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Research Paper

The association of Enamelin, Lactoferrin, and Tumour necrosis factor alpha gene polymorphisms with high caries susceptibility in Chinese children under 4 years old

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ABSTRACT

Objective: The aim of this study was to assess the role of *ENAM* rs3796703, *LTF* rs1126478, and *TNF-α* rs1800629 in high caries susceptibility.
Design: The present case–control study included 1005 unrelated children under 4 years old: 505 with severe caries (dmft index ≥4) and 500 who were caries-free (dmft index = 0 and without white-spot lesions). Questionnaires were obtained from parents and gardians about the children's diet and oral behavioural habits. All the children received dental examinations and oral swabbing for human genomic DNA collection. *ENAM* rs3796703, *LTF* rs1126478, and *TNF-α* rs1800629 were genotyped by Sanger sequencing.
Results: The frequency of the *ENAM* rs3796703 T allele (6.7% in the caries group and 4.2% in the caries-free group), CT genotype (12.7% in the caries group and 8.4% in the caries-free group), *TNF-α* rs1800629 A allele (4.8% in the caries group and 6.8% in the caries-free group), and AG genotype (8.7% in the caries group and 13.2% in the caries-free group) were significantly different between the caries and caries-free groups ($p < 0.05$). No significant difference was found in the *LTF* rs1126478 allele frequency and genotype distribution between the two groups. The *ENAM* rs3796703 CT genotype increased caries susceptibility by 60.9% compared to the CC genotype ($\beta = 0.746$, OR = 1.609), and the *TNF-α* rs1800629 AG genotype reduced caries susceptibility by 47.4% compared to the GG genotype ($\beta = -0.642$, OR = 0.526). In terms of habits covariates, prolongation of night feeding time by 1 month increased caries susceptibility by 3.3% ($\beta = 0.033$, OR = 1.033); additionally, sweets and acidic drinks consumption 1–2 times per day increased caries susceptibility by 218.2% ($\beta = 1.158$, OR = 3.182), and consumption 3 or more times pe/r day increased susceptibility by 883.5% ($\beta = 2.286$, OR = 9.835) compared to non-consumption. Topical fluoride application decreased caries susceptibility by 43.0% ($\beta = -0.562$, OR = 0.570).
Conclusions: The *ENAM* and *TNF-α* genes are likely associated with caries experience in Chinese children. The *ENAM* rs3796703 CT genotype might be involved in caries susceptibility, while *TNF-α* rs1800629 AG genotype might be involved in caries protection.

1. Introduction

In China, caries remains the biggest threat for the oral health of children. Caries prevalence for children aged 1 to 6 years was 0.3%, 17.3%, 40.2%, 54.4%, 66.1%, and 70.7%, respectively (Zhang et al., 2016). If left untreated, caries decay may lead to intense pain and occlusion disorder, decrease masticatory performance, incur focal infection and affect general health. However, the prevention of this lifestyle-related disease is not an easy matter because of the complexity of its aetiology. Dietary habits, hygiene practices, and fluoride intake were all relevant factors for this disease (Kuriakose, Prasannan, Remya, Kurian, & Sreejith, 2015). Individual susceptibility also played a role. The higher occurrence of caries in specific individuals (termed polarisation) had been widely discussed (Tanner et al., 2013). As early as the 20th century, scholars had already noticed the phenomenon of family aggregation and

Abbreviations: ENAM, enamlin; LTF, lactoferrin; TNF-α, tumor necrosis factor alpha; SNP, single nucleotide polymorphism; dmft, decayed, missing due to caries, filled teeth; MAF, minor allele frequency.

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heritability of caries (Klein & Bethesda, 1946). Twin studies found high concurrence of caries status in both dizygotic and monozygotic twins, further verifying the influence of heredity on caries (Boraas, Messer, & Till, 1988). These findings all raised possibilities for associations between genes and caries. In recent years, thanks to progresses in molecular genetics research, genetic association studies were applied to detect the hereditary characteristics of caries, and gradually revealed how various genes might affect caries. Generally, four kinds of genes were involved in caries susceptibility: enamel formation genes, immune response genes, saliva protein genes, and taste genes (Werneck, Mira, & Trevilatto, 2010).

Enamelin (ENAM) is the largest enamel matrix protein. The protein was coded by the *ENAM* gene and was exclusively expressed by ameloblasts at the secretory and early maturation stages. It concentrated near the mineralisation front by the C-terminus and facilitates crystal elongation and organisation through self-assembling nanostructures (Hu et al., 2014). *ENAM* mutations could cause hereditary enamel hypoplasia. Polymorphisms of *ENAM* gene probably influenced enamel thickness (Daubert et al., 2016) and were also associated with magnesium and calcium concentrations in teeth (Halusic et al., 2014), further facilitated the progression of the carious process. *ENAM* rs3796704, rs12640848 and rs7671281 were reported to be associated with caries susceptibility (Gerreth, Zaorska, Zabel, Borysewicz-Lewicka, & Nowicki, 2016; Abbasoğlu et al., 2015). *ENAM* rs12640848 was an intron variant and had no effect on gene expression. The functional locus within exon sequence including rs3796703 deserve more attention.

Lactoferrin (LTF) is an iron-binding glycoprotein that has a broad spectrum of antimicrobial activity and is associated with immunoreaction and inflammation processes. High concentrations of this multifunctional protein in saliva might enable oral tissues to withstand the attack of bacteria, viruses, and fungi. It also displayed bioactivities against other acid-producing microbes (Velliyagounder et al., 2003) and regulated the aggregation and development of dental plaque biofilm, thus influenced caries susceptibility. An *in vitro* function study suggested that lactoferrin could exert anti-*Streptococcus mutans* effect in saliva (Fine et al., 2013) by killing Gram-positive bacteria, both directly (through direct ion-binding interactions with bacteria by its strongly basic N-terminal region) and indirectly (through sequestering the iron that bacteria require for growth). *LTF* rs1126478 exhibited stronger antibacterial effect and was involved with some oral infectious diseases, such as localized juvenile periodontitis and caries, by applying modifications of inflammatory response (Azevedo, Pecharki, Brancher, & Cordeiro, 2010; Velliyagounder et al., 2003). The association between rs1126478 and sever caries in Chinese children is still in need of verification.

Tumour necrosis factor alpha (TNF-α) is a pleiotropic cytokine produced mainly by monocytes and macrophages. Caries was a chronic infectious disease, which could elicit immune responses (Gómez, Jaramillo, Moreno, Roa, & Rodríguez, 2015). Elevated TNF-α had been detected in unstimulated whole saliva of caries patients as one of the initial responses of the host immune system to pathological insults (Gornowicz et al., 2012). Under the actions of cariogenic bacteria, TNF-α was highly expressed in caries-affected dental pulp and/or odontoblasts (Gornowicz et al., 2012). *TNF-α* rs1800629 was thought to be involved in systemic inflammation and autoimmune diseases. Associations were found between *TNF-α* rs1800629 allele A frequency and aggressive periodontitis patients with clinical attachment level ≥4 mm in Turkish population (Özer Yücel et al., 2015). But it is still unknown whether *TNF-α* rs1800629 affects caries susceptibility.

In this study, we investigated the distributional difference of *ENAM* rs3796703, *LTF* rs1126478, and *TNF-α* rs1800629 polymorphic locus in severe caries and caries-free children, with the aim of assessing the role of these genetic factors in high caries susceptibility.

2. Materials and methods

2.1. Sample size calculation

Caries prevalence was 47% for 3-year-old children living in Beijing (Li, Miao, & Zhang, 2012). When the alpha level set as 0.05 and expected odds ratio at 1.6, a sample size approaching 470 children in each group was required in order to achieve an enough statistics power (β = 0.8). Sample size calculation was carried out using Quanto software (http://biostats.usc.edu/Quanto.html).

2.2. Participants

All the children in the caries group were selected from the outpatients who came to the Pediatric Department in the Peking University Hospital of Stomatology during the period of August 2015 to September 2016. The caries-free children came from 16 kindergarderns in Haidian District, Beijing, at the same time.

The inclusion criteria were: child under 4 years old; the child's mother had lived in Beijing during the whole pregnancy and the child was brought up in Beijing; mother had no pregnancy-related disease; child was born by normal full-term delivery (range 37 to 42 weeks in pregnancy); child without systemic diseases; child's teeth without enamel hypoplasia or dentin hypoplasia; child's dmft index (number of decayed, missing, filled teeth) ≥4 in the caries group; child's dmft index = 0 in the caries-free group. Written informed consents were obtained from the parents or guardians of all the participants prior to the enrolment. Questionnaires were filled by guardians about the children's basic information, diet, oral behavioural habits and application of topical fluoride.

Finally, a total of 505 children with severe caries (dmft index ≥4) and 500 caries-free children were incorporated.

The study design, protocol and informed consent details were approved by the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-201628050).

2.3. Dental examination

Teeth that were decayed, missing due to caries, or filled were recorded according to the modified World Health Organization [1997] caries diagnostic criteria. At the beginning of the oral examination, possible food debris was removed by a piece of sterilized cotton, and the teeth surfaces were gently dried by air scavenging before taking record of the caries condition. In the caries group, white-spot lesions were also recorded but not calculated in the dmft index. No radiographs were taken. Caries examination and diagnosis was performed by a single examiner and the examination consistency was ensured by examining 20 children before the initiation of the study. The κ value for intra-examiner agreement was 0.861.

2.4. DNA collection

Sterile buccal swabs were used to obtain DNA by swabbing the buccal mucosa, and were stored at room temperature before transporting to laboratory within one day. Genomic DNA was extracted with a TIANamp Swab DNA Kit (TIANGEN BIOTECH, BEIJING, China), according to the manufacturer's instructions. The isolated DNA was stored at −20 °C until use.

2.5. Single nucleotide polymorphism (SNP) selection and genotyping

We chose tag SNPs with minor allele frequency (MAF) exceeding 0.05 using Haploview 4.2 according to the HapMap database and filtered the potential functional SNPs by F-snp (http://compbio.cs.queensu.ca/F-SNP/). *ENAM* rs3796703, *LTF* rs1126478 and *TNF-α* rs1800629 were selected as tag SNPs (Table 1).

Primers were designed by Primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/) (Table 2). Genes were amplified by 2 × Taq PCR Master Mix (TIANGEN BIOTECH, BEIJING, China). Polymerase chain reaction was performed in a Mastercycler Gradient thermal cycler (Eppendorf, Hamburg, Germany) with the following temperature procedures: denaturation at 94 °C for 3 min, 29 cycles of 30 s at 94 °C, 30 s at annealing temperature (see Table 2), and 30 s at 72 °C, with a 5-min extension step at 72 °C. The products were sequenced using an ABI 3730XL Automatic Sequencer (Applied Biosystems, Foster City, CA, USA).

2.6. Statistical analysis

Hardy-Weinberg equilibrium in both two groups was determined by the chi-square test. The frequency of sweets and acidic drinks consumption was enumeration data, and analysis was conducted by chi-square. The distribution of alleles and genotypes was detected by chi-square or Fisher's exact test. Night feeding and first tooth brushing time were measurement data and didn't conform to a normal distribution, and the analysis was conducted by Mann-Whitney *U* test. Meanwhile, the associations of genes and behavioural factors with caries susceptibility were assessed by the binary logistic regression test. A p-value < 0.05 was considered significant.

The statistical analysis was done using SPSS 20.0 and PLINK 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/). Statistical power was verified by Quanto (http://biostats.usc.edu/Quanto.html).

3. Results

3.1. General information of the participants

The children in the caries group were at an average of 41.7 ± 7.0 months old (ranged from 15 months to 48 months old), while the caries-free children were 43.7 ± 4.0 months old (ranged from 21 months to 48 months old). The percentages of males were 52.7% in the caries group and 49.0% in the caries-free group. The two groups exhibited no significant difference in terms of age (p = 0.211) or gender (p = 0.244). (Table 3) The dmft index in caries group ranged from 4 to 20, with a mean index of 13.046 ± 3.298.

3.2. Behavioural habits analysis

Behavioural habits differences were summarised in Table 4. Children in caries group remained being fed at night until 19.8 ± 9.2 months old, while caries-free children remained being fed at night until 16.6 ± 10.4 months old (p = 0.000). The first time for tooth brushing in caries group was at 22.8 ± 10.3 months old, while caries-free group was at 21.0 ± 10.4 months old (p = 0.004). The frequency of sweets and acidic drinks consumption differed significantly between two groups (p = 0.000). The application of topical fluoride differed significantly between two groups (p = 0.000).

3.3. Genetic analysis

Allele frequency and genotype distribution were summarised in Table 5. All the three SNPs were in Hardy–Weinberg equilibrium. The *ENAM* rs3796703 T allele frequency was 6.7% in the caries group and 4.2% in the caries-free group, and the CT genotype frequency was 12.7% in the caries group and 8.4% in the caries-free group; showing significant differences (T allele: $\chi^2 = 6.231$, p = 0.013; CT genotype: $\chi^2 = 4.683$, p = 0.026). The *TNF-α* rs1800629 A allele frequency was 4.8% in the caries group and 6.8% in the caries-free group, and the AG genotype frequency was 8.7% in the caries group and 13.2% in the caries-free group; these differences were also significant (A allele: $\chi^2 = 3.847$, p = 0.049; AG genotype: $\chi^2 = 5.142$, p = 0.023). No significant difference was found in *LTF* rs1126478 allele frequency or genotype distribution between the two groups.

3.4. Multivariate analysis

All the genotypes of SNPs and oral behavioural habits factors mentioned above were included as variables in a logistic regression model to evaluate the combined impacts of genetic and environmental factors.

Result showed *ENAM* rs3796703 CT genotype increased caries susceptibility by 60.9% compared to the CC genotype (β = 0.746, OR = 1.609), and the *TNF-α* rs1800629 AG genotype reduced caries susceptibility by 47.4% compared to the GG genotype (β = −0.642, OR = 0.526).

Habit factors were also related with caries susceptibilities. When night feeding was prolonged by one month, caries susceptibility was increased by 3.3% (β = 0.033, OR = 1.033). Furthermore, compared to no consumption of sweets and acidic drinks at all, when consumption frequency was at 1–2 times per day, caries susceptibility was increased by 218.2% (β = 1.158, OR = 3.182); when consumption frequency was ≥3 times per day, caries susceptibility was increased by 883.5% (β = 2.286, OR = 9.835). Topical fluoride application decreased caries susceptibility by 43.0% (β = −0.562, OR = 0.570) (Table 6).

4. Discussion

In recent years, the worldwide caries prevalence declined, which benefited from the reduced consumption of sugar, effective oral hygiene control to remove plaque and fluoride application (Tanner et al., 2013). According to the third Chinese national epidemiological survey, 79.3% of caries occurrence concentrated in only one third of the 5-year-old population group (Qi, 2008). These individuals had much higher mean dmft index (dmft = 8.3) than the average dmft index of all the caries children (dmft = 3.5). Thus

Table 1
SNPs selected in this study.

Candidate gene	Polymorphism position	Base change	Minor allele frequency	Function
ENAM rs3796703	Exon 10	C/T	T = 0.089[a]	Enamel formation
LTF rs1126478	Exon 2	G/A	A = 0.3726[a]	Saliva protein
TNF-α rs1800629	Promoter region	G/A	A = 0.0903[b]	Immune response

[a] Minor allele frequency in Han Chinese in Beijing, China (according to the HapMap database).
[b] Minor allele frequency in global samples (according to the HapMap database).

Table 2
Primers and annealing temperature for polymerase chain reaction.

Gene	Forward primer	Reverse primer	Annealing temp
ENAM rs3796703	5′-AGAGGACCCAGTTGATCCAA-3′	3′-AAATGTGTTGCCTGATCCCA-5′	58 °C
LTF rs1126478	5′-TGTGGAGAATGGCTGGACAT-3′	3′-CCATTCAGCTTGGTCCCAAC-5′	60 °C
TNF-α rs1800629	5′-GAAGCCCCTCCCAGTTCTAG-3′	3′-CGGGGATTTGGAAAGTTGGG-5′	56 °C

Table 3
General information of the participants.

	Caries group	Caries-free group	U/χ^2	p-value
Minimum age (month)	15.0	21.0		
Maximum age (month)	48.0	48.0		
Mean age ± SD (month)	41.7 ± 7.0	43.7 ± 4.0	120498.000	0.211
Proportion of male (%)	52.7	49.0	1.356	0.244

Table 4
Univariate analysis of behavioural habits of the participants.

	Caries group	Caries-free group	U/χ^2	p-value
Mean night feeding time ± SD (month)	19.8 ± 9.2	16.6 ± 10.4	95691.000	*0.000*
Mean first tooth brushing time ± SD (month)	22.8 ± 10.3	21.0 ± 10.4	113242.000	*0.004*
Frequency of sweets and acidic drinks consumption			121.832	*0.000*
Never	5 (1%)	15 (3%)		
1–2 times per week	133 (26.3%)	286 (57.2%)	0.403	0.526[a]
1–2 times per day	248 (49.1%)	183 (36.6%)	8.218	*0.004*[a]
≥3 times per day	124 (24.6%)	31 (6.2%)	27.655	*0.000*[a]
Topical fluoride application			14.097	*0.000*
Yes	224	281		
No	281	219		

[a] Chi-square test compared to nonconsumption of sweets and acidic drinks.

these individuals could be considered as a population that was extremely susceptible to caries. The screening of these high dmft index individuals would be both important and valuable for caries prevention and diagnosis. In our study, the caries participants had an average dmft score of 13.0 ± 3.3, with the range of 4 to 20 decayed, missing or filled teeth. This large average score and their early age (under 4 years old) meant these children suffered a much more severe caries oral condition at an much earlier age, compared to those 1/3 children who suffered from severe caries in the national epidemiological survey. Of these children, we hypothesized that host factors may play a more important role in the aetiology of caries, although differences of diet and oral hygiene habits might also exist.

Table 5
Univariate analysis of alleles and genotypes of the participants.

Gene polymorphism	Caries group	Caries-free group	χ^2	p-value
EANM rs3796703				
Allele C	942 (93.3%)	958 (95.8%)	6.231	*0.013*
Allele T	68 (6.7%)	42 (4.2%)		
Genotype CC	439 (86.9%)	458 (91.6%)		
Genotype CT	64 (12.7%)	42 (8.4%)	4.683[a]	*0.026*[a]
Genotype TT	2 (0.4%)	0		0.240[a,b]
LTF rs1126478				
Allele G	660 (57.1%)	663 (65.3%)	0.203	0.652
Allele A	350 (42.9%)	337 (34.7%)		
Genotype GG	219 (43.4%)	227 (45.4%)		
Genotype AG	222 (43.9%)	209 (41.8%)	0.507[a]	0.476[a]
Genotype AA	64 (12.7%)	64 (12.8%)	0.032[a]	0.858[a]
TNF-α rs1800629				
Allele G	962 (95.2%)	932 (93.2%)	3.847	*0.049*
Allele A	48 (4.8%)	68 (6.8%)		
Genotype GG	459 (90.9%)	433 (86.6%)		
Genotype AG	44 (8.7%)	66 (13.2%)	5.142[a]	*0.023*[a]
Genotype AA	2 (0.4%)	1 (0.2%)		>0.999[a,b]

[a] Chi-square test compared to wild-type genotype.
[b] When cells had expected count <5, Fisher's exact test was used.

Some previous studies presented no significant difference of the influences of various gene polymorphisms on caries between caries-free people and caries population. But the caries state of these people were with a DMFT/dmft score around 5 at most (Doetzer et al., 2015; Karayasheva, Glushkova, Boteva, Mitev, & Kadiyska, 2016; Patir et al., 2008)

Based on these results, we believed that correlation analysis carried out on the most susceptible participants in our study would either detect the strongest caries-related gene polymorphism or better confirm the non-significant difference on the gene polymorphism influences. Both aspects of the results would be conductive to further prevention and diagnosis of caries disease.

Improper diet habits and poor oral hygiene behaviours were linked to caries (Congiu, Campus, & Lugliè, 2014). Sugar intake as a recognized risk factor for caries was widely accepted. WHO suggested that non-milk products and extrinsic sugar should not exceed 10% of the total daily caloric intake of children (Organization, 2015). The lactose in milk and internal sugar in fruit-vegetable had low cariogenic potency, while extrinsic sugar was the major cariogenic factor. We found that high frequency (more than once a day) of sweets and acidic drinks consumption increased the risk of severe caries occurrence. Reasonable diet was of great importance to maintain oral health of children, the frequency of sweets and drinks should be controlled below 1 time/day according to our

Table 6
Multivariate analysis of genotypes and behavioural factors in the subjects.

Covariates	β	OR	Lower 95% CI	Upper 95% CI
ENAM rs3796703				
Genotype CC				
Genotype CT	0.746	1.609	1.029	2.518
Genotype TT	21.762	22907.418	0.000	0.000
LTF rs1126478				
Genotype GG				
Genotype AG	0.106	1.112	0.724	1.708
Genotype AA	0.062	1.604	0.794	1.425
TNF-α rs1800629				
Genotype GG				
Genotype AG	−0.642	0.526	0.338	0.820
Genotype AA	0.676	1.966	0.114	33.946
Night feeding time (month)	0.033	1.033	1.019	1.048
First tooth brushing time (month)	0.008	1.008	0.995	1.022
Frequency of sweets and acidic drinks consumption				
Never				
1–2 times per week	0.079	1.082	0.377	3.102
1–2 times per day	1.158	3.182	1.114	9.088
≥3 times per day	2.286	9.835	3.252	29.749
Topical fluoride application	−0.562	0.570	0.434	0.749

results. Teeth cleaning should be started along with the eruption of the first primary tooth. We also found that the average age of first booth brushing in the caries children was significantly later than the caries-free children. A study done by of Abbasoğlu showed that brushing teeth after the window of *S. mutans* infectivity (19–31 months of age) was a risk factor for caries occurrence (Abbasoğlu et al., 2015). Topical fluoride application was a simple and effective method for the prevention of caries. An in vitro study proved that fluoride solution could inhibit the demineralization of caries lesion, increase enamel and dentin remineralization (ten Cate, Buijs, & Damen, 1995). A randomized clinical trial showed the caries incidence in young children could be reduced by oral hygiene instructions associated with fluoride varnish applications (Mahtab, Shorangize, Ebrahim, & Mehrdad, 2016).

Gene polymorphisms also exhibited important relationships with caries occurrence.

ENAM gene was involved in enamel formation and was considered as a caries candidate gene. Several studies have revealed the associations of some *ENAM* gene SNPs to caries. Patir et al. found that *ENAM* rs3796704 might interact with *S. mutans*, contributing to caries susceptibility (Patir et al., 2008). *ENAM* rs12640848 was reported as a protective factor against caries (Abbasoğlu et al., 2015; Gerreth et al., 2016; Shimizu et al., 2012). Other SNPs, such as rs2609428 and rs7671281, were also found to be associated with caries susceptibility or protection. However, the correlations were inconsistent in some literature (Chaussain et al., 2014; Shimizu et al., 2012). This *ENAM* rs3796703 polymorphism was located in the variable region of exon 10 of the *ENAM* gene according to the evolutionary chart of mammalian *ENAM* (Al-Hashimi, Sire, & Delgado, 2009), and it induced a shift from proline to leucine at amino acid position 724. In the present study, we found that the CT genotype of the *ENAM* rs3796703 SNP distributed much higher occurrence rate in the caries children than caries-free children. CT genotype increased caries susceptibility by 60.1% compared to the CC genotype. Chaussain's data suggested a potential modification of the *ENAM* function in the region of rs3796704 and rs1280484, leaded to change of enamel microstructure, which were very close to rs3796703, based on genome sequence information from the PubMed database (Chaussain et al., 2014). But how did this polymorphism loci affect *ENAM* function and enamel formation was not reported.

LTF is one of the saliva protein genes. The rs1126478 polymorphism produced a shift from arginine to lysine at amino acid position 47 in the antimicrobial region, and exhibited transcriptional activation activity (Velliyagounder et al., 2003). The lysine form of lactoferrin had greater capability to kill early plaque-forming, acid-producing bacteria, including *S. mutans*, and played a prominent role in the host defence against caries (Fine et al., 2013). We found no significant difference in the distribution of the rs1126478 polymorphism between severe caries and caries-free children. This result was in accordance with those of Volckova, who detected no difference in the frequencies of the *LTF* rs1126478 variant among 637 Czech children with and without caries (Volckova et al., 2014). In contrast, a case–control study involving 120 12-year-old children found that allele A increased salivary flow ($p = 0.06$, $OR = 2.48$) and was a protective factor against caries ($p = 0.01$, $OR = 0.16$) (Azevedo et al., 2010). *LTF* polymorphisms differed among genetic backgrounds. Unlike the cases in European populations, A is the minor allele in Asian populations (Videm, Dahl, Wålberg, & Wiseth, 2012). These ethnic differences might also contribute to the heterogeneity of these results.

TNF-α gene affects immunoreaction. The biological activity of TNF-α is controlled by gene polymorphisms. Rs1800629 was located in the promoter region, upstream from the transcription initiation site. Its functions included causing modification of transcriptional regulation and influencing the serum level of TNF-α (Kroeger, Steer, Joyce, & Abraham, 2000). It might be responsible for the progression of aggressive periodontitis (Özer Yücel et al., 2015). Previous study among 186 African-American women showed *TNF-α* microsatellite loci was associated with level of *Lactobacillus acidophilus*, which was one of the causative microorganisms of dental caries (Acton et al., 1999). Cariogenic bacteria could enter the bloodstream and cause transient bacteraemia (Lockhart et al., 2009). Although *S. mutans* was found to stimulate the expression of system TNF-α through the activation of p38/JNK and ERK/p38/JNK MAPK in an *in vivo* study (Kim et al., 2012), the precise mechanism of TNF-α actions in dental caries immunity had not been completely discovered. Our study revealed an association

between the *TNF-α* rs1800629 AG genotype and caries-free experience, suggesting that the *TNF-α* polymorphism may affect caries susceptibility.

However, dental caries was an especially complex phenotype that cannot be readily summarised using a single metric (Wang et al., 2010). The selected candidate genes may play meaningful, but not principal, roles.

Besides gene polymorphisms, previous studies documented that epigenetic events could modify gene expression and might affect oral diseases (Seo et al., 2015). The molecular mechanisms of epigenetics involve DNA methylation, histone modification and small non-coding RNAs, but do not lead to DNA sequence change. Twin studies showed epigenetic and environmental factors could influence tooth shape and emergence (Hughes et al., 2017). Epigenetic events also regulated inflammatory response to cariogenic bacteria in the pulp (Hui et al., 2016) and periapical tissues (Okehie, 2015). Until now we didn't find any results in the literature illustrating if there was confirmed influence by epigenetic factors to caries development. However, considering that the host and bacteria are both important factors in the etiology of caries, future researches are in demand to further explore their relationships.

5. Conclusion

In this study, we investigated both oral behavioural habits and the distributional difference of *ENAM* rs3796703, *LTF* rs1126478, and *TNF-α* rs1800629 polymorphic locus in severe caries and caries-free children.

Low frequency of sweets and acidic drinks consumption, to start tooth brushing at an early age, and fluoride application were of great importance for caries prevention.

The *ENAM* and *TNF-α* genes are likely associated with caries experience in Chinese children. The *ENAM* rs3796703 CT genotype may be involved in caries susceptibility, while *TNF-α* rs1800629 AG genotype may be involved in caries protection. Future studies are still in need to explore the host genetic factors in caries aetiology.

Conflict of interests

The authors declare no conflict of interest.

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Ethical approval

The ethics protocol was approved by the Ethics Committee of Peking University School and Hospital of Stomatology (PKUSSIRB-201628050).

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