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Early Childhood Caries Is Associated with Genetic Variants in Enamel Formation and Immune Response Genes

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Key Words
Early childhood caries · Gene-environment interaction · Risk and protective factors

Abstract
Early childhood caries (ECC) is a chronic, infectious disease that affects the primary dentition of young children. It is the result of an imbalance of risk factors and protective factors that influence the disease. The aim of this study was to assess genetic and environmental factors that may contribute to ECC. Two hundred and fifty-nine unrelated children were evaluated using a cross-sectional design. Data on oral habits were obtained through a questionnaire, and caries experience data were collected by clinical examination. Twenty-three markers in 10 genes were studied. Genotyping of the selected polymorphisms was carried out by real-time PCR. Regression analyses were performed comparing individuals with and without caries experience. Of 259 subjects, 123 were caries free. The genotype TT in *ALOX15* (rs7217186) was a risk factor for ECC, whereas the genotypes GG in *ENAM* (rs1264848), AG and GG in *KLK4* (rs198968), CT in *LTF* (rs4547741), and GG in *TUFT1* (rs3790506) were protective for EEC. In conclusion, environmental factors and gene interactions can act as protective or risk factors for ECC. These factors together contribute to the presence and severity of the disease.

Early childhood caries (ECC) is defined as 'the presence of one or more decayed (noncavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces' in any primary tooth in a 71-month or younger child [Drury et al., 1999]. ECC represents one of the major diseases that impact on children's health and remains a public health problem in many communities. It results from a chronic imbalance between multiple risk factors and protective factors [American Academy of Pediatric Dentistry, 2008].

It is well established that environmental factors such as diet, oral hygiene, other oral habits, and socioeconomic factors are risk or protective factors for caries [Levy et al., 2003; Ferreira et al., 2007; Menghini et al., 2008; Tan-

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nure et al., 2012c]. However, the factors related to the host are genetically controlled, and environmental factors can overcome the genetic component of this complex disease. Our more recent studies continue to demonstrate that genetic variation in the host is associated with caries experience, and these variations can play a role in caries etiology as risk factors or as protective factors [Patir et al., 2008; Deeley et al., 2008; Vieira et al., 2008; Ozturk et al., 2010; Shaffer et al., 2011; Tannure et al., 2012a, b; Shimizu et al., 2012; Wang et al., 2012; Briseño-Ruiz et al., 2013; Shimizu et al., 2013]. However, one criticism regarding these studies is the incomplete information concerning environmental factors to be included as covariates in the genetics analysis. In this study, we evaluated the association between genes involved in enamel formation and genes involved in immune response and their interaction with environmental factors in ECC experience.

Subjects and Methods

The Human Ethics Committee of Marmara University, Turkey, 2011, and the University of Pittsburgh Institutional Review Board approved this study. Informed consent was obtained from all parents/legal guardians.

Healthy unrelated children with no chronic illnesses from 2 to 5 years of age who had no systemic fluoride consumption were enrolled in this cross-sectional study. All children sought dental treatment at the Pediatric Dental Clinics of Marmara University from 2011 to 2012, and all parents/caregivers answered a questionnaire about the children's diet and oral hygiene habits.

Determination of Caries Experience

The examiner (Z.A.) carried out the clinical examination after being trained by an experienced specialist (M.K.) in pediatric dentistry. Caries was diagnosed by visual examination and was recorded if there was definite visual evidence of a breach in the enamel with or without extension into dentin. Visible presence of white spot lesions due to enamel demineralization was also recorded. 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's guidelines. Caries experience was assessed using the dmft and dmfs indexes for each individual. Calculations excluded teeth lost to trauma or primary teeth lost to exfoliation.

Subjects were classified according to caries experience level. They were categorized into two groups: caries free (children with dmft = 0) and children with caries experience (dmft ≥1).

DNA Samples and Genotyping

Genomic DNA was extracted from buccal cells using a QIAmp DNA isolation protocol. Twenty-four markers in 10 genes (7 involved in enamel formation and 3 involved in immune response) were included in this study (table 1). Genotyping was performed by PCRs using the TaqMan method [Ranade et al., 2001] with an ABI PRISM® 7900HT Sequence Detection System (Foster City, Calif., USA). Predesigned probes were supplied by Applied Biosystems (Foster City, Calif., USA). Markers were chosen based on a previous association with caries experience, allele frequency, position on the gene and linkage disequilibrium relationships to maximize information content.

Table 1. Genes and markers included in this study

Genetic marker	Gene	Chromosome	Base change	Minor allele frequency
rs2619112	*ALOX15*	17	A/G	0.447
rs7217186	*ALOX15*	17	C/T	0.472
rs4694075	*AMBN*	4	C/T	0.479
rs34538475	*AMBN*	4	G/T	0.187
rs17878486	*AMELX*	X	C/T	0.111
rs946252	*AMELX*	X	A/G	0.300
rs11362	*DEFB1*	8	A/G	0.405
rs1800972	*DEFB1*	8	C/G	0.154
rs12640848	*ENAM*	4	A/G	0.357
rs3796704	*ENAM*	4	A/G	0.120
rs2235091	*KLK4*	19	C/T	0.340
rs198968	*KLK4*	19	A/G	0.313
rs2269436	*LTF*	3	A/G	0.103
rs743658	*LTF*	3	A/G	0.103
rs4547741	*LTF*	3	C/T	0.059
rs17078878	*LTF*	3	A/C	0.146
rs1784418	*MMP20*	11	A/G	0.407
rs5997096	*TFIP11*	22	C/T	0.468
rs134136	*TFIP11*	22	C/T	0.337
rs7526319	*TUFT1*	1	C/T	0.338
rs4970957	*TUFT1*	1	A/G	0.240
rs3828054	*TUFT1*	1	C/T	0.105
rs3790506	*TUFT1*	1	C/T	0.248
rs2337360	*TUFT1*	1	A/G	0.250

Statistical Analysis

Data was subsequently processed and analyzed using the Epi Info 3.3.2 statistical software package (http://www.cdc.gov/epiinfo). Student's t test was used to assess mean differences, and χ^2 or Fisher's exact tests were used to find the difference in frequencies between caries-free children and children with caries experience. Logistic regression analysis of each genetic marker was performed. The environmental factors identified as possible modifiers for ECC experience were included as covariates during the multivariate analyses to detect gene-environment interactions. The established α was 5%, and the Hardy-Weinberg equilibrium was evaluated by χ^2 test with 1 degree of freedom within each marker.

Results

Of the 259 children included in this study, 123 (47.5%) were caries free and 136 (52.5%) were children with caries experience. The mean age was 4.6 years (standard de-

viation, SD, 0.61). Caries-free children (4.14 years old, SD 0.9) were younger than children with caries experience (4.45 years old, SD 0.32, p = 0.0001). Among the affected children, the dmft varied from 2 to 19 and the mean dmft was 5.16 (SD 5.5). In this group, the dmfs varied from 2 to 62 and the mean dmfs was 10.44 (SD 13.17). All children with caries experience had a carious lesion in at least 1 posterior tooth, and almost all children had additional lesions in an anterior tooth (130; 95.6%). Demographic data and environmental risk factors for ECC are summarized in table 2. Two environmental factors were associated with ECC in this population. Brushing a child's teeth for the first time after the window of infectivity (19–31 months of age [Caufield et al., 1993]) was a risk factor for ECC (odds ratio 1.33; 95% confidence interval 0.67–2.65). The frequency of sugar and/or acidic drink consumption each day increased the risk for ECC almost 3 times.

The environmental factors for ECC identified and described above were included in the multivariate analyses in order to identify gene-environment interactions. The results of the univariate and multivariate analyses of the association of genotypes with ECC are presented in table 3. The genotype TT in *ALOX15* (rs7217186) was a risk factor for ECC in the multivariate analysis. The genotype GG in *ENAM* (rs1264848) was a protective factor for ECC in the multivariate analysis. The genotypes AG and GG in *KLK4* (rs198968) were associated as protective factors with ECC in the multivariate analysis. The genotype CT in *LTF* (rs4547741) was a protective factor for EEC in the univariate and in the multivariate analyses. Finally, the genotype GG in *TUFT1* (rs3790506) was a protective factor for ECC in the univariate and in the multivariate analyses.

Discussion

Although it is well established that multiple factors contribute to an individual's risk for caries, not many studies evaluated the interactions between environmental factors and genetic factors. The genome-wide scan of caries experience in primary dentition [Shaffer et al., 2011] included children 3–12 years of age as well as an analysis of genetic associations based on having sufficient or deficient home fluoride exposure. However, no statistically significant associations were found despite some borderline suggestive results. To the best of our knowledge, this is the first work to look for interactions between genetic variants and environmental factors in ECC. It is not difficult to propose that genetic mechanisms that modulate the enamel development and the immune response are involved with ECC experience and are influenced by factors such as oral hygiene, diet and possibly other environmental factors.

It is important to emphasize that both of the groups analyzed here had a similar lifestyle and relied on the same health service. Among all self-reported environmental factors analyzed here, only the frequency of sugar and/or acid drink consumption and the time of first toothbrushing were associated with ECC. Also, more caries-free children were among the 2- and 3-year-olds. For this reason, these factors were included as covariates in the multivariate analysis. Multivariate analyses are useful to elucidate the interactions of environmental factors and genetic variants influencing a given trait [Leboyer et al., 1998].

We studied genes involved in enamel development [ameloblastin *(AMBN)*, amelogenin *(AMELX)*, enamelin *(ENAM)*, kallikrein 4 *(KLK4)*, matrix metalloproteinase 20 *(MMP20)*, tuftelin *(TUFT1)* and tuftelin-interacting protein 11 *(TFIP11)*] and genes related to the immune response of the host [β-defensin 1 *(DEFB1)* and lactoferrin *(LTF)*]. Arachidonate 15-lipoxygenase *(ALOX15)* was associated with bone mineralization [Vilella et al., 2009], and it is plausible that this gene is involved in the formation of the hard structures of teeth. This gene has also been related to inflammatory response [Kelavkar and Badr, 1999]. Based on the complex and multifactorial nature of caries, it was not surprising that we found associations between some of these genes and ECC.

Dental enamel is a highly mineralized tissue with 85% of its volume occupied by hydroxyapatite crystals. This structure is rigorously controlled in ameloblasts through the interaction of a number of organic matrix molecules such as ENAM, AMELX, AMBN, TUFT1 and TFIP11. ENAM is the largest protein in the enamel matrix during development and comprises approximately 5% of total enamel matrix protein [Pavlic et al., 2007]. In our results, the multivariate analyses demonstrated that the GG in *ENAM* (rs1264848) was protective for ECC. Our previous study also demonstrated the association of this gene with caries experience in Turkish children when the presence of *Streptococcus mutans* was modeled with the T allele of rs3796704 [Patir et al., 2008]. Another study of our group demonstrated that the mechanism through which *ENAM* is possibly involved with caries consists in contributing to an enamel surface more susceptible to demineralization [Shimizu et al., 2012].

Table 2. Demographic characteristics and environmental risk factors for ECC in the studied population

Variables	Total children (n = 259)	Caries experience[a] (n = 136)	Caries free (n = 123)	Odds ratio	p value
Sex					0.573
Male	129 (49.8)	70 (51.5)	59 (48.0)	0.89 [0.53–1.41]	
Female	130 (50.2)	66 (48.5)	64 (52.0)	ref.	
Age group					0.0001
2 years old	6 (2.3)	0 (0.0)	6 (4.9)	–	
3 years old	34 (13.1)	9 (6.6)	25 (20.3)	0.08 [0.03–0.17]	
4 years old	74 (28.6)	36 (26.5)	38 (30.9)	0.56 [0.31–0.99]	
5 years old	145 (56.0)	91 (66.9)	54 (43.9)	ref.	
Mean birth weight ± SD, g	3,228±599.2	3,202±627.0	3,257±568.1	–	0.463
Milk bottle					0.431
Yes	185 (71.4)	100 (73.5)	85 (69.1)	ref.	
No	74 (28.6)	36 (26.5)	38 (30.9)	0.80 [0.46–1.38]	
Mean duration of milk bottle usage ± SD, months	22.1±11.22	22.5±10.4	21.7±12.12	1.0 [0.98–1.02]	0.635
Milk ingredient					0.439
No milk consumption	70 (27.0)	35 (25.7)	35 (28.5)	–	
No sugar	71 (27.4)	34 (25.0)	37 (30.1)	0.76 [0.42–1.38]	
With sugar	118 (45.6)	67 (49.3)	51 (41.5)	ref.	
Milk consumption before sleeping					0.171
Yes	171 (66.0)	95 (69.9)	76 (61.8)	0.69 [0.41–1.16]	
No	88 (34.0)	41 (30.1)	47 (38.2)	ref.	
Mean snack consumption ± SD, n/day	2.50±1.10	2.59±1.11	2.39±1.08	–	0.150
Sugar and/or acidic drink consumption					0.001
Never	90 (34.7)	36 (26.5)	54 (43.9)	–	
Occasional	123 (47.5)	14 (10.3)	15 (12.2)	1.40 [0.60–3.24]	
Once per day	17 (6.6)	80 (58.8)	43 (35.0)	2.69 [1.59–4.89]	
Twice or more per day	29 (11.2)	6 (4.4)	11 (8.9)	0.81 [0.27–2.41]	
Time of first toothbrushing					0.023
Before the window of infectivity	6 (2.3)	0 (0.0)	6 (4.87)	–	
During the window of infectivity	206 (79.5)	109 (80.1)	97 (78.9)	ref.	
After the window of infectivity	40 (15.4)	24 (17.6)	16 (13.0)	1.33 [0.67–2.65]	
Did not know	7 (2.8)	3 (2.3)	4 (3.23)	–	
Toothbrushing before sleeping					0.995
Every day	97 (37.5)	51 (37.5)	46 (37.4)	ref.	
Sometimes	138 (53.3)	73 (53.7)	65 (52.8)	0.90 [0.34–2.36]	
Never	20 (7.7)	10 (7.4)	10 (8.1)	0.89 [0.34–2.27]	
Did not know	4 (1.5)	2 (1.4)	2 (1.7)	–	
Toothbrushing frequency					0.671
No brushing	11 (4.2)	6 (4.4)	5 (4.1)	ref.	
Twice or 3 times per week	18 (6.9)	7 (5.1)	11 (8.9)	0.90 [0.25–3.19]	
Once per day	138 (53.3)	75 (55.1)	63 (51.2)	1.71 [0.61–4.81]	
Twice per day	92 (35.5)	48 (35.3)	44 (35.8)	0.91 [0.54–1.55]	

Figures in parentheses indicate percentages; figures in square brackets indicate 95% confidence intervals. p values <0.05 are considered statistically significant.

[a] Caries lesions were defined as a definite breakdown of enamel with or without an extension to dentin and visible white spot lesions due to demineralization of enamel.

Table 3. Univariate and multivariate analyses of the genotypes

Gene	Genetic marker	Genotype	Univariate analysis		Multivariate analysis	
			p value	odds ratio	p value	odds ratio
ALOX15	rs2619112	CC	ref.	–	ref.	–
		AG	0.864	0.95 (0.50–1.79)	0.869	1.06 (0.54–2.09)
		GG	0.521	0.79 (0.39–1.61)	0.886	0.95 (0.44–2.02)
	rs7217186	CC	ref.	–	ref.	–
		CT	0.368	0.63 (0.23–1.72)	0.239	0.52 (0.18–1.54)
		TT	0.061	2.57 (0.96–6.92)	*0.050*	2.97 (1.00–8.86)
AMBN	rs4694075	CC	ref.	–	ref.	–
		CT	0.151	1.99 (0.78–5.08)	0.265	1.74 (0.66–4.61)
		TT	0.153	0.51 (0.21–1.28)	0.258	0.58 (0.22–1.49)
	rs34538475	GG	ref.	–	ref.	–
		GT	0.606	0.84 (0.43–1.64)	0.439	0.75 (0.36–1.57)
		TT	0.170	0.42 (0.12–1.45)	0.186	0.41 (0.11–1.54)
AMELX	rs17878486	CC	ref.	–	ref.	–
		CT	0.823	1.10 (0.46–2.65)	0.931	1.04 (0.41–2.64)
		TT	0.506	1.28 (0.62–2.67)	0.534	1.28 (0.59–2.77)
	rs946252	CC	ref.	–	ref.	–
		CT	0.189	1.54 (0.80–2.96)	0.188	1.59 (0.79–3.18)
		TT	0.172	1.59 (0.81–3.13)	0.383	1.37 (0.67–2.78)
DEFB1	rs11362	CC	ref.	–	ref.	–
		CT	0.956	0.98 (0.56–1.74)	0.818	0.93 (0.51–1.71)
		TT	0.934	0.97 (0.49–1.92)	0.667	0.85 (0.41–1.77)
	rs1800972	CC	ref.	–	ref.	–
		CG	0.432	1.89 (0.39–9.22)	0.287	2.57 (0.45–14.7)
		GG	0.701	1.35 (0.29–6.18)	0.700	1.39 (0.26–7.34)
ENAM	rs12640848	AA	ref.	–	ref.	–
		AG	0.221	0.65 (0.32–1.30)	0.200	0.61 (0.29–1.29)
		GG	0.100	0.53 (0.25–1.13)	*0.032*	0.41 (0.18–0.92)
	rs3796704	AG	ref.	–	ref.	–
		CT	0.963	–	0.962	–
		GG	0.247	0.63 (0.29–1.37)	0.217	0.58 (0.25–1.37)
KLK4	rs2235091	AA	ref.	–	ref.	–
		AG	0.531	1.58 (0.38–6.55)	0.518	1.65 (0.36–7.57)
		GG	0.401	1.78 (0.46–6.88)	0.467	1.70 (0.40–7.18)
	rs198968	AA	ref.	–	ref.	–
		AG	0.265	0.43 (0.09–1.90)	*0.037*	0.15 (0.03–0.89)
		GG	0.275	0.45 (0.11–1.87)	*0.040*	0.17 (0.03–0.92)
LTF	rs2269436	f	ref.	–	ref.	–
		AG	0.787	1.12 (0.50–2.50)	0.521	1.34 (0.55–3.26)
		GG	0.396	2.68 (0.27–26.2)	0.627	1.77 (0.18–17.5)
	rs743658	AA	ref.	–	ref.	–
		AG	0.438	0.38 (0.03–4.24)	0.769	0.69 (0.06–7.8)
		GG	0.403	0.37 (0.03–3.69)	0.657	0.59 (0.06–5.89)
	rs4547741	CC	ref.	–	ref.	–
		CT	*0.036*	0.47 (0.23–0.95)	*0.038*	0.44 (0.21–0.96)
		TT	0.427	0.38 (0.03–4.21)	0.257	0.24 (0.02–2.79)

Table 3. (continued)

Gene	Genetic marker	Genotype	Univariate analysis		Multivariate analysis	
			p value	odds ratio	p value	odds ratio
	rs17078878	AA	ref.	–	ref.	–
		AC	0.382	0.35 (0.03–3.67)	0.676	0.60 (0.06–6.53)
		CC	0.441	0.41 (0.04–3.99)	0.669	0.61 (0.06–6.01)
MMP20	rs1784418	CC	ref.	–	ref.	–
		CT	0.484	1.25 (0.66–2.37)	0.598	1.2 (0.61–2.39)
		TT	0.919	1.04 (0.52–2.05)	0.947	1.02 (0.49–2.12)
TFIP11	rs5997096	CC	ref.	–	ref.	–
		CT	0.414	0.75 (0.37–1.51)	0.242	0.64 (0.31–1.35)
		TT	0.683	1.19 (0.52–2.68)	0.950	1.03 (0.44–2.39)
	rs134136	CC	ref.	–	ref.	–
		CT	0.568	1.18 (0.67–2.06)	0.185	1.58 (0.80–3.11)
		TT	0.383	1.39 (0.66–2.9)	0.860	1.06 (0.58–1.91)
TUFT1	rs7526319	CC	ref.	–	ref.	–
		CT	0.255	1.38 (0.79–2.40)	0.344	1.34 (0.73–2.43)
		TT	0.473	1.32 (0.62–2.85)	0.465	1.36 (0.60–3.09)
	rs4970957	AA	ref.	–	ref.	–
		AG	0.577	0.85 (0.48–1.50)	0.952	0.98 (0.53–1.79)
		GG	0.358	0.64 (0.25–1.63)	0.249	0.57 (0.22–1.48)
	rs3828054	AA	ref.	–	ref.	–
		AG	0.649	1.17 (0.59–2.29)	0.866	1.06 (0.52–2.17)
		GG	0.579	0.51 (0.05–5.66)	0.511	0.44 (0.04–5.06)
	rs3790506	AA	ref.	–	ref.	–
		AG	0.881	0.93 (0.34–2.53)	0.442	0.64 (0.21–1.98)
		GG	*0.039*	0.34 (0.12–0.94)	*0.014*	0.23 (0.07–0.74)

The analyses were adjusted for frequency, sugar and/or acid drink consumption and time of first toothbrushing. Figures in parentheses indicate 95% confidence intervals; italics indicate $p \leq 0.05$. The markers rs17878486, rs946252, rs3796704, rs2337360 were not in Hardy-Weinberg equilibrium and were not further tested.

We also found that the GG genotype in *TUFT1* rs3790506 was protective for EEC in the univariate and multivariate analyses. Previous studies also found an association between this gene and caries experience in children [Slayton et al., 2005; Patir et al., 2008; Shimizu et al., 2012] and in adults [Deeley et al., 2008; Shimizu et al., 2012]. Slayton et al. [2005] suggested that two polymorphisms in *TUFT1* interacted with the *S. mutans* present and explained 27% of the variability of caries experience in children from Iowa, USA. In Patir et al. [2008], the CT genotype of *TUFT1* rs3790506 was overrepresented in cases with dmft scores >5. Shimizu et al. [2012] showed that the G allele of *TUFT1* rs4970957 was overrepresented in populations both from Argentina and Brazil. In Guatemala, Deeley et al. [2008] showed that *TUFT1* rs2337360 genotype distribution was different depending on whether individuals had DMFT scores of 2 or lower versus 3, 4, 5 or 6 and higher. Similarly to *ENAM*, the mechanism *TUFT1* may predispose to caries by forming an enamel structure more susceptible to demineralization [Shimizu et al., 2012].

Mutations in *MMP20* and *KLK4* have been previously implicated in amelogenesis imperfecta [Ozdemir et al., 2005]. Our hypothesis is that common genetic variations of these genes may be involved in subclinical changes of the enamel and, as a consequence, may be involved in differences in caries experience. In the study presented here, the AG and GG genotypes in *KLK4* (rs198968) are protective for ECC. We found no evidence of an association between EEC and *MMP20*.

Regarding our findings related to the immune response of the host, we did not find an association with *DEFB1*, a gene that we previously associated with caries in adults [Ozturk et al., 2010]. However, we found that a polymorphism in *LTF* is associated with ECC. The fact that *DEFB1* was not associated with ECC and *LTF* can be explained by the age of children affected by ECC and differences in microbiota of ECC. *LTF* is a glycoprotein that is present in various secretory fluids, including saliva, and has been previously associated with caries [Azevedo et al., 2010; Brancher et al., 2011]. It is one of the components of the immune system and has antimicrobial activity, particularly in human infants. *LTF* plays an important role in fighting against *Candida albicans* [Viejo-Díaz et al., 2004] and, interestingly, *C. albicans* is an important component of dental biofilm associated with ECC [Yang et al., 2012]. Since *C. albicans* produces, and is also very tolerant of, acids, it has the potential to induce or exacerbate carious lesions [Klinke et al., 2011].

In spite of all that is known about preventing ECC, there are still children who appear to be more susceptible and some who are extremely resistant, regardless of the environmental risk factors to which they are exposed. In summary, our results suggest that genetic variation in genes involved in enamel formation and genes involved in immune response may contribute to ECC and that susceptibility results from gene-environment interactions.

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References

- American Academy of Pediatric Dentistry: Policy on early childhood caries (ECC): classifications, consequences, and preventive strategies. Pediatr Dent 2008;30:40–43.
- Azevedo LF, Pecharki GD, Brancher JA, Cordeiro CA Jr, Medeitors KG, Antunes AA, Arruda ES, Werneck RI, Azevedo LR, Mazur RF, Moysés ST, Faucz FR, Trevilatto PC: Analyis of the association between lactotransferrin (LTF) gene polymorphism and dental caries. J Appl Oral Sci 2010;18:166–170.
- Brancher JA, Pecharki GD, Doetzer AD, Medeitors KG, Cordeiro CA Jr, Sotomaior VS, Bauer P, Trevilatto PC: Analysis of polymorphisms in the lactotransferrin gene promoter and dental caries. Int J Dent 2011;2011:571726.
- Briseño-Ruiz J, Shimizu T, Deeley K, Dizak PM, Ruff TD, Faraco IM Jr, Poletta FA, Brancher JA, Pecharki GD, Küchler EC, Tannure PN, Lips A, Vieira TC, Patir A, Koruyucu M, Mereb JC, Resick JM, Brandon CA, Letra A, Silva RM, Cooper ME, Seymen F, Costa MC, Granjeiro JM, Trevilatto PC, Orioli IM, Castilla EE, Marazita ML, Vieira AR: Role of TRAV locus in low caries experience. Hum Genet 2013;132:1015–1025.
- Caufield PW, Cutter GR, Dasanayake AP: Initial acquisition of mutans streptococci by infants: evidence for a discrete window of infectivity. J Dent Res 1993;72:37–45.
- 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.
- Drury TF, Horowitz AM, Ismail AI, Maertens MP, Rozier RG, Selwitz RH: Diagnosing and reporting early childhood caries for research purposes. A report of a workshop sponsored by the National Institute of Dental and Craniofacial Research, the Health Resources and Services Administration, and the Health Care Financing Administration. J Public Health Dent 1999;59:192–197.
- Ferreira SH, Béria JU, Kramer PF, Feldens EG, Feldens CA: Dental caries in 0- to 5-year-old Brazilian children: prevalence, severity, and associated factors. Int J Paediatr Dent 2007;17:289–296.
- Kelavkar UP, Badr KF: Effects of mutant p53 expression on human 15-lipoxygenase-promoter activity and murine 12/15-lipoxygenase gene expression: evidence that 15-lipoxygenase is a mutator gene. Proc Natl Acad Sci U S A 1999;96:4378–4383.
- Klinke T, Guggenheim B, Klimm W, Thurnheer T: Dental caries in rats associated with *Candida albicans*. Caries Res 2011;45:100–106.
- Leboyer M, Bellivier F, Nosten-Bertrand M, Jouvent R, Pauls D, Mallet J: Psychiatric genetics: search for phenotypes. Trends Neurosci 1998;21:102–105.
- Levy SM, Warren JJ, Broffitt B, Hillis SL, Kanellis MJ: Fluoride, beverages and dental caries in the primary dentition. Caries Res 2003;37:157–165.
- Menghini G, Steiner M, Imfeld T: Early childhood caries – facts and prevention. Ther Umsch 2008;65:75–82.
- Ozdemir D, Hart PS, Ryu OH, Choi SJ, Ozdemir-Karatas M, Firatli E, Piesco N, Hart TC: MMP20 active-site mutation in hypomaturation amelogenesis imperfecta. J Dent Res 2005;84:1031–1035.
- Ozturk A, Famili P, Vieira AR: The antimicrobial peptide DEFB1 is associated with caries. J Dent Res 2010;89:631–636.
- 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.
- Pavlic A, Petelin M, Battelino T: Phenotype and enamel ultrastructure characteristics in patients with ENAM gene mutations g.13185–13186insAG and 8344delG. Arch Oral Biol 2007;52:209–217.
- 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.
- Shaffer JR, Wang X, Feingold E, Lee M, Begum F, Weeks DE, Cuenco KT, Barmada MM, Wendell SK, Crosslin DR, Laurie CC, Doheny KF, Pugh EW, Zhang Q, Feenstra B, Geller F, Boyd HA, Zhang H, Melbye M, Murray JC, Weyant RJ, Crout R, McNeil DW, Levy SM, Slayton RL, Willing MC, Broffitt B, Vieira AR, Marazita ML: Genome-wide association scan for childhood caries implicates novel genes. J Dent Res 2011;90:1457–1462.
- Shimizu T, Ho B, Deeley K, Briseño-Ruiz J, Faraco IM Jr, Schupack BI, Brancher JA, Pecharki GD, Küchler EC, Tannure PN, Lips A, Vieira TC, Patir A, Yildirim M, Poletta FA, Mereb JC, Resick JM, Brandon CA, Orioli IM, Castilla EE, Marazita ML, Seymen F, Costa MC, Granjeiro JM, Trevilatto PC, Vieira AR: Enamel formation genes influence enamel microhardness before and after cariogenic challenge. PLoS One 2012;7:e45022.

Shimizu T, Deeley K, Briseño-Ruiz J, Faraco IM Jr, Poletta FA, Brancher JA, Pecharki GD, Küchler EC, Tannure PN, Lips A, Vieira TC, Patir A, Yildirim M, Mereb JC, Resick JM, Brandon CA, Cooper ME, Seymen F, Costa MC, Granjeiro JM, Trevilatto PC, Orioli IM, Castilla EE, Marazita ML, Vireira AR: Fine-mapping of 5q12.1–13.3 unveils new genetic contributors to caries. Caries Res 2013;47:273–283.

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

Tannure PN, Küchler EC, Falagan-Lotsch P, Amorim LMF, Luiz RR, Costa MC, Vieira AR, Granjeiro JM: MMP13 polymorphism decreases risk for dental caries. Caries Res 2012a;46:401–407.

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

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

Vieira AR, Marazita ML, McHenry TG: Genome-wide scan finds suggestive caries loci. J Dent Res 2008;87:435–439.

Viejo-Díaz M, Andrés MT, Fierro JF: Modulation of in vitro fungicidal activity of human lactoferrin against *Candida albicans* by extracellular cation concentration and target cell metabolic activity. Antimicrob Agents Chemother 2004;48:1242–1248.

Vilella AJ, Severin J, Ureta-Vidal A, Heng L, Durbin R, Birney E: EnsemblCompara GeneTrees: complete, duplication-aware phylogenetic trees in vertebrates. Genome Res 2009;19:327–335.

Wang X, Shaffer JR, Zeng Z, Begum F, Vieira AR, Noel J, Anjomshoaa I, Cuenco KT, Lee M-K, Beck J, Boerwinkle E, Cornelis MC, Hu FB, Crosslin DR, Laurie CC, Nelson SC, Doheny KF, Pugh EW, Polk DE, Weyant RJ, Crout R, McNeil DW, Weeks DE, Feingold E, Marazita ML: Genome-wide association scan of dental caries in the permanent dentition. J Dent Res 2012;92:432–437.

Yang XQ, Zhang Q, Lu LY, Yang R, Liu Y, Zou J: Genotypic distribution of *Candida albicans* in dental biofilm of Chinese children associated with severe early childhood caries. Arch Oral Biol 2012;57:1048–1053.