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Association study for the role of Matrix metalloproteinases *2* and *3* gene polymorphisms in dental caries susceptibility

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ABSTRACT

Objective: Various exogenous and endogenous risk factors have been described as contributing to dental caries susceptibility. In the last decade it has been established that both pro and active forms of host derived Matrix metalloproteinases (MMPs) are present in the oral cavity. MMPs role in caries development has been hypothesized. The aim of this study was to analyse *MMP2* (rs2287074) and *MMP3* (rs679620) single nucleotide polymorphisms (SNPs) and their role in caries susceptibility.
Design: The two SNPs were analysed by PCR- restriction fragment length polymorphism (RFLP) in a sample of 102 ethnic Bulgarian volunteers (42 males and 60 females), all students in Sofia Medical University.
Results: Statistical analysis of the *MMP2* SNP showed significant differences for the genotype frequencies between the caries free (CF, DMFT = 0) and low caries experience (LCE, DMFT $\leq$ 5) groups. Analysis for the non-synonymous *MMP3* SNP found significant differences between both CF vs caries experience groups (LCE+ high caries experience (HCE, DMFT $\geq$ 5)) and LCE vs HCE groups. The presence of allele G decreased the risk of HCE about 4 times.
Conclusions: MMP2 and *MMP3* genes are likely to be involved in caries susceptibility in our population. However, as dental caries is a multifactorial disorder and several genes are likely to have influence on it, it is reasonable to expect that SNPs, even those proven to be functional like rs679620, potentially play a significant, but not major role in the disease outcome.

1. Introduction

Dental caries is a complex disease characterized by demineralization of inorganic portion and destruction of organic portion of teeth, eventually resulting in cavitation. This process is caused by certain cariogenic bacteria of the oral cavity, and various exogenous and endogenous risk factors have been described as contributing to dental caries development. In addition, the impact of individual genetic variation on the development of dental caries, including its severity and extent has been demonstrated in twin studies (Rintakoski, Kaprio, & Murtomaa, 2010).

In the last decade it has been established that both pro and active forms of host derived MMPs are present in the oral cavity. They are activated on-site by the lower pH, caused by the presence of the cariogenic bacteria, suggesting their importance in dental lesion process (Tjäderhane et al., 1998). MMPs are a family of zinc-dependent endopeptidases that degrade extracellular matrix proteins. Twenty five members of the MMP family have been identified in humans. On the basis of substrate specificity and homology, MMPs can be divided into six groups: collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs (MT-MMPs), and other MMPs (Murphy & Knäuper, 1997; Murphy & Nagase, 2008; Visse & Nagase, 2003).

MMPs contribute to various physiological processes, e.g. embryonic development, tissue turnover, and wound healing, as well as to pathological processes, e.g. cancer, cardiovascular diseases, arthritis, periodontitis, and fibrosis (Hadler-Olsen, Winberg, & Uhlin-Hansen, 2013; Agewall, 2006; Malemud, 2006; Sorsa, Tjäderhane, & Salo, 2004). Several MMPs are found to have a role in tooth development. It was suggested that they may regulate mineralization by controlling the proteoglycan turnover (Hannas,

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Pereira, Granjeiro, & Tjäderhane, 2007). Although most MMPs are expressed during normal dentin-pulp complex formation and maintenance, MMP-20 was identified as the first proteinase secreted into the developing enamel matrix (Bartlett, 2013).

The important role of genetics in the multifactorial etiology of dental caries is well established. However, Genome wide association studies (GWAS) on caries risk are limited in the literature. A recent published paper by Wang et al. in 2013 identified 53 potential susceptibility genes for dental caries and their protein-protein interactions. Functional analyses of these 53 genes revealed three major clusters: cytokine network relevant genes, matrix MMPs family, and transforming growth factor-beta (TGF-β) family (Wang, Jia, & Cuenco, 2013). Therefore the aim of the present study was to analyse single nucleotide polymorphisms (SNPs), located into two MMP genes- *MMP2* (rs2287074, p. Thr460Thr, c.1380G > A) and *MMP3* (rs679620, p.Lys45Glu, c.133A > G) and to evaluate their role in dental caries susceptibility in a clinically selected group of 102 Bulgarian students.

2. Materials and methods

More than 140 subjects, all of them students in Sofia Medical University completed a questionnaire about their ethnicity, parent's education and social environment, oral hygiene habits, dietary habits, snacking between meals, previous preventive fluoride treatments, orthodontic treatment, frequency of preventive dental examinations, self-evaluation of salivary flow, medical history. Of them, 102 fulfilled the initial requirements for being clinically healthy, without systemic diseases or generalized periodontal inflammations, perfect oral hygiene habits and at least annual dental examinations.

The selected participants were ethnic Bulgarians, aged from 20 to 32 years (42 males and 60 females). Subsequently all 102 subjects were clinically examined. Clinical examinations were performed with dental mirrors, explorers, artificial light and photo-polymerizing lamps. Radiographs were not performed.

The study was conducted according to the World Medical Association Declaration of Helsinki and additionally approved by the Ethics Committee of Sofia Medical University. Written informed consent was obtained from all participants prior to genetic testing.

The studied subjects were classified according to the caries experience level using the DMFT indexes. According to recently published papers, the different genetic loci and SNPs could lead to either increased or decreased activities and thus contribute differently to caries experience. In this context, the genetic factors have been divided into caries protective genes (associated with CF phenotype) and caries susceptibility genes (associated with LCE or HCE phenotypes) (Tannure et al., 2012; Vieira, Modesto, & Marazita, 2014).

The subjects were divided into three groups: CF (with DMFT = 0, n = 20), LCE (with DMFT $\leq$ 5, n = 41) and HCE (with DMFT $\geq$ 5, n = 41). Epithelial buccal cells were collected in sterile, Ependorf-type plastic containers of 2 ml. Each container was labeled indicating the name and individual number. The samples were collected after normal hygiene and breakfast individual habits of the participants, between 9 and 11.30 a.m.

DNA was extracted from buccal cells by the *Chelex® 100* (*Bio-Rad* Laboratories, Hercules, CA) extraction technique. The vials with buccal cells were centrifuged at 7500 rpm for 15 min, and the supernatant was removed. Then the pellet was resuspended thorough mixing in 150 μl of 5% Chelex 100 resin and Proteinase K solution, followed by incubation at 56 °C for 2 h in a dry heat block. The mixture was boiled for 10 min and then chilled on ice for 5 min. It was then centrifuged at 12,000 rpm for 10 min. The supernatant was carefully removed, with the Chelex avoided. The DNA was stored at −20 °C prior to analysis.

For *MMP2* SNP genotyping, DNA fragments were amplified by the use of primer pairs MMP2F 5′-GTCCAGGCATCTTCTTGTTA-3′ and MMP2R 5′-GAGGACAAGAAGCAAGCTCC-3′ (322 bp). PCR amplification was performed in 25 μl volume containing 100 ng of genomic DNA. The thermal cycles were initiated for 5 min at 95C, followed by 30 cycles of 40 s at 95C, 30 s at 58C, and 30 s at 72 °C, and a final extension at 72 °C for 10 min. The PCR products were subjected to restriction fragment length polymorphism (RFLP) with 5 U of BseYI (NEB, Germany) at 37 °C overnight, and the products were separated on a 3% agarose gel and stained with Ethidium bromide in order to yield G (122 and 200 bp) and A (322 bp) alleles, allowing, therefore, the determination of the GG, GA, and AA genotypes.

For *MMP3* SNP genotyping, DNA fragments were amplified by the use of primer pairs MMP3F 5′-GATTAAGAAGTGAGCAACTGCA-3′ and MMP3R 5′-CCTCCAATCCAAGGAACTTC-3′ (212 bp). PCR amplification was performed using the conditions for the MMP2 SNP. The PCR products were then subjected to RFLP with 5 U of TaqI (NEB, Germany) and incubated overnight at 65 °C. The products were separated on a 3% agarose gel and stained with Ethidium bromide in order to yield A (212 bp) and G (120 and 92 bp) alleles, allowing, therefore, the determination of the AA, AG, and GG genotypes.

Genetic data was entered and processed with SPSS 16.0 software (SPSS, Chicago, IL). Frequencies of alleles and genotypes were compared using χ^2 test. OR and their corresponding 95% CI were determined as per standard statistical methods. The Hardy-Weinberg equilibrium among the CF volunteers (used as control group) was also tested using standard χ^2 statistics. P values of <0.05 are considered statistically significant.

3. Results

All 102 volunteers were successfully genotyped for the *MMP2* and *MMP3* SNPs. The distribution of the allele and genotype frequencies is shown in Table 1. There are no differences in age and gender distribution between the groups. Considering the two SNPs independently, genotype distributions do not deviate from Hardy-Weinberg equilibrium among the groups (p > 0,05). There were no significant differences of the allele and genotype frequencies between the subjects from our study and the reported in dbSNP database for other European populations. For *MMP2* (rs2287074), the reported minor allele frequency (MAF) is 35%, while in our study is 39%. For *MMP3 (*rs679620), the reported MAF is 37%, while in our study is 47%.

The statistical analysis for the *MMP2* gene showed significant differences for the genotype frequencies between the CF and LCE groups. We found that the presence of genotype AA increased the risk for dental caries development about 3,5 times. The comparison between the other investigated groups of CF vs HCE and CF vs LCE + HCE showed differences close the borderline of p = 0.05 (Table 1). However, after applying the Yates correction for the small sample sizes, the p-values showed no statistical differences between the three groups.

Our study for the non-synonymous *MMP3* SNP found significant differences between both CF vs LCE+ HCE and LCE vs HCE groups. The presence of genotype GG decreased the risk of HCE about 4 times (Table 1).

When the three groups were divided according to their sex (data not shown), no significant differences were found between the groups and *MMP2* allele and genotype frequencies. After statistical analysis of *MMP3*, it was noted that the significant difference between the groups of CF or LCE and HCE cases was due to the high impact of female, but not male cases.