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Genetic Susceptibility to Periapical Disease: Conditional Contribution of *MMP2* and *MMP3* Genes to the Development of Periapical Lesions and Healing Response

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Abstract

Introduction—It has been proposed that individual genetic predisposition may contribute to a persistent apical periodontitis condition. Matrix metalloproteinases (MMPs) are associated with levels of inflammation and are involved in caries, pulpal and periapical tissue destruction. MMPs also play a major role in bone resorption. In this study, we hypothesized that polymorphisms in *MMP* genes and their regulators may contribute to an individual's increased susceptibility to apical tissue destruction in response to deep carious lesions.

Methods—Sixteen hundred radiographic records obtained through the University of Pittsburgh School of Dental Medicine Dental Registry and DNA Repository were screened for subjects with deep carious lesions in dentin with or without periapical lesions (≥3mm). DNA samples of 268 patients were sorted into two groups: 158 cases with deep carious lesions but no periapical lesions (controls), and 110 cases with periapical lesions and deep carious lesions (cases). Sixteen SNP markers in *MMP2*, *MMP3*, *MMP9*, *MMP13*, *MMP14*, *and TIMP2*, were selected for genotyping. Genotypes were generated by end-point analysis in a Real Time PCR instrument. Analyses were performed comparing cases and controls. Allele and genotypic frequencies and haplotype analysis were calculated using PLINK program.

Results—Association was found for *MMP3* rs639752 (p=0.03) and rs679620 (p=0.004) genotypes in individuals with periapical lesions. We also observed altered transmission of *MMP2* marker haplotypes (p=0.000004) in these individuals.

Conclusion—Variations in *MMP2* and *MMP3* are associated with periapical lesion formation in individuals with untreated deep carious lesions. Future studies could help predict host susceptibility to developing periapical lesions.

INTRODUCTION

Apical periodontitis is an inflammatory disorder of periradicular tissues caused by persistent microbial infection within the root canal system of the affected tooth (1, 2). Problems that

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lead to persistent apical periodontitis include: inadequate aseptic control, poor access cavity design, missed canals, inadequate instrumentation, debridement and leaking temporary or permanent restorations (3). However, It has been recently proposed that genetic predisposition in certain genes can contribute to persistent apical periodontitis (4, 5).

Matrix metalloproteinases (MMPs) are strongly associated with levels of inflammation and play a major role in bone remodeling and bone resorption (6). They are secreted as proenzyme forms, require extracellular activation and are regulated by endogenously secreted inhibitors, the tissue inhibitor of metalloproteinases (TIMPs) (6). Clinical and experimental evidence support a role for the MMPs to promote osteoclastic bone resorption and bone metastasis. The precise molecular mechanisms appear to involve proteolytic cleavage of substrates and subsequent activation of prometastatic factors—such as TGF-β, IGFs, and vascular endothelial growth (VEGFs)—and ultimate activation of the RANKL pathway. Bone matrix is comprised mostly of mineralized fibrillar type I collagen. MMPs are capable of cleaving native, nondenatured collagens with long uninterrupted triple helices and can function as collagenases in vivo (7). Several MMPs are expressed in bone function in endochondral ossification during embryonic development and in modeling and remodeling of bone postnatally and later in life (8). MMPs act in the degradation of the organic matrix (mainly type I collagen) (9), and their expression is frequently induced by cytokines with bone-resorbing activity such as IL-1 and IL-6 (10).

MMPs play an important role during periapical pathology development (11– 13), and it has been suggested that the modality of root canal treatment could interfere with MMPs expression (14). Moreover, elevated MMP levels have also been reported to correlate with non-healing (15, 16). Due to their various roles in bone remodeling, immune responses, caries, and dental development, we hypothesize that variations in MMP and TIMP genes may alter the level of bone destruction and remodeling and contribute to the formation of more extensive periapical lesions in teeth affected by deep carious lesions. For this experiment, we purposely selected individuals with several deep carious lesions that did not evolve to formation of periapical lesions and compared them to individuals with several deep carious lesions that developed periapical pathology measuring more than 3 mm. The underlying hyothesis here is that genetic variation in *MMP* genes influence progression of carious lesions in dentin and development of periapical pathology, since MMPs are involved with dentin and bone degradation. Human genetic polymorphisms appear to play a role in the disease susceptibility of the host. Hence, combined bacterial/host genotyping may provide an important tool in defining disease risk and targeting bacteria eradication to high-risk individuals like it has been proposed to H. pylori in gastric carcinoma (17).

MATERIALS AND METHODS

Sample Population

Individual samples and clinical history were obtained through the Dental Registry and DNA Repository of the School of Dental Medicine, University of Pittsburgh. This study was approved the University of Pittsburgh Institutional Review Board (IRB #0606091). Sixteen hundred radiographic records were screened for subjects with deep carious lesions with or without periapical lesions (≥3mm in diameter). All participants signed an informed consent and provided a saliva sample as source of genomic DNA.

DNA samples of 268 white individuals with deep carious lesions in dentin were sorted into two groups: 158 control individuals (65 males, 93 females, average age 58 ± 8 SD) with deep carious lesions but no periapical lesions, and 110 case individuals (57 males, 53 females, average age 57 ± 10 SD) with periapical lesions and deep carious lesions.

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All individuals selected to this study had thermal and electric pulpal vitality tests performed previously as attested in their dental records. Cases presenting apical periodontitis were diagnosed as pulp necrosis; and cases without apical periodontitis as vital pulps. Cases that did not present this information were excluded from the study.

Selection of Candidate Genes and Single Nucleotide Polymorphisms

We selected 16 polymorphisms spanning the *MMP2, MMP3, MMP9, MMP13, MMP14*, and *TIMP2* genes. Some of the SNPs were selected based on published reports and/or their locations within the genes. Additional SNPs were selected based on their likelihood to have functional consequences (i.e., located in the promoters, exons, or near exon/intron boundaries), or considered tag-SNPs as surrogates for the linkage disequilibrium blocks surrounding the candidate gene. We used information from the NCBI dbSNP (http://www.ncbi.nlm.gov/SNP/) and HapMap Project (http://www.hapmap.org) databases. Details of selected genes and polymorphisms are presented in Table 1.

Genotyping

Genomic DNA was extracted from saliva using established protocols. Genotypes were generated using Taqman chemistry (18). Reactions were carried out in 5-μL volumes in an ABI PRISM Sequence Detection System 7900 (Applied Biosystems, Foster City, CA). Assays and reagents were supplied by Applied Biosystems. The results were analyzed using SDS software version 1.7 (Applied Biosystems, Foster City, CA). In order to ensure quality control of genotyping reactions, we used a non-template control (using water instead of DNA) as a negative control and a sample of DNA of previously known genotype as a positive control.

Association analyses

Allele and genotypic frequencies of each polymorphism in cases and controls were calculated using PLINK software version 1.06 (19). Analyses were performed comparing cases and controls. We used Bonferroni correction

as implemented in PLINK to adjust for multiple testing. Haplotype analyses were performed using 2-, 3-, and 4-SNP sliding windows in genes where two or more SNPs were investigated.

RESULTS

Hardy-Weinberg Equilibrium (HWE) is used to describe the genotype distribution of a population when it is large, self-contained, and randomly mating. Testing for HWE in the control group is commonly used to detect genotyping errors in genetic association studies (20). There was no evidence of deviation from Hardy-Weinberg equilibrium for any of the investigated markers between the groups (data not shown).

The results of the association analysis in the study groups are presented in Tables 2 and 3. We assessed genotype and allelic associations between the investigated genes and individuals affected with deep carious lesions and periapical disease. We observed a significant association of two *MMP3* marker genotypes with individuals presenting deep caries and periapical lesions, namely rs679620 (p=0.004) and rs639752 (p=0.03) (Table 3). Under a dominant model, genotypes containing the minor allele A in SNP rs679620 also showed borderline association (p=0.05) in cases with deep carious lesions with periapical disease (Table 2). Additional haplotype analyses also showed strong altered transmission of *MMP2* marker alleles in cases with deep carious lesions with periapical disease (Table 4).

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DISCUSSION

MMPs and TIMPs have been shown to play important roles in dentin formation, caries progression, and hybrid layer degradation (21, 22) as well as in pulpal and periapical pathologies (11, 12, 14, 23–26). We hypothesized that MMPs and TIMPs influence the formation of more extensive periapical lesions in teeth affected by deep carious lesions. Our control group was composed by individuals presenting deep carious lesions that did not develop periapical bone destruction, meaning that they could represent a group that is not susceptible to the development of pulpal or periapical pathology even in the presence of caries-prone microorganisms.

We observed significant association between *MMP2* and *MMP3* and the presence of large periapical lesions. This suggests that MMPs may be associated with periapical lesion formation due to untreated deep carious lesions, and may contribute to varying levels of bone remodeling, regeneration, and inflammation in response to the deep carious condition.

It is well understood that endodontic failure is mainly due to an infected root canal system, acting as a reservoir for microbial cells, virulence products and antigens, which collectively evoke and maintain apical periodontitis (27). The microbial components, especially lipopolysaccharides, activate macrophages to synthesize and secrete a variety of pro-inflammatory molecules, including the cytokines IL-1 and TNF-α, prostaglandins, and hydrolytic enzymes (28). Similarly, bacterial substances activate T lymphocytes to produce IL-1 and lymphotoxin, a molecule with similar properties to TNF-α. These cytokines manifest potent pro-inflammatory and catabolic activities, and play key roles in periodontal tissue breakdown through collagenolytic enzymes such as matrix metalloproteinases (MMPs) (28). IL-1 alpha also induces pulpal tissue destruction by differentially regulating MMPs and TIMPs (29). In addition, in cases of periapical biofilms, where neutrophils cannot engulf and digest bacteria, large quantities of pro-inflammatory cytokines are released leading to a chronic inflammatory condition (30) and subsequently stimulating MMPs to participate in the tissue breakdown process in the periapical area.

MMP-3, also called stromelysin, is involved in the breakdown of extra-cellular matrix in normal physiological processes, such as embryonic development, reproduction, tissue remodeling, as well as in disease processes (31). Regarding the two MMP3 SNPS associated in this study, noteworthy is the *MMP3* gene variant, SNP rs679620, which is a missense mutation shown to alter wild type gene function (32), and results in a change from a Lysine to Glutamine in the final protein. Taken the strong association of this variant in individuals with deep carious lesions and periapical disease identified in our study, it is plausible to propose that this variant could serve as a marker for susceptibility of faster carious lesion progression in dentin and periapical pathologies.

We also performed haplotype analysis in *MMP2*, *MMP3*, and *MMP9* genes, for which two or more SNPs were investigated. Although not individually associated, several haplotypes in *MMP2* showed significant association in this study. Particularly the combination of alleles for rs243847 and rs2287074, and also rs2287074 and rs9923304 yielded strong association results and warrants further investigations. MMP-2, also known as gelatinase A, is a membrane-bound protein that is important for extracellular matrix turnover, preferentially cleaving collagen types IV, V, VII, and XI and gelatin (33). The *MMP2* variant rs2287074 is a synonymous mutation, resulting in the same amino acid (threonine) at codon 460 regardless of the allele present. Although this variant does not appear to create a new splice site or alter an existing one, it has been shown that variation at synonymous sites could result in allele-specific structural differences in mRNA that could affect mRNA structure-dependent mechanisms, which could have biologic consequences (34). Furthermore,

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although *MMP2 rs2287074* may not have a direct effect on periapical disease formation, it may be physically linked to other genetic regions showing a causal effect.

In summary, our findings suggest that markers in *MMP3* and *MMP2* genes could help predict host susceptibility to developing periapical lesions and healing response. Additional studies with other populations should focus on these genes to further investigate their participation in human periapical disease.

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Table 1

Details of the genes and SNPs investigated in this study.

Gene	dbSNP ID	Location (chromosome, base position)	SNP Function	Position in sequence (nucleotide change)[a]	Alleles[b]
MMP2	rs243865	chr. 16 54069297	5′ near gene	−1306 C/T	C/T
	rs2285053	chr. 16 54069878	5′ near gene	−735 C/T	C/T
	rs243847	chr. 16 54081499	Intron		T/C
	rs2287074	chr. 16 54084614	Coding synonymous	Thr460Thr (exon 9)	G/A
	rs9923304	chr. 16 54087802	Intron		C/T
	rs11639960	chr. 16 54090771	Intron		A/G
MMP3	rs639752	chr. 11 102212549	Intron		T/G
	rs650108	chr. 11 102213997	Intron		G/A
	rs679620	chr. 11 102218830	Missense	Lys45Glu (exon 2)	G/A
	rs522616	chr. 11 102220258	5′ near gene	−709 A/G	A/G
MMP9	rs3918253	chr. 20 44072918	Intron	49 5′exon 4	C/T
	rs17576	chr. 20 44073632	Missense	Gln279Arg	A/G
	rs17577	chr. 20 44076518	Missense	Gln668Arg(exon 12)	G/A
MMP13	rs2252070	chr. 11 102331749	5′ near gene	−77 A/G	G/A
MMP14	rs1042704	chr. 14 22382434	Missense	Asn273Asp	A/G
TIMP2	rs9894526	chr. 17 74438681	5′ near gene		C/T

[a] According to NCBI Reference Assembly.

[b] Ancestral allele listed first.

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Table 2

Results of the allele frequency comparisons.

Gene	SNP	Minor allele frequency	P-value
MMP2	rs243865	0.1667	0.8689
MMP2	rs2285053	0.1471	0.4610
MMP2	rs243847	0.4151	0.9767
MMP2	rs2287074	0.4333	0.3115
MMP2	rs9923304	0.3029	0.1019
MMP2	rs11639960	0.2474	0.1536
MMP3	rs639752	0.4762	0.6514
MMP3	rs650108	0.2788	0.4397
MMP3	rs679620	0.4952	0.9664
MMP3	rs522616	0.1990	0.4066
MMP9	rs3918253	0.4567	0.9563
MMP9	rs17576	0.4286	0.4891
MMP9	rs17577	0.1474	0.3437
MMP13	rs2252070	0.2584	0.3083
MMP14	rs1042704	0.1875	0.6723
TIMP2	rs9894526	0.4300	0.2771

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Table 3

Results of case-control comparisons for the associated SNPs in *MMP3*. Comparisons were performed for genotypic association under a normal distribution, and under dominant and recessive allele models.

SNP/Genotypes	Test/Model	Cases	Controls	Chi-square	P-value
MMP3 rs679620					
AA/AG/GG	Genotype	20/63/21	44/59/46	10.8	**0.004**
AA + AG vs. GG	Dominant Model	83/21	103/46	3.588	**0.05**
AA vs. AG + GG	Recessive Model	20/84	44/105	3.438	0.06
MMP3 rs639752					
TT/TG/GG	Genotype	20/60/25	43/60/44	6.818	**0.03**
TT + TG vs. GG	Dominant Model	80/25	103/44	1.155	0.28
TT vs. TG + GG	Recessive Model	20/85	43/104	3.401	0.06

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Table 4

Results of haplotype analysis using sliding windows between markers in *MMP2*, *MMP3*, and *MMP9*, where two or more SNPs were investigated. For *MMP2* markers, note that the combination of haplotype alleles including allele A of SNP rs2287074 yields significant association results across haplotype blocks.

MMP2					
rs243865	**rs2285053**	**rs243847**	**rs2287074**	**rs9923304**	**rs11639960**
P=0.59 CT					
	P=0.39 TT				
		P=0.0009 C<u>A</u>			
			P=4.4E-06 <u>A</u>C		
				P=0.13 CA	
P=0.57 CTT					
	P=0.002 CC<u>A</u>				
		P=7.96E-05 C<u>A</u>C			
			P=9.12E-06 <u>A</u>CA		
P=0.003 CCC<u>A</u>					
	P=0.0002 CC<u>A</u>C				
		P=5.32E-05 C<u>A</u>CA			
P=0.0006 CCC<u>A</u>CA					

MMP3			
rs639752	**rs650108**	**rs679620**	**rs522616**
P=0.29 AG			
	P=0.27 GC		
		P=0.73 CT	
P=0.43 AGC			
	P=0.32 GCT		
P=0.32 AGCT			

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MMP9		
rs3918253	**rs17576**	**rs17577**
P=0.42 CG		
	P=0.27 GG	
	P=0.64 CGG	