Original Paper

Caries Research

Caries Res 2012;46:401–407
DOI: 10.1159/000339379

Received: November 1, 2011
Accepted after revision: April 17, 2012
Published online: June 15, 2012

MMP13 Polymorphism Decreases Risk for Dental Caries

P.N. Tannure[a, c] E.C. Küchler[d] P. Falagan-Lotsch[f] L.M.F. Amorim[e]
R. Raggio Luiz[b] M.C. Costa[a] A.R. Vieira[g] J.M. Granjeiro[e, f]

[a]Department of Pediatric Dentistry and Orthodontics, School of Dentistry, and [b]Institute of Public Health Studies, Federal University of Rio de Janeiro, and [c]Discipline of Pediatric Dentistry, School of Dentistry, Veiga de Almeida University, Rio de Janeiro, [d]Unit of Clinical Research, and [e]Department of Cellular and Molecular Biology, Biology Institute, Fluminense Federal University, Niterói, and [f]Institute of Metrology, Quality and Technology (INMETRO), Duque de Caxias, Brazil; [g]Departments of Oral Biology and Pediatric Dentistry, and Center for Craniofacial and Dental Genetics, School of Dental Medicine, Department of Human Genetics, Graduate School of Public Health, and Clinical and Translational Science Institute, University of Pittsburgh, Pittsburgh, Pa., USA

Key Words
Dental caries · Genetic susceptibility · Matrix metalloproteinases · Matrix metalloproteinase inhibitors, polymorphisms

Abstract
Recent evidence suggests that genetic studies may contribute to a better understanding of individual susceptibility to caries. Matrix metalloproteinases (MMPs) and their tissue inhibitors have been suggested to be involved in the caries process. The purpose of this study was to determine if polymorphisms in *MMP2* (rs243865), *MMP9* (rs17576), *MMP13* (rs2252070), and *TIMP2* (rs7501477) were associated with caries. Eligible unrelated children and adolescents were evaluated using a cross-sectional design. Data on oral health habits was obtained through a questionnaire and caries data was collected by clinical examination. Genotyping of the selected polymorphisms was carried out by real-time PCR. Allele and genotype frequencies were compared between individuals with and without caries experience. Of 505 subjects, 212 were caries-free and most subjects (61.2%) had mixed dentition. Allele frequency of *MMP2, MMP13* and *TIMP2* was different between caries-affected and caries-free individuals, with significant association for *MMP13* ($p = 0.004$). Mutant allele carriers for *MMP13* demonstrated a significantly decreased risk for caries (OR = 0.538, 95% CI 0.313–0.926); this result remained significant after adjustment for candidate genes, type of dentition and dietary factors. Allelic and genotype frequencies of the polymorphism in MMP9 were similar in caries-affected and caries-free individuals. Genetic variations in *MMP13* may contribute to individual differences in caries susceptibility. Our findings reinforce that susceptibility to caries results from gene-environment interactions.

Matrix metalloproteinases (MMPs) are major regulators of extracellular matrix (ECM) turnover and constitute an important family of zinc-dependent endopeptidases, which are able to degrade most components of ECM. MMPs have been well documented to have an important function in the organization of enamel and den-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0008–6568/12/0464–0401$38.00/0

Accessible online at:
www.karger.com/cre

Jose Mauro Granjeiro
R. Marques de Paraná, 303 – Centro
24030-215 Niterói, RJ (Brazil)
Tel. +55 21 2629 9255
E-Mail jmgranjeiro@gmail.com

tin organic matrix, suggesting their participation in the control and progression of caries [Tjaderhane et al., 1998; Sulkala et al., 2001; Chaussain-Miller et al., 2006; Shimada et al., 2009].

The MMP family is composed of 23 enzymes that share significant sequence homologies. They can be classified into subfamilies such as collagenases, stromelysins, gelatinases and membrane-type MMPs [Hannas et al., 2007]. MMP2 and MMP9 are gelatinases that can degrade denatured collagens (gelatins) and type IV collagen, which is the major structural component of the ECM [Crouch et al., 1980; Steffensen et al., 1995]. MMP13 is a collagenase 3 that can degrade ECM components, such as collagens, gelatin, aggrecan, perlecan and fibronectin [Sulkala et al., 2004].

Tissue inhibitors of metalloproteinases (TIMPs) are endogenous inhibitors of activated MMPs that play a role in normal processes such as tissue development [Brew and Nagase, 2010]. The TIMP family consists of four members. The balance between activated MMPs and TIMPs controls the extent of ECM remodeling [Hannas et al., 2007; Brew and Nagase, 2010]. TIMP2 has been shown to interact with MMP2 contributing to many biological functions [Hernandez-Barrantes et al., 2000].

MMPs and TIMPs have an important function in dental development and they have been proposed to be involved in caries lesion progression [Chaussain-Miller et al., 2006; Hannas et al., 2007]. MMP2 can cleave amelogenin, the major structural protein component of the enamel matrix, into several fragments and therefore, play an important role during tooth development [Caron et al., 2001]. A recent study revealed increased MMP2 expression in caries-affected dentine compared to sound dentine [Toledano et al., 2010]. MMP9 is the major MMP in whole saliva and appears to be the predominant gelatinolytic enzyme in dentin caries lesions [Tjaderhane et al., 1998]. MMP13 is involved in bone development and repair [Yamagiwa et al., 1999; Inada et al., 2004] and is expressed in pulp dental tissue [Sulkala et al., 2004]. TIMP2 was detected in enamel matrix [Yoshiba et al., 2003] and is coexpressed with *MMP2* and *MMP9* during mouse tooth morphogenesis [Sahlberg et al., 1999]. TIMP2 is also an endogenous inhibitor of MMP13 [Knauper et al., 1996].

Genes related to enamel development and mineralization such as *amelogenin, ameloblastin*, *tuftelin* and *enamelysin* have been associated with caries [Slayton et al., 2005; Deeley et al., 2008; Patir et al., 2008; Tannure et al., 2012b]. Also, an association between caries and saliva levels of specific proline-rich proteins (encoded by *PRH1* and *PRH2* genes), a saliva component that influences the attachment of bacteria, was reported [Zakhary et al., 2007]. Genetics also controls saliva function as there is evidence of association between buffer capacity and polymorphisms in the carbonic anhydrase 6 gene in children [Peres et al., 2010]. Host response to bacterial colonization is also controlled by individual genetic background. Variation in the bacterial ligand *CD14* was associated with the presence of four or more carious lesions in children [De Soet et al., 2008]. Beta-defensins have also been associated with high caries experience in adults [Ozturk et al., 2010].

Functional polymorphisms in the *MMP* and *TIMP* genes could lead to either increased or decreased activities and can affect the enamel development and caries establishment. The positive hypothesis of this study is that polymorphisms in *MMP2, MMP9, MMP13* and *TIMP2* are associated with caries susceptibility.

Materials and Methods

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

Eligible unrelated children and adolescents from 3 to 21 years of age were enrolled using a cohort design in the Pediatric Dental Clinics of Federal University of Rio de Janeiro during the period of February 2009 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 people of African descent (obviously of mixed European, 33.6% or not obviously mixed Africans, 12.3%). The remaining 0.5% of the population is of Amerindian or Asian descent [IBGE, 2007].

All subjects or parents/caregivers answered a questionnaire about fluoride exposure history and oral hygiene habits. 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 [Tannure et al., 2012a].

Determination of Caries Experience

Two pediatric dentists (P.N.T. and E.C.K.) conducted the clinical examinations [Tannure et al., 2012a]. 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. Caries was assessed using the DMFT and/or dmft indexes. The subjects were classified according to caries

Table 1. Candidate gene markers studied

Gene and base change	Location in the gene	SNP	Allele	Alteration	Locus	References
MMP2 (**C**/T)	promoter	rs243865	T	down-regulation[a]	16q13–q21	Rodriguez-Lopez et al. [2006]
MMP9 (**A**/G)	exon 6	rs17576	–	Gln279Arg[b]	20q1–q13	Singh et al. [2010]
MMP13 (**A**/G)	promoter	rs2252070	A	up-regulation[a]	11q22.3	Rodriguez-Lopez et al. [2006]
TIMP2 (**G**/T)	promoter	rs7501477	T	down-regulation[a]	17q25	Peterson et al. [2009]

[a] Change in transcription; reported regulation of gene expression determined by the mentioned allele.
[b] Missense; boldface indicates ancestral allele.

experience level. They were categorized into two groups: caries-free (subjects with dmft/DMFT = 0) and caries experience (dmft/DMFT ≥1).

Determination of Genotypes

Genomic DNA for molecular analysis was extracted from buccal cells based on the modified reported method [Küchler et al., 2012]. Genetic polymorphisms in the *MMP2, MMP9, MMP13* and *TIMP2* were genotyped by real-time polymerase chain reactions using the Taqman assay [Ranade et al., 2001] (Agilent Technologies, Stratagene Mx3005P). Primers, probes and universal master mix were provided by Applied Biosystems (Foster City, Calif., USA).

Three single nucleotide polymorphisms (SNPs) in the promoters of the genes encoding *MMP2, MMP13 and TIMP2* [Rodriguez-Lopez et al., 2006; Peterson et al., 2009] and one SNP in a coding region in *MMP9* [Singh et al., 2010] were selected for this study (table 1).

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS 16.0). The Student's test, odds ratio calculations and χ^2 test were used to analyze the age, ethnicity, gender, preventive habits between groups with caries experience and caries-free groups. Odds ratio calculations and χ^2 test at a level of significance of 0.05 were also performed to evaluate if subgroups presented preferential genotype and allele associations. The binary logistic regression was adjusted for genotype, type of dentition and dietary factor. Gene-gene interactions were also ascertained with binary logistic regression analysis. The standard χ^2 test was used to test for deviation from Hardy-Weinberg equilibrium.

Results

Of 505 subjects recruited in this study, 212 (41.9%) were caries-free. Caries-free subjects were older than subjects with caries experience (p = 0.044). The mean DMFT was 0.73 (SD ±1.53) and the dmft was 2.35 (SD ±2.85). A significant difference for factors like age, type of dentition and dietary factors amongst caries experience and caries-free were observed in the present study. The institution where the study was conducted, as well as children's residence, is in a geographic area with fluoridated water supply, and all subjects/guardians reported the use of fluoridated dentifrice. The demographic and clinical details of the subjects are presented in table 2.

The genotype and allele frequency distribution of four polymorphic sites in genes selected amongst caries-free subjects and subjects with caries experience is presented in table 3. All SNPs were in Hardy-Weinberg equilibrium in unaffected individuals. The variant allele frequencies of *MMP2, MMP13* and *TIMP2* were different between groups with caries experience and caries-free groups, with significant association in *MMP13* (p = 0.004). For *MMP13* a significant difference in genotype distribution was also observed between subjects with caries experience and the caries-free group (p = 0.042). Allelic and genotype frequencies of the polymorphism in MMP9 were similar in caries-affected and caries-free individuals (table 3). No difference was observed when we analyzed the genotype and allele distribution according to caries experience in deciduous teeth (dmft) and caries experience in permanent teeth (DMFT; data not shown, $p > 0.05$).

In order to verify if the association between genetic variants and caries susceptibility could be affected by other factors, we performed a binary logistic regression adjusted for genotypes, type of dentition and dietary factor (table 4). In *MMP13*, the genotype GG showed significant association with caries experience (p = 0.025), demonstrating a decreased risk for caries (OR = 0.538, 95% CI 0.313–0.926). In the multivariate analysis, this result together with caries experience in mixed dentition and ingestion of sweets between meals remained significant when adjusted for variables selected. When *TIMP2* genotype alone was used as the dependent variable, no

Table 2. Demographic data and risk factors for caries in the study subjects (n = 505)

	Caries experience (n = 293)	Caries-free (n = 212)	OR (95% CI)	p value
Mean age ± SD, years	8.83 ± 2.95	9.40 ± 3.39	0.943 (0.891–0.999)	0.044
Type of dentition (%)				0.001
Permanent dentition	52 (17.7)	71 (33.5)	reference	
Mixed dentition	201 (68.6)	108 (50.9)	2.541 (1.657–3.896)	
Primary dentition	40 (13.7)	33 (15.6)	1.655 (0.923–2.966)	
Gender (%)				0.528
Female	141 (48.1)	96 (45.3)	1.120 (0.786–1.598)	
Male	152 (51.9)	116 (54.7)	reference	
Ethnicity (%)				0.533
Caucasian	166 (56.7)	126 (59.4)	0.892 (0.623–1.277)	
Afro-descendants	127 (43.3)	86 (40.6)	reference	
Tooth-brushing (%)				0.385
1×	20 (6.8)	16 (7.5)	1.126 (0.566–2.238)	
2×	109 (37.2)	68 (32.1)	1.312 (0.892–1.931)	
3× or more	149 (50.9)	122 (57.6)	reference	
No answer	13 (4.4)	6 (2.8)		
Use of dental floss daily (%)				0.181
Yes	81 (27.7)	71 (33.5)	reference	
No	199 (67.9)	134 (63.2)	1.302 (0.884–1.916)	
No answer	13 (4.4)	7 (3.3)		
Dietary factors (sweets between meals) (%)				0.001
Yes	186 (63.5)	90 (42.5)	2.356 (1.641–3.383)	
No	107 (36.5)	122 (57.5)	reference	

χ^2 test, $p \leq 0.05$ indicates statistical significance.

Table 3. Summary of allele and genotype frequencies

Subjects	Alleles (%)		p value	OR (95% CI)	Genotypes (%)			p value
MMP2 rs243865 (C>T)	T	C			CC	CT	TT	
Caries experience	172 (29.6)	408 (70.4)	0.059	1.31 (0.98–1.76)	157 (54.1)	94 (32.4)	39 (13.5)	0.232
Caries-free	103 (24.2)	321(75.8)			130 (61.3)	61 (28.7)	21 (10.0)	
MMP9 rs17576 (A>G)	G	A			AA	AG	GG	
Caries experience	186 (31.8)	398 (68.2)	0.785	1.04 (0.79–1.37)	153 (52.3)	92 (31.5)	47 (16.2)	0.115
Caries-free	131 (31.0)	291 (69.0)			104 (49.3)	83 (39.3)	24 (11.4)	
MMP13 rs2252070 (A>G)	G	A			AA	AG	GG	
Caries experience	160 (27.5)	422 (72.5)	0.004	0.67 (0.51–0.89)	163 (56.0)	96 (33.0)	32 (11.0)	0.042
Caries-free	150 (36.0)	266 (64.0)			96 (45.4)	80 (37.9)	35 (16.7)	
TIMP2 rs7501477 (G>T)	T	G			GG	GT	TT	
Caries experience	119 (20.5)	459 (79.5)	0.059	1.38 (0.97–1.95)	181 (62.6)	97 (33.5)	11 (3.9)	0.201
Caries-free	65 (15.8)	345 (84.2)			145 (69.0)	55 (26.2)	10 (4.8)	

$p \leq 0.05$ indicates statistical significance.

Table 4. Regression analysis of children and adolescent sample

Variables	Univariate analysis		Multivariate analysis	
	p value	OR (95% CI)	p value	OR (95% CI)
MMP2				
CC	reference		reference	
CT	0.229	1.276 (0.858–1.898)	0.344	1.229 (0.802–1.885)
TT	0.145	1.538 (0.862–2.744)	0.214	1.503 (0.791–2.856)
MMP9				
AA	reference		reference	
AG	0.152	0.753 (0.511–1.110)	0.164	0.744 (0.490–1.129)
GG	0.309	1.1331 (0.767–2.310)	0.320	1.354 (0.745–2.458)
MMP13				
AA	reference			
AG	0.081	0.707 (0.479–1.043)	0.100	0.705 (0.464–1.070)
GG	0.025	0.538 (0.313–0.926)	0.046	0.554 (0.310–0.990)
TIMP2				
GG	reference		reference	
GT	0.088	1.413 (0.950–2.100)	0.048	1.532 (1.004–2.339)
TT	0.779	0.881 (0.364–2.133)	0.782	0.872 (0.332–2.293)
Type of dentition				
Permanent dentition	reference		reference	
Mixed dentition	0.001	2.541 (1.657–3.896)	0.001	2.639 (1.676–4.155)
Primary dentition	0.091	1.655 (0.923–2.966)	0.259	1.427 (0.770–2.644)
Sweets between meals				
Yes	0.001	2.356 (1.641–3.383)	0.001	2.412 (1.641–3.547)
No	reference		reference	

$p \leq 0.05$ indicates statistical significance.

significant association was observed but it became significant after adjustment for genotypes, type of dentition and diet (p = 0.048; OR = 1.532, 95% CI 1.004–2.339).

Gene-gene interactions that could involve any of the four ECM protease SNPs were ascertained with binary logistic regression analysis. Different genotype combinations of studied polymorphisms were tested and no influence of combinations on caries experience was observed (data not show).

Discussion

To the best of our knowledge this is the first report that investigates gene polymorphisms in MMPs and their associations in caries experience. Our present work provided evidence that the polymorphisms studied in the *MMP13* gene is associated with alterations in risk for caries experience in a group of Brazilian children and adolescents with similar access to oral care and fluoride exposure.

In animal models, specifically in growing rat incisors, it was shown that MMPs 2, 3, 9, and 20 play a critical role [Goldberg et al., 2003]. Another study found differential expression of MMPs 2, 9 and RECK in the different phases of amelogenesis, signifying that tissue remodeling is rigorously controlled during dental mineralization [Paiva et al., 2009]. These previous results suggested potential candidate genes for caries susceptibility.

In line with our results, other studies demonstrated that genes involved in enamel development were associated with increased caries susceptibility [Slayton et al., 2005; Deeley et al., 2008; Patir et al., 2008; Tannure et al., 2012b]. Genetic variations in these genes contribute to structural alterations of the enamel that may cause enamel porosity, decreased mineral content, presence of enamel crystal inhibitory proteins, higher levels of mineral loss under acid conditions and/or facilitate bacterial attachment and biofilm deposition [Shuler, 2001; Patir et al., 2008].

Considering the multifactorial nature of the caries disease, environmental factors such as low socioeconomic

status, poor oral hygiene, and cariogenic diet are variables contributing to its development. Human saliva contains gelatinases [van Strijp et al., 2003] and indeed, MMP2, MMP8 and MMP9 can be activated at low pH level followed by neutralization [Tjaderhane et al., 1998]. According to our results, ingestion of sweets between meals was the environmental factor that interacted with MMPs, resulting in an increased risk to caries experience. Following this pattern, subjects with poor oral hygiene and cariogenic diet ingestion have intermittent pH fluctuations. These fluctuations could lead to MMP activation (under acidic conditions) and MMP action (at neutral pH) that destroy the dentin matrix [Chaussain-Miller et al., 2006].

We assume that MMPs and TIMPs are highly expressed in two key moments that modify caries susceptibility. First, these genes participate in enamel formation, in histomorphogenesis and cytodifferentiation [Yoshiba et al., 2003]. Both events are accompanied by changes in cellular organization and remodeling of the ECM [Yoshiba et al., 2003]. MMP2 is expressed by ameloblasts and odontoblasts and plays a role in the biomineralization processes responsible for the formation of enamel and dentin [Caron et al., 2001]. Second, these enzymes are activated by pH fluctuations in saliva and again could influence caries experience.

Results of the present study indicate that MMP13 polymorphism shows an association with dental caries susceptibility. It is known that the polymorphism selected in our study is located in the promoter region of MMP13 and seems to alter the expression of the gene [Fernandez-Cadenas et al., 2011]. However, the exact role of MMP13 in enamel and/or dentin cannot be explained by the current knowledge of tooth development. *MMP13*-null mice have profound defects in growth plate cartilage and delay in endochondral ossification and formation and vascularization of primary ossification centers, however, no description of the dentition status was provided [Inada et al., 2004]. It is reasonable to hypothesize that tooth mineralization may also be affected in these animals.

Considering the limits of this genetic epidemiological approach, our study did not evaluate other mechanisms of enzyme expression in the oral environment. We can speculate that a high ratio of MMP13 to TIMP2 is the result of a cariogenic oral environment, mainly due to a low pH level after the ingestion of sweets between meals. In our regression analysis, genetic markers in *MMP13* and *TIMP2* in combination with other genes, type of dentition and dietary factors were associated with caries susceptibility. *MMP13* is expressed during the bud stage of dental development in the mouse [Morris-Wiman et al., 2000] and variation in expression of *MMP13* in humans due to hypomorphic alleles could generate enamel that is more susceptible to high cariogenic challenges.

MMP9 was shown by zymography and Western blot to be the main gelatinase in whole saliva, in gingival crevicular fluid and several caries lesions [Tjaderhane et al., 1998; van Strijp et al., 2003]. On the other hand, a lack of association between *MMP9* polymorphism and dental caries was verified and no gene-gene interactions could be observed in our findings.

In summary, our results suggest that genetic variations in *MMP13* may contribute to caries susceptibility and that susceptibility to caries results from gene-environment interactions. Further studies that include genes involved in enamel development, salivary function and immune response may lead to a better understanding of genetic determinants of caries risk.

Acknowledgments

The authors are indebted to the participants of the study. Support for this work was provided by CAPES (E.C.K. and P.N.T. fellowship), FAPERJ (M.C.C., J.M.G.), FINEP (J.M.G.), MS/DECIT (J.M.G.), and CNPq (J.M.G.).

Disclosure Statement

No conflicts of interest.

References

Brew K, Nagase H: The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. Biochim Biophys Acta 2010;1803:55–71.

Caron C, Xue J, Sun X, Simmer JP, Bartlett JD: Gelatinase A (MMP-2) in developing tooth tissues and amelogenin hydrolysis. J Dent Res 2001;80:1660–1664.

Chaussain-Miller C, Fioretti F, Goldberg M, Menashi S: The role of matrix metalloproteinases (MMPs) in human caries. J Dent Res 2006;85:22–32.

Crouch E, Sage H, Bornstein P: Structural basis for apparent heterogeneity of collagens in human basement membranes: type IV procollagen contains two distinct chains. Proc Natl Acad Sci USA 1980;77:745–749.

Deeley K, Letra A, Rose EK, Brandon CA, Resick JM, Marazita ML, Vieira AR: Possible association of amelogenin to high caries experience in a Guatemalan-Mayan population. Caries Res 2008;42:8–13.

De Soet JJ, van Gemert-Schriks MC, Laine ML, van Amerongen WE, Morre SA, van Winkelhoff AJ: Host and microbiological factors related to dental caries development. Caries Res 2008;42:340–347.

Fernandez-Cadenas I, Mendioroz M, Domingues-Montanari S, Del Rio-Espinola A, Delgado P, Ruiz A, Hernandez-Guillamon M, Giralt D, Chacon P, Navarro-Sobrino M, Ribo M, Molina CA, Alvarez-Sabin J, Rosell A, Montaner J: Leukoaraiosis is associated with genes regulating blood-brain barrier homeostasis in ischaemic stroke patients. Eur J Neurol 2011;18:826–835.

Goldberg M, Septier D, Bourd K, Hall R, George A, Goldberg H, Menashi S: Immunohistochemical localization of MMP-2, MMP-9, TIMP-1, and TIMP-2 in the forming rat incisor. Connect Tissue Res 2003;44:143–153.

Hannas AR, Pereira JC, Granjeiro JM, Tjaderhane L: The role of matrix metalloproteinases in the oral environment. Acta Odontol Scand 2007;65:1–13.

Hernandez-Barrantes S, Toth M, Bernardo MM, Yurkova M, Gervasi DC, Raz Y, Sang QA, Fridman R: Binding of active (57 kDa) membrane type 1-matrix metalloproteinase (MT1-MMP) to tissue inhibitor of metalloproteinase (TIMP)-2 regulates MT1-MMP processing and pro-MMP-2 activation. J Biol Chem 2000;275:12080–12089.

IBGE: Contagem da população. Instituto Brasileiro de Estatística, 2007.

Inada M, Wang Y, Byrne MH, Rahman MU, Miyaura C, Lopez-Otin C, Krane SM: Critical roles for collagenase-3 (Mmp13) in development of growth plate cartilage and in endochondral ossification. Proc Natl Acad Sci USA 2004;101:17192–17197.

Knauper V, Lopez-Otin C, Smith B, Knight G, Murphy G: Biochemical characterization of human collagenase-3. J Biol Chem 1996;271:1544–1550.

Küchler EC, Tannure PN, Falagan-Lotsch P, Lopes TS, Granjeiro JM, Amorin LMF: Buccal cells DNA extraction to obtain high quality human genomic DNA suitable for polymorphism genotyping by PCR-RFLP and real time PCR. J Appl Oral Sci 2012, in press.

Morris-Wiman J, Burch H, Basco E: Temporospatial distribution of matrix metalloproteinase and tissue inhibitors of matrix metalloproteinases during murine secondary palate morphogenesis. Anat Embryol (Berl) 2000;202:129–141.

Ozturk A, Famili P, Vieira AR: The antimicrobial peptide DEFB1 is associated with caries. J Dent Res 2010;89:631–636.

Paiva KB, Zambuzzi WF, Accorsi-Mendonca T, Taga R, Nunes FD, Sogayar MC, Granjeiro JM: Rat forming incisor requires a rigorous ECM remodeling modulated by MMP/RECK balance. J Mol Histol 2009;40:201–207.

Patir A, Seymen F, Yildirim M, Deeley K, Cooper ME, Marazita ML, Vieira AR: Enamel formation genes are associated with high caries experience in Turkish children. Caries Res 2008;42:394–400.

Peres RC, Camargo G, Mofatto LS, Cortellazzi KL, Santos MC, Nobre-dos-Santos M, Bergamaschi CC, Line SR: Association of polymorphisms in the carbonic anhydrase 6 gene with salivary buffer capacity, dental plaque pH, and caries index in children aged 7–9 years. Pharmacogenomics J 2010;10:114–149.

Peterson NB, Beeghly-Fadiel A, Gao YT, Long J, Cai Q, Shu XO and Zheng W: Polymorphisms in tissue inhibitors of metalloproteinases-2 and -3 and breast cancer susceptibility and survival. Int J Cancer 2009;125:844–850.

Ranade K, Chang MS, Ting CT, Pei D, Hsiao CF, Olivier M, Pesich R, Hebert J, Chen YD, Dzau VJ, Curb D, Olshen R, Risch N, Cox DR, Botstein D: High-throughput genotyping with single nucleotide polymorphisms. Genome Res 2001;11:1262–1268.

Rodriguez-Lopez J, Perez-Pampin E, Gomez-Reino JJ, Gonzalez A: Regulatory polymorphisms in extracellular matrix protease genes and susceptibility to rheumatoid arthritis: a case-control study. Arthritis Res Ther 2006;8:R1.

Sahlberg C, Reponen P, Tryggvason K, Thesleff I: Timp-1, -2 and -3 show coexpression with gelatinases A and B during mouse tooth morphogenesis. Eur J Oral Sci 1999;107:121–130.

Shimada Y, Ichinose S, Sadr A, Burrow MF, Tagami J: Localization of matrix metalloproteinases (MMPs-2, 8, 9 and 20) in normal and carious dentine. Aust Dent J 2009;54:347–354.

Shuler CF: Inherited risks for susceptibility to dental caries. J Dent Educ 2001;65:1038–1045.

Singh R, Srivastava P, Srivastava A, Mittal RD: Matrix metalloproteinase (MMP-9 and MMP-2) gene polymorphisms influence allograft survival in renal transplant recipients. Nephrol Dial Transplant 2010;25:3393–3401.

Slayton RL, Cooper ME, Marazita ML: Tuftelin, mutans streptococci, and dental caries susceptibility. J Dent Res 2005;84:711–714.

Steffensen B, Wallon UM, Overall CM: Extracellular matrix binding properties of recombinant fibronectin type II-like modules of human 72-kDa gelatinase/type IV collagenase. High affinity binding to native type I collagen but not native type IV collagen. J Biol Chem 1995;270:11555–11566.

Sulkala M, Paakkonen V, Larmas M, Salo T, Tjaderhane L: Matrix metalloproteinase-13 (MMP-13, collagenase-3) is highly expressed in human tooth pulp. Connect Tissue Res 2004;45:231–237.

Sulkala M, Wahlgren J, Larmas M, Sorsa T, Teronen O, Salo T, Tjaderhane L: The effects of MMP inhibitors on human salivary MMP activity and caries progression in rats. J Dent Res 2001;80:1545–1549.

Tannure PN, Costa Mde C, Küchler EC, Romanos HF, Granjeiro JM, Vieira AR: Caries experience in individuals with cleft lip and palate. Pediatr Dent 2012a;34:127–131.

Tannure PN, Küchler EC, Lips A, Costa Mde C, Luiz RR, Granjeiro JM, Vieira AR: Genetic variation in MMP20 contributes to higher caries experience. J Dent 2012b;40:381–386.

Tjaderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, Salo T: The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. J Dent Res 1998;77:1622–1629.

Toledano M, Nieto-Aguilar R, Osorio R, Campos A, Osorio E, Tay FR, Alaminos M: Differential expression of matrix metalloproteinase-2 in human coronal and radicular sound and carious dentine. J Dent 2010;38:635–640.

van Strijp AJ, Jansen DC, DeGroot J, ten Cate JM, Everts V: Host-derived proteinases and degradation of dentine collagen in situ. Caries Res 2003;37:58–65.

Yamagiwa H, Tokunaga K, Hayami T, Hatano H, Uchida M, Endo N, Takahashi HE: Expression of metalloproteinase-13 (collagenase-3) is induced during fracture healing in mice. Bone 1999;25:197–203.

Yoshiba N, Yoshiba K, Stoetzel C, Perrin-Schmitt F, Cam Y, Ruch JV, Lesot H: Temporospatial gene expression and protein localization of matrix metalloproteinases and their inhibitors during mouse molar tooth development. Dev Dyn 2003;228:105–112.

Zakhary GM, Clark RM, Bidichandani SI, Owen WL, Slayton RL, Levine M: Acidic proline-rich protein Db and caries in young children. J Dent Res 2007;86:1176–1180.