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SHORT COMMUNICATION

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

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Abstract

Objective. Caries is a common disease in humans and has a multifactorial etiology. It has been suggested that children born with cleft lip and/or palate (CL/P) have a higher susceptibility to caries, but data from several independent cohorts does not support this assumption. Previous work from our group suggested *DEFB 1* is associated with higher caries experience. Since it is suspected that children born with CL/P have the same risk factors predisposing them to caries as other children of the same ages, the aim was to test if *DEFB 1* was associated with caries experience in children born with CL/P. ***Materials and methods.*** Sixty-nine children born with CL/P (aged 2–12 years) were included. Twenty-seven males and seven females had cleft lip and palate (CLP), six males and seven females had cleft lip (CL) and 13 males and nine females had cleft palate (CP). Caries was evaluated with the DMFT/dmft index by a calibrated evaluator. Two single nucleotide polymorphisms in *DEFB 1* were selected (rs11362 and rs1800972) based on being associated with higher caries experience in previous work. Genotyping were carried out by real-time PCR using the Taqman assay method. The statistical analysis was performed between 'low-to-moderate caries experience group' and the 'high caries experience group'. Odds ratio calculations between caries experience and variant alleles and chi-square of Fisher exact tests at a level of significance of 0.05 were used. ***Results.*** There was no significant difference for caries experience between cleft types ($p = 0.551$). An association was found for the marker rs11362 and genotype distribution ($p = 0.047$). When analyzed in a recessive model, the genotype GG in this polymorphism increased the risk for caries susceptibility by more than 3-times ($p = 0.031$; OR = 3.16; 95% CI = 0.97–10.62). ***Conclusion.*** The genetic variant rs11362 in *DEFB 1* influences caries susceptibility in CL/P children. The results support the hypothesis that expression of *DEFB 1* in saliva may serve as a biomarker for future caries risk.

Key Words: *Beta defensin, dental caries, cleft lip and palate*

Introduction

Caries is a common chronic, widespread, complex and multifactorial non-contagious infectious disease in children and is a serious oral health problem [1–4]. Although caries is a largely preventable disease, it affects a large proportion of the world's population and is more prevalent in certain population groups [5,6]. Some studies attempted to evaluate caries incidence in groups of children born with cleft lip and/or palate (CL/P) [7–14]. While many studies suggest that they have a higher susceptibility for caries than children without this condition, a systematic review evaluating case-control studies with CL/P children was unable to find conclusive evidence that these children exhibit more caries [15].

Numerous contributing factors are known to be involved with caries, such as quality of dental hygiene, microflora, fluoride exposure and genetic components [13,14,16–20]. Moreover we evaluated two

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Table I. Definition of caries experience based on age and DMFT/dmft (Decayed, Missing due to caries, Filled Teeth) scores.

	Sub-groups						
		Sex					
Caries experience level	Number of individuals	Males	Females	DMFT/dmft, Mean (SD)	DMFT/dmft, Average	DMFS/dmfs, Mean (SD)	DMFS/dmfs, Average
Toddlers (2–3 years of age)	37	28	9	2.97 (3.88)	0/15	5.68 (11.99)	0/65
Low and moderate: dmft = 0–1	17	11	6				
High caries: dmft = 2 or higher	20	17	3				
Children (6–12 years of age)	32	18	14	7.25 (3.2)	0/14	13.12 (9.01)	0/35
Low and moderate: DMFT/dmft = 0–7	18	13	5				
High caries: DMFT/dmft = 8 or higher	14	5	9				

promoter polymorphisms within the gene *DEFB 1* and they demonstrated association with caries susceptibility [18]. This gene is expressed in the oral cavity and is related with resistance to microbial colonization, thereby playing a role in the host defense against infection by oral bacteria [21–23].

Since polymorphisms in the promoter region of *DEFB 1* demonstrated to be potentially involved in caries susceptibility, we aimed to test if *DEFB 1* was associated with caries experience in children born with CL/P.

Materials and methods

Subjects

We used the case-only design to test if genetic variation in *DEFB 1* is associated with caries experience in children born with CL/P. The study sample consisted of 69 children aged 2–12 years (average age = 5.1 years). Of all 69 children born with CL/P included in this study, 46 were males and 23 were females. Twenty-seven males and seven females had cleft lip and palate (CLP), six males and seven females had cleft lip (CL) and 13 males and nine females had cleft palate (CP). All subjects had surgery to correct the cleft defect. Subjects were recruited at the Riga Cleft Lip and Palate Centre of the Institute of Stomatology, Riga Stradins University, the only referral unit for cleft children in Latvia. Ethical approval for the study was obtained from the Central Medical Ethics Committee of Latvia. Appropriate informed consent was signed by all participants or their parents.

Evaluation of caries

All children were examined in a dental chair under standard examination conditions, using a dental mirror and a dull probe. Only one calibrated examiner (K.K.) collected caries experience data. Caries was diagnosed using WHO recommendation for oral health surveys [24]. The DMFT/dmft and DMFS/dmfs scores for each individual were calculated with teeth lost to trauma or primary tooth lost to exfoliation excluded for the calculation. Lesions were recorded as present when a carious cavity was apparent on visual inspection.

The caries experience was categorized according to the DMFT/dmft scores in the studied population. Children were from two distinct age groups: 2–3 year olds and 6–12 year olds. Caries experience distribution was evaluated based on these two age groups. Children were divided into lower-to-moderate caries experience and higher caries experience and the cut-off was based on the mean DMFT/dmft in each age group. Children of 2–3 years of age with dmft scores 0, 1 and 2 were considered as having low-to-moderate caries experience. Children with dmft scores of 3 and higher were considered as having more severe caries experience. Similarly, children of 6–12 years of age with DMFT scores of 7 or lower were considered as having low-to-moderate caries experience. Children with dmft scores of 8 and higher were consideredas having more severe caries experience. The definitions of caries experience are presented in Table I and Figure 1.

DNA samples and genotyping

The genomic DNA for the molecular analysis was extracted from venous blood [25]. Based on the results of our recently published study about genetic susceptibility to caries [18], we selected two single nucleotide polymorphisms (SNPs) of beta-defensin 1 (*DEFB 1*): rs11362(G-20-A) and rs1800972 (C-44G).

Genotyping was carried out by real-time PCR using the Taqman chemistry method [26], and performed on an Applied Biosystems 7900 HT Sequence Detection System machine (Applied Biosystems Inc., Foster City, CA) in the University of Pittsburgh School of Dental Medicine by a single operator. Each sample was tested once blindly, but a second attempt was made in the case of samples that PCR initially failed.

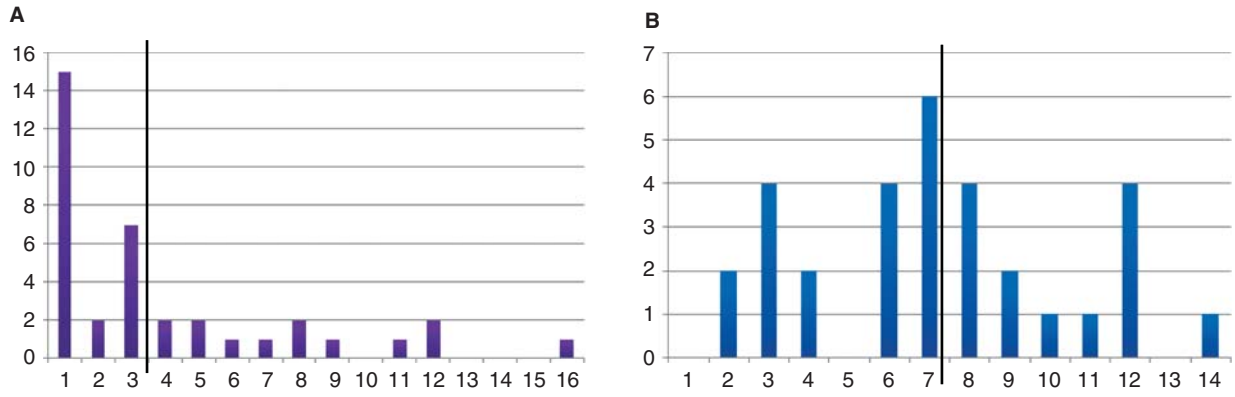

Figure 1. Distribution of DMFT/dmft scores of 2–3 years old (A) and 6–12 years old children (B). The *x*-axis indicates the number of subjects and the *y*-axis the distribution of DMFT/dmft scores. The bar shows the DMFT/dmft mean, which was used as the parameter for defining children with low-to-moderate caries experience (DMFT/dmft lower than the mean) vs children with high caries experience (DMFT/dmft higher than the mean).

Statistical analysis

Statistical analyses were conducted using the PLINK 1.05 (http://pngu.mgh.harvard.edu/~purcell/plink/) and Epi Info3.5.3 statistical software package (http://www.cdc.gov/epiinfo/). The statistical analysis was performed between a combination of the low and moderate caries experience group and the high caries group, combining children of both sexes and age groups. Caries experience (low and moderate vs high) was defined as described above based on distribution of the dmft/DMFT scores of the data. Odds ratio calculations and chi-square of Fisher exact tests at a level of significance of 0.05 were used to determine if caries experience was associated with any allele or genotype. The standard chi-square test was used to test for deviations from Hardy-Weinberg equilibrium [27]. ANOVA test was used for DMFT/dmft mean evaluation between cleft types.

Results

A statistically significant difference between sexes and caries experience was not found. The mean dmft (SD) was 3.2 (3.9) for male toddlers (2–3 year olds) and 2.2 (3.8) for female toddlers (p = 0.51 for caries distribution by sex); the mean DMFT/dmft was 6.55 (3.1) for male children (6–12 year olds) and 8.1 (3.3) for female children (p = 0.17 for caries distribution by sex). There was also no significant difference in the caries experience between cleft types. The mean DMFT/dmft for CLP was 4.6 (3.9), for CL was 5.1 (4.1) and for CP was 5.4 (4.7) (p = 0.785). The mean DMFs/dmfs for CLP was 8.1 (8.7), for CL was 8.2 (9.5) and for CP was 11.3 (15.3) (p = 0.55) for caries distribution by cleft type.

Both analyzed SNPs were in Hardy-Weinberg equilibrium (data not shown). Table II shows the distribution of the genotypes of the SNPs between the groups. There were no significant differences between allele distribution and caries experience. A statistically significant difference was found for the genotype distribution of marker rs11362 considering caries experience (p = 0.047). When we analyzed the data in a recessive model (CC vs CT+TT), the genotype CC increased more than 3-times the odds for a higher caries experience (p = 0.031; OR = 3.16; 95% CI = 0.97–10.62).

Discussion

One of the challenges of studying a complex, multifactorial disease, such as caries, is the fact that many genetic and environmental factors contribute to the susceptibility of the disease. Our previous study of *DEFB 1* in caries susceptibility highlighted the importance of the genes involved in host-microbial interaction and their contribution to caries experience [18]. Indeed, the results reported here reinforce this view: variation in the promoter region of *DEFB 1* potentially plays a role in the etiology of caries.

DEFB 1 is an oral anti-microbial peptide, which provides the first line of defense against a wide spectrum of pathogens [23,28–31]. The great variation of defensin concentrations in saliva and in oral tissues could be attributed to the genetic variations in the host [29,30]. Previous studies demonstrated that polymorphisms in the promoter region of *DEFB 1* alter transcriptional activity compared with that region in the wild type [32,33]. This variation can be related with differences in caries experience between subjects. We previously showed that carrying a copy of the variant allele of the *DEFB 1* marker rs11362 (G-20A) increased the DMFT and DMFS scores more than 5-fold. A high-caries-experience haplotype (GCA), which increased DMFT scores 2-fold, and a low-caries-experience haplotype (ACG), which decreased DMFT scores 2-fold, exist in the *DEFB 1* promoter [18]. It is remarkable that this study

Table II. Distribution of *DEFB 1* markers based on caries experience.

Caries experience groups	Genotypes, *n* (%)			*p*	Allele, *n* (%)		*p*	OR (95% CI)
DEFB 1 rs11362	CC	CT	TT		C	T	0.224	1.52 (0.77–2.98)
Low and moderate	7 (20.0)	21 (60.0)	7 (20.0)	0.047*	35 (50.0)	35 (50.0)		
High	15 (44.1)	11 (32.4)	8 (23.5)		41 (60.3)	27 (39.7)		
DEFB 1 rs1800972	CC	CG	GG	0.703	C	G	0.910	1.05 (0.44–2.51)
Low and moderate	1 (2.9)	14 (40.0)	20 (57.1)		16 (22.9)	54 (77.1)		
High	2 (5.9)	11 (32.4)	21 (61.8)		15 (22.1)	53 (77.9)		

*Statistically significant result.

with a relatively small sample suggests a similar effect of *DEFB 1* in caries than our previous publication. If this was the first study, one could easily argue this study was under-powered and the results may be due just to chance but, as a second study, the results may indicate the effect of *DEFB 1* promoter variants in caries not only exist, but their effect sizes might be important and these variants may serve as biomarkers for the disease risk.

Environmental factors, such as dietary factors, adequate education on tooth brushing technique specific to CL/P toddlers and children, the use of intraoral appliance and the high prevalence of numerous dental anomalies (e.g. tooth agenesis, supernumerary teeth, misalignment of teeth and enamel hypoplasia) can contribute to an increased caries susceptibility in children born with CL/P [34]. Our previous studies that analyzed four geographically independent sites, Philippines, Guatemala, Argentina [3], and Brazil [13], did not support the hypothesis that subjects born with CL/P have higher caries experience than the general population.

Since this study was performed in a group of children born with clefts we compared their caries experience with previously reported caries experience in Latvian children [35,36]. These analyses allowed us to observe that the dmft/DMFT means, as well as the prevalence of caries-free children in the group of children born with clefts we studied, were similar to those reported in the general population for many ages (Table III).

Previous epidemiological studies showed high caries experience in Latvian children [35–37]. Although the prevalence of caries-free children between 2–6 years of age in the group we studied was similar to the general population, the dmft mean was slightly higher in the children born with CL/P we studied. However, it is important to emphasize that, while the comparison group is from kindergartens in Riga, the capital of Latvia, our sample is from the only referral unit for cleft children in Latvia, and represents the whole country. In the study performed by Berzina and Care [36] for 11 and 13 years old children the mean values for DMFT and DMFS were significantly lower in Riga than in the rest of Latvia.

We attempted to analyze caries experience based on the distribution of the scores in the sample. Our approach allowed us to identify that carriers of the C allele of rs11362 have higher caries experience. It

Table III. Caries experience in our CL/P population and in the general population reported by two studies performed in Latvia.

Age	CL/P children	General population	Reference	Study design
Relative frequency of caries-free children				
2 years	50.0%	20%	[36]	Kindergartens were randomly selected and 638 children, aged from 2–6-years-old, attending kindergarten in Riga, were examined for caries experience.
3 years	34.8%	36%		
DMFT means (SD)				
2 years	1.1 (1.3)	0.7 (1.7)		
3 years	4.0 (4.4)	1.6 (2.7)		
6 years	6.8 (3.6)	3.6 (3.4)		
Relative frequency of caries-free children			[37]	Caries experience was assessed in a representative national sample of Latvians consisting of 705 schoolchildren, aged 11 and 13 years, that were randomly included in the study. This study was a part of a National Oral Health survey.
11–13 years	0%	8.8%		
DMFT means (SD)				
11 and 12 years	4.7 (2.3)	—		
11 years	—	3.9 (3.0)		
13 years	—	6.1 (4.0)		

would have been interesting to study caries-free individuals in comparison with individuals with very high caries experience, but our sample size did not allow for this approach. Aside from the possible limitation of our small sample size, our study cannot provide direct evidence for how *DEFB 1* may influence caries. DEFB 1 could influence the disease by modifying the composition of biofilm, but without plaque samples these analyses are not possible. We were also not able to test if environmental factors potentially modify the genetic influence and future studies will focus on gene–gene and gene–environmental interactions.

Briefly, our results demonstrated that the polymorphism G-20-A (C-T if one looks at the other DNA strain) in the promoter region of *DEFB 1* was associated with caries experience in children born with CL/P. Variation in the expression of *DEFB 1* within the oral cavity can be associated with individual susceptibility for caries [38]. In our study we did not analyze the gene and/or protein expressions in saliva; however, future studies should consider including these analyses in order to clarify the correlation between genetic variation in the promoter region of *DEFB 1*, defensin concentration in saliva, and susceptibility to caries.

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