueça de circular as tabelas entre os co-autores.
- Antes da submissão para a revista, os resultados deverão ser submetidos à Comissão de Estatística, para aprovação.
- O banco de dados disponibilizado deverá ser destruído após a aceitação para publicação definitiva do artigo.
- Variáveis novas (criadas para a análise) deverão ser entregues à coorte correspondente, com os respectivos logs.
- Novos artigos com o banco já disponibilizado deverão ser submetidos como nova proposta à Comissão de Publicações.

1- Identificação

Autor principal Nome: Marcos Britto Correa

Instituição: Faculdade de odontologia UFPel

E-mail: marcosbrittocorrea@hotmail.com

Telefone: 53 981155031

Endereço: Gonçalves chaves 457, Pelotas, RS

Co-autores

Da coorte:

Nomes: Flávio Fernando Demarco, Bernardo Horta

Externos à coorte:

Nomes: Luiz Alexandre Chisini

Luciana Tovo-Rodrigues:

- 1- Título provisório do artigo:** Influência de polimorfismos genéticos na experiência de cárie: evidências a partir de um estudo prospectivo em uma coorte de nascimentos

- a. Data de início 01 / 05 / 2019
- b. Data de conclusão 01 / 12 / 2019
(6 meses para apresentação do comprovante de submissão do artigo ao Comitê de Publicações)

2- Financiamento

O projeto tem ou terá financiamento:* (X) Sim (2) Não

Qual é o prazo para a submissão do projeto: __ __ / __ __ / __ __ __ __

Liste as fontes de financiamento: : O estudo de saúde bucal da coorte de 1982 aos 31 anos foi financiado pelo CNPq (grants #403257/2012-3-FFD e #475979/2013-3-MBC).

***Propostas deverão ser revisadas pelo Comitê de Publicações antes de serem submetidas a uma agência de fomento e deverão ser enviadas ao Comitê pelo menos três semanas antes do esgotamento do prazo da agência de financiamento.**

3- Variáveis

Marque a fonte das variáveis que serão utilizadas nesta proposta e dê maiores detalhes na seção Resumo da Proposta (item 7)

- a. Existentes nos bancos de dados:
- i. Entrevista (X)
 - ii. Amostras biológicas ()
 - iii. Medidas (X)
 - iv. DNA* (X)
- b. Listar as variáveis e os acompanhamentos de onde se originam

Coorte: 1982
Acompanhamento: <u>Questionário Perinatal – Levantamento 1982:</u>
Nome/descrição das variáveis Escolaridade Materna 21-22. Anos de estudo completados com sucesso: anos 23. Renda familiar do casal 24. Raça 64. Sexo
Ancestralidade Genômica: Proporção de ancestralidade genética europeu, africano e ameríndio
Acompanhamento: <u>Questionário 2004</u>

Nome/descrição das variáveis:

20. Bolacha doce ou recheada

224. Sorvete

225. Açúcar

226. Balas

227. Chocolate em pó ou Nescau

228. Chocolate em barra ou bombom

229. Pudim ou doces

230. Refrigerantes

283: No mês passado, quanto receberam as pessoas que moram na casa?

284. A família teve alguma outra fonte de renda?

285. SE SIM: De quanto foi?

Acompanhamento: Questionário de saúde bucal 2006:**Nome/descrição das variáveis**

6. Alguma vez na vida foste ao consultório do dentista?

7. Desde <mês> do ano passado tu consultaste com dentista?

8. Onde consultaste na última vez?

Variáveis d17, d16, d15, ... d47

Variáveis S17, S16, S15, ... S47

Variáveis C17, C16, C15, ... C47

Variáveis B17, B16, B15, ... B47

Acompanhamento: Questionário Levantamento 2013 30 anos:**Nome/descrição das variáveis**

441b. Grau:

444b. Grau:

477. No total, quanto recebeste no mês passado (se trabalha: sem contar o que recebeu no teu trabalho)

477a. Em reais? _ _ _ _ _

477b. Em salários mínimos? _ _ _ . _

Acompanhamento: Questionário Avaliação de saúde bucal aos 31 anos 2013**Nome/descrição das variáveis**

Bloco A:

- 01- Tu costumas escovar os dentes com pasta de dentes?
 02- Qual o tipo de água você bebe geralmente?
 03- Você usa fio dental?
 Bloco B
 04- Alguma vez na vida foste ao consultório do dentista?
 05 - Quando você consultou o dentista pela última vez?
 07 - Onde você foi atendido?

Acompanhamento: Variáveis Cárie e Periodontia 31, 24 e 15 anos

Nome/descrição das variáveis

Cárie aos 31 anos: conjunto de variáveis cpo

CPOS aos 31 anos: zcpo_31

Doença periodontal aos 31 anos: Profundidade de sondagem (conjunto de variáveis "ps")

Nível gengival (conjunto de variáveis "ng")

Cálculo dentário (conjunto variáveis "cal")

Sangramento Gengival (conjunto de variáveis "isg")

Cárie aos 24 anos: variável cpod

Cárie aos 15 anos: variáveis bcpod, bc, bp e bo

Doença periodontal aos 24 anos: conjunto de variáveis os, oc e ob

DNA

SNPs e genes:

AMBN	rs34538475 (G/T)	TAS1R2	rs3935570 (G/T)
	rs4694075 (C/T)		rs4920566 (G/A)
	rs496502 (G/T)		rs9701796 (G/C)
AMELX	rs17878486 (C/T)	TAS2R38	rs35874116 (T/C)
	rs2106416 (C/T)		rs713598 (C/G)
	rs5933871 (T/C)		rs1726866 (G/A)
	rs5934997 (T/C)	TAS1R3	rs10246939 (C/T)
	rs6639060 (C/T)		rs307355 (C/T)
	rs946252 (C/T)		rs1499821 (NR)

	s7052450 (T/C)		rs5398 (NR)
BMP2	rs1884302 (T/C)		rs5400 (G/A)
BMP4	rs2761887 (A/C)	GLUT2	rs5400 (C/T)
BMP7	rs388286 (T/C)		rs11924032 (NR)
	rs10459948 (T/G)		
	rs11656951 (T/C)		rs2274327 (C/T)
	rs12452477 (T/C)		rs2274327 (A/G)
DLX3	rs16948563 (A/G)		rs2274328 (A/C)
	rs2278163 (C/T)		rs2274333 (A/G)
	rs2303466 (A/G)		rs142460367 (A/G)
	rs3891034 (A/G)		rs142460368 (A/C)
	rs12640848 (A/G)	CA6	rs17032907 (C/T)
	rs2609428 (T/C)		rs11576766 (A/C)
ENAM	rs3796703 (C/T)		rs10864376 (T/C)
	rs3796704 (A/G)		rs3765964 (T/C)
	rs198968 (A/G)		rs6680186 (A/G)
	rs198969 (C/G)		rs923911 (A/C)
KLK4	rs2235091 (A/G)	AQP5	rs1996315 (A/G)
	rs2242670 (A/G)		rs3759129 (A/C)
	rs2978642 (A/T)	AQP2	rs467323 (A/C)
	rs2978643 (C/G)		rs10875989 (C/T)
MMP13	rs2252070 (A/G)		rs1800450 G/A
	rs243847 (T/C)		rs7096206 G/C
MMP2	rs243865 (C/T)	MBL2	rs7096206 C/G
			rs11003125 C/G
	rs1711437 (G/A)		rs1126478 A/G
MMP20	rs1784418 (C/T)		rs1126477 G/A
MMP3	rs522616 (A/G)		rs2269436 A/G
MMP9	rs17576 (A/G)	LTF	rs743658 A/G
	rs3790506 (A/G)		rs4547741 C/T
TFIP1	rs3828054 (A/G)		rs6441989 A/G
	rs7526319 (C/T)		

<i>TIMP1</i>	rs4898 (T/C)		rs2073495 C/G
<i>TIMP2</i>	rs7501477 (G/T)		rs11716497 A/G
	rs233736 (A/G)		
<i>TUFT1</i>	rs4970957 (A/G)	<i>MASP2</i>	rs72550870 A/G
	rs5997096 (C/T)		
<i>TFIP11</i>	rs134136 (C/T)		rs11362 G/A
			rs11362 C/T
		<i>DEFB1</i>	rs1800972 C/G
			rs1799946 G/A
			rs1799946 C/T
		<i>FCN2</i>	rs17514136 A/G
			rs3124953 G/A
			rs2735733 C/T
			rs2249073 C/T
		<i>MUC5B</i>	rs2672812 A/G
			rs2672785 A/G
			rs2857476 C/T

***Toda a análise envolvendo DNA necessita voltar ao Comitê de Ética da UFPEL para aprovação. O projeto deverá estar em português para ser submetido ao Comitê de Ética.**

4- Justificativa para a utilização das coortes de Pelotas no estudo (máximo 100 palavras)

A descoberta de novos genes ou a confirmação dos já identificados e que foram associados com uma maior susceptibilidade à cárie são de extrema importância para ampliar a identificação de indivíduos com risco aumentado. Além disso, o entendimento dos caminhos complementares que podem influenciar o risco à doença cárie podem ser evidenciados com tais abordagens, principalmente quando ajustados pelos fatores de risco já conhecidos. A compreensão dos mecanismos genéticos e das vias genéticas podem prover uma interessante abordagem para discriminar de forma mais detalhada as diferenças observadas entre indivíduos com os mesmos determinantes sociais, ambientais e comportamentais, porém, com experiências de cárie diferentes.

5- Aprovação no Comitê de Ética

O estudo já foi aprovado por um Comitê de Ética

() Sim () Não (X) NSA

SE SIM: Anexe cópia do parecer do Comitê de Ética.

Para análises de dados que não envolvam DNA, não é necessário submeter a proposta a um Comitê de Ética.

6- Resumo da proposta

Em no máximo duas páginas, descreva a sua proposta, indicando claramente os dados que serão obtidos na coorte de Pelotas. No caso de análises a serem realizadas em material biológico, indique claramente o volume/quantidade necessário(a) de cada espécime e o tamanho da amostra. Para novas coletas ou visitas aos participantes das coortes, é importante indicar o tamanho da amostra e como será feita a visita, citando todos os procedimentos (entrevista, medidas, coleta de material biológico). O resumo também deverá indicar os resultados de estudos já publicados sobre o tema, com dados das coortes, além de objetivos, plano de análise e dummy tables.

Não é questionável que fatores biológicos, socioeconômicos e comportamentais sejam as principais variáveis que explicam a ocorrência e a distribuição da cárie dentária na população. Entretanto, em alguns casos, indivíduos que apresentam a mesma proteção - como a fluoretação da água - ou fatores de risco, e com comportamento semelhante relacionado à saúde bucal, apresentam diferentes padrões de ocorrência de cárie dentária [Slade et al., 2013; van Loveren e Duggal, 2001]. Para esses indivíduos, fatores genéticos poderiam ser uma influência intrínseca para fornecer resistência adicional ou suscetibilidade à cárie dentária [Vieira et al., 2014]. Nesse contexto, estudos propuseram que uma parte dessas variações na prevalência de cárie dentária pode ser explicada por fatores genéticos [Deeley et al., 2008; Vieira et al., 2014]. De fato, uma ampla gama de genes e Single Nucleotide Polymorphisms (SNPs) foram identificados, mostrando um papel importante no desenvolvimento e progressão da cárie [Vieira et al., 2014]. Assim, a compreensão de quais SNPs e genes estão envolvidos na suscetibilidade dos indivíduos à doença cárie poderia apoiar o desenvolvimento de uma abordagem viável para melhor compreender esses mecanismos complexos.

Objetivo: Investigar a associação dentre genes do desenvolvimento dental, gustação, composição salivar e resposta imune e experiência de cárie dental dos indivíduos da coorte de 1982 de Pelotas.

Métodos: A variável desfecho do presente estudo será a cárie dental dos participantes que será avaliada em 3 pontos da vida dos indivíduos (15, 24 e 31 anos). Foram colheradas os CPO-D aos 15 e 24 anos. Aos 31 anos de idade, a cárie dental foi avaliada através do índice CPO-S (WHO, 1997), proporcionando assim, um maior detalhamento em relação às exatas superfícies acometidas pela cárie.

O Group-Based trajectory modelling será utilizado para identificar grupos com trajetórias semelhantes do componente “C” (Traj-cárie) e do CPO-D (Traj-CPOD) no percurso da vida (ESB-97, ESB-06 e ESB-13). Assim, os modelos serão estimados com o comando “traj” no programa Stata 12.0 (JONES et al., 2001) Identificando a similaridade da trajetória entre os indivíduos avaliados. Os parâmetros para a trajetória de modelos serão determinados baseada na máxima verossemelhança pelo método de quasi-Newton (DENNIS et al., 1981; JONES E NAGIN, 2007). A seleção dos modelos será considerada e estimada pelo número latentes de categorias e pela ordem polinomial de cada trajetória latente. O número de trajetórias será determinado quando através das comparações sequenciais do Bayesian information criterion (BIC) e com seus critérios de ajustes entre o modelo com K e K+1 trajetórias não produzirá diferença substancial no score BIC do modelo k + 1. Assim, será definido o número de grupos de trajetórias para as variáveis desfecho Traj-Cárie e Traj-CPOD.

A coleta de material genético dos participantes da coorte de nascimentos de 1982 de Pelotas foi coletada durante o período de outubro de 2004 a agosto de 2005. Todos os participantes localizados na área urbana da cidade foram visitados. Assim, os participantes (de 22 a 23 anos) foram entrevistados e examinados em casa e convidados a visitar o laboratório de

pesquisa para doar uma amostra de sangue, coletada por punção venosa. O DNA e o soro foram extraídos e congelados a -70°C . As amostras de DNA já foram genotipadas usando Illumina HumanOmni2.5-8v1 array (VICTORA E BARROS, 2006; HORTA et al., 2015). Além disso, a ancestralidade genômica foi avaliada usando ADMIXTURE (ALEXANDER et al., 2009) baseado em aproximadamente 370 000 SNPs disponíveis na coorte de nascimentos de 1982 de Pelotas compatíveis com os projetos HapMap e Human Genome Diversity para a população brasileira (LIMA-COSTA et al., 2015).

As variáveis independentes principais a serem relacionadas com o fenótipo serão os SNPs.

As variáveis independentes de ajuste que serão utilizadas no estudo foram obtidas dos levantamentos realizados no nascimento, aos 22, 24 e 31 anos de idade.

O sexo dos indivíduos foi coletado no primeiro levantamento em 1982, logo após o nascimento dos indivíduos. A ancestralidade genômica será utilizada sendo definida por dez componentes na análise de componentes principais.

O software STATA versão 12.0. (StataCorp, College Station, TX, EUA) será utilizado para organização do banco de dados. Posteriormente o Programa PILNK será utilizado para realização da análise dos dados. Será realizada uma análise descritiva para determinar a frequência relativa e absoluta das variáveis independentes e dependentes em relação aos genótipos avaliados. SNPs que não estiverem em equilíbrio de Hardy-Weinberg serão excluídos das análises de associação. Para controle dos possíveis fatores confundidores na associação entre os genótipos e fenótipos serão utilizados modelos de regressão logística para variáveis categóricas (CPOD-alto/médio/baixo, CPOD-cárie, Traj-CPOD, Traj-cárie) e modelos de regressão linear para variáveis contínuas (CPOD-total, CPOS-Oclusal, CPOS Livres). Correções de Bonferroni serão utilizadas para correções por múltiplos testes. Além disso, dois modelos de efeitos genéticos

serão utilizados para cada um dos SNPs: aditivo e dominante. Modelos serão controlados por sexo e ancestralidade.

7- Contrapartida orçamentária (liste claramente a contrapartida do projeto para as coortes)

Os estudos de saúde bucal na sub amostra da coorte de 1982 têm sido financiados pela equipe proponente deste trabalho. No último levantamento, realizado em 2013, foram utilizados recursos do CNPq captados pelos pesquisadores Flávio Fernando Demarco e Marcos Britto Correa. As contrapartidas foram acertadas na época da realização do estudo.

8- Termo de compromisso

A informação coletada em qualquer etapa das coortes de Pelotas é altamente confidencial. É essencial que o proponente leia as regras de utilização do banco de dados e assine concordando com as mesmas.

Regras de utilização dos bancos de dados

1. Deverá ser mantida a confidencialidade do banco de dados e eu não terei acesso a identificação dos participantes do estudo.
2. Todos os artigos científicos ou resumos baseados em dados das coortes de Pelotas devem ser enviados ao Comitê de Publicação das coortes, para aprovação, pelo menos duas semanas antes da data de submissão.
3. Os dados fornecidos pela coorte de Pelotas serão utilizados somente para as análises aprovadas nesta proposta.
4. Novas análises poderão ser realizadas somente após serem aprovadas pelo Comitê de Publicações.
5. Ao final das análises propostas neste formulário, o banco de dados deverá ser destruído. As instruções necessárias para a criação das novas variáveis utilizadas nesta análise serão enviadas a gerência de banco de dados do(s) acompanhamento(s) envolvido(s).
6. O banco de dados não poderá ser fornecido para outros pesquisadores, que não estejam envolvidos na presente proposta.
7. O uso das variáveis em outras análises, com produção de outros artigos ou mesmo para apresentação em congressos, sem o consentimento da Comissão de Publicações, será considerado falta grave e impedirá futuras solicitações de dados ao Comitê.

X Li e concordo.

9- Local, data e assinatura do proponente principal:

Pelotas, 18/04/2019



Anexo A – Parecer do comitê de ética

FACULDADE DE MEDICINA DA
UNIVERSIDADE FEDERAL DE
PELOTAS



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Condições de saúde geral, socioeconômicas, comportamentais, clínicas e de acesso a serviços ao longo do ciclo vital: associação com saúde bucal em uma coorte de nascidos vivos no Sul do Brasil

Pesquisador: Flávio Fernando Demarco

Área Temática:

Versão: 1

CAAE: 19551713.9.0000.5317

Instituição Proponente: Faculdade de Medicina da Universidade Federal de Pelotas

Patrocinador Principal: CONS NAC DE DESENVOLVIMENTO CIENTIFICO E TECNOLÓGICO

DADOS DO PARECER

Número do Parecer: 384.332

Data da Relatoria: 29/08/2013

As condições bucais mais prevalentes e importantes são cumulativas e crônicas na sua natureza, sendo necessário um longo período para a sua ocorrência. Os estudos com delineamento de coorte prospectiva suportam a perspectiva do ciclo vital e parte do pressuposto de que o estado de saúde em qualquer idade é o resultado não só de condições atuais, mas também de um acúmulo de condições que foram incorporadas ao longo da vida. No entanto, os estudos de coorte de nascidos vivos são escassos no mundo e no Brasil, a coorte de Pelotas, RS, é a única localizada em países de renda média, avalia desfechos bucais e são essenciais para a verificação de etiologia do processo saúde-doença. O presente trabalho tem como objetivo estudar a influência da trajetória socioeconômica ao longo da vida na saúde bucal na vida adulta e a associação entre condições de saúde bucal com condições de saúde geral em adultos. Serão reavaliados todos os indivíduos nascidos em 1982 aos 30 anos (sub-amostra N=720). Eles foram avaliados anteriormente aos 15 anos e aos 24 anos de idade, respectivamente, em 1997 e 2006. As variáveis do exame clínico incluem a presença de cárie dentária coronária; edentulismo, dentição funcional e arco dentário reduzido; uso e necessidade de próteses dentárias; sangramento gengival; doença periodontal; qualidade das restaurações e lesões de tecido mole. Os exames serão realizados nos domicílios dos participantes, com uso de luz artificial (fotóforos acoplados à cabeça), material de exame (espelho plano, sondas periodontais, espátulas de madeira e gaze) devidamente esterilizado. Todos os examinadores, cirurgiões dentistas, pós-graduandos em Odontologia ou Epidemiologia, estarão

devidamente paramentados respeitando as normas de biossegurança preconizadas pela Organização

Mundial da Saúde. Outras variáveis do estudo, como as perinatais, demográficas, socioeconômicas,

demográficas, comportamentais, de saúde bucal (higiene bucal, dor de origem dentária, dificuldades de alimentação em razão de condições bucais, xerostomia, o impacto dos desfechos de saúde bucal na qualidade de vida dos indivíduos e a utilização de serviços) serão coletadas pela aplicação de questionário padronizado e pré-testado previamente em outros estudos epidemiológicos. As condições de saúde geral, como peso, altura, circunferência abdominal, pressão arterial, densidade óssea, espessura da carótida medial, uso de medicamentos, morbidades auto-referidas, uso de serviços de saúde e auto-avaliação de saúde serão obtidas do levantamento de saúde geral em andamento no ano de 2012. Os entrevistadores serão alunos de graduação da Faculdade de Odontologia (UFPel), também com experiência neste tipo de atividade. A equipe de trabalho de campo será composta por 8 examinadores e 8 entrevistadores, além dos supervisores do trabalho de campo e auxiliares para digitação e arquivamento de material. Será elaborado um manual de instruções para a equipe de campo. Estima-se a realização de uma média de 50-60 entrevistas e exames completos por semana, o que totaliza aproximadamente quatro meses de trabalho de campo, incluindo o treinamento, pré-teste e estudo piloto. Estão previstas reuniões semanais de avaliação entre a equipe de campo e os supervisores e coordenadores do estudo. Todos os dados serão avaliados pelo software Stata versão 11.0 e análises descritivas (frequências absolutas e relativas); univariada (teste Qui-quadrado para variáveis categóricas nominais e Qui-quadrado de tendência linear para variáveis ordinais) e multivariável (adoção de modelos hierárquicos onde as variáveis independentes foram ordenadas em blocos que determinarão a entrada das mesmas na análise estatística. Estes modelos devem descrever a relação hierárquica existente entre os possíveis fatores de risco aos desfechos estudados. Somente as variáveis que na análise bivariada apresentarem valor $p < 0,25$ serão incluídas nos modelos e as finais com $p < 0,05$. Em síntese, os estudos de coorte de saúde bucal são raros, mas oferecem valiosas contribuições para a compreensão dos antecedentes e da história natural de desfechos de saúde bucal e do processo saúde-doença. Além disso, auxiliam na tomada de decisões no campo da Saúde Pública, pois permitem a avaliação da interrelação entre Saúde Bucal e Sistêmica, buscando otimizar tanto recursos humanos quanto materiais e estão de acordo com a teoria do ciclo vital, pois a saúde bucal é resultante de interação de fatores socioeconômicos, biológicos e psicológicos.

Objetivo da Pesquisa:

Objetivos

Geral

- Estudar a condição de saúde bucal em adultos e seus desfechos ao longo da vida, assim

como a associação entre a saúde bucal e condições de saúde geral nesta população. Específicos

- Estimar a incidência e a trajetória de ocorrência dos principais agravos à saúde bucal em adultos, como a cárie dentária e a doença periodontal;
- Estimar o impacto das condições de saúde bucal sobre a qualidade de vida;
- Estimar a prevalência de medo frente a tratamentos odontológicos e seu impacto na qualidade de vida;
- Avaliar longitudinalmente a longevidade e a qualidade das restaurações;
- Verificar o uso e a necessidade de próteses dentárias;
- Estimar o acesso aos serviços de saúde geral e odontológico ao longo da vida;
- Estimar a prevalência das lesões em tecidos moles;
- Estudar a relação entre doença periodontal e sinais sub-clínicos de aterosclerose;
- Estudar a relação entre doença periodontal, perdas dentárias e pressão arterial;
- Verificar a associação entre níveis sanguíneos de proteína C reativa, colesterol e doença periodontal e perda dentária;
- Investigar a associação entre edentulismo e sobrepeso, obesidade central, abdominal e consumo de alimentos ultraprocessados;
- Relacionar a prevalência de lesões cervicais não-cariosas e características oclusais, como presença e facetas de desgaste;
- Avaliar se a experiência de lesão cariiosa coronária ao longo da vida predispõe a ocorrência de lesão cariiosa de raiz aos 31 anos de idade;
- Estudar a relação entre traumatismos dentários e traumatismos gerais ao longo da vida;
- Estimar a prevalência de desgaste dentário nesta população.
- Avaliar se a perda dentária está associada a trajetória socioeconômica dos indivíduos da coorte.

Avaliação dos Riscos e Benefícios:

A participação no estudo prevê um exame bucal que será realizado por dentistas e não oferece nenhum risco, não causa dor alguma e todos os instrumentos utilizados estarão esterilizados ou serão descartáveis.

Benefícios: conhecer a realidade da saúde dos moradores de Pelotas, a qual poderá melhorar os serviços de saúde nas comunidades. Além disso, se for identificada alguma necessidade de tratamento dentário, ele será realizado na Faculdade de Odontologia da UFPel, sem custo algum ao participante.

Comentários e Considerações sobre a Pesquisa:

O delineamento deste estudo será de uma coorte prospectiva de nascimentos. Em 1982, todos os nascimentos hospitalares que ocorreram na cidade de Pelotas, RS, foram identificados e os 5.914 nascidos vivos, cuja família residia na área urbana da cidade, foram pesados e as mães entrevistadas. O estudo de saúde bucal de 2013 (ESB-13) compreenderá os 900 indivíduos da

amostra selecionada para o primeiro estudo de saúde bucal de 1997. Assim como os levantamentos anteriores, este estudo constará de aplicação de questionário com questões relacionadas à saúde bucal e uso de serviços e exame clínico, onde além da avaliação das restaurações serão avaliadas outras condições bucais. As entrevistas dos participantes e exame clínico de saúde bucal serão realizadas nas casas dos indivíduos. Uma secretária agendará o dia de visita da equipe à residência. A coleta de dados será realizada por equipes compostas por examinadores (cirurgiões-dentistas), anotadores e entrevistadores (acadêmicos de Odontologia – UFPel).

Considerações sobre os Termos de apresentação obrigatória:

OK

Recomendações:

OK

Conclusões ou Pendências e Lista de Inadequações:

OK

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

PELOTAS, 04 de Setembro de 2013

Assinador por:
Patricia Abrantes Duval
(Coordenador)