Article

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Association between *DEFB1* rs11362 and Caries Susceptibility in Permanent Dentition: A Cross Sectional Study

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Background: Dental caries is a multifactorial chronic bacterial infectious disease. Variations in the predisposition of the general population to dental cavities suggest that genetic and immunological factors play significant roles in its pathogenesis. This study aims to explore the impact of the Beta-Defensin 1 (*DEFB1*) rs11362 polymorphism on caries susceptibility in permanent dentition among the Bai Kuyao and Zhuang ethnic groups in China.

Methods: A sample of 754 adolescents aged 12–15 was randomly selected from primary and junior high schools in Nandan County, Guangxi, China. All adolescents underwent clinical examinations, and DNA samples were collected. The genotype of *DEFB1* rs11362 was determined using single nucleotide polymorphism (SNP) typing. The concentration of human β Defensin 1 (hBD-1) protein in saliva was measured using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA).

Results: The distribution of the *DEFB1* rs11362 T allele was lower in the Bai Kuyao group compared to the Zhuang group. The disparity in the rs11362 genotype was statistically significant in the superficial dentin caries subgroup of the Bai Kuyao population ($p = 0.017$). Following adjustment for all potential confounding variables, the analysis revealed a heightened risk of superficial dental caries among CT genotype carriers in the Bai Kuyao population under a co-dominant model (odds ratios (OR) = 2.70; 95% confidence intervals (CI) [1.35–5.44]; $p = 0.005$), and an increased risk among CC genotype carriers in the Bai Kuyao population under a dominant model (OR = 2.35; 95% CI [1.18–4.67]; $p = 0.015$). A significant difference ($p < 0.05$) was noted in the distribution of rs11362 genotypes and salivary hBD-1 levels among the Bai Kuyao group. Salivary hBD-1 levels were notably higher in the CC genotype group (4.12 $\pm$ 2.07 ng/mL) compared to both the CT (2.77 $\pm$ 1.62 ng/mL) and TT genotype groups (2.32 $\pm$ 0.98 ng/mL).

Conclusion: The *DEFB1* rs11362 polymorphism showed an association with caries susceptibility in permanent teeth and influenced hBD-1 protein expression in saliva. Consequently, the *DEFB1* polymorphism likely represents a concealed risk factor for caries.

Keywords: *DEFB1* rs11362; caries susceptibility; permanent dentition; salivary hBD-1

Introduction

Dental caries is a multifactorial, chronic bacterial infectious disease influenced by factors such as sugar consumption, saliva composition, genetics, and other variables [1].

Differences in the susceptibility of the general population to dental cavities suggest significant involvement of genetic and immunological factors in its development. Studies on heritability [2,3] have revealed that genetic factors contribute to 35–55% of the variation in dental caries incidence in permanent teeth. Despite similar dietary habits, exposure to cariogenic bacteria, and other environmental risk factors, individuals may exhibit varying predispositions to caries [4]. Our research group noted that although oral hygiene among the Bai Kuyao population was inferior to that of the Zhuang population, the prevalence of dental caries was lower among the Bai Kuyao [5,6]. Understanding the genetic influences on caries susceptibility is crucial for effective caries prevention.

Numerous studies involving subjects have demonstrated that gene polymorphisms involved in immune re-

actions contribute to caries susceptibility [7–9], including Beta-Defensin 1 (*DEFB1*) [10–12]. *DEFB1* is a genome-wide association study (GWAS) immune response-associated gene encoding human β defensin 1 (hBD-1). hBD-1 is linked to the innate immune response [13], capable of directly or indirectly inactivating or killing a wide range of bacteria by inducing both innate and adaptive immune reactions [14,15]. It serves as the primary defense against infection and colonization by various oral microorganisms, such as *Streptococcus* [16,17]. Polymorphisms in the *DEFB1* gene, particularly in the initiator region (rs11362), have been associated with caries susceptibility [18,19].

The *DEFB1* rs11362 genotype exhibits three variants (CC/CT/TT). Carriers of the *DEFB1* rs11362 T allele face a higher risk of caries compared to carriers of the C allele [19]. Single nucleotide polymorphisms (SNPs) located in non-coding DNA sequences can impact protein transcription levels and serve as disease risk factors [20]. The *DEFB1* rs11362 polymorphism regulates the level of hBD-1 protein, consequently influencing disease susceptibility and the immune system's response to infection [16,21,22]. Therefore, this study aims to investigate the correlation between *DEFB1* rs11362 and caries susceptibility in the permanent teeth of Chinese adolescents, as well as explore the association between *DEFB1* rs11362 and salivary hBD-1 expression.

Materials and Methods

Reagents and Instruments

Reagents:

- dNTP mix (2.5 mM) (Takara, Otsu, Shiga, Japan, 4030Q)
- GeneScanTM-500 (ABI, Waltham, MA, USA, 4310361), HI-DI (ABI, Waltham, MA, USA, 4311320)
- imLDRTM typing kit (Genesky, Shanghai, China, S1006L)
- Tag DNA ligase (50 U/μL) (Genesky, Shanghai, China, P1008L)
- Tag DNA ligase Buffer (10×) (Genesky, Shanghai, China, P1008LA)
- Takara HotStart Taq (5 U/μL) (Takara, Otsu, Shiga, Japan, R007A)
- TIANamp Genomic DNA Kit (Tiangen, Beijing, China, DP304)
- SNP scan kit (Tianhao, Shanghai, China, G0104KS)
- Enzyme-linked immunosorbent assay kit (Abclonal, Wuhan, China, RK00211)

Instruments:

- Centrifuge5810R (Eppendorf, Hamburg, Germany,)
- 2720 Thermal Cycler (ABI, Waltham, MA, USA), 1–10 μL pipette (discovery, Warsaw, Poland)
- 3730xl genetic analyze (ABI, Waltham, MA, USA), Milli-Q Academic (Millipore, New York, NY, USA)
- Microsampler (Eppendorf, Hamburg, Germany)

Subjects

The recruited adolescents were permanent residents of Nandan County, Guangxi Province, where water lacked fluoride supplementation. To qualify as a participant in this study, individuals must: (1) possess permanent dentition, (2) be adolescents aged 12–15 years, (3) exhibit no systemic or genetic diseases, (4) have no other oral diseases such as periodontitis, and (5) have not received antibiotics in the last six months.

The sample size was estimated using an online calculation tool (https://www.stat.ubc.ca/~rollin/stats/ssize/caco.html) with a power of 0.8 and a significance level of 0.05. Based on the lowest minor allele frequency of T = 0.341 at *DEFB1* rs11362 in the Thousand Genomes Project and a locus corresponding to a relative risk value of RR = 2.0, the size for each group was calculated using the formula: $p2 = p0 \times RR/(1 + p0 \times (RR - 1))$. The calculation predicted that a minimum of 136 subjects was needed to detect an effect size for the caries group or caries-free group. A total of 291 subjects (148 Bai Kuyao and 143 Zhuang) in the caries group and 463 participants (314 Bai Kuyao and 149 Zhuang) in the caries-free group were recruited. Written informed consent was obtained from the caretaker of each adolescent. The study received approval from the Ethics Committee of Guangxi Medical University (approval number: 20200043).

Questionnaire

The questionnaire, designed based on the Fourth National Oral Health Epidemiological Survey [23], was utilized to explore the socio-demographic characteristics of the participants (including gender, ethnicity, age, and parents' education), along with details regarding dietary and oral hygiene practices. Data from the questionnaire were collected through face-to-face interviews.

Clinical Oral Examination

Three trained dentists conducted the oral examinations, achieving a high level of inter-observer reliability (kappa = 0.85). The examinations were conducted under artificial light using a disposable stomatoscope and a Community Periodontal Index (CPI) probe. The criteria recommended by the modified International Caries Detection and Assessment System II (ICDAS-II) [24] were adhered to during the examination process. According to the modified ICDAS-II system, the caries status was recorded as follows: 0 = Sound, 1 = Enamel decay, 2 = Dentin decay, 3 = Dentin decay close to the pulp, 4 = Filled, Sound, 5 = Missing due to caries, 6 = Sealed closure, 7 = Unerupted, 9 = Unrecorded.

Table 1. Frequency distribution of characteristics in caries and caries-free group n (%).

Variable	Bai Kuyao				Zhuang			
	Caries group (N = 148)	Caries-free group (N = 314)	χ^2/t	p	Caries group (N = 143)	Caries-free group (N = 149)	χ^2/t	p
Gender			1.41	0.235			1.64	0.201
Male	69 (46.6)	165 (52.5)			68 (47.6)	82 (55.0)		
Female	79 (53.4)	149 (47.5)			75 (52.4)	67 (45.0)		
Age	13.83 ± 0.91	13.79 ± 0.94	0.53	0.465	13.38 ± 0.77	13.58 ± 0.83	2.53	0.113
Parental education level			0.55	0.459			0.37	0.542
Primary school and below	104 (70.3)	231 (73.6)			35 (24.5)	32 (21.5)		
Secondary school and above	44 (29.7)	83 (26.4)			108 (75.5)	117 (78.5)		
The frequency of teeth-brushing			0.32	0.570			0.12	0.730
≤1/d	30 (20.3)	71 (22.6)			40 (28.0)	39 (26.2)		
≥2/d	118 (79.7)	243 (77.4)			103 (72.0)	110 (73.8)		
Gum bleeding			9.37	0.020*			0.03	0.855
No	43 (29.1)	138 (43.9)			85 (59.4)	87 (58.4)		
Yes	105 (70.9)	176 (56.1)			58 (40.6)	62 (41.6)		
Dental calculus			4.08	0.043*			7.45	0.006*
No	15 (10.1)	16 (5.1)			59 (41.3)	39 (26.2)		
Yes	133 (89.9)	298 (94.9)			84 (58.7)	110 (73.8)		
The frequency of dessert/candy intake			0.25	0.620			7.29	0.007*
≤1/w	96 (64.9)	211 (67.2)			46 (32.2)	71 (47.7)		
≥2/w	52 (35.1)	103 (32.8)			97 (67.8)	78 (52.3)		

Note: * means significant level $p < 0.05$ (two-sided test).

Table 2. Genotype and allele distributions of the Beta-Defensin 1 (*DEFB1*) rs11362 polymorphisms between Bai Kuyao and Zhuang n (%).

Type	Bai Kuyao, n (%)	Zhuang, n (%)	χ^2	p
Genotype				
CC	322 (69.7)	126 (43.2)	56.51	<0.001*
CT	124 (26.8)	133 (45.5)		
TT	16 (3.5)	33 (11.3)		
Allele				
C	768 (83.1)	385 (65.9)	58.76	<0.001*
T	156 (16.9)	199 (34.1)		

Note: p values indicate the difference in genotype distributions (or allele frequency) between Bai Kuyao and Zhuang populations by the χ^2 test, * means a significant level $p < 0.05$ (two-sided test).

Preparation of DNA Sample

Before collecting shed cells containing the buccal mucosa, participants were instructed to abstain from food for 30 minutes and rinse their mouth with water. Four sterile buccal swabs were used for each participant, with a minimum of 20 repetitions on each cheek inside the mouth. The swabs were then placed in 5 mL angled-picking tubes. All samples were collected by a trained collector.

DNA Extraction and SNP Genotyping

According to the manufacturer's instructions of the TIANamp Genomic DNA Kit (Tiangen, Beijing, China, DP304), DNA was extracted from detached buccal mucosal cells. Two allele-specific probes (sequences: GAGGTTGTGCAATCCACCAGTCT and TACGGTTATTCGGGCTCCTGTCCCAGTTC-CTGAAATCCAGA) were utilized, along with a universal probe for the locus (sequence: GTGTTGC-CTGCCAGTCGCCATTTTTTTTTT), which was designed using a SNP scan kit (Tianhao, Shanghai, China, G0104KS). Genotyping was conducted employing the iMLDR multiple SNP typing technique.

Non-Stimulating Whole Saliva Collection and Enzyme-Linked Immunosorbent Assay (ELISA)

Non-stimulated whole saliva samples were obtained from Bai Kuyao (N = 36) and Zhuang (N = 37) subjects, who were randomly selected from the entire participant pool using the random number table method. Subjects were instructed to abstain from eating or drinking for one hour prior to the sampling procedure and to rinse their mouths with water 10 minutes beforehand. Participants were then instructed to tilt slightly forward with the lower lip against the opening of the collection tube, allowing saliva to naturally flow into the tube until 3 mL was collected. The concentration of hBD-1 protein in the saliva was measured using a human BD-1 enzyme-linked immunosorbent assay kit (Abclonal, Wuhan, China, RK00211).

Table 3. The relationship between the genotype and allele distribution of *DEFB1* rs11362 and the severity of dental caries in Bai Kuyao and Zhuang n (%).

Ethnic	Genotype/ Allele	Enamel caries group				Superficial dentin group				Deep dentin group			
		Enamel caries group	Caries-free group	χ^2	p	Superficial dentin group	Caries-free group	χ^2	p	Deep dentin group	Caries-free group	χ^2	p
Bai Kuyao	CC	31 (68.9)	221 (70.4)	1.96	0.376	22 (53.7)	221 (70.4)	8.13	0.017*	48 (77.4)	221 (70.4)	5.77	0.056
	CT	14 (31.1)	82 (26.1)			19 (46.3)	82 (26.1)			9 (14.5)	82 (26.1)		
	TT	0 (0.0)	11 (3.5)			0 (0.0)	11 (3.5)			5 (8.1)	11 (3.5)		
	C	76 (84.4)	524 (83.4)	0.06	0.810	63 (76.8)	524 (83.4)	2.21	0.137	105 (84.7)	524 (83.4)	0.12	0.733
	T	14 (15.6)	104 (16.6)			19 (23.2)	104 (16.6)			19 (15.3)	104 (16.6)		
Zhuang	CC	9 (32.1)	70 (47.0)	2.10	0.350	15 (44.1)	70 (47.0)	1.32	0.518	32 (39.5)	70 (47.0)	1.21	0.546
	CT	15 (53.6)	62 (41.6)			17 (50.0)	62 (41.6)			39 (48.1)	62 (41.6)		
	TT	4 (14.3)	17 (11.4)			2 (5.9)	17 (11.4)			10 (12.3)	17 (11.4)		
	C	33 (58.9)	202 (67.8)	1.66	0.198	47 (69.1)	202 (67.8)	0.05	0.832	103 (63.6)	202 (67.8)	0.83	0.362
	T	23 (41.1)	96 (32.2)			21 (30.9)	96 (32.2)			59 (36.4)	96 (32.2)		

Note: p values indicate the difference for comparison of genotype distributions (or allele frequency) between caries and caries-free group by the χ^2 test in Bai Kuyao, and, Zhuang, * means a significant level $p < 0.05$ (two-sided test).

Table 4. The relationship between *DEFB1* rs11362 and the severity of dental caries under different genetic models of Bai Kuyao and Zhuang.

Degree of caries	Model	Genotype	Enamel caries		Superficial dentin		Deep dentin	
Ethnic			OR adj (95% CI)	p adj	OR adj (95% CI)	p adj	OR adj (95% CI)	p adj
Bai Kuyao	Codominant	CC (ref)						
		CT	1.06 (0.51–2.22)	0.870	2.70 (1.35–5.44)	0.005*	0.52 (0.24–1.15)	0.106
		TT	————	0.999	————	0.999	1.63 (0.49–5.40)	0.423
	Dominant	CC (ref)						
		CT+TT	0.93 (0.45–1.92)	0.836	2.35 (1.18–4.67)	0.015*	0.68 (0.35–1.34)	0.268
	Recessive	CC+CT (ref)						
		TT	————	0.999	————	0.999	1.85 (0.56–6.07)	0.312
	Additive	——	0.82 (0.43–1.57)	0.550	1.66 (0.93–2.95)	0.084	0.88 (0.52–1.48)	0.629
Zhuang	Codominant	CC (ref)						
		CT	2.16 (0.82–5.69)	0.118	1.47 (0.64–3.41)	0.365	1.27 (0.68–2.36)	0.454
		TT	1.51 (0.37–6.13)	0.561	0.45 (0.09–2.36)	0.344	1.04 (0.40–2.70)	0.939
	Dominant	CC (ref)						
		CT+TT	1.99 (0.79–4.99)	0.142	1.21 (0.54–2.71)	0.646	1.22 (0.67–2.19)	0.517
	Recessive	CC+CT (ref)						
		TT	1.01 (0.28–3.63)	0.988	0.37 (0.07–1.83)	0.221	0.92 (0.37–2.27)	0.857
	Additive	——	1.40 (0.75–2.59)	0.287	0.92 (0.51–1.68)	0.797	1.09 (0.71–1.67)	0.698

Note: Adjusted for sex, age, parental education, frequency of tooth brushing, gingival bleeding, dental calculus, and frequency of dessert and candy intake; * indicates a significance level $p < 0.05$ (two-sided test). OR, odds ratios; CI, confidence intervals.

Statistical Analysis

Subjects with a Modified ICDAS-II Index of 0 or 6 were classified as caries-free. Participants with a Modified ICDAS-II Index of 1–3 were categorized as having caries. The caries group was further stratified into subgroups based on different levels of caries severity: enamel caries group (Modified ICDAS-II Index = 1), superficial dentin caries group (Modified ICDAS-II Index = 2), and deep dentin caries group (Modified ICDAS-II Index = 3). Subjects with Modified ICDAS-II Indices of 4, 5, 7, and 9 were excluded from the analysis (Index 4 represents filled without caries and cannot determine caries severity; Index 5 indicates missing due to caries and does not precisely reflect the severity of caries before loss; Indices 7 and 9 do not correlate with caries severity).

Categorical data were analyzed using either the chi-square test or Fisher's exact test. The Hardy-Weinberg equilibrium (HWE) was assessed for both ethnic groups with or without caries using PLINK 1.07 software (MA, USA) (https://www.cog-genomics.org/plink/1.9/). The chi-square test was employed to compare differences in the distribution of allele and genotype frequencies of *DEFB1* rs11362 between the caries and caries-free groups. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using unconditional logistic regression models adjusted for confounders, including sex, age, parental education, frequency of tooth brushing, gingival bleeding, dental calculus, breakfast, and candy intake frequencies. The levels of hBD-1 (Mean $\pm$ SD) across different groups were determined using a nonparametric *T*-test. A significance level of $p < 0.05$ was considered statistically significant. Statistical analysis was performed using the SPSS software package (ver. 25.0; SPSS Inc., Chicago, IL, USA).

Results

Participants Features

The caries prevalence among the Bai Kuyao and Zhuang ethnic groups was 33.9% and 52.7%, respectively. Table 1 displays the sociodemographic characteristics, dietary habits, and oral hygiene practices of the study participants.

Genotype Distributions

The *DEFB1* rs11362 genotype was observed to be in HWE within both the caries and caries-free groups among the two populations. Additionally, a statistically significant difference ($p < 0.05$) was observed in the distribution of alleles and genotypes for the *DEFB1* rs11362 polymorphism between the two ethnicities under study. Specifically, the distribution of the *DEFB1* rs11362 T allele was lower in the Bai Kuyao population compared to the Zhuang population ($p < 0.05$) (Table 2).

The associations between the *DEFB1* rs11362 polymorphism and subgroups with varying levels of caries (enamel caries, superficial dentin caries, and deep dentin caries) are outlined in Table 3. Notably, a statistically significant difference in the rs11362 genotype was observed within the superficial dentin caries group among the Bai Kuyao population ($p = 0.017$), while no significant difference was detected in the Zhuang population.

Genotypic Association of rs11362 with Different Levels of Dental Caries

After adjusting for all potential confounding factors, the analysis revealed a higher risk of superficial dental caries among CT carriers in the Bai Kuyao population in the co-dominant model (OR = 2.70; 95% CI [1.35–5.44]; $p = 0.005$) and among CC carriers in the Bai Kuyao population in the dominant model (OR = 2.35; 95% CI [1.18–4.67]; $p = 0.015$) (Table 4). However, no statistically significant differences were observed in the subgroups with different levels of caries compared to the caries-free group in the Zhuang population.

Association between Salivary hBD-1 Expression Levels and rs11362

A statistically significant difference ($p < 0.05$) was found in the distribution of rs11362 genotypes and salivary hBD-1 levels among the Bai Kuyao population. Specifically, the level of salivary hBD-1 was significantly higher in the CC genotype group (4.12 $\pm$ 2.07 ng/mL) compared to both the CT (2.77 $\pm$ 1.62 ng/mL) and TT genotype groups (2.32 $\pm$ 0.98 ng/mL). However, no significant differences were observed in the Zhuang population (Table 5). Furthermore, no statistically significant differences were noted between the caries subgroups (enamel caries, superficial dentin, and deep dentin) and the caries-free group in either the Bai Kuyao or Zhuang population.

Discussion

The investigation aimed to explore the correlation between *DEFB1* rs11362 and susceptibility to caries in the permanent teeth of 12–15-year-old adolescents. Our findings indicated that Bai Kuyao adolescents exhibited a lower prevalence of caries compared to Zhuang adolescents, and the distribution of the T allele of *DEFB1* rs11362 was lower among Bai Kuyao adolescents than among Zhuang adolescents. This observation suggests that the T allele of *DEFB1* rs11362 may serve as a risk indicator for caries development in our study. A meta-analysis has further shown that carriers of the TT genotype have a sevenfold increased risk of developing caries in permanent dentition compared to carriers of the CC genotype [25]. Additionally, an association between the *DEFB1* rs11362 mutant genotype (TT/AA) and elevated caries risk has been reported in North American [10], Turkish [12], and Indian Tamil adults [26]. However, Navarra [18] reported that individuals with the wild-type GG genotype had higher decayed, missing, filled

Table 5. Comparison of salivary hBD-1 (ng/mL) concentrations among groups with different genotype, caries status, and degree of caries.

	Bai Kuyao				Zhuang			
Group	Number	Mean ± SD	H	*p*	Number	Mean ± SD	H	*p*
Genotype								
CC	13	4.12 ± 2.07	6.89	0.032*	13	3.73 ± 1.74	0.31	0.857
CT	13	2.77 ± 1.62			18	3.82 ± 2.32		
TT	10	2.32 ± 0.98			6	3.89 ± 0.87		
Degree of caries								
Caries-free	12	3.18 ± 2.16	1.85	0.604	14	4.19 ± 2.37	1.34	0.720
Enamel caries	2	3.69 ± 0.03			5	3.52 ± 1.94		
Superficial dentin	4	2.72 ± 1.78			7	3.25 ± 1.63		
Deep dentin	15	2.89 ± 1.31			10	3.96 ± 1.47		

Note: hBD-1 (ng/mL) concentrations are expressed as mean ± standard deviation (Mean ± SD), differences between groups were compared using the Kruskal-Wallis H test, * represents $p < 0.05$. hBD-1, human β defensin 1.

teeth (DMFT) scores than those with the heterozygous GA and mutant AA genotypes in a northeastern Italian population. Discrepancies between the Navarra study and our results may arise from differences in study design, examination methods, survey area, study ethnicity, or dental criteria. Therefore, further validation of the association between *DEFB1* rs11362 and caries susceptibility in a larger population is warranted to support the findings of our study.

This study revealed an association between *DEFB1* rs11362 and the risk of superficial dentin caries, but not with enamel or deep dentin caries, within the Bai Kuyao population. The lack of association with enamel caries may be attributed to the predominant influence of environmental factors, such as bacteria, diet, saliva composition, enamel demineralization, tooth type, and oral hygiene, in the early stages of enamel caries pathogenesis [27]. It's possible that *DEFB1* may play a role in mediating long-term or chronic disease processes rather than the relatively short-term development of caries. However, these hypotheses require validation in future studies. The absence of an association with deep dentin caries could be explained by the likelihood that individuals with deep dentin caries experience them for extended periods compared to those with superficial dentin caries. Consequently, the cariogenic bacteria present in their saliva might have developed resistance to hBD-1 [28]. In our study, participants aged 12–15 were in the early stages of permanent dentition, and the observation period might have been insufficient. Therefore, a follow-up study is warranted to further investigate these associations. In a previous study, we observed that *DEFB1* rs11362 was associated with the risk of caries in a co-dominant, dominant, and additive model in a 12-year-old Zhuang population [29].

However, this association was not observed in the Zhuang population in the present study. The variance in results could stem from variances in caries screening methods utilized during the caries examination. The World Health Organization (WHO) standard method of caries examination solely assesses caries present in the enamel and dentin, where cavities have already formed. Conversely, the modified ICDAS-II system encompasses early enamel caries, where cavities have not yet developed. Hence, it encompasses the early stages of caries in its assessment.

DEFB1 rs11362 is situated in the promoter region of this gene. SNPs within promoter regions have the potential to alter the transcriptional activity of wild-type genes [30]. Our investigation revealed a significantly higher level of salivary hBD-1 in CC genotype groups compared to CT and TT groups in the Bai Kuyao population ($p < 0.05$), but not in the Zhuang population. Consequently, we hypothesize that the T allele of *DEFB1* rs11362 might decrease hBD-1 expression levels in the Bai Kuyao, resulting in diminished antimicrobial capacity and an elevated risk of dental caries. Salivary hBD-1 levels appear to be influenced by the interplay between environmental factors (such as distinct dietary habits in the Bai Kuyao and Zhuang populations) and genetic factors (*DEFB1*). The magnitude of these interactions could potentially offset the differential effects of genes. Moreover, the transcription of *DEFB1* is not solely governed by SNPs but also by various transcription factors [31]. Consequently, we aim to investigate the correlation between salivary hBD-1 and cariogenic microorganisms in subsequent studies.

Our findings suggest that the *DEFB1* rs11362 polymorphism correlates with dental caries in permanent teeth among 12–15-year-old Chinese adolescents and impacts the levels of hBD-1 protein expression in saliva. *DEFB1* rs11362 holds promise as a potential biomarker for permanent teeth. Exploring *DEFB1* polymorphisms could offer novel insights into the pathogenesis, risk assessment, and clinical diagnosis of dental caries at the genetic level. This implies the potential development of genetic test kits for screening and predicting high caries risk, facilitating targeted caries prevention based on these results. Moreover, such findings could inform government policies and measures based on predictive outcomes, thereby extending ben-

efits to a larger population and reducing the disease burden of caries. However, the limited and homogeneous sample size in this study necessitates further expansion of sample size and the conduct of comprehensive and systematic investigations across diverse ethnic and age groups to validate our findings. Additionally, establishing a gene-knockout model using animal models of dental caries would help eliminate the influence of environmental factors such as diet and microbiology, thereby verifying the relationship between the *DEFB1* rs11362 polymorphism and the expression of salivary hBD-1 and dental caries.

Conclusion

The *DEFB1* rs11362 polymorphism was associated with susceptibility to caries in permanent teeth and influenced hBD-1 protein expression in saliva. Therefore, the *DEFB1* polymorphism likely represents a latent caries risk factor.

Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Author Contributions

XJZ designed the research study. QLL, LL, SYC, XTY, SC and SQC performed the research. QLL, LL, SYC and XJZ participated in acquisition of data, and resource sharing. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The research covered in this paper has been conducted in strict accordance with the Declaration of Helsinki. The study received approval from the Ethics Committee of Guangxi Medical University (approval number: 20200043). Written informed agreement was received from the caretaker of each adolescent.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Pitts NB, Zero DT, Marsh PD, Ekstrand K, Weintraub JA, Ramos-Gomez F, *et al*. Dental caries. Nature Reviews. Disease Primers. 2017; 3: 17030.

[2] Conry JP, Messer LB, Boraas JC, Aeppli DP, Bouchard TJ, Jr. Dental caries and treatment characteristics in human twins reared apart. Archives of Oral Biology. 1993; 38: 937–943.

[3] Wang X, Shaffer JR, Zeng Z, Begum F, Vieira AR, Noel J, *et al*. Genome-wide association scan of dental caries in the permanent dentition. BMC Oral Health. 2012; 12: 57.

[4] Sheiham A. Dental caries affects body weight, growth and quality of life in pre-school children. British Dental Journal. 2006; 201: 625–626.

[5] Lin WY, Si GA, Li H, Chen BL, Zeng XJ. Survey report on 359 cases of oral health status of Baiku yao residents in Guangxi. Journal of Guangxi Medical University. 2014; 31: 321–324. (In Chinese)

[6] Ma F, Chen SY, Yu XT, Liu QL, Zeng XJ. Comparison of the status and analysis of the impact factors of caries in children aged 12 years old between Baikuyao and Zhuang as well as Han population in Guangxi. Chinese Journal of Practical Stomatology. 2019; 12: 729–734. (In Chinese)

[7] Baltimore D, Boldin MP, O'Connell RM, Rao DS, Taganov KD. MicroRNAs: new regulators of immune cell development and function. Nature Immunology. 2008; 9: 839–845.

[8] Azevedo LF, Pecharki GD, Brancher JA, Cordeiro CA, Jr, Medeiros KGDS, Antunes AA, *et al*. Analysis of the association between lactotransferrin (LTF) gene polymorphism and dental caries. Journal of Applied Oral Science: Revista FOB. 2010; 18: 166–170.

[9] Fine DH, Toruner GA, Velliyagounder K, Sampathkumar V, Godboley D, Furgang D. A lactotransferrin single nucleotide polymorphism demonstrates biological activity that can reduce susceptibility to caries. Infection and Immunity. 2013; 81: 1596–1605.

[10] Ozturk A, Famili P, Vieira AR. The antimicrobial peptide DEFB1 is associated with caries. Journal of Dental Research. 2010; 89: 631–636.

[11] Krasone K, Lāce B, Akota I, Care R, Deeley K, Küchler EC, *et al*. Genetic variation in the promoter region of beta-defensin 1 (DEFB 1) is associated with high caries experience in children born with cleft lip and palate. Acta Odontologica Scandinavica. 2014; 72: 235–240.

[12] Yildiz G, Ermis RB, Calapoglu NS, Celik EU, Türel GY. Gene-environment Interactions in the Etiology of Dental Caries. Journal of Dental Research. 2016; 95: 74–79.

[13] Piekoszewska-Ziętek P, Turska-Szybka A, Olczak-Kowalczyk D. Single Nucleotide Polymorphism in the Aetiology of Caries: Systematic Literature Review. Caries Research. 2017; 51: 425–435.

[14] Harder J, Gläser R, Schröder JM. Human antimicrobial proteins effectors of innate immunity. Journal of Endotoxin Research. 2007; 13: 317–338.

[15] Aerts AM, François IEJA, Cammue BPA, Thevissen K. The mode of antifungal action of plant, insect and human defensins. Cellular and Molecular Life Sciences. 2008; 65: 2069–2079.

[16] Krisanaprakornkit S, Kimball JR, Weinberg A, Darveau RP, Bainbridge BW, Dale BA. Inducible expression of human beta-defensin 2 by Fusobacterium nucleatum in oral epithelial cells: multiple signaling pathways and role of commensal bacteria in

innate immunity and the epithelial barrier. Infection and Immunity. 2000; 68: 2907–2915.

[17] Komatsuzawa H, Ouhara K, Kawai T, Yamada S, Fujiwara T, Shiba H, *et al*. Susceptibility of periodontopathogenic and cariogenic bacteria to defensins and potential therapeutic use of defensins in oral diseases. Current Pharmaceutical Design. 2007; 13: 3084–3095.

[18] Navarra CO, Robino A, Pirastu N, Bevilacqua L, Gasparini P, Di Lenarda R, *et al*. Caries and Innate Immunity: DEFB1 Gene Polymorphisms and Caries Susceptibility in Genetic Isolates from North-Eastern Italy. Caries Research. 2016; 50: 589–594.

[19] Wu L, Li Z, Zhou J, Ma B, Yu F, Zheng X, *et al*. An association analysis for genetic factors for dental caries susceptibility in a cohort of Chinese children. Oral Diseases. 2022; 28: 480–494.

[20] Buckland PR. The importance and identification of regulatory polymorphisms and their mechanisms of action. Biochimica et Biophysica Acta. 2006; 1762: 17–28.

[21] Prado-Montes de Oca E. Human beta-defensin 1: a restless warrior against allergies, infections and cancer. The International Journal of Biochemistry & Cell Biology. 2010; 42: 800–804.

[22] Polesello V, Zupin L, Di Lenarda R, Biasotto M, Ottaviani G, Gobbo M, *et al*. Impact of DEFB1 gene regulatory polymorphisms on hBD-1 salivary concentration. Archives of Oral Biology. 2015; 60: 1054–1058.

[23] 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. The Chinese Journal of Dental Research. 2018; 21: 181–193.

[24] Wu S, Zhang T, Liu Q, Yu X, Zeng X. Effectiveness of fluoride varnish on caries in the first molars of primary schoolchildren: a 3-year longitudinal study in Guangxi Province, China. International Dental Journal. 2020; 70: 108–115.

[25] Hatipoğlu Ö, Saydam F. Association between rs11362 polymorphism in the beta-defensin 1 (DEFB1) gene and dental caries: A meta-analysis. Journal of Oral Biosciences. 2020; 62: 272–279.

[26] Subbiah HV, Subbiah U, Ajith A. Association of β-defensin 1 gene Polymorphism and dental caries susceptibility in Tamil Ethnicity. Research Journal of Pharmacy and Technology. 2021; 14: 4731–4735.

[27] Palti DG, Machado MADAM, Silva SMBD, Abdo RCC, Lima JEDO. Evaluation of superficial microhardness in dental enamel with different eruptive ages. Brazilian Oral Research. 2008; 22: 311–315.

[28] Phattarataratip E. The role of salivary antimicrobial peptides in shaping Streptococcus mutans ecology [PhD's thesis]. The University of Iowa. 2010.

[29] Ma F, He H, Chen S, Yu X, Liu Q, Zeng X. Associations of PART1 and DEFB1 polymorphisms with Dental Caries in twelve-year-old children in Southern China: a cross-sectional study. BMC Pediatrics. 2023; 23: 6.

[30] Milanese M, Segat L, Crovella S. Transcriptional effect of DEFB1 gene 5' untranslated region polymorphisms. Cancer Research. 2007; 67: 5997; author reply 5997.

[31] Flores Saiffe Farías A, Jaime Herrera López E, Moreno Vázquez CJ, Li W, Prado Montes de Oca E. Predicting functional regulatory SNPs in the human antimicrobial peptide genes DEFB1 and CAMP in tuberculosis and HIV/AIDS. Computational Biology and Chemistry. 2015; 59: 117–125.