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Gingival overgrowth in cyclosporine, tacrolimus, or sirolimus-based immunosuppressive regimens and the single nucleotide IL-6 (–174 G/C) gene polymorphism

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ABSTRACT

Objective: Interleukin-6 (IL-6) may be involved in drug-induced gingival overgrowth (GO). The present study was conducted to assess the association between IL-6 (–174 G/C) gene polymorphism and GO in renal transplant recipients under cyclosporine (CsA), tacrolimus (Tcr), or sirolimus (Sir)-based regimens.

Methods: Within an eligible population, 45 unrelated subjects were selected for each CsA, Tcr, and Sir group, totaling a sample of 135 subjects. GO was visually assessed and subjects were assigned as controls (non-responders) or cases (responders) in a post hoc definition. IL-6 gene polymorphism was assessed using the polymerase chain reaction amplification and digestion. The distribution of genotypes and allele frequencies in responders and non-responders were compared using the Chi-squared test.

Results: The number of responders was 27 (60.0%), 13 (28.9%), and 7 (15.6%) in the CsA, Tcr, and Sir groups, respectively. No differences could be observed at frequencies of –174GG, –174CG, and –174CC genotypes when comparing responders to non-responders in the CsA, Tcr, and Sir groups. Similar to genotypes, allele frequencies showed no differences between responders and non-responders in all groups.

Conclusions: No association between IL-6 (–174 G/C) gene polymorphism and gingival overgrowth was observed in renal transplant recipients under CsA, Tcr, or Sir-based immunosuppressive maintenance regimens.

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1. Introduction

Fibrotic enlargement of the gingiva is commonly viewed as a side effect of different drug categories, including antic-

onvulsivants, calcium channel blockers (CCB), and immunosuppressants.¹ This factor is frequently present in renal transplant recipients under maintenance therapies based on cyclosporine (CsA) or tacrolimus (Tcr) when applied as the

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main immunosuppressive agent.^{2–8} Recently, this undesirable side-effect, called gingival overgrowth (GO), was also described in transplant subjects under sirolimus (Sir)-based regimens, but within a non-significant clinical threshold.⁹ The management of drug-induced gingival enlargement in this group of solid organ recipients is a concern among dental health care professionals due to its potential functional, aesthetic, and systemic complications.

Mechanisms underlying gingival tissue response to medications inducing GO are still not fully understood. As only a subset of subjects develop GO, it has been hypothesized that they present a fibroblast profile with abnormal susceptibility.^{1,10,11} There is also evidence that gingival cytokines are involved in the pathogenesis of the disease. Subjects presenting GO tend to have an unbalanced cytokine profile with abnormal levels of specific cytokines in the gingival tissues.¹⁰ It has been proposed that the proinflammatory interleukin-6 (IL-6) may play a role in the fibrogenic events of drug-induced GO. IL-6 commonly targets fibroblasts both by enhancing proliferation as well as by exerting a positive regulation on collagen and glycosaminoglycan synthesis.^{12,13} In fact, an increased IL-6 expression within the gingival connective tissue has been reported to be a histological feature of CsA-induced GO.^{12,14} Nevertheless, there is no available data concerning IL-6 in Tcr and Sir-based regimens.

It is recognized that the variation in cytokine and cell receptor production can be at least partially explained by the presence of genetic polymorphisms, which may result in either altered expression or functional changes of the encoded molecules, possibly making individuals more susceptible to a given disease or even resulting in increased disease severity.^{15,16} Polymorphisms in the promoter region of the IL-6 gene may result in interindividual variation in the transcription and the expression of this cytokine. A single nucleotide –174 G/C polymorphism, located within the IL-6 promoter, has been reported to influence IL-6 expression, with heterozygote and homozygote subjects presenting different cytokine profiles.^{16,17}

Variations in individual susceptibility to GO and differences in individual responses may well be a result of intra-subjects' pharmacological and demographic variables, oral conditions, and/or genetic predisposition. Different studies have investigated potential risk factors for the development of GO. While many studies have shown some degree of association between GO and variables, such as gender, time since transplant, papillary bleeding index, the use of calcium channel blockers (CCB), and immunosuppressant dosage, other have failed to demonstrate significant associations for these GO-related variables.^{4–7,9,18} Different polymorphisms of specific genes have also been investigated as potential risk factors for GO in other studies,^{19–22} the findings of which have been diverse. One study which assessed IL-6 polymorphisms demonstrated no association with GO in subjects of Polish origin who had received CsA.²⁰

In this light, GO is thought to be multifactorial, and it remains unclear why some subjects develop GO, whereas others are unaffected. For this reason, the search for potential risk factors is still indispensable. The terms “responders” and “non-responders” have been proposed to describe subjects who positively react to the drugs and develop GO at different levels.

Therefore, given that: (a) IL-6 may be an important cytokine in drug-induced GO; (b) IL-6 polymorphism may determine subjects with altered cytokine expression levels; (c) only one previous study²⁰ investigated the association between IL-6 (–174 G/C) gene polymorphism and GO in renal transplant recipients under CsA-based regimens; (d) there is no data concerning IL-6 (–174 G/C) polymorphisms and GO in subjects under Tcr or Sir regimens, the aim of the present study was to assess the possible association between single nucleotide IL-6 (–174 G/C) polymorphisms and GO in renal transplant recipients under CsA, Tcr, and Sir immunosuppressive maintenance regimens.

2. Materials and methods

2.1. Study design and sampling strategy

The present study employed a cross-sectional design and involved a subset of unrelated renal transplant recipients under immunosuppressive maintenance therapy from the city of Belo Horizonte, Brazil. This study has been approved by the Research Ethics Committee from the Federal University of Minas Gerais (ETIC 514/05). All participants were informed of the goals of the study and were provided with a written informed consent form prior to their participation in the study. Subjects' rights were protected at all times.

An eligible sample was selected and recruited from the organ transplant unit of 2 public hospitals. Patients were seen on a regular basis at the respective hospitals to monitor drug therapy and graft survival. During the data collection period (from September 2007 to October 2008), patients meeting the inclusion criteria were randomly invited to participate in the study in accordance with the accessibility and availability of the subjects in the post-transplant maintenance routine.

In this approach, every eligible subject was examined and included in the study groups according to the main immunosuppressive agent. They were also assigned as controls (non-responders/GO– subjects) or cases (responders/GO+ subjects) in a post hoc definition. Hence, a convenience sample of 135 subjects was formed and divided into the CsA group ($n = 45$), the Tcr group ($n = 45$), and the Sir group ($n = 45$).

Participants were all at least 2-month post-renal transplant recipients under an immunosuppressive therapy based on CsA, Tcr, or Sir as the main immunosuppressive agent. At the time of examination, they were 18 years of age or older and had a minimum of 6 of the 12 most anterior teeth in the upper or lower dental arches. These subjects also came from the same geographic area and living conditions, were of a low socioeconomic status, and made up a multiethnic group. Subjects under combined therapies of CsA, Tcr, and Sir, as well as current and former smokers, were excluded from the study. Ethnicity was not established, as the hazards of judging Brazilians by color and race has been cautioned in a previous report.²³

2.2. Medical and pharmacological variables

Medical and pharmacological data were obtained from each subject's medical records. As part of long-term management,

transplant recipients were screened regularly for whole blood and serum concentrations of the main immunosuppressive agent. Data from the most recent assessment, usually collected in the last medical examination (0–30 days), were recorded. Gender, age, time since transplant, main immunosuppressive agent dosage, and serum level, as well as the concomitant use of calcium channel blockers (CCB), were used in the analysis. Patients' medical records were thoroughly examined, and data were confirmed (or updated when pertinent) by the organ transplant medical group.

2.3. Gingival assessments

After the examination of patient medical records and after applying exclusion and inclusion criteria, subjects were scheduled for gingival evaluation. Examinations were performed in a separated room within the hospital units, under proper lighting and infection control conditions. Inflammation and oral hygiene status were assessed through the plaque index²⁴ and the papillary bleeding index.²⁵ This methodological approach was supported by cross-sectional studies that demonstrated that gingival bleeding has been a good indicator of the role of inflammation induced by bacterial plaque in GO severity.^{4,5,7,9}

The score for the level of oral hygiene was obtained according to the plaque index.²⁴ Measurements were performed on the lingual, labial, and interproximal surfaces of the six most anterior teeth in the upper and lower arches. Scores for each site were summed and the mean values attained. The papillary bleeding index was recorded in the interproximal papilla of the six most anterior teeth in both arches.²⁵

Gingival evaluation was performed by one trained periodontist who was blinded to each patient's identity, medical history, and immunosuppressive regimen. To determine intra-examiner reliability, the papillary bleeding index and GO scores of 10 subjects were evaluated at the beginning of the study and repeated 1 month later. All unweighted *Kappa* scores were greater than 0.92, and intraclass correlation coefficients were greater than 0.90.

The present study assessed the GO in the 12 most anterior teeth, as previously described and justified.^{4,5,7,9} Briefly, upper and lower anterior teeth were evaluated through visual inspection. Scores between 0 and 5, depending on the amount of both horizontal and vertical enlargement, were assigned to each buccal and lingual papilla of the 6 most anterior upper and lower teeth. Likewise, according to the number of anterior teeth available on each dental arch, a total of 20 such papilla could be selected and examined. A potential maximum GO score of 100 could be assigned and expressed as a percentage. Subjects with GO scores ≥ 30 were classified as having clinically significant overgrowth, as previously suggested.²⁶

2.4. Sample collection and DNA isolation

Oral mucosa swabs were taken once from each subject. The swabs were performed using sterile plastic tips. After gently scraping the oral mucosa, the tip was immediately immersed in 2 ml sterile microtubes containing 1500 μ l of Krebs buffer (NaCl 20%, KCl 2%, CaCl₂ 2%, H₂O 2%, 0.29 g/l MgSO₄, 5.95 g/l KH₂PO₄, 1.80 g/l C₆H₁₂O₆). DNA extraction was performed as

previously described.¹⁶ A pellet of epithelial cells was obtained by centrifugation at 200 \times *g* for 5 min. The supernatant was removed and 20 μ l of silica (SiO₂)¹ and 450 μ l of lysis buffer (6.0 M GuSCN, 65 mM Tris-HCl pH 6.4, 25 mM EDTA, and 1.5% Triton X-100) were added. Samples were homogenized using a vortex and incubated for 30 min at 56 °C. After this incubation, samples were submitted to another centrifugation (200 \times *g*) and the supernatant was discharged. The pellet obtained (with the DNA adsorbed onto the silica) was washed twice with 450 μ l of washing buffer (6.0 M GuSCN, 65 mM Tris-HCl, pH 6.4), twice with 450 μ l of 70% ethanol, once with 450 μ l of acetone, and then dried at 56 °C for 20 min. Finally, 100 μ l of TE buffer (10 mM Tris-HCl pH 8.0 and 1 mM EDTA) was added and incubated at 56 °C for 12 h. After incubation, the solution was homogenized and centrifuged, and the supernatant containing DNA was obtained.

2.5. Polymerase chain reaction (PCR) and restriction endonuclease digestion

IL-6 (–174G/C) polymorphism was assessed by PCR amplification and digestion. The sense primer²⁷ 5'-CAGAAGAACTCAGATGACTG-3' and the antisense primer²⁷ 5'-GTGGGGC-TGATTGGAAACC-3' were used with a product size of 431 base pairs (bp). PCR was carried out in a total volume of 50 μ l, containing 10 μ l (~400 ng) of DNA, pre-mixed buffer solution (50 mM KCl, 10 mM Tris-HCl pH 8.4, 0.1% Triton X-100, 1.5 mM MgCl₂, deoxynucleotide triphosphates, *Taq* DNA polymerase²) and primers (20 pmol/reaction). The amplification conditions consisted of 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 56 °C for 35 s, and 72 °C for 30 s. The run was terminated by final elongation at 72 °C for 5 min.

The products were digested with 6 units of *Hsp92II*³ at 37 °C for 4 h, and digestion products of 229 + 122 + 51 + 29 bp and 229 + 173 + 29 bp were obtained for the C and G alleles, respectively. Therefore, genotypes were determined as follows: (a) CC = 229 + 122 + 51 + 29 bp; (b) GC = 229 + 173 + 122 + 51 + 29 bp; (c) GG = 229 + 173 + 29 bp. The separation and visualization of the digestion products was performed in a 10 cm \times 7 cm 6.5% polyacrylamide gel electrophoresis with silver staining.

2.6. Statistical analysis

From each single patient, data were collected and analyzed using statistical software.⁴ The normality of the data was assessed by means of the Lilliefors test. The total sample and immunosuppressant groups were described in relation to variables of interest. First, the sample was divided according to the presence (responders) or absence (non-responders) of gingival overgrowth, regardless of the main immunosuppressive agent (CsA, Tcr, or Sir). Responders and non-responders were compared using the Student's *t* and Mann-Whitney tests, when appropriate. Next, the presence/absence of GO was

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² Phoneutria, Brazil.

³ Promega, Madison, WI, USA.

⁴ Statistical Package for Social Sciences, Version 16.0 for Windows—SPSS Inc., Chicago, IL, USA.

Table 1 – Characteristics of the sample in relation to gingival overgrowth.

Variables	GO+ subjects (responders)			
	Total sample	CsA group	Tcr group	Sir group
Number of subjects	47 (34.8%)	27 (60.0%)	13 (28.9%)	7 (15.6%)
Number of subjects with GO scores				
From 1 to 10%	24 (17.8%)	13 (28.8%)	7 (15.5%)	6 (13.3%)
From 11 to 20%	5 (3.7%)	2 (4.4%)	2 (4.4%)	1 (2.2%)
From 21 to 29%	8 (5.9%)	8 (17.8%)	0 (0.0%)	0 (0.0%)
≥30% (clinically significant)	10 (7.4%)	6 (13.3%)	4 (8.9%)	0 (0.0%)
Mean GO score (%)	16.88 ± 13.71 (2.00–52.00)	19.20 ± 13.95 (3.00–52.00)	17.08 ± 15.25 (2.00–44.00)	7.57 ± 2.44 (4.00–22.00)
Mean GO score per papilla	0.84 ± 0.69 (0.1–2.60)	0.96 ± 0.70 (0.15–2.60)	0.85 ± 0.76 (0.10–2.20)	0.38 ± 0.12 (0.2–0.6)
% of papilla affected by GO	34.31 ± 23.15 (5.00–95.00)	41.20 ± 24.25 (10.00–95.00)	30.77 ± 20.40 (5.00–80.00)	14.29 ± 4.50 (10.00–20.00)

CsA = cyclosporine; Tcr = tacrolimus; Sir = sirolimus; GO = gingival overgrowth. Mean ± SD unless specified; percents in relation to the total number of subjects in the group.

evaluated within each immunosuppressant group. For multiple comparisons of parametric data, the one-way ANOVA was used to estimate overall significance, followed by a post hoc Student's t-test. For multiple comparisons of non-parametric data, a Kruskal–Wallis test was used to estimate overall significance, followed by a post hoc Dunn's test. The distribution of genotypes and allele frequencies in responders and non-responders were compared using the Chi-squared test. The Yates correction was used when appropriate. The study groups were tested for the Hardy–Weinberg equilibrium,

comparing the expected with the observed genotype frequencies.²⁸ All estimates were determined to be significant when the p-value < 0.05.

3. Results

Characteristics of the sample in relation to GO are presented in Table 1. In the total sample, when considering subjects, regardless of the main immunosuppressive agent, the number

Table 2 – Characteristics of the sample in relation to variables of interest according to the presence/absence of gingival overgrowth (GO).

Variables	Responders (GO+)	Non-responders (GO–)	p
Total sample (n = 135)			
Male (n)	30 (63.8%)	49 (55.7%)	
Female (n)	17 (36.2%)	39 (44.3%)	0.360
Age (years)	42.64 ± 9.94	43.02 ± 11.21	0.844
Time since transplant (months)	85.72 ± 51.62	50.03 ± 46.52	<0.001
Concomitant CCB use	31 (66.0%)	16 (18.2%)	<0.001
CsA group (n = 45)			
Male (n)	17 (63.0%)	9 (50.0%)	
Female (n)	10 (37.0%)	9 (50.0%)	0.388
Age (years)	42.29 ± 10.27	43.72 ± 9.99	0.891
Daily dosage of CsA (mg)	182.78 ± 49.87	169.44 ± 55.94	0.407
Time since transplant (months)	111.85 ± 47.67	107.67 ± 52.16	0.782
Concomitant CCB use	18 (66.7%)	5 (27.8%)	0.011
Tcr group (n = 45)			
Male (n)	9 (69.2%)	17 (53.1%)	
Female (n)	4 (30.8%)	15 (46.9%)	0.321
Age (years)	41.23 ± 9.47	43.72 ± 11.20	0.485
Daily dosage of Tcr (mg)	6.69 ± 4.21	5.00 ± 2.88	0.202
Time since transplant	53.31 ± 34.66	34.56 ± 31.44	0.085
Concomitant CCB use	9 (69.2%)	5 (15.6%)	<0.001
Sir group (n = 45)			
Male (n)	4 (57.1%)	23 (60.5%)	
Female (n)	3 (42.9%)	15 (39.5%)	0.867
Age (years)	42.71 ± 10.67	42.11 ± 11.95	0.901
Daily dosage of Sir (mg)	2.57 ± 1.13	2.60 ± 1.03	0.938
Time since transplant	45.14 ± 30.15	35.76 ± 31.29	0.468
Concomitant CCB use	4 (57.1%)	6 (15.8%)	0.016

GO = gingival overgrowth; CCB = calcium channel blockers; CsA = cyclosporine; Tcr = tacrolimus; Sir = sirolimus; mean ± SD unless specified; statistically significant p-values are shown in bold.

of responders [(GO+) subjects presenting any score of GO] was 47 (34.8%), with a mean GO score of 16.88 ± 13.71 . Out of these 47 subjects with GO, 10 subjects (7.4%) were classified as presenting clinically significant GO (scores $\geq 30\%$).

When GO was evaluated within the immunosuppressant groups, the number of responders was 27 (60.0%), 13 (28.9%), and 7 (15.6%) in the CsA, Tcr, and Sir groups, respectively. The CsA group presented a mean GO score of 19.20 ± 13.95 , and 6 subjects (13.3%) were classified as presenting clinically significant GO. The Tcr group presented a mean GO score of 17.08 ± 15.25 , and 4 subjects (8.9%) were classified as presenting clinically significant GO. The Sir group presented a mean GO score of 7.57 ± 2.44 , and no subjects were classified as presenting clinically significant GO.

Characteristics of the sample in relation to variables of interest are presented in Table 2. Responders, as compared to non-responders, presented a higher time since transplant ($p < 0.001$) and a higher frequency of concomitant CCB use ($p < 0.001$) in the total sample. No differences between responders and non-responders were observed in relation to gender and age. Responders within CsA ($p = 0.011$), Tcr ($p < 0.001$), and Sir ($p = 0.016$) groups presented a higher frequency of concomitant CCB use when compared to their controls (non-responders within the same immunosuppressant group). Furthermore, no differences could be observed in relation to gender, age, time since transplant, and daily dosage of the main immunosuppressive agent.

Distribution of IL-6 genotypes, as well as allele frequencies, is presented in Table 3. Responders and non-responders were characterized by a similar distribution of IL-6 genotypes. Considering the CsA, Tcr, and Sir groups in an isolated manner, no differences could be observed in the frequency of -174GG, -174CG, and -174CC genotypes between the GO+ and GO- subjects, nor could they be observed when all groups were considered together, regardless of the main immunosuppressant. Similar to genotypes, the G and C allele frequencies showed no differences between responders and non-responders in all groups.

When genotypes and allele frequencies were compared between the subjects who presented clinically significant GO and non-responders in the total sample, no differences could be observed (data not shown).

4. Discussion

No available data could be found regarding the association between IL-6 (-174 G/C) polymorphisms and GO in renal transplant recipients under Tcr and Sir-based regimens. In relation to CsA-based regimens, the absence of such an association was described in one prior study.²⁰ The present study provides preliminary evidence that the IL-6 (-174 G/C) polymorphism has no effects on GO in renal transplant recipients under Tcr and Sir immunosuppressive maintenance regimens and corroborates with the available data on the absence of association in CsA regimens.

IL-6 is a pleiotropic cytokine capable of regulating proliferation, differentiation, and activity in a variety of cell types. It has been shown to positively affect the growth and metabolism of connective tissue cells, including fibroblasts,¹⁰ playing

Table 3 – Distribution of -174C/G IL6 genotype and allelic frequency in immunosuppressant groups according to the presence/absence of gingival overgrowth.

IL6	Responders (GO+)	Non-responders (GO-)	p
Total sample			
Genotypes			
-174GG	29 (61.7%)	43 (48.9%)	0.228
-174CG	16 (34.0%)	35 (39.8%)	
-174CC	2 (4.3%)	10 (11.4%)	
Alleles			
-174G	78.7%	68.8%	0.081
-174C	21.3%	31.2%	
Cyclosporine group			
Genotypes			
-174GG	18 (66.7%)	11 (61.1%)	0.913
-174CG	8 (29.6%)	6 (33.3%)	
-174CC	1 (3.7%)	1 (5.6%)	
Alleles			
-174G	81.5%	77.8%	0.667
-174C	18.5%	22.2%	
Tacrolimus group			
Genotypes			
-174GG	6 (46.2%)	15 (46.9%)	0.270
-174CG	7 (53.8%)	12 (37.5%)	
-174CC	0 (0.0%)	5 (15.6%)	
Alleles			
-174G	73.1%	65.6%	0.493
-174C	26.9%	34.4%	
Sirolimus group			
Genotypes			
-174GG	5 (71.4%)	17 (44.7%)	0.316
-174CG	1 (14.3%)	17 (44.7%)	
-174CC	1 (14.3%)	4 (10.5%)	
Alleles			
-174G	78.6%	67.1%	0.394
-174C	21.4%	32.9%	

GO = gingival overgrowth.

an important regulatory role in the turnover of periodontal tissues^{10,29} and an important pathogenic role in fibrotic events.^{30–33} Its secretion levels are determined by the producing cell type, the genetic background, and the nature of the stimulus.²⁹ There is evidence that CsA stimulates IL-6 secretion by fibroblasts within the gingiva^{13,14,34} and other tissues,³⁵ thus modifying extracellular matrix synthesis and degradation.

Molecular mechanisms underlying GO in Tcr and Sir-based immunosuppressive regimens have not been described, but similarities with CsA-induced GO could be hypothesized since ultrastructural findings have been reported to be analogous in all drug categories.^{1,11,36,37} In addition, similarities of CCB and CsA effects on collagenous extracellular metabolism have been reviewed in prior studies, showing that both drugs influence the production of fibroblast cytokines.¹⁰

It has been advocated that GO is the preferred term for all drug-related gingival lesions, formerly known as gingival hypertrophy or hyperplasia, since these early terminologies did not precisely reflect the histology of the drug-modified

gingival tissue.¹ Although the pharmacological effect of each drug is unique, all of these seem to produce similar impacts on the connective gingival tissue, causing common clinical and ultrastructural findings.^{1,36} Moreover, the clinical and pathological features in the drug-induced GO occur regardless of the drug administered. The histopathology of the lesion is similar in all drug categories. Ultrastructural analysis of gingival specimens demonstrated that the increased tissue volume is due to a connective tissue response, which is characterized by an excessive accumulation of the extracellular matrix, especially collagen. An increase in the number of fibroblasts has been reported, but this finding is still controversial.^{1,10,11,36,37}

In humans, the IL-6 gene is located in the short arm of chromosome 7 (7p21) and displays a single nucleotide polymorphism in the promoter region (–174 G/C), which has been found to be associated with variations of IL-6 expression and serum levels. Genotypes of the IL-6 polymorphism have been evaluated in relation to the transcription and levels of cytokine in serum or tissues. Some evidence has shown that increased levels of cytokine tend to occur in GG genotypes. G allele carriers, when compared with CC individuals, tend to present increased plasma levels of IL-6, as well as higher IL-6 transcriptional activity, and tend to develop higher inducible IL-6 responses.^{17,38–40} Nevertheless, discrepancies in these profiles were previously reported⁴¹ and C allele were associated with higher IL-6 levels.

Evidence linking the genetic IL-6 polymorphism to certain diseases or conditions, to disease severity, and/or to clinical findings of diseases, including fibrosis-related events, has been reported in prior literature^{15,39,40,42}. The role of IL-6 polymorphisms in periodontal diseases has also been documented. Studies have shown associations with incidence, severity, extent, and response to treatment.^{16,38,39,43–45}

For this reason, the altered transcription as well as levels of IL-6, due to the variant IL-6 genotype, may well influence the local inflammation response and may in turn impact the GO. Based on this assumption, Drozdziak et al.²⁰ evaluated renal transplant recipients of Polish origin and showed no significant association between the –174 G/C polymorphism of the IL-6 gene and GO. The authors reported that the distribution of genotypes and G/C alleles between subjects with and without GO presented no significant difference. Findings from the present study, the first of its kind describing the –174 G/C polymorphism of the IL-6 gene in renal recipients of Brazilian origin, were analogous to the findings from Drozdziak et al.,²⁰ with no associations between the IL-6 polymorphism and GO for subjects under CsA regimens. In addition, no associations could be observed in the present study when GO was considered for Tcr and Sir regimens, nor for the overall sample.

Considering that most evidence suggested a higher level of IL-6 associated with the G allele, we expected to find a higher frequency of subjects with the GG genotype or the G allele among subjects with GO. However, frequencies of the GG, GC, and CC genotypes, as well as frequencies of the G and C alleles, were not significantly different among responders and non-responders. Further studies, involving different and larger populations, proved to be necessary to confirm these findings, especially as regards Tcr and Sir regimens. One significant feature of association data from genetic studies that investi-

gate the relationship between gene polymorphisms and specific diseases or conditions is that they can differ among populations.²⁸

In accordance with findings from Drozdziak et al.,²⁰ responders and non-responders in the present study presented similar regimen-related factors that could be implicated in GO, such as main immunosuppressant dosage, time since transplant, gender, and age. However, responders presented a higher frequency of concomitant CCB use. CCB have been implicated as a causal agent for GO^{1,10,36} and as a risk factor for GO in CsA,^{2,4,8} Tcr,^{4,8} and Sir⁹ immunosuppressive regimens.

It has been speculated that the occurrence of GO among renal transplant recipients under CsA or Tcr regimens could be strongly attributed to CCB use.⁸ Greenberg et al.⁸ evaluated 115 subjects under immunosuppressive regimens with and without CCB use and reported an overall GO prevalence of 34%, with the highest figure occurring among those subjects under CsA and CCB regimens (76%). The results of the present study are quite similar, with an overall GO prevalence of 34.8% and 66.7% for those under the CsA and CCB regimen. Prevalence data for CsA, Tcr, and Sir groups in the present study are also in agreement with other prevalence reports.^{4,5,7,9} The evaluation of the IL-6 (–174 G/C) polymorphism in relation to GO among subjects medicated solely with CCB should be considered in further studies.

Based on the findings from this study, a single nucleotide IL-6 (–174 G/C) gene polymorphism was found not to be associated with gingival overgrowth in renal transplant recipients under cyclosporine, tacrolimus, or sirolimus-based immunosuppressive maintenance regimens. Future studies should consider investigating the association of different cytokine gene polymorphisms with GO in multivariate models, taking into consideration the influence of demographic and pharmacological variables.

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Conflict of interest statement

The authors declare that there are no conflicts of interest related to the present study.

Ethical approval

Not required.

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