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Association of *ENAM*, *TUFT1*, *MMP13*, *IL1B*, *IL10 and IL1RN* gene polymorphism and dental caries susceptibility in Chinese children

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Abstract

Objective: To investigate the association between single nucleotide polymorphisms (SNPs) in six candidate genes (enamelin [*ENAM*]; tuftelin 1 [*TUFT1*]; matrix metallopeptidase 13 [*MMP13*]; interleukin 1 beta [*IL1B*]; interleukin 10 [*IL10*]; interleukin 1 receptor antagonist [*IL1RN*]) and dental caries in children from northwest China.

Methods: This case–control study enrolled children (12–15 years) who underwent routine dental examinations. The children were divided into two groups based on the presence of dental caries. A saliva sample was collected and seven SNPs (rs3806804A/G in *ENAM*, rs3811411T/G in *TUFT1*, rs2252070A/G and rs597315A/T in *MMP13*, rs1143627C/T in *IL1B*, rs1800872A/C in *IL10* and rs956730G/A in *IL1RN*) were genotyped.

Results: A total of 357 children were enrolled in the study: 161 with dental caries and 196 without dental caries. No significant difference was found in the alleles and genotypes of five genes (*ENAM*, *TUFT1*, *MMP13*, *IL10* and *IL1RN*) between those with and without dental caries. A significant relationship was found between the *IL1B* rs1143627C/T polymorphism and dental caries susceptibility with those carrying the rs1143627CT genotype having a lower risk of dental caries compared with those carrying the CC genotype (odds ratio 0.557; 95% confidence interval 0.326, 0.952).

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Conclusion: The *IL1B* rs1143627C/T polymorphism may be associated with dental caries susceptibility in children from northwest China.

Keywords
Dental caries, single nucleotide polymorphisms (SNPs), *ENAM*, *TUFT1*, *MMP13*, *IL1B*, *IL10*, *IL1RN*

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Introduction

Dental caries is a multifactorial infectious disease, which is highly prevalent throughout the world, affecting 60–90% of school-aged children.[1] If it does not get treated in a timely manner, the disease continues to develop and results in many negative clinical consequences, such as decayed crowns, ulceration in tissues due to tooth fragments from decayed crowns, pulpal involvement, periapical periodontitis and inflammation of the alveolar bone.[2] Dental caries can lead to discomfort, pain and poor quality of life, which seriously affects people's health.[1] Although increasing numbers of prevention and treatment strategies have been applied in childhood dentistry, dental caries remains one of most common childhood diseases.[3,4]

People that like sugary food and drinks, and those with poor oral hygiene, are regularly seen in our clinical practice, but dental caries is not necessarily evident in their oral cavities. Conversely, some people who seldom consume sugary food and beverages, and have good oral hygiene, still experience tooth decay. In addition, we have also observed that if parents experienced dental caries more often, then their children were more likely to experience dental caries. These observations suggest that genetic factors may play an important role in the occurrence of dental caries.[5,6]

Along with the considerable progress in molecular biology and the in-depth research in genomics, evidence from the published literature demonstrated that dental caries was closely associated with genetic polymorphisms.[6–11] However, some results were inconsistent, especially in people from different geographical locations and ethnic minorities.[12–14] Therefore, the aim of this current study was to analyse seven single nucleotide polymorphisms (SNPs; rs3806804A/G, rs3811411T/G, rs2252070A/G, rs597315A/T, rs1143627C/T, rs1800872A/C and rs956730G/A) in six candidate genes (enamelin [*ENAM*]; tuftelin 1 [*TUFT1*]; matrix metallopeptidase 13 [*MMP13*]; interleukin 1 beta [*IL1B*]; interleukin 10 [*IL10*]; interleukin 1 receptor antagonist [*IL1RN*]) and to evaluate their association with the susceptibility for dental caries in children from the northwest part of China.

Patients and methods

Study population

This case–control study sequentially recruited children from Dibu county, Gannan Tibetan autonomous prefecture, Gansu Province, which is located in the northwest of China. The children were permanent residents in this district and aged 12–15 years. All of the children were recruited during a general health examination at the Key Laboratory of Oral Diseases of Gansu Province, Lanzhou, Gansu Province, China between June 2016 and August 2016. The dental check was performed by two examiners (X.P.H. and

T.Z.S.) who had been trained by an experienced dentist (Z.Q.L.). The Kappa value between the two examiners reached 0.8. The dental caries examination followed the World Health Organization criteria.[15] Exclusion criteria included the following: (i) any systemic disease; (ii) any other dental diseases such as periodontitis, recurrent oral ulcer and oral genetic disorders. The children had a similar socioeconomic background and oral hygiene status. The children were divided into two groups based on the Decayed, Missing, and Filled Teeth (DMFT) index:[11] the case group had a DMFT ≥ 1; and the control group had a DMFT $= 0$.

Ethical approval was obtained by the Ethics Committee of the Northwest Minzu University, Lanzhou, Gansu Province, China (no. XBMU-YX-2019001) and written informed consent was provided by all of the children and their parents/carers/legal guardians.

DNA preparation and SNP genotyping

All of children were asked not to eat and not to brush their teeth in the morning of their oral examination. Non-irritating saliva (2 ml) was collected from each child and added to saliva protection fluid (Olegene saliva DNA collector; Guangzhou Deep Blue Gene Technology, Guangzhou, China). The saliva samples were stored at –20°C until processing. Genomic DNA was extracted from each saliva sample using a Genomic DNA Isolation Kit (BioMiao Biological Technology, Beijing, China) according to the manufacturer's instructions. All of the seven SNPs in the six candidate genes were genotyped using iPLEX MassARRAY® system (Agena Bioscience, San Diego, CA, USA), which was similar to that used in previous research.[16] The main steps included: (i) polymerase chain reaction (PCR): PCR volume in the MassARRAY® system contained 0.625 μl of $15 \times$ PCR buffer, 0.325 μl of 25 mM $MgCl_2$, 0.1 μl of 25 mM dNTP Mix, 1 μl of 500 nM primer Mix, 0.1 μl of 5 U/μl Hot Star Taq enzyme, 1 μl of 20 ng/μl DNA template and 1.85 μl of water; (ii) Shrimp alkali enzyme purification (SAP) reaction: The volume contained 0.17 μl of $10 \times$ SAP buffer, 0.3 μl of 1 U/μl SAP enzyme and 1.53 μl of water; (iii) single-base extension reaction included 0.2 μl of iPLEX BufferPlus, 0.2 μl of iPLEX Termination Mix, 0.94 μl of iPLEX Extend Primer Mix, 0.041 μl of iPLEX Enzyme, 0.619 μl of water and 7 μl of PCR + SAP product; and (iv) resin purification: 30 samples were randomly selected for duplicate detection to verify the genotyping results. The PCR primers were designed using AssayDesigner3.1 software (Agena Bioscience) and then synthesized by BioMiao Biotechnology Company. The primer sequences are shown in Table 1.

Statistical analyses

All statistical analyses were performed using the SPSS® statistical package, version 17.0 (SPSS Inc., Chicago, IL, USA) for Windows®. The data were entered and validated by double entry. The Hardy–Weinberg equilibrium for the seven SNPs in the six candidate genes was assessed using χ^2-test. The differences in allele and genotype frequencies between the two groups were tested using χ^2-test. An unconditional logistic regression analysis was used to test each gene polymorphism, in which the potential risk factors were adjusted. A P-value < 0.05 was considered statistically significant.

Results

A total of 357 children (178 males and 179 females) were enrolled in the study. The children were divided into two groups based on the DMFT index score (Table 2). The caries-affected group (DMFT ≥ 1, case group) included 161 children (84 males and

Table 1. Polymerase chain reaction primer sequences for seven single nucleotide polymorphisms (SNPs) in six candidate genes (enamelin [*ENAM*]; tuftelin 1 [*TUFT1*]; matrix metallopeptidase 13 [*MMP13*]; interleukin 1 beta [*IL1B*]; interleukin 10 [IL10]; interleukin 1 receptor antagonist [*IL1RN*]) that were genotyped using a MassARRAY® system.

SNP	Tm, °C	Amplicon length, bp	Primer sequence
rs3806804A/G in *ENAM*	48.5	109	F:5′- ACGTTGGATGTTTGCCATTGTACCCAACTC -3′ R:5′- ACGTTGGATGGCTAGCAGGACATAGTGTTC -3′
rs3811411T/G in *TUFT1*	49	88	F:5′- ACGTTGGATGTCTGTTCTAAAGGGCCTCTG -3′ R:5′- ACGTTGGATGACTGTACAGCTTAGGAGCCG -3′
rs2252070A/G in *MMP13*	47	101	F:5′- ACGTTGGATGTATAGGCCTGCAATGGTGAG -3′ R:5′- ACGTTGGATGGCCACGTAAGCATGTTTACC -3′
rs597315A/T in *MMP13*	52	101	F:5′- ACGTTGGATGTACCCATTTCGTACTCACCC -3′ R:5′- ACGTTGGATGAAAATGCTGCTCAGGTCAGG -3′
rs1143627C/T in *IL1B*	49.8	101	F:5′- ACGTTGGATGCCTCGAAGAGGTTTGGTATC -3′ R:5′- ACGTTGGATGATTTCTCAGCCTCCTACTTC -3′
rs1800872A/C in *IL10*	45.6	115	F:5′- ACGTTGGATGTCCTCAAAGTTCCCAAGCAG -3′ R:5′- ACGTTGGATGAAAGGAGCCTGGAACACATC -3′
rs956730G/A in *IL1RN*	48.2	92	F:5′- ACGTTGGATGCAGGCTCTTGTTCTCGTAAC -3′ R:5′- ACGTTGGATGGGCTCAGGTTACCTCAATTC -3′

Tm, melting temperature; bp, base pairs; F, forward; R, reverse.

Table 2. Demographic characteristics of the study population of children ($n = 357$) who were enrolled in this case–control study to examine the role of single nucleotide polymorphisms on the susceptibility to dental caries.

Characteristics	Case group DMFT ≥ 1 $n = 161$	Control group DMFT $= 0$ $n = 196$
Age range, years	12–15	12–15
Age, years	13.8	14.1
Sex		
Male	84 (52.2)	94 (48.0)
Female	77 (47.8)	102 (52.0)
DMFT score	3.46 ± 2.1	0.0 ± 0.0

Data presented as mean ± SD or *n* of subjects (%). DMFT, Decayed, Missing, and Filled Teeth.

77 females), while the caries-free group (DMFT = 0, control group) had 196 children (94 males and 102 females). There was no statistical difference in age and sex distribution between the two groups.

A total of seven different SNPs in six candidate genes were analysed. The characteristics of the distribution of the seven SNPs are shown in Table 3. The Hardy–Weinberg equilibrium for the allele frequencies in the control group are also presented in Table 3.

The differences in allele frequencies and genotype frequencies between groups were analysed to evaluate the effects of the seven SNPs on the susceptibility for dental caries and the results are presented in Table 4. Individuals with the rs1143627CT genotype in the *IL1B* gene were less likely to have dental caries compared with those subjects with the CC genotype (odds ratio [OR] 0.557; 95% confidence interval [95% CI] 0.326, 0.952; $P = 0.032$). However, an association was not found between the susceptibility for dental caries and any of the other six SNPs (rs3806804, rs3811411, rs2252070, rs597315, rs1800872 and rs956730).

Discussion

The development of dental caries is known to result from interactions between the

Table 3. Characteristics of distribution of the seven single nucleotide polymorphisms (SNPs) in six candidate genes (enamelin [*ENAM*]; tuftelin 1 [*TUFT1*]; matrix metallopeptidase 13 [*MMP13*]; interleukin 1 beta [*IL1B*]; interleukin 10 [IL10]; interleukin 1 receptor antagonist [*IL1RN*]) and the Hardy–Weinberg equilibrium (HWE) for the allele frequencies in the control group.

Gene	Type of alteration	SNP	Major/minor allele[a]	MAF in the study population	*P*-value for HWE in the control group
ENAM	Upstream	rs3806804	A/G	0.3927	NS
TUFT1	Downstream	rs3811411	T/G	0.4928	NS
MMP13	Upstream	rs2252070	A/G	0.4971	$P = 0.001$
MMP13	Upstream	rs597315	A/T	0.4167	NS
IL1B	Upstream	rs1143627	C/T	0.4316	NS
IL10	Upstream	rs1800872	A/C	0.416	NS
IL1RN	Intron	rs956730	G/A	0.2977	NS

[a]Major allele was the wild-type allele and the minor allele was the mutant-type allele.
MAF, minor allele frequency; NS, not statistically significant ($P \geq 0.05$).

presence of oral bacteria, host characteristics, dietary choices and length of exposure time.[17] The host's genetic makeup appears to play an important role the development of dental caries.[5] In order to minimize the impact of dietary, environmental and other factors, permanent residents from an autonomous county in Gansu Province were chosen for this current study. The geographical area in which they live is small and population mobility is limited, which should have reduced sample heterogeneity. To avoid trauma and reduce the chances of being infected, a non-irritating saliva sample, rather than a blood sample, was chosen for DNA analysis in the present study, although blood samples would have been easier to test than saliva samples.

Enamel is a hard, highly mineralized substance, covering the crown that acts as a barrier to protect the tooth.[18] The formation of enamel is controlled by genetic factors.[18] The susceptibility for dental caries could also be influenced by genetic inheritance.[19] For example, *TUFT1* genotypes appear to interact with the levels of *Streptococcus mutans* in children.[20] Some previous studies showed that genetic polymorphisms of the amelogenin (rs5933871, rs5934997 and rs17878486), *ENAM* (rs12640848 and rs7671281) and *TUFT1* (rs2337360 and rs3790506) genes were closely associated with the susceptibility for dental caries, while other studies did not find the same association.[21–25] The present study investigated the association between the rs3806804 polymorphism in *ENAM* and the rs3811411 polymorphism in *TUFT1* with dental caries susceptibility, but no correlations were found in Chinese children.

Matrix metalloproteinases (MMPs) play an important role in the control and progression of dental caries because they are involved in enamel and dentin formation.[26,27] Polymorphisms of the related MMP genes, including *MMP2, MMP3, MMP9, MMP10, MMP13, MMP14, MMP16* and *MMP20*, were reported to be associated with dental caries.[28–32] These studies showed that rs1711437 and rs1784418 in *MMP20*, rs2046315 in *MMP16*, rs2252070 in *MMP13*, rs17576 in *MMP9*, rs679620 in *MMP3* and rs2287074 in *MMP2* might decrease or increase dental caries susceptibility.[28–32] According to one of the studies,[32] carriers of the mutant allele (G) for *MMP13* (rs2252070A/G) showed a significantly decreased risk of dental caries (OR 0.538;

Table 4. Distribution of allele and genotype frequencies in the seven single nucleotide polymorphisms (SNPs) in six candidate genes (enamelin [*ENAM*]; tuftelin 1 [*TUFT1*]; matrix metallopeptidase 13 [*MMP13*]; interleukin 1 beta [*IL1B*]; interleukin 10 [IL10]; interleukin 1 receptor antagonist [*IL1RN*]) and unconditional logistic regression analysis.

Gene	SNP	Genotypes and alleles[a]	Case group DMFT $\geq$ 1 $n = 161$[b]	Control group DMFT = 0 $n = 196$[b]	Statistical significance	Odds ratio (95% confidence interval)[c]
ENAM	rs3806804 (A/G)	AA (reference)	56 (40.6)	56 (33.9)		
		AG	63 (45.7)	81 (49.1)	NS	0.778 (0.474, 1.277)
		GG	19 (13.8)	28 (17.0)	NS	0.679 (0.340, 1.353)
		AG+GG	82 (59.4)	109 (66.1)	NS	0.752 (0.471, 1.202)
		A	175 (63.4)	193 (58.5)		
		G	101 (36.6)	137 (41.5)	NS	0.813 (0.585, 1.129)
TUFT1	rs3811411 (T/G)	TT (reference)	35 (23.0)	41 (21.0)		
		TG	89 (58.6)	111 (56.9)	NS	0.939 (0.553, 1.596)
		GG	28 (18.4)	43 (22.1)	NS	0.763 (0.396, 1.470)
		TG+GG	117 (77.0)	154 (79.0)	NS	0.890 (0.534, 1.484)
		T	159 (52.3)	193 (49.5)		
		G	145 (47.7)	197 (50.5)	NS	0.893 (0.662, 1.206)
MMP13	rs2252070 (A/G)	AA (reference)	38 (24.4)	32 (16.6)		
		AG	92 (59.0)	119 (61.7)	NS	0.651 (0.378, 1.121)
		GG	26 (16.7)	42 (21.8)	NS	0.521 (0.265, 1.027)
		AG+GG	118 (75.6)	161 (83.4)	NS	0.617 (0.364, 1.045)
		A	168 (53.8)	183 (47.4)		
		G	144 (46.2)	203 (52.6)	NS	0.773 (0.573, 1.042)
MMP13	rs597315 (A/T)	AA (reference)	58 (37.7)	78 (40.0)		
		AT	79 (51.3)	99 (50.8)	NS	1.073 (0.684, 1.684)
		TT	17 (11.0)	18 (9.2)	NS	1.270 (0.603, 2.675)
		AT+TT	96 (62.3)	117 (60.0)	NS	1.103 (0.715, 1.703)
		A	195 (63.3)	255 (65.4)		
		T	113 (36.7)	135 (34.6)	NS	1.095 (0.801, 1.495)
IL1B	rs1143627 (C/T)	CC (reference)	44 (34.6)	42 (26.6)		
		CT	56 (44.1)	96 (60.8)	$P = 0.032$	0.557 (0.326, 0.952)
		TT	27 (21.3)	20 (12.7)	NS	1.289 (0.630, 2.638)
		CT+TT	83 (65.4)	116 (73.4)	NS	0.683 (0.411, 1.135)
		C	144 (59.0)	180 (57.0)		
		T	110 (41.0)	136 (43.0)	NS	1.011 (0.724, 1.411)
IL10	rs1800872 (A/C)	AA (reference)	54 (33.5)	61 (31.1)		
		AC	81 (50.3)	106 (54.1)	NS	0.863 (0.541, 1.377)
		CC	26 (16.2)	29 (14.8)	NS	1.013 (0.532, 1.928)
		AC+CC	107 (66.5)	135 (68.9)	NS	0.895 (0.573, 1.398)
		A	189 (58.7)	228 (58.2)		
		C	133 (41.3)	164 (41.8)	NS	0.978 (0.725, 1.319)
IL1RN	rs956730 (G/A)	GG (reference)	74 (48.4)	90 (46.6)		
		GA	70 (45.8)	88 (45.6)	NS	0.967 (0.623, 1.501)
		AA	9 (5.9)	15 (7.8)	NS	0.730 (0.302, 10762)
		GA+AA	79 (51.6)	103 (53.4)	NS	0.933 (0.610, 1.427)
		G	218 (71.2)	268 (69.4)		
		A	88 (28.8)	118 (30.6)	NS	0.917 (0.660, 1.274)

Data presented as *n* of subjects or alleles (%).

[a]Wild-type homozygote genotypes or alleles were used as references.

[b]Total sample sizes for each SNP are different because the genotypes of some SNPs were unreadable.

[c]Adjusted for sex and age.

NS, not statistically significant ($P \geq 0.05$).

95% CI 0.313, 0.926). In the current study, no relationship was found with *MMP13* (rs2252070A/G) (OR 0.773; 95% CI 0.573, 1.042) or *MMP13* (rs597315A/T) (OR 1.095; 95% CI 0.801, 1.495). Therefore, the precise nature of the relationship between dental caries and MMPs needs to be studied further in the future.

There is evidence that cytokines are important in regulating and controlling the inflammatory response to bacterial infection.[33,34] Genetic and immunological differences between hosts may also be an important risk factor for dental caries. Although the role that cytokines play in the aetiology and mechanism of dental caries remains unclear, it was found that *S. mutans*, an important factor in dental caries development, could stimulate proinflammatory cytokine production.[35,36] To date, only one study has evaluated the association between interleukin (IL)-1β, IL-1 receptor antagonist and IL-10 and dental caries susceptibility.[37] It found that the level of *S. mutans* was positively correlated with the saliva IL-1β concentration and inversely correlated with saliva IL-1 receptor antagonist concentration; but there were no associations between *IL1B, IL1RN* and *IL10* gene polymorphisms and dental caries.[37] In the current study, individuals with the *IL1B* rs1143627CT genotype had a lower risk of dental caries compared with those with the CC genotype (OR 0.557; 95% CI 0.326, 0.952; $P = 0.032$). However, the sample size in this current study was limited. Therefore, whether it really affects the susceptibility for dental caries and its related mechanisms requires further investigation.

In conclusion, the findings of the current case–control study suggest that individuals from the northwest part of China with the *IL1B* rs1143627CT genotype had a lower risk of dental caries compared with those with the CC genotype. However, other candidate genes that are related to dental caries susceptibility need to be studied in different populations from a range of geographical locations.

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Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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