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ORIGINAL ARTICLE

Association between molar hypomineralization, genes involved in enamel development, and medication in early childhood: A preliminary study

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Abstract

Background: Molar hypomineralization (MH) is defined as a multifactorial condition, and thus, its presence may be defined by interactions between environmental and genetic factors.

Aim: To evaluate the association between MH, genes involved in enamel development, and the use of medication during pregnancy in early childhood.

Design: One hundred and eighteen children, 54 with and 64 without MH, were studied. The data collected included demographics, socioeconomic data, and the medical history of mothers and children. Genomic DNA was collected from saliva. Genetic polymorphisms in ameloblastin (AMBN; rs4694075), enamelin (ENAM; rs3796704, rs7664896), and kallikrein (KLK4; rs2235091) were evaluated. These genes were analyzed by real-time polymerase chain reaction using TaqMan chemistry. The software PLINK was used to compare allele and genotype distributions of the groups and to assess the interaction between environmental variables and genotypes ($p < .05$).

Results: The variant allele KLK4 rs2235091 was associated with MH in some children (odds ratio [OR]: 3.75; 95% confidence interval [CI] = 1.65–7.81; $p = .001$). Taking medications in the first 4 years of life was also associated with MH (OR: 2.94; 95% CI = 1.02–6.04; $p = .041$) and specifically in association with polymorphisms in ENAM, AMBN, and KLK4 ($p < .05$). The use of medications during pregnancy was not associated with MH (OR: 1.37; 95% CI = 0.593–3.18; $p = .458$).

Conclusion: The results of this study suggest that taking medication in the postnatal period appears to contribute to the etiology of MH in some evaluated children. There may be a possible genetic influence of polymorphisms in the KLK4 gene with this condition.

KEYWORDS

dental enamel, enamel defects, gene, molar hypomineralization, polymorphisms

1 | INTRODUCTION

Molar hypomineralization (MH) involves qualitative developmental defects of enamel with an approximate frequency of 20% in children throughout the world.[1–3] Clinically, this condition is characterized by idiopathic demarcated opacities, which may vary from white/yellowish to brown and, in severe cases, may present posteruptive enamel breakdown and carious lesions, resulting in atypical restorations.[4,5] First permanent molars (FPMs) are the most affected teeth, with or without affected permanent incisors, and so this phenotype has been commonly referred to as molar incisor hypomineralization.[4] In addition, similar hypomineralization lesions have also been described in permanent canines and primary second molars.[6,7]

Nowadays, MH is defined as an acquired condition with multiple risk factors, meaning its clinical occurrence may be defined by interactions between environmental factors (i.e., use of medication, occurrence of systemic diseases, exposure to chemicals and others) and a genetic predisposition (probably related to polymorphisms in enamel development genes).[5,8–10] The proposed mechanism for the specific occurrence of the enamel disturbance leading to enamel hypomineralization has been recently accounted on a lack of complete removal of albumin from enamel during the late stages of amelogenesis, disturbing crystal growth and resulting in less mineralized enamel.[11,12] The hypomineralization phenotype would be thus probably influenced by the interaction between environmental and genetic factors.[13,14]

A relatively small but potentially important genetic influence in MH was indicated by identical twin studies.[15,16] Other genetic studies have shown that polymorphisms in various genes involved in enamel development may also be associated with this condition.[17,18] These studies also reported that multiple genes may be involved together in the occurrence of hypomineralization defects but the specific pathomechanisms by which the polymorphisms interact or are triggered by environmental factors to result in protein absorption disruptions are not known yet.[11,12–14,17]

In view of the current evidence, it has been hypothesized that polymorphisms in genes acting during amelogenesis may contribute to the etiology of hypomineralization defects. Added to the fact that some prenatal and postnatal factors are also generally listed as "predisposing factors," the aim of this study was to contribute to the knowledge of possible associations between hypomineralization defects in FPMs and (1) genes that act in the enamel development and (2) the use of medication in the first 4 years of life or during pregnancy.

Why this paper is important to paediatric dentists

- This study showed the information that may contribute to future studies regarding the etiology of MH.
- The present manuscript reports the results and discussions regarding the gene–environment interaction in some individuals with MH allowing a better understanding of its etiology.

2 | MATERIALS AND METHODS

2.1 | Study design and ethical aspects

This report follows the STROBE guidelines, and data used for this study were obtained from a case–control study carried out between July 2015 and December 2016. Children and adolescents aged 7–14 years, who sought care at the Pediatric Dentistry Clinic, were examined by a trained and calibrated examiner (F.M.F.S.). The study was approved by the local research ethics committee (Hospital Universitario Clementino Fraga Filho [HUCFF] under the protocol number: 44598514.7.00005257). All subjects/guardians read and signed a written informed consent form before their participation in the study.

2.2 | Participants

The inclusion criterion was children/adolescents between 7 and 14 years old, presenting fully erupted FPMs. The eligible participants were defined as patients who had at least one permanent first molar affected by hypomineralization defects (case group) or those without signs of hypomineralization (control group). The exclusion criteria were children/adolescents presenting with syndromes, and other enamel defects (hypoplasia, fluorosis, and amelogenesis imperfecta), those undergoing orthodontic treatment, and those whose parents/caregivers could not provide information on the prenatal and postnatal periods until the first 4 years of the child's life.

2.3 | Training and calibration exercise

To assess the intrareliability of the identification of hypomineralization defects, a calibration exercise was carried out through two stages: theorical and practical/clinical. The theorical stage consisted of a discussion

about MH criteria diagnosis. A senior researcher (M.C.C.) coordinated this step and instructed the examiner on how to perform the clinical examination. The practical/clinical step was performed firstly with 20 clinical images of enamel lesions, including fluorosis, hypoplasia, amelogenesis imperfecta, and hypomineralization defects in different locations, with different discolorations, and the presence of enamel breakdown. The examiner (F.M.F.S.) examined independently these images for intraexaminer agreement evaluation. Two weeks after the first assessment, the examiner carried out a new assessment of the images to calculate the intraexaminer agreement (Kappa = 0.88).

2.4 | General health data collection

The health aspects were collected during the anamnesis, and data were annotated in a form. The form was filled out by the interviewer with the accompanying parent. The following variables were collected: demographic data (child's age, sex, place of birth, residence, and socioeconomic status), mother's health during pregnancy (medications used and complications during childbirth), and child's medical history (antibiotics or corticoids use), systemic diseases, and severe infections.

2.5 | Clinical examination

Clinical examinations were performed by a paediatric dentist (F.M.F.S.), with the children sitting in the dental chair under artificial light and after professional prophylaxis. The diagnosis of MH was determined by using the European Academy of Paediatric Dentistry (EAPD) guidelines.[4] Mild hypomineralization was considered when the tooth presented demarcated opacity ≥1.0 mm, whereas severe cases were defined as teeth affected with a lesion that required treatment, enamel breakdown, the presence of atypical dental caries lesions or restorations.[4,5] At the end of the clinical examination, each patient received oral hygiene instructions and was referred for further dental treatment if needed.

2.6 | Sample collection/genotyping and allele analyses

Saliva samples were collected with the children comfortably seated in the dental chairs, and DNA was extracted according to the protocol of DNA Oragene™. Before sample collection, all the patients were informed not to eat for up to 30 min. The amount of DNA concentration and the purity of each sample were determined by spectrophotometry (NanoDrop 1000; Thermo Fisher Scientific, USA). Four single-nucleotide polymorphisms (SNPs) in the genes *AMNB* (rs*4694075*), *enamelin (ENAM)* (rs3796704, rs7664896), and kallikrein (KLK4) (rs2235091) were included in that study.

Genotyping was performed using real-time polymerase chain reaction (PCR). All PCR reactions were prepared with a final volume of 3.0 μL (1 μL of DNA with a concentration of 1 ng/μL reaction; 1.5 μL master mix; 0.075 μL of the TaqMan of each SNP; and 0.425 μL of deionized water q.s.q.). Reactions were carried out using TaqMan chemistry in a total volume of 3.0 μL in an ABI PRISM Sequence Detection System 7900.[18] The genotyping results were analyzed using the SDS software program version 1.7 (Applied Biosystems). PCRs were repeated twice when necessary, and allele frequencies were also calculated.

2.7 | Statistical analyses

Data were analyzed using the SPSS software [v.21.0; Statistical Package for Social Sciences (SPSS), Chicago, IL, USA]. The chi-squared test was used to compare frequencies among the following categorical variables: sex, ethnicity, and socioeconomic level (low, moderate, and high). Student's *t*-test was applied to compare the mean age of patients between cases and controls, and odds ratio calculations were used to assess the associations between prenatal and postnatal risk factors and hypomineralization defects. The analysis did not adjust for any subject characteristics, and the statistical significance for all tests was determined by $p \leq .05$. In addition, the PLINK software[19] was used to compare the allele and genotype frequencies distributed between groups (with hypomineralization versus without hypomineralization defects) and to assess the association between the environmental variables (medications taken during pregnancy and early childhood up to 4 years old) and the genotypes. The Hardy–Weinberg equilibrium was evaluated using the chi-squared test within each polymorphism.

3 | RESULTS

All the subjects recruited were included in this study ($n = 118$). The participants were divided into children with MH ($n = 54$) and those without MH ($n = 64$), with mean ages of 9.9 (±1.9) and 9.7 (±1.7) years, respectively. Most of the participants were male ($n = 70$; 59.3%) and had low socioeconomic status ($n = 105$; 89%). There were no statistically significant differences between the groups

in relation to demographic characteristics (sex, age, and socioeconomic status; $p \geq .05$; Table 1). Of the 54 patients with MH, 267 FPMs presented demarcated opacities. Most of the affected teeth were located in the maxillary arch ($n = 154$; 57.6% teeth, versus $n = 113$; 42.4% in the mandibular arch), whereas most of the lesions occurred in FPMs ($n = 148$) followed by the maxillary central incisors ($n = 71$). Considering all affected teeth, 76% ($n = 203$) of the teeth were mildly affected whereas 24% ($n = 64$) presented severe lesions. Hypomineralization defects in FPMs were associated with children taking medication up to 4 years old (odds ratio [OR] = 2.94; 95% confidence interval [CI] = 1.02–6.01; $p = .041$). From 27 individuals that used medications in the first 4 years of life, 17 (66.7%) were affected by hypomineralization defects in FPMs. No association, however, was observed between taking medication during pregnancy and the presence of defects ($p \geq .05$; Table 2).

There was statistical evidence for the association of polymorphisms in *KLK4* rs2235091 (A>G) with FPM hypomineralization defects (OR = 3.60; 95% CI = 1.84–7.62, $p \leq .01$). This means that children presenting with this risk allele may be more susceptible to develop hypomineralization defects in FPMs, whereas no association was observed with the polymorphisms located in *ENAM* and *ameloblastin (AMBN)* ($p \geq .05$; Table 3). Polymorphisms in *KLK4* rs2235091 were also the only ones showing a statistically significant genetic-only influence in the presence of MH opacities (Table 4). All markers studied (located in ENAM, *AMB*, and *KLK4*) showed an association between the use of medication in the first years of life and the presence of molar incisor hypomineralisation (MIH) (Table 4).

TABLE 1 Characteristics of the sample.

Total sample, $n = 118$		**Absence of molar hypomineralization (MH), $n = 64$ (%)**	**Presence of MH, $n = 54$ (%)**	**p[b]**
Gender, *n* (%)	Female	25 (40.3)	23 (41.1)	.542[b]
	Male	37 (59.7)	33 (58.9)	
Mean age (standard deviation)		9.84 (±1.67)	10.06 (±1.7)	.495[c]
Ethnicity, *n* (%)	Caucasian	37 (57.8)	29 (53.7)	.833[b]
	White	17 (26.6)	15 (27.8)	
	Black	10 (15.6)	10 (18.5)	
CCEB *n* (%)[a]	High	3 (4.7)	6 (11.1)	.412[b]
	Middle income	59 (92.2)	46 (85.2)	
	Low income	2 (3.1)	2 (3.7)	
molar incisor hypomineralisation severity	Mild	–	32 (57.1)	–
	Severe	–	22 (42.9)	

[a]Brazilian Economic Classification Criteria = CCEB (Critério de Classificação Econômica Brasil).

[b]Chi-squared ($p < .05$).

[c]Student's *t*-test ($p < .05$).

TABLE 2 Association between risk factors and the presence of molar hypomineralization (MH) during the prenatal and postnatal periods.

Medication taken during pregnancy				
	Yes, $n = 29$ (%)	**No, $n = 89$ (%)**	**p***	**Odds ratio (OR) (95% confidence interval [CI])[a]**
Presence of MH, $n = 54$	15 (51.7)	39 (43.8)	.361	1.37 (0.593–3.18)
Absence of MH, $n = 64$	14 (48.3)	50 (56.2)		
Medication taken up in early childhood (Until 4 years)				
	Yes, $n = 27$ (%)	**No, $n = 91$ (%)**		
Presence of MH, $n = 64$	17 (63)	37 (40.7)	.041	2.94 (1.02–6.01)
Absence of MH, $n = 64$	10 (37)	54 (59.3)		

*p = chi-squared ($p \leq .05$).

[a]Odds ratio; 95% confidence intervals.

TABLE 3 Genotype and allele frequency in the presence of molar hypomineralization (MH)/without MH phenotype.

Gene/ polymorphism	Patients ($n = 118$) Presence of MH = 54 Without MH = 64	Genotype n (%)				Allele wild-type frequency (%)	Allele risk frequency (%)	p value for allelic association*	OR (95% confidence interval [CI])[a]
AMNB rs4694075 *C>T*	Presence of MH	CC 6 (11.1%)	CT 9 (16.7%)	TT 10 (18.5%)	Undetermined 29 (53.7%)	C (42%)	T (58%)	.465	0.724 (0.35–1.48)
	Without MH	CC 10 (15.6%)	CT 18 (28.1%)	TT 10 (15.6%)	Undetermined 26 (40.6%)	C (50%)	T (50%)		
Enamelin (*ENAM*) rs3796704 *G>A*	Presence of MH	AA	AG 6 (11.1%)	GG 19 (35.2%)	Undetermined 29 (53.7%)	A (12%)	G (88%)	1	0.981 (0.33–2.88)
	Without MH	AA	AG 10 (15.6%)	GG 31 (48.4%)	Undetermined 23 (35.9%)	A (12.3%)	G (87.7%)		
ENAM rs7664896 *C>G*	Presence of MH	GG 4 (7.4%)	GC 9 (16.7%)	CC 15 (27.8%)	Undetermined 26 (48.1%)	C (30.4%)	G (69.6%)	.154	1.83 (0.82–4.07)
	Without MH	GG 3 (4.7%)	GC 9 (14.1%)	CC 27 (42.2%)	Undetermined 25 (39%)	C (19.2%)	G (80.8%)		
Kallikrein rs2235091 *A>G*	Presence of MH	AA 5 (9.3%)	AG 8 (14.8%)	GG 14 (25.9%)	Undetermined 27 (50%)	A (33.3%)	G (66.7%)	**$p < .001$**	**3.75 (1.84–7.62)**
	Without MH	AA 19 (29.7%)	AG 22 (34.4%)	GG 5 (7.8%)	Undetermined 18 (28.1%)	A (65.2%)	G (34.8%)		

*p value = Chi-squared test ($p < .05$).

Note: Wild-type allele = common allele found in the population. Risk allele = more chance to develop MH.

[a]Odds ratio; 95% confidence intervals.

TABLE 4 Association between gene and medications taken during pregnancy and postnatal periods (up to 4 years of age) and its association with molar hypomineralization (MH).

			Genetics and medications taken up at 4 years of age		Genetics and medications taken up during pregnancy	
GENE	SNP	Risk allele	Odds ratio (OR) (95% confidence interval [CI])	*p**	OR (95% CI)	*p**
Ameloblastin	rs4694075	C	10.04 (2.33–43.14)	.001	2.52 (0.785–8.08)	.120
Enamelin (*ENAM*)	rs3796704	A	7.58 (2.02–28.4)	.002	2.76 (0.867–8.81)	.085
ENAM	rs7664896	G	6.16 (1.45–26.02)	.013	2.73 (0.705–10.63)	.145
Kallikrein	*rs2235091*	*G*	4.72 (1.27–17.56)	.020	2.74 (0.789–9.54)	.112

Note: Logistic regression is statically significant ($p < .05$). Risk allele = More chance to develop the alteration (MH).

4 | DISCUSSION

The present study found associations between polymorphisms in genes involved in enamel development and MH. All the genes (*AMNB, ENAM*, and *KLK4*) included in the present study have different functions during amelogenesis. Moreover, the variant allele in the studied markers of these genes may promote structural and mineralization defects in the dental enamel.[20,21] Differently from a previous study from Brazil,[10] we did not find an association of MH with AMBN and ENAM.[10] Also, the present study did find an association between MH and *KLK4* rs2235091. These disparities could be related to different demographic and socioeconomic characteristics since the Brazilian population is considered genetically heterogeneous[22] and the studies were conducted in different geographic populations and cities. It is also noteworthy that KLK4 mutations can cause amelogenesis imperfecta with hypomineralized phenotypes resembling those of MH, such as color alteration and susceptibility to posteruptive breakdown.[20,23]

Regarding the influence of environmental factors, aspects such as interferences during pregnancy (severe infections), complications during childbirth (hypoxia), and medications taken during pregnancy and during early childhood have been associated with MIH.[8,16] Although relatively few subjects used medications during childhood (24/118), the present study observed a higher prevalence of MH in this subgroup (67%) than in non-MH controls (33%).

Some studies have reported the potential interaction between genes and environmental factors related to enamel development defects.[14,18] For the analysis carried out in the present study, which tested gene–environment interactions, statistically significant associations were found between all markers in *AMBN, KLK4*, and *ENAM* and taking medication up to 4 years old with MH. Thus, individuals with MH who reported taking medications in the first 4 years of life (postnatal) were more likely to carry the risk alleles tested than the individuals without MH. This was not seen for the use of medications in the gestational period and the markers in these genes.

Most children diagnosed with hypomineralization defects in FPMs in this study were within a middle socioeconomic status. These data are in line with the study carried out in Southeast Brazil,[24] where most of the individuals with MIH belonged to the middle socioeconomic group. Balmer et al.[25] also reported an association of MIH with middle and high socioeconomic status in families from Northern England. Ethnicity, on the contrary, which is a confounder for socioeconomic status, was not associated with MIH in the studied sample. A previous study in a Brazilian sample, however, suggested a positive association between ethnicity and MIH,[26] although the study was done in a different state.

Among the limitations of this study, one could cite the lack of knowledge of the type of medication used by the MH group. Moreover, while it is acknowledged that most of the demarcated opacities are visible on the occlusal half of FPMs and, thus, any tissue injury may probably have happened until the child was up to 1 year of age, the variable "medications taken at 4 years of age" was used in the present study due to the evidence that mineralization of the whole FPM crown is not completed until past 3 years of age[27] Although the FPM susceptibility window for MH may go a little beyond the first year of age, further studies could probably narrow this time stamp during interviews with the parents.

A larger sample size could probably allow comparisons of the relationship between the different clinical phenotypes and the genetic polymorphisms. Also, in the present study, only half of the individuals were genotyped, which may not have allowed the detection of some associations. In contrast, the strengths of this study included the well-characterized phenotype, obtained using an established clinical criterion, and evaluated by an experienced paediatric dentist, increasing the confidence in the results obtained. Finally, the genes and SNPs selected were those with known and specific functions during amelogenesis. For these reasons, we consider that, although the results

of this study should not be viewed as definitive, the associations found can be considered for further studies.

Based on the results of the present study and those that have already been reported in the literature, MH is an acquired condition influenced by the presence of environmental factors (i.e., use of medications) associated with genetic factors. It means that patients who present certain genetic variants, under certain conditions, are more likely to develop MH.

Additional studies to analyze gene–environment interactions in diverse populations should obtain more detailed information about what specific medication is involved in the development of MH. It is also important to explore hypomineralization lesions further based on different demographic, ethnic, and socioeconomic characteristics and different groups of affected teeth.

In the present study, the polymorphism *rs2235091* located in *KLK4* was associated with MH in some children. In addition, genes responsible for enamel development and the use of medications in the first 4 years of life (postnatal) may act in association, leading to different MH phenotypes. Future studies, with larger sample sizes, are needed to further unravel the specific contributions of genetic and environmental factors in the etiology of hypomineralization lesions.

AUTHOR CONTRIBUTIONS

FMFS worked on study design, collected the data, analyzed the data, drafted the initial manuscript, and approved the final manuscript as submitted. FMC established the study design, analyzed the data, reviewed the manuscript, and approved the final manuscript as submitted. ALMMF analyzed the data, reviewed the manuscript, and approved the final manuscript as submitted. TRCS collected the data, reviewed the manuscript, and approved the final manuscript as submitted. AFG established the study design, reviewed the manuscript, and approved the final manuscript as submitted. ARV conducted the final analysis, contributed to the final version of the manuscript, and approved the final manuscript as submitted. AAN established the study design, contributed to the final version of the manuscript, and approved the final manuscript as submitted. MCC established the study design, conducted the analysis, contributed to the final version of the manuscript, and approved the final manuscript as submitted.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

1. Zhao D, Dong B, Yu D, Ren Q, Sun Y. The prevalence of molar incisor hypomineralization: evidence from 70 studies. *Int J Paediatr Dent*. 2018;28(2):170-179. doi:10.1111/ipd.12323
2. Weerheijm KL, Duggal M, Mejàre I, et al. Judgement criteria for molar incisor hypomineralisation (MIH) in epidemiologic studies: a summary of the European meeting on MIH held in Athens, 2003. *Eur J Paediatr Dent*. 2003;4(3):110-113.
3. Hubbard MJ, Perez VA, Ganss B. 100 years of chalky teeth research: from pioneering histopathology to social good. *Front Dent Med*. 2021;1:632534. doi:10.3389/fdmed.2020.632534
4. Lygidakis N, Wong F, Jälevik B, Vierrou AM, Alaluusua S, Espelid I. Best clinical practice guidance for clinicians dealing with children presenting with molar-incisor-hypomineralisation (MIH): an EAPD policy document. *Eur Arch Paediatr Dent*. 2010;11(2):75-81.
5. Ghanim A, Silva MJ, Elfrink MEC, et al. Molar incisor hypomineralisation (MIH) training manual for clinical field surveys and practice. *Eur Arch Paediatr Dent*. 2017;18(4):225-242. doi:10.1007/s40368-017-0293-9
6. Elfrink ME, ten Cate JM, Jaddoe VW, Hofman A, Moll HA, Veerkamp JS. Deciduous molar hypomineralization and molar incisor hypomineralization. *J Dent Res*. 2012;91(6):551-555. doi:10.1177/0022034512440450
7. Schmalfuss A, Stenhagen KR, Tveit AB, Crossner CG, Espelid I. Canines are affected in 16-year-olds with molar-incisor hypomineralisation (MIH): an epidemiological study based on the Tromsø study: "fit futures". *Eur Arch Paediatr Dent*. 2016;17(2):107-113. doi:10.1007/s40368-015-0216-6
8. Fatturi AL, Wambier LM, Chibinski AC, et al. A systematic review and meta-analysis of systemic exposure associated with molar incisor hypomineralization. *Community Dent Oral Epidemiol*. 2019;47(5):407-415. doi:10.1111/cdoe.12467
9. Jeremias F, Koruyucu M, Küchler EC, et al. Genes expressed in dental enamel development are associated with molar-incisor

hypomineralization. *Arch Oral Biol*. 2013;58(10):1434-1442. doi:10.1016/j.archoralbio.2013.05.005
10. Jeremias F, Pierri RA, Souza JF, et al. Family-based genetic association for molar-incisor hypomineralization. *Caries Res*. 2016;50(3):310-318. doi:10.1159/000445726
11. Bartlett JD. Dental enamel development: proteinases and their enamel matrix substrates. *ISRN Dent*. 2013;2013:684607. doi:10.1155/2013/684607
12. Williams R, Perez VA, Mangum JE, Hubbard MJ. Pathogenesis of molar hypomineralisation: Hypomineralised 6-year molars contain traces of fetal serum albumin. *Front Physiol*. 2020;11:619. doi:10.3389/fphys.2020.00619
13. Bussaneli DG, Restrepo M, Fragelli CMB, et al. Genes regulating immune response and amelogenesis interact in increasing the susceptibility to molar-incisor hypomineralization. *Caries Res*. 2019;53(2):217-227. doi:10.1159/000491644
14. Bezamat M, Souza JF, Silva FMF, et al. Gene-environment interaction in molar-incisor hypomineralization. *PLoS One*. 2021;16(1):241898. doi:10.1371/journal.pone.0241898
15. Teixeira RJPB, Andrade NS, Queiroz LCC, et al. Exploring the association between genetic and environmental factors and molar incisor hypomineralization: evidence from a twin study. *Int J Paediatr Dent*. 2018;28(2):198-206. doi:10.1111/ipd.12327
16. Silva MJ, Scurrah KJ, Craig JM, Manton DJ, Kilpatrick N. Etiology of molar incisor hypomineralization—a systematic review. *Community Dent Oral Epidemiol*. 2016;44(4):342-353. doi:10.1111/cdoe.12229
17. Vieira AR, Manton DJ. On the variable clinical presentation of molar-incisor Hypomineralization. *Caries Res*. 2019;53(4):482-488. doi:10.1159/000496542
18. Ranade K, Chang MS, Ting CT, et al. High-throughput genotyping with single nucleotide polymorphisms. *Genome Res*. 2001;11(7):1262-1268.
19. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81(3):559-575.
20. Seymen F, Lee KE, Koruyucu M, et al. ENAM mutations with incomplete penetrance. *J Dent Res*. 2014;93(10):988-992. doi:10.1177/0022034514548222
21. Jedeon K, Houari S, Loiodice S, et al. Chronic exposure to Bisphenol a exacerbates dental fluorosis in growing rats. *J Bone Miner Res*. 2016;31(11):1955-1966. doi:10.1002/jbmr.2879
22. Alves-Silva J, da Silva Santos M, Guimarães PE, et al. The ancestry of Brazilian mtDNA lineages. *Am J Hum Genet*. 2000;67:444-461.
23. Jedeon K, De la Dure-Molla M, Brookes SJ, et al. Enamel defects reflect perinatal exposure to bisphenol A. *Am J Pathol*. 2013;183(1):108-118. doi:10.1016/j.ajpath.2013.04.004
24. Tourino LF, Correa-Faria P, Ferreira RC, Bendo CB, Zarzar PM, Vale MP. Association between molar incisor hypomineralization in schoolchildren and both prenatal and postnatal factors: a population-based study. *PLoS One*. 2016;11(6):e0156332. doi:10.1371/journal.pone.015633
25. Balmer R, Toumba J, Godson J, Duggal M. The prevalence of molar incisor hypomineralisation in northern England and its relationship to socioeconomic status and water fluoridation. *Int J Paediatr Dent*. 2012;22(4):250-257.
26. Fatturi AL, Menoncin BL, Reyes MT, et al. The relationship between molar incisor hypomineralization, dental caries, socioeconomic factors, and polymorphisms in the vitamin D receptor gene: a population-based study. *Clin Oral Investig*. 2020;24:3971-3980. doi:10.1007/s00784-020-03263-y
27. Reid DJ, Dean MC. Variation in modern human enamel formation times. *J Hum Evol*. 2006;50(3):329. doi:10.1016/j.jhevol.2005.09.003

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