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Genetic Variation in *MMP20* Contributes to Higher Caries Experience

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Summary

Matrix metalloproteinases play an important role during the initial process of enamel development and therefore may play a role in caries.

Objectives—To evaluate the association between *MMP20* and caries experience in Brazilian children.

Methods—Eligible unrelated children with or without caries were evaluated using a cohort design. Demographic data and oral health habits were obtained though a questionnaire. Caries data was collected by clinical examination. Genotyping of the selected polymorphism was carried out by real-time PCR from genomic DNA. Allele and genotype frequencies were compared between groups with distinct caries experience and oral health habits.

Results—Of 388 subjects, 161 were caries free children. There were no differences between caries levels and genotype distribution in the total cohort. When ethnic background was considered, differences in genotype distribution were observed in caries free children versus children with caries in Caucasians (p=0.03). Differences could also be seen when poor oral hygiene was used to stratify the analysis (p=0.02). Regression analysis, adjusted for genotype and ethnicity, confirmed that ingestion of sweets between meals increases the risk of presenting carious lesions (p=0.00001; OR=2.33; 95%CI 1.53–3.54).

Conclusion—Variation in *MMP20* may be associated with caries experience mainly in Caucasian subjects with poor oral health habits.

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Keywords

dental caries; matrix metalloproteinases; enamelysin; polymorphisms; SNPs; genetic susceptibility

Introduction

Caries is a complex, chronic and infectious disease and one of the most common illnesses worldwide. Children diagnosed with severe caries complain of toothache, have problems eating certain foods, and miss school, showing the disease can have a very negative impact on children's oral health-related quality of life.[1] Several caries risk factors have been identified during the last years; however, these variables alone may not entirely explain the disease development.[2] There is evidence for a genetic component in caries susceptibility and studies in humans have suggested that variation in enamel formation genes may contribute to caries development.[3–5]

Enamel consists of about 95% mineral which makes it harder than other mineralized tissues. *Tuftelin* genotypes appeared to interact with levels of *Streptococcus mutans* colonization in children with early childhood caries.[5] In adults, genetic variation in *amelogenin* was associated with higher caries experience.[4] Variation in *ameloblastin* and *tuftelin* was associated with caries experience in Turkish children and *enamelin* may interact with the presence of Streptococcus mutans colonization.[3]

Matrix metalloproteinases (MMPs) form a multigene family within the metalloproteinase class of endopeptidades that mediate the degradation of practically all extracellular matrix molecules.[6–8] Prior to mineralization, MMPs may participate in the organization of enamel and dentin organic matrix, or they may regulate mineralization by controlling the proteoglycan turnover.[7] During the enamel matrix development, the early protease secreted is named enamelysin (MMP20).[9] Therefore, we tested the hypothesis that a single nucleotide polymorphism in *MMP20* was associated with caries experience in Brazilian children.

Materials and Methods

The Human Ethics Committee of the Health Department of the city of Rio de Janeiro, Rio de Janeiro, Brazil (113/09) approved this study. Informed consent was obtained from all participating individuals or parents/legal guardians.

Eligible unrelated children from 5 to 14 years of age were recruited at the Pediatric Dental Clinics, Federal University of Rio de Janeiro, during the period of February 2010 to February 2011.

The ethnicity definition was ascertained based on self-reported information. The institution where the subjects were recruited is located in the Southeast of Brazil, the most densely populated and industrialized region of the country. The Southeast region of Brazil comprises an ethnic admixture of Caucasians (European descent; 53.6%) and African descents (obviously of mixed European, 33.6% or not obviously mixed Africans, 12.3%). The remaining 0.5% of the population is Amerindian or Asian descents.[10]

All subjects or parents/caregivers answered a questionnaire about fluoride exposure history (the use of fluoride mouthwashes and the use of fluoride toothpaste) and oral hygiene habits (the frequency of tooth brushing and use of tooth floss). Information was also sought on the child's frequency of ingesting cakes, cookies, and sweets between meals on the day prior to completing the questionnaire.[11]

Determination of caries experience

Two pediatric dentists (P.N.T. and E.C.K.) conducted the clinical examinations. Cohen's kappa values for agreement between examiners were 0.91. Caries was diagnosed in primary and permanent teeth by visual examination and was registered if there was definite visual evidence with a breach in the enamel and extension into dentine. Subjects were seated in a dental chair, and the examiner used a probe and dental mirror according to the criteria recommended by the World Health Organization guidelines. Dental caries was assessed using the DMFT and/or dmft indexes. We also evaluated the presence or absence of visible dental plaque.

The studied subjects were classified according to the caries experience level. The subjects were divided in two groups: caries free (subjects with dmft/DMFT=0) and caries affected (dmft/DMFT 1). In addition, the caries affected group was stratified based on the criteria of Tannure et al.[11] as follows: *low caries experience*: dmft/DMFT=1; *moderate caries experience*: 2≤dmft/DMFT≤3; *high caries experience*: dmft/DMFT≥4.

According to the information about oral hygiene and dietary habits extracted from the questionnaire, we stratified the children according to their oral health habits, based upon the frequency of tooth brushing and the frequency of ingesting cakes, cookies and sweets between meals. The subjects classified as having poor oral health habits reported ingesting cakes, cookies, and sweets and/or brush their teeth no more than one time per day.[11]

DNA samples and genotyping

Genomic DNA for molecular analysis was extracted from buccal cells based on a modification of a published protocol.[12] A polymorphism in the *MMP20* gene (Intron 1 region, rs1784418, 11q22.3-q23 locus) was genotyped by real-time polymerase chain reaction using the Taqman method[13] (Agilent Technologies, Stratagene Mx3005P). Applied Biosystems supplied the assays and reagents (Foster City, CA). In these analyses, two TaqMan probes were used in the allelic discrimination assay, one probe for each allele in a two-allele system. Each probe consists of an oligonucleotide with a 5′-reporter dye and a 3′-quencher dye. During PCR, forward and reverse primers hybridize to a specific sequence of the target DNA. The TaqMan probe hybridizes to a target sequence within the PCR product. The separation of the reporter dyes from the quencher dye results in increasing fluorescence for each of the reporters. The increase in fluorescence is measured and is a direct consequence of target amplification during PCR.

All quantitative real-time polymerase chain reactions were performed in a total volume of 3ul (4 ng DNA/reaction, 1,5 ul Taqman PCR master mix, 0,075 SNP assay; Applied Biosystems, Foster City, CA). The thermal cycling was carried out by starting with a hold cycle of 95°C for 10 minutes, followed by 40 amplification cycles of 92°C for 15 seconds and 60°C for 1 minutes.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS – 16.0). The *t* test, odds ratio calculations and chi-square test were used to compare age, ethnicity, gender, and preventive habits between caries affected and caries free groups. Odds ratio calculations and chi-square tests at a level of significance of 0.05 were also used to evaluate if subgroups presented higher frequency of specific genotypes or alleles. Binary logistic regression adjusted for genotype, ethnicity and variables related to oral health habits was also used. Two interaction terms of multivariate analysis at a level of significance of 0.10

were used. Moreover, the standard chi-square test was used to test for deviation from Hardy-Weinberg.

Results

Of the 388 individuals included in this study, 161 (41.5%) were caries free children, 41 (10.6%) were classified as low, 63 (16.2%) as moderate and 123 (31.7%) as children with high caries experience. Caries free children were older than caries affected children (p=0.01). Most children (n=245; 63.1%) were in mixed dentition. A similar percentage of children were in primary (n=53; 13.7%) and permanent dentition (n=90; 23.2%). The mean DMFT was 0.73 (sd±1.5) and the dmft was 2.45 (sd±2.8). The most affected region was the posterior dentition (n=195; 50.3%). A total of 111 (28.6%) subjects were classified as having poor oral health habits. Demographic data and risk factors for caries were summarized in Table 1.

Table 1 shows that in general, there were no major differences in demographics, adherence to preventive oral health habits, such as tooth brushing, presence of visible plaque, and use of fluoride mouthwash between caries free and caries affected children. The exceptions for preventive oral health habits were the use of dental floss and ingestion of sweets between meals, which were significantly more common in caries free children (p=0.02; OR=0.62; 95% C.I. 0.39–0.97) and in children with caries (p=0.001; OR=2.27; 95% C.I. 1.47–3.51), respectively. All genotypes were in Hardy-Weinberg equilibrium. The C allele frequency was 0.58 among the caries affected group and 0.56 among the caries free group, while the T allele frequencies were 0.42 among caries affected and 0.44 among caries free children.

There were no differences between caries levels and genotype distribution (p=0.38). Allele and genotype frequency comparisons between subgroups are summarized in Table 2. Differences in allele and genotype distributions between subgroups were observed only in Caucasians (p=0.032). Following this pattern, Caucasian subjects that reported poor oral health habits presented differences in genotype distribution depending on caries status (p=0.02) (Table 2).

Table 3 shows the results for the regression analysis of subjects with caries. When the poor oral hygiene alone was used as the outcome variable, the OR for caries was further increased (p=0.06; OR=1.53–95%CI 0.97–2.42). When adjusted for other variables included in multivariate analysis, the poor oral hygiene was not positively associated with caries experience.

Binary logistic regression analysis revealed that subjects that ingest sweets between meals have a significant increase OR for caries. These results remained significant when adjusted for genotype and ethnicity (p=0.000001; OR=2.33, 95%CI 1.53–3.54) (Table 3).

Table 4 shows the predictive values for caries considering the multivariate regression analysis. There is a 74.61% chance that a Caucasian subject that ingests sweets between meals and carries the TT genotype has caries.

Discussion

This is the first report that investigates a role for the *MMP20* gene in children's caries experience. Variation in genes involved in enamel development may lead to a higher susceptibility to caries and the oral habits acquired in infancy could act by modulating the genetic factor.

Introns are non-coding DNA sequences that separate neighboring exons in a gene. During gene expression, introns are transcribed into messenger RNA but then, intron sequences are removed from the pre-mRNA by splicing. Although our polymorphism is located in an intronic region, it could alter the enzymatic functional activities regulating gene expression. Previous studies demonstrated that this polymorphic variant is involved with tooth agenesis[14] and kidney aging[15]. We hypothesize that this genetic variant (rs1784418) could affect the transcription processes and ultimately MMP20 protein amounts. MMP20 possibly contributes to degradation of amelogenin, the most common enamel protein.[16] A mildly defective amelogenin or slightly smaller amounts of this protein could lead to some degree of disorganization of the enamel prisms that increases the individual's susceptibility to caries.[4] However, testing the hypothesis that an intronic single nucleotide polymorphism (SNP) changes protein expression is not a simple task. Testing amino acid-altering coding SNPs for their effect on gene transcription often requires elaborate expression constructs and analysis using an *in vitro* system, however this approach does not allow testing the functionality of SNPs in intronic regions, unknown regulatory elements, or intergenic regions.[17] Furthermore, our underlying hypothesis is that enamel development is affected to the point it increases caries risk, and it is difficult to conceive an experiment that allow to directly test if enamel that developed under variable levels of MMP20 expression will be more susceptible to demineralization, without generating several lines of hypomorphic Mmp20 mice.

Gender, age and ethnicity have been described as additional risk factors for caries progress. In our study, differences in *MMP20* genotype distribution in Caucasians were observed in two instances: between caries free children and children with caries ($p=0.03$), and when children reported poor oral health habits and had caries ($p=0.02$), but these differences were not seen in African-descents. Previous data from Brazil has suggested that Caucasian children may have higher levels of caries experience in comparison to other ethnic groups[18]. Also, people from the African continent have historically lower levels of caries experience[19]. Differences in socioeconomic status, access to dental care, and dietary habits may explain at least part of these results. We were unable to include in our analysis a direct measurement of socioeconomic status, although all subjects that participated in our study were being treated at the same site. This site serves a population with similar socioeconomic background. The differences in genotype distribution seen in Caucasians in comparison to African-descents reflect a higher frequency of the allele T in the latter. It is possible that this allele has a protective effect against caries progression.

It is important to emphasize that all subjects of our studied population had a similar life style and were dependent on the public health service. The institution where the study was conducted, as well as children's residence, is in a geographic area with fluoridated water supplied, and all subjects/guardians reported the use of fluoridated dentifrice. There is evidence that fluoride can alter the expression of MMP20 in ameloblasts[20], however, the hypothesis that fluoride exposure covary with genetic variation in increasing susceptibility to caries could not be tested due to the design implemented of matching subjects based on fluoride exposure.

Environmental factors such as, low socioeconomic status, poor oral hygiene habits, cariogenic diet, and unmet needs for dental care are variables contributing to the development of caries. However, the combination of all these factors mentioned above does not entirely explain disease outcome.[2] There is evidence that individuals exposed to the same levels of environmental risk factors present differences in the DMFT index suggesting an influence of genetic factors in the etiopathogenesis of the disease.[21] According to our results, we can speculate that *MMP20* genotypes may be involved with caries susceptibility depending on the ethnic background and dietary habits. We observed that Caucasians that

ingest sweets between meals and two copies of the T rs1784418 allele have 74.61% of risk to developing caries.

MMP20 is the enzyme that processes ameloblastin during the secretory stage of amelogenesis[22] and its expression is regulated by ODAM (odontogenic ameloblast-associated protein) and RUNX2 (runt related transcription factor 2)[23]. Mice lacking functional Mmp20 produce enamel that is thin and structurally abnormal[24]. In humans, *MMP20* haploinsufficiency possibly leads to amelogenesis imperfecta[25] and in light of the association of genetic variation in *MMP20* and higher caries susceptibility, it is possible to propose that hypomorphic MMP20 may increase caries susceptibility in humans by altering the development of the enamel and its consequent microstructure.

Several MMPs and their inhibitors play important roles in dentine formation, caries progression, and hybrid layer degradation. MMP2, MMP3, MMP9, TIMP1, and TIMP2 are differently expressed along different dentine depths, suggesting differential collagen degradation potentials may be expected depending upon the depth in which dentine is exposed[26,27]. Hence the suggestion of the use of non-toxic MMP inhibitors, such as chlorhexidine, as an appropriate additional step in bonding procedures, with the idea this can increase the longevity of the adhesive restorations[28].

Being able to predict an individual's risk for caries would be a great advancement for the field. Previous caries experience continues to be the best predictor of future carious lesions, although work using data mining approaches and decision tree techniques may prove to provide new insight[29]. Also, studies using more sophisticated caries scores than the DMFT may improve the quality of the data[30]. But these methods still rely on the identification of visible lesions and we hope our work on the identification of genetic variants that may modify caries susceptibility will allow the identification of individuals at risk before they develop irreversible loss of teeth structure.

Conclusion

Present results suggest that even when caries experience is multifactorial, it may be associated with *MMP20* genotypes, mainly in Caucasian subjects with poor oral health habits.

References

1. Feitosa S, Colares V, Pinkham J. The psychosocial effects of severe caries in 4-year-old children in Recife, Pernambuco, Brazil. Caderno de Saude Publica. 2005; 21:1550–6.
2. Werneck RI, Mira MT, Trevilatto PC. A critical review: an overview of genetic influence on dental caries. Oral Diseases. 2010; 16:613–23. [PubMed: 20846151]
3. Patir A, Seymen F, Yildirim M, Deeley K, Cooper ME, Marazita ML, et al. Enamel formation genes are associated with high caries experience in Turkish children. Caries Research. 2008; 42:394–400. [PubMed: 18781068]
4. Deeley K, Letra A, Rose EK, Brandon CA, Resick JM, Marazita ML, et al. Possible association of amelogenin to high caries experience in a Guatemalan-Mayan population. Caries Research. 2008; 42:8–13. [PubMed: 18042988]
5. Slayton RL, Cooper ME, Marazita ML. Tuftelin, mutans streptococci, and dental caries susceptibility. Journal of Dental Research. 2005; 84:711–4. [PubMed: 16040727]
6. Chaussain-Miller C, Fioretti F, Goldberg M, Menashi S. The role of matrix metalloproteinases (MMPs) in human caries. Journal of Dental Research. 2006; 85:22–32. [PubMed: 16373676]
7. Hannas AR, Pereira JC, Granjeiro JM, Tjaderhane L. The role of matrix metalloproteinases in the oral environment. Acta Odontologica Scandinavica. 2007; 65:1–13. [PubMed: 17354089]

8. Birkedal-Hansen, H.; Yamada, S.; Windsor, J.; Pollard, AH.; Lyons, G.; Stetler-Stevenson, W., et al. Current Protocols in Cell Biology. Vol. Chapter 10. 2008. Matrix metalloproteinases; p. 8
9. Lu Y, Papagerakis P, Yamakoshi Y, Hu JC, Bartlett JD, Simmer JP. Functions of KLK4 and MMP-20 in dental enamel formation. Biological Chemistry. 2008; 389:695–700. [PubMed: 18627287]
10. Instituto Brasileiro de Geografia e Estatistica. Contagem da populacao. 2007.
11. Tannure PN, Kuchler EC, Romanos HF, Vieira AR, Costa MC, Granjeiro JM. Caries experience in individuals with cleft lip and palate. Pediatric Dentistry. In press.
12. Aidar M, Line SR. A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. Brazilian Dental Journal. 2007; 18:148–52. [PubMed: 17982556]
13. Ranade K, Chang MS, Ting CT, Pei D, Hsiao CF, Olivier M, et al. High-throughput genotyping with single nucleotide polymorphisms. Genome Research. 2001; 11:1262–8. [PubMed: 11435409]
14. Kuchler EC, Menezes R, Callahan N, Costa MC, Modesto A, Meira R, et al. MMP1 and MMP20 contribute to tooth agenesis in humans. Archives of Oral Biology. 2011; 56:506–11. [PubMed: 21144496]
15. Wheeler HE, Metter EJ, Tanaka T, Absher D, Higgins J, Zahn JM, et al. Sequential use of transcriptional profiling, expression quantitative trait mapping, and gene association implicates MMP20 in human kidney aging. PLoS Genetics. 2009; 5:e1000685. [PubMed: 19834535]
16. Bourd-Boittin K, Septier D, Hall R, Goldberg M, Menashi S. Immunolocalization of enamelysin (matrix metalloproteinase-20) in the forming rat incisor. Journal of Histochemistry and Cytochemistry. 2004; 52:437–45. [PubMed: 15033995]
17. Olivier M. From SNPs to function: the effect of sequence variation on gene expression. Physiological Genomics. 2004; 16:182–83. [PubMed: 14726602]
18. Souza JMP, Arrillaga AN, Ochoa FV, Rocha O. Prevalence of dental caries in Whites and non-Whites. Revista de Saude Publica. 1967; 1:38–43. [PubMed: 5603523]
19. World Health Organization. The World Oral Health Organization Report 2003. 2003. p. 38
20. Zhang Y, Yan Q, Li W, DenBesten PK. Fluoride down-regulates the expression of matrix metalloproteinase-20 in human fetal tooth ameloblast-lineage cells in vitro. European Journal of Oral Sciences. 2006; 114:105–10. [PubMed: 16674670]
21. Pine, C. Caries Risk: Individual and Population Perspectives. Community Dentistry and Oral Epidemiology; Proceedings of a Symposium at the 81st Annual Meeting of the International Association for Dental Research (IADR); Gothenburg, Sweden. 28 June 2003; 2005. p. 239-279.
22. Chun YH, Yamakoshi Y, Yamakoshi F, Fukae M, Hu JC, Bartlett JD, Simmer JP. Cleavage site specificity of MMP-20 for secretory-stage ameloblastin. Journal of Dental Research. 2010; 89:785–790. [PubMed: 20400724]
23. Lee HK, Lee DS, Ryoo HM, Park JT, Park SJ, Bae HS, Cho MI, Park JC. The odontogenic ameloblast-associated protein (ODAM) cooperates with RUNX2 and modulates enamel mineralization via regulation of MMP-20. Journal of Cell Biochemistry. 2010; 111:755–767.
24. Smith CE, Richardson AS, Hu Y, Bartlett JD, Hu JC, Simmer JP. Effect of kallikrein 4 loss on enamel mineralization: comparison with mice lacking matrix metalloproteinase 20. Journal of Biology & Chemistry. 2011; 286:18149–18160.
25. Lee SK, Seymen F, Kang HY, Lee KE, Gencay K, Tuna B, Kim JW. MMP20 hemopexin domain mutation in amelogenesis imperfecta. Journal of Dental Research. 2010; 89:46–50. [PubMed: 19966041]
26. Mazzoni A, Papa V, Nato F, Carrilho M, Tjaderhane L, Ruggeri A Jr, Gobbi P, Mazzotti G, Tay FR, Pashley DH, Breschi L. Immunohistochemical and biochemical assay of MMP-3 in human dentine. Journal of Dentistry. 2011; 39:231–237. [PubMed: 21215789]
27. Niu LN, Zhang L, Jiao K, Li F, Ding YX, Wang DY, Wang MQ, Tay FR, Chen JH. Localization of MMP-2, MMP-9, TIMP-1, and TIMP-2 in human coronal dentine. Journal of Dentistry. 2011; 39:536–542. [PubMed: 21641958]
28. Mazzoni A, Carrilho M, Papa V, Tjaderhane L, Gobbi P, Nucci C, Di Lenarda R, Mazzotti G, Tay FR, Pashley DH, Breschi L. MMP-2 assay within the hybrid layer created by a two-step etch-and-rinse adhesive: Biochemical and immunohistochemical analysis. Journal of Dentistry. 2011; 39:470–477. [PubMed: 21554921]

29. Ito A, Hayashi M, Hamasaki T, Ebisu S. Risk assessment of dental caries by using Classification and Regression Trees. Journal of Dentistry. 2011; 39:457–463. [PubMed: 21514355]

30. Mitropoulos P, Rahiotis C, Stamatakis H, Kakaboura A. Diagnostic performance of the visual caries classification system ICDAS II versus radiography and microcomputed tomography for proximal caries detection: An in vitro study. Journal of Dentistry. 2010; 38:859–867. [PubMed: 20654681]

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Table 1

Demographic data and risk factors for caries in the study subjects.

	Total of Children (n=388)	Caries experience (n=227)	Caries free (n=161)	OR (95%CI)	p-value
Mean Age (SD)	9.03 (2.75)	8.73(0.17)	9.45(0.22)	0.94 (0.89–0.99)	**0.01**
Gender (%)					
Female	186 (48.0)	113 (49.8)	73 (45.3)	1.19 (0.78–1.83)	0.38
Male	202 (52.0)	114 (50.2)	88 (54.7)	Reference	
Ethnicity (%)					
Caucasian	223 (57.5)	125 (55.1)	98 (60.9)	0.79 (0.51–1.21)	0.25
Afro-descendents	165 (42.5)	102 (44.9)	63 (39.1)	Reference	
Visible plaque (%)					
yes	127 (32.7)	79 (34.8)	48 (29.8)	1.26 (0.80–1.99)	0.30
no	261 (67.3)	148 (65.2)	113 (70.2)	Reference	
Tooth-brushing (%)					
1X	34 (8.7)	20 (8.8)	14 (8.7)	1.09 (0.52–2.29)	0.69
2X	149 (38.4)	91 (40.1)	58 (36.0)	1.20 (0.78–1.85)	
3X or more	205 (52.8)	116 (51.1)	89 (52.2)	Reference	
Use of dental floss daily (%)					
yes	123 (31.7)	62 (27.3)	61 (37.9)	0.62 (0.39–0.97)	**0.02**
no	265 (68.3)	165 (72.7)	100 (62.1)	Reference	
Use of fluoride mouthwash daily (%)					
yes	82 (21.1)	46 (20.3)	36 (22.4)	0.88 (0.52–1.49)	0.61
no	306 (78.9)	181 (79.7)	125 (77.6)	Reference	
Dietary factors (Ingest sweets between meals) (%)					
yes	205 (52.8)	139 (61.2)	66 (41.0)	2.27 (1.47–3.51)	**0.001**
no	183 (47.2)	88 (38.8)	95 (59.0)	Reference	

Note: p≤0.05; OR (95% C.I.)= Odds ratios; 95% confidence intervals; bold forms indicated statistical significance

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Table 2

Summary of the allele and genotype frequency comparisons.

	rs1784418 Alleles				rs1784418 Genotypes			
Subjects	**C**	**T**	**p-value**	**OR (95%CI)**	**CC**	**CT**	**TT**	**p-value**
In all subjects								
caries free vs. caries experience	181/264	141/190	0.59	0.92 (0.69–1.25)	50/84	81/96	30/47	0.28
caries free vs. moderate + high	181/218	141/154	0.52	0.91 (0.66–1.24)	50/69	81/80	30/37	0.37
caries free + low vs. moderate + high	227/218	177/154	0.49	0.91 (0.67–1.22)	65/69	97/80	40/37	0.54
Only Caucasians								
caries free vs. caries experience	113/144	83/106	0.99	1.00 (0.67–1.49)	30/49	53/46	15/30	**0.03**
caries free vs. caries experience in subjects that related poor oral health habits	35/44	19/32	0.42	1.34 (0.61–2.94)	10/17	15/10	2/11	**0.02**
caries free vs. caries experience in permanent dentition	28/20	30/24	0.77	1.12 (0.47–2.65)	4/6	20/8	5/8	0.06
caries free vs. caries experience in primary dentition	21/23	9/9	0.87	0.91 (0.27–3.13)	7/9	7/5	1/2	0.64
caries free vs. caries experience in mixed dentition	64/101	44/73	0.84	1.06 (0.63–1.76)	19/34	26/33	9/20	0.44
Only Afro-descendent								
caries free vs. caries experience	68/120	58/84	0.38	0.82 (0.51–1.32)	20/35	28/50	15/17	0.52
caries free vs. caries experience in subjects that related poor oral health habits	12/43	10/27	0.56	0.75 (0.26–2.20)	4/13	4/17	3/5	0.58
caries free vs. caries experience in permanent dentition	27/12	27/12	1.00	1.00 (0.34–2.91)	7/3	13/6	7/3	0.99
caries free vs. caries experience in primary dentition	8/20	8/8	0.15	0.40 (0.09–1.71)	3/7	2/6	3/1	0.20
caries free vs. caries experience in mixed dentition	23/88	23/64	0.34	0.73(0.36–1.48)	10/25	13/38	5/13	0.94

Note: p≤0.05; OR (95% C.I.)= Odds ratios; 95% confidence intervals; bold forms indicated statistical significance

Table 3

Regression analysis of children studied.

	Univariate analysis			Multivariate analysis		
Variables	**B**	**p-value**	**OR (95%CI)**	**B**	**p-value**	**OR (95%CI)**
rs1784418 *Genotype*					**0.041**	
CT	−0.349	0.140	0.705 (0.446–1.116)	−0.63	**0.043**	0.530 (0.286–0.980)
TT	−0.070	0.810	0.933 (0.524–1.660)	0.18	0.650	1.199 (0.547–2.627)
Afro-descendent	0.238	0.255	1.269 (0.842–1.914)	0.13	0.719	1.143 (0.552–2.369)
Ingest sweets between meals	0.821	**0.001**	2.274 (1.506–3.434)	0.85	**0.001**	2.330 (1.531–3.545)
Absence of use dental floss daily[#]	0.485	**0.028**	1.623 (1.054–2.501)			
Poor oral hygiene[#]	0.428	**0.067**	1.534 (0.970–2.426)			
Interaction Genotype[*]Ethnicity					**0.057**	
Interaction Genotype CT[*] Afro-descendent				0.621	0.203	1.862 (0.715–4.847)
Interaction GenotypeTT[*]Afro-descendent				−0.740	0.226	0.477 (0.144–1.581)

Note: The analyses were adjusted for genotype (with CC as reference), ethnicity (with Caucasian as reference), dental floss daily (with use as reference), sweets between meals (with no ingest as reference), poor oral hygiene (with absence as reference). OR (95% C.I.)= Odds ratios; 95% confidence intervals. Bold forms indicated statistical significance; p≤0.10.

[#] These variables were removed in multivariate analysis because when adjusted for other variables, the p-value was not significant.

Table 4

Predictive values for caries susceptibility considering multivariate regression analysis.

		rs1784418 Genotype (%)		
Ethnicity	**Ingest sweets between meals**	**CC**	**CT**	**TT**
Caucasian	Yes	71.02	56.50	74.61
	No	51.27	35.79	55.78
Afro-descendents	Yes	73.70	73.44	61.58
	No	54.60	54.27	40.76