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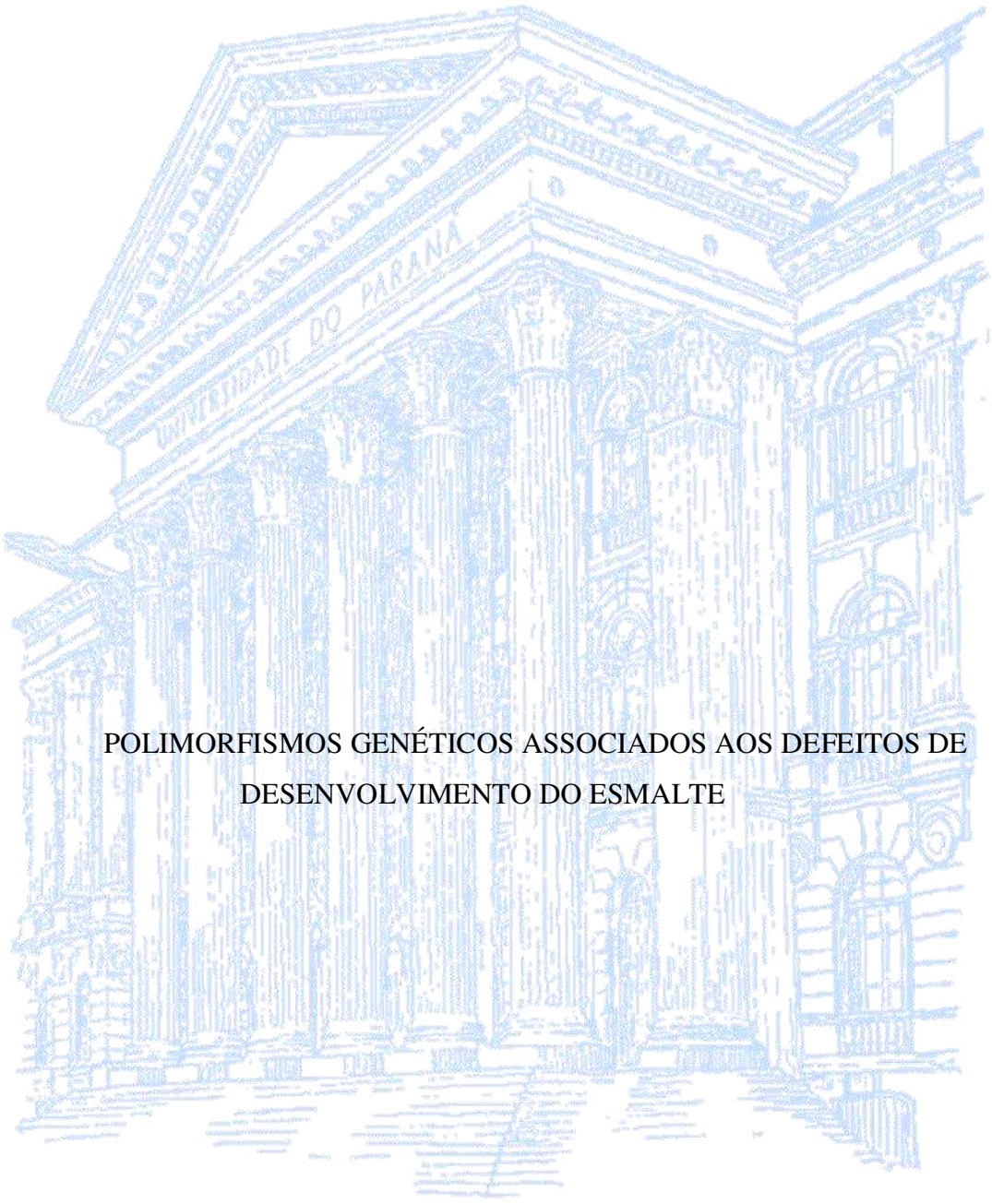
ALUHÊ LOPES FATTURI



POLIMORFISMOS GENÉTICOS ASSOCIADOS AOS DEFEITOS DE DESENVOLVIMENTO DO ESMALTE

CURITIBA

2021

ALUHÊ LOPES FATTURI

POLIMORFISMOS GENÉTICOS ASSOCIADOS AOS DEFEITOS DE DESENVOLVIMENTO DO ESMALTE

Tese apresentada ao Programa de Pós-graduação em Odontologia, área de concentração em Odontopediatria, nível Doutorado, Setor de Ciências da Saúde, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutora em Odontologia.

Orientadora: Profa. Dra. Juliana Feltrin de Souza Caparroz

CURITIBA
2021

Fatturi, Aluhê Lopes
Polimorfismos genéticos associados aos defeitos de desenvolvimento do esmalte [recurso eletrônico] / Aluhê Lopes Fatturi – Curitiba, 2021.

Tese (doutorado) – Programa de Pós-Graduação em Odontologia. Setor de Ciências da Saúde, Universidade Federal do Paraná, 2021.

Orientadora: Profa. Dra. Juliana Feltrin de Souza Caparroz

1. Esmalte dentário. 2. Fluorose dentária. 3. Hipoplasia do esmalte dentário. 4. Desmineralização do dente. 5. Polimorfismo genético. I. Caparroz, Juliana Feltrin de Souza. II. Universidade Federal do Paraná. III. Título.

CDD 617.634

Maria da Conceição Kury da Silva CRB 9/1275

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS DA SAÚDE
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO ODONTOLOGIA - 40001016065P8

ATA Nº122

ATA DE SESSÃO PÚBLICA DE DEFESA DE DOUTORADO PARA A OBTENÇÃO DO GRAU DE DOUTOR EM ODONTOLOGIA

No dia dezesseis de julho de dois mil e vinte e um às 09 horas, na sala PLATAFORMA TEAMS, Campus Botanico, foram instaladas as atividades pertinentes ao rito de defesa de tese da doutoranda **ALUHÊ LOPES FATTURI**, intitulada**: POLIFORMISMOS GENETICOS ASSOCIADOS AOS DEFEITOS DE DESENVOLVIMENTO DO ESMALTE**, sob orientação da Profa. Dra. JULIANA FELTRIN DE SOUZA. A Banca Examinadora, designada pelo Colegiado do Programa de Pós-Graduação em ODONTOLOGIA da Universidade Federal do Paraná, foi constituída pelos seguintes Membros: JULIANA FELTRIN DE SOUZA (UNIVERSIDADE FEDERAL DO PARANÁ), LETICIA MAIRA WAMBIER (UNIVERSIDADE POSITIVO), JULIANA LUCENA SCHUSSEL (UNIVERSIDADE FEDERAL DO PARANÁ), RICARDO DELLA COLETTA (UNIVERSIDADE ESTADUAL DE CAMPINAS/PIRACICABA), YASMINE MENDES PUPO (UNIVERSIDADE FEDERAL DO PARANÁ). A presidência iniciou os ritos definidos pelo Colegiado do Programa e, após exarados os pareceres dos membros do comitê examinador e da respectiva contra argumentação, ocorreu a leitura do parecer final da banca examinadora, que decidiu pela APROVAÇÃO. Este resultado deverá ser homologado pelo Colegiado do programa, mediante o atendimento de todas as indicações e correções solicitadas pela banca dentro dos prazos regimentais definidos pelo programa. A outorga de título de doutor está condicionada ao atendimento de todos os requisitos e prazos determinados no regimento do Programa de Pós-Graduação. Nada mais havendo a tratar a presidência deu por encerrada a sessão, da qual eu, JULIANA FELTRIN DE SOUZA, lavrei a presente ata, que vai assinada por mim e pelos demais membros da Comissão Examinadora.

CURITIBA, 16 de Julho de 2021.

Assinatura Eletrônica
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JULIANA FELTRIN DE SOUZA
Presidente da Banca Examinadora

Assinatura Eletrônica
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LETICIA MAIRA WAMBIER
Avaliador Externo (UNIVERSIDADE POSITIVO)

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Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

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RICARDO DELLA COLETTA
Avaliador Externo (UNIVERSIDADE ESTADUAL DE CAMPINAS/PIRACICABA)

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YASMINE MENDES PUPO
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS DA SAÚDE
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PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO ODONTOLOGIA - 40001016065P8

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ODONTOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **ALUHÊ LOPES FATTURI** intitulada: **POLIFORMISMOS GENETICOS ASSOCIADOS AOS DEFEITOS DE DESENVOLVIMENTO DO ESMALTE**, sob orientação da Profa. Dra. JULIANA FELTRIN DE SOUZA, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 16 de Julho de 2021.

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DEDICATÓRIA

Dedico minha tese de doutorado a toda a minha família, que sempre me deu suporte e incentivo para que eu alcançasse meus sonhos.

AGRADECIMENTOS

Agradeço ao Divino por me permitir vir a esse mundo e ter essa experiência de vida com tantas conquistas e alegrias. Agradeço a minha mãe Arlete Lopes por tanto amor a mim dedicado, e por ser meu exemplo e inspiração, ao meu pai Arani Fatturi por me dar a vida, a minha irmã Aluhine Lopes Fatturi pelo companheirismo, ao meu Amor Cesar de Ramos Junior por toda compreensão e incentivo. Aos meus avós Nelson Fatturi (in memorian) e Nayr Weissemer pela educação que sempre prezaram, e Abílio Lopes e Evanir Lopes por sempre estarem prontos para dar um cafuné e um carinho. A toda minha família por sempre estar ao meu lado torcendo pelo meu crescimento. As minhas amigas, Thalita Casarotti, Michelle Meger e Mariana Rinaldi por tornarem os dias mais leves e por compartilharem comigo momentos alegres e difíceis. Aos meus colegas de trabalho, alunos e pacientes pela compreensão da minha agendada apertada nesse momento. Aos colegas de turma com quem eu pude compartilhar momentos de aprendizado. A Universidade Federal e ao Programa de Pós Graduação em odontologia da UFPR pela oportunidade de realizar meu estudo nessa instituição. Aos Professores por todo o ensinamento, exemplo e dedicação, vocês fizeram toda a diferença nessa trajetória. A professora Juliana Feltrin, minha orientadora, por quem eu tenho admiração muito grande, obrigada por sempre acreditar que é possível e me incentivar a querer mais.

EPÍGRAFE

Viva como se fosse morrer amanhã. Aprenda como se fosse viver para sempre.

Gandhi

RESUMO

Os defeitos de desenvolvimento de esmalte (DDE) são resultados de alterações durante a amelogênese. Clinicamente, esses defeitos são classificados em opacidade demarcada, opacidade difusa e hipoplasia. Dentre os DDE, os mais prevalentes são a fluorose dentária (FD), hipomineralização de molares e incisivos (HMI) e hipomineralização de segundos molares decíduos (HSMD). A FD é caracterizada por opacidades difusas, as quais podem se apresentar em linhas isoladas ou confluentes. A HMI é um defeito do esmalte específico, que se caracteriza clinicamente por opacidades demarcadas de coloração variada, nos primeiros molares permanentes e frequentemente em incisivos permanentes, quando essa hipomineralização está presente em segundos molares decíduos, esse defeito é denominado HSMD. Estudos anteriores consideravam os fatores sistêmicos como fatores de risco para defeito de desenvolvimento do esmalte (DDE), mas não explicavam totalmente sua etiologia. Considerando que a amelogênese está sob influência de fatores genéticos, é possível que a variação genética possa contribuir para o aumento do risco dos DDE, assim os objetivos desse estudo foram: (1) avaliar em uma amostra de base populacional relação entre HSMD com polimorfismos genéticos no gene *HIF-1* (regulador de hipóxia); (2) por meio de estudo de revisão sistemática, avaliar os polimorfismos genéticos associados aos DDEs como FD, HMI e HSMD. (1) Para investigar a relação entre polimorfismos no gene *HIF-1* e fatores ambientais, relacionados à hipóxia, com HSMD foi realizado um estudo observacional transversal com 731 escolares de 8 anos selecionados aleatoriamente em Curitiba-PR. A prevalência de HSMD nesta população foi de 9,4%, representando 69 casos (HSMD) e 662 sem HSMD. Os fatores ambientais foram coletados por meio de questionário e o HSMD foi diagnosticado por examinadores calibrados utilizando o critério DDE index modificado da FDI. Dois polimorfismos genéticos (*rs2301113* e *rs2057482*) no gene *HIF-1* foram genotipados por reação em cadeia da polimerase em tempo real. As associações foram testadas por análise de regressão de Poisson (Razão de Prevalência ajustada - RP_a; P <0,05). No modelo múltiplo, incluindo os polimorfismos genéticos e fatores ambientais, o uso materno de droga (RP_a = 4,52; P <0,001; IC 95% = 2,38-8,53), presença de doenças maternas durante a gravidez (RP_a = 1,97; P = 0,034; IC 95% = 1,05 a 3,71), e doenças respiratórias durante a infância (RP_a = 2,66; P = 0,003; IC 95% = 1,41 a 5,03) aumentaram significativamente a prevalência de HSMD. Na presença de fatores ambientais, indivíduos portadores de pelo menos um alelo C em *rs2057482* apresentaram menor prevalência de HSMD (RP_a = 0,51; P = 0,048; IC 95% = 0,27 a 0,99). (2) Um estudo de revisão sistemática foi realizado afim de avaliar os polimorfismos genéticos associados aos DDE, sob registro PROSPERO (CRD42018115270). As buscas foram realizadas nas bases de dados PubMed, Scopus, Web of Science, LILACS, BBO e Biblioteca Cochrane, bem como na literatura cinzenta. Os estudos incluídos foram observacionais, transversais, caso-controle e coorte que avaliaram a associação entre DDE e polimorfismos genéticos. O risco de viés dos estudos foi avaliado por meio do critério de Newcastle-Ottawa. A busca inicial resultou em 1.076 estudos, desses foram incluídos 22 estudos nessa revisão. O risco de viés dos estudos variou de 4 a 8 pontos. A idade dos participantes incluídos variou de 1 a 38 anos e o número de participantes incluídos variou de 52 a 1065. Foram investigados 23 genes cujas proteínas estão relacionadas ao desenvolvimento do esmalte (*AMBN, AMELX, AMTN, ENAM, FAM83H, MMP, TFIP, TUFT*), crescimento e morfogênese craniofacial (*DLX, IRF, BGLAP, BMP, COL, CTR, FGFR, OPG, RANK*), resposta imune (*IL, MBL, DEFB*), e transcrição/recepção hormonal (*ESR, GHR, PTH*). Observou-se que a HMI e/ou HSMD esteve associada com 18 polimorfismos de genes responsáveis pelo desenvolvimento do esmalte, resposta imune e crescimento e morfogênese. Encontrou-se associação

significante entre as diversas aparências clínicas da fluorose dentária com 7 polimorfismos de genes responsáveis pelo desenvolvimento do esmalte, desenvolvimento craniofacial e transcrição/ recepção hormonal. Conclui-se (1) com base no estudo observacional, que crianças com fatores sistêmicos relacionados à hipóxia apresentaram maior prevalência de HSMD. Polimorfismo no gene no gene *HIF-1* foi significantemente associado a prevalência de HSMD, sendo os indivíduos com alelo C em *rs2057482* tiveram menor prevalência de HSMD em condições de hipóxia. (2) Os estudos incluídos na revisão sistemática observaram que os DDE, como HMI, HSMD e FD, reportados como etiologia complexa, também estão significativamente associados a polimorfismos genéticos. Estudos prospectivos e em animais são necessários para evidenciar de forma aprofundada o papel dos fatores sistêmicos/ambientais e genéticos na etiologia complexa dos DDEs.

Palavras-chaves: esmalte dentário; fluorose dentária; hipoplasia do esmalte dentário; desmineralização do dente; polimorfismo genético.

ABSTRACT

Developmental defect of enamel (DDE) are the result of changes during amelogenesis. Clinically, these defects are classified as: demarcated opacity, diffuse opacity and hypoplasia. Among the DDE, the most prevalent are dental fluorosis (DF), molar-incisor hypomineralization (MIH) and hypomineralization of primary second molar (HPSM). The DF is characterized by diffuse opacities, which can appear in isolated or confluent lines. MIH is a specific defect of enamel, which is clinically characterized by demarcated opacities of varying color, in the first permanent molars and often in permanent incisors, when this hypomineralization is present in primary second molars, this defect is called hypomineralization of primary second molars (HPSM). Previous studies considered systemic factors as risk factors for developmental defect of enamel (DDE), but did not fully explain their etiology. Considering that amelogenesis is under the influence of genetic factors, it is possible that genetic variation may contribute to the increased risk of DDE, thus the objectives of this study were: (1) to evaluate in a population-based sample the relationship between HPSM with genetic polymorphisms in the HIF-1 gene (hypoxia regulator), (2) systematically evaluate the genetic polymorphisms associated with DDE such as DF, MIH and HPSM. (1) To investigate whether polymorphisms in the HIF-1 gene and environmental factors, related to hypoxia, were associated with HPSM, an observational cross-sectional study was carried out with 731 schoolchildren aged 8 years randomly selected in Curitiba-PR. The prevalence of HPSM in this population was 9.4%, representing 69 cases (HPSM) and 662 controls. The environmental factors were collected through a questionnaire and calibrated examiners, using the modified DDE index by FDI, diagnosed the HPSM. Two genetic polymorphisms (rs2301113 and rs2057482) in the HIF-1 gene were genotyped by polymerase chain reaction in real time. Associations were tested by Poisson regression analysis (Prevalence Ratio adjusted - PRa; $P < 0.05$). In the multiple model, including genetic polymorphisms and environmental factors, maternal use of illicit drugs (PRa = 4.52; $P < 0.001$; 95% CI = 2.38-8.53), maternal diseases during pregnancy (PRa = 1.97; P = 0.034; 95% CI = 1.05 to 3.71), and respiratory diseases during childhood (PRa = 2.66; P = 0.003; 95% CI = 1.41 to 5.03) significantly increased the prevalence of HPSM. In the presence of environmental factors, individuals with at least one C allele in rs2057482 had a lower prevalence of HPSM (PRa = 0.51; P = 0.048; 95% CI = 0.27 to 0.99). (2) In order to conduct a systematic review to assess the available literature on genetic polymorphisms associated with DDE, it was first registered in the PROSPERO database (CRD42018115270). The searches were performed in the PubMed, Scopus, Web of Science, LILACS, BBO and Cochrane Library databases, as well as in the gray literature. The included studies were observational, cross-sectional, case-control and cohort that evaluated the association between DDE and genetic polymorphism. The risk of bias in the studies was assessed using the Newcastle-Ottawa criteria. The searches led to the retrieval of 1076 studies, of these, 22 studies were included in this review. The risk of bias in the studies ranged from 4 to 8 points. The age of participants included in the studies ranged from 1 to 38 years and the number of participants included in the studies varied from 52 to 1065. It was investigated 23 genes whose proteins are related to enamel development (*AMBN, AMELX, AMTN, ENAM, FAM83H, MMP, TFIP, TUFT*), craniofacial patterning and morphogenesis (*DLX, IRF, BGLAP, BMP, COL, CTR, FGFR, OPG, RANK*), immune response (*IL, MBL, DEFB*), and hormone transcription/receptor (*ESR, GHR, PTH*). It was observed that MIH and / or HSPM was associated with 18 polymorphisms of genes responsible for enamel development, immune response and growth and morphogenesis craniofacial. A significant association was found between the different clinical appearances of dental fluorosis with 7 polymorphisms of genes

responsible for enamel development, craniofacial development and hormonal transcription / reception. It is concluded that (1) based on the observational study, that children who had hypoxia-related factors presented with a higher prevalence of HSPM, and individuals with C allele in rs2057482 had a lower prevalence of HSPM in hypoxia conditions. (2) The studies included in the systematic review found that DDE, such as MIH, HSPM and DF, reported as a complex etiology, are also significantly associated with genetic polymorphisms. Prospective and animal studies are necessary to demonstrate in depth the role of systemic / environmental and genetic factors in the complex etiology of DDEs.

Keywords: dental enamel; fluorosis dental; dental enamel hypoplasia; tooth demineralization; polymorphism, genetic

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LISTA DE SIGLAS

AMBN: Ameloblastin

AMELX: Amelogenin, X-linked

AMTN: Amelotin

BBO: Biblioteca Brasileira de Odontologia

BGLAP: Bone gamma-carboxyglutamate protein

BMP2: Bone morphogenetic protein 2

BMP7: Bone morphogenetic protein 7

BMP4: Bone morphogenetic protein 4

CDH5: Cadherin 5

COL1A2: Collagen type I alpha 2 chain

COL14A1: Collagen type XIV alpha 1 chain

CTR: Calcitonin receptor

DDE: Defeito de desenvolvimento de esmalte

DEFB1: Defensin beta 1

DF: Dental Fluorosis

DLX1: Distal-less homeobox 1

DLX2: Distal-less homeobox 2

DLX3: Distal-less homeobox 3

DNA: Ácido desoxirribonucleico

EAPD: Academia Europeia de Odontologia Pediátrica

ENAM: Enamelin

ESR1: Estrogen receptor 1

ESR2: Estrogen receptor 2

ESRB: Estrogen related receptor beta

FAM83H: Family with sequence similarity 83 member H

FD: Fluorose Dental

FGFR1: Fibroblast growth factor receptor 1

GHR: Growth hormone receptor

HIF: Hypoxia-inducible factor

HMI: Hipomineralização de molares e incisivos

HSMD: Hipomineralização de Segundos Molares decíduos

IADR: International Association for Dental Research

IL1A: Interleukin1 alpha

IL1B: Interleukin1 beta

IL4: Interleukin 4

IL6: Interleukin 6

IL8: Interleukin 8

IL10: Interleukin 10

IL17A: Interleukin 17A

IRF6 : Interferon regulatory factor 6

KCNG1: Potassium voltage-gated channel modifier subfamily G member 1

LTF: Lactotransferrin

MASP2: MBL associated serine protease 2

MBL2: Mannose binding lectin 2

MIH: Molar incisor hypomineralization

MBL2: Mannose binding lectin 2

MIR17HG: miR-17-92a-1 cluster host gene

MMP2: Matrix metallopeptidase 2

MMP9: Matrix metallopeptidase 9

MMP13: Matrix metallopeptidase 13

MMP20: Matrix metallopeptidase 20

HPSM: Hypomineralization of primary second molar

LILACS: Literatura Latino Americana e do Caribe em ciências da Saúde

OPG: Osteoprotegerin

PCR: Polymerase chain reaction

PPARGC1A: PPARG coactivator 1 alpha

PR: Paraná

PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-analyses

PROSPERO – International Prospective Register of Systematic Reviews

PTH: Parathyroid hormone

RANK: Receptor activator of nuclear factor kappa-B

RANK L: Receptor activator of nuclear factor kappa-B ligand

RMI1: RecQ mediated genome instability 1

SIGLE: System for Information on Grey Literature in Europe

STREGA: Strengthening the Reporting of Genetic Association Studies

SPARC: Secreted protein acidic and cysteine rich

STAT: Signal transducer and activator of transcription 1

TCLE: Termo de Consentimento Livre e Esclarecido

TFIP11: Tuftelin interacting protein 11

TGFA: Transforming growth factor alpha

TGFB1: Transforming growth factor beta 1

TGFBR1: Transforming growth factor beta receptor 1

TIMP1: TIMP metallopeptidase inhibitor 1

TIMP2: TIMP metallopeptidase inhibitor 2

TNF: Tumor necrosis factor

TTLL12: Tubulin tyrosine ligase like 12

TUFT1: Tuftelin 1

UFPR – Universidade Federal do Paraná

VDR: Vitamin D receptor

SUMÁRIO

1.INTRODUÇÃO

A amelogênese é um processo complexo de interação ectomesenquimal, o qual é regulado por diversos genes e fatores de crescimento, sendo dividida em dois estágios principais: secreção e mineralização/maturação (Thesleff, 2000, 2006). No estágio secretor, os ameloblastos têm a função de secretar as proteínas da matriz do esmalte, como a enamelina, ameloblastina e amelogenina, que se depositam de forma progressiva e estruturada em prismas, principalmente, de hidroxiapatita, dando origem a uma matriz proteica parcialmente mineralizada do esmalte (Cate, 1998). Após o período secretor ocorre uma alteração geneticamente programada e inicia-se a secreção de proteinases, incluindo as Metaloproteinases (MMP20, MMP45, MMP41) e as Calicreínas (KLK4, KLK34 e KLK31), enzimas responsáveis pela degradação e remoção de proteínas da matriz do esmalte, cessando então, o crescimento dos cristais do esmalte em comprimento e favorecendo seu crescimento em espessura, dando início ao período de mineralização da amelogênese.

Enquanto a matriz orgânica é degradada ocorre a deposição de minerais, consolidando a mineralização do esmalte, resultando em um tecido mineralizado com 95% de seu conteúdo composto por material inorgânico (Cate, 1998; Lacruz et al. , 2013).

Distúrbios durante o processo de amelogênese podem alterar a espessura ou a mineralização / maturação do esmalte, podendo levar a defeitos qualitativos ou quantitativos de desenvolvimento do esmalte dentário (DDE) (Seow, 1997), dependendo do estágio da formação do esmalte em que a alteração ocorre, as diferentes variações clínicas de DDE se manifestam.

Distúrbios ocorridos durante o estágio da secreção da matriz do esmalte podem ocasionar alteração na sua espessura, conhecida como hipoplasia do esmalte e que, clinicamente, caracteriza-se pela redução na espessura do esmalte em formato de lesões hipoplásicas como fossetas, sulcos, linhas ou até grandes

regiões de ausência do esmalte (Suckling, 1989; Seow, 1997). Por sua vez, distúrbios ocorridos durante o estágio de mineralização/maturação do esmalte podem ocasionar alteração na translucidez do mesmo (hipomineralização), que se apresentam clinicamente como opacidades (Suckling, 1989; Seow, 1997)

As hipomineralizações podem ser demarcadas ou difusas (Suckling, 1989; Seow, 1997). As opacidade difusas, não apresentam um limite demarcado entre o esmalte normal e o esmalte afetado, e podem se apresentar em linhas, isoladas ou confluentes, sendo manchas brancas ou amarelo a marrom, podendo ter uma porosidade pronunciada do esmalte, a opacidade difusa é denominada fluorose dentária (FD) (Figura 1) (Suckling, 1989; Seow, 1997).

A etiologia da FD está associada a ingestão excessiva e crônica de flúor durante o período de amelogênese (Huang et al., 2008).

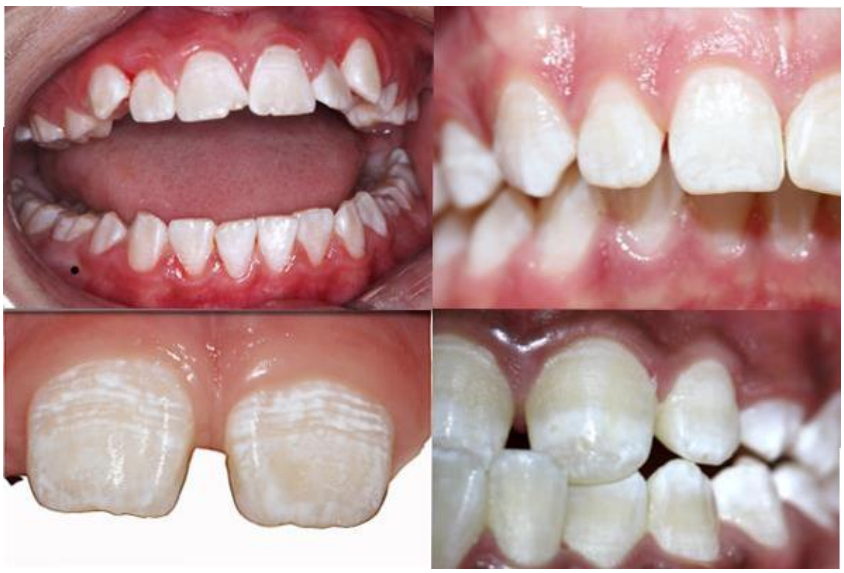

Figura 1: Características clínicas da FD (Autoria: Equipe odontopediatria (UFPR)

As opacidades demarcadas se apresentam clinicamente com limites bem definidos entre o esmalte normal e o esmalte hipomineralizado. A coloração pode variar do branco, amarelo ao

marrom (Suckling, 1989; FDI, 1992; Seow, 1997). Opacidades demarcadas em primeiros molares permanentes e em incisivos foram definidas em 2001 pela Academia Europeia de Odontopediatria como hipomineralização de molares e incisivos (HMI) (Figura 2) como um tipo específico de DDE, que se apresenta clinicamente como opacidades demarcadas envolvendo de um a quatro primeiros molares permanentes, podendo afetar também os incisivos permanentes (Weerheijm, Jalevik, & Alaluusua, 2001).

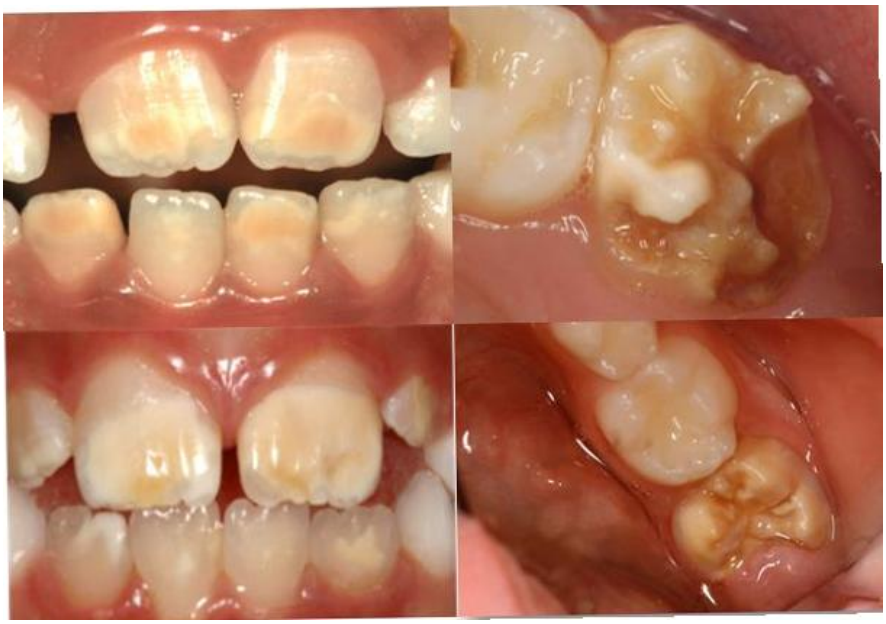

Figura 2: Características clínicas da HMI (Autoria: Equipe odontopediatria UFPR)

Defeito semelhante à HMI tem sido observado em segundos molares decíduos, por isso chamado de hipomineralização de segundos molares decíduos (HSMD) (Figura 3) e foi descrito, em 2008, por Elfrink e colaboradores. Os autores classificaram a HSMD como hipomineralização demarcada que afeta de um a quatro segundos molares decíduos (Elfrink et al., 2008). Sugere-se que essa semelhança entre os defeitos ocorre devido à coincidência cronológica de desenvolvimento dos primeiros molares permanentes e segundos molares decíduos. Assim, HMI e HSMD poderiam compartilhar os mesmos fatores etiológicos, principalmente, na vida pré-natal até o primeiro ano de idade (Ghanim et al., 2012; Ghanim,

et al., 2013; Elfrink et al., 2014; Elfrink, Ghanim etal., 2015).

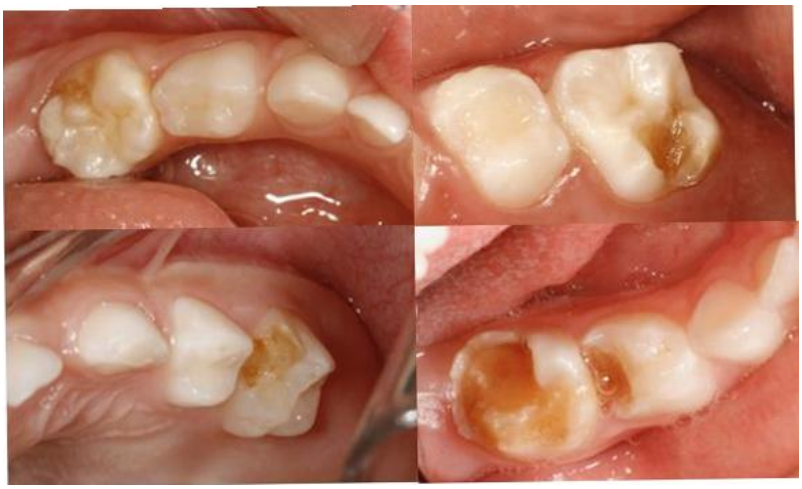

Figura 3: Características clínicas da HSMD (Autoria: Equipe odontopediatria UFPR)

A etiologia sistêmica de HMI e HSMD já foi pesquisada em diferentes populações e ainda não está totalmente esclarecida (Alaluusua, 2010; Ghanim et al., 2012 Elfrink et al., 2015; Fatturi et al., 2019). Alguns achados indicaram uma associação entre esses defeitos de esmalte e situações de hipóxia, parto prematuro e / ou complicações no nascimento (Lygidakis, Dimou, & Marinou, 2008; Garot, Manton, & Rouas, 2016; Fatturi et al., 2019), bem como potencial associação entre HMI e doenças infantis (doenças respiratórias e icterícia), febre e uso de medicamentos (Alaluusua, 2010; Silva et al., 2016; Fatturi et al., 2019). Apesar das revisões sistemáticas observarem o envolvimento de fatores sistêmicos ou ambientais na etiologia da HMI, o papel de cada um desses fatores na amelogênese não é totalmente compreendido (Alaluusua, 2010; Silva et al., 2016; Fatturi et al., 2019)

Por outro lado, soma-se aos fatores sistêmicos e ambientais anteriormente descritos, o componente genético, uma vez que a amelogênese está sob a influência de uma série de genes (Thesleff, 2006). Sendo assim, é razoável supor que alterações em genes que codificam as proteínas envolvidas na formação do esmalte dentário podem contribuir para o aumento do risco de defeitos de

desenvolvimento do esmalte (Lacruz etal., 2013, Jeremias et al., 2016).

Estudos anteriores já demonstraram essa possível associação entre polimorfismos genéticos relacionados aos defeitos de esmalte (Gerreth etal., 2018; Arid et al., 2019; Fatturi et al., 2021;), como HMI (Jeremias et al., 2013; Kuhnisch et al., 2014; Jeremias et al., 2016; Bussaneli et al., 2019; Fatturi et al., 2020; Hocevar et al., 2020; Pang et al., 2020; Bezamat et al., 2021) e fluorose dentária (Huang et al., 2008; Ba et al., 2011; Wen et al., 2012; Jiang et al. , 2015; Kuchler et al., 2017; Kuchler et al., 2018; Romualdo et al., 2019; Dalledone et al., 2019; Abbasoglu et al., 2020; Saha et al., 2021). Embora esse processo seja geneticamente controlado, fatores ambientais podem afetar a amelogênese, como alteração do pH, suporte sanguíneo, febre, ingestão de alta concentração de fluoreto, medicamentos antimicrobianos e dioxinas (Alaluusua, 2010; Sonmez, Yildirim, & Bezgin, 2013). Assim, atualmente, aceita-se a teoria de uma origem multifatorial na HMI, em que os eventos sistêmicos, ambientais e genéticos atuam em sinergismo durante o desenvolvimento dentário.

Estudos epidemiológicos também observaram que fatores ambientais relacionados à hipóxia, como parto prolongado, uso materno de nicotina e drogas ilícitas durante a gravidez, problemas respiratórios na primeira infância foram associados a maior prevalência de opacidades demarcadas na dentição permanente e decídua (Alaluusua, 2010; Sidaly et al., 2015). Um estudo in situ observou que a hipóxia alterou a amelogênese, o que poderia resultar clinicamente em um defeito do esmalte (Sidaly et al., 2015), em estudo in vitro, observou-se que condições de hipóxia em ameloblastos promoveram aumento da expressão do fator induzível por hipóxia (*HIF*) (Tung, Fujita, Yamashita, & Takagi, 2006).

O *HIF*-1 é um fator de transcrição que regula a homeostase do oxigênio. É composto por duas subunidades (*HIF-1α e HIF-1β*)

que coordenam a resposta à hipóxia em tecidos normais e tumores, permitindo a adaptação e a sobrevivência celular em um ambiente hostil (Guo et al., 2015). O gene *HIF-1α* codifica o fator 1 induzível por hipóxia subunidade alfa, que desempenha um papel fundamental na homeostase do oxigênio, ativando a transcrição de aproximadamente 100 genes, que estão envolvidos no metabolismo energético, angiogênese e apoptose, cujos produtos proteicos aumentam a liberação de oxigênio ou facilitam a adaptação metabólica à hipóxia (Hu, Fang, & Zheng, 2014). No estresse oxidativo, as células respondem por meio do *HIF-1*, mediando os mecanismos de reparo e adaptação (Hlatky et al., 2007; Sidaly et al., 2015). Como o *HIF-1* é o principal componente que detecta a hipóxia em organismos, é possível que os polimorfismos do *HIF-1* influenciem na adaptação às situações de hipóxia (Zhang, Song, Li, & Tian, 2009) durante o desenvolvimento do esmalte.

2. OBJETIVO

Avaliar os polimorfismos genéticos que podem estar associados aos Defeitos de Desenvolvimento de Esmalte (DDE)

2.1. OBJETIVOS ESPECÍFICOS

1. Avaliar por meio de um estudo observacional transversal se polimorfismos genéticos no *HIF1α* e fatores ambientais relacionados à hipóxia estão associados à prevalência de HSMD.
2. Realizar uma revisão sistemática para verificar os polimorfismos genéticos associados aos DDE como FD, HMI e HMSD.

3. ARTIGO 1

Interaction between environmental factors and polymorphisms in a hypoxia-related gene (*HIF-1*) associated with hypomineralized primary second molars

Abstract

Purpose: This study's purpose was to investigate whether polymorphisms in the HIF-1 encoding gene and hypoxia-related environmental factors were associated with hypomineralized second primary molars (HPSMs). Methods: From a total of 731 children from Curitiba, Paraná, Brazil, were selected, the prevalence of HSPMs in this population was 9.4%, representing 69 cases (HPSMs) and 662 controls. The environmental factors were collected via questionnaire. HSPMs were evaluated by calibrated examiners. Two genetic polymorphisms (*rs2301113* and *rs2057482*) in the *HIF-1* gene were genotyped by polymerase chain reaction in real time. Associations were tested by Poisson regression analysis (PR_a; $P<0.05$). Results: In the multiple variable model, including the environmental factors and genetic polymorphisms, maternal use of an illicit drug ($PR_a = 4.52$; $P<0.001$; 95% CI =2.38-8.53), maternal diseases during pregnancy ($PR_a =1.97$; $P=0.034$; 95% CI = 1.05 to 3.71), and respiratory diseases during childhood ($PR_a = 2.66$; $P=0.003$; 95% CI = 1.41 to 5.03) increased significantly the prevalence of HPSMs. In the presence of environmental factors, individuals carrying at least one C allele in *rs2057482* had a lower prevalence of HPSMs ($PR_a = 0.51$; $P=0.048$; 95% CI = 0.27 to 0.99). Conclusions: Children who had hypoxia-related factors presented with a higher prevalence of hypomineralized second primary molars. A C allele in *rs2057482* served as protection against HPSMs in hypoxia conditions.

Keywords: dental enamel; enamel hypoplasia; hypomineralized second primary molars; deciduous teeth; genetic polymorphism; hif-1 gene

Introduction

Hypomineralized primary second molars (HPSMs) are clinically represented by demarcated opacities in enamel involving one to four primary second molars.[1] The presence of HPSMs is associated with a higher prevalence of molar-incisor hypomineralization (MIH).[2] It is suggested that this association occurs due to the chronological coincidence between dental development of permanent first molars and primary second molars. Thus, MIH and HPSMs could share the same etiological factors, especially in prenatal life until the age of one year.[3] Recent studies show that these demarcated opacities have a multifactorial and complex origin in which systemic or environmental factors act in synergism with the individual genetic aspects.[4,5]

Amelogenesis is a complex process of ectomesenchyme interactions. The ameloblasts perform the synthesis function, enamel protein matrix secretion, mineralization, and maturation of the enamel matrix.[6] Although this process is genetically controlled, environmental factors could affect the amelogenesis, such as pH alteration, blood support, fever, intake of high fluoride concentration, antimicrobial medications, and dioxins.[7-11]

An in situ and in vitro studies observed that hypoxia altered the amelogenesis, which could clinically result in an enamel defect.[7,12] Epidemiological studies also observed that environmental factors related to hypoxia, such as prolonged birth delivery, maternal use of nicotine and illicit drugs during pregnancy, and respiratory problems in early childhood, were associated with a higher prevalence of demarcated opacities in both the permanent and primary dentition.[7,13-15] Sidaly et al.,[15] in a case-control study, analyzed if a lower Apgar score could be associated with a higher frequency of MIH. The authors did not find a significant association between children who had an Apgar score greater than five at five minutes and MIH. They suggested that ameloblasts could be affected by hypoxia; however, in mild hypoxia, compensatory mechanisms were observed, including increased expression of HIF-1α, which is positively regulated in ameloblasts after hypoxia.[16]

Hypoxia-inducible factor (*HIF-1*) is a transcriptional factor regulating oxygen homeostasis. It consists of two subunits (*HIF-1α and HIF-1β*) that coordinate

the response to hypoxia in normal tissues and tumors, allowing adaptation and cell survival in a hostile environment.[17] The *HIF-1 α* gene encodes the hypoxia-inducible factor one alpha subunit, which plays a key role in oxygen homeostasis by activating the transcription of approximately 100 genes involved in energetic metabolism, angiogenesis, and apoptosis, whose protein products increase oxygen release or facilitate metabolic adaptation to hypoxia.[18]

During oxidative stress, cells respond through *HIF-1*, mediating the repair and adaptation mechanisms.[7,19] In an in vitro study, conditions of hypoxia in ameloblasts promoted an increase of *HIF* expression.[7] In addition, alterations in the expression of cytokines, such as IL -1 α, IL-1 β, IL-6, IL-10, IFN-γ, and MCP-1, that could affect the expression of enamel proteins are also observed.[16]

Evidence from twin studies,[20,21] epidemiological studies,[22,23] and genetic studies (phenotype-genotype design)[24-26] suggests that HPSM/MIH has a multifactorial etiology in which, environmental factors and genetic factors play a role in the etiology of HPSMs. In fact, some phenotype-genotype studies have already demonstrated that genetic polymorphisms in many genes with a small effect are involved in HPSMs/MIH phenotype.[24,25,27]

The actual evidence supports the hypothesis that genetic polymorphisms in a hypoxia-related gene are candidates for HPSM.[7,15,16]. *HIF-1* is the main component that detects hypoxia in organisms; it is possible that *HIF-1* polymorphisms influence adaptation to hypoxic events[28] during enamel development. The present study's authors hypothesize that interactions between hypoxia-related environmental factors and genetic polymorphisms in the *HIF-1α* are involved in the etiology of HPSM.

Therefore, the purpose of this cross-sectional study was to analyze whether genetic polymorphisms in the *HIF-1 α* and hypoxia-related environmental factors were associated with the prevalence of HPSMs.

Methods

Ethical approval. This cross-sectional study was approved by the Health Sciences Research Ethics Committee of the Federal University of Parana (UFPR), Curitiba, Paraná, Brazil, and the Municipal Education Secretary of Curitiba, Paraná,

Brazil. The protocol was approved by the human research committee of UFPR before the research had begun. Previous to the study, children and their caregivers agreed to participate in the study and signed the informed consent form. This article was reported according to strengthening the Reporting of Genetic Association Studies (STREGA).[29]

Recruitment and eligibility criteria. Eight-year-old school children were included in the study. Children who had orthodontic appliances that would impair clinical examination, with the absence of one to four primary second molars, and with syndromes or imperfect amelogenesis were excluded.

Sample. For a representative sample of the city, the participants were selected from public schools in the city of Curitiba, with a population of 1,908,359 inhabitants and a Human Development Index of 0.823. The determination of the sample size and the sampling procedures were described by Lopes-Fatturi et al.[3]

Clinical data collection. Clinical data collection was performed in a school environment by four calibrated examiners using artificial light, a dental mirror, dental probe, and sterile gauze. Four examiners were trained and calibrated to diagnose HPSMs using a modified developmental defects of enamel (DDE) index. The examiner's calibration was described by Lopes-Fatturi et al.[3] Data collection was performed from November 2016 to September 2017. The dependent variable was HPSMs, the case was defined as children with at least one primary second molar affected by HPSMs, and the control was defined as children without HPSMs on the clinical exam.

Evaluation of environmental factors. Environmental factors were collected through a structured questionnaire completed by the children's caregivers.[3] The questionnaire was prepared using previous studies in literature and contained questions regarding medical history during pregnancy, the perinatal period, and the postnatal period, up to the age of three years.[30]

For this study, the hypoxia-related environmental factors were selected according to reports in the literature as producing hypoxia situations.[31] Hypoxia-related factors were considered as follows: drug use was considered the maternal use of alcohol, tobacco, and illicit drugs during the pregnancy; maternal problems were

considered disease or health conditions previously related to hypoxia, such as diabetes, hypertension, and prolonged labor during the delivery; and respiratory diseases were considered the presence of bronchitis, asthma, and pneumonia until the first three years of life.

Genetic polymorphisms in *HIF-1α*. For the genetic polymorphism's analysis, 352 children from the included sample were randomly selected in a case-control design as previously described in Fatturi et al.[24] In the previous study, all children were selected for the case group; for the control group, children were randomly selected, matching gender, age, and ethnicity at a ratio of three-to-one (control-to-case; Figure 1).[24]

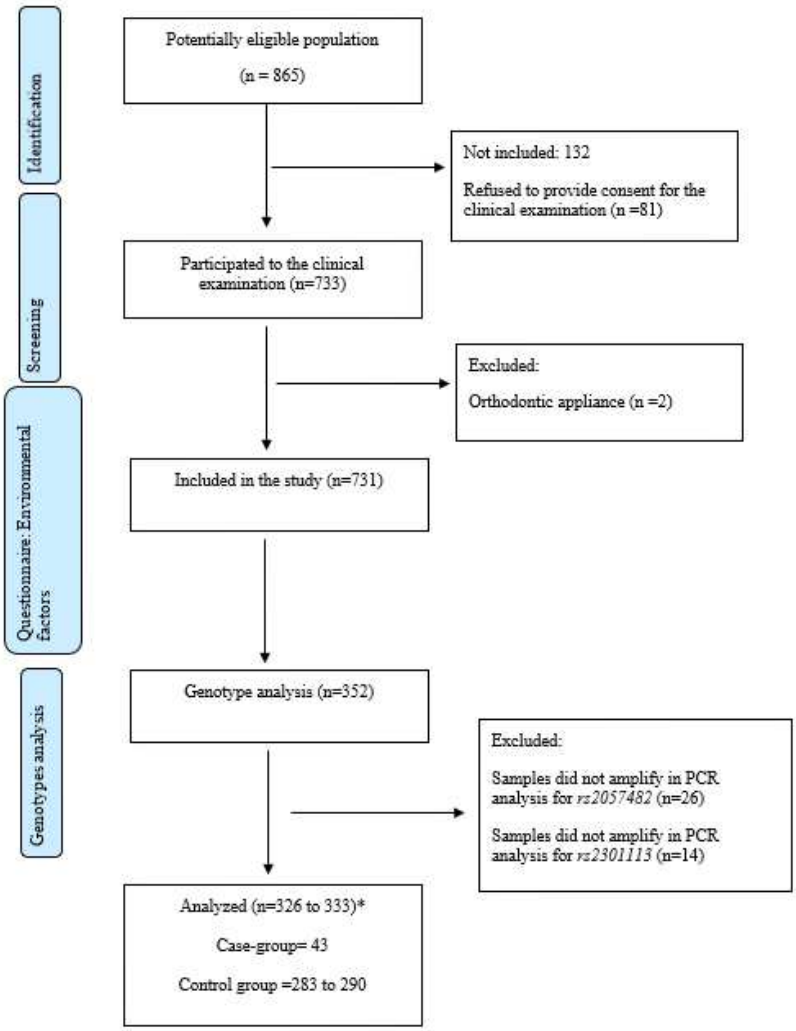

Figure 1 – Flow diagram of study. Case-group included children with at least one second primary molar affected by HSPM. Control group included children without HSPM.

* The ranges is because some of the exclusions were not complete, some samples only did not amplify for *rs2057482* and others only for *rs2301113*.

Therefore, the schoolchildren's DNA was obtained from oral mucosal epithelial cells using a five ml mouthwash of three percent glucose solution, according to a previously published protocol.[32,33] Functional genetic polymorphisms in the HIF-1α were screened according to Cariaso and Lennon[34] based on their lowest allele frequency, which must have been greater than 30%, and linkage disequilibrium. Therefore, two genetic polymorphisms (*rs2301113* and *rs2057482*) that were previously associated with different conditions,[35-37] indicating their possible clinical relevance, were selected. The description of the selected genetic polymorphisms is provided in Table 1.

Table 1. Candidate gene description and its polymorphisms: hardy-weinberg equilibrium and the distribution of observed frequency of the genotype on the study population

Gene	Polymorphism	Locus	Change of base*	Minimum allele frequency	Genotype	N (%)	Hardy-Weinberg equilibrium chi-square
HIF-1	*rs2301113*	61739830	[A/C]	0.47	AA AC CC	157 (46.4) 130 (38.5) 51 (15.1)	1.712
	rs2057482	61747130	[C/T]	0.24	CC CT TT	226 (70.0) 86 (26.6) 11 (3.4)	0.256

*Nucleotide base change, adenine (A) to cytosine (C) in the *rs2301113*; cytosine (C) to thymine (T) in the *rs2057482*

Allelic discrimination analysis using real-time polymerase chain reaction and the Taqman test (StepOnePlus Real-Time PCR System, Thermo Fisher Scientific, Waltham, Mass., USA) were performed for genotyping.

Statistical analysis of data. The HPSM dependent variable was categorized as "case group" and "control group." The presence of HPSMs was calculated according to Ghanim et al.,[1] when at least one of the primary second molars is affected by demarcated hypomineralization. Genotypes were categorized as additive, dominant allele, and recessive allele. The Hardy-Weinberg equilibrium was evaluated by the chi-square test. The association among HPSMs, *HIF-1α* genotypes, and hypoxia-related environmental factors were analyzed by univariable and multiple

Poisson regression analysis, with a robust variance used with its respective prevalence ratio (PR at a significance level of five percent. To perform a Poisson regression analysis for the multiple variables, independent variables with P<0.20 in the association with HPSMs were added to the multiple model. Statistical analysis was performed using the SPSS 16.0 software for Windows (SPSS Inc., Chicago, Ill., USA) and STATA 14.0 software (StataCorp, Texas city, USA).

Results

Among the potentially eligible population of 865 schoolchildren, a total of 784 children and their caregivers agreed to participate in the study (response rate equals 90.6%); 733 children were clinically examined for eligibility and two children were excluded from the study due to the use of an orthodontic appliance), resulting in a final sample of 731 children.3 The prevalence of HPSMs in this population was 9.4% , representing 69 cases (HPSMs) and 662 controls. [3] The present study is a sequential analysis of the same cohort.[3,24] For genotyping analysis, 43 cases and 279 to 287 controls were included (Figure). The genotype distribution was within the Hardy-Weinberg equilibrium (chi-square equals 0.256 and 1.712 for *HIF rs2057482* and *HIF rs2301113*, respectively; Table 1).

Of this sample, 374 children were males (51.16%). Caucasian was the predominant ethnicity (617 children; 84.40%; Table 2).

Table 2. Demographic characteristics of study population (Curitiba, Paraná, Brazil, 2017, N=731)*

Variables	Categories	N (%)
Gender	Male	374 (51.6)
	Female	357 (48.8)
Age	7	267 (36.5)
	8	464 (63.5)
Ethnicity	Caucasian	617 (84.4)
	African descendant	89 (12.2)
	Asiatic	11 (1.5)
	Indian	14 (1.9)

*N=absolute frequency

In a univariable analysis, a significant increase in the occurrence of HPSMs was observed in children whose mothers used drugs (tobacco, alcohol, and illicit drugs) during pregnancy (PR_c = 2.14; P = 0.004) or had some health problem during pregnancy (PR_c = 1.99; P = 0.006) and children who had respiratory diseases (PR_c = 1.78; P = 0.016). In this unadjusted analysis, the polymorphisms in *rs2057482* and *rs2301113* were not significantly associated with HPSMs (Table 3).

Table 3. Crude prevalence ratio of the environmental factors and genotype polymorphisms for hypomineralized primary second molars (HSPMs)

		HSPMs		**Total**	**PR_c* (95% confidence interval)**	***P*-value****
		Case N (%)	**Control N (%)**			
Drug use during pregnancy†	Yes	17 (17.0)	83 (83.0)	100	2.14 (1.28-3.57)	**0.004**
	No	47 (7.9)	545 (92.1)	592	1.00	
Maternal problems‡	Yes	30 (14.5)	177 (85.5)	207	1.99 (1.21-3.25)	**0.006**
	No	27 (7.3)	344 (92.7)	371	1.00	
Respiratory diseases in childhood§	Yes	24 (14.5)	142 (85.5)	166	1.78 (1.11-2.85)	**0.016**
	No	42 (8.1)	476 (91.9)	518	1.00	
HIF-1α rs2057482 Genotypes	CT/CC	33 (11.0)	282 (89.0)	315	0.38 (0.13-1.06)	0.066
	TT	3 (27.3)	8 (72.7)	11	1.00	
HIF-1α rs2301113 Genotypes	AC/CC	24 (13.4)	155 (86.6)	179	0.87 (0.46-1.64)	0.671
	AA	16 (10.3)	138 (89.6)	154	1.00	

*Crude Prevalence rate (PR_c) calculated by univariable Poisson regression analysis.

** Level of significance for P value=0.05.

Bold text indicates statistical significance difference for P value..

†Drug use was considered the mother's use of tobacco, alcohol, and illicit drugs during the pregnancy.

‡Maternal problems were considered the presence of diabetes, hypertension during pregnancy, and prolonged labor delivery.

§Respiratory diseases were considered the presence of bronchitis, asthma, and pneumonia until the first three years of life.

Analyzing the factors in a multiple variables model (Table 4), children whose mothers used drugs during pregnancy were 3.52 times more likely to have HPSM than children whose mothers did not use drugs (PR_a = 4.52; P< 0.01). In the same way, children whose mothers had suffered from health problems (such as diabetes and/or hypertension during pregnancy or prolonged labor delivery; (PR_a = 1.97; P = 0.03) had a 97% higher prevalence of HPSMs than mothers without these health problems. During childhood, children who suffered from respiratory diseases were 1.66 times more likely to have HPSMs (PR_a = 2.66; P = 0.03) than children without respiratory diseases. In the presence of hypoxia-related environmental factors, individuals who carried at least one C allele (with allele CC/CT genotype) in the *rs2057482* polymorphism had a 50% lower prevalence of HSPMs (PR_a = 0.50; P = 0.04) than allele TT children (Table 4).

Table 4. Adjusted prevalence ratio of the environmental factors and genotype polymorphisms for hypomineralized primary second molars (HSPMs; N=323)

		PR_a*(95% confidence interval)	***P*-value****
Drug use during pregnancy	Yes	4.52 (2.38-8.53)	<0.001
	No	1.00	
Maternal problems	Yes	1.97(1.05-3.71)	0.034
	No	1.00	
Respiratory disease during childhood	Yes	2.66 (1.41-5.03)	0.003
	No	1.00	
HIF-1α rs2057482 genotype	CT/CC	0.51 (0.27-0.99)	0.048
	TT	1.00	

*Adjusted prevalence rate (PR_a) calculated by multiple variables Poisson regression analysis.

**Level of significance for P value =0.05.

To perform the multiple Poisson regression analysis, independent variables (with P<0.20 in the association with HSPMs) were added to the multiple model.

The PR_a was adjusted by the independent variables presented on the table: drug use during pregnancy, maternal problems, respiratory disease during childhood, and *HIF-1α rs2057482* genotype.

Discussion

HPSM is an enamel development defect that is clinically similar to MIH, since the formation period of the permanent first molars and the primary second molars have a chronological coincidence. In this study, the authors assumed that, from the fourth month of intrauterine life to the first year, environmental factors could result in hypomineralization in the primary second molars as well as the permanent molars.[38] The present study analyzed the association between environmental factors related to hypoxia, as well as polymorphisms in the *HIF-1α* gene, which could be associated with a prevalence of HPSMs.

Although the MIH and HPSM etiologies have not been completely elucidated, previous studies have already noted some environmental and genetic factors associated with these defects.[3,30,39] Besides these environmental factors, the association of the mother's use of tobacco and alcohol during pregnancy,[3,39,40] maternal diseases,[30,41,42] complications in childbirth,[30,42] low birth weight, prematurity,[43-46] and respiratory problems in children up to three years of age[3,4,30,47] were observed. The present study's results corroborate previous studies, with a higher prevalence of HPSMs in children whose mothers had a health problem during pregnancy or mothers who were on drugs during pregnancy as well as a higher prevalence of children who had respiratory problems up to the age of three years. The presence of fever, dioxin factors, antibiotic use, nicotine, and alcohol may lead to changes in cell differentiation and enamel mineralization, and, consequently, be associated with enamel hypomineralization.[8,10,48,49]

Hypoxia may be caused by systemic problems during birth, prematurity, Caesarean section, respiratory problems, and prolonged labor.[31] An increased expression of *HIF-1α* has also been observed in pregnant women with preeclampsia,[50] which reinforces the hypothesis that maternal problems during pregnancy can lead to hypoxia-generating factors.

In the present study, hypoxia-related environmental factors significantly increased the prevalence of HPSMs. Some studies have already proposed that hypoxia at birth is a risk factor for enamel defects development.[42,51,52] It is possible to assume

that some environmental factors associated with hypomineralization are hypoxia-generating situations.

The authors observed through multiple analyses that, when environmental factors analyze *HIF-1α* polymorphism (*rs2057482*), the strength of association increased significantly between the environmental factors and the *HIF-1α rs2057482* polymorphism with HPSMs. This suggests a positive interaction between these events. In the presence of hypoxia-related environmental factors, individuals with C allele (CC/CT) at the *HIF-1α rs2057482* polymorphism presented with a 50% lower prevalence of HPSMs, indicating that the C allele in the *rs2057482* polymorphism acts as a protective factor for HPSMs in hypoxia conditions.

Adaptation to hypoxia in cells and tissues leads to the transcriptional induction of several genes that participate in angiogenesis, iron metabolism, glucose metabolism, and cell proliferation/survival. The main factor mediating this response is the transcription factor *HIF-1α*, an oxygen-sensitive transcriptional activator.[53] In a rat study, Sidaly et al.[7] found that 24- and 48-hour hypoxia situations increased the expression of the structural genes: amelogenin (*AMELX*); ameloblastine (*AMBN*); enamelin (*ENAM*); and the matrix metalloproteinase-20 (*MMP20*). This suggests that hypoxia situations may disrupt amelogenesis-related protein expression and, thus, cause an enamel disturbance.[7] Another factor that corroborates with this hypothesis is the fact that previous studies have shown that hypomineralized enamel has a substantial protein content higher than normal and a decreased mineral content,[54,55] suggesting alteration on these enamel proteins that were not removed in the mineralization stage.

Although retrospective design studies have memory bias limitations, this bias is likely not common in important health events such as pregnancy disorders, complications during childbirth, and illnesses in the child's early years.[56] It is believed that this bias was not a limitation in this study, and biological factor related to hypoxia was also analyzed. Thus, future studies should analyze both environmental factors and genetic variants for the etiology of HPSMs. Moreover, future investigations should consider the prevalence of HPSMs in a subset of children with medically documented hypoxia.

Conclusions

Based on this study's results, the following conclusions can be made:

1. Children who were exposed to hypoxia-related environmental factors presented with a higher prevalence of hypomineralized primary second molars.

2. Children carrying the C allele in the *rs2057482* presented with a lower prevalence of HPSMs in the presence of hypoxia-related environmental factors.

3. These findings suggest a possible interaction between environmental and genetic factors on the etiology of HPSMs.

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4. ARTIGO 2

Genes and Genetic polymorphisms associated to developmental defect of enamel: A scoping review

Abstract

Previously studies considered the developmental defect of enamel (DDE) with a complex etiology, with involvement of environmental and genetical factors. However, its etiology is not fully evidenced. Considering that amelogenesis is under genetical, it is possible that the genetic variation may contribute to the increased risk of DDE. Thus, the aim of the present scoping review was to assess the available literature on genetic polymorphisms associated with DDE. This review was registered in the PROSPERO database (CRD42018115270). Searches were conducted in the PubMed, Scopus, Web of Science, LILACS, BBO, and Cochrane Library databases as well as the grey literature. The included studies were observational, cross-sectional, case control and cohort that evaluated the association between DDE and genetic polymorphism. The risk of bias of the studies was assessed using the Newcastle-Ottawa Scale. The searches led to the retrieval of 1076 papers, 22 studies were included. The age of participants included in the studies ranged from 1 to 38 years and the number of participants included in the studies varied from 52 to 1065. It was investigated 23 genes whose proteins are related to enamel development (*AMBN, AMELX, AMTN, ENAM, FAM83H, MMP, TFIP, TUFT*), craniofacial patterning and morphogenesis (*DLX, IRF, BGLAP, BMP, COL, CTR, FGFR, OPG, RANK*), immune response (*IL, MBL, DEFB*), and hormone transcription/receptor (*ESR, GHR, PTH*). It was observed that MIH and / or HSPM was associated with 18 polymorphisms of genes responsible for enamel development, immune response and growth and morphogenesis craniofacial. A significant association was found between the different clinical appearances of dental fluorosis with 7 polymorphisms of genes responsible for enamel development, craniofacial development and hormonal transcription / reception. The studies included in the systematic review found that DDE, such as MIH, HSPM and DF, reported as a complex etiology, are also significantly associated with genetic polymorphisms. Prospective and animal studies are necessary to demonstrate in depth the role of systemic and/or environmental and genetic factors in the complex etiology of DDEs.

Keywords: Tooth hypomineralization; Molar incisor hypomineralization; Fluorosis dental; Genetic Polymorphism.

Introduction

Amelogenesis involves the secretion of enamel proteins, followed by the mineralization of the enamel matrix that occurs by the action of the enzymes. In the secretory period of amelogenesis, ameloblasts secrete enamel proteins such as amelogenin, ameloblastin, and enamelin, after this secretory period, a genetically programmed change occurs and starts the secretion of enzymes, including Matrix Metalloproteinases (MMP20, MMP 45, MMP 41) and Kallikrein-related Peptidases (KLK4, KLK34 and KLK31), which become responsible for the degradation and removal of proteins from the enamel matrix, then ceasing the growth of the enamel crystals in length and favoring their growth in thickness, starting then the period of mineralization of amelogenesis (1). For example, Matrix metalloproteinase (*MMPs*) and Tissue inhibitors of metalloproteinases (*TIMPs*) are involved in the remodeling of dental tissue and in the degradation of the extracellular matrix (2). The Distal Less (*DLX)* genes are expressed during the initial stages of amelogenesis, taking part in the organization of dental epithelium and in the control of enamel morphogenesis via regulation of amelogenin expression (3).

The amelogenesis is also involved by other local or systemic environmental metabolic activities, such as the ions transport, degradation and removal of enamel matrix proteins, and pH balance to reach dental enamel tissue around 95% of inorganic mineral content. The overall process is finely regulated by a myriad of genes and is influenced by environmental disorders. So, if the homeostasis was broken in any stage of amelogenesis a permanently biological mark, defined as a developmental defect of enamel (DDE) occurs (1).

Disturbances during the amelogenesis process impact on the enamel thickness or mineralization/ maturation, it could lead to qualitative or quantitative defects on the dental enamel (4), the different types of enamel defects can occur depending on the stage of amelogenesis affected. Developmental defect of enamel (DDE) are categorized into two main groups: hypomineralization and hypoplasia (5). Hypomineralization is a qualitative defect of enamel observed clinically as a defect in translucency. That could present as demarcated or diffuse opacities (5). While, hypoplasia is a quantitative defect on the enamel surface related to the reduction of its thickness (5).

The molar incisor hypomineralization (MIH) and the hypomineralization of primary second molars (HPSM) are a specific demarcated hypomineralization, which affects the first permanent molars and primary second molars, respectively (6, 7). In 2001,

the European Academy of Pediatric Dentistry (EAPD) classified MIH as an enamel defect that affects at least one permanent molar and often permanent incisors (6). In 2008, Elfrink et al. classified HPSM as demarcated hypomineralization that affects one to four primary second molars (7). Another common enamel defect is dental fluorosis (DF), is also a specific hypomineralization of enamel clinically characterized by diffuse opacities, presenting white stains or yellow to brown discoloration, and may have a pronounced enamel porosity with the stains (8).

The systemic etiology of DF is associated with a disturbance in tooth formation caused by excessive fluoride intake during the period of amelogenesis (8). However, the systemic etiology of MIH and HPSM remains unclear (9-11). Some findings have indicated an association between these enamel defects and hypoxia situations, premature birth and/or complications at birth (11-13), a potential association with childhood diseases, fever, and medication use (9, 11, 14). Despite of the systematic reviews observed the involvement of systemic or environmental factors on the MIH etiology, the pathway of these factors in the amelogenesis is not fully comprehended (9, 11, 14, 15).

It is known that amelogenesis is genetically controlled. Thus, the size, shape, and composition of dental enamel are influenced by genetic variations (1). This genetic involvement has already been extensively studied in the Amelogenesis Imperfecta (AI), where genetic mutations were already observed in genes encoding the enamel matrix proteins, such as *AMBN, AMELX, ENAM, MMP20, KLK4, ITGB6, LAMA3, LAMB3, COL17A1, AMTN, FAM83H* and *DLX3* (16).

Once previous studies have already demonstrated the possible association between genetic polymorphisms related to enamel defects (17-19), such as MIH (4, 20-26) and DF (8, 27-35), it is reasonable to assume that the genetic variation in genes that encode the proteins involved in the formation of dental enamel or genes from immune response may contribute to the increased risk of developmental defects of enamel.

Based on this, the objective of this scoping review was to review systematically the literature about the genes and genetic polymorphisms associated with developmental defect of enamel.

Materials and methods

Protocol and registration

This study protocol was registered in the PROSPERO database (CRD 42018115270) and the recommendations of the MOOSE (36) statement for observational

studies was followed. This study was conducted from November 2018 to March 2021 at the Federal University of Paraná, Paraná, Brazil

Information sources and search strategy

The search strategy was based on controlled vocabulary (MeSH terms) of the PubMed database along with free keywords, the terms were combined with the Boolean operator "OR" and "AND". In addition, the search included other electronic databases (Scopus, Web of Science, the Latin American and Caribbean Health Sciences Literature database [LILACS], the Brazilian Library in Dentistry [BBO] and the Cochrane Library). The search strategy was first developed in PubMed and was adapted for each of these databases (Table 1). No restrictions on publication date or languages were involved. Abstracts of the International Association for Dental Research (IADR) and its regional divisions (1990–2020) were used and the grey literature and the database System for Information on Grey Literature in Europe (SIGLE) and Google Scholar was also verified, dissertations and theses were searched using the ProQuest databases and Capes Periodical databases.

The study question was formulated according to the "PECO acronym (population, exposure, comparison and outcome)" strategy, in which (P) were children or adolescents or adults, (E) presence of the genetic polymorphism, (C) absence of the genetic polymorphism, and (O) presence of developmental defect of enamel.

Eligibility Criteria

The included studies were observational studies with comparison group, such as a cross-sectional, case control and cohort that evaluated the association between developmental defect of enamel and genetic polymorphism. Randomized clinical trials, pilot study, review studies, editorial letter, case reports, animal and in vitro studies were not included. Observational studies without comparison group and studies that associated amelogenesis imperfecta or other syndrome with the genetic polymorphisms were excluded.

Study selection and data collection process

The articles were selected by title and abstracts according to the eligibility criteria previously described. Those found in more than one database were considered

only once. The full text of the articles was obtained when the information presented in the abstracts or title was insufficient to decide about the inclusion of the article.

Subsequently three reviewers (A.L.F, J.F.S and L.M.W) evaluated the full-text articles and selected those that were included in the eligibility criteria. Relevant study information about were extracted using previously tested extraction forms by the authors (A.L.F and J.F.S) (Table 2). The collection form was tested on a sample of study reports used as a pilot sample to ensure that the form was consistent with the research question.

Risk of bias

The risk of bias in the included studies was evaluated independently by three reviewers (A.L.F, J.F.S and L.M.W) using the Modified Newcastle - Ottawa Scale for Risk of Bias criteria (37). This scale was adapted according to epidemiological study design, cross sectional, case-control or cohort studies. The risk of bias evaluation criteria presented a maximum score of 9 points. These score points were divided between the following domains: patient selection (generalization and applicability - 4 points), comparability of groups (2 points) and exposure measurements in studies designed (3 points). Discrepancies between the examiners were solved in a consensus. Subsequently, the studies were classified as “high” risk of bias (0-3 points), “moderate” risk of bias (4-6 points) and “low” risk of bias (≥7 points) (38).

Results

Study Selection

The initial screening in the databases resulted in 1076 studies. After the removal of the duplicates 804 studies was maintained. A hundred and eight studies remained after the examination of the title. Seventy-eight studies were excluded after the abstract streaming, result in 31 full text articles for the eligibility evaluation (Figure 1).

Nine out of these 31 studies were excluded for: 1) assessed only skeletal fluorosis (39), 2) lacking a comparison group (40-42), 3) evaluated another syndrome (43), 4) assessed amelogenesis imperfecta (44, 45), 5) being a case report (46), 6) being an animal study (47).

Characteristics of included articles

The characteristics of the 22 selected studies are presented in Table 2. Twelve were cross-sectional studies (18-20, 22-25, 27, 29, 31, 33, 48), six were case control studies (8, 17, 28, 30, 34, 35) and four were cohort (4, 21, 26, 32).

The studies were performed in 7 countries: 11 in the Brazil (18-20, 22, 23, 27, 29, 31-33, 48), 5 in China (8, 21, 28, 30, 35), 1 in Slovenia (25), 1 in Poland (17), 1 in Germany (26), 1 in India (34), and two studies included population from Brazil and Turkey in their sample (4, 24).

The age of participants included in the studies ranged from 1 to 38 years. The number of participants included in the studies varied from 52 to 1065, Bezamat et al presented the higher population between the included studies (24).

Six studies enrolled participants from community (8, 21, 28, 30, 34, 35), six studies enrolled participants from schools (17-19, 23, 27, 29), five studies enrolled participants from University dental clinics (4, 20, 31, 33, 48), two studies enrolled participants from school + university clinic (24, 32) one study enrolled participant from Hospital (25) and two studies did not reported this information (22, 26).

Most of the included studies (n=8) uses diagnostic criteria for dental fluorosis by Dean's criteria (8, 27-33, 35), seven studies used de European Academy of Pediatric Dentistry (EAPD) criteria for MIH evaluation (4, 20-22, 24-26), four studies used the modified-DDE index (17-19, 48), one study used Fluorosis Research and Rural Development Foundation criteria (34) and one study used EAPD + DDE index criteria (23).

The samples of DNA were collected by buccal cells samples in 15 studies (4, 17-24, 27, 29, 31-33, 48), and blood samples in 7 studies (8, 25, 26, 28, 30, 34, 35).

Genetic analysis

The investigated genes and polymorphisms are presented on Table 2, and their characteristics, protein functions are described on Table 3. It was investigated genes whose proteins are related to enamel development (*AMBN, AMELX, AMTN, ENAM, FAM83H, MMP, TFIP, TUFT*), craniofacial patterning and morphogenesis (*DLX, IRF, BGLAP, BMP, COL, CTR, FGFR, OPG, RANK*), immune response (*IL, MBL, DEFB*), and hormone transcription/receptor (*ESR, GHR, PTH*) (Table 3). The major of the studies investigated the association between polymorphisms and DDE by univariate model, that is, they assessed only the association between DDE and the polymorphism individually

(4, 17, 20, 21, 26-35, 48), while others evaluated an interaction between the polymorphisms, DDE and environmental factors (8, 19, 22-25, 34).

The genotypes of subjects in the SNP loci were tested for deviation from Hardy-Weinberg equilibrium (4, 8, 17-22, 24, 27, 29, 31, 33, 48).

Analysis of genetic samples was carried out through familiar association in 2 studies (20, 22), polymorphism analysis in 18 studies (4, 8, 17-19, 21, 23-25, 28-35, 48), miRNA analysis in 1 study (27) and principal component analysis of genetic data in 1 study (26).

Assessment of the risk of bias

The risk of bias of the included studies is presented in Table 2. None study presented high risk of bias, 18 studies presented moderate risk of bias (4, 8, 18, 20, 22, 24-29, 31-35, 48), and 4 studies presented low risk of bias (17, 19, 21, 23), according to the Newcastle-Ottawa Scale quality assessment. For the comparability domain, it is important to note that majority of studies did not had a control group selected from the population.

Discussion

In this study our proposal was to review the literature looking for genes and genetic polymorphisms associated with developmental defect of enamel. The main results point to 23 most cited genes and 110 polymorphisms. Between genes, the most studied were the estrogen receptor gene (*ESR*) and Enamelin gene (*ENAM),* while *rs3796704* (*ENAM)* and rs4694075 *(AMBN)* were the most cited.

Although the genetic polymorphisms are common, they do not necessarily alter the aminoacids sequence in the encoded proteins (49). So, they have been studied in terms of susceptibility to health outcomes (49). The present review study aimed to analyze primary studies which investigated the association between genetic polymorphisms and DDE with complex etiology, including DF, MIH and HPSM. Particularly, Amelogenesis Imperfecta, whose etiology is only genetic, is not included in the present review.

Considering the association between genes and genetic polymorphisms and all types of DDE (hypoplasia, diffuse or demarcated hypomineralization), two studies were included: the cross-sectional study of Arid et al. (18), carried out in Brazil with a sample of 216 children, and the case-control study conducted by Gerreth et al (17) in a Poland sample of 52 children. The studies found significant association between DDE in primary

and/or permanent teeth with genes responsible for enamel matrix secretion, such as *AMBN, AMELX* (17), and genes of the estrogen receptor (18). This data suggests that the polymorphism in these genes could impact on the DDE susceptibility.

Regarding these protein functions, it is known that amelogenin is a tooth-specific protein that acts as a pH buffer of the enamel matrix and participates in the growth of the enamel crystals (4). Ameloblastin is expressed in the secretory stage and also in the mineralization/maturation stages of amelogenesis. Its function is also related to crystal growth (20). During the amelogenesis process, estrogen can also act in the mineralization enamel. In an animal model, it was observed that estrogen deficiency resulted in a significant reduction in the enamel micro hardness (50).

Regarding to MIH defect, it is known its complex etiology, involving environmental and genetic factors. MIH is a quantitative enamel defect, represented clinically by enamel hypomineralization. Traces of amelogenin were found in its dental samples (51). This finding suggests that incomplete degradation of amelogenin can be involved in the MIH pathogenesis. Jeremias et al. (20) and Bussaneli et al. (22) have found association between *AMELX* gene and susceptibility of MIH. Moreover, Bussaneli et al. (22) also observed a gene interaction between *AMELX* and interleukin (*IL*) genes. *IL* gene is involved in inflammatory modulation, suggesting an involvement of MIH with inflammatory conditions. Animal studies could explain its involvement, showing that inflammatory mediators present in the serum can negatively affect the function of tight junctions in salivary gland cells (52, 53). Some animal studies also observed that local and systemic inflammatory situations could alter the blood-enamel ion transport during the enamel mineralization. Another finding is the presence of blood proteins in the enamel mineralization (54), which could be related to the inflammatory cytokines and angiogenesis induction that possibly increase the risk of blood proteins through the enamel development (55).

Another view of MIH pathogenesis is a disorder in proteolytic agents such as Kallikrein-related Peptidases (KLK4), Matrix Metalloproteinases (MMP2, MMP3, MMP9, MMP20) (54, 56), and also Tuftelin, which play an important role during the mineralization stage of amelogenesis (57). The studies analyzed in the present review reported that polymorphisms (*rs1711399, rs1711423, rs2245803, rs1784418, rs4970957, rs3790506*) in the *MMP20* gene and *TUFT1* were associated to MIH (4, 20, 21, 25).

Other studies have also observed the association between polymorphism in genes responsible for Bone Morphogenetic Proteins (*BMP, FGFR, IRF and DLX*) (20,

24) and tissue growth factors (*TGF*) (22, 24) with MIH, suggesting that its pathogenesis may not only be restricted to proteins of enamel development. The TGF is a multifunctional cytokine secreted by T cells, which plays a role not only in the immune system, but also in cell differentiation, and bone formation (58). Although TGF is not expressed during the amelogenesis, it is expressed during the stages of enamel organ differentiation and in the pre-secreting period of amelogenesis (59, 60), being involving indirectly on the enamel development.

The genes responsible for codified bone morphogenetic proteins are also expressed in pre-ameloblasts and ameloblasts (20). The *IRF* gene participates in the formation of oral structures and its influence on amelogenesis has been reported, with reduced enamel density being observed in IRF6 knockout mice (61). The *BMP4* gene encodes a specific protein that regulates the development and induction of mesoderm in tooth development, limb formation and bone repair induction (62). An animal study showing that knock-out mice for *BMP4* gene had decrease differentiation of mature odontoblasts (63). Another possible explanation for the *BMP* gene influence on the MIH pathogenesis is the interaction of *BMP* and *DLX* genes. In the absence of the *BMP* gene also affects the expression of the *DLX* gene, which is also expressed in ameloblasts and controls many of the genes involved in amelogenesis (63).

Endocrine factors can also influence the amelogenesis, affecting the enamel formation (64). On this line, in a cross-section study, Fatturi et al (23) observed a significant association between MIH with *VDR* gene and suggest that individual who carry at least one G allele in *rs739837* presented higher prevalence of the most severe phenotype MIH in molars and incisors. It is already known that vitamin D stimulates the enamel mineralization through the *VDR* gene expressed in dental cells (65)

In the case of HPSM, it is evidenced the association of HPSM and MIH in primary and permanent teeth, respectively. Thus, it can be assumed both defects presented coincidence regarding to the risk factors etiology. Fatturi et al (19) observed a significant association between the prevalence of HSPM and the genotypes of polymorphism (*rs2057482*) in *HIF1α* gene, in the presence of hypoxia conditions. These findings evidence a possible interaction of the systemic factors already known (66-68) with the genetic factors. Abnormal oxygen levels can inhibit the function of proteolytic enzymes and the development of hydroxyapatite crystals, resulting in hypomineralized enamel (69).

The etiology of DF involves the chronic excessive exposition to fluoride, and it is already known that fluoride influences ameloblasts leading to a decrease in production and removal of the enamel matrix. Its fact seems to be related to negative regulation of KLK4 during the maturation period (53). Moreover, fluoride can lead to delayed amelogenin removal and enamel demineralization (70). Although the major etiology factor of DF is a chronically excessive exposition to fluoride, it has been studied the genetic polymorphism involved on the DF etiology, mainly in genes responsible to proteins from enamel development, such as AMBN, TFIP, TIMP and TUFT (28, 31, 32). The studies found a significant association between genetic polymorphism in these gene with DF. (28, 31, 32). A limitation of these studies that is important to note is not evaluated the confounders variables associated to fluoride exposition.

Polymorphisms in estrogen receptor gene (*ESR*) were significant associated with DF in four studies, its known that *ESR* is expressed in osteoblasts and ameloblasts, acting on bone remodeling and promoting the deposition of calcium and phosphate (71). Thus, this genetic variability could promote alterations in the estrogen pathway affecting the capacity of the enamel mineralization in the presence of fluoride (71).

Based on previous studies we can assume that there are several genetic polymorphism can be associated to different types of DDE, suggesting that are different pathogenesis involving in the enamel defects, represented by a complex interaction between systemic and genetic factors (15), what can be evaluated with more profundity with futures studies that involves the genetic interaction analysis, such as, haplotype and diplotype analysis. Moreover epigenetics analysis could be applied on DDE studies. It has been shown to be increasingly relevant in the understanding of diseases with complex etiology and reports genetic modifications associated with differences in DNA expression in different tissues and the environmental influence on gene expression (72), which can be applied to DDE.

Conclusion

Based on this scooping review its possible conclude that DDE were significantly associated with genetic polymorphisms in 23 genes, whose proteins are related to enamel development (*AMBN, AMELX, AMTN, ENAM, FAM83H, MMP, TFIP, TUFT*), craniofacial patterning and morphogenesis (*DLX, IRF, BGLAP, BMP, COL, CTR, FGFR, OPG, RANK*), immune response (*IL, MBL, DEFB*), and hormone transcription/receptor

(*ESR, GHR, PTH*). Further studies involving systemic and genetic factors in large population are needed to improve the evidence of the DDE complex etiology.

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Table 1 – Electronic databases and search strategy**.**

Pubmed= 649 (18/02/2021)	
#1 ((((((((((((((((((((((((Tooth calcification[MeSH Terms]) OR dental enamel[MeSH Terms]) OR Dental enamel Hypoplasia[MeSH Terms]) OR Fluorosis, Dental[MeSH Terms]) OR Amelogenesis[MeSH Terms]) OR "Tooth calcification"[Title/Abstract]) OR "dental enamel"[Title/Abstract]) OR "Dental enamel Hypoplasia"[Title/Abstract]) OR "Fluorosis dental"[Title/Abstract]) OR Amelogenesis[Title/Abstract]) OR "molar incisor hypomineralization"[Title/Abstract]) OR "molar incisor hypomineralisation"[Title/Abstract]) OR MIH[Title/Abstract]) OR "hypomineralized molars"[Title/Abstract]) OR "cheese molars"[Title/Abstract]) OR "Enamel Hypoplasia"[Title/Abstract]) OR "enamel hypomineralization"[Title/Abstract]) OR "Developmental dental defects"[Title/Abstract]) OR "Demarcated opacities"[Title/Abstract]) OR "Demarcated opacity"[Title/Abstract]) OR “enamel defects"[Title/Abstract]) OR "enamel defect”[Title/Abstract]))))	#2 ((((((((((((((Polymorphism, Genetic[MeSH Terms]) OR Epigenomics[MeSH Major Topic]) OR gene-environment interaction[MeSH Major Topic]) OR genetic variations[MeSH Terms]) OR Alleles[MeSH Major Topic]) OR Genetic Association Studies[MeSH Terms]) OR "Polymorphism Genetic"[Title/Abstract]) OR Epigenomics[Title/Abstract]) OR "gene environment interaction"[Title/Abstract]) OR "genetic variations"[Title/Abstract]) OR Alleles[Title/Abstract]) OR "Genetic Association Studies"[Title/Abstract]) OR "Genetic factors"[Title/Abstract]) OR "Genetic makers"[Title/Abstract]))
#1 AND #2	
Scopus= 337 (18/02/2021)	
#1 ((TITLE-ABS-KEY ("t??th calcification") OR TITLE-ABS-KEY ("dental enamel") OR TITLE-ABS-KEY ("dental enamel hypoplasia") OR TITLE-ABS-KEY ("fluorosis dental") OR TITLE-ABS-KEY (amelogenesis) OR TITLE-ABS-KEY ("molar incisor hypomineralization") OR TITLE-ABS-KEY (mih) OR TITLE-ABS-KEY ("hypomineralized molar") OR TITLE-ABS-KEY ("cheese molar") OR TITLE-ABS-KEY ("enamel hypoplasia") OR TITLE-ABS-KEY ("enamel hypomineralization") OR TITLE-ABS-KEY ("developmental dental defects") OR TITLE-ABS-KEY ("demarcated opacity") OR TITLE-ABS-KEY ("enamel defect")))	#2 ((TITLE-ABS-KEY ("polymorphism genetic") OR TITLE-ABS-KEY (epigenomic) OR TITLE-ABS-KEY ("gene environment interaction") OR TITLE-ABS-KEY ("genetic variation") OR TITLE-ABS-KEY (allele) OR TITLE-ABS-KEY ("genetic association study") OR TITLE-ABS-KEY ("genetic factor") OR TITLE-ABS-KEY ("genetic maker")))
#1 AND #2	
Web of Science- 77 (18/02/2021)	
#1 TÓPICO: ("t??th calcification") OR TÓPICO: ("dental enamel") OR TÓPICO: ("dental enamel hypoplasia") OR TÓPICO: ("fluorosis dental") OR TÓPICO: (amelogenesis) OR TÓPICO: ("molar incisor hypomineralization") OR TÓPICO: (MIH) OR TÓPICO: ("hypomineralized molar") OR TÓPICO: ("cheese molar") OR TÓPICO: ("enamel hypoplasia") OR TÓPICO: ("enamel hypomineralization") OR TÓPICO: ("developmental dental defects") OR TÓPICO: ("demarcated opacity") OR TÓPICO: ("enamel defect$") Índices=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI Tempo estipulado=Todos os anos	#2 TÓPICO: ("polymorphism genetic") OR TÓPICO: (epigenomic) OR TÓPICO: ("gene environment interaction") OR TÓPICO: ("genetic variation") OR TÓPICO: (allele) OR TÓPICO: ("genetic association study") OR TÓPICO: ("genetic factor") OR TÓPICO: ("genetic maker") Índices=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI Tempo estipulado=Todos os anos

<table>
<tr><td colspan="2">Lilacs and BBO = 13 (18/02/2021)</td></tr>
<tr><td>#1 (tw:((mh:"tooth calcification" OR mh:"dental enamel" OR mh:"dental enamel hypoplasia" OR mh:"fluorosis dental" OR mh:amelogenesis OR tw:"molar incisor hypomineralisation" OR tw:"hipomineralização de molares e incisivos" OR tw:"hipomineralización del incisivo molar" OR tw:"molar incisor hypomineralization" OR tw:"hipomineralização dos incisivos e molares" OR tw:"incisivo molar hipomineralacción" OR tw:mih OR tw:hmi OR tw:"hypomineralized molars" OR tw:"molares hipomineralizados" OR tw:"cheese molars" OR tw:"molares de queijo" OR tw:"molares de queso" OR tw:"enamel hypoplasia" OR tw:"hipoplasia do esmalte" OR tw:"hipoplasia del esmalte" OR tw:"enamel hypomineralization" OR tw:"hipomineralização do esmalte" OR tw:"hipomineralización del esmalte" OR tw:"developmental dental defects" OR tw:"defeitos de desenvolvimento dentário" OR tw:"defectos de desarrollo dental" OR tw:"demarcated opacities" OR tw:"opacidades demarcadas" OR tw:"demarcated opacity" OR tw:"opacidade demarcada" OR tw:"opacidad demarcada" OR tw:"enamel defects" OR tw:"defeitos de esmalte" OR tw:"defectos del esmalte" OR tw:"enamel defect" OR tw:"defeito de esmalte" OR tw:"defecto del esmalte")))</td><td>#2 (tw:((mh:"polymorphism genetic" OR mh:epigenomic OR mh:"gene environment interaction" OR mh:"genetic variations" OR mh:alleles OR mh:"genetic association studies" OR tw:"genetic factors" OR tw:"fatores genéticos" OR tw:"factores genéticos" OR tw:"genetic factor" OR tw:"fator genético" OR tw:"factor genético" OR tw:"genetic makers" OR tw:"marcadores genéticos" OR tw:"genetic maker" OR tw:"marcador genético")))</td></tr>
<tr><td colspan="2">#1 AND #2 AND (db:("LILACS" OR "BBO"))</td></tr>
<tr><td colspan="2">Cochrane Library = 0 (18/02/2021)</td></tr>
<tr><td>#1 MeSH descriptor: [Tooth Calcification] explode all trees
#2 MeSH descriptor: [Dental Enamel] explode all trees
#3 MeSH descriptor: [Dental Enamel Hypoplasia] explode all trees
#4 MeSH descriptor: [Fluorosis, Dental] explode all trees
#5 MeSH descriptor: [Amelogenesis] explode all trees
#6 (molar incisor hypominerali?ation OR MIH OR "hypomineralized molar" OR "cheese molar" OR "enamel hypoplasia"):ti,ab,kw
#7 ("enamel hypomineralization" OR "developmental dental defect" OR "demarcated opacity" OR enamel near defect):ti,ab,kw
#8 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7</td><td>#10 MeSH descriptor: [Polymorphism, Genetic] explode all trees
#11 MeSH descriptor: [Gene-Environment Interaction] explode all trees
#12 MeSH descriptor: [Genetic Variation] explode all trees
#13 MeSH descriptor: [Alleles] explode all trees
#14 MeSH descriptor: [Genetic Association Studies] explode all trees
#15 genetic near factor OR genetic next maker):ti,ab,kw
#16 #10 OR #11 OR #13 OR #14 OR #15</td></tr>
<tr><td colspan="2">#8 AND #16</td></tr>
</table>

Table 2 -Summary of the studies selected for this systematic review.

Study ID	Study design	Country	Patient's age [mean ± SD] (years)	Number of patients [response rate]	Recruitment of patients	Methods to obtain DNA – Analysis	Outcomes evaluated			Newcastle–Ottawa Scale		
							Investigated genes	Prevalence of DDE (%)	Evaluation Criteria	S	C	O
Abbasoglu et al, 2020 (27)	Cross sectional	Brazil	10 – 14 [12 ± 0.47]	527	Public and private school	Buccal cells – PCR miRNA	*MIR17HG (rs4284505)*	27.3% (DF)	Dean's criteria	****		**
Arid et al, 2019 (18)	Cross sectional	Brazil	9-12 [NR]	216 [NR]	Public school	Buccal cells – PCR real time	*ESR1 (rs1884051)* *ESR1 (rs12154178)* *GHR (rs2973015)* *GHR (rs2940913)* *GHR (rs2910875)* *GHR (rs1509460)*	19.9% (DDE)	DDE index (FDI)	****		**
Ba et al, 2011(28)	Case control	China	8-12 [NR]	240 [NR]	Community	Blood – PCR, RFLP	*COL1A2 RsaI (rs1256049)* *XbaI (rs2234693)* *COL1A2 PvuII (rs9340799)*	31.3 % (DF)	Dean's criteria	****		**
Bezamat et al, 2021 (24)	Cross sectional	Brazil/ Turkey	6 – 12 [NR]	1065 [NR]	Public/ private school and University dental clinic	Buccal cells – PCR real time	*IRF6 (rs17015215)* *IRF6 (rs2013162)* *IRF6(rs861019)* *IRF6 (rs2073487)* *IRF6 (rs642961)* *TGFA (rs2166975)* *TGFA (rs1523305)* *TGFA (rs2902345)* *TGFA (rs930655)*	40.2% (MIH)	EAPD criteria for MIH	**		**
Bussaneli et al, 2019 (22)	Cross sectional		6-18 [10]	391 [NR]	NR	Buccal cells – PCR familiar association	*IL10 (rs1800872)* *IL1A (rs1800587)* *IL1B (rs1143634)* *STAT (rs3771300)* *TGFBR1 (rs10733708)*	33.5% (MIH)	EAPD criteria for MIH	***		**

		Brazil					*IL8 (rs4073)* *IL4 (rs207074)* *TNF (rs1800629)* *IL17A (rs2275913)* *IL6 (rs1800795)* *TGFB1 (rs1800470)*					
Dalledone et al, 2019 (29)	Cross sectional	Brazil	12	538 [NR]	Public and private school	Buccal cells – PCR real time	*ESR1(rs12154178)* *ESR1 (rs1884051)* *ESR1 (rs9340799)* *ESR1 (rs2234693)* *ESR2 (rs4986938)* *ESR2 (rs1256049)* *ESRRB (rs745011)* *ESRRB (rs6574293)* *ESRRB (rs10132091)* *ESRRB (rs4903399)* *ESRRB (rs1077430)* *ESRRB (rs676303)* *ESRRB (rs2860216)* *ESRRB (rs1997532)*	27.3% (DF)	Dean's criteria for DF	****		**
Fatturi et al, 2020 (23)	Cross sectional	Brazil	8	731 [90.6%]	Public school	Buccal cells – PCR real time	*VDR (rs739837)* *VDR (rs2228570)*	12.0% (MIH) 5.0% (HPSM)	EAPD criteria for MIH and DDE index (FDI)	****	*	***
Fatturi et al, 2021 (19)	Cross sectional	Brazil	8	731 [90.6%]	Public school	Buccal cells – PCR real time	*HIF1A (rs2057482)* *HIF1A (rs2301113)*	9.4% (HPSM)	DDE index (FDI)	****	*	***
Gerreth et al, 2018 (17)	Case control	Poland	1-4	52 [81.6%]	Primary School	Buccal cells – PCR real time	*AMBN (rs4694075)* *AMELX (rs17878486)* *TUFT1 (rs3790506)* *TFIP11 (rs134136)* *TFIP11 (rs5997096)* *ENAM (3796704)*	11.0% (DDE)	DDE index (FDI)	****	*	***
Hocevar et al, 2020 (25)	Cross sectional	Slovenia	12.3 – 14.1 [13.2]	113 [25.6%]	Hospital	Blood – PCR, polymorphism	*AMBN (rs546778141)* *AMELX (rs2106416)* *AMTN (rs35286445)*	19.5% (MIH)	EAPD criteria for MIH	***		**

						and haplotypes	*AMTN (rs7660807)* *COL14A1 (rs4870723)* *ENAM (rs3796704)* *MMP20 (rs2245803)* *TUFT1 (rs3828054)*					
Huang et al, 2008 (8)	Case control	China	8-12	240 [81.0%]	Community	Blood – PCR, RFLP	*COL1A2 PvuII (rs414408)* *COL1A2 RsaI (rs4266)*	31.2% (DF)	Dean's criteria for DF	***		***
Jeremias et al, 2013 (4)	Cohort	Brazil/ Turkey	5-38	405 [NR]	University dental clinic	Buccal cells – PCR real time	*AMBN (rs4694075)* *ENAM (rs3796704)* *ENAM (rs3796704)* *TFIP11 (rs5997096)* *TFIP11 (rs134136)* *TUFT1 (rs2337360)* *TUFT1 (rs4970957)* *TUFT1 (rs3790506)*	57.7% (MIH)	EAPD criteria for MIH	***		**
Jeremias et al, 2016 (20)	Cross sectional	Brazil	NR [10]	391 [NR]	University dental clinic	Buccal cells – PCR familiar association	*FAM83H (rs7821494)* *AMBN (rs34367704)* *BMP2 (rs3789334)* *BMP7 (rs6099486)* *BMP4 (rs762642)* *ENAM (rs7664896)* *MMP20 (rs1711399)* *MMP20 (rs1711423)* *DLX3 (rs2278163)* *FGFR1 (rs6996321)* *AMELX (rs5979395)*	33.2% (MIH)	EAPD criteria for MIH	***		**
Jiang et al, 2015 (30)	Case Control	China	8-12 [NR]	321 [NR]	Community	Blood – PCR, RFLP	*CTR AluI*	37.7% (DF)	Dean's criteria for DF	***	*	**
Kuchler et al, 2017 (31)	Cross sectional	Brazil	6-18 [9.8 ± 2.7]	481 [98.3%]	University dental clinic	Buccal cells – PCR real time	*DLX1 (rs788173)* *DLX2 (rs743605)* *MMP13 (rs2252070)* *TIMP1 (rs4898)* *TIMP2 (rs7501477)*	22.4% (DF)	Dean's criteria for DF	***		***

Kuchler et al, 2018 (32)	Cohort	Brazil	6-18 [NR]	1017 [NR]	Public and private Schools + University dental clinic	Buccal cells – PCR real time	*AMELX (rs946252)* *AMBN (rs4694075)* *ENAM (rs12640848)* *TFIP11 (rs5997096)* *TUFT1 (rs4970957)*	25.0% (DF)	Dean's criteria for DF	***		**
Kuchler et al, 2020 (48)	Cross sectional	Brazil	4-12 [7.6 ± 2.8]	248 [NR]	University dental clinic	Buccal cells – PCR real time	*RANK (rs3826620)* *RANKL (rs9594738)* *OPG (rs2073618)*	22.2% (DDE)	DDE index (FDI)	***		**
Kuhnisch et al, 2014 (26)	Cohort	Germany	NR [10.2 ± 0.2]	668 [NR]	NR	Blood – PCA analysis	*MIR573 (rs17650401)* *SLC28A3(rs13288553)* *CDH5 (rs1126179)* *NFACT2 (rs4811117)* *SCUBE1 (rs13058467)*	13.2% (MIH)	EAPD criteria for MIH	***		**
Pang et al, 2020 (21)	Cohort	China	12-13 [NR]	430 [NR]	Community	Buccal cells – PCR real time	*DEFB1 (rs11362)* *DEFB1 (rs1800972)* *LTF (rs4547741)* *MBL2 (rs1800450)* *MASP2 (rs10779570)* *AQP5 (rs1996315)*	8.1% (MIH)	EAPD criteria for MIH	****	*	**
Romualdo et al 2019 (33)	Cross sectional	Brazil	6-18 [NR]	481 [NR]	University dental clinic	Buccal cells – PCR real time	*MMP2 (rs243865)* *MMP2 (rs243867)* *MMP9 (rs17576)* *MMP20 (rs1784418)* *MMP20 (rs1711437)*	22.4 % (DF)	Dean's criteria for DF	***		**
Saha et al, 2021(34)	Case control	India	12-15 [NR]	87 [85.3%]	Community	Blood – PCR, RFLP	*ESR1 (rs2234693)* *ESR1 (rs2228480)* *ESR1 (rs3798577)* *ESR1 (rs2077647)* *ESR1 (rs9340799)* *COL1A2 (rs42524)* *COL1A2 (rs412777)* *BGLAP (rs1800247)* *SPARC (rs6579885)* *SPARC (rs4958278)*	41.4% (DF)	Fluorosis Research and Rural Development Foundation criteria	***		***

Wen et al, 2012 (35)	Case control	China	8-12 [NR]	225 [NR]	Community	Blood – PCR, RFLP	*PTH Bst BI*	50.4% (DF)	Dean's criteria for DF	****		**

Note: MIH: Molar-incisor hypomineralization; DF: Dental fluorosis; DDE: developmental defect of enamel; PCR: polymerase chain reaction; RFLP: Restriction Fragment Length Polymorphism; EAPD; European academy of pediatric Dentistry; NR: not reported

Table 3 -Summary of the genes investigated on the developmental defect of enamel.

Gene	Official full name	Locus	Protein Function	Polymorphism	Summary of reported results
AMBN	Ameloblastin	4q13.3	Control the elongation of enamel crystals and direct enamel mineralization.	*rs34367704*	• Significant association with MIH in Transmission/disequilibrium test with family based association (Jeremias et al., 2016)
				rs4694075	• T allele may be protective against DDE in children (17) • The T allele suggesting a protective role against MIH (4) • Allele T increases the risk for DF (32)
				rs546778141	• No association with MIH (25)
				rs13115627	• GG genotype may be protective against MIH in children (21)
AMELX	Amelogenin, X-linked	Xp22.2	Encodes for the structural modeling protein, amelogenin, to direct the mineralization of enamel.	*rs946252*	• No association with MIH (4) • No association with DF (32)
				rs5979395	• 97% of G alleles were transmitted to MIH-affected individuals, (20)
				rs17878486	• Associated with DDE in primary dentition, the rare T allele may be a strong risk variant for DDE in children (17) • No association with MIH (4)
				rs2106416	• No association with MIH (25)
				rs6654939	• Gene interaction association in the IL4 (rs2070874), IL17A (rs2275913), IL10 (rs1800872), IL1A (rs1800587), and STAT1 (rs3771300), in MIH (22)
AMTN	Amelotin	4q13.3	Promote calcium phosphate mineralization, rolling in the formation of the, mineralized and aprismatic enamel.	*rs35286445*	• No association with MIH (25)
				rs7660807	• No association with MIH (25)
BGLAP	Bone gamma-carboxyglutamate protein	1q22	Encodes bone protein secreted by osteoblasts that regulates bone remodeling	*rs1800247*	• No association with DF (34)
BMP2	Bone morphogenetic protein 2	20p12.3	Induce osteoblast differentiation in a variety of cell types.	*rs3789334*	• Significant association with MIH in Transmission/disequilibrium test with family based association. (20)

BMP7	Bone morphogenetic protein 7	20q13.31	Participates in the transformation of mesenchymal cells into bone and cartilage.	*rs6099486*	• Significant association with MIH in Transmission/disequilibrium test with family based association. (20)
BMP4	Bone morphogenetic protein 4	14q22.2	Encodes a secreted ligand of the transforming growth factor-beta proteins.	*rs762642*	• Significant association with MIH in Transmission/disequilibrium test with family based association. (20)
CDH5	Cadherin 5	16q21	Plays a role in endothelial adherent's junction assembly and maintenance.	*rs1126179*	• No association with MIH (26)
COL1A2	Collagen type I alpha 2 chain	7q21.3	Is a fibril-forming collagen found in most connective tissues and is abundant in bone, cornea, dermis and tendon.	*rs42524* *rs412777*	• No association with DF (34) • No association with DF (34)
COL14A1	Collagen type XIV alpha 1 chain	8q24.12	Interacts with the fibril surface and is involved in the regulation of fibrillogenesis.	*rs4870723*	• No association with MIH (25)
CTR	Calcitonin receptor	7q21.3	Binds the peptide hormone calcitonin and is involved in maintenance of calcium homeostasis.	*Alu I*	• One of the genetic components associated with DF (30)
DEFB1	Defensin beta 1	8p23.1	Encodes defensin, beta 1, an antimicrobial peptide implicated in the resistance of epithelial surfaces to microbial colonization.	*rs1800972* *rs11362*	• The C allele and CC genotype were associated with MIH cases (21) • No association with MIH (21)
DLX1	Distal-less homeobox 1	2q31.1	The encoded protein plays a role in craniofacial patterning.	*rs788173*	• Significant association with DF (31)
DLX2	Distal-less homeobox 2	2q31.1	Plays a role in craniofacial patterning and morphogenesis.	*rs743605*	• Significant association with DF (31)
DLX3	Distal-less homeobox 3	17q21.33	Regulates hair follicle differentiation and cycling.	*rs2278163*	• Significant association with MIH in Transmission/disequilibrium test with family based association. (20)
ENAM	Enamelin	4q13.3	Encodes the largest protein in the enamel matrix of developing teeth, involved in the mineralization and structural organization of enamel.	*rs7664896* *rs12640848* *rs3796704*	• Significant association with MIH in Transmission/disequilibrium test with family based association. (20) • No association with DF (32) • No association with DDE (17) • 17-fold more likely to be affected by MIH. (25) • Associated with MIH severity, since the G allele was more frequent in the severe MIH subgroup (4)

ESR1	Estrogen receptor 1	6q25.1	Plays a role in growth, metabolism, sexual development, gestation, and other reproductive functions.	*XbaI (rs9340799)*	• Children carrying X allele had a significantly decreased risk of DF (28) • No association with DF (29) • No association with DF (34)
				RsaI	• Children who carried R allele had a significantly increased risk of DF (28) • No association with DF (8)
				PvuII (rs2234693)	• No association with DF (28) • Children who carried homozygous P allele had a significantly increased risk of DF (HUANG 2008) • No association with DF (29) • No association with DF (34)
				rs1884051	• No association with DF (29) • No association with DDE (18)
				rs12154178	• The CC allele is a protective factor for DF (29) • Significant association with DDE (18)
				rs2228480	• No association with DF (34)
				rs3798577	• No association with DF (34)
				rs2077647	• No association with DF (34)
ESR2	Estrogen receptor 2	14q23.2	Transduce extracellular signals into transcriptional responses.	*rs4986938*	• No association with DF (29)
				rs1256049	• No association with DF (29)
ESRB	Estrogen related receptor beta	14q24.3	Encodes a protein with similarity to the estrogen receptor.	*rs745011*	• No association with DF (29)
				rs6574293	• No association with DF (29)
				rs10132091	• No association with DF (29)
				rs4903399	• No association with DF (29)

				rs1077430	• No association with DF (29)
				rs1676303	• No association with DF (29)
				rs2860216	• No association with DF (29)
				rs1997532	• No association with DF (29)
FAM83H	Family with sequence similarity 83 member H	8q24.3	Plays an important role in the structural development and calcification of tooth enamel.	*rs7821494*	• Significant association with MIH in Transmission/disequilibrium test with family based association. (20)
FGFR1	Fibroblast growth factor receptor 1	8p11.23	Critical for the truncation of embryonic structures and formation of muscle and bone tissues.	*rs6996321*	• Significant association with MIH in Transmission/disequilibrium test with family based association. (20)
GHR	Growth hormone receptor	5p13.1	This gene encodes a protein that is a trans membrane receptor for growth hormone	*rs2973015*	• No association with DDE (18)
				rs2940913	• No association with DDE (18)
				rs2910875	• No association with DDE (18)
				rs1509460	• Significant association with DDE (18)
HIF1A	Hypoxia inducible factor 1 alpha subunit	14q23.2	Regulator of cellular and systemic homeostatic response to hypoxia.	*rs2057482*	• Individuals who carried at least one C allele had a lower occurrence of HPSM (19)
				rs2301113	• No association with HPSM (19)
IL1A	Interleukin1 alpha	2q14.1	Involved in various immune responses, inflammatory processes, and hematopoiesis.	*rs1800587*	• No association with MIH (22)
IL1B	Interleukin1 beta	2q14.1	Involved in modulation of autoimmune inflammation.	*rs1143634*	• No association with MIH (22)
IL4	Interleukin 4	5q31.1	Mediates and regulates a variety of human host responses such as allergic and acute inflammation.	*rs2070874*	• Significant association with the SNPs in the TUFT1 (rs7526319) and BMP2 (rs2355767) genes in MIH. (22)

IL6	Interleukin 6	7p15.3	The protein is primarily produced at sites of acute and chronic inflammation.	*rs1800795*	• No association with MIH (22)
IL8	Interleukin 8	4q13.3	It induces chemo taxis in target cells, primarily neutrophils and other granulocytes.	*rs4073*	• No association with MIH (22)
IL10	Interleukin 10	1q32.1	Effects in immunoregulation and inflammation.	*rs1800872*	• No association with MIH (22)
IL17A	Interleukin 17A	6p12.2	Mediated downstream pathways induce the production of inflammatory molecules.	*rs2275913*	• No association with MIH (22)
IRF6	Interferon regulatory factor 6	1q32.2	Related to the formation of connective tissue, for example that of the palate.	*rs17015215*	• Association with MIH (24)
				rs2013162	• Association with MIH (24)
				rs861019	• Association with MIH (24)
				rs2073487	• Interaction with TGFA rs2902345 in MIH (24)
				rs642961	• Association with MIH (24)
KCNG1	Potassium voltage-gated channel modifier subfamily G member 1	20q13.13	Regulates neurotransmitter release, heart rate, insulin secretion and neuronal excitability.	*rs4811117*	• No association with MIH (26)
LTF	Lactotransferrin	3p21.31	Regulation of iron homeostasis and cellular growth	*rs4547741*	• No association with MIH (21)
MASP2	MBL associated serine protease 2	1p36.22	Plays a role in the coagulation cascade through cleavage of prothrombin to form thrombin.	*rs10779570*	• No association with MIH (21)
MBL2	Mannose binding lectin 2	10q21.1	Calcium-dependent lectin involved in innate immune defense,	*rs1800450*	• No association with MIH (21)
MIR17HG	miR-17-92a-1 cluster host gene	13q31.3	Involved in cell survival, proliferation, differentiation, and angiogenesis.	*rs4284505*	• Associated with an increased risk of DF. (27)
MMP2	Matrix metallopeptidase 2	16q13	Involved in remodeling of the vasculature, angiogenesis, tissue repair, tumor invasion, inflammation, and atherosclerotic plaque rupture	*rs243865*	• No association with DF (33)
				rs243867	• No association with DF (33)

MMP9	Matrix metallopeptidase 9	20q1-q13	Play a role in bone osteoclastic resorption	*rs17576*	• No association with DF (33)
MMP13	Matrix metallopeptidase 13	11q22.2	Involved in the embryonic development, reproduction, and tissue remodeling.	*rs2252070*	• No association with DF (31)
MMP20	Matrix metallopeptidase 20	11q22.2	The protein encoded by this gene degrades amelogenin, the major protein component of dental enamel matrix, and thus thought to play a role in tooth enamel formation.	*rs1711399*	• Significant association with MIH in Transmission/disequilibrium test with family based association. (20)
				rs1711423	• Significant association with MIH in Transmission/disequilibrium test with family based association. (20)
				rs2245803	• Related to a higher probability of MIH development. (25)
				rs1784418	• The TT genotype was significantly associated with MIH (21)
OPG	Osteoprotegerin	8q24.12	Plays an important role in bone metabolism inhibiting osteoclast genesis and bone resorption.	*rs2073618*	• No association with DDE (48)
PPARGC1A	PPARG coactivator 1 alpha	4p15.2	Is a transcriptional coactivator that regulates the genes involved in energy metabolism.	*rs17650401*	• No association with MIH (26)
PTH	Parathyroid hormone	11p15.3	Parathyroid hormone-related peptide receptor regulates blood calcium and phosphate levels.	*Bst BI*	• No association with DF (35)
RANK	Receptor activator of nuclear factor kappa-B	18q21.33	Related to osteoclast genesis and differentiation and activation of osteoclasts pathways.	*rs3826620*	• No association with DDE (48)
RANK L	Receptor activator of nuclear factor kappa-B ligand	13q14.11	Functions as a key factor for osteoclast differentiation and activation.	*rs9594738*	• No association with DDE (48)
RMI1	RecQ mediated genome instability 1	9q21.32	Plays an important role in the processing of homologous recombination intermediates to limit DNA crossover formation in cells.	*rs13288553*	• No association with MIH (26)
SPARC	Secreted protein acidic and cysteine rich	5q33.1	The encoded protein is required for the collagen in bone to become calcified.	*rs6579885*	• No association with DF (34)
				rs4958278	• No association with DF (34)
STAT	Signal transducer and activator of transcription 1	2q32.2	Has a key role in many gene expressions that cause survival of the cell.	*rs3771300*	• No association with MIH (22)

TFIP11	Tuftelin interacting protein 11	22q12.1	Play a role in the differentiation of ameloblasts and odontoblasts or in the forming of the enamel extracellular matrix.	*rs5997096*	• No association with DDE (17) • TT genotype marker was seen in cases with severe MIH diagnosis (4) • Significant association with DF (32)
				rs134136	• No association with DDE (17)
TGFA	Transforming growth factor alpha	2p13.3	Encodes a growth factor which activates a signaling pathway for cell proliferation, differentiation and development.	*rs2166975*	• Association with MIH (24)
				rs1523305	• Interaction with IRF6 rs642961 in MIH (24)
				rs2902345	• Association with MIH (24)
				rs930655	• Association with MIH (24)
TGFB1	Transforming growth factor beta 1	19q13.2	Regulates cell proliferation, differentiation and growth.	*rs1800470*	• No association with MIH (22)
TGFBR1	Transforming growth factor beta receptor 1	9q22.33	The encoded protein is a serine/threonine protein kinase.	*rs10733708*	• Severe MIH cases associated with the G allele (22)
TIMP1	TIMP metallopeptidase inhibitor 1	Xp11.23	Natural inhibitors of the matrix metalloproteinase, involved in degradation of the extracellular matrix.	*rs4898*	• Significant association with DF (31)
TIMP2	TIMP metallopeptidase inhibitor 2	17q25.3	Are natural inhibitors of the matrix metalloproteinase, involved in degradation of the extracellular matrix.	*rs7501477*	• No association with DF (31)
TNF	Tumor necrosis factor	6p21.33	Is involved in processes of cell proliferation, differentiation and apoptosis.	*rs1800629*	• No association with MIH (22)
TTLL12	Tubulin tyrosine ligase like 12	22q13.2	Plays a role in mitosis and in maintaining chromosome number stability	*rs13058467*	• Association with MIH (26)
TUFT1	Tuftelin 1	1q21.3	Plays a role in dental enamel mineralization. It is also thought to be involved with adaptation to hypoxia and mesenchymal stem cell function.	*rs4970957*	• Significant association with protection against MIH (4) • The A allele increases the risk for DF (32)
				rs3790506	• No association with DDE (17) • Significant association with protection against MIH (4)
				rs3828054	• No association with MIH (25)

VDR	Vitamin D receptor	12q13.11	Vitamin D3 receptor are principally involved in mineral metabolism.	*rs739837* *rs2228570*	Children with the GT genotype had a higher prevalence of MIH. No association with HPSM (23) • No association with MIH and HPSM (23)

Table 4 -Summary of the genes associated with developmental defect of enamel.

DDE	Gene	rs associated to DDE
DF	*AMBN*	*rs4694075* (32)
	CTR	*Alu I* (30)
	ESR1	*XbaI (rs9340799)* (28) *RsaI* (28) *PvuII (rs2234693) (8)* *rs12154178* (29)
	MIR17HG	*rs4284505* (27)
	TFIP11	*rs5997096* (32)
	TIMP1	*rs4898* (31)
	TUFT1	*rs4970957* (32)
DDE**	*AMBN*	*rs4694075* (17)
	AMELX	*rs17878486* (17)
	ESR1	*rs12154178* (18)
HPSM	*HIF1A*	*rs2057482* (19)
MIH	*AMBN*	*rs34367704* (20) *rs4694075* (4) *rs13115627* (21) *rs5979395* (20) *rs6654939* (22)
	AMLX	*rs5979395* (20) *rs6654939* (22) *Gene interaction association in the *IL4 - rs2070874, IL17A - rs2275913, IL10 - rs1800872, IL1A - rs1800587, and STAT1 -rs3771300*
	BMP2	*rs3789334* (20)
	BMP7	*rs6099486* (20)
	BMP4	*rs762642* (20)
	DEFB1	*rs1800972* (21)
	DLX3	*rs2278163* (20)
	ENAM	*rs7664896* (20) *rs3796704* (4,25)
	FAM83H	*rs7821494* (20)

FGFR1	*rs6996321* (20)
IL4	*rs2070874* (22) *Gene interaction with the SNPs in the *TUFT1 - rs7526319* and *BMP2 - rs2355767*
IRF6	*rs17015215* (24) *rs2013162* (24) *rs861019* (24) *rs2073487* (24) *Interaction with *TGFA rs2902345* *rs642961* (24)
MMP20	*rs1711399* (20) *rs1711423* (20) *rs2245803* (25) *rs1784418* (21)
TFIP11	*rs5997096* (4)
TGFA	*rs2166975* (24) *rs1523305* (24) *Gene Interaction with *IRF6 rs642961* *rs2902345* (24) *rs930655* (24)
TGFBR1	*rs10733708* (22)
TUFT1	*rs4970957* (4) *rs3790506* (4)
VDR	*rs739837* (23)

Note: MIH: Molar-incisor hypomineralization; DF: Dental fluorosis; DDE: developmental defect of enamel; HPSM: hypomineralization of Primary second molars

**DDE composed by demarcated and diffuse hypomineralization and hypoplasia, according to FDI DDE index.

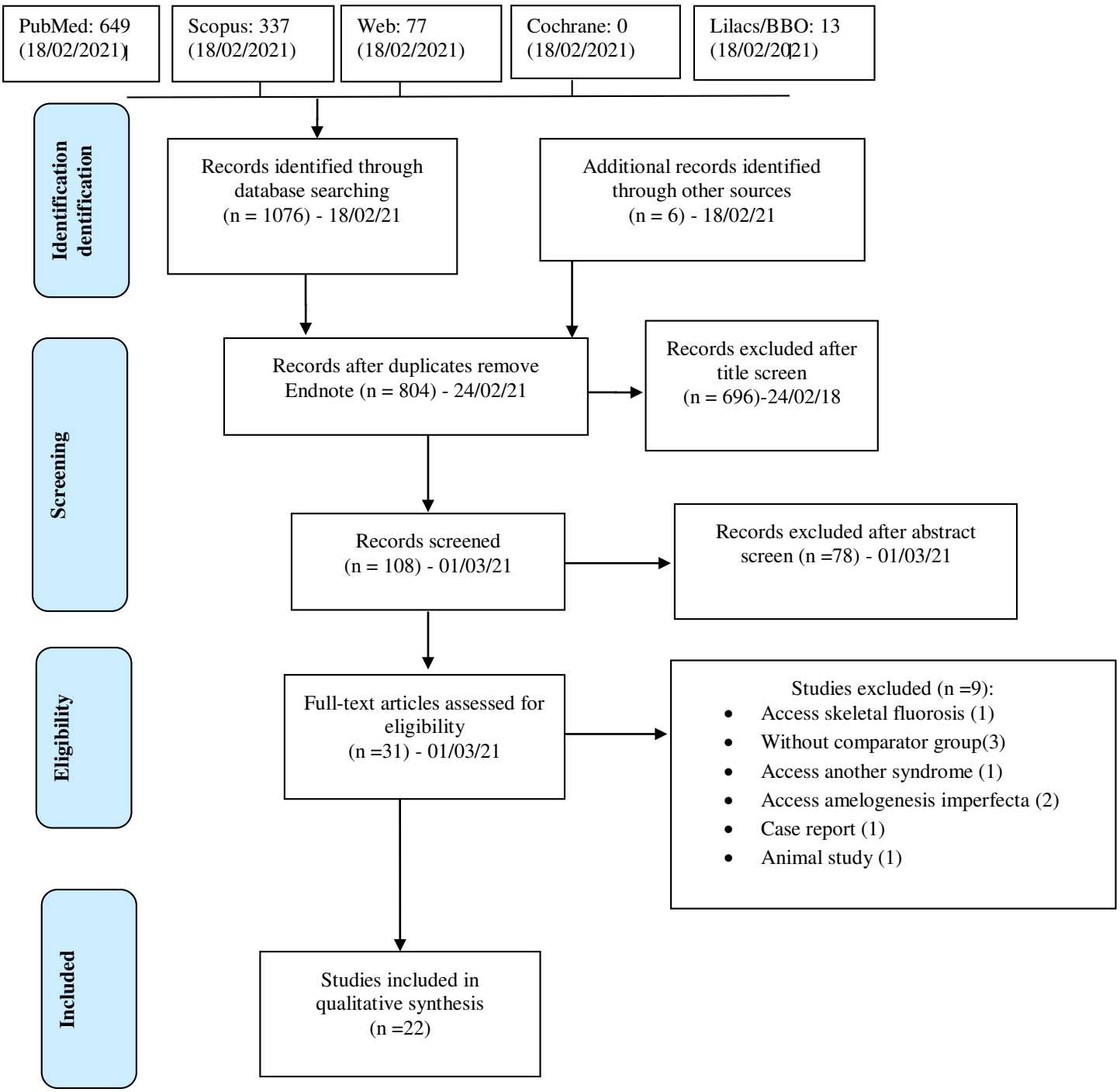

Figure 1 – Flow diagram of study.

5. CONSIDERAÇÕES FINAIS

Quanto à HSMD, baseado no estudo epidemiológico observacional observou-se que crianças expostas aos fatores ambientais relacionados à hipóxia apresentaram maior prevalência de HSMD. Ademais, crianças que apresentam o alelo C no *rs2057482* apresentaram uma prevalência menor baixa de HSMD na presença de fatores ambientais relacionados à hipóxia, do que crianças com alelo T. Esses achados sugerem uma possível interação entre fatores ambientais e genéticos relacionados à hipóxia na etiologia dos HSMD.

Com base no estudo de revisão sistemática podemos concluir que DDE foi significativamente associado a polimorfismos genéticos em 23 genes, cujas proteínas estão relacionadas ao desenvolvimento do esmalte (*AMBN, AMELX, AMTN, ENAM, FAM83H, MMP, TFIP, TUFT*), crescimento e morfogênese craniofacial (*DLX, IRF, BGLAP, BMP, COL, CTR, FGFR, OPG, RANK*), resposta imune (*IL, MBL, DEFB*), e transcrição/ recepção hormonal (*ESR, GHR, PTH*).

Diante desses estudos, nota-se que os DDEs estão associados às exposições sistêmicas (fatores ambientais), bem como a polimorfismos genéticos, e portanto, evidenciando uma etiologia multifatorial com complexas interações entre os eventos. Sugere-se que estudos prospectivos e experimentos em animais são necessários compreender de forma profunda a patogênese dos diversos DDE.

6. REFERÊNCIAS

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7. APÊNDICES

APÊNDICE 1 – TLCE

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Nós, Juliana Feltrin de Souza e Magdalena Raquel Torres Reys da Universidade Federal do Paraná, estamos convidando **VOCÊ pai/mãe responsável e SEU (SUA) FILHO** escolares matriculados nas escolas estaduais de Curitiba a participar de um estudo intitulado **"Hipomineralização Molar-Incisivo em uma população sul-brasileira: Prevalência, fatores sistêmicos associados e polimorfismos genéticos"**. Este estudo é importante para que possamos avaliar alterações no esmalte dentário, cárie dentária e suas possíveis causas.

1. O objetivo desta pesquisa é observar a frequência de crianças afetadas pela Hipomineralização (defeito no esmalte dentário), cárie dentária e avaliar as possíveis causas.
2. Caso você participe da pesquisa, será necessário preencher um questionário, que será enviado a sua casa e seu(sua) filho(a) participará de um exame bucal realizado no ambiente escolar, onde seu filho(a) estuda, em que será realizado também a coleta de saliva do mesmo, para avaliar as possíveis causas genéticas dos defeitos de esmalte. O exame bucal e coleta de saliva terão a duração entre 5 e 10 minutos. Para tanto você deverá enviar o questionário preenchido à escolar do seu filho.
3. O exame bucal é como qualquer exame odontológico de rotina, mas o exame não terá nenhuma consequência para seu filho(a). Se seu filho(a) mostrar-se contrário(a) à realização do exame ou constrangido(a), este será interrompido imediatamente. Se você apresentar algum constrangimento ao preencher o questionário, mesmo preenchendo em sua casa, poderá deixar a resposta em branco. O uso das amostras de saliva serão unicamente para essa pesquisa e salienta que logo após essa análise, as amostras de saliva serão descartadas de forma apropriada.
4. Dentre os benefícios diretos da pesquisa, caso de seu(sua) filho(a) apresentar alguma alteração na boca que indique tratamento, você será informado(a) e orientado(a) a levar seu(sua) filho(a) para buscar atendimento nas clínicas de Odontologia da Universidade Federal do Paraná.
5. A sua participação e de seu (sua) filho(a) neste estudo é voluntária, e se você não quiser mais fazer parte da pesquisa poderá desistir a qualquer momento. As informações relacionadas ao estudo poderão ser conhecidas por pessoas autorizadas (professora orientadora e aluna). O material obtido – questionários e ficha do exame clínico odontológico– será utilizado unicamente para esta pesquisa e será incinerado após 03 anos do término do estudo. As amostras de saliva serão descartadas de forma apropriada como material biológico logo após a análise.
6. Os pesquisadores responsáveis por este estudo poderão ser localizados no Campus Botânico da Universidade Federal do Paraná (Av. Prefeito Lothário Meissner, 632 - Jardim Botânico, Curitiba/PR, CEP 80210-170), no Departamento de Estomatologia ou pelo e-mail: julianafeltrin@hotmail.com e/ou magdalentorres@hotmail.es, para esclarecer eventuais dúvidas que você possa ter e fornecer-lhe as informações que queira, antes, durante ou depois de encerrado o estudo. Se você tiver dúvidas sobre seus direitos como participante de pesquisa, você pode contatar também o Comitê de Ética em Pesquisa em Seres Humanos (CEP/SD) do Setor de Ciências da Saúde da Universidade Federal do Paraná, pelo telefone (41)3360-7259.

Eu,_____, li esse Termo de Consentimento e compreendi a natureza e objetivo do estudo do qual EU concordei em participar. Eu também concordo com a participação DO(A) MEU FILHO(A) _____ neste estudo. A explicação que recebi menciona os riscos e benefícios. Eu entendi que sou livre para interromper MINHA participação e a participação do(a) MEU FILHO(A) a qualquer momento, sem justificar minha decisão e sem qualquer prejuízo para mim nem para meu filho(a). EU concordo voluntariamente em participar deste estudo e consinto a participação voluntária do(a) meu FILHO(A).

Curitiba, ____ de _____ de _____.

_____ Assinatura do pai / responsável

_____ Assinatura do pesquisador responsável

Responsável legal pelo participante da pesquisa _____
Pesquisador responsável ou quem aplicou o TCLE _____
Orientador:

Comitê de Ética em Pesquisa com Seres Humanos do Setor de Ciências da Saúde da UFPR | CEP/SD Rua Padre Camargo, 285 | térreo | Alto da Glória | Curitiba/PR | CEP 80060-240 | cometica.saude@ufpr.br - telefone (041) 3360-7259

APENDICE 2 – Questionário eventos sistêmicos

Para completar os dados da pesquisa, precisamos que você responda às questões abaixo. **Não existe resposta certa ou errada, mas a resposta verdadeira é muito importante para que se conheça a realidade das crianças** (marque X).

Nome da criança:_____

Data de nascimento da criança:____/___/___

Qual o gênero da Criança? **( ) Feminino** **() Masculino**

Qual a etnia da Criança? **( ) Branca** **( ) Negra** **( ) Amarela** **( ) Indígena** **( ) Parda**

Qual o seu nome:_____**Qual é a sua idade?** _____

Qual o seu endereço:_____**CEP**_____**Qual o bairro?**_____

Qual o seu telefone para contato? _____

- **Qual a sua relação com a criança?**

( ) Sou a mãe
( ) Sou o pai
( ) Outro: Qual?_____

- **Qual o seu estado civil?**

( ) Solteira(o)
( ) Separada(o)
( ) Viúva(o)
( ) Casada(o) ou relação estável (Morando junto há 5 anos)

- **A criança mora com você?**

( ) Sim
( ) Não

- **Quantas pessoas moram na mesma casa da criança (número total de moradores)?** _____

- **Qual é a renda mensal da sua casa?**

R$_____(reais)
(Incluir o total da casa: salários mínimos, Bolsa Família, Seguro desemprego e "bicos" de todos os moradores da sua casa.)

Vamos falar um pouco sobre VOCÊ:

- **VOCÊ trabalha**:

( ) Em casa/Aposentado ( ) Fora de casa

- **VOCÊ estudou até qual série?**

( ) Não estudou
( ) Primário(1ª a 4ª série) incompleto
( ) Primário (1ª a 4ª série) completo
( ) Ginasial (5ª a 8ª série) incompleto
( ) Ginasial (5ª a 8ª série) completo
( ) Colegial (ensino médio) incompleto
( ) Colegial (ensino médio) completo
( ) Superior (faculdade) incompleto
( ) Superior (faculdade) completo

- **Mãe/ pai ou responsável, quando foi a SUA última visita ao dentista?**

( ) Há menos de 1 mês
( ) De 1 a 6 meses
( ) De 6 meses a 1 ano
( ) Há mais de 1 ano
( ) Nunca foi
( ) Não lembra

- **Por que VOCÊ procurou o dentista pela última vez?**

( ) Para consulta preventiva
( ) Para resolver algum problema ou dor
( ) Nunca foi ao dentista

- **Como você considera a situação da saúde de SUA boca e de seus dentes?**

( ) Boa
( ) Razoável
( ) Ruim

- **VOCÊ teve dor de dentes nos últimos 6 meses?**

( ) Sim
( ) Não

- **Se VOCÊ tiver que ir ao dentista amanhã, como você se sentiria?**

( ) Eu estaria esperando uma experiência razoavelmente agradável
( ) Eu não me importaria
( ) Eu me sentiria ligeiramente desconfortável
( ) Eu acho que eu me sentiria desconfortável e teria dor
( ) Eu estaria com muito medo do que o dentista me faria

- **Quando VOCÊ está esperando na sala de espera do dentista, como você se sente?**

( ) Relaxado
( ) Meio desconfortável
( ) Tenso
( ) Ansioso
( ) Tão ansioso que começo a suar ou começo a me sentir mal

- **Quando VOCÊ está na cadeira odontológica esperando o dentista preparar o motor para trabalhar nos seus dentes, como você se sente?**

( ) Relaxado
( ) Meio desconfortável
( ) Tenso
( ) Ansioso
( ) Tão ansioso que começo a suar a me sentir mau

- **Você está na cadeira odontológica. Enquanto você aguarda o dentista pegar os instrumentos para raspar os seus dentes (perto da gengiva), como você se sente?**

( ) Relaxado
( ) Meio desconfortável
( ) Tenso
( ) Ansioso
( ) Tão ansioso que começo a suar ou começo a me sentir mal

<u>Agora vamos falar um pouco sobre seu Filho(a)</u>

- **Seu FILHO(A) já foi ao dentista alguma vez**?

( ) Sim
( ) Não
( ) Não Lembro

- **Se sim, onde?**

( ) Serviço público
( ) Serviço particular
( ) Serviço Público e também no Particular
( ) Nunca foi ao dentista

- **Você acha que o seu FILHO(A) tem medo de ir ao dentista?**

( ) Não tem medo
( ) Um pouco de medo
( ) Tem medo
( ) Sim, muito medo

- **O que você acha da saúde bucal do seu FILHO(A)?**

( ) Ruim
( ) Razoável
( ) Boa
( ) Não sei

- **Quantas vezes seu FILHO(A) escova os dentes por dia?**

( ) Nenhuma
( ) Uma
( ) Duas
( ) Mais de duas

- **O seu FILHO(A) já teve dor de dente?**

( ) Sim
( ) Não
( ) Não Lembro

- **Você fez alguma coisa para o aliviar a dor de dente do seu FILHO(A)?**

( ) Não
( ) Sim, dei um remédio que tinha em casa
( ) Sim, levei ao dentista
( ) Nunca teve dor de dente

- **Seu FILHO(A) já faltou à escola / CMEI porque estava com dor de dente?**

( ) Sim
( ) Não
( ) Não Lembro

- **O que VOCÊ já parou de fazer para estar com seu filho(a) que estava com dor de dente, que você considera mais relevante?**

( ) Faltei ao trabalho
( ) Parei de fazer tarefas domésticas
( ) Deixei de cuidar de outra criança
( ) Outras atividades
( ) Meu filho(a) nunca teve dor de dente

- **Quando foi a ultima vez que seu filho (a) foi ao dentista?**

( ) Menos de 1 ano
( ) Entre 1 e 3 anos
( ) Mais que 3 anos
( ) Nunca foi

- **Nessa última consulta por qual motivo você levou seu filho (a) ao dentista?**

( ) Para consulta preventiva
( ) Para resolver algum problema ou dor
( ) Nunca foi ao dentista
( ) Não sei ou não Lembro

- **Alguma vez você precisou de algum tratamento odontológico e não teve como pagar por este tratamento ou não conseguiu vaga para este atendimento na rede pública?**

( ) Sim
( ) Não
( ) Não Lembro

<u>Queremos saber sobre a história médica do seu filho(a). Tente recordar se durante a GESTAÇÃO a Mãe da criança teve alguns desses problemas:</u>

- **Episódios Múltiplos de Febre alta?**

( ) Sim
( ) Não
( ) Não Lembro

- **Virose?**

( ) Sim
( ) Não
( ) Não Lembro

- **Tomou remédios por tempo prolongado?**

( ) Sim, qual?_____
( ) Não
( ) Não Lembro

- **Diabetes?**

( ) Sim
( ) Não
( ) Não Lembro

- **Vômitos Prolongados?**

( ) Sim
( ) Não
( ) Não Lembro

- **Má nutrição?**

( ) Sim
( ) Não
( ) Não Lembro

- **Anemia?**

( ) Sim
( ) Não
( ) Não Lembro

- **Varicela (catapora)?**

( ) Sim
( ) Não
( ) Não Lembro

- **Pressão alta?**

( ) Sim
( ) Não
( ) Não Lembro

- **Consumo de álcool?**

( ) Sim
( ) Não
( ) Não Lembro

- **Consumo de cigarro?**

( ) Sim
( ) Não
( ) Não Lembro

- **Consumo de drogas Ilícitas?**

( ) Sim
( ) Não
( ) Não Lembro

- **Outros problemas ?**

( ) Não
( ) Sim, Qual_____

<u>Tente recordar sobre o PARTO, do seu filho(a)</u>

- **O parto de seu filho(a) foi**

( ) Natural
( ) Cesariana

- **Foi necessário usar fórceps?**

( ) Sim
( ) Não

- **O parto foi prolongado?**

( ) Sim, Quantas horas?_____
( ) Não

- **Houveram complicações no parto?**

( ) Sim, Qual? _____
( ) Não

- **Foram Gêmeos?**

( ) Sim
( ) Não

- **O Nascimento foi prematuro?**

( ) Sim, Quantas semanas?_____
( ) Não

- **Seu filho nasceu com qual peso?**

Nasceu com: _____
( ) Não Lembro

- **Outros problemas ?**

( ) Não
( ) Sim, Qual_____

<u>Tente recordar sobre o PRIMEIRO MÊS DE VIDA seu filho(a) teve alguns desses problemas</u>

- **Teve que ficar na Incubadora?**

( ) Sim, Quantos dias?_____
( ) Não
( ) Não Lembro

- **Problemas cardíacos?**

( ) Sim
( ) Não
( ) Não Lembro

- **Icterícia (amarelão)?**

( ) Sim
( ) Não
( ) Não Lembro

- **Diabetes?**

( ) Sim
( ) Não
( ) Não Lembro

- **Vômito Prolongado?**

( ) Sim
( ) Não
( ) Não Lembro

- **Anemia?**

( ) Sim
( ) Não
( ) Não Lembro

- **Varicela (catapora)**

( ) Sim
( ) Não
( ) Não Lembro

- **Outros problemas ?**

( ) Não
( ) Sim, Qual_____

Tente recordar sobre TRÊS PRIMEIROS ANOS DE VIDA seu filho(a) teve alguns desses problemas

- **Episódios múltiplos de febre alta?**

( ) Sim
( ) Não
( ) Não Lembro

- **Infecção no ouvido?**

( ) Sim
( ) Não
( ) Não Lembro

- **Bronquite?**

( ) Sim
( ) Não
( ) Não Lembro

- **Asma?**

( ) Sim
( ) Não
( ) Não Lembro

- **Pneumonia?**

( ) Sim
( ) Não
( ) Não Lembro

- **Infecção de garganta?**

( ) Sim
( ) Não
( ) Não Lembro

- **Uso de Antibiótico?**

( ) Sim
( ) Não
( ) Não Lembro
Se sim, quantas vezes?
( ) 1vez ( ) 3vezes ( ) 5vezes ( ) mais 10 de vezes

Qual antibiótico?_____

- **Uso de outra medicação?**

( ) Sim, Qual?_____
( ) Não
( ) Não Lembro

- **Convulsão?**

( ) Sim
( ) Não
( ) Não Lembro

- **Infecção Urinária?**

( ) Sim
( ) Não
( ) Não Lembro

- **Anemia?**

( ) Sim
( ) Não
( ) Não Lembro

- **Má Nutrição?**

( ) Sim
( ) Não
( ) Não Lembro

- **Outros problemas ?**

( ) Não
( ) Sim, Qual?_____

- **Seu filho é celíaco (intolerante ao glúten)?**

( ) Sim
( ) Não
( ) Não Lembro

- **Seu filho tem alguma intolerância alimentar?**

( ) Sim, Qual? _____
( ) Não
( ) Não Lembro

Muito Obrigada pela sua participação!!

UFPR

Examinador:_____Anotador: _____

Escola:_____Classe:_____Data:_____/___/___

Nome:_____Idade:_____Gênero:_____Raça ( ) Branca ( ) Negra ( ) Asiática ( ) Indígena

	17	16	55 / 15	54 / 14	53 / 13	52 / 12	51 / 11	61 / 21	62 / 22	63 / 23	64 / 24	65 / 25	26	27
ceo-d / CPO-D														
DDE														
HMI														
HMI														
DDE														
ceo-d / CPO-D														
	46	46	45 / 85	44 / 84	43 / 83	42 / 82	41 / 81	31 / 71	32 / 72	33 / 73	34 / 74	35 / 75	36	37

c	e	o	ceo-d

C	P	O	CPO-D

ÍNDICE DE CÁRIE		
CPO	*CEO*	
0	A	HÍGIDO
1	B	CARIADO
2	C	REST. + CÁRIE
3	D	REST. SEM CÁRIE
4	E	PERDIDO CÁRIE
5	F	PERDIDO OUTRA RAZÃO
6	G	SELANTE
8	I	Ñ ERUPCIONADO
9	J	EXCLUÍDO
T	T	TRAUMA

HMI			
1. MANCHAS	1 – branca	2- amarela	3-marrom
2. PERDA ESTRUTURA	1- apenas esmalte	2 – esmlate e dentina	
3. RESTAURAÇÃO ATÍPICA	1- satisfatória	2- insatisfatória	
4. EXODONTIA POR HMI			

DDE
0 NORMAL
1 OPACIDADE DEMARCADA
2 OPACIDADE DIFUSA
3 HIPOPLASIA
4 OUTRO
5 OP. DEMARCADA + DIFUSA
6 OP. DEMARCADA + HIPOPLASIA
7 OP. DIFUSA + HIPOPLASIA
8 TODAS AS 3
9 NÃO REGISTRADO

Extensão do DDE	Escore
Normal	0
Menor que 1/3	1
Até 2/3	2
Mais 2/3	3

Má oclusão	
Classe	
Mordida Aberta Ant	
Mordida Cruzada Ant	
Mordida Cruzada Post	
Mordida Profunda	
Sobressaliência Excessiva	
Apinhamento Ant	

Critérios Má Oclusão:
1-Mordida Aberta: Ausência de trespasse vertical cobrindo os incisivos inf
2-Mordida Cruzada Ant: Dois ou mais incisivos superiores ocluindo lingualmente em relação aos incisivos inferiores
3-Mordida Cruzada Post: 2 ou mais molares superiores ocluindo lingualmente em relação aos molares inferiores
4-Mordida Profunda: Trespasse vertical com o incisivo superior cobrindo metade ou mais do compr. Total do incisivo inferior
5-Sobressaliência Excessiva: Distância entre a vestibular do inci. Supe/inf mais que 4mm
6-Apinhamento ant: falta de espaço menor do que a metade do diâmetro medio-distal de um incisivo.

9. ANEXOS

ANEXO 1 – Parecer Consubstanciado do CEP

UNIVERSIDADE FEDERAL DO PARANÁ - SETOR DE CIÊNCIAS DA SAÚDE/ SCS -

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Hipomineralização Molar-Incisivo em uma população sul-brasileira: Prevalência, fatores sistêmicos associados e polimorfismos genéticos

Pesquisador: Juliana Feltrin de Souza Caparroz

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 3

CAAE: 56829516.0.0000.0102

Instituição Proponente: Departamento de Estomatologia

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.689.362

Apresentação do Projeto:

Trata-se de estudo que visa estudar a presença de hipomineralização molar-incisivo em uma população de 960 crianças matriculadas em escolas da rede municipal de ensino de Curitiba. O estudo tem como pesquisadora a Professora Juliana Feltrin de Souza Caparroz e uma equipe de pesquisa composta por Magdalena Raquel Torres Reyes, João Armando Brancher,
Luciana Reichert da Silva Assunção,Lupe Furtado Alle. O estudo será realizado com crianças de oito anos de idade, nas escolas municipais de Curitiba. ALém da coleta de amostras da mucosa bucal para estudo de polimorfismos genéticos e exame bucal para avaliação das condições de saúde bucal das crianças, também será utilizado um questionário a ser aplicado aos pais das crianças a fim de obter informações pertinentes á pesquisa em tela. O estudo terá a duração de 30 meses a partir da aprovação pelo CEP/SD.

Objetivo da Pesquisa:

Objetivo Geral: avaliar a prevalência da Hipomineralização Molar-Incisivo e de hipomineralização em dentes decíduos na população de Curitiba –PR, bem como avaliar a associação dessas condições com cárie dentária, fatores etiológicos sistêmicos, polimorfismos genéticos e o impacto na qualidade de vida das crianças portadoras. Além disto a pesquisadora responsável informa que

Endereço: Rua Padre Camargo, 285 - Térreo
Bairro: Alto da Glória **CEP:** 80.060-240
UF: PR **Município:** CURITIBA
Telefone: (41)3360-7259 **E-mail:** cometica.saude@ufpr.br

UNIVERSIDADE FEDERAL DO PARANÁ - SETOR DE CIÊNCIAS DA SAÚDE/ SCS -

Continuação do Parecer: 1.689.362

Outros	Modelo2_encaminhamento_ATA.pdf	17:05:15	Souza Caparroz	Aceito
Folha de Rosto	folhaDeRosto.pdf	29/04/2016 16:09:21	Juliana Feltrin de Souza Caparroz	Aceito
Declaração de Pesquisadores	Carta_encaminhamento_projetoCEP.pdf	28/04/2016 17:09:57	Juliana Feltrin de Souza Caparroz	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CURITIBA, 22 de Agosto de 2016

Assinado por:
IDA CRISTINA GUBERT
(Coordenador)

Endereço: Rua Padre Camargo, 285 - Térreo
Bairro: Alto da Glória **CEP:** 80.060-240
UF: PR **Município:** CURITIBA
Telefone: (41)3360-7259 **E-mail:** cometica.saude@ufpr.br

ANEXO 2 – Autorização Secretaria de Educação

CURITIBA

EDUCAÇÃO

Prefeitura Municipal de Curitiba
Secretaria Municipal da Educação
Superintendência de Gestão Educacional
Departamento de Ensino Fundamental
Av. João Gualberto, 623 7º Andar Torre A
Alto da Glória
80030-000 Curitiba PR
Tel 41 33503076
Fax 41 3350 3047
www.curitiba.pr.gov.br

Curitiba, 05 de abril de 2017.

AUTORIZAÇÃO

Informamos que as pesquisadoras **Aluhe Lopes, Magdalena Torres, Bruna Menoncin e Paula Deschi**, alunas de Mestrado em Odontologia da UFPR, orientadas pela Professora Drª. Juliana Feltrin de Souza, estão autorizadas a realizar pesquisa sobre "Hipomineralização Molar-Incisivo em uma população sul-brasileira: Prevalência, fatores sistêmicos associados e polimorfismos genéticos".

O objetivo é avaliar a prevalência da HMI e hipomineralização em dentes decíduos na população de Curitiba,PR, bem como avaliar a associação dessas condições com cárie dentária, fatores etiológicos sistêmicos, polimorfismos genéticos e o impacto na qualidade de vida das crianças portadoras. Ainda pretende-se investigar se os componentes e propriedades salivares estão relacionados coma severidade da HMI.

As pesquisadoras pretendem:

- Avaliar a prevalência da HMI e hipomineralização em dentes decíduos na população de Curitiba,PR, bem como avaliar a associação desses defeitos de esmalte com a cárie dentária;
- Avaliar o impacto da hipomineralização molar-incisivo na qualidade de vida das crianças;
- Avaliar os fatores sistêmicos associados a HMI nessa população;
- Avaliar a presença de polimorfismosgenéticos relacionados às proteínas de interesse em crianças com HMI e controle;
- Relaciona componentes salivares como cálcio, fosfato, ferro, magnésio e flúor, e propriedades salivares com a severidade da HMI.

Os instrumentos de pesquisa serão a coleta de material biológico para análise bioquímica salivar, questionários aplicados aos pais e entrevista com as crianças para análise de qualidade de vida.

CURITIBA

EDUCAÇÃO

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Secretaria Municipal da Educação
Superintendência de Gestão Educacional
Departamento de Ensino Fundamental
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Alto da Glória
80030-000 Curitiba PR
Tel 41 33503076
Fax 41 3350 3047
www.curitiba.pr.gov.br

As escolas sorteadas para a pesquisa serão:

SANTA FELICIDADE:

EM Nympha – 3 turmas

EM Pedro Dallabona

EM João Stival

PORTÃO

EM Nova Esperança – 3 turmas

EM São Luiz

EM Itacellina Bittencourt

CIC

EM Álvaro Borges – 3 turmas

EM Pro Morar Barigui – 3 turmas

EM Dom Bosco

BOA VISTA

EM Jaguariaiva – 4 turmas

EM Doutel de Andrade – 3 turmas

EM Anisio Teixeira – 1 turma

PINHEIRINHO

EM Maria Lenkot – 4 turmas

EM José Lamartini – 3 turmas

EM Umuarama – 1 turma

BOQUEIRÃO

EM Germano Pacionik – 3 turmas

EM Rolandia – 2 turmas

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EDUCAÇÃO

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Fax 41 3350 3047
www.curitiba.pr.gov.br

EM David Carneiro – 1 turma

BAIRRO NOVO

EM Pedro Viriato – 3 turmas

EM Bairro Novo do CAIC/ GLBS – 3 turmas

EM Paulo Freire – 1 turma

CAJURU

EM Prof Maria Marli – 2 turmas

EM Guilherme Braga – 3 turmas

EM Eneas Marques – 2 turmas

EM Linneu F do Amaral – 1 turma

MATRIZ

EM Batel – 2 turmas

EM Vila Torres II – 1 turma

EM Caramuru – 1 turma

A pesquisa será realizada entre os meses de janeiro de 2017 e dezembro de 2018.

Informamos ainda que a decisão final de participar da referida pesquisa caberá aos estudantes e profissionais envolvidos.

Ressaltamos também que o pesquisador deverá entregar **uma cópia impressa e encadernada dos resultados da investigação** para a escola e outra para o Departamento de Ensino Fundamental – Gerência Pedagógica.

Atenciosamente,

Simone Zampier da Silva
Diretora
Departamento de Ensino Fundamental

ANEXO 3 – Critério para diagnóstico DDE

Critério para classificação dos defeitos de desenvolvimento de esmalte (DDE) segundo FDI, 1992.

Escore	**Características clínicas**
0.Normal	Esmalte com espessura normal e sem alterações de coloração
1.Opacidade demarcada	Esmalte de espessura normal e com superfície intacta, existe uma alteração na translucidez do esmalte, de grau variável. Ela é demarcada a partir do esmalte normal adjacente com limites nítidos e claros, e pode ter a coloração branca, bege, amarela ou marrom.
2.Opacidade difusa	Também uma anormalidade envolvendo uma alteração na translucidez do esmalte, de grau variável, e de coloração branca. Não existe um limite claro entre o esmalte normal adjacente e a opacidade. Pode ser linear ou em placas ou ter uma distribuição confluente.
3.Hipoplasia	Um defeito envolvendo a superfície de esmalte e associado com uma redução localizada na espessura do esmalte. Pode ocorrer de forma de (a) fóssulas únicas ou múltiplas, rasas ou profundas, difusas ou alinhadas, disposta horizontalmente na superfície do dente; (b) sulcos únicos ou múltiplos, estreitos ou amplos (máximo de 2 mm); (c) ausência parcial ou total de esmalte sobre uma área considerável de dentina. O esmalte afetado pode ser translúcido ou opaco.
4.Outros defeitos	
5.Opacidade demarcada e difusa	
6.Opacidade demarcada e hipoplasia	
7.Opacidade difusa e hipoplasia	
8.Todas as três condições juntas	
9. Não registrado	

ANEXO 4 – Registro da revisão sistemática no prospero

NIHR | National Institute for Health Research

PROSPERO
International prospective register of systematic reviews

What genes are associated with defects in the development of enamel in children? A systematic review

Leticia Wambier, Juliana Feltrin, Aluhe Lopes, João Armando Brancher, Alessandra Reis

Citation

Leticia Wambier, Juliana Feltrin, Aluhe Lopes, João Armando Brancher, Alessandra Reis. What genes are associated with defects in the development of enamel in children? A systematic review. PROSPERO 2018 CRD42018115270 Available from: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42018115270

Review question

What genes are associated with defects in the development of enamel in children? A systematic review

Searches

To identify trials to be included for this review, we will search on the electronic databases MEDLINE via PubMeb, Scopus, Web of Science, Latin American and Caribbean Health Sciences Literature database (LILACS), Brazilian Library in Dentistry (BBO) and Cochrane Library. We will hand-search the reference lists of all primary studies for additional relevant publications and the related articles link of each primary study in the PubMed database without restrictions to publication date or languages. No restrictions will be placed on the publication date or languages, and all relevant studies will be translated and reviewed. We will search the abstracts of the annual conference of the International Association for Dental Research (IADR) and their regional divisions (1990–2018) and will get in touch with authors of relevant abstracts for further information. We will explore the grey literature using the database System for Information on Grey literature in Europe (SIGLE), and dissertations and theses using the ProQuest Dissertations and Theses Fulltext database, as well as the Periódicos Capes Theses database. To locate unpublished and ongoing trials related to the review question, we will search the following clinical trials registry:Current Controlled Trials (www.controlled-trials.com), International Clinical trials registry platform

(http://apps.who.int/trialsearch/), the ClinicalTrials.gov (www.ClinicalTrials.gov), Rebec (www.rebec.gov.br), and EU Clinical Trials Register (https://www.clinicaltrialsregister.eu). The search strategy will be appropriately modified for each database and performed by two reviewers to identify eligible studies. Full text versions of the papers that appeared to meet the inclusion criteria will be retrieved for further assessment and data extraction.

Types of study to be included

Observational studies

retrospective observational studies

case-control studies

cross-sectional studies

cohort studies

Clinical trial, editorial letters, pilot studies, case reports, historical reviews, in vitro studies, experimental in animals and case series will be excluded.

Condition or domain being studied

Polymorphism, Genetic; Genetic Variations.

Participants/population

Inclusion: children with polymorphisms of genes for enamel defects.

Exclusion: children with polymorphisms of genes without enamel defects.

Intervention(s), exposure(s)
Children with polymorphisms of genes for enamel defects.

Comparator(s)/control
Children with polymorphisms of genes without enamel defects.

Context
Randomized clinical trials and observational (full-text articles or abstracts published in dental meetings).

Main outcome(s)
Evaluation of the genes associated with enamel defects.

Additional outcome(s)
Main gene associated with enamel defect.

Data extraction (selection and coding)
Articles will be selected by title and abstracts according to the previously described search strategy. Articles that appear in more than one database will be considered only once. Full-text articles will also be obtained when the title and abstract have insufficient information to make a clear decision. Subsequently, two reviewers will classify those which met the inclusion criteria. To handle such a large number of studies, we will use a study ID for each eligible study, combining first author and year of publication. Any disagreement between the reviewers over the eligibility of particular studies will be resolved through discussion with a third reviewer.

Risk of bias (quality) assessment

The risk of bias among included studies will be evaluated by two independent reviewers using the Modified Newcastle - Ottawa Scale for Risk of Bias criteria (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp). Discrepancies between the examiners were solved in a consensus. The evaluation criteria have a maximum score of 9 points. These score points are divided between the following domains: patient selection (generalization and applicability -4 points), comparability of groups (2 points), exposure measurements in studies designed (3 points).The studies will be later dichotomized at high risk of bias (0-3 points), moderate risk of bias (4-6 points) and low risk of bias (?7 points).

Strategy for data synthesis
The extracted data will be analyzed using Revman (Review Manager version 5.3 softwar, Cochrane Collaboration,Copenhagen, Denmark). Data from eligible studies will be dichotomous (survival rate). Only studies classified at "low" risk of bias in the key domains will enter into the meta-analysis. The outcomes will be summarized by calculating the

odds ratio and the 95% confidence interval. The random-effects models will be employed. Heterogeneity will be assessed using the Cochran Q test and I^2 statistics. All analyses will conducted using CMA software (version 3, Biostat Englewood, USA).

Analysis of subgroups or subsets
None planned.

Contact details for further information
Leticia Wambier
marcelo_gumy@hotmail.com

Organisational affiliation of the review
UEPG

Review team members and their organisational affiliations

Miss Leticia Wambier. UEPG
Professor Juliana Feltrin. UFPR
Miss Aluhe Lopes. UFPR
Professor João Armando Brancher. UP
Professor Alessandra Reis. UEPG

Type and method of review
Systematic review

Anticipated or actual start date
28 November 2018

Anticipated completion date
01 November 2019

Funding sources/sponsors
None

Conflicts of interest

Language
English

Country
Brazil

Stage of review
Review Ongoing

Subject index terms status
Subject indexing assigned by CRD

Subject index terms
Child; Dental Enamel; Dental Enamel Hypoplasia; Humans

Date of registration in PROSPERO
20 November 2018

Date of publication of this version
20 November 2018

Details of any existing review of the same topic by the same authors

Stage of review at time of this submission
The review has not started

Stage	Started	Completed
Preliminary searches	No	No
Piloting of the study selection process	No	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

The record owner confirms that the information they have supplied for this submission is accurate and complete and they understand that deliberate provision of inaccurate information or omission of data may be construed as scientific misconduct.

The record owner confirms that they will update the status of the review when it is completed and will add publication details in due course.

Versions

20 November 2018

ANEXO 5 – Critério para risco de viés do artigos incluídos na Revisão Sistemática

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort
   a) truly representative of the average _____ (describe) in the community*
   b) somewhat representative of the average _____ in the community
   c) selected group of users eg nurses, volunteers
   d) no description of the derivation of the cohort
2) Selection of the non exposed cohort
   a) drawn from the same community as the exposed cohort*
   b) drawn from a different source
   c) no description of the derivation of the non exposed cohort
3) Ascertainment of exposure
   a) secure record (e.g. surgical records)*
   b) structured interview*
   c) written self report
   d) no description
4) Demonstration that outcome of interest was not present at start of study
   a) yes
   b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis
   a) study controls for _____ (select the most important factor) *
   b) study controls for any additional factor* (This criteria could be modified to indicate specific control for a second important factor.)

Outcome

1) Assessment of outcome
   a) independent blind assessment*
   b) record linkage*
   c) self report
   d) no description
2) Was follow-up long enough for outcomes to occur
   a) yes (select an adequate follow up period for outcome of interest) *
   b) no
3) Adequacy of follow up of cohorts
   a) complete follow up - all subjects accounted for*
   b) subjects lost to follow up unlikely to introduce bias - small number lost - > ____ % (select an adequate %) follow up, or description provided of those lost) *
   c) follow up rate < ____% (select an adequate %) and no description of those lost
   d) no statement

Wells, G. A, Shea, B., O'Connel, D. et al. The Newcastle-Ottawa scale (NOS) for assessing the quailty of nonrandomised studies in meta-analyses. http://www ohri ca/programs/clinical_epidemiology/oxford htm 2009 Feb 1

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
CASE-CONTROL STUDIES

Note: A study can be awarded a maximum of one star (*) for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?
   a) yes, with independent validation *
   b) yes, e.g., record linkage or based on self reports
   c) no description
2) Representativeness of the cases
   a) consecutive or obviously representative series of cases *
   b) potential for selection biases or not stated
3) Selection of Controls
   a) community controls *
   b) hospital controls
   c) no description
4) Definition of Controls
   a) no history of disease (endpoint) *
   b) no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
   a) study controls for _____ (Select the most important factor.) *
   b) study controls for any additional factor * (This criteria could be modified to indicate specific control for a second important factor.)

Exposure

1) Ascertainment of exposure
   a) secure record (eg surgical records) *
   b) structured interview where blind to case/control status *
   c) interview not blinded to case/control status
   d) written self report or medical record only
   e) no description
2) Same method of ascertainment for cases and controls
   a) yes *
   b) no
3) Non-Response rate
   a) same rate for both groups *
   b) non respondents described
   c) rate different and no designation