



## Rapid Communication

# Mannose-binding lectin gene polymorphisms are not associated with susceptibility to severe early childhood caries

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## ABSTRACT

Our purpose was to investigate a possible relationship between severe early childhood caries (S-ECC) and polymorphism of the mannose binding lectin gene and investigate the role of allele variant as a possible factor in the susceptibility to S-ECC. Sixty-two Chinese children with S-ECC and 68 caries-free control children were included in this study. Genomic DNA was extracted from buccal epithelial cells of each individual. The identification of MBL B allele was performed by restriction fragment length polymorphism using Ban I restriction enzyme. The frequency of MBL mutant genotype (GGC/GAC and GAC/GAC) was more frequent among children with S-ECC compared with control groups, but did not significantly differ between two groups ( $\chi^2 = 2.82, p > 0.05$ ). There was no significant difference in the allele frequency of codon54 wild type (allele A) between two groups ( $\chi^2 = 2.76, p > 0.05$ ). The present study did not find evidence of MBL codon54 polymorphisms being associated with S-ECC in the population studied, but a larger sample size is necessary to confirm the present results.

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## 1. Introduction

Early childhood caries (ECC) is a particular type of dental caries that can destroy the primary dentition of infants and pre-school children. More specifically, severe-ECC (S-ECC) is to designate caries considered progressive, acute or rampant. S-ECC has been described as nursing caries or baby bottle tooth decay. Demineralization starts in the maxillary incisors followed by the maxillary and mandibular first molars. S-ECC is a serious public health problem in very young children, it may lead to pain, compromised chewing ability, malocclusion in permanent dentition, speech problems, suboptimal health, iron deficiency and lower self-esteem [1,2]. Children who have caries in their primary teeth are more likely to develop dental caries in their permanent dentition [3]. Rapid caries progression usually causes complications such as pulpitis and apical periodontitis. It has been shown that chronically dental infection may cause systemic infections and affect immunity [4].

Although the etiology of caries is complex and multifactorial, with contributions from microbial ecology, tooth morphology, dietary habit, oral hygiene, the role of genetics in caries etiology has been verified. Genetic research applied to dental decay began in the 1930s with observational research involving familial aggregation analysis and twin studies. The most convincing data on the

role of genetics in the pathogenesis of dental caries has been developed by analyzing the caries incidence in twins [5,6]. Recently, evaluation of genetic polymorphisms also provides answers as to specific contributions of genetics in the etiology of dental caries. Other genetics approaches as genome-wide association studies on large populations also implicate genetic influence in caries [7].

Mannose-binding lectin (MBL) is a C-type serum lectin, it has been shown to bind to a wide range of microorganisms and also mediates lectin-dependent activation of the complement pathway [8]. There are two human MBL genes (*MBL-1* and *MBL-2*) which are closely positioned on the long arm of chromosome 10 at q11.2–21 [9]. *MBL-1* is a pseudo gene and only *MBL-2* encodes a protein product. Three polymorphic sites have been identified in exon1 of the *MBL-2* gene, at codon52 (CGT to TGT), codon54 (GGC to GAC) and codon57 (GGA to GAA) [8]. These mutations are frequently referred to as variants D, B and C, respectively, with A indicating wild-type. Particularly, codon54 mutation of the *MBL-2* gene is associated with recurrent infections and autoimmune diseases [10,11].

Up to the present, there are few studies investigating the relationship between *MBL-2* gene polymorphisms and dental caries [12]. There is no study investigating the relationships of *MBL* and S-ECC. However, while the general etiology of S-ECC appears similar to that of other types of caries, to characterize the genetic contribution to etiology and susceptibility of S-ECC, we analyzed the polymorphisms in exon1 codon54 of *MBL-2* gene in children with S-ECC. The single nucleotide polymorphisms (SNPs) detected in

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patients was also evaluated in controls to compare genotypes and allele frequencies between the two groups.

## 2. Material and methods

### 2.1. Study participants

Subjects were recruited from the Department of Pediatric Dentistry at Peking University School and Hospital of Stomatology during September 2010 to August 2011. Eligible Chinese children from 1 to 5 years of age were included in this study. All individuals received an oral and dental examination performed by a single experienced examiner. Examination for dental caries was carried out using conventional dental chairs, artificial light, flat mirror and explorer. Caries experience was scored by the number of decayed, missing or filling tooth (dmft) according to WHO criteria [13].

The disease of ECC has been defined as the presence of 1 or more decayed, missing (due to caries), or filled tooth surface in any primary tooth in a child 71 months of age or younger. In children younger than 3 years of age, any sign of smooth-surface caries is indicative of S-ECC. From ages 3–5, one or more cavitated, missing, or filled smooth surfaces in primary maxillary anterior teeth or dmft  $\geq 4$  (age 3),  $\geq 5$  (age 4), or  $\geq 6$  (age 5) surfaces constitutes S-ECC [14]. According to the dmft, the study subjects were divided into two groups: cases and controls. Cases were defined as children diagnosed with S-ECC, while controls were defined as children with no evidence of caries (including white-spot lesions) and no history of caries.

This study was approved by the Ethics Committee of Peking University Health Science Center and carried out according to the guidelines of the World Medical Association Declaration of Helsinki. Informed consent was obtained from all individuals and their parents.

### 2.2. SNP analysis of MBL-2 gene codon 54

Genomic DNA was extracted from buccal epithelial cells. To obtain buccal epithelial cells, buccal swabs were placed in the patients' mouth and rotated against the inside of the cheek for approximately 20 rounds. Then, the swabs were dried at room temperature overnight, placed in the swab holder, and stored at  $-20^{\circ}\text{C}$ . Genomic DNA was extracted from buccal swabs of each individual with the use of a TIANamp Swab DNA mini kit (Tiangen, Beijing, China) according to the manufacturer's instructions.

Exon 1 of MBL-2 was identified by polymerase chain reaction (PCR) using the primers as described previously [15]. The sequences of the primers were: 5'-AGGGCATGCTCGTAAAT-3' (forward primer) and 5'-CTCATATCCCCAGGCAGTT-3' (reverse primer). Sample DNA was amplified in a 25  $\mu\text{l}$  volume containing 50 ng DNA, 0.5  $\mu\text{M}$  of each primer, GoTaq DNA Polymerase (Promega, Madison, WI, USA). PCR conditions were an initial denature at  $95^{\circ}\text{C}$  for 5 min, 35 cycles of denaturation at  $95^{\circ}\text{C}$  for 45 s, annealing at  $53.7^{\circ}\text{C}$  for 45 sec, extension at  $72^{\circ}\text{C}$  for 1 min, and a final elongation at  $72^{\circ}\text{C}$  for 5 min.

Genotyping of MBL-2 exon 1 was carried out by restriction digestion of the PCR products with restriction enzyme *Ban* I (NEB, Beverly, MA, USA) to identify codon 54 variants. According to the manufacturer's instruction, the digestion reaction was performed in a 20  $\mu\text{l}$  volume at  $37^{\circ}\text{C}$  for 2 h. After enzyme digestion, the genotypes were determined by electrophoresis on 3% agarose gel.

### 2.3. SNP analysis in control group

To compare genotypes and allele frequencies of MBL-2 between patients and controls, 68 control individuals were evaluated. Col-

lection of buccal epithelial cells, extraction of genomic DNA, performance of PCR and genotyping of MBL-2 exon 1 were done as described earlier.

### 2.4. Statistical analysis

Mean age ( $\pm\text{SD}$ ) was calculated for the two groups. The statistical significance of the difference between the means was tested by unpaired Student's *t*-test. The difference of gender between two groups was compared with chi-square test.

Genotypes and allele frequencies in patients and controls were compared with chi-square tests. The results were considered statistically significant when *p*-values  $<0.05$ .

## 3. Results

Totally 140 children were included in this study, 70 children in each group respectively, but failed to extract genomic DNA from 10 buccal swabs samples. Finally, 62 children (33 boys and 29 girls) were designated as S-ECC, and 68 children (32 boys and 36 girls) as controls. The differences of gender and mean ages between two groups were not significant ( $p > 0.05$ ) (Table 1). Ninety-five percent of children with S-ECC had decayed or filled teeth more than six, and the mean dmft was 12.29.

The distribution of MBL-2 genotypes among the study groups is presented in Fig. 1. The electrophoresis showed that the wild type GGC/GGC was cleaved into two bands (232 and 93 bp), while the homozygous mutant type GAC/GAC showed one band (325 bp), and heterozygous mutant GGC/GAC showed three bands (325, 232 and 93 bp).

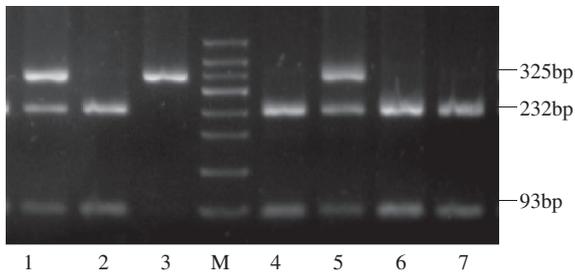
The comparison of the MBL-2 codon54 genotype and allele frequencies between two groups is given in Table 2. In 62 S-ECC children, the frequency of MBL-2 mutant genotype GAC was increased compared with caries-free groups. However, the difference of the frequency of wild genotype GGC/GGC compared with mutant genotype (GGC/GAC and GAC/GAC) between the two groups was not significant ( $\chi^2 = 2.82$ ,  $p > 0.05$ ). The frequency of codon54 (allele B) mutation was found 21% in S-ECC group and 13.2% in caries-free group. There was no significant difference in the allele frequency of codon54 wild type (allele A) between two groups ( $\chi^2 = 2.76$ ,  $p > 0.05$ ). The results showed no association between the dental caries susceptibility and SNPs of MBL-2 gene in Chinese population.

## 4. Discussion

ECC is one of the most common chronic childhood diseases. Like many medical and dental diseases, it depends on a complex interaction between the genetic structure of an individual and the superimposed environmental factors. To date, only a few specific genes have been associated with caries risk. Genes involved in enamel formation, including *amelogenin* (AMELX), *ameloblastin* (AMBN), and *tuftelin* (TUFT1), have been associated with susceptibility or resistance to dental caries [16–18]. Human leukocyte anti-

**Table 1**  
Demographic characteristics of the sample.

Characteristics	S-ECC	Controls
Number	62	68
Boys	33	32
Girls	29	36
	$p > 0.05$	
Age (months, mean $\pm$ SD)	42.61 $\pm$ 10.19	42.99 $\pm$ 10.04
	$p > 0.05$	



**Fig. 1.** Agarose gel electrophoresis results of PCR-RFLP analysis of *MBL-2* codon 54. M: molecular size marker; lane 1, 5: GGC/GAC heterozygote; lane 2, 4, 6, 7: GGC/GGC homozygous; lane 3: GAC/GAC homozygous.

**Table 2**

Genotype and allele frequencies of *MBL* codon54 in patients with S-ECC and caries-free controls.

<i>MBL</i> codon54	Cases		Controls		Chi-square test, $\chi^2$	p value
	n	%	n	%		
<i>Genotype frequency</i>						
GGC/GGC	38	61.3	51	75.0	2.82	0.093
GGC/GAC	22	35.5	16	23.1		
GAC/GAC	2	3.2	1	1.5		
Total	62	100	68	100		
<i>Allele frequency</i>						
A	98	79.0	118	86.8	2.76	0.097
B	26	21.0	18	13.2		
Total	124	100	136	100		

gen allele (*HLA-DRB1*) was found significant association with the susceptibility to ECC [19]. Polymorphisms in the *MBL-2* gene were analyzed in Turkish children, but did not find significant differences between caries-free and children with carious teeth [12].

*MBL-2* gene contains four exons. Exon 1 encodes the signal peptide, a cysteine-rich region and part of the glycine-rich collagenous region. Exon 2 encodes the remainder of the collagenous region, Exon 3 encodes a helical coil structure, and Exon 4 encodes the carbohydrate-recognition domain [20]. MBL is an important constituent of the innate immune system and one of the proteins of the complement system [21]. MBL plays a critical role in the immune response in early childhood before specific immune protection develops [22]. There are increasing evidences that MBL has a complex role in many diseases. Several studies have related *MBL-2* gene codon54 mutation to recurrent infections, progression of chronic viral diseases, acquired immunodeficiency syndrome and autoimmune diseases [10,11,23].

S-ECC is rampant caries affecting the primary dentition of young children. Might there be a link between mannose-binding lectin polymorphism and S-ECC? Our hypothesis was that: firstly, MBL has been shown to bind to a wide range of micro-organisms. It primarily recognizes and binds to specific sugar groups that are displayed on the surface of microorganisms. It has been reported that *Staphylococcus aureus* and beta-hemolytic group A streptococci have strong MBL-binding capacity [24]. Recent studies showed that *MBL-2* gene mutation at codon 54 was related to Behcet's disease, and *MBL* gene mutation may lead to susceptibility to streptococcal bacterial infections such as tonsillitis and dental caries in patients with Behcet's disease [23]. Like other types of Caries, ECC is a bacterial infectious disease, the key bacteria associated with ECC is recognized as *mutans streptococci* (MS). Mutations of MBL may result poor binding to the antigenic peptides of the MS, and the variations in immune responses against microorganisms may influence children's susceptibility to ECC. Secondly, MBL play an important role in humans between 6 and 18 months of age when the adaptive system is still immature. In children with

ECC, MS can be found as early as 12 months of age. At this age children did not have so much contact with environmental factors related with the development of dental decay. This may cause a better possibility to detect the genetic factors related with dental decay. MBL protein may play a role in the early onset of ECC.

In this preliminary study, we investigated the relationship between *MBL-2* gene polymorphism and children with severe early onset caries. The frequencies of three polymorphic sites in the *MBL-2* gene vary between different ethnic groups. Mutant alleles at codon54 are more common in Caucasian populations, Eskimos and Chinese with gene frequencies ranging from 0.11 to 0.17. The mutant allele 57 is found only in populations of African origin. The mutation in codon52 is the least common structural mutation and is present in white and Caucasian populations [25]. In our study, *MBL-2* gene codon 57 and codon 52 rare alleles were not detected, these genotypes seem to be absent or extremely rare in Chinese population. In this respect, the data of this study were analyzed only for the *MBL-2* codon 54 genotype distributions and rate of allele carriage of the study groups.

In this study, the frequency of *MBL-2* A/B genotype was higher among children with S-ECC compared with caries-free children, but the difference was found statistically insignificant. However, ECC is a complex and multifactorial disease, functions of other genes and environmental factors could not be excluded in our patients. To the best of our knowledge, this is the first reported study on the possible association between *MBL* gene polymorphism and S-ECC. However, our study has some limitations. We did not consider other environmental factors. Moreover, numbers of experiments and controls were not perfectly matched. Considering the relative low number of subjects of the present study, further research is needed to investigate the etiology of S-ECC.

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