AMBN gene polymorphism Alters the Caries Susceptibility of Adolescents in South China

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Abstract

The aim of this study was to identify genetic factors under additive and dominance models that contribute to caries susceptibility, and to investigate into the interactions between these genes. A cross-sectional study was conducted among 1055 adolescents in Foshan, South China. The International Caries Detection and Assessment System (ICDAS) was used to identify caries. Demographic and environmental variables were analyzed. Twenty-three single nucleotide polymorphisms (SNPs) in 14 genes were identified from saliva samples. Regression analysis was used for the evaluation of direct and epistasis of genes under the hypothesis of an additive model and using the minor allele as the reference allele. After the adjustment by environmental factors, the G allele in AMBN (rs13115627) was a protective factor for caries under both additive model ($P=0.007$; OR=0.728; 95% CI, 0.579-0.916) and dominance model ($P=0.021$; OR=0.728; 95% CI, 0.556-0.953). No interactions between selected genes met the Bonferroni correction significance cutoff for multiple testing. Our results suggests that gender, one-child family, Cariostat score and Plaque Index have independent protective effects on dental caries. The polymorphisms in AMBN (rs13115627), under both additive and dominance models alters caries susceptibility of Adolescents in South China. No epistatic effect has been found between selected genes.

1. Introduction

Dental caries is one of the most common chronic diseases among children and adults worldwide. The cost of direct treatment was estimated to account for an average of 4.6% of global health expenditures(1). Dental caries is a major public health problem due to the increasing prevalence in developing countries and the heavy disease burden(2). According to the 4th National Oral Health Survey in China, the prevalence of caries in 12-year-old children increased from 28.9% to 38.5% in the last decade, which has attracted the attention of the government(3).

Dental caries is a multifactorial disease, and environmental risk factors such as addiction to sugary snacks and drinks, poor oral hygiene, high levels of cariogenic bacteria, salivary dysfunction, and insufficient fluoride exposure are critical to its development(2). However, even when exposed to the same level of environmental risks, some people are more susceptible to caries than others. The high prevalence of caries among certain groups has motivated research towards identifying genetic contributors to caries(4)(5).

Genes involved in enamel formation, the immune response, saliva proteins and food preferences have been considered to be involved in the etiology of dental caries(6)(7). Evidences found in studies regarding twins estimated that 40-60% of caries susceptibility is genetically determined (8)(9). Some studies have identified large numbers of single nucleotide polymorphisms (SNPs) associated with caries(6). However, few studies have taken different genetic models into consideration, particularly in Chinese population. The present study investigated environmental factors and genetic factors related with dental caries, focusing on the genes involved in enamel formation, immune response, saliva proteins, and taste preferences under different genetic models.

2. Methods And Materials

2.1 Subject

The study was approved by the Ethical Review Committee of Guanghua School of Stomatology, Sun Yat-Sen University (ERC-[2018]01). A written informed consent was obtained from each student's guardian before the study.

The oral examination and saliva tests were performed in March 2018 in Foshan. Foshan is a medium-sized city in Guangdong Province in southern China with a population of 7.6 million. The GDP was CNY 124,324 (USD 18,018) per capita in 2017 (10). The water fluoride concentration is 0.16 mg/L (11).

According to Peduzzi, for logistic regression analysis, the number of events (death or illness) should be 5-10 times the number of independent variables (12). In total, 43 independent variables were investigated via logistic regression in this study, and the caries prevalence in 12-year-old adolescents was 38.5%; thus, the total sample size should be between 559 and 1117 (12)(3).

A random cluster sampling technique was employed in 6 middle schools according to the number of children that we needed to recruit. All Grade 7 students living in Foshan for the previous 2 years who reported no systematic illness and no antibiotic use for at least the preceding 2 weeks were invited to participate in the study.

2.2 Questionnaire

The questionnaire was mainly designed with reference to the Fisher-Owens conceptual model of influence on children's oral health (2). The structured questionnaire recorded demographic information, socioeconomic status, diet, self-reported oral health behaviors and past dental experiences. The questionnaire was self-administered and completed in schools by students with the guidance of guardians. The reliability of the questionnaire was assessed by an internal consistency test, and the Cronbach's alpha was 0.75.

2.3 Oral examination

The oral examination was conducted in the classrooms. The examinee was lying in a portable dental chair. The teeth were examined visually with the help of a plain mouth mirror, a CPI probe and compressed air. The International Caries Detection and Assessment System (ICDAS) criteria were used (13). All tooth surfaces were first examined with a wet surface and then reexamined after the teeth were dried with compressed air. Filled surfaces and missing teeth due to caries (the reasons for missing teeth were obtained from the questionnaire) were also recorded. The DMFT was calculated both at the early clinical stage of decay, with enamel and dentine caries (at the D_1 level, as in the ICDAS method), and at the later stages of decay, with dentine-only caries (at the D_3 level, as in the ICDAS method), and these values were recorded as D_1 MFT and D_3 MFT, respectively. Individuals with lesions scoring from 3-6 were

classified into the caries group, and the others were classified into the caries-free group in this study(14). The Plaque Index (PII) was recorded according to the Silness and Le scale (15).

The three examiners had taken a 12-hour e-learning course on ICDAS before the study. Ten percent of the subjects were randomly selected to obtain a duplicate examination in order to monitor the reliability of the diagnoses and calculate the interexaminer consistency. The Cohen's kappa values were 0.85, 0.83 and 0.91 for caries examination.

2.4 Microbiological examination

The Microbiological assessment was performed by the Cariostat method, which is a colorimetric test for measuring caries activity based on the presence of acidogenic microorganisms (16)(Ramesh et al. 2013). Plaque was collected using the sterile swab included in the Cariostat kit (GangDa Medical Technology Co. LTD., Beijing, China) package. According to the Cariostat kit instructions, the examiners scrubbed the buccal surfaces of the maxillary molars and mandibular incisors 3-5 times and immersed the swab into the culture medium ampule. The samples were incubated at 37C for 48 hours. The color of the culture medium was compared with the reference color on the color chart supplied with the Cariostat kit. The reference color was scored from 0 to 3 with every 0.5 grade as an interval. The color turns from blue to green and, ultimately, to yellow, indicating an increased acid production ability of the plaque in the sample.

2.5 Saliva tests

All saliva was collected after the students had rinsed their mouths with tap water. The unstimulated saliva was collected for 15 minutes. Students were asked to spit the saliva through a funnel into a scaled tube every 3 minutes. Then, the unstimulated saliva flow rate (ml/min) was calculated. The saliva buffering capability was measured according to the Ericsson method (17). One milliliter of saliva was added to 3 ml of 3.3 mmol HCl within 5 minutes after collection and then allowed to stand for 20 minutes to remove CO₂. The final pH of the saliva was evaluated by an electrical pH meter. The buffering capability of unstimulated saliva was recorded as 'low', 'normal' and 'high' when the pH value fell into the ranges of <4.25, 4.25-4.75 and >4.75, respectively.

2.6 Candidate Genes Selection

The major candidate gene categories identified to date include enamel formation genes, immune response genes, genes related to saliva, and genes related to taste and dietary habits (6). The candidate genes selected for this study are listed in Table 1. The Haploview 4.2 software package (<http://www.broad.mit.edu/mpg/haploview/>) was used to select tag SNPs in each candidate gene. SNPs reported having associations with dental caries in previous studies or located in a gene fragment that could have functional effects were selected.

2.7 DNA analysis

Genomic DNA was extracted from 1-ml saliva samples according to the manufacturer's instructions (Saliva DNA Sample Collection Kit, ZEESAN, Xiamen, China). The DNA concentration and purity of each sample were determined by spectrophotometry. Twenty-three SNPs in 14 genes were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

2.8 Statistical analysis

The data were analyzed by SPSS version 22 for Windows (IBM Inc., Chicago, IL, USA). The differences in the characteristics between the caries and caries-free groups were compared by binary regression analysis and an independent samples *T* test. A binary regression model was also applied to analyze the environmental risk factors. For both the characteristics and environmental risk factors, each risk indicator with a *P* value of less than 0.2 in the univariate analysis was applied to stepwise multivariate analysis. Risk factors with a final *P* value in the multivariate analysis of less than 0.05 were used as covariates in further analysis.

The Hardy-Weinberg equilibrium was estimated by the *chi-square* test. Genotypes of the candidate genes were analyzed under the hypothesis of an additive model and using the major allele as the reference. Each SNP was treated as a continuous exposure under additive model, coded 0,1, or 2(minor alleles). For direct associations, a binary regression model was applied. Each SNP with a *P* value of less than 0.2 in the univariate analysis would be applied to stepwise multivariate analysis. All possible pairwise SNP-SNP interactions between the selected SNPs were assessed by a bivariate logistic regression model. Significant characteristics and environmental risk indicators were used as covariables. The interaction between two SNPs was defined as the additional effect of their concomitant carriage on caries beyond the addition of their independent effects (departure from additivity): $\beta_{\text{interaction}} = \beta_{\text{observed}} - (\beta_{\text{SNP1}} + \beta_{\text{SNP2}})$, where β_{observed} is the observed effect of the concomitant carriage of SNP1 and SNP2 (versus the carriage of neither) in bivariate binary regression and β_{SNP1} and β_{SNP2} are the independent effect of SNP1 and SNP2, respectively, in the same bivariate regression framework (bivariate refers only to the number of pairwise SNPs, as nongenetic covariables were systematically included for all analyses)(18).The highly conservative Bonferroni correction considering the number of tested SNPs was applied. There were 253 possible SNP-SNP pairs in total, so the cutoff value for multiple testing significance was set at $P < 0.0001$ (0.05/253).

3. Results

3.1 Characteristics

In total, 1055 students participated in the survey. The mean D_{1-6} MFT was 4.42 ± 0.11 , and the mean D_{3-6} MFT was 0.72 ± 0.40 . A total of 382 (36.2%) students had caries scored from 3-6 (categorized into the caries group in this study), and 673 (63.8%) students had caries scored from 0-2 (categorized into the caries-free group in this study), among which 129 (12.2%) scored 0. The characteristics are summarized in Table 2. Girls, and students from families with more than one child had a higher risk of having caries

experience. As a result, gender and family children number were taken as covariables and used in further analysis.

3.2 Environmental factors

According to multivariate analysis, Plaque Index and the Cariostat score were associated with caries in this population (Table 3). Students with a Plaque Index score of 2-3 or 1-2 had a higher risk of having caries experience than those with a score of 0-1. Students with a Cariostat score of 2-3 or 1-2 had a higher risk of having caries experience than those with a score of 0-1.

3.3 Linkage disequilibrium

A total of 23 SNPs distributed across the 14 genes were investigated (Table 1), among which rs3796703 (*ENAM*), rs946252 (*AMLEX*), rs11003125 (*MBL2*) and rs1126478 (*LTF*) were inconsistent with the Hardy-Weinberg equilibrium distribution and were thus excluded from further analysis.

3.3.1 Direct associations

The results of univariate analyses of the association between genotypes and caries under additive model and dominance model are presented in Table 4&5. Only one *P*-value for SNPs was less than 0.2 in both models, so the multivariable analysis was omitted. For additive model, SNP rs13115627 in *AMBN* were observed significantly associated to caries in both the unadjusted and adjusted analyses (Table 4). Individuals with the G allele in rs13115627 (*AMBN*) had a lower risk of having caries experience than those without the G allele. For dominance model, SNP rs13115627 in *AMBN* were observed significantly associated to caries after adjusted by gender, family children number, Cariostat score and Plaque Index (Table 5). Individuals carries GG or AG in rs13115627 (*AMBN*) had a lower risk of having caries experience than those carries AA.

3.3.2 Interactions

After pairwise SNP-SNP interactions analyzed by binary regression adjusted for gender, the number of children in the family, the Plaque Index and the Cariostat score, 11 interactions were significant under addition model, and 5 interactions were significant under dominance model (Table 6&7). None of them met the significance cut-off *P* value (0.0001) after the Bonferroni correction for multiple testing. No epistaic effect was found between these genes to be related to caries risk in this population.

4. Discussion

The present study gives a profile of dental caries of adolescents in South China using the scales of ICDAS. To our knowledge, this work is the first to show the caries situation of Chinese adolescent by ICDAS, and is the first to examine the contribution of caries related genes under different genetic models.

For demographic and environmental factors, girls, families with more than one child, a high Plaque Index and a high Cariostat score were associated with caries experience which was consistent with the previous studies(19) (20). Therefore, these factors were included as covariates in the multivariate analysis to elucidate the independent contributions of genes.

For genetic factors, we found significant associations between rs13115627 in *AMBN* and caries under additive model and dominance model. Previous studies examined the relationship between other SNPs in *AMBN* and caries. Shimizu et al. found that the frequency of the C allele of rs4694075 in *AMBN* was significantly higher in individuals with high caries experience than in those with low caries experience in five different populations(4). Ergöz et al. found that a variation in *AMBN* (rs4694075) was related to lower caries experience among asthmatic children in Turkey(21).

AMBN encodes ameloblastin, which is believed to control the elongation of enamel crystals and generally directs enamel mineralization during tooth development(22)(23)(24). Our previous study found that the GG genotype of *AMBN* (rs13115627) was significantly associated with an increased calcium-phosphorus ratio in adolescents in southern China(25). Das et al. found that a lower calcium-phosphorus ratio may lead to higher caries risk (26). Thus, *AMBN* (rs13115627) was assumed to affect caries susceptibility through altering enamel composition.

In the present study, *AMBN*(rs13115627) was significantly associated with dental caries under both additive and dominance model, so we attempted to check the epistasis between *AMBN* and other genes related with dental caries. Our results show that the *P* values of interaction between *AMBN*(rs13115627) and *TFIP11*(rs134143) were less than 0.05 under both additive and dominance model, but didn't meet the Bonferroni correction for multiple testing. So no epistatic effect was found between *AMBN* and *TFIP11*. Neither were other SNP-SNP pairwise. A further genome-wide association and sequencing study should be done to check the epistatic effects.

Overall, our results show the situation of dental caries and provide a more comprehensive view of genetic factors related to caries of adolescents in South China. The polymorphism of gene *AMBN*(rs13115627) can affect the caries susceptibility under both additive model and dominance model. No epistatic effect was found between selected genes. Further genome-wide association and sequencing study should be done to check the epistasis between genes and larger independent populations should be performed to support the preliminary findings. Understanding the contribution of a network of genes to variations in dental caries risk may lead to new approaches to prevention, early identification of high-risk patients, and therapeutic targets.

Declarations

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Ketian Wang and Liangyue Pang substantially contributed to conception and design of this study. Luoping Yin, Xia Li, Jianming Zhang, Tianqiang Cui, Ye Tao and Huancai Lin contributed to acquisition, analysis, and interpretation of data. Ketian Wang drafted the manuscript, Liangyue Pang, Luoping Yin, Xia Li, Jianming Zhang, Tianqiang Cui, Ye Tao and Huancai Lin revised the manuscript for important intellectual content critically. All authors gave final approval to this article, and all Agreed to be accountable for all aspects of the work in ensuring that questions relating to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Declarations

The datasets generated or analysed during this study are available from the corresponding author on reasonable request.

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Tables

Table 1 Candidate genetic markers evaluated in this study

Gene	Locus	Marker public ID	Base pair exchange (MAF)	Most severe consequence	HWE test	
					χ^2	<i>P</i>
Enamel formation genes						
<i>ENAM</i>	4q13.3	rs12640848	A/G (0.33)	intron variant	0.206	0.650
		rs3796703	C/T (0.01)	missense variant	88.863	<0.001
<i>AMBN</i>	4q21	rs13115627	A/G (0.30)	intron variant	0.680	0.410
<i>AMELX</i>	Xp22.2	rs946252	C/T (0.31)	intron variant	425.798	<0.001
<i>TFIP11</i>	22q12.1	rs134143	T/C (0.35)	noncoding transcript exon variant	1.199	0.273
<i>MMP20</i>	11q22.3-q23	rs2097470	C/T (0.29)	intron variant	3.478	0.062
		rs1612069	G/T (0.48)	intron variant	2.959	0.085
<i>TUFT1</i>	1q21	rs1784418	C/T (0.42)	intron variant	0.846	0.358
		rs17640579	A/G (0.22)	intron variant	0.039	0.844
		rs3790506	G/A (0.25)	intron variant	2.862	0.091
Immune response genes						
<i>DEFB1</i>	8p23.1	rs11362	C/T (0.40)	5' UTR variant	0.331	0.565
<i>LTF</i>	3p21.31	rs1800972	G/C (0.14)	5' UTR variant	0.367	0.545
		rs4547741	C/T (0.07)	intron variant	0.905	0.314
<i>MBL2</i>	10q21.1	rs1126478	C/T (0.37)	missense variant	4.117	0.042
		rs1800450	C/T (0.12)	missense variant	2.421	0.120
<i>MASP2</i>	1p36.22	rs11003125	G/C (0.31)	regulatory region variant	4.255	0.039
		rs10779570	G/T (0.36)	intron variant	3.280	0.070
Saliva genes						
<i>AQP5</i>	12q13.12	rs1996315	G/A (0.43)	intron variant	0.480	0.488
<i>CA6</i>	1p36.23	rs923911	C/A (0.22)	intron variant	0.182	0.670
		rs2274327	C/T (0.27)	missense variant	0.226	0.634
Taste receptor genes						
<i>TAS1R2</i>	1p36.13	rs35874116	T/C (0.27)	missense variant	2.879	0.090
<i>TAS2R38</i>	7q34	rs9701796	G/C (0.20)	missense variant	0.016	0.901
		rs713598	C/G (0.50)	missense variant	0.705	0.401

Table 2 Caries distribution among characteristics

	Caries-free (n=673) <i>N</i> (%)	Caries (n=382) <i>N</i> (%)	Univariate		Multivariate	
			<i>P</i> *	<i>OR</i> (95% <i>CI</i>)	<i>P</i> *	<i>OR</i> (95% <i>CI</i>)
Gender			0.011 ^{a,b}		0.036 ^a	
Female	261 (38.8)	179 (46.9)		1.392 (1.080,1.794)		1.316 (1.018,1.702)
Male	412 (61.2)	203 (53.1)		1		1
Residence			0.743			
Suburban	333 (49.5)	185 (48.4)		1.043 (0.811,1.341)		
Urban	340 (50.5)	197 (51.6)		1		
Caregiver			0.688			
Other people	187 (27.8)	99 (25.9)		0.893 (0.668,1.195)		
Father	76 (11.3)	40 (10.5)		0.888 (0.587,1.344)		
Mother	410 (60.9)	243 (63.6)		1		
Education of caregiver			0.379			
≥9 years	300 (44.6)	181 (47.4)		1.120 (0.870,1.440)		
<9 years	373 (55.4)	201 (52.6)		1		
Household income			0.034 ^{a,b}		0.051	
(RMB)						
<3000	157 (23.3)	89 (23.3)		1.484 (0.970,2.269)		1.471 (0.960,2.255)
3000-7000	393 (58.4)	246 (64.4)		1.638 (1.129,2.376)		1.593 (1.095,2.318)
≥7000	123 (18.3)	47 (12.3)		1		1
One-child family			0.030 ^{a,b}		0.049 ^a	
No	507 (75.3)	310 (81.2)		1.410 (1.033,1.923)		1.370 (1.001,1.875)
Yes	166 (24.7)	72 (18.8)		1		1
	<i>Mean (SD)</i>	<i>Mean (SD)</i>	<i>P</i> **	<i>OR</i> (95% <i>CI</i>)	<i>P</i> **	<i>OR</i> (95% <i>CI</i>)
Age (years)	13.16 (0.02)	13.18 (0.02)	0.345	0.026 (-0.075,0.026)		

**binary logistic analysis*

**Independent samples t test

^a*P*<0.05

^b*P*<0.2 (in univariate analysis)

Table 3 Binary logistic regression analysis of environmental factors

Environmental factors	Caries-free (n=673) N (%)	Caries (n=382) N (%)	Univariate		Multivariate	
			<i>P</i> *	<i>OR</i> (95% <i>CI</i>)	<i>P</i> *	<i>OR</i> (95% <i>CI</i>)
Frequency of toothbrushing			0.638			
<2 times per day	276 (41.0)	151 (39.5)		0.940 (0.728,1.215)		
≥2 times per day	397 (59.0)	231 (60.5)		1		
Dental flossing			0.202			
No	612 (90.9)	356 (93.2)		1.365 (0.847,2.200)		
Yes	61 (9.1)	26(6.8)		1		
Toothpaste			0.377			
Nonfluoride	170 (25.3)	106 (27.7)		1.136 (0.856,1.509)		
Fluoride	503 (74.7)	276 (72.3)		1		
Frequency of snacking			0.122 ^b		0.268	
≥2 per day	68 (10.1)	51 (13.4)		1.625 (1.020,2.589)		1.497 (0.919,2.439)
1 per day	462 (68.6)	265 (69.4)		1.243 (0.895,1.726)		1.158 (0.822,1.632)
<1 per day	143 (21.2)	66 (17.3)		1		1
Frequency of drinking sweet drinks			0.768			
≥2 per day	59 (8.8)	36 (9.4)		1.157 (0.715,1.874)		
1 per day	430 (63.9)	249 (65.2)		1.098 (0.821,1.470)		
<1 per day	184 (27.3)	974 (25.4)		1		
Pit and fissure sealant			0.177 ^b		0.311	
No	640 (95.1)	370 (96.9)		1.590 (0.811,3.116)		1.435 (0.714,2.885)
Yes	33 (4.9)	12 (3.1)		1		1
Orthodontics appliance			0.470			
Yes	17 (2.5)	7 (1.8)		0.720 (0.296,1.753)		
No	656 (97.5)	375 (98.2)		1		
Saliva secretion(ml/min)			0.465			
<0.25	178 (26.4)	109 (28.5)		1.110 (0.839,1.470)		
≥0.25	495 (73.6)	273 (71.5)		1		
Saliva buffering capability (pH)			0.191 ^b		0.359	
<3.5	213 (31.6)	126 (33.0)		1.201 (0.888,1.623)		1.103 (0.803,1.516)
3.5-4.25	186 (27.6)	121 (31.7)		1.320 (0.970,1.797)		1.265 (0.917,1.744)
≥4.25	274 (40.7)	135 (35.3)		1		1
Plaque Index			0.001 ^{a,b}		0.003 ^a	
2-3	49 (7.3)	51 (13.4)		2.212 (1.437,3.405)		2.174 (1.389,3.402)
1-2	252 (37.4)	156 (40.8)		1.316 (1.006,1.721)		1.215 (0.918,1.609)
0-1	372 (55.3)	175 (45.8)		1		1
Cariostat score			<0.001 ^{a,b}		<0.001 ^a	
2-3	79 (11.7)	124 (32.5)		5.012 (3.326,7.552)		4.851 (3.200,7.355)
1-2	412 (61.2)	201 (52.6)		1.558 (1.107,2.193)		1.537 (1.087,2.173)
0-1	182 (27.0)	57 (14.9)		1		1

**binary logistic* analysis

^a $P<0.05$

^b $P<0.2$ (in univariate analysis)

Table 4 Univariate logistic regression analysis of SNPs under additive model

Gene	SNP	Caries-free	Caries	Unadjusted		Adjusted [#]		
		(n=673)	(n=382)	<i>P</i> *	<i>OR</i>	<i>P</i> *	<i>OR</i>	
		N (%)	N (%)		(95% <i>CI</i>)		(95% <i>CI</i>)	
Enamel formation genes								
<i>ENAM</i>	rs12640848			0.766	1.034 (0.831,1.286)	0.993	0.999 (0.795,1.256)	
		AA	429 (63.7)					243 (63.6)
		AG	217 (32.2)					120 (31.4)
		GG	27 (4.1)					19 (5.0)
<i>AMBN</i>	rs13115627			0.033 ^a	0.788 (0.633,0.981)	0.007 ^a	0.728 (0.579,0.916)	
		AA	382 (56.8)					239 (62.6)
		AG	253 (37.6)					130 (34.0)
		GG	38 (5.6)					13 (3.4)
<i>TFIP11</i>	rs134143			0.541	0.944 (0.783,1.137)	0.425	0.924 (0.761,1.122)	
		TT	304 (45.2)					175 (45.8)
		CT	285 (42.3)					167 (43.7)
		CC	84 (12.5)					40 (10.5)
	rs2097470			0.309	0.897 (0.728,1.106)	0.170	0.858 (0.690,1.068)	
		CC	353 (52.5)					209 (54.7)
		CT	277 (41.2)					155 (40.6)
		TT	43 (6.4)					18 (4.7)
<i>MMP20</i>	rs1612069			0.572	1.054 (0.878,1.266)	0.916	0.990 (0.817,1.200)	
		GG	191 (28.4)					102 (26.7)
		GT	350 (52.0)					202 (52.9)
		TT	132 (19.6)					78 (20.4)
	rs1784418			0.741	0.970 (0.810,1.162)	0.833	1.021 (0.844,1.234)	
		CC	189 (28.1)					111 (29.1)
		CT	345 (51.3)					194 (50.8)
		TT	139 (20.6)					77 (20.1)
<i>TUFT1</i>	rs17640579			0.701	0.961 (0.786,1.175)	0.655	0.954 (0.774,1.175)	
		AA	360 (53.5)					200 (52.4)
		AG	258 (38.3)					161 (42.1)
		GG	55 (8.2)					21 (5.5)
	rs3790506			0.354	1.097 (0.902,1.335)	0.514	1.071 (0.872,1.314)	
		GG	375 (55.7)					213 (55.8)
		AG	255(37.9)					130 (34.0)
		AA	43 (6.4)					39 (10.2)
Immune response genes								
<i>DEFB1</i>	rs11362			0.552	1.056 (0.883,1.263)	0.388	1.086 (0.901,1.309)	
		CC	240 (35.7)					133 (34.8)
		CT	322 (47.8)					179 (46.9)
		TT	111 (16.5)					70 (18.3)
	rs1800972			0.516	0.906 (0.672,1.221)	0.416	0.879 (0.644,1.200)	
		GG	543 (80.7)					316 (82.7)
		GC	123 (18.3)					61 (16.0)
		CC	7 (1.0)					5 (1.3)
<i>LTF</i>	rs4547741			0.986	0.997 (0.682,1.457)	0.790	0.948 (0.638,1.407)	
		CC	600 (89.2)					341 (89.3)
		CT	70 (10.4)					39 (10.2)

<i>MBL2</i>	TT	3 (0.4)	2 (0.5)	0.880	1.020 (0.791,1.314)	0.970	0.995 (0.765,1.295)
	rs1800450						
	CC	508 (75.5)	288 (75.4)				
	CT	150 (22.3)	84 (22.0)				
<i>MASP2</i>	TT	15 (2.2)	10 (2.6)	0.704	1.040 (0.851,1.270)	0.746	1.035 (0.840,1.274)
	rs10779570						
	TT	391 (58.1)	219 (57.3)				
	TG	236 (35.1)	134 (35.1)				
Saliva genes	GG	46 (6.8)	29 (7.6)	0.982	0.998 (0.831,1.199)	0.993	1.001 (0.827,1.212)
	<i>AQP5</i>						
	rs1996315						
	GG	240 (35.7)	135 (35.3)				
	GA	328 (48.7)	189 (49.5)	0.592	1.062 (0.852,1.325)	0.346	1.117 (0.887,1.408)
	AA	105 (15.6)	58 (15.2)				
	rs923911						
	CC	429 (63.7)	254 (66.5)				
<i>CA6</i>	CA	228 (33.9)	101 (26.4)	0.372	0.914 (0.749,1.114)	0.279	0.891 (0.724,1.098)
	AA	16 (2.4)	27 (7.1)				
	rs2274327						
	CC	336 (49.9)	194 (50.8)				
Taste receptor genes	CT	276 (41.0)	164 (42.9)	0.269	0.848 (0.632,1.136)	0.251	0.837 (0.617,1.135)
	TT	61 (9.1)	24 (6.3)				
	<i>TAS1R2</i>						
	rs35874116						
	TT	521 (77.4)	303 (79.3)	0.431	1.091 (0.878,1.357)	0.466	1.088 (0.867,1.365)
	TC	144 (21.4)	79 (20.7)				
	CC	8 (1.2)	0 (0.0)				
	rs9701796						
	CC	419 (62.3)	242 (63.4)	0.989	0.999 (0.827,1.206)	0.791	1.027 (0.843,1.252)
	CG	235 (34.9)	114 (29.8)				
	GG	19 (2.8)	26 (6.8)				
	<i>TAS2R38</i>						
	rs713598			0.989	0.999 (0.827,1.206)	0.791	1.027 (0.843,1.252)
	GG	317 (47.1)	183 (47.9)				
	GC	287 (42.6)	157 (41.1)				
	CC	69 (10.3)	42 (11.0)				

*binary logistic analysis

#The results are shown adjusted for the covariates of gender, one-child family, Cariostat score and Plaque Index.

^a $P < 0.05$

Table 5 Univariate logistic regression analysis of SNPs under dominance model

Gene	SNP	Caries-free (n=673)	Caries (n=382)	Unadjusted		Adjusted [#]		
				<i>P</i> *	<i>OR</i>	<i>P</i> *	<i>OR</i>	
					(95% <i>CI</i>)		(95% <i>CI</i>)	
Enamel formation genes								
<i>ENAM</i>	rs12640848			0.966	1.006 (0.775,1.306)	0.766	0.960 (0.731,1.260)	
		AA	429 (63.7)	243 (63.6)				
<i>AMBN</i>	rs13115627	AG+GG	244 (36.3)	139 (36.4)	0.066	0.785 (0.607,1.016)	0.021 ^a	0.728 (0.556,0.953)
		AA	382 (56.8)	239 (62.6)				
<i>TFIP11</i>	rs134143	AG+GG	291 (43.2)	143 (37.4)	0.841	0.974 (0.757,1.254)	0.743	0.957 (0.736,1.245)
		TT	304 (45.2)	175 (45.8)				
	rs2097470	CT+CC	369 (54.8)	207 (54.2)	0.479	0.913 (0.710,1.175)	0.363	0.885 (0.680,1.152)
		CC	353 (52.5)	209 (54.7)				
<i>MMP20</i>	rs1612069	CT+TT	320 (47.5)	173 (45.3)	0.559	1.088 (0.821,1.442)	0.823	0.967 (0.720,1.299)
		GG	191 (28.4)	102 (26.7)				
	rs1784418	GT+TT	482 (71.6)	280 (73.3)	0.736	0.953 (0.722,1.258)	0.905	1.018 (0.761,1.362)
		CC	189 (28.1)	111 (29.1)				
<i>TUFT1</i>	rs17640579	CT	484 (71.9)	271 (79.0)	0.722	1.047 (0.814,1.346)	0.669	1.059 (0.815,1.376)
		AA	360 (53.5)	200 (52.4)				
	rs3790506	AG+GG	313 (46.5)	182 (47.6)	0.990	0.998 (0.775,1.286)	0.909	0.985 (0.756,1.282)
		GG	375 (55.7)	213 (55.8)				
		AG+AA	298 (37.9)	169 (34.0)				
		Immune response genes						
<i>DEFB1</i>	rs11362			0.783	1.038 (0.798,1.350)	0.762	1.043 (0.793,1.371)	
		CC	240 (35.7)	133 (34.8)				
	rs1800972	CT+TT	433 (64.3)	249 (65.2)	0.413	0.872 (0.629,1.210)	0.315	0.839 (0.597,1.181)
		GG	543 (80.7)	316 (82.7)				
<i>LTF</i>	rs4547741	GC+CC	130 (19.3)	66 (17.3)	0.954	0.988 (0.659,1.482)	0.799	0.946 (0.620,1.444)
		CC	600 (89.2)	341 (89.3)				
<i>MBL2</i>	rs1800450	CT+TT	73 (10.8)	41 (10.7)	0.974	1.005 (0.751,1.345)	0.997	0.999 (0.737,1.355)
		CC	508 (75.5)	288 (75.4)				
<i>MASP2</i>	rs10779570	CT+TT	165 (24.5)	94 (24.6)	0.808	1.032 (0.800,1.331)	0.761	1.042 (0.799,1.359)
		TT	391 (58.1)	219 (57.3)				
		TG+GG	282 (41.9)	163 (42.7)				
		Saliva genes						
<i>AQP5</i>	rs1996315			0.917	1.014	0.921	1.014	

					(0.780,1.318)		(0.771,1.334)
	GG	240 (35.7)	135 (35.3)				
	GA+AA	433 (64.3)	247 (64.7)				
	rs923911			0.369	0.886	0.555	0.920
					(0.680,1.154)		(0.699,1.213)
	CC	429 (63.7)	254 (66.5)				
	CA+AA	244 (36.3)	128 (33.5)				
CA6	rs2274327			0.788	0.966	0.470	0.908
					(0.752,1.242)		(0.698,1.180)
	CC	336 (49.9)	194 (50.8)				
	CT+TT	337 (50.1)	188 (49.2)				
Taste receptor genes							
TAS1R2	rs35874116			0.472	0.894	0.481	0.891
					(0.658,1.214)		(0.648,1.227)
	TT	521 (77.4)	303 (79.3)				
	TC+CC	152 (22.6)	79 (20.7)				
	rs9701796			0.724	0.954	0.720	0.952
					(0.736,1.238)		(0.725,1.249)
	CC	419 (62.3)	242 (63.4)				
	CG+GG	254 (34.9)	140 (29.8)				
TAS2R38	rs713598			0.802	0.968	0.932	0.989
					(0.753,1.245)		(0.760,1.286)
	GG	317 (47.1)	183 (47.9)				
	GC+CC	287 (42.6)	157 (41.1)				

*binary logistic analysis

#The results are shown adjusted for the covariates of gender, one-child family, Cariostat score and Plaque Index.

^a $P < 0.05$

Table 6 Univariate analysis of gene-gene interaction under additive model

Gene 1	SNP 1	Gene 2	SNP 2	<i>P</i> *	OR (95%CI)
DEFB1	rs1800972	AQP5	rs923911	0.010 [#]	1.966 (1.178,3.283)
TAS2R38	rs713598	TAS1R2	rs9701796	0.011	0.633 (0.445,0.901)
TUFT1	rs3769506	AQP5	rs923911	0.012	1.532 (1.097,2.139)
MASP2	rs10779570	LTF	rs4547741	0.018	2.288 (1.155,4.779)
DEFB1	rs1800972	LTF	rs4547741	0.018	2.698 (1.183,6.153)
TUFT1	rs3769506	TAS1R2	rs9701796	0.023	1.487 (1.058,2.092)
AQP5	rs1996315	CA6	rs2274327	0.029	0.705 (0.515,0.965)
TAS1R2	rs9701796	DEFB1	rs11362	0.029	0.697 (0.505,0.963)
TFIP11	rs134143	AMBN	rs13115627	0.038	1.405 (1.019,1.936)
MMP20	rs1612069	AQP5	rs1996315	0.043	0.736 (0.547,0.991)
AQP5	rs1996315	TAS1R2	rs9701796	0.047	0.712 (0.510,0.996)

* Binary logistic regression

SNP and gene indicate the individual polymorphism within multiple gene-gene interaction model. *P* values are shown adjusted for the covariates, including gender, one-child family, Cariostat score and Plaque Index.

Table 7 Univariate analysis of gene-gene interaction under dominance model

Gene 1	SNP 1	Gene 2	SNP 2	<i>P</i> *	<i>OR</i> (95% <i>CI</i>)
<i>TFIP11</i>	rs1800972	<i>TAS2R38</i>	rs713958	0.015	0.518 (0.305,0.881)
<i>MASP2</i>	rs10779570	<i>LTF</i>	rs4547741	0.030	2.608 (1.100,6.184)
<i>TFIP11</i>	rs2097470	<i>TAS2R38</i>	rs713598	0.032	0.560 (0.330,0.952)
<i>TAS2R38</i>	rs713598	<i>TAS1R2</i>	rs9701796	0.039	0.563 (0.326,0.973)
<i>TFIP11</i>	rs134143	<i>AMBN</i>	rs13115627	0.040	1.760 (1.025,3.023)

* Binary logistic regression

SNP and gene indicate the individual polymorphism within multiple gene-gene interaction model. *P* values are shown adjusted for the covariates, including gender, one-child family, Cariostat score and Plaque Index.