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

Ketian Wang
Sun Yat-Sen University

Liangyue Pang
Sun Yat-Sen University

Luoping Yin
Sun Yat-Sen University

Xia Li
Foshan University

Jianming Zhang
Foshan University

Tianqiang Cui
Foshan University

Ye Tao
Sun Yat-Sen University

Huan Cai Lin linhc@mail.sysu.edu.cn
Sun Yat-Sen University

Corresponding Author
ORCiD: 0000-0002-7222-5570

DOI: 10.21203/rs.2.12670/v1

SUBJECT AREAS
Head & Neck Surgery, Dentistry

KEYWORDS
Dental caries, Gene interaction, Epistasis, SNP, Adolescent, ICDAS
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 (PlI) was recorded according to the Silness and Löe 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 37°C 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 Haploviev 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 $\beta_{\text{observed}}$ is the observed effect of the concomitant carriage of SNP1 and SNP2 (versus the carriage of neither) in bivariate binary regression and $\beta_{\text{SNP1}}$ and $\beta_{\text{SNP2}}$ 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 $4.42\pm0.11$, and the mean $D_{3-6}MFT$ was $0.72\pm0.40$. 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 el 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 el 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 epistatasis 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.

Funding

The present study was supported by the National Natural Science Foundation of China (no. 81570967) and China’s Forth National Oral Health Epidemiology Survey (no. 201502002).

Acknowledgments

We gratefully acknowledge the assistance of the staff at the Preventive Dentistry at the Hospital of Stomatology of Sun Yat-sen University and the staff at Foshan Stomatology Hospital for their assistance during the oral examination and sample collection process.
Declarations

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

References

1. Listl S, Galloway J, Mossey PA, Marcenes W. Global Economic Impact of Dental Diseases. J Dent Res. 2015 Oct;94(10):1355–61.

2. Fisher-Owens SA, Gansky SA, Platt LJ, Weintraub JA, Soobader M-J, Bramlett MD, et al. Influences on Children’s Oral Health: A Conceptual Model. Pediatrics. 2007;120(3):e510–e520.

3. Quan JK, Wang XZ, Sun XY, Yuan C, Liu XN, Wang X, et al. Permanent Teeth Caries Status of 12- to 15-year-olds in China: Findings from the 4th National Oral Health Survey. Chin J Dent Res Off J Sci Sect Chin Stomatol Assoc CSA. 2018;21(3):181–93.

4. Shimizu T, Ho B, Deeley K, Briseño-Ruiz J, Faraco IM, Schupack BI, et al. Enamel formation genes influence enamel microhardness before and after cariogenic challenge. PloS One. 2012;7(9):e45022.

5. Slayton RL, Cooper ME, Marazita ML. Tuftelin, Mutans Streptococci, and Dental Caries Susceptibility. J Dent Res. 2005 Aug;84(8):711–4.

6. Vieira AR, Modesto A, Marazita ML. Caries: Review of Human Genetics Research. Caries Res. 2014;48(5):491-506.

7. Piekoszewska-Ziętek P, Turska-Szybka A, Olczak-Kowalczyk D. Single Nucleotide Polymorphism in the Aetiology of Caries: Systematic Literature Review. Caries Res. 2017;51(4):425-35.

8. Bretz WA, Corby PM, Schork NJ, Robinson MT, Coelho M, Costa S, et al.
Longitudinal Analysis of Heritability for Dental Caries Traits. J Dent Res. 2005 Nov;84(11):1047-51.

9. Finn SB, Caldwell RC. DENTAL CARIES IN TWINS--I. A COMPARISON OF THE CARIES EXPERIENCE OF MONOZYGOTIC TWINS, DIZYGOTIC TWINS AND UNRELATED CHILDREN. Arch Oral Biol. 1963 Jul;8:571-85.

10. Foshan Municipal Bureau of Statistics. Statistical Communique of 2017 National Economic and Social Development of Foshan City - China Statistics Information Network [Internet]. Statistical Communique of 2017 National Economic and Social Development of Foshan City - China Statistics Information Network. 2018 [cited 2019 Jan 11]. Available from: http://www.tjcn.org/tjgb/19gd/35445.html

11. Foshan Water Conservancy Bureau. Foshan Municipal Water Affairs Bureau - Foshan City Water Supply Monthly Announcement (June 2017) [Internet]. Foshan Municipal Water Affairs Bureau - Foshan City Water Supply Monthly Announcement (June 2017). 2017 [cited 2019 Jan 11]. Available from: http://www.fswater.gov.cn/zhengwu/ywxx/gongshui/gs_szgb/201707/t20170714_6258352.html

12. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol. 1996 Dec 1;49(12):1373-9.

13. Pitts NB, Ekstrand KR, ICDAS Foundation. International Caries Detection and Assessment System (ICDAS) and its International Caries Classification and Management System (ICCMS) - methods for staging of the caries process and enabling dentists to manage caries. Community Dent Oral Epidemiol. 2013 Feb;41(1):e41-52.

14. Banting D, Eggertsson H, Ekstrand KR, Ferreira-Zandoná A, Ismail AI,
Longbottom C, et al. Rationale and evidence for the international caries detection and assessment system (ICDAS II). Ann Arbor. 2005;1001:48109-1078.

15. Löe H. The gingival index, the plaque index and the retention index systems. J Periodontol. 1967;38(6P2):610–616.

16. Ramesh K, Kunjappan S, Ramesh M, Shankar S, Reddy S. Comparative evaluation of predictive value of three caries activity tests-snyder, lactobacillus count and cariostat in mixed dentition children with and without caries. J Pharm Bioallied Sci. 2013 Jun;5(Suppl 1):S63-68.

17. Ericsson Y. Clinical investigations of the salivary buffering action. Acta Odontol Scand. 1959;17(2):131-165.

18. Correa-Rodríguez M, Viatte S, Massey J, Schmidt-RioValle J, Rueda-Medina B, Orozco G. Analysis of SNP-SNP interactions and bone quantitative ultrasound parameter in early adulthood. BMC Med Genet [Internet]. 2017 Dec [cited 2019 Jan 10];18(1). Available from: http://bmcmedgenet.biomedcentral.com/articles/10.1186/s12881-017-0468-6

19. Martinez-Mier EA, Zandona AF. The impact of gender on caries prevalence and risk assessment. Dent Clin North Am. 2013 Apr;57(2):301–15.

20. Axelsson P, Lindhe J. The effect of a preventive programme on dental plaque, gingivitis and caries in schoolchildren. Results after one and two years. J Clin Periodontol. 1974;1(2):126-138.

21. Ergöz N, Seymen F, Gencay K, Tamay Z, Deeley K, Vinski S, et al. Genetic variation in Ameloblastin is associated with caries in asthmatic children. Eur Arch Paediatr Dent Off J Eur Acad Paediatr Dent. 2014 Jun;15(3):211-6.

22. Nanci A, Zalzal S, Lavoie P, Kunikata M, Chen W, Krebsbach PH, et al.
Comparative immunochemical analyses of the developmental expression and distribution of ameloblastin and amelogenin in rat incisors. J Histochem Cytochem Off J Histochem Soc. 1998 Aug;46(8):911–34.

23. Iwata T, Yamakoshi Y, Hu JC-C, Ishikawa I, Bartlett JD, Krebsbach PH, et al. Processing of ameloblastin by MMP-20. J Dent Res. 2007 Feb;86(2):153–7.

24. Fukumoto S, Kiba T, Hall B, Iehara N, Nakamura T, Longenecker G, et al. Ameloblastin is a cell adhesion molecule required for maintaining the differentiation state of ameloblasts. J Cell Biol. 2004 Dec 6;167(5):973–83.

25. Pang L, Zhi Q, Zhuang P, Yu L, Tao Y, Lin H. Variation in Enamel Formation Genes Influences Enamel Demineralization In Vitro in a Streptococcus mutans Biofilm Model. Front Physiol [Internet]. 2017 Oct 30 [cited 2019 Jan 11];8. Available from: http://journal.frontiersin.org/article/10.3389/fphys.2017.00851/full

26. Das B, Muthu MS, Farzan JM. Comparison of the chemical composition of normal enamel from exfoliated primary teeth and teeth affected with early childhood caries: an in vitro study. Int J Paediatr Dent. 2016 Jan 1;26(1):20–5.

Tables

Table 1 Candidate genetic markers evaluated in this study
| Gene         | Locus         | Marker public ID | Base pair exchange (MAF) | Most severe consequence |
|--------------|---------------|------------------|--------------------------|-------------------------|
| **Enamel formation genes** | | | | |
| *ENAM*      | 4q13.3        | rs12640848       | A/G (0.33)               | intrc                   |
|             |               | rs3796703        | C/T (0.01)               | misse                   |
| *AMBN*      | 4q21          | rs13115627       | A/G (0.30)               | intrc                   |
| *AMELX*     | Xp22.2        | rs946252         | C/T (0.31)               | intrc                   |
| *TFIP11*    | 22q12.1       | rs134143         | T/C (0.35)               | intrc                   |
|             |               | rs2097470        | C/T (0.29)               | intrc                   |
| *MMP20*     | 11q22.3-q23   | rs1612069        | G/T (0.48)               | intrc                   |
|             |               | rs1784418        | C/T (0.42)               | intrc                   |
| *TUFT1*     | 1q21          | rs17640579       | A/G (0.22)               | intrc                   |
|             |               | rs3790506        | G/A (0.25)               | intrc                   |
| **Immune response genes** | | | | |
| *DEFB1*     | 8p23.1        | rs11362          | C/T (0.40)               | 5’ UTR                  |
|             |               | rs1800972        | G/C (0.14)               | 5’ UTR                  |
| *LTF*       | 3p21.31       | rs4547741        | C/T (0.07)               | intrc                   |
|             |               | rs1126478        | C/T (0.37)               | misse                   |
| *MBL2*      | 10q21.1       | rs1800450        | C/T (0.12)               | misse                   |
|             |               | rs11003125       | G/C (0.31)               | regulatory              |
| *MASP2*     | 1p36.22       | rs10779570       | G/T (0.36)               | intrc                   |
| **Saliva genes** | | | | |
| *AQP5*      | 12q13.12      | rs1996315        | G/A (0.43)               | intrc                   |
|             |               | rs923911         | C/A (0.22)               | intrc                   |
| *CA6*       | 1p36.23       | rs2274327        | C/T (0.27)               | misse                   |
| **Taste receptor genes** | | | | |
| *TAS1R2*    | 1p36.13       | rs35874116       | T/C (0.27)               | misse                   |
|             |               | rs9701796        | G/C (0.20)               | misse                   |
| *TAS2R38*   | 7q34          | rs713598         | C/G (0.50)               | misse                   |

Table 2 Caries distribution among characteristics
| Environmental factors | Caries-free (n=673) N (%) | Caries (n=382) N (%) | Univariate | M |
|-----------------------|---------------------------|---------------------|------------|---|
| Frequency of toothbrushing |                           |                     |            |   |
| <2 times per day       | 276 (41.0)                | 151 (39.5)          | 0.940      |   |
| ≥2 times per day       | 397 (59.0)                | 231 (60.5)          | 1          |   |
| Dental flossing        |                           |                     |            |   |
| No                    | 612 (90.9)                | 356 (93.2)          | 1.365      |   |
| Yes                   | 61 (9.1)                  | 26 (6.8)            | 1          |   |
| Toothpaste             |                           |                     |            |   |
| Nonfluoride            | 170 (25.3)                | 106 (27.7)          | 1.136      |   |
| Fluoride               | 503 (74.7)                | 276 (72.3)          | 1          |   |
| Frequency of snacking  |                           |                     |            |   |
|                       | 0.122^b                   | 0.268               |            |   |

*binary logistic analysis
**Independent samples t test
^P<0.05
_bP<0.2 (in univariate analysis)
Table 4 Univariate logistic regression analysis of SNPs under additive model

| Gene                        | SNP          | Caries-free (n=673) N (%) | Caries (n=382) N (%) | Unadjusted |
|-----------------------------|--------------|---------------------------|----------------------|------------|
|                            |              |                           |                      | p*         | OR (95% CI) | p*         |
| Enamel formation genes      | ENAM         |                            |                      |            |             |            |
| rs12640848                  |              | 68 (10.1)                 | 51 (13.4)            | 0.766      | 1.034 (0.831, 1.286) | 0.993      |

*binary logistic analysis
aP<0.05
bP<0.2 (in univariate analysis)
| Gene | Marker | Minor Allele | N  | Minor Allele Frequency | p-value | Odds Ratio | 95% CI |
|------|--------|--------------|----|------------------------|---------|------------|--------|
| AMBN | rs13115627 | AA | 429 (63.7) | 0.033 \(^a\) | 0.788 (0.633, 0.981) | 0.007 \(^z\) |
|      |        | AG | 217 (32.2) | | | |
|      |        | GG | 27 (4.1) | | | |
| TFIP11 | rs134143 | TT | 304 (45.2) | 0.541 | 0.944 (0.783, 1.137) | 0.425 |
|       |        | CT | 285 (42.3) | | | |
|       |        | CC | 84 (12.5) | 0.309 | 0.897 (0.728, 1.106) | 0.170 |
| MMP20 | rs1612069 | GG | 191 (28.4) | 0.572 | 1.054 (0.878, 1.266) | 0.916 |
|       |        | GT | 350 (52.0) | | | |
|       |        | TT | 132 (19.6) | 0.741 | 0.970 (0.810, 1.162) | 0.833 |
| TUFT1 | rs17640579 | AA | 360 (53.5) | 0.701 | 0.961 (0.786, 1.175) | 0.655 |
|       |        | AG | 258 (38.3) | | | |
|       |        | GG | 55 (8.2) | 0.354 | 1.097 (0.902, 1.335) | 0.514 |
| Immune response genes | DEFBI | rs11362 | CC | 240 (35.7) | 0.552 | 1.056 (0.883, 1.263) | 0.388 |
|       |        | CT | 322 (47.8) | | | |
|       |        | TT | 111 (16.5) | 0.516 | 0.906 (0.672, 1.221) | 0.416 |
| Gene   | SNP       | Gene frequency | Odds ratio | 95% CI     |
|--------|-----------|----------------|------------|------------|
| LTF    | rs4547741 |                | 0.986      | 0.997      |
|        |           |                | (0.682,1.457) | (0.790,1.314) |
|        | CC        | 600 (89.2)     | 341 (89.3) |
|        | CT        | 70 (10.4)      | 39 (10.2)  |
|        | TT        | 3 (0.4)        | 2 (0.5)    |
|        |           |                | 0.880      | 1.020      |
|        | (0.791,1.314) | (0.851,1.325) |
|        | CC        | 508 (75.5)     | 288 (75.4) |
|        | CT        | 150 (22.3)     | 84 (22.0)  |
|        | TT        | 15 (2.2)       | 10 (2.6)   |
| MBL2   | rs1800450 |                | 0.704      | 1.040      |
|        |           |                | (0.851,1.325) | (0.815,1.320) |
|        | TT        | 391 (58.1)     | 219 (57.3) |
|        | TG        | 236 (35.1)     | 134 (35.1) |
|        | GG        | 46 (6.8)       | 29 (7.6)   |
| MASP2  | rs10779570|                | 0.746      |            |
|        |           |                |            |            |
|        | TG        | 236 (35.1)     | 134 (35.1) |
|        | GG        | 46 (6.8)       | 29 (7.6)   |
| Saliva genes |   |               |            |            |
| AQP5   | rs1996315 |                | 0.982      | 0.998      |
|        |           |                | (0.831,1.199) | (0.811,1.199) |
|        | GG        | 240 (35.7)     | 135 (35.3) |
|        | GA        | 328 (48.7)     | 189 (49.5) |
|        | AA        | 105 (15.6)     | 58 (15.2)  |
|        | rs923911  |                | 0.592      | 1.062      |
|        |           |                | (0.852,1.325) | (0.815,1.320) |
|        | CC        | 429 (63.7)     | 254 (66.5) |
|        | CA        | 228 (33.9)     | 101 (26.4) |
|        | AA        | 16 (2.4)       | 27 (7.1)   |
| CA6    | rs2274327 |                | 0.372      | 0.914      |
|        |           |                | (0.749,1.114) | (0.749,1.114) |
|        | CC        | 336 (49.9)     | 194 (50.8) |
|        | CT        | 276 (41.0)     | 164 (42.9) |
|        | TT        | 61 (9.1)       | 24 (6.3)   |
| Taste receptor genes |   |               |            |            |
| TAS1R2 | rs35874116|                | 0.269      | 0.848      |
|        |           |                | (0.632,1.136) | (0.827,1.206) |
|        | TT        | 521 (77.4)     | 303 (79.3) |
|        | TC        | 144 (21.4)     | 79 (20.7)  |
|        | CC        | 8 (1.2)        | 0 (0.0)    |
|        | rs9701796 |                | 0.431      | 1.091      |
|        |           |                | (0.878,1.357) | (0.878,1.357) |
|        | CC        | 419 (62.3)     | 242 (63.4) |
|        | CG        | 235 (34.9)     | 114 (29.8) |
|        | GG        | 19 (2.8)       | 26 (6.8)   |
| TAS2R38| rs713598  |                | 0.989      | 0.999      |
|        |           |                | (0.827,1.206) | (0.827,1.206) |
|        | 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.

$p<0.05$

## Table 5 Univariate logistic regression analysis of SNPs under dominance model

| Gene       | SNP       | Caries-free (n=673) N (%) | Caries (n=382) N (%) | Unadjusted | $P^*$ | OR (95% CI) | $P^*$ |
|------------|-----------|---------------------------|----------------------|------------|-------|-------------|-------|
| Enamel formation genes |           |                           |                      |            |       |             |       |
| ENAM       | rs12640848|                           |                      |            |       |             |       |
|            | AA        | 429 (63.7)                | 243 (63.6)           | 0.966      | 1.006 | (0.775,1.306) | 0.766 |
|            | AG+GG     | 244 (36.3)                | 139 (36.4)           |            |       |             |       |
| AMBN       | rs13115627|                           |                      |            |       |             |       |
|            | AA        | 382 (56.8)                | 239 (62.6)           | 0.066      | 0.785 | (0.607,1.016) | 0.021 |
|            | AG+GG     | 291 (43.2)                | 143 (37.4)           |            |       |             |       |
| TFIP11     | rs134143  |                           |                      |            |       |             |       |
|            | TT        | 304 (45.2)                | 175 (45.8)           | 0.841      | 0.974 | (0.757,1.254) | 0.743 |
|            | CT+CC     | 369 (54.8)                | 207 (54.2)           |            |       |             |       |
|            | rs2097470 |                           |                      |            |       |             |       |
|            | CC        | 353 (52.5)                | 209 (54.7)           | 0.479      | 0.913 | (0.710,1.175) | 0.363 |
|            | CT+TT     | 320 (47.5)                | 173 (45.3)           |            |       |             |       |
| MMP20      | rs1612069 |                           |                      |            |       |             |       |
|            | GG        | 191 (28.4)                | 102 (26.7)           | 0.559      | 1.088 | (0.821,1.442) | 0.823 |
|            | GT+TT     | 482 (71.6)                | 280 (73.3)           |            |       |             |       |
|            | rs1784418 |                           |                      |            |       |             |       |
|            | CC        | 189 (28.1)                | 111 (29.1)           | 0.736      | 0.953 | (0.722,1.258) | 0.905 |
|            | CT        | 484 (71.9)                | 271 (79.0)           |            |       |             |       |
| TUFT1      | rs17640579|                           |                      |            |       |             |       |
|            | AA        | 360 (53.5)                | 200 (52.4)           | 0.722      | 1.047 | (0.814,1.346) | 0.669 |
|            | AG+GG     | 313 (46.5)                | 182 (47.6)           |            |       |             |       |
|            | rs3790506 |                           |                      |            |       |             |       |
|            | GG        | 375 (55.7)                | 213 (55.8)           | 0.990      | 0.998 | (0.775,1.286) | 0.909 |
|            | AG+AA     | 298 (37.9)                | 169 (34.0)           |            |       |             |       |
| Immune response genes |           |                           |                      |            |       |             |       |
| DEFB1      | rs11362   |                           |                      |            |       |             |       |
|            | CC        | 240 (35.7)                | 133 (34.8)           | 0.783      | 1.038 | (0.798,1.350) | 0.762 |
|            | CT+TT     | 433 (64.3)                | 249 (65.2)           |            |       |             |       |
| Gene      | SNP       | Odds Ratio | 95% CI      | P Value |
|-----------|-----------|------------|-------------|---------|
| rs1800972 | GG        | 0.413      | (0.629, 1.210) | 0.315   |
| LTF       | GC+CC     | 0.413      | (0.629, 1.210) | 0.315   |
| rs4547741 | CC        | 0.954      | (0.659, 1.482) | 0.799   |
| MBL2      | CT+TT     | 0.974      | (0.751, 1.345) | 0.997   |
| MASP2     | TT        | 0.808      | (0.600, 1.331) | 0.761   |
| Saliva genes |         |            |             |         |
| AQP5      | GG        | 0.917      | (0.780, 1.318) | 0.921   |
| rs1996315 | GA+AA     | 0.369      | (0.680, 1.154) | 0.555   |
| rs923911  | CC        | 0.788      | (0.572, 1.248) | 0.470   |
| CA6       | CA+AA     | 0.472      | (0.658, 1.214) | 0.481   |
| Taste receptor genes | |            |             |         |
| TAS1R2    | TT        | 0.472      | (0.658, 1.214) | 0.481   |
| rs35874116 | TC+CC     | 0.724      | (0.576, 1.238) | 0.720   |
| TAS2R38   | GG        | 0.802      | (0.753, 1.245) | 0.932   |

*binary logistic analysis

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

aP<0.05
Table 6: Univariate analysis of gene-gene interaction under additive model

| Gene 1  | SNP 1      | Gene 2    | SNP 2      | $P^*$ | OR (95%CI) |
|---------|------------|-----------|------------|-------|------------|
| DEFBI   | rs1800972  | AQP5      | rs923911   | 0.010*| 1.966 (1.178,3.28) |
| TAS2R38 | rs713598   | TAS1R2    | rs9701796  | 0.011 | 0.633 (0.445,0.90)  |
| TUFT1   | rs3769506  | AQP5      | rs923911   | 0.012 | 1.532 (1.097,2.13)  |
| MASP2   | rs10779570 | LTF       | rs4547741  | 0.018 | 2.288 (1.155,4.77)  |
| DEFBI   | rs1800972  | LTF       | rs4547741  | 0.018 | 2.698 (1.183,6.15)  |
| TUFT1   | rs3769506  | TAS1R2    | rs9701796  | 0.023 | 1.487 (1.058,2.09)  |
| AQP5    | rs1996315  | CA6       | rs2274327  | 0.029 | 0.705 (0.515,0.96)  |
| TAS1R2  | rs9701796  | DEFB1     | rs11362    | 0.029 | 0.697 (0.505,0.96)  |
| TFIP11  | rs134143   | AMBN      | rs13115627 | 0.038 | 1.405 (1.019,1.93)  |
| MMP20   | rs1612069  | AQP5      | rs1996315  | 0.043 | 0.736 (0.547,0.99)  |
| AQP5    | rs1996315  | TAS1R2    | rs9701796  | 0.047 | 0.712 (0.510,0.99)  |

* 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       | $P^*$ | OR (95%CI)          |
|----------|-------------|----------|-------------|-------|---------------------|
| TFIP11   | rs1800972   | TAS2R38  | rs713958    | 0.015 | 0.518 (0.305, 0.88) |
| MASP2    | rs10779570  | LTF      | rs4547741   | 0.030 | 2.608 (1.100, 6.18) |
| TFIP11   | rs2097470   | TAS2R38  | rs713598    | 0.032 | 0.560 (0.330, 0.95) |
| TAS2R38  | rs713598    | TAS1R2   | rs9701796   | 0.039 | 0.563 (0.326, 0.97) |
| TFIP11   | rs134143    | AMBN     | rs13115627  | 0.040 | 1.760 (1.025, 3.02) |

* 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.