POLYMORPHISMS IN THE ANTIMICROBIAL PEPTIDE DEFB1 ARE NOT ASSOCIATED WITH CARIES IN PRIMARY DENTITION

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BACKGROUND: Single nucleotide polymorphisms (SNPs) in the promoter region of the β-defensin gene DEFB1 have recently been shown to be associated with altered risk of developing caries in permanent dentition.

OBJECTIVE: We therefore sought to establish whether DEFB1 SNPs conferred a similar risk in primary dentition.

METHODOLOGY: DEFB1 genotypes (rs11362 and rs1800972) were studied in 178 children, of whom 92 had caries.

CONCLUSION: No association between genotype and phenotype was detected, even when more severe caries was considered as the phenotype. These and other data suggest that non-genetic factors may be greater modifiers of caries risk in primary dentition.

DECLARATION OF INTERESTS: There are no conflicts of interest to declare.

KEY WORDS: Dental caries, primary dentition, β-defensin, single nucleotide polymorphism.


INTRODUCTION

Dental caries is a model multifactorial disease. While the cariogenic microorganism Streptococcus mutans plays a dominant role in the pathogenesis of dental caries, the development of the disease is also intimately linked to both substrate (amount of fermentable carbohydrate, protective foods, pH) and host (tooth anatomy, enamel quality, immune competence, and saliva quantity and quality) factors [1]. As a component of host-related factors, the importance of genetic susceptibility has been recognised for decades, with caries being attributable to an estimated 30-60% heritable component [2]. However, although a few candidate genes (such as tuftelin [3] and ameloblastin [4] are postulated to contribute to the pathogenesis via plausible mechanisms, these associations have not been rigorously replicated and there are no useful genetic biomarkers for use in practice. The results of genome-wide association studies (GWAS) may provide an unbiased appraisal of susceptibility loci, and these have started to report in both adults [5] and children [6]. However, the results for children in particular have been disappointing, with no loci meeting criteria for genome-wide significance.

However, a recent study measuring three single nucleotide polymorphisms (SNPs) in the promoter region of the β-defensin gene DEFB1 demonstrated a significant association with dental caries in the permanent dentition.
of nearly three hundred unrelated individuals [7]. The variant allele rs11362 conferred a five-fold increased risk in both decayed, missing teeth due to caries, filled teeth (DMFT) and decayed, missing teeth due to caries, filled surface of tooth (DMFS) scores, while the variant rs119946 genotype was associated with a reduced risk. The mechanistic hypothesis is plausible: defensins are key mediators of innate immunity and are a first line defence against pathogens such as S. mutans, and therefore any genetic variability that alters the efficacy of defensins may also influence microbial colonisation or pathogenicity [8]. In addition, DEFB1 has been shown to be constitutively expressed in oral cells and tissues when oral micro-organisms are present [9-11], and DEFB1 is secreted in crevicular fluid [12] and saliva [13], suggesting importance in oral health.

These promising results in adults prompted us to investigate the association between two SNPs in DEFB1, (rs11362 and rs1800972) and dental caries in the primary dentition of children aged three to six years of age. We aimed to establish whether DEFB1 polymorphisms contribute to early disease pathogenesis and therefore what clinical role, if any, genetic testing of DEFB1 might play in early detection and active management of dental caries in children.

SUBJECTS AND METHODS

Study subjects and definitions
A total of 178 children aged between three and six years of age were recruited into the study at the Department of Pedodontics, Istanbul University. All parents provided written informed consent for the participation of their children in the study and this protocol was approved by University of Pittsburgh Institutional Review Boards (PRO11070236).

Individuals were categorised as either controls (no caries; n=86) or as having dental caries (n=92); dental caries was defined as individuals with either a DMFT or DMFS of >1. For more stringent analysis of the caries phenotype, ‘high caries’ was defined as DMFT of >8, or DMFS equal to or greater than their age, as described previously [7].

Genotyping
Unstimulated saliva samples were obtained from all participants and stored in Otagene DNA self-collection kits (DNA Genotek Inc., Ottawa, ON, Canada) at room temperature until processed. DNA was extracted according to the manufacturer's instructions.

Two polymorphisms (rs11362 (G-20A) and rs1800972 (C-44G)) in the promoter region of DEFB1 were selected for study, as previously described [Ozturk et al., 2010]. The variants were genotyped using a validated Taqman assay on an ABI Prism 7900HT Sequence Detection System and automated software (Applied Biosystems, Foster City, CA).

Statistical analysis
Goodness of fit to Hardy-Weinberg expected proportions was examined using the chi-squared test. Pairwise linkage disequilibrium was estimated with D’ [14]. Differences between groups were tested using the Student’s t-test or chi-square test depending on whether the data were continuous or categorical, respectively. Odds ratios were determined using the 2-way contingency table chi-square test. Odds ratios were calculated by comparing individuals with zero copies of the variant allele and individuals with 1 or 2 copies of the variant allele. Statistical analysis was performed using SPSS® for Windows (IBM® SPSS® Statistics 19, Chicago, Ill, USA), and a p-value of <0.05 was considered statistically significant.

RESULTS
The mean age of the participants was 5.37 years, and ranged from three to six years of age. Average age of children in the caries group was 4.83 years old. Both groups were equally matched for gender (Table 1). Children with dental caries had DMFT scores ranging from 1 to 15 (average 7.0) and DMFS scores ranging from 1 to 34 (average 9.9).

Legends
Table 1. Demographics data of the Sample according to caries status.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Caries Status</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present (N=87)</td>
<td>Absent (N=74)</td>
</tr>
<tr>
<td>Age (mean ± standard deviation), years</td>
<td>4.83±0.81</td>
<td>5.99±0.21</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>48</td>
<td>38</td>
</tr>
<tr>
<td>Males</td>
<td>39</td>
<td>36</td>
</tr>
<tr>
<td>Mean DMFT</td>
<td>7.0±2.3</td>
<td>0</td>
</tr>
<tr>
<td>Mean DMFS</td>
<td>9.9±7.7</td>
<td>0</td>
</tr>
</tbody>
</table>

The rs11362 and rs1800972 polymorphisms were in linkage disequilibrium (p=xxx) and the genotypes in Hardy-Weinberg equilibrium. Allele frequencies were similar to previously described [7]. There was no evidence...
of an association between polymorphisms in *DEFB1* and the presence or absence of dental caries (Table 2), with the odds ratios for rs11362 (G-20A) and rs1800972 (C-44G) 0.93 (0.51-1.69) and 1.08 (0.55-2.01), respectively. Even when considering the extremes of caries phenotype, there was no association between genotype and phenotype.

### DISCUSSION

Polymorphisms in the promoter region of *DEFB1* have recently been shown to be associated with the caries experience in the permanent dentition of adults [7]. This prompted us to investigate whether *DEFB1* SNPs were also associated with caries in primary dentition, in order to provide insights into the role that *DEFB1* might play in the pathogenesis of dental caries and establish in which patient groups *DEFB1* SNP testing might be clinically useful. We did not find an association between the two SNPs tested and caries in the primary dentition in this cohort of unrelated children, suggesting that either *DEFB1* does not influence disease progression in children, other factors predominate in the pathogenesis of dental caries in children, or that *DEFB1* mediates long-term or chronic disease progression. For instance, in addition to their role in innate immunity, defensins also mediate the cross-talk between innate and acquired immune responses, such as by acting as chemotaxtants for T-cells and dendritic cells [15]. Their effects may therefore not be evident over the relatively short time-frames represented in the genesis of caries in children.

The results of GWAS studies into dental caries have largely been disappointing, especially in children. In a large GWAS study of 1305 children aged 3-12 years old, there were no single SNPs that were definitively associated with childhood dental caries, although a few regions on chromosomes 1, 11, and 17 did highlight weak associations with caries and contained a few plausible candidate genes of interest, including *ACTN2*, *MTR*, *EDARADD*, *MPPED2*, and *LPO* [6]. Weak associations with caries have also been observed for regions near the *DEFB1* locus at 8p23.1 in another small GWAS study, although the locus itself was not significant [16]. Adult studies have been slightly more encouraging, with two homologous genes (*BCOR* and *BCORL1*) found to be associated with the phenotype [5]. Although the heritability of caries scores in primary dentition has been reported to be greater than in permanent dentition [17], this observation does not appear to be borne out when tested by modern genotyping methodologies that have proven to be so successful for determining susceptibility to other complex genetic traits, such as for cardiovascular disease. Together with the current study, however, it appears that the bacterial, dietary, salivary, morphological, hygienic, and exposure-related factors may dominate in children.

This study is not without limitations. The sample size is relatively small and data on diet, oral hygiene, bacterial colonisation, and fluoride exposure, as well as other significant demographic details such as ethnicity and socioeconomic status are not available. These factors are known to influence risk and might provide a greater insight into the influencing factors in this cohort. Since gene-environment interactions are likely to be important in the pathogenesis of caries, the genetic risk is likely to be environment-specific, and further attempts to establish genetic risk stratifiers will need to take this into account.

In conclusion, although polymorphisms in the promoter region of *DEFB1* appear to be associated with caries risk in adults, they do not appear to be associated with risk of caries in primary dentition in children, consistent with genome-wide studies. Other non-genetic factors appear to be greater modifiers of risk and are likely to be more useful for stratification purposes.

### REFERENCES