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Matrix metalloproteinase-3 gene polymorphism in renal transplant patients with gingival overgrowth

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Background and Objective: Gingival enlargement frequently occurs in transplant patients receiving immunosuppressive drugs. It was hypothesized that gingival enlargement associated with cyclosporine use results from reduced degradation of extracellular matrix in the gingiva. Matrix metalloproteinase-3 (MMP-3) is involved in biodegradation of the extracellular matrix, and its inhibition may contribute to an abnormal accumulation of fibronectin and proteoglycans, which are MMP-3 substrates. The aim of this study was to investigate whether an association exists between *MMP-3* genotypes and gingival enlargement in kidney transplant patients medicated with cyclosporine A.

Material and Methods: Sixty-four unrelated kidney transplant patients suffering from gingival overgrowth, as well as 111 control transplant patients without gingival overgrowth, were enrolled in the study. Gingival overgrowth was assessed 6 mo after transplantation. During the post-transplant period all patients were given cyclosporine A as a principal immunosuppressive agent. *MMP-3* polymorphism was determined using a PCR restriction fragment length polymorphism assay.

Results: In kidney transplant patients suffering from gingival overgrowth the mean gingival overgrowth score was 1.35 ± 0.57, whereas in control subjects the mean gingival overgrowth score was 0.0. The distribution of *MMP-3*-1178A/*dupA alleles among all kidney transplant patients, as well as in the two study subgroups, did not differ significantly from Hardy–Weinberg equilibrium. The frequency of the *MMP-3-1171*A/*A* genotype (28.1% for gingival overgrowth vs. 26.1% for controls) and of the *MMP-3-1171*dupA/*dupA* genotype (32.8% for gingival overgrowth vs. 22.5% for controls) was similar for both study groups. The risk of gingival overgrowth was lowest among patients carrying the *MMP-3-1171*A/*dupA* genotype (odds ratio 0.52), but this did not differ markedly from the other genotypes.

Conclusion: No association between *MMP-3* gene polymorphism and gingival overgrowth was revealed in kidney transplant patients administered cyclosporine A.

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Gingival overgrowth frequently occurs in transplant patients receiving immunosuppressive drugs, such as cyclosporine A (INN cyclosporine), which have been widely used since the 1970s. The incidence of gingival overgrowth among renal transplant patients treated with cyclosporine A ranges from 13 to 84.6% (1–4). Gingival overgrowth induced by treatment with cyclosporine A is probably the consequence of alterations in the turnover of epithelial and connective tissues, resulting in an increase in the amount of structural proteins sometimes associated with epithelial thickening. Numerous studies have investigated potential risk factors in the development of the condition, including the extent of renal disease, interval since transplantation, duration of renal replacement therapy, dose of cyclosporine A, gingival inflammation, plaque indices and recipient human leukocyte antigen phenotype (4–7). Whilst some studies have suggested various associations between the incidence and severity of gingival overgrowth and gender, pre-transplant diagnosis, age at transplantation, duration of therapy and cyclosporine dosage, others have failed to find any significant drug-related risk factors for the development of the condition, as previously reported (4–7). However, it remains unclear why a proportion of patients are susceptible to gingival overgrowth while others remain unaffected.

It was hypothesized that gingival overgrowth induced by cyclosporine results in a disturbance in the homeostatic balance, which is characterized by an increase in both the number of fibroblasts and the volume of the extracellular matrix. This loss of growth control results in an accumulation of redundant tissue of relatively normal composition (8). Matrix metalloproteinases are very important factors implicated in extracellular matrix processing. Immunofluorescent studies showed expression of MMP-3 within gingival tissue, predominantly concentrated in the connective tissue matrix subadjacent to the basement membrane and in the cores of the papillary connective tissue (9). Further studies demonstrated that the production of MMP-3 by gingival fibroblasts was significantly inhibited by cyclosporine at concentrations found in the serum of patients undergoing treatment with cyclosporine A (10). There is also strong evidence that MMP-3 is required for efficient activation of human MMP-1 and MMP-9 (other important players of extracellular matrix turnover), 'super-activating pro-MMP-1 to a specific activity 12 times higher in the absence' (11,12). The reports cited above suggest that inhibition of MMP-3 activity may contribute to accumulation of the extracellular matrix components and hence trigger gingival overgrowth in cyclosporine A-medicated patients. An *in vitro* promoter activity assay showed that the activity of MMP-3 is genetically determined. It was demonstrated that the *MMP-3-1171*A* allele has a twofold greater promoter activity than the *MMP-3-1171*dupA* allele, which can bind a transcriptional repressor. This difference has been observed in several cell types, including macrophages, smooth muscle cells and fibroblasts (13).

Therefore, it was decided to investigate the possible association between the *MMP-3-1171*A/*dupA* gene polymorphism and susceptibility to gingival overgrowth in kidney transplant patients medicated with cyclosporine A.

Material and methods

Patients of Polish origin from Western Pomerania (Poland) were included in the study after giving informed consent to participate. The protocol of the study was approved by the ethics committee of the Pomeranian Medical University, Szczecin, Poland. A total of 64 unrelated kidney transplant patients suffering from gingival overgrowth (40 men and 24 women; 22–68 years of age; mean age 38.6 ± 11.9 years) were enrolled in the study during 2004–2006. All patients were examined by two independent consultant periodontal specialists 6 mo after kidney transplantation. The patients were assessed using a clinical scoring method according to Pernu *et al*. (14). The patients were ascribed a general whole-mouth score of between 0 and 3, as follows: 0 = no overgrowth seen; 1 = mild gingival overgrowth (thickening of the marginal gingiva and/or lobular granulation of the gingival pocket as well as overgrowth covering the gingival third of the crown or less); 2 = moderate gingival overgrowth (overgrowth extending to the middle of the crown); and 3 = severe gingival overgrowth (overgrowth covering two thirds of the crown or affecting the whole attached gingiva).

Control samples were from 111 kidney transplant patients (60 men and 51 women; 20–72 years of age; mean age 42.4 ± 10.8 years), who were free from gingival overgrowth signs at 6 mo after transplantation, as evaluated by consultant periodontal specialists.

During the study period all subjects were administered cyclosporine A, azathioprine, prednisone and one of two calcium-channel blockers (i.e. diltiazem or verapamil); eight patients without gingival overgrowth were given atenolol or prazosine. The serum concentrations of cyclosporine A were measured using the fluorescence polarization immunoassay (TDx; Abbott Laboratories, Abbott Park, IL, USA) in all patients. The medication regimen administered in both groups of the study was comparable (Table 1).

Genotyping

An *in vitro* promoter activity assay showed that the *MMP-3-1171*A* allele has a twofold greater promoter activity than the *MMP-3-1171*dupA* allele, which can bind a transcriptional repressor. This difference has been observed in several types of cell, including macrophages, smooth muscle cells and fibroblasts (13). In general, the **dupA/*dupA* genotype was associated with increased matrix protein deposition, whereas the **A/*A* and **A/*dupA* genotypes were associated with increased matrix protein degradation. The *MMP-3-1171dupA* polymorphism was previously associated with various cardiovascular conditions and disease activity in rheumatoid arthritis (13,15,16).

Genomic DNA was extracted from 450 μL of whole-blood samples using

Table 1. Characteristics of medication at monthly intervals after transplantation

	1 mo	2 mo	3 mo	4 mo	5 mo	6 mo
Patients with gingival overgrowth (n = 64)						
Cyclosporine concentration (ng/ml)	430 ± 226	407 ± 228	337 ± 200	311 ± 207	294 ± 177	266 ± 207
Cyclosporine dose (mg/d)	302 ± 89	279 ± 79	261 ± 80	257 ± 86	241 ± 80	234 ± 68
Diltiazem dose (mg/d)	202 ± 54	202 ± 54	202 ± 54	202 ± 54	202 ± 54	202 ± 54
Verapamil dose (mg/d)	174 ± 72	174 ± 72	174 ± 72	174 ± 72	174 ± 72	174 ± 72
Prednisone dose (mg/d)	19 ± 7	17 ± 6	15 ± 7	14 ± 4	12 ± 4	11 ± 4
Patients without gingival overgrowth (n = 111)						
Cyclosporine concentration (ng/ml)	460 ± 266	411 ± 326	354 ± 276	323 ± 247	297 ± 183	292 ± 181
Cyclosporine dose (mg/d)	297 ± 96	280 ± 90	255 ± 84	246 ± 81	244 ± 78	236 ± 72
Diltiazem dose (mg/d)	186 ± 51	186 ± 51	184 ± 51	189 ± 51	187 ± 51	186 ± 51
Verapamil dose (mg/d)	184 ± 60	184 ± 60	184 ± 60	184 ± 60	184 ± 60	185 ± 60
Prednisone dose (mg/d)	17 ± 4	17 ± 6	14 ± 5	13 ± 5	12 ± 4	11 ± 4

the GeneMatrix kit (EURx, Gdansk, Poland). Genotyping of each subject for the presence of the *MMP-3-1171dupA* polymorphism (rs3025058) in *MMP-3* promoter regions was performed using the previously described PCR–restriction fragment length polymorphism assay with minor modifications (17). The PCR conditions were as follows: forward primer, 5′-GAT TAC AGA CAT GGG TCA CA-3′, reverse primer, 5′-TTT CAA TCA GGA CAA GAC GAA GTT T-3′, initial denaturation of 2 min at 95°C, followed by 35 cycles of 40 s at 94°C, 1 min at 53°C, 1 min at 72°C, and a final extension at 72°C for 2 min, using *Xmn*I PCR product digestion for allele identification.

Statistical analysis

The allele and genotype frequencies were determined by direct counting of *MMP-3* alleles. Concordance of the genotype distribution with Hardy–Weinberg equilibrium and associations between categorical variables were assessed by Fisher's exact test using STATISTICA 7.0 (Statsoft, Warsaw, Poland). Odds ratios (ORs) and confidence intervals were calculated using the Newcombe–Wilson method without the continuity correction.

Results

Out of 64 patients with gingival overgrowth, 44 subjects had a gingival overgrowth score of 1, 16 subjects had a score of 2 and four subjects had a score of 3. The mean gingival overgrowth score was 1.35 ± 0.57. Control transplant patients were characterized by having healthy gingiva (i.e. were scored as 0).

The distribution of *MMP-3-1171*A* alleles among all kidney transplant patients, as well as in two study subgroups, did not differ significantly from Hardy–Weinberg equilibrium. The frequency of the *MMP-3-1171*A/*A* genotype (28.1% vs. 26.1) and of the *MMP-3-1171*dupA/*dupA* genotype (32.8% vs. 22.5%) was similar among individuals with gingival overgrowth and in the control group. The risk of gingival overgrowth was lowest among patients carrying the *MMP-3-1171*A/*dupA* genotype (OR 0.52), but did not differ markedly from the other genotypes.

As with genotypes, the distribution of the *MMP-3-1171* allele was similar in patients with gingival overgrowth and healthy gingiva. The risk of gingival overgrowth was lowest among patients carrying the *MMP-3-1171*A* allele (OR 0.59), but did not differ significantly from *MMP-3-1171*dupA* allele carriers (Table 2).

Discussion

Several studies have investigated specific polymorphisms as risk factors for the development of gingival overgrowth. While studies on MMP-1, interleukin-6 and the drug transporter *MDR1* gene have failed to identify any association with gingival overgrowth (18–21), other studies have identified clear associations between gingival overgrowth and polymorphisms in α2 integrin, transforming growth factor-β1 and interlukin-1 (22–24). Serum levels of transforming growth factor-β1 have been shown to be a significant

Table 2. Distribution of kidney transplant patients with and without overgrowth according to *MMP-3* genotype

	Gingival overgrowth (n = 64) n (%)	Healthy gingiva (n = 111) n (%)	p value[a]	OR (95% CI)
Genotype				
A*/A*	18 (28.1)	29 (26.1)	0.531	0.74 (0.32–1.69)
A*/dupA*	25 (39.1)	57 (51.4)	0.124	0.52 (0.24–1.10)
dupA*/dupA*	21 (32.8)	25 (22.5)	–	–
**A* allele carriers	43 (67.2)	86 (77.5)	0.156	0.59 (0.30–1.18)[b]
**dupA* allele carriers	46 (71.9)	82 (73.9)	0.859	0.90 (0.45–1.80)[b]
Allele frequencies				
**A*	61 (47.7)	115 (51.8)	0.51	
**dupA*	67 (52.3)	107 (48.2)	–	

[a]Calculated in relation to the 'low-activity' allele (**dupA*) and genotype (**dupA*/**dupA*), except for [b]calculated in relation to noncarriers of the respective allele.

indicator of the risk of developing gingival overgrowth (25).

There are no data available on the effects of the *MMP-3* gene polymorphism on gingival overgrowth in kidney transplant patients medicated with cyclosporine A. However, some information suggests a potential role of the polymorphism in the pathogenesis of the overgrowth. A disturbance in connective tissue homeostasis has been suggested as one of the key events occurring during the development of cyclosporine-induced gingival overgrowth. Matrix metalloproteinase-3 is involved in biodegradation of the extracellular matrix, and its inhibition may result in an abnormal accumulation of fibronectin and proteoglycans, substrates for MMP-3, in the cyclosporine-A-treated gingival tissue. Former studies indicated that genetically determined disturbances in extracellular matrix turnover may contribute to gingival pathology. The association between an MMP-1 promoter region polymorphism and severe chronic periodontitis in a Chinese population was revealed by Cao *et al*. (26). Similarly, Keles *et al*. (27) reported that an MMP-9 promoter gene polymorphism seemed to be associated with severe generalized chronic periodontitis in Turkish subjects, and Astolfi *et al*. (28) found an MMP-3 gene polymorphism to contribute to periodontal tissue destruction during periodontitis in Brazilian subjects. However, the latter authors reported no significant association for the MMP-1 polymorphisms with susceptibility to periodontitis. Likewise, no genetic polymorphisms of the MMP-9 gene were associated with susceptibility to chronic periodontitis in the Czech population (29).

The present study revealed a similar distribution of *MMP-3* alleles and genotypes in kidney transplant patients medicated with cyclosporine A, with and without gingival overgrowth. The distribution of *MMP-3-1171*A* alleles among all kidney transplant patients, as well as in two study subgroups, did not differ significantly from Hardy–Weinberg equilibrium. The frequencies of the *MMP-3 -1171*A/*A* genotype (28.1% vs. 26.1) and the *MMP-3-1171*dupA/*dupA* genotype (32.8% vs. 22.5%) were similar among individuals with gingival overgrowth and in the control group. The risk of gingival overgrowth was lowest among patients carrying the *MMP-3-1171*A/*dupA* genotype (OR 0.52), but did not differ markedly from the other genotypes.

As with genotypes, the distribution of the *MMP-3-1171* allele was similar in patients with gingival overgrowth and healthy gingiva. The risk of gingival overgrowth was lowest among patients carrying the *MMP-3-1171*A* allele (OR 0.59), but did not differ significantly from *MMP-3-1171*dupA* allele carriers.

Interactions between simultaneously administered drugs affecting gingival enlargement have been reported. Cyclosporine A-treated patients are often co-medicated with azathioprine and prednisolone, which can modify the severity of gingival overgrowth (30). By contrast, patients on cyclosporine A who are also receiving calcium-channel blockers present with a greater risk of gingival lesions than patients treated with cyclosporine alone (31). In the present study, both groups (i.e. patients with and without gingival overgrowth) received similar medication during the observation period. So, the effect of treatment modality on gingival pathology could be disregarded in the data analysis.

Another factor that could potentially influence the study conclusions is the number of subjects evaluated. As in the present study the groups analysed comprised 64 and 111 subjects, the conclusions drawn should be considered as preliminary data, and the results of the study should be confirmed by observations from other populations involving larger groups of patients.

In the present study, no association was documented between MMP-3 gene polymorphism and the development of gingival overgrowth in kidney transplant patients administered cyclosporine A as a principal immunosuppressive agent. The conclusion from the study also supports the notion that the pathology of drug-induced gingival overgrowth is complex with interplay of many tissue components. Complexity of pathological processes is often behind the negative results of genetic association studies.

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