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Role of *MDR1* gene polymorphisms in gingival overgrowth induced by cyclosporine in transplant patients

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Background and Objective: The aim of the present study was to determine the association between genotypes of the *MDR1* gene, encoding P-glycoprotein, and gingival overgrowth in transplant patients treated with cyclosporine, and to evaluate the effect of periodontal treatment in these patients.

Material and Methods: Fifty transplant patients receiving therapy with cyclosporine and suffering from gingival overgrowth were subjected to nonsurgical periodontal treatment and received oral hygiene instructions. Hyperplastic index, periodontal probing depths, bleeding and plaque scores were recorded at baseline and after 3 and 6 mo. Patients were dichotomized into two groups: those with a hyperplastic index of $< 30\%$ (minimal gingival overgrowth) and those with a hyperplastic index of $\geq 30\%$ (clinically significant gingival overgrowth). *MDR1* C3435T and G2677T polymorphisms were evaluated in all patients and in 100 controls.

Results: At baseline, 32 patients (64%) had minimal gingival overgrowth and 18 patients (36%) had clinically significant gingival overgrowth. The mutated C3435T genotype was significantly more frequent in the second group ($p < 0.019$). The significant association between gingival overgrowth and the 3435TT genotype was confirmed by logistic regression analysis ($p < 0.031$). The differences in hyperplastic index, observed at baseline between patients with the TT genotype and those with the CC/CT genotype disappeared in the second and third evaluation. The mean monthly change of the square root of the gingival overgrowth scores for all patients, assessed using linear models, was significantly different from baseline (-0.17 points per month, $p < 0.00001$); and this was particularly evident in subjects with renal transplant (-1.62, $p < 0.01$).

Conclusion: Aetiological periodontal and self-performed maintenance therapy is effective in reducing gingival overgrowth, particularly in subjects with the 3435TT genotype and in patients with renal transplant.

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Cyclosporine A is the first-choice immunosuppressant in the prevention of transplant rejection and for the treatment of autoimmune diseases. Among the various side effects induced by this drug, gingival overgrowth is an important clinical problem. The frequency of this complication, which affects the quality of life of patients, is

high, with a variable prevalence in different studies that ranges from 13 to 84% (1–4).

Several factors have been associated with the between-subject variation in susceptibility to cyclosporine, including age, gender, periodontal conditions before transplantation, pharmaceutical preparations and association with other drugs, in particular calcium-channel blockers (1–10). By contrast, there is no definite consensus on the impact of dosage (11), blood levels (12), oral hygiene and level of plaque control (2,13–15), and, at present, it is not possible to identify patients at risk of developing this side effect.

Cyclosporine is a substrate of the drug efflux pump P-glycoprotein, a member of the ABC (ATP binding cassette) family of transporters. This protein is overexpressed in multidrug-resistant tumor cells, but is also present in normal tissues and has a role in excreting drugs, xenobiotics and metabolites into the urine, bile and intestinal lumen, and in protecting tissues such as the brain. P-glycoprotein is expressed in the ducts of salivary glands (16) and is therefore a potential determinant of cyclosporine excretion and hence salivary concentration. The protein is expressed also in the endothelial layers of blood vessels in the gingival tissue (17) and is particularly abundant in the healthy gingiva, while it is expressed less in the granulation tissue of periodontal inflamed structures.

Among the causes of the different response to cyclosporine in gingival tissue, the variability of P-glycoprotein expression observed in humans could be important. The protein is encoded by the *MDR1* gene (ABCB1), and a number of polymorphisms of this gene have been described (18). In particular, alterations of the expression and function of the protein have been associated with the G2677T/A polymorphism in exon 21 and with the C3435T polymorphism in exon 26 (19).

Previous studies have evaluated the importance of these polymorphisms in drug-induced gingival overgrowth, but the results have been inconsistent. The first study (16) was conducted in 54 patients with cyclosporine-induced overgrowth and did not show any relationship with the presence of the C3435T polymorphism; however, Meisel *et al*. (17) found an association between the G2677T/A genotype and gingival hyperplasia induced by calcium antagonists.

The present study was therefore conducted in 50 transplant patients under maintenance therapy with cyclosporine, who had been transplanted more than 6 mo before examination and exhibited gingival overgrowth. The aim of our research was to clarify the relationship between polymorphisms in the *MDR1* gene and the severity of gingival overgrowth; the effects of aetiological nonsurgical periodontal therapy in these patients were also evaluated.

Material and Methods

Study population

A prospective longitudinal study was performed in 50 allograft patients undergoing immunosuppressive therapy with cyclosporine A. Patients were recruited from the Periodontal Unit of the Dental School of the University of Trieste, Italy; written, informed consent was obtained from each subject before enrolment in the study, and the research was conducted in full accordance with ethical principles. Twenty-six patients had undergone renal transplant, 21 had undergone heart transplant and three had undergone liver transplant. Demographic and medical data and smoking history were recorded from all patients at the time of first examination, at least 6 mo after transplantation. The presence of all anterior teeth was considered among the inclusion criteria.

At the beginning of the study, all recruited patients underwent an initial visit to a dental hygienist from whom they received oral hygiene instructions; they were then subjected to periodontal debridment and nonsurgical periodontal therapy, consisting of supragingival and subgingival scaling with both ultrasonic and hand instruments, under antibiotic coverage with amoxicillin, 2 g orally or clarithromycin 500 mg orally, 1 h before the intervention. The whole oral cavity was examined radiologically as part of the periodontal screening process and to exclude other possible oral or maxillofacial diseases.

After the completion of therapy, lasting 4–6 weeks, depending on oral status and motivation, all patients received additional oral hygiene instructions and were placed on a recall maintenance program. All evaluations were performed by an expert dental hygienist, under the supervision of a dentist.

A baseline periodontal examination, confined to the six anterior teeth in each arch, was performed, and the following parameters were investigated and scored. Plaque index was assessed using the system described by O'Leary *et al*. (20) and bleeding index was also recorded (21). Periodontal probing depths were determined, and the percentage of sites exhibiting probing depths of ≥ 3 mm was calculated.

The hyperplastic index was evaluated (22): in brief, for each vestibular and lingual interdental papilla of the six upper and lower anterior teeth, the vertical and horizontal gingival enlargement were measured. For the vertical component, a score between 0 and 3 (0, no gingival hyperplasia; 1, blunting of the gingival margin; 2, overgrowth covering less than one-half of the crown; 3, overgrowth covering more than one-half of the crown) was used. A three-point score was used (0, normal width; 1, thickening up to 2 mm; 2, thickening more than 2 mm) in the evaluation of the horizontal component, which measures the degree of gingival thickening in both labial and lingual directions. The maximum overgrowth score obtained when adding the two components for each interdental unit is five; a total of 20 interdental papillae were examined, giving a potential maximum score of 100, and the hyperplastic index was therefore expressed as a percentage. Patients were dichotomized into two groups: those with a hyperplastic index of $< 30\%$, who had minimal gingival overgrowth, and those with a hyperplastic index of $\geq 30\%$ and clinically significant gingival overgrowth (23).

The same expert hygienist evaluated patients after 3 and 6 mo; during every

visit, reinforcement of the hygiene instruction program was administered. Thirty-four patients attended both control examinations.

A complete drug history was obtained for each subject; the serum concentrations of cyclosporine A and creatinine were measured on the day of each visit for periodontal examination.

Genotyping

Genomic DNA was purified from the buccal swabs of all patients or from the anonymous buffy coats of 100 consecutive healthy blood donors of the same geographic area, by using a standard phenol/chloroform extraction procedure, and the *MDR1* polymorphisms were determined by polymerase chain reaction–restriction fragment length polymorphism assays (24). The polymerase chain reaction consisted of an initial denaturation step at 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, and 1 cycle of 72°C for 7 min. DNA fragments, generated after digestion with restriction enzymes, were separated on a 3% agarose gel for G2677T and on a 2% gel for C3435T. The primers and restriction enzymes used are shown in Table 1.

Statistical analysis

The patients' characteristics were compared between the two groups (hyperplastic index < 30% and hyperplastic index ≥ 30%), using the unpaired *t*-test. Any possible association between sensitivity to cyclosporine and the polymorphisms in each gene was investigated by calculating the odds ratio and 95% confidence intervals from contingency tables and using the two-sided Fisher's exact test. To control for confounding variables, multivariate logistic regression was performed with a model considering the hyperplastic index as the dependent variable and patients' age, gender, education level (lower than high school and high school or higher), genotypes, organ transplanted (heart, kidney or liver), cyclosporine dose and other administered drugs (in particular calcium antagonists, azathioprine, statins, or steroids) as the independent variables.

Associations between the change over time in the hyperplastic index and in the *MDR1* genotypes were assessed using linear mixed models allowing both random intercepts and slopes. To make the hyperplastic index more normally distributed, the square root values of this parameter were calculated. All models were adjusted for gender, education (dichotomized as above), type of transplant and the use or not of other drugs. One-hundred and twenty nine observations at different time points were available for this analysis. At baseline, complete data were available for 50 patients; after 3 mo hyperplastic index values were missing for six patients; and after 6 mo hyperplastic index values were missing for 15 patients. The linear mixed-effect model used for the longitudinal analysis allows data with missing observations to be handled appropriately (25). *p*-values lower than 0.05 were considered to be statistically significant.

Results

One-hundred healthy subjects and 50 transplant patients who required treatment with cyclosporine A and presented with gingival overgrowth were included in the study. The demographic characteristics and periodontal and pharmacological details of the patients are shown in Table 2.

Table 1. Primers used for amplification of polymerase chain reaction fragments and restriction enzymes used for restriction fragment length polymorphism analysis

SNP	Primers	Restriction enzyme
C3435T	5′-TGTTTTCAGCTGCTTGATGG-3′ 5′-AAGGCATGTATGTTGGCCTC-3′	*Dpn*II
G2677T	5′-TGCAGGCTATAGGTTCCAGG-3′ 5′-TTTAGTTTGACTCACCTTCCCG-3′	*Ban*I

At baseline, patients were dichotomized into two groups according to gingival changes: 32 patients (64%) had a hyperplastic index of < 30% and 18 (36%) had a hyperplastic index of ≥ 30% and were considered to have clinically significant gingival overgrowth. There was no statistically significant difference between the two groups in mean age, gender ratio, organ transplanted, interval between transplant and baseline evaluation, cyclosporine dosage, and cyclosporine and creatinine blood levels. Most patients were receiving therapy with drugs in addition to cyclosporine, but there was no statistical difference in the type and number of the other drugs used by the two groups. Only 6.25% of patients were smokers and, of these, all were in the minimal gingival overgrowth group (Table 2).

There was a significant difference between the two groups dichotomized on the basis of hyperplastic index in terms of percentage of sites exhibiting probing depths of ≥3 mm ($p < 0.001$), bleeding index ($p < 0.01$) and plaque index ($p < 0.05$) (Table 2).

Transplant patients receiving cyclosporine were characterized by *MDR1* genotypes similar to those of the nonmedicated controls (C3435T: odds ratio = 0.44, 95% confidence interval = 0.18–1.06, $p = 0.07$; G2677T: odds ratio = 0.62, 95% confidence interval = 0.23–1.68, $p = 0.48$).

When the influence of the genotype on the hyperplastic index was considered, a significant association with the C3435T polymorphism was found, and the mutated 3435TT genotype was significantly more frequent in the group with clinically significant gingival overgrowth (odds ratio = 7.5, 95% confidence interval = 1.323–42.52, $p = 0.019$). Also, the carriage of the polymorphic allele differed significantly between subjects with a hyperplastic index of ≥ 30% (61.1%) and those with a hyperplastic index of < 30% (35.9%; odds ratio = 2.80, 95% confidence interval = 1.21–6.51, $p = 0.021$). No differences between the two groups were observed for the G2677T polymorphism (Table 3).

The results of the logistic regression analysis are reported in

Table 2. Demographic, pharmacological and periodontal details of the transplant patients

Patients' characteristics	Overgrowth < 30% (n = 32)	Clinically significant overgrowth ≥ 30% (n = 18)
Age[a]	56.3 (34–73)	57.9 (35–75)
Gender[b]		
Female	8 (25.0)	28 (58.2)
Male	24 (75.0)	39 (41.8)
Organ transplanted[b]		
Heart	13 (40.6)	8 (44.4)
Kidney	17 (53.1)	9 (50.0)
Liver	2 (6.30)	1 (5.60)
Interval in months between transplant and baseline evaluation[a]	68.81 (4–165)	49.50 (6–136)
Cyclosporine dose at first clinical evaluation[a]	189.8 (125–275)	192.6 (150–350)
Cyclosporine blood levels at first clinical evaluation[a]	152.05 (101–263)	154.81 (101–207)
Creatinine blood levels at first clinical evaluation[a]	1.62 (1.25–2.78)	1.63 (1.2–2.43)
Other drugs[b]		
Ca antagonists	6 (18.7)	6 (33.3)
Steroids	11 (34.3)	7 (38.9)
Statins	7 (21.9)	6 (33.3)
Azathioprine	5 (15.6)	3 (16.7)
Smokers	2 (6.25)	0 (0.00)
Hyperplastic index[c]	13.34 ± 1.670	53.94 ± 3.362***
Sites ≥ 3 mm	23.28 ± 3.181	60.99 ± 6.545***
Bleeding index	16.36 ± 2.605	33.15 ± 6.137**
Plaque index	47.38 ± 4.646	64.96 ± 6.886*

[a]In years: mean (range).
[b]*n* (percentage).
[c]Mean ± SE.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, Student's *t*-test for independent data.

Table 3. *MDR1* genotype distribution in patients with a hyperplastic index of < 30% and a hyperplastic index of ≥ 30%

	Genotype			MUT vs. WT + HET OR (95% CI) *p* value
	WT	HET	MUT	
C3435T				
Hyperplastic index ≥ 30% (18)	2 (11.1)	10 (55.5)	6 (33.4)	7.5 (1.32–42.52)
Hyperplastic index < 30% (32)	11 (34.4)	19 (59.4)	2 (6.20)	0.019*
G2677T				
Hyperplastic index ≥ 30% (18)	8 (44.4)	7 (38.89)	3 (16.7)	1.93 (0.35–10.77)
Hyperplastic index < 30% (32)	14 (43.7)	15 (46.9)	3 (9.40)	0.654

*Fisher's exact test.
The values given in parentheses indicate percentage.
HET, heterozygous; MUT, mutated; WT, wild type.

Table 4. This analysis confirmed an independent, significant association between gingival overgrowth and the variable C3435T genotype (adjusted odds ratio = 9.99, 95% confidence interval = 1.24–80, $p = 0.031$). The other variables were not significantly associated.

Table 4. Adjusted odds ratios (OR) with confidence intervals (CI) and *p*-values for the independent variables in the logistic regression model for the effect of the *MDR1* genotype on the hyperplastic index

Variable	OR	95% CI	*p*-value
Age (years)	1.00	0.96–1.09	0.50
Gender			
Female	1.00		
Male	1.17	0.19–7.04	0.87
Education level			
Lower than high school	1.00		
High school or higher	3.02	0.74–12.34	0.12
Organ transplanted			
Heart	1.00		
Kidney	0.64	0.15–2.76	0.55
Liver	1.88	0.091–38.94	0.68
Use of azathioprine			
No	1.00		
Yes	0.89	0.13–5.81	0.90
Use of calcium antagonists			
No	1.00		
Yes	2.81	0.47–16.73	0.26
Use of steroids			
No	1.00		
Yes	1.57	0.34–7.18	0.56
MDR-1 C3435T genotype			
WT/HET	1.00		
MUT	9.99	1.24–80.55	**0.031**
MDR-1 G2677T genotype			
WT/HET	1.00		
MUT	0.73	0.36–8.18	0.80

The separation of the subjects according to their *MDR1* exon 26 genotype revealed that, at baseline, the mean hyperplastic index was significantly higher in patients carrying the 3435TT genotype compared to those with the 3435CC/CT genotype ($p = 0.019$). No difference was observed at baseline between the two groups of patients in sites exhibiting probing depths of ≥ 3 mm, bleeding index and plaque index (Fig. 1). In both groups of patients, all clinical variables decreased in the second and third clinical examination compared with baseline values. After 6 mo, no significant difference was evident between the two groups in any of the parameters investigated (Fig. 1).

Associations between the change over time in the hyperplastic index and *MDR1* genotypes were assessed using linear models allowing both random intercepts and slopes. The mean monthly change in the square root of

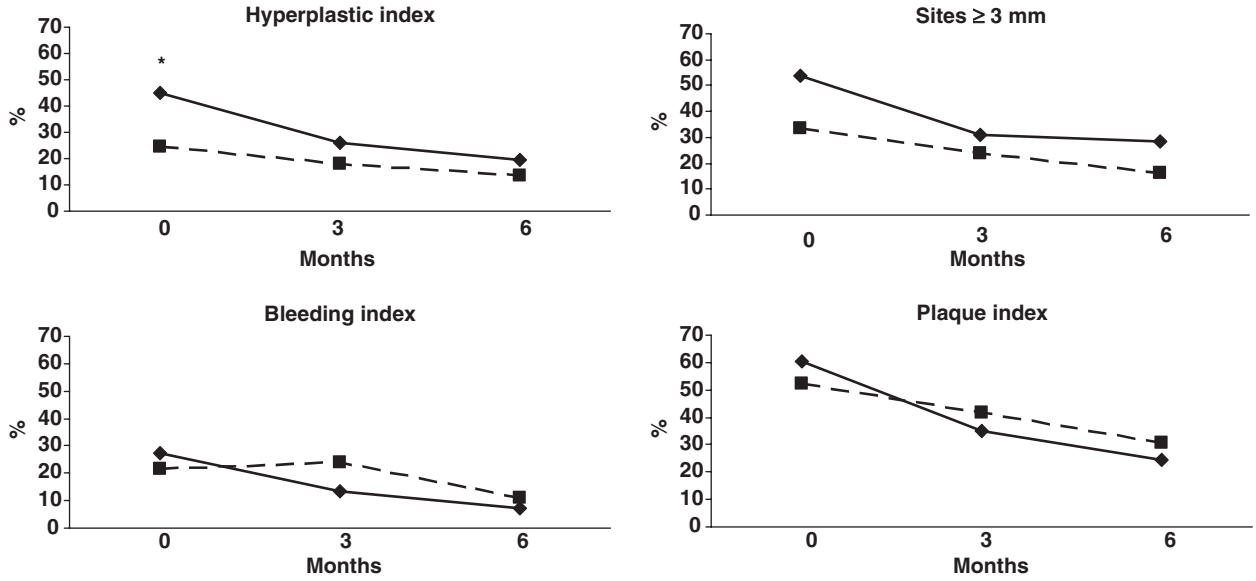

Fig. 1. Hyperplastic index, bleeding index, percentage of sites exhibiting a probing depth of ≥ 3 mm, and plaque index at baseline and after 3 and 6 mo in patients with the 3435TT mutated genotype (diamond) and with the 3435CT/CC genotype (square). *$p < 0.05$, Student's *t*-test for independent data.

Table 5. Monthly change in the square root value of the hypertrophy score between transplant subjects treated with cyclosporine and with different transplant type and *MDR1* genotypes, assessed using the linear mixed model

	Estimate	Standard error	*p*-value
Monthly change	−0.17	0.040	< 0.00001
Liver transplant	−0.34	1.13	> 0.05 (0.76)
Renal transplant	−1.63	0.53	< 0.01 (0.0039)
C3435T TT genotype	1.07	0.70	> 0.05 (0.14)
G2677T TT genotype	−0.010	0.82	> 0.05 (0.99)

Estimates indicate the monthly change in the square root of the hypertrophy index in patients with the specified transplant type compared to patients with heart transplant. The values are adjusted for *MDR1* genotypes, gender, education, use of calcium antagonists, azathioprine and statins: 129 observations for 50 patients were available for this analysis.

the hyperplastic index scores for all patients was significantly different from baseline (−0.22 points per month, $p < 0.00001$; Table 5). Subjects with a renal transplant had a significantly accelerated decrease in the square root value of the hyperplastic index (−1.65, $p < 0.01$), compared to subjects with a heart transplant or a liver transplant (Fig. 2). Adjustment for the *MDR1* genotypes and for gender, education, use of calcium antagonists, azathioprine, statins or steroids, did not alter the observed association (Table 5). In heart transplant patients the cyclosporine dose at different evaluation time points was significantly higher than in subjects with renal transplant or liver transplant (Table 6).

Discussion

The incidence of gingival overgrowth among transplant patients receiving cyclosporine A is high, ranging from 13 to 84.6% in different series (4,26,27), but it is still unclear why some patients are susceptible to this side effect, while others remain unaffected. Among the factors that can play a role in differences in susceptibility, age, gender, smoking habits and type of transplant could be important (1–10); however, in the present study, no difference in these parameters was observed between the two groups of patients. Other drugs concomitantly administered could also play a role in inducing hypertrophy. Calcium antagonists, and in particular nifedipine, are frequently used in patients undergoing therapy with cyclosporine (27) and are also implicated as a cause of gingival overgrowth (28–30). On the contrary, immunosuppressants (such as corticosteroids and azathioprine) have been shown to reduce the severity of this side effect as a result of their anti-inflammatory action on plaque-induced inflammation (9,26,31). However, no difference in the percentage of patients taking these drugs was evident between the two groups in the present study. Cyclosporine dose and blood levels are other potential risk factors for gingival overgrowth; although there is no general agreement on their role, some researchers have found a correlation with gingival overgrowth (12,26). In agreement with the observations reported in other publications (6,31,32), no significant correlation of cyclosporine dose and blood levels with gingival overgrowth was observed in our study.

Cyclosporine is a substrate of P-glycoprotein (33), which plays an important role in the transport of a number of drugs across cell membranes (34). A wide interindividual variability in P-glycoprotein expression and activity has been described in

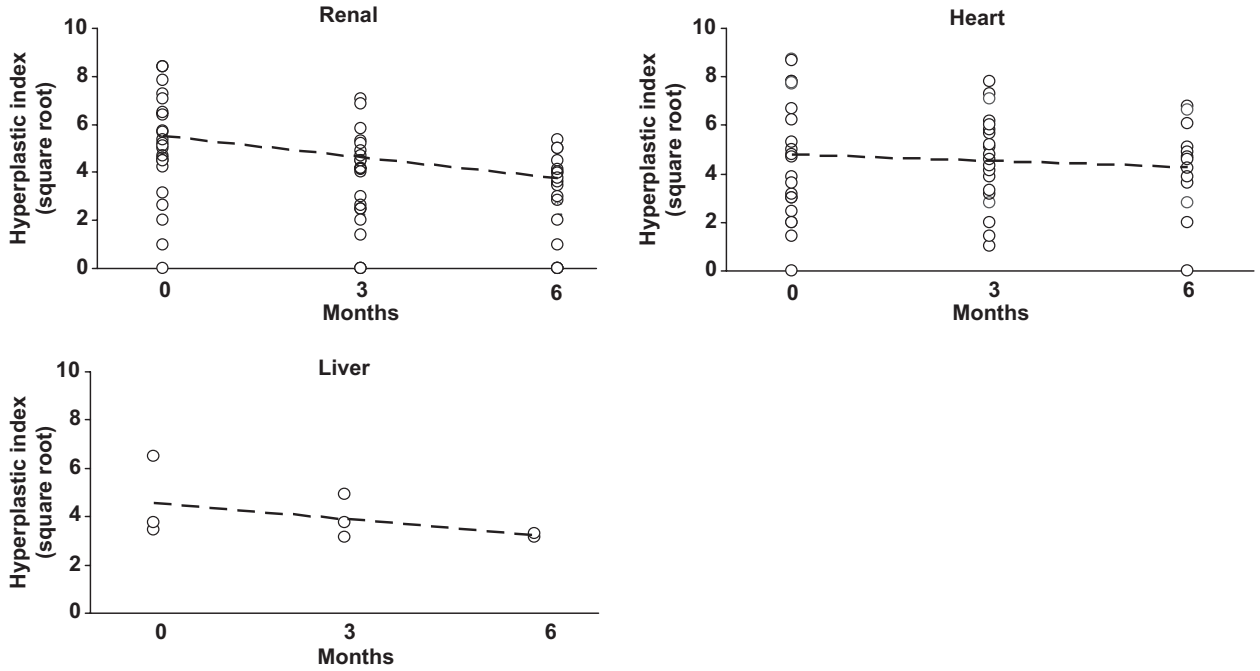

Fig. 2. Display of the square root value of the hyperplastic index and the time after treatment for patients with renal, heart and liver transplant (broken line: least-squares line of the plot).

Table 6. Dose of cyclosporine (mean and range) taken by patients at different evaluation time points (0, 3, 6 mo)

Organ transplanted	Cyclosporine dose mg/d (mean and range)		
	At baseline	1° evaluation	2° evaluation
Kidney	166.3 (125–275)	158.3 (125–275)	160.0 (125–275)
Heart	219.3 (150–350)***	220.0 (220–250)***	227.5 (150–350)***
Liver	166.7 (150–175)	166.7 (150–175)	182.3 (150–175)

The dose taken by patients with heart transplant was, at each time point, significantly higher than the dose taken by liver and renal transplant patients.
***$p < 0.0001$, Student's *t*-test for independent data.

humans, and these differences have been related to variable pharmacokinetics and drug effects (35).

P-glycoprotein is the product of the *MDR1* gene, and a number of polymorphisms of this gene have been described in recent years (18). Among these, a polymorphism in exon 26 (C3435T) and a polymorphism in exon 21 (G2677T/A) have been correlated with the expression and activity of P-glycoprotein (18). In our study, the C3435T mutated genotype was significantly more frequent in patients who exhibited a higher hyperplastic index. Even though this mutation is synonymous (and hence does not change the amino acid sequence) and is not located in the promoter, it has been associated with lower expression and activity of P-glycoprotein (18,35). A recent study (36) has suggested that the presence of a rare codon, marked by the synonymous polymorphism, could affect the timing of cotranslational folding and insertion of P-glycoprotein into the membrane, thereby altering its structure. In addition, a strong association exists between this polymorphism and the G2677T/A missense mutation in exon 21 (37); this mutation has also been associated with differences in the expression of P-glycoprotein, but no association with gingival overgrowth was observed in our study.

Our results differ from those of Drozdzik *et al*. (16), who failed to find any association between the C3435T polymorphism and gingival overgrowth. It is difficult to make a direct comparison between the two studies; indeed, in the article of Drozdzik *et al*., all patients with different degrees of gingival overgrowth were considered and compared with patients without this complication. By contrast, in our study, patients with mild gingival overgrowth (hyperplastic index < 30%) were compared with subjects with a hyperplastic index of ≥ 30%, and the mutated genotype was significantly more frequent in these patients.

It is unclear how P-glycoprotein could contribute to cyclosporine-induced hypertrophy; however, as P-glycoprotein is expressed in the endothelial layers of blood vessels in gingival tissue (17), the C3435T mutated genotype, which is associated with a lower expression and activity of P-glycoprotein, could result in an increased concentration of the drug in this tissue, and hence in the increased hypertrophy. P-glycoprotein has also been found in duct cells of the salivary gland, and it has been suggested that it could participate in the excretion of cyclosporine into saliva (16). It has

also been suggested that cyclosporine salivary concentrations are related to gingival overgrowth; however, the data are not univocal and other research has failed to find any such association (2,38). Our observations, which indicate that subjects with this polymorphism have a greater tendency to develop gingival overgrowth, do not support this hypothesis; it should be noted that P-glycoprotein expression might differ significantly from tissue to tissue, and studies should be performed to evaluate directly the effect of *MDR1* polymorphisms on cyclosporine salivary concentrations.

It has also been suggested that the *MDR1* genotype may modulate inflammatory reactions (17) and a significant association has indeed been observed between polymorphisms in this gene and some inflammatory disorders, in particular inflammatory bowel disease. A recent meta-analysis demonstrated that the 3435T allele and 3435TT genotype are more frequent in patients with ulcerative colitis (39), and the same genotype has been shown in this study to be a risk factor for gingival overgrowth.

The present study showed, in addition, that a proper self-performed plaque-control program, carefully monitored and with renewed motivation for oral hygiene, combined with professional care, leads to a decrease of gingival overgrowth and of all other parameters investigated, after 3 and 6 mo, in transplant patients receiving cyclosporine. The reduction in gingival overgrowth was particularly evident in patients carrying the 3435TT genotype, and the significant difference between the two groups of patients, observed at baseline, disappeared at the second and third examination. A significantly accelerated decrease in gingival overgrowth (square root value of hyperplastic index) was observed also in renal transplant: these patients received a lower cyclosporine dose compared with heart transplant patients. While there is no definite consensus on the impact of dosage (11) on cyclosporine-induced gingival overgrowth, it seems probable that the dose might condition the response to periodontal therapy.

In conclusion, our study has demonstrated that patients with a C3435T mutation have a greater tendency to exhibit a higher hyperplastic index at baseline when treated with cyclosporine. However, a self-performed plaque-control program, after initial nonsurgical therapy, allows all patients (and in particular those with the mutated C3435T genotype) to reduce the hypertrophy. Patients with the C3435T mutation should therefore be examined early and included in these type of protocols. In these subjects, in particular, this approach should decrease the need for surgical therapy, which is often followed by a recurrence of hypertrophy (40,41). The response to this treatment is very effective in patients with renal transplant and less satisfactory in subjects with heart and liver transplant, who should therefore be treated with more intensive periodontal therapy.

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