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Association between *HLA-DRB1** allele polymorphism and caries susceptibility in Han Chinese children and adolescents in the Xinjiang Uygur Autonomous Region

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Abstract

Objective: The rate of caries and the mean number of decayed, missing, or filled teeth were reported to be significantly higher in children in the Xinjiang Uygur Autonomous Region than in children in eastern China. Little is known regarding the genetic basis of caries among residents of the Xinjiang Uygur Autonomous Region. This study investigated the association between *HLA-DRB1* alleles and caries susceptibility in Han Chinese children and adolescents in the Xinjiang Uygur Autonomous Region.

Methods: *HLA-DRB1* allele frequency was assessed in DNA samples from buccal swabs of 42 patients with caries and 123 healthy control participants using a polymerase chain reaction method with sequence-specific primers. The chi-squared test or Fisher's exact test, followed by Bonferroni correction, was used to calculate differences in allele frequencies between groups.

Results: Compared with the healthy controls, the allele frequency of *HLA-DRB1*13* was significantly higher in patients with caries in the Xinjiang Uygur Autonomous Region (35.71% vs. 18.70%). The allele frequency of *HLA-DRB1*09* was significantly lower in patients with caries than in healthy controls (4.76% vs. 25.20%).

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Conclusions: *HLA-DRB1*13* alleles could confer greater caries susceptibility, whereas *HLA-DRB1*09* could be protective against caries pathogenesis, in Han Chinese children in the Xinjiang Uygur Autonomous Region.

Keywords
HLA-DRB1, Dental Caries, Xinjiang, Polymerase Chain Reaction, Adolescent, Child, Gene Frequency, China, Han Chinese

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Introduction

Dental caries in permanent teeth is the second most common human disease.[1] With advances in oral medicine, the incidence of caries has significantly decreased worldwide.[2] However, caries continues to impact the quality of life of a large number of school-aged children and adults.[3] Notably, the rate of untreated dental caries in 5-year-old children in China was 97.1% in 2005.[4] The rate of caries and the mean number of decayed, missing, or filled teeth were reported to be significantly higher in children in the Xinjiang Uygur Autonomous Region than in children in eastern China.[5] Therefore, potential causes for this increased incidence and severity must be investigated.

Thus far, extensive studies have elucidated the roles of genetic factors in the etiology of caries.[6–8] Four major factors—host, dental plaque, food, and time—are reportedly associated with the pathogenesis of caries.[9] Genes encoding the major histocompatibility complex (MHC) could modulate host immune responses against typical cariogenic bacteria, bacterial adhesion to dental surfaces, and dental surface colonization, as well as enamel development.[10,11] Variations in MHC class II-DR antigens are presumed to influence caries susceptibility.[12] MHC polymorphisms are crucial for immune responses to colonization by oral microorganisms, thereby influencing individual variations in caries susceptibility. The human leukocyte antigen (HLA) *II-DRB1* gene has been associated with the onset of oral diseases.[7,13] Lehner et al.[14] reported that individuals with the *HLA-DRB1*04* allele exhibited greater caries susceptibility. Moreover, the *HLA-DRB1*04* allele was positively associated with the number of pathogenic *Streptococcus mutans* bacteria in the oral cavity.

In 2014, Mauramo et al.[15] reported an association between HLA alleles and susceptibility to caries in Caucasian adults; *HLA-DRB1*01*, *HLA-DRB1*04*, *HLA-DRB1*07*, and *HLA-DRB1*13* alleles were more frequently detected in patients with caries. Additional HLA alleles have been associated with the onset of other oral diseases. For example, *HLA-B15* and *HLA-DRB1*12* alleles were associated with periodontal diseases, whereas the *HLA-A32* allele was associated with temporomandibular dysfunction.[16] Recently, Opal et al.[8] proposed that the *HLA-DRB1*04* and *HLA-DQB1* alleles may confer caries susceptibility. These prior studies suggest an association between *HLA* alleles and caries formation.

To the best of our knowledge, little is known regarding the genetic basis of caries among residents of the Xinjiang

Uygur Autonomous Region. In this study, we investigated the association between MHC (*HLA-DRB1*) alleles and caries susceptibility in Han Chinese children and adolescents in the Xinjiang Uygur Autonomous Region.

Materials and methods

Participants

Adolescent and pediatric patients with caries who presented for outpatient consultations in the Department of Pediatric Dentistry in the First Affiliated Hospital of Xinjiang Medical University in 2015 were recruited for this study. The inclusion criteria were as follows: (i) patients with at least 10 caries; (ii) patients aged 6 to 12 years; (iii) patients from Han Chinese families living in the Xinjiang Uygur Autonomous Region for more than two generations who were not related to other participants in this study; (iv) patients without systemic, immune, or hereditary diseases; (v) patients with similar lifestyle, eating habits, and oral hygiene habits; and (vi) patients without orthodontic devices in the mouth. The exclusion criteria were as follows: (i) patients with enamel hypoplasia or incomplete enamel mineralization; (ii) patients with tooth loss from other causes; (iii) patients who underwent caries prevention using a sealant or fluoride. The caries severity was evaluated using the World Health Organization scale for decayed/missing/filled teeth: 0.0 to 1.1, very low; 1.2 to 2.6, low; 2.7 to 4.4, moderate; 4.5 to 6.5, high; and >6.6, very high. Han Chinese children with a value of 0 on the decayed/missing/filled teeth scale, who were randomly selected from data in the Blood Transfusion Department of the First Affiliated Hospital of Xinjiang Medical University, served as healthy controls; all other inclusion criteria were identical to those used for the patients in this study.

Ethics statement

Informed consent was obtained from all participants. The study protocol was approved by the Ethics Review Committee of the First Affiliated Hospital of Xinjiang Medical University.

Sample collection and DNA preparation

All participants rinsed their mouths to remove any food remnants. Then, buccal swabs were obtained using one sterile cotton swab for each participant. The swab was allowed to dry naturally and then suspended in 5% Chelex-100 (Sigma-Aldrich, St. Louis, MO, USA); this was followed by the addition of 4 μL Proteinase K (20 mg/mL; Bio-Rad, Hercules, CA, USA) and vortexing of the sample tube. The tube containing the swab in solution was incubated in a water bath for 3 hours at 55°C, then incubated in an ice bath for 3 minutes. The sample tube was centrifuged at 17,000 × g for 2 minutes, using a centrifuge supplied by Sigma-Aldrich, and the supernatant was collected. Subsequently, the DNA product was purified using phenol-chloroform extraction. DNA purity was determined using a NanoDrop Spectrophotometer ND-100 (Thermo Fisher Scientific Inc., Rockford, IL, USA); DNA was then preserved at –80°C until further analysis.

HLA-DRB allele analysis

HLA alleles were measured using the commercial HLA-DRB1* cyclerplate reagent kit (Cat. No. 200 030; Protrans, Hockenheim, Germany) for polymerase chain reaction (PCR) with sequence-specific primers, in accordance with the manufacturer's instructions. In brief, DNA samples (2.025–3.375 ng) were added to each well of the plate, and genotyping was performed for 23 genetic loci: DRB1 (i.e., *DR1*, *DR103*, *DR15*, *DR16*,

DR17, *DR18+*, *DR4*, *DR7*, *DR8*, *DR9*, *DR10*, *DR11*, *DR12*, *DR13.1*, *DR13.3*, *DR13.4*, *DR14.1*, *DR14.2*, *DR14.3*, and *DR14.4*), DRB3, DRB4, and DRB5. PCR reaction mixtures were prepared containing Pre Master Mix (70 μL Buffer R, 140 μL Buffer Y, and 1.6 μL of 5 U/μL Taq polymerase) and 50 µL DNA template. PCR amplifications were performed on the G-Storm PCR instrument (Agilegene Technologies, Ltd., Somerset, UK) under the following conditions: denaturation for 1 minute at 96°C; five amplification cycles at 96°C for 25 seconds, 70°C for 50 seconds, and 72°C for 45 seconds; 21 amplification cycles of 96°C for 25 seconds, 65°C for 50 seconds, and 72°C for 45 seconds; four amplification cycles of 96°C for 25 seconds and 55°C for 60 seconds; and final extension at 72°C for 2 minutes. PCR products were subjected to 1.5% agarose gel electrophoresis (GELDOC XR170-8170, Bio-Rad) followed by ethidium bromide staining. The results were visualized using the GelDoc XR system (BioRad). The frequencies of *HLA* alleles were calculated in patients with caries and healthy controls using the direct calculation method: (individual number of a given allele/total sample number)×100%.

Statistical analysis

Statistical analysis was conducted using the IBM SPSS Statistics for Windows, version 19.0 (IBM Corp., Armonk, NY, USA). The chi-squared test or Fisher's exact test, followed by Bonferroni correction, was used to calculate differences in allele frequencies between groups. Differences with $P<0.05$ were considered to be statistically significant.

Results

This study included 42 patients with caries and 123 healthy controls. Genotyping was performed for 23 genetic loci of *DRB1*, *DRB3*, *DRB4*, and *DRB5*. Thirteen *DRB1* alleles were assessed for single nucleotide polymorphisms: *DRB1*010x*, *DRB1*15*, *DRB1*160x*, *DRB1*03*, *DRB1*04*, *DRB1*070x*, *DRB1*08*, *DRB1*0901/02*, *DRB1*1001*, *DRB1*11*, *DRB1*120x*, and *DRB1*13*. The DRB1 serotypes assessed were as follows: DRB1-DR1, DR103, DR15, DR16, DR17, DR18+, DR4, DR7, DR8, DR9, DR10, DR11, DR12, DR13.1, DR13.3, DR13.4, DR14.1, DR14.2, DR14.3, and DR14.4.

The allele frequencies of *HLA-DRB1** for patients with caries and healthy controls are shown in Table 1 and Figure 1. The allele frequency of *HLA-DRB1**13 was significantly higher in patients with caries than in healthy controls ($P=0.024$), while the allele frequency of *HLA-DRB1**09 was significantly lower in patients with caries than in healthy controls ($P=0.004$). Among the healthy controls, the most frequent *HLA-DRB1* alleles were *DRB1*15*, *DRB1*09*, *DRB1*12*, *DRB1*04*, *DRB1*13*, *DRB1*07*, *DRB1*08*, *DRB1*14*, *DRB1*01*, *DRB1*11*, *DRB1*03*, *DRB1*10*, and *DRB1*16*.

Discussion

Caries prevalence varies among populations and the potential mechanisms remain unclear. Further studies are needed to identify the underlying etiological factors. The familial aggregation of caries indicates a degree of genetic susceptibility.[17] In this study, we focused on caries-related susceptibility genes among Han Chinese children to improve our understanding of the pathophysiological processes of caries formation, which may aid in early diagnosis, prevention, and treatment of caries.

Genetic contributions to the development of caries have been recognized since the 1920s. A comparison of dental

Table 1. *HLA-DRB1* allele frequencies in healthy controls and in patients with caries.

HLA-DRB1 Allele	Healthy controls (N = 123)		Patients with caries (N = 42)		
	n	Frequency (%)	n	Frequency (%)	P
*DRB1*01*	14	11.38	3	7.14	0.564
*DRB1*03*	11	8.94	7	16.67	0.249
*DRB1*04*	28	22.76	14	33.33	0.175
*DRB1*07*	17	13.82	5	11.90	0.752
*DRB1*08*	15	12.20	3	7.14	0.567
DRB1*09	31	25.20	2	4.76	0.004
*DRB1*10*	7	5.69	3	7.14	0.716
*DRB1*11*	12	9.76	8	19.05	0.111
*DRB1*12*	28	22.76	8	19.05	0.615
DRB1*13	23	18.70	15	35.71	0.024
*DRB1*14*	15	12.20	7	16.67	0.462
*DRB1*15*	38	30.89	13	30.95	0.994
*DRB1*16*	6	4.88	6	14.29	0.077

N: total number of samples; n: number of positive samples.
Alleles in bold font indicate significantly different allele frequencies between groups ($P < 0.05$).

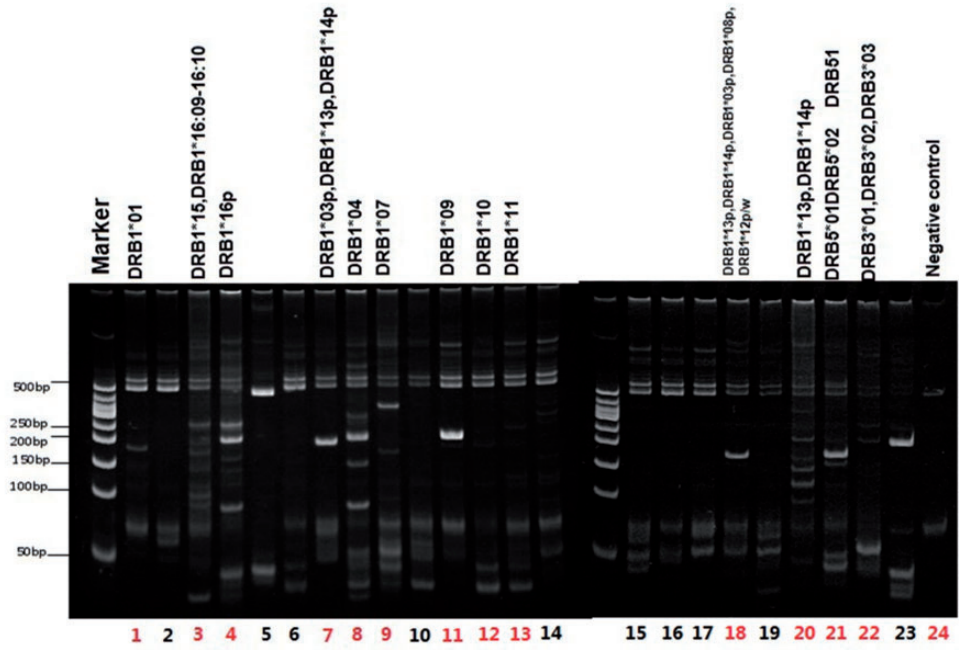

Figure 1. *HLA-DRB** allele frequencies in patients with caries and healthy controls, as demonstrated by electrophoresis of PCR products. Positive bright bands are present in the following lanes (numbers shown in red), indicated with the corresponding HLA allele: 1 (*DRB1*01*), 3 (*DRB1*15* and *DRB1*16*), 4 (*DRB1*16*), 7 (*DRB1*03*, *DRB1*13*, and *DRB1*14*), 8 (*DRB1*04*), 9 (*DRB1*03*), 11 (*DRB1*09*), 12 (*DRB1*10*), 13 (*DRB1*11*), 18 (*DRB1*13*, *DRB1*14*, *DRB1*08*, and *DRB1*12*), 20 (*DRB1*13* and *DRB1*14*), 21 (*DRB5**), and 22 (*DRB3**). Lane 24 contains the blank control.

characteristics in pairs of identical and fraternal twins revealed that the prevalence of similar characteristics was greater in monozygotic twins than in dizygotic twins, and that differences in characteristics were greater in dizygotic twins of different sexes.[18] In a previous study, Gustafsson et al.[19] reported that some participants were able to maintain a caries-free oral cavity despite the ingestion of a large amount of highly cariogenic foods. Thus, an individual's susceptibility or resistance to caries is affected by their genotype.

Ozawa et al.[20] investigated the genetic predisposition of individuals to the accumulation of oral microorganisms, together with the associations of *HLA-DQA1*, *HLA-DQB1*, and *HLA-DRB1* alleles with the numbers of salivary *Streptococcus mutans* and *Lactobacillus* bacteria in young Japanese adults. They found that *HLA-DQA1*0102*, *HLA-DQB1*0604*, *HLA-DRB1*0802*, and *HLA-DRB1*1302* were weakly associated with the numbers of *Lactobacillus* bacteria in saliva samples, while *HLA-DQB1*0601* was associated with the numbers of *S. mutans* bacteria in saliva samples. Moreover, Bagherian et al.[21] found that the allele frequency of *HLA-DRB1*04* was 10-fold greater in children with caries than in caries-free children, which suggested that the *HLA-DRB1*04* allele might confer susceptibility to caries. Valarini et al.[22] performed a comparative analysis of 15- to 19-year-old students with or without caries; their findings confirmed a close relationship between *HLA* genes and caries. In particular, the *HLA-DQ* gene was more frequently found in students with caries. Similarly, McCarlie et al.[23] reported that patients without the *HLA-DRB1*04* allele exhibited reduced reactivity to *S. mutans* antigens, along with reduced secretory immunoglobulin A and total immunoglobulin A activities.

The primary loci in residents of northern China were *DRB1*15, DRB1*09*, *DRB1*04*, *DRB1*12*, and *DRB1*07*; in contrast, the primary loci in residents of southern China were *DRB1*09*, *DRB1*15*, *DRB1*12*, *DRB1*04*, and *DRB1*08*.[24] The most frequent HLA-DRB1 alleles of the healthy controls in our study represented a combination of the major alleles of *DRB1* observed in Han individuals from the northern and southern regions of China. This genetic trend reflects the complex north-south migration of the Han Chinese population in the Xinjiang Uygur Autonomous Region and the influences of specific environmental features in this region. The relatively high incidence of caries in the Han Chinese population in the Xinjiang Uygur Autonomous Region presumably results from the combination of various environmental factors under this specific genetic background, and children at higher risk for caries appeared to have distinct genotypes. Specifically, higher frequencies of alleles conferring greater susceptibility to infectious factors can cause altered immune responses to infectious factors, thereby affecting caries susceptibility. Our results suggested that the risk of caries may be increased in children and adolescents carrying the *DRB1*13* allele. Conversely, the *DRB1*09* allele was expressed at a relatively lower frequency in patients with caries than in healthy controls, which indicated that this allele may be protective against caries. Wallengren et al.[24] and Yildiz et al.[25] independently reported an association between the *DRB1*04* allele and caries development. In the present study, the allele frequency of *DRB1*04* was higher in patients with caries than in healthy controls; however, the difference was not statistically significant. Our results differed from those of the studies by Valarini et al. and McCarlie et al.,[22,23] possibly because of the following factors: (i) There are nationality, regional, and ethnic influences that affect the relationship between dental caries and HLA

alleles. (ii) Variations in sample size may impact the study results. (iii) There might be differences in the *HLA-DRB1* alleles between adolescents and adults at high risk of caries, which lead to differences in dominant cariogenic bacteria in saliva.

There were some limitations in this study: our sample size was small and we could not identify the specific gene or allele responsible for caries susceptibility in the Han Chinese population in the Xinjiang Uygur Autonomous Region. Our future studies will address these aspects.

In conclusion, we highlighted a potential association between *HLA* alleles and caries susceptibility. Importantly, we found allele associations in the Han Chinese population in the Xinjiang Uygur Autonomous Region that differed from associations found in Han Chinese populations in other parts of China. This finding suggests that the pathogenesis of caries is not induced in a simple manner by one or more genetic factors, and that it may involve the interactions of multiple genes within a specific environmental context.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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