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P-glycoprotein drug transporter MDR1 gene polymorphism in renal transplant patients with and without gingival overgrowth

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Abstract

Objective: To determine whether there is association between genotypes of drug transporter multidrug resistant (MDR)1 gene coding drug transporter P-glycoprotein and gingival overgrowth in kidney transplant patients.

Methods: Fifty-four unrelated kidney transplant patients suffering from gingival overgrowth as well 120 control transplant patients without overgrowth were enrolled into the study. Gingival overgrowth was assessed by two independent periodontal specialists at 6 months after transplantation. During the post-transplant period all patients were given medication, which included cyclosporine A, diltiazem or verapamil, prednisone, azathioprine. MDR1 C3435T polymorphism was determined using the polymerase chain reaction-restriction fragment length polymorphism assay.

Results: In kidney transplant patients suffering from gingival overgrowth mean score of gingival overgrowth was 1.43 ± 0.63, whereas in control subjects was 0.0. Patients with gingival overgrowth induced by immunosuppressive medication were characterized by similar distribution of MDR1 genotypes. There were no significant differences of 3435CC, 20.4% and 22.5%, 3435CT, 61.1% and 54.2% and 3435TT, 18.5% and 23.3% genotypes (frequencies) between patients with and without gingival overgrowth. The risk of gingival overgrowth was the highest among patients carrying 3435CT genotype (OD 1.33), but did not differ markedly from the other genotypes, i.e. 3435CC (OD 0.88) and 3435TT (OD 0.75). Likewise to genotypes, distribution of alleles was similar in patients with gingival overgrowth and healthy gingiva. The wild-type allele 3435C was found in 50.9% and 49.6% of subjects whereas the mutated allele 3435T was revealed in 49.1% and 50.4% of patients with and without gingival overgrowth, respectively. The evaluated risk of gingival overgrowth in patients with 3435C allele was 1.06 versus 0.95 in those with healthy gingiva. The medication regimen administered in both groups of the study was comparable. Immuno-histochemical studies revealed expression of P-glycoprotein in ducts of the salivary gland.

Conclusion: No association between the MDR1 gene polymorphism and gingival overgrowth was revealed in kidney transplant patients administered cyclosporine A as a principal immunosuppressive agent. Further studies are needed to elucidate the role of P-glycoprotein in drug transport in salivary glands.

Key words: gingival overgrowth; multidrug resistant 1; P-glycoprotein; polymorphism

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Gingival overgrowth frequently occurs in transplant patients receiving immunosuppressive drugs such as cyclosporine (INN cyclosporin), which have been widely used since 1970s. The incidence of gingival overgrowth among renal transplant patients treated with cyclosporine A ranges from 13% to 84.6% (Pernu et al. 1992, Somacarrera et al.

1994, Margiotta et al. 1996, Afonso et al. 2003). Gingival overgrowth induced by cyclosporine A is probably the consequence of alterations in the turnover of epithelial and connective tissues, resulting in an increase in 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 antigens phenotype. While some studies have suggested some associations between the incidence and severity of gingival overgrowth and sex, 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 disease as previously reported (Seymour et al. 1996, Thomas et al. 2000, Thomas et al. 2001, Afonso et al. 2003). It was hypothesized that gingival overgrowth due to cyclosporine A administration is related to stimulation of TGF-β_1 transcription and modulation of interleukin expression (Shin et al. 1998, Myrillas et al. 1999). Gingival inflammation and plaque are believed to influence gingival overgrowth by increasing sequestration of cyclosporine A into the gingival crevice (Ellis et al. 1995). The pharmacokinetics of cyclosporine A and its bioavailability are clearly of importance in mediating its clinical effects including its side effects, such as gingival overgrowth.

The use of cyclosporine A in combination with a calcium channel blocker has been shown to be associated with increased prevalence and severity of gingival overgrowth compared with monotherapy with cyclosporine (Spratt et al. 1999). However, it remains unclear why a proportion of patients are susceptible to gingival overgrowth while others remain unaffected.

Cyclosporine A and calcium channel blockers are substrates of P-glycoprotein, encoded by the multidrug resistant (MDR)1 gene, ABCB1 (according to the official gene code) (Schwab et al. 2003). The protein acts as an adenosine triphosphate-dependent membrane efflux pump. Human P-glycoprotein is present in high levels in normal tissues including the luminal membranes of renal proximal tubules, the biliary canalicular membrane of hepatocytes, the apical surface of mucosal cells in the small and large intestine, the capillary endothelial cells of brain and testis, the adrenal gland and the endometrium of the pregnant uterus (Tanigawara 2000). This tissue distribution suggests that P-glycoprotein plays a role in excreting toxic xenobiotics and metabolites into urine and bile and into the intestinal lumen, and in preventing their accumulation in the brain. There is discrepancy concerning the expression of P-glycoprotein in the salivary glands, which in turn could influence secretion of its substrates into saliva. Early reports negate the presence of P-glycoprotein in salivary glands, but recent data do suggest expression of the protein (Thiebaut et al. 1987). Uematsu et al. (2001) found the expression of P-glycoprotein on the basolateral membrane of serous acinar cells in the major and minor salivary glands, particularly in the anterior lingual gland. So, P-glycoprotein in the salivary glands might contribute to the rate of excretion of drugs being its substrates, among them cyclosporine A. Hoffmeyer et al. (2000) have reported a naturally occurring MDR1 gene polymorphism, which is associated with the expression of P-glycoprotein. This polymorphism consists of a C-to-T exchange at position 3435 in exon 26 of the MDR1 gene. Individuals with the 3435TT genotype had significantly lower duodenal MDR1 expression and function than those with the 3435CC genotype. The difference in P-glycoprotein levels between two groups was greater than 65-fold, and affected the pharmacokinetics of the glycoprotein substrate, i.e. digoxin. Thus specific genotype, i.e. 3435CC leading to an increased expression of P-glycoprotein, mediating excretion of cyclosporine A and calcium channel blockers into saliva, might be a risk factor for development of gingival overgrowth in transplant patients. The frequency of MDR1 genotypes found in a Polish population, 3435CC 23.3%, 3435CT 56.3% and 3435TT 20.4% (Drozdzik et al. 2003), is similar to the data reported from German population (Cascorbi et al. 2001) as well as other white populations, and differ from West Africans (3435CC, frequency 83%), African Americans (3435CC, 61%) or Japanese population (3435CC, 36%) (Schaeffler et al. 2001).

No study has to date explored the possible interaction between individual susceptibility to gingival overgrowth induced by drugs being substrates for P-glycoprotein and polymorphism of MDR1 gene.

Materials and Methods

Patients of Polish origin from the Western Pomerania (Poland) were included in the study after giving informed consent. The protocol of the study was approved by the ethics committee of the Pomeranian Medical University, Szczecin, Poland. A total of 54 unrelated kidney transplant patients suffering from gingival overgrowth (36 males, 18 females) aged 22–68 years (mean 39.5 ± 11.52 years) were enrolled into the study. All patients were examined by two independent consultant periodontal specialists 6 months after kidney transplantation. The patients were assessed using a clinical scoring method according to Pernu et al. (1992). The patients were ascribed a general whole-mouth score of between 0 and 3: 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); 3 = severe gingival overgrowth (overgrowth covering two-thirds of the crown or affectation of the whole attached gingiva).

Control samples were from 120 kidney transplant patients (61 males, 59 females), aged 21–72 years (mean 43.7 ± 10.6 years), who were free from gingival overgrowth signs at 6 months 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; seven patients without gingival overgrowth were given atenolol or prazosine. The serum concentrations of cyclosporine A were measured by fluorescence polarization immunoassay using TDx analyzer (Abbott, Abbott Park, IL, USA) in all patients. The characteristics of medication with drugs that are substrates for glycoprotein P or could affect its function is given in Table 1.

Genotyping

Genomic DNA was from leukocytes contained in 450 μl of venous blood

Table 1. Characteristics of medication at monthly intervals after transplantation

	1 Month	2 Months	3 Months	4 Months	5 Months	6 Months
Patients with gingival overgrowth ($n = 54$)						
cyclosporine concentration (ng/ml)	428.1 ± 220.1	403.9 ± 227.3	339.2 ± 199.9	308.9 ± 204.7	294.2 ± 178.9	266.3 ± 207.8
cyclosporine dose (mg/day)	300.6 ± 88.6 ($n = 54$)	277.4 ± 77.7 ($n = 54$)	261.9 ± 78.9 ($n = 54$)	251.2 ± 84.1 ($n = 54$)	238.1 ± 79.5 ($n = 54$)	233.9 ± 67.7 ($n = 54$)
diltiazem dose (mg/day)	201.6 ± 54.5 ($n = 39$)	201.6 ± 54.5 ($n = 39$)	201.6 ± 54.5 ($n = 39$)	201.6 ± 54.5 ($n = 39$)	201.6 ± 54.5 ($n = 39$)	201.6 ± 54.5 ($n = 39$)
verapamil dose (mg/day)	174.5 ± 72.2 ($n = 15$)	174.5 ± 72.2 ($n = 15$)	174.5 ± 72.2 ($n = 15$)	174.5 ± 72.2 ($n = 15$)	174.5 ± 72.2 ($n = 15$)	174.5 ± 72.2 ($n = 15$)
prednisone dose (mg/day)	18.5 ± 7.3 ($n = 52$)	16.9 ± 6.1 ($n = 52$)	15.9 ± 6.9 ($n = 52$)	13.8 ± 4.7 ($n = 52$)	11.6 ± 3.4 ($n = 52$)	10.8 ± 3.8 ($n = 52$)
Patients without gingival overgrowth ($n = 120$)						
cyclosporine concentration (ng/ml)	460.4 ± 267.2	408.2 ± 324.9	353.5 ± 275.9	325.5 ± 246.8	297.9 ± 186.7	290.8 ± 180.9
cyclosporine dose (mg/day)	295.0 ± 96.7 ($n = 120$)	279.4 ± 89.6 ($n = 120$)	253.4 ± 83.6 ($n = 120$)	247.1 ± 81.9 ($n = 120$)	242.5 ± 76.4 ($n = 120$)	234.7 ± 71.4 ($n = 120$)
diltiazem dose (mg/day)	186.1 ± 50.9 ($n = 81$)	186.1 ± 50.9 ($n = 81$)	186.1 ± 50.9 ($n = 81$)	186.1 ± 50.9 ($n = 81$)	186.1 ± 50.9 ($n = 81$)	186.1 ± 50.9 ($n = 81$)
verapamil dose (mg/day)	184.3 ± 60.5 ($n = 30$)	184.3 ± 60.5 ($n = 30$)	184.3 ± 60.5 ($n = 30$)	184.3 ± 60.5 ($n = 30$)	184.3 ± 60.5 ($n = 30$)	184.3 ± 60.5 ($n = 30$)
prednisone dose (mg/day)	16.94 ± 4.5 ($n = 120$)	15.5 ± 5.2 ($n = 120$)	13.7 ± 4.4 ($n = 120$)	12.7 ± 4.4 ($n = 120$)	11.4 ± 3.6 ($n = 120$)	10.8 ± 3.7 ($n = 120$)

with ethylene diamine tetra-acetic acid as an anticoagulant. DNA was then precipitated in 95% ethanol, dissolved in distilled water and stored at -20°C until analysis. MDR1 C3435T polymorphism was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay (Cascorbi et al. 2001). A 197-bp fragment of P-glycoprotein situated in exon 26 of MDR1 gene was amplified from genomic DNA with the primer pair P1 and P2. The sense primer: 5′-TGTTTTCAGCTGCTTGATGG-3′ and antisense: 5′-AAGGCATGTATGTTGGCCTC-3′ were used. PCR amplification was performed in a total volume of 100 μl that contained 200 ng genomic DNA (dATP, dCTP, dGDP and TTP, 200 μmol/l each, MBI Fermantas, Vilnius, Lithuania), 250 ng of each primer; 1.5 mmol/l magnesium chloride and 2 U Taq DNA polymerase (Gibco-BRL Life Technologies, Glasgow, UK). The amplification reaction was performed using the Mastercycler 5330 (Eppendorf, Hamburg, Germany). PCR amplification consisted of an initial denaturation for 2 min at 94°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 30 s. The terminal elongation was performed at 72°C for 7 min. In an amplified 197 bp fragment the C3435T polymorphism destroys a restriction enzyme cleavage site for Sau3AI such that after digestion with this enzyme for 16 h at 37°C, the 3435C allele can be detected by the presence of two fragments, which are 158 and 39 bp long. The presence of 3435T allele in the amplified segment remains uncut, and the presence of a heterozygous genotype results in the presence of all three bands. DNA fragments generated after restriction enzyme digestion were separated on a 3.5% agarose gel. Restriction fragments were visualized after ethidium bromide staining of the agarose gel with the use of an ultraviolet transilluminator (Biometra, Gottingen, Germany).

Immunohistochemistry

A 4-μm section was cut from paraffin blocks of human salivary gland, deparaffinized in xylene and rehydrated. Endogenous peroxidase activity was blocked with DAKO Peroxidase Blocking Reagent (DAKO, Hamburg, Germany), according to the manufacturer's instructions. A mouse monoclonal anti-P-glycoprotein, Clone F4 (Kamiya

Biochemical Company, Seattle, WA, USA), was applied for 2 h at 37°C. The primary antibody was visualized using the DAKO LSAB+System HRP (DAKO) according to the instruction manual. The slide was counterstained with hematoxylin.

Statistical analysis

Differences of medication between studied groups were compared with the use of the unpaired Student's *t*-test. Frequencies of genotypes were given with their 95% confidence intervals (95% CIs). The distribution of genotypes in renal transplant patients with and without gingival overgrowth statistically evaluated by the use of the χ^2 test with Yate's correction (Epi Info 6 program, version 6.2, World Health Organization, Geneva, Switzerland).

Results

Out of 54 patients with gingival overgrowth, 35 subjects were classified as score 1 of gingival overgrowth, 15 patients were ascribed score 2 and four subjects score 3. Mean score of gingival overgrowth was 1.43 ± 0.63 according to Pernu's scoring system (Pernu et al. 1992). Control transplant patients were characterized by healthy gingiva, i.e. were scored 0. The medication regimen administered in both groups of the study was comparable, except for one time point of prednisone dosage. This difference was, however, of no clinical importance for the present study (Table 1).

The distribution of MDR1 gene genotypes and alleles in kidney transplant patients is shown in Table 2. The patients with gingival overgrowth induced by immunosuppressive medication were characterized by similar distribution of MDR1 genotypes. There were no significant differences in the frequency of 3435CC, 3435CT and 3435TT genotypes between patients with and without gingival overgrowth. The risk of gingival overgrowth was the highest among patients carrying 3435CT genotype (OD 1.33), but did not differ markedly from the other genotypes, i.e. 3435CC (OD 0.88) and 3435TT (OD 0.75). Likewise to genotypes, distribution of alleles was similar in patients with gingival overgrowth and healthy gingiva. The evaluated risk of gingival overgrowth in patients with 3435C allele was 1.06 versus 0.95 in those with healthy gingiva.

Immunohistochemical studies revealed expression of P-glycoprotein in ducts of the salivary gland as it is demonstrated in Fig. 1.

Discussion

The present study revealed expression of P-glycoprotein in duct cells of the salivary gland. The functional role of P-glycoprotein in salivary glands has not been determined yet. However, it is well established that the glycoprotein plays an important role in drug transport across cellular membranes, acting as an efflux pump, thus preventing their accumulation. P-glycoprotein mediates drug excretion into urine and bile and into the intestinal lumen, and in preventing their accumulation in the brain. So, it can be speculated that it also participates in drug excretion into saliva. Among substrates of P-glycoprotein are drugs, whose clinical use is associated with gingival overgrowth, i.e. cyclosporine A, tacrolymus, phenytoin, calcium channel blockers (verapamil, diltiazem) (Seymour et al. 2000, Schwab et al. 2003). Some studies with cyclosporine A revealed that serum concentrations of the drug are not good determinants for gingival overgrowth, whereas a positive correlation was found between cyclosporine concentrations in stimulated saliva and the extent of gingival overgrowth (McGaw et al. 1987, Hefti et al. 1994). These data suggest that P-glycoprotein may play a role in the pathogenesis of cyclosporine A-induced gingival overgrowth in transplant patients, by influencing the drug concentration in the saliva. Other drugs concomitantly administered in those patients may modulate the effects of cyclosporine A on gingival overgrowth. Some of them are also substrates and/or modulators of P-glycoprotein activity, such as calcium channel blockers and prednisone (Wilson et al. 1998, Spratt et al. 1999). However, it remains unclear why a proportion of patients are susceptible to gingival overgrowth while others remain unaffected.

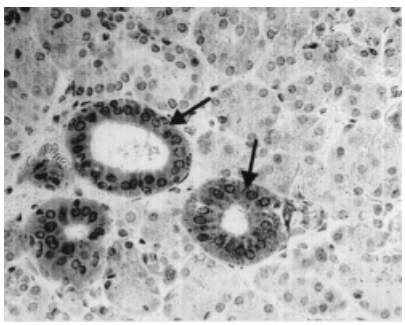

Fig. 1. Immunohistochemical identification of P-glycoprotein in human salivary glands. P-glycoprotein in the gland is dyed as dark gray.

A naturally occurring MDR1 polymorphism have been described and correlated with potential clinical effects. The C3435T polymorphism was found to significantly correlate with the function of MDR1 and the expression of P-glycoprotein (Hoffmeyer et al. 2000). This polymorphism consists of a C-to-T exchange at position 3435 in exon 26 of the MDR1 gene. Individuals with the T/T genotype had significantly lower duodenal MDR1 expression, than those with the C/C genotype. The difference in P-glycoprotein levels between two

Table 2. Distribution of kidney transplant patients with gingival overgrowth and without overgrowth according to MDR1 genotypes

	Gingival overgrowth ($n = 54$)		Healthy gingiva ($n = 120$)		OD (95% CI)	χ^2-Value	*p*-Value
	n	% (95% CI)	*n*	% (95% CI)			
Genotype							
3435CC	11	20.4 (10.6–33.5)	27	22.5 (15.9–30.8)	0.88 (0.18–1.58)	0.10	>0.75
3435CT	33	61.1 (46.9–74.1)	65	54.2 (45.3–62.8)	1.33 (0.35–2.31)	0.73	>0.39
3435TT	10	18.5 (9.2–31.4)	28	23.3 (19.7–28.7)	0.75 (0.14–1.36)	0.51	>0.47
Allele							
3435C	55	50.9 (40.6–60.3)	119	49.6 (43.3–55.9)	1.06 (0.58–1.54)	0.05	>0.81
3435T	53	49.1 (39.7–58.5)	121	50.4 (44.1–56.7)	0.95 (0.52–1.38)	0.05	>0.81

groups was greater than 65-fold, and affected the pharmacokinetics of the glycoprotein substrate, i.e. digoxin. Because C3435T does not change the amino acid sequence and is not located at a promoter position in the MDR1 gene, it is unlikely that this polymorphism directly influences P-glycoprotein expression. Interestingly, a strong association between the C3435T and G2677 (A, T) allele was revealed by Tanabe et al. (2001). Since G2677 (A, T) is a missense mutation, it is likely to be causative for differences in expression. So, activity of P-glycoprotein, which is genetically determined, could be related to gingival overgrowth in renal transplant patients, by influencing an extent of drugs secreted into saliva, which in turn may be responsible for the overgrowth.

The aim of this study was to evaluate an association between MDR1 gene polymorphism, encoding P-glycoprotein and gingival overgrowth in renal transplant patients administered cyclosporine A as an immunosuppressive agent, based on the assumption that C3435TT genotype underlay lower P-glycoprotein expression in the salivary glands. Reduced expression of P-glycoprotein may play a protective role by less effective excretion of cyclosporine into saliva leading to decreased salivary concentrations of the drug, and thus reducing the risk of gingival overgrowth. On the contrary, high activity of P-glycoprotein encoded by 3435CC genotype may promote gingival overgrowth. In the present study, no significant correlation between C3435T polymorphism of the gene and the disease was found. The distribution of wild-type alleles and mutated ones was similar in both groups of transplant patients, i.e. with and without gingival overgrowth, and a risk of overgrowth was comparable in these two groups of patients. Likewise, distribution of the MDR1 gene alleles 3435C and 3435T did not differ markedly between the analyzed kidney transplant patients. Other drug factors, i.e. cyclosporine A dose and serum concentrations, diltiazem and prednisone dose were similar in both groups of the study, thus did not influence the results. However, there is now a considerable body of evidence that the combination of calcium channel blocker (e.g. nifedipine, diltiazem) and cyclosporine A in organ transplant patients produces more gingival overgrowth than if either drug was used singularly (Margiotta et al. 1996, Thomasson et al. 1996). Perhaps, interaction of diltiazem at the P-glycoprotein level (nifedipine is not a substrate for human P-glycoprotein) may promote cyclosporine excretion in the salivary glands, and thus increasing cyclosporine salivary concentration stimulates gingival overgrowth. Inhibitory effects on the activity of P-glycoprotein demonstrate corticosteroids (Lo & Burckart 1999). However, corticosteroids due to their anti-inflammatory actions on plaque-induced gingival inflammation reduce severity of drug-induced gingival overgrowth in organ transplant patients (Hassel & Hefti 1991, Somacarrera et al. 1994). So, drugs administered to renal transplant patients could interact at the P-glycoprotein level in the salivary glands and thus abolish the importance of genetically determined differences in the glycoprotein activity. Furthermore, other gene polymorphism could contribute to the observed results. Cyclosporine A is principally metabolized by CYP3A4/5, whose activity may relay on its genetic polymorphism (Kuehl et al. 2001). So, this polymorphism could be a factor affecting gingival overgrowth. There are also suggestions that functional consequences of the MDR 3435T allele are tissue specific, which in turn may modulate drug disposition (Yates et al. 2003). Therefore, the effect of MDR1 gene polymorphism on activity of P-glycoprotein in salivary glands, and thus on salivary drug concentration should be evaluated in the further studies.

The frequency of MDR1 genotypes found in kidney transplant patients (3435CC, 21.8%, 3435CT, 56.4% and 3435TT, 21.8%) is similar to those in a healthy Polish population (3435CC, 23.3%, 3435CT, 56.3% and 3435TT, 20.4%) (Drozdzik et al. 2003).

In summary, no association between the MDR1 gene polymorphism and gingival overgrowth was revealed in renal transplant patients administered cyclosporine A as a principal immunosuppressive agent.

References

Afonso, M., de Oliveira Bello, V., Shibli, J. A. & Sposto, M. R. (2003) Cyclosporine A-induced gingival overgrowth in renal transplant recipients. *Journal of Periodontology* **74**, 51–56.

Cascorbi, I., Gerloff, T., Johne, A., Meisel, Ch., Hoffmeyer, S. & Schwab, M. (2001) Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. *Clinical Pharmacology and Therapeutics* **69**, 169–174.

Drozdzik, M., Bialecka, M., Mysliwiec, K., Honczarenko, K., Stankiewicz, J. & Sych, Z. (2003) Polymorphism in the P-glycoprotein drug transporter MDR1 gene – a possible link between environmental and genetic factors in Parkinson's disease. *Pharmacogenetics* **13**, 259–263.

Ellis, J. S., Seymour, R. A., Thomasson, J. M. & Idle, J. R. (1995) Periodontal factors affecting crevicular fluid sequestration of nifedipine in drug-induced gingival overgrowth. *Journal of Periodontal Research* **30**, 272–276.

Hassel, T. M. & Hefti, A. F. (1991) Drug-induced gingival overgrowth: old problem, new problem. *Critical Reviews in Oral Biology and Medicine* **2**, 103–137.

Hefti, A., Eshenaur, A. E., Hassell, T. M. & Stone, C. (1994) Gingival overgrowth in cyclosporine A treated multiple sclerosis patients. *Journal of Periodontology* **65**, 744–749.

Hoffmeyer, S., Burk, O., von Richter, O., Arnold, H. P., Brockmoller, J. & Johne, A. (2000) Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proceedings of the National Academy of Sciences USA* **97**, 3473–3478.

Kuehl, P., Zhang, J., Lin, Y., Lambda, J., Assem, M., Schuetz, J., Watkins, P. B., Daly, A., Wrighton, S., Hall, S., Maurel, P., Relling, M., Brimer, C., Yasuda, K., Venkataramanan, R., Storm, S., Thummel, K., Boguski, M. & Schuetz, S. (2001) Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nature Genetics* **27**, 383–391.

Lo, A. & Burckart, G. J. (1999) P-glycoprotein and drug therapy in organ transplantation. *Journal of Clinical Pharmacology* **39**, 995–1005.

Margiotta, V., Pizzo, I., Pizzo, G. & Barbaro, A. (1996) Cyclosporine- and nifedipine-induced gingival overgrowth in renal transplant patients: correlations with periodontal and pharmacological parameters, and HLA-antigens. *Journal Oral Pathology and Medicine* **25**, 128–134.

McGaw, T., Lam, S. & Coates, J. (1987) Cyclosporine-induced gingival overgrowth: correlation with dental plaque scores, gingivitis scores, and cyclosporine levels in serum and saliva. *Oral Surgery, Oral Medicine, Oral Pathology* **64**, 293–297.

Myrillas, T., Linden, G. J., Marley, J. J. & Irwin, C. R. (1999) Cyclosporine A regulates interleukin-1b and interleukin-6 expression in gingiva: implications for gingival overgrowth. *Journal of Periodontology* **70**, 294–300.

Pernu, H. E., Pernu, H. M. L., Huttunen, K. R. H., Nieminen, P. A. & Knuuttila, M. L. E. (1992) Gingival overgrowth among renal

transplant recipients related to immunosuppressive medication and possible local background factors. *Journal of Periodontology* **63**, 548–553.

Schaeffler, E., Eichelbaum, M., Brinkmann, U., Penger, A., Asante-Poku, S. & Zanger, U. M. (2001) Frequency of C3435T polymorphism of MDR1 gene in African people. *Lancet* **358**, 383–384.

Schwab, M., Eichelbaum, M. & Fromm, M. F. (2003) Genetic polymorphisms of the human MDR1 drug transporter. *Annual Review of Pharmacology and Toxicology* **43**, 285–307.

Seymour, R. A., Ellis, J. S. & Thomasson, J. M. (2000) Risk factors for drug-induced gingival overgrowth. *Journal of Clinical Periodontology* **27**, 217–223.

Seymour, R. A., Thomason, J. M. & Ellis, J. S. (1996) The pathogenesis of drug-induced gingival overgrowth. *Journal of Clinical Periodontology* **23**, 165–175.

Shin, G. T., Khanna, A., Ding, R., Sharma, V. K., Lagman, M., Li, B. & Sunathiran, M. (1998) In vivo expression of transforming growth factor-β1. *Transplantation* **65**, 313–318.

Somacarrera, M. L., Hernandez, G., Acero, J. & Moskow, B. S. (1994) Factors relating to the incidence and severity of cyclosporine-induced gingival overgrowth in transplant patients. A longitudinal study. *Journal of Periodontal Research* **65**, 671–675.

Spratt, H., Boomer, S., Irwin, C. R., Marley, J. J., James, J. A. & Maxwell, P. (1999) Cyclosporine-associated gingival overgrowth in renal transplant recipients. *Oral Disease* **5**, 27–31.

Tanabe, M., Ieiri, I., Nagata, N., Inoue, K., Ito, S. & Kanamori, Y. (2001) Expression of p-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. *Journal of Pharmacology and Experimental Therapeutics* **297**, 1137–1143.

Tanigawara, Y. (2000) Role of P-glycoprotein in drug disposition. *Therapeutic Drug Monitoring* **22**, 137–140.

Thiebaut, F., Tsuruo, T., Hamada, H., Gottesman, M. M., Pastan, I. & Willingham, M. C. (1987) Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proceedings of the National Academy of Sciences USA* **84**, 7735–7738.

Thomas, D. W., Baboolal, K., Subtamanian, N. & Newcombe, R. G. (2001) Cyclosporine A-induced gingival overgrowth is unrelated to allograft function in renal transplant recipients. *Journal of Clinical Periodontology* **28**, 706–709.

Thomas, D. W., Newcombe, R. G. & Osborne, G. (2000) Risk factors in the development of cyclosporine-induced gingival overgrowth. *Transplantation* **70**, 552–556.

Thomasson, J. M., Seymour, R. A., Ellis, J. S., Kelly, P. J., Parry, G., Wilkinson, R. & Idle, J. R. (1996) Determinants of gingival overgrowth severity in organ transplant patients: an examination of the role of HLA phenotype. *Journal of Clinical Periodontology* **23**, 628–634.

Uematsu, T., Yamaoka, M., Matsuura, T., Doto, R., Hotomi, H., Yamada, A., Hasumi-Nakayama, Y. & Kayamoto, D. (2001) P-glycoprotein expression in human major and minor salivary glands. *Archives of Oral Biology* **46**, 521–527.

Wilson, R. F., Morel, A., Smith, D., Koffman, C. G., Ogg, C. S. & Rigden, S. P. (1998) Contribution of individual drugs to gingival overgrowth in adult and juvenile renal transplant patients treated with multiple therapy. *Journal of Clinical Periodontology* **25**, 457–464.

Yates, Ch., Zhang, W., Song, P., Li, S., Gaber, O., Kotb, M., Honaker, M., Alloway, R. & Meilohm, B. (2003) The effect of CYP3A5 and MDR1 polymorphic expression on cyclosporine oral disposition in renal transplant patients. *Journal of Clinical Pharmacology* **43**, 555–564.

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