Relationship Between Fcγ Receptor and Interleukin-1 Gene Polymorphisms and Post-treatment Apical Periodontitis

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Abstract

Introduction: Genetic polymorphisms have been reported to act as modifiers of diverse diseases and, as such, might theoretically influence the severity and response to treatment of apical periodontitis. The purpose of this study was to investigate the association of Fcγ receptor and interleukin (IL)-1 gene polymorphisms with post-treatment apical periodontitis in Brazilian individuals. **Methods:** The study population consisted of 18 patients with post-treatment apical periodontitis and 44 individuals with root canal–treated teeth exhibiting healthy/healing periradicular tissues (controls). Patients were typed for the following genes (alleles): FcγRIIA (R131 or H131), FcγRIIIB (NA1 or NA2), IL-1A (1 or 2), and IL-1B (1 or 2). **Results:** No significant statistical differences were observed for all specific genotypes and almost all allele carriage rates of the test genes as well as combinations thereof with regard to association with disease ($P > .05$). Actually, only 2 genetic conditions were found to be associated with post-treatment apical periodontitis: carriage of allele H131 of the FcγRIIa gene ($P = .04$) and a combination of this allele with allele NA2 of the FcγRIIIb gene ($P < .01$). **Conclusions:** Data from the present study suggest that some conditions associated with polymorphism of Fcγ receptor genes might influence the patient's response to endodontic treatment of teeth with apical periodontitis. *(J Endod 2009;35:1186–1192)*

Key Words

Fcγreceptor, genetic polymorphism, interleukin-1, post-treatment apical periodontitis

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Post-treatment apical periodontitis is caused by microorganisms involved in persistent or secondary intraradicular infections or in extraradicular infections (1). Although it is widely recognized that emergence or persistence of post-treatment disease is dependent on infection (2), the possibility exists that other factors might influence the development, severity, or response to treatment of apical periodontitis lesions. These factors are usually known as disease modifiers or susceptibility/severity factors (3, 4).

Interindividual variations in the response to infection might be caused by systemic conditions or the genetic background of the individual. For instance, diabetes has been demonstrated to influence the development, course, and healing of apical periodontitis (5, 6). As for the genetic influence, gene polymorphism has been demonstrated to result in differences in the expression of molecules involved in inflammation and cellular activation (4). Existence of genetic polymorphisms might help explain the different courses of the same disease and the different responses to treatment in different patients (7).

Genetic polymorphisms are recognized when different alleles of a gene are found in the population. In a biallelic locus, the most common allele is termed normal (N-allele or allele 1) and must occur in <99%, whereas the rarer allele (R-allele or allele 2) is present in >1% in the population. The simplest type of polymorphism involves a change from one nucleotide to another and is referred to as single nucleotide polymorphism (SNP). Occurrence of SNP within the coding region of a gene might result in production of an altered protein, which might present altered function. Occurrence of SNP within the promoter region of the gene might alter gene regulation. This might lead to reduction/inhibition of gene expression or, conversely, overexpression of the gene (8). If the product of the polymorphic gene relates to inflammation or repair, the response might vary among individuals presenting different genotypes and carrying specific alleles.

FcγR are surface receptors expressed by leukocytes for the constant (Fc) region of immunoglobulin G (IgG) (9). IgG binds bacteria or bacterial soluble products and acts as opsonins, favoring the phagocytosis via FcγR by neutrophils or internalization by antigen-presenting cells, including macrophages and B cells. T cells and natural killer cells might also become activated when their FcγRs bind IgG-opsonized bacteria (9). As a consequence of the interaction between IgG-opsonized bacteria and FcγRs on immune cells, proinflammatory cytokines can be produced and released (9). Polymorphisms in the FcγR genes might lead to enhancement or inhibition of events mediated by FcγR and consequently might influence the susceptibility/severity to inflammatory diseases. The FcγR genes are found on chromosome 1 and encode 3 main classes of receptors: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). These classes are subdivided into the following subclasses: FcγRIa and b, FcγRIIa, b, and c, and FcγRIIIa and b. Polymorphism in the FcγRIIa gene (or FcγRIIA) is characterized by a G to A transition that leads to the substitution of histidine (H) (N-allele) for arginine (R) (R-allele) at amino acid position 131 of the receptor. The FcγRIIA-H131 allotype binds IgG2 immune complexes efficiently, whereas the FcγRIIA-R131 allotype cannot mediate this interaction (10). The FcγRIIIb gene (or FcγRIIIB) polymorphism is caused by 4 amino acid substitutions, which cause differences in receptor glycosylation with resulting effects on receptor affinity. Polymorphism in the FcγRIIIb gene results in the FcγRIIIB-neutrophil antigens NA1 (N-allele) or NA2 (R-allele). The FcγRIIIB-NA1 allotype promotes phagocytosis of IgG1- and IgG3-opsonized bacteria or bacterial products and binds IgG3 complexed to antigens more efficiently than FcγRIIIB-NA2 (9). Studies have found associations between polymorphisms in FcγRIIA and FcγRIIIB and the severity of periodontal diseases (11–15).

IL-1α and IL-1β are key cytokines involved with mediation of inflammation and bone resorption (7, 16). High levels of these cytokines have been demonstrated in apical periodontitis lesions, and a role in the immunopathogenesis of the disease has been ascribed (17–20). The genes encoding IL-1α and IL-1β (IL-1A and IL-1B, respectively) are located in close proximity on the long arm of chromosome 2. A biallelic polymorphism of the IL-1B gene at position +3954 (C/T, formerly +3953) was found to result in production of greater amounts of this cytokine by monocytes (21). Actually, homozygous individuals for the allele 2 (T) and heterozygous individuals (alleles 1 and 2) might produce, respectively, 4-fold and about 2-fold more IL-1β than individuals homozygous for allele 1 (C) (21). It has also been shown that the carriage of allele 2 of IL-1A –889 was associated with an almost 4-fold increase in the levels of this cytokine (22). Kornman et al (23) were the first to report on the role of IL-1 polymorphisms as a severity factor of marginal periodontitis. The combined presence of the R-allele of IL-1A at position –889 (within the promoter region) and the R-allele of IL-1B at position +3954 (within exon 5) was associated with severity of periodontitis in nonsmoking white patients (23). This combined carriage rate of the R-alleles of both IL-1A and IL-1B was designated the IL-1 composite genotype (23). Other investigators have reported similar findings in cross-sectional studies (24–26).

Apical periodontitis is primarily caused by bacteria, but potential disease modifiers, such as diabetes, acquired factors, and certain genetic polymorphisms, might alter the host defense to infection. So far in endodontic research, only one study has evaluated the influence of genetic polymorphism on the development of symptomatic infections (27), and a series of abstracts have been published on the association of SNP in some cytokine genes with treatment outcome (28–30). Given the scarcity of data reporting on the association of gene polymorphisms with endodontic inflammatory diseases, this study investigated the relationship between FcγRIIA, FcγRIIIB, IL-1A, and IL-1B genotypes and allele carriage rates and the occurrence of post-treatment disease in a Brazilian population.

Material and Methods

Individuals and Inclusion Criteria

The protocol for this study was approved by the Ethics Committee of the Estácio de Sá University. The population sample that met the inclusion criteria described below involved 62 adult individuals, 37 female and 25 male, from the cities of Rio de Janeiro, RJ, and Juiz de Fora, MG, both in the southeastern region of Brazil. A questionnaire was given to all individuals participating in the study to obtain information regarding their general health and habits.

Treatment outcome was determined on the basis of radiographic and clinical evaluations. Immediate postoperative radiographs at the time of treatment available in the Dental School records and follow-up radiographs of treated teeth were taken by using film holders, and treatment outcome was categorized as previously reported (31):

(1) Healed: Contour and width of the periodontal ligament space (PDL) were normal, or PDL contour was widened mainly around excess filling. Appearance of surrounding bone was normal.
(2) Healing: Periradicular radiolucency was clearly decreasing in size. These teeth were included in controls, because a significant level of healing was observed. Cases with uncertain healing were excluded.
(3) Not healed: Periradicular radiolucency was unchanged or increased in size.

In teeth with more than 1 root, the least favorable outcome was registered. Two experienced endodontists who had not been involved in the treatment or follow-up appointments analyzed independently the radiographs under magnification. In the only 2 cases in which disagreement occurred, a third observer was consulted. Observers were calibrated against a set of 100 reference teeth. Clinical examination was also performed to check for other signs/symptoms of treatment failure, ie, pain, swelling, or sinus tracts.

Rigid inclusion criteria were used to select patients adequate for the study purpose. To be enrolled, each individual should have only 1 root canal–treated tooth or more than 1 treated teeth with the very same periradicular status at the follow-up examination (ie, healthy/healing or diseased). All individuals should have presented with pulp necrosis and apical periodontitis at the time of treatment, which in turn should have been concluded for at least 1 year. Treated teeth should have both adequate root canal fillings and adequate coronal restorations. Root canal treatment was ranked as adequate when all canals were obturated, no voids were present, and fillings ended from 0–2 mm short of the radiographic apex. A coronal restoration was ranked as adequate when it appeared clinically and radiographically intact. Inadequate root canal treatment displayed fillings ending more than 2 mm short of the radiographic apex or grossly overfilled and/or with voids, inadequate density, unfilled canals, or poor condensation. Inadequate restoration was any permanent restoration with detectable radiographic signs of overhangs, open margins or recurrent caries, or presence of temporary coronal restoration. Teeth with inadequate treatment and/or inadequate coronal restoration were excluded from the study. Teeth with no coronal restorations, permanent or temporary, were also excluded. Because these rigid inclusion criteria significantly reduced the number of patients for the post-treatment group, individuals were recruited from 4 teaching institutions. All of them were treated either by graduate students or by specialists.

Following these inclusion criteria, the group of post-treatment disease was composed of 18 patients, whereas 44 individuals with root canal–treated teeth evincing healthy/healing periradicular tissues were included as controls.

Sample Taking and DNA Extraction

Sampling and DNA extraction procedures were performed according to Laine et al (32). Briefly, each individual rinsed out his/her mouth with 10 mL of sterile 0.9% saline solution for 1 minute. Samples were stored frozen at –20°C until DNA extraction procedures. For DNA extraction, samples were thawed at 37°C for 10 minutes, and buccal epithelial cells were centrifuged at 300*g* for 10 minutes. The pellet was washed twice in Tris–ethylenediaminetetraacetic acid (EDTA) buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 7.6), resuspended in 100 μL of 50 mmol/L NaOH, and boiled for 10 minutes. Samples were neutralized with 14 μL of 1 mol/L Tris (pH 7.5) and centrifuged at 14,000*g* for 3 minutes. Supernatants were collected and stored at –20°C until analysis.

FcγRIIA Genotyping

FcγRIIA–R131/H131 genotyping was performed as described elsewhere (14, 33). Aliquots of 10 μL of DNA extract was added to 50 μL polymerase chain reaction (PCR) mixture containing 5 μL of 10 × PCR buffer (Fermentas, Burlington, Canada), 1 μmol/L each of primer P63 (5′-CAA GCC TCT GGT CAA GGT C–3′) and P52 (5′-GAA GAG CTG CCC ATG CTG–3′), 1 U *Taq* DNA polymerase (Fermentas), 25 mmol/L $MgCl_2$, and 0.2 mmol/L of each deoxyribonucleoside triphosphate (Biotools, Madrid, Spain). The first PCR conditions were as follows: 1 cycle at 95°C/5 min, 55°C/5 min, and 72°C/5 min. This was followed by 35 cycles at 95°C/1 min, 55°C/1 min, and 72°C/2 min, ending with an extension step at 72°C/10 min. One microliter of the first PCR product was subsequently reamplified by using a combination

of the common reverse primer P13 (5′-CTA GCA GCT CAC CAC TCC TC–3′) and the G-specific forward primer P5G (5′-GAA AAT CCC AGA AAT TTT TCC G–3′) or the A-specific forward primer P4A (5′-GAA AAT CCC AGA AAT TTT TCC A–3′), both of which are located at the polymorphic site on exon 4. The reaction mixture was as above, and temperature conditions for the second PCR were as follows: 95°C/5 min followed by 30 cycles of 95°C/15 s, 60°C/30 s, and 72°C/30 s, with an extension step at 72°C/10 min. PCR reactions yielded a 278 base pair (bp) product for both FcγRIIA alleles. Thus, 2 separate PCR reactions were performed for genotyping of each individual.

FcγRIIIB Genotyping

FcγRIIIB–NA1/NA2 genotyping was performed as described by Haas et al (34). A common reverse primer (5′–ATG GAC TTC TAG CTG CAC–3′) was used in association with a NA1-specific forward primer (5′-CAG TGG TTT CAC AAT GTG AA–3′), yielding a 141-bp fragment, and a NA2-specific forward primer (5′-CAA TGG TAC AGC GTG CTT–3′), generating a 219-bp fragment. Because there was a substantial difference in length between the NA1-specific and NA2-specific reaction products, both alleles could be detected in the same duplex PCR reaction. The reaction mixture (50 μL) contained 10 μL of DNA extract, 5 μL of 10 × PCR buffer (Fermentas), 0.6 μmol/L of the NA1 and NA2 primers, 1 μmol/L of the reverse primer, 1 U *Taq* DNA polymerase (Fermentas), 25 mmol/L $MgCl_2$, and 0.2 mmol/L of each deoxyribonucleoside triphosphate (Biotools). PCR conditions were as follows: 94°C/3 min followed by 30 cycles of 94°C/1 min, 57°C/2 min, and 72°C/1 min, and a final extension step at 72°C/10 min.

IL-1A (–889) Genotyping

Primers used for IL-1A (–889) genotyping were IL-1Af (5′–AAG CTT GTT CTA CCA CCT GAA CTA GGC-3′) and IL-1Ar (5′–TTA CAT ATG AGC CTT CCA TG-3′) (23). PCR amplifications were performed in 50 μL of reaction mixture containing 5 μL of 10 × PCR buffer (Fermentas), 0.8 μmol/L concentration of each primer, 1.25 U of *Taq* DNA polymerase (Fermentas), 2 mmol/L $MgCl_2$, and 0.2 mmol/L of each deoxyribonucleoside triphosphate (Biotools). Thermocycling conditions were as follows: 95°C/2 min followed by 45 cycles of 94°C/1 min, 50°C/1 min, 72°C/1 min, and final extension at 72°C/10 min. To confirm amplification, 8 μL of each PCR product was electrophoresed on a 2% agarose gel in Tris-borate/EDTA buffer. Gels were stained with ethidium bromide, and DNA was visualized under ultraviolet light. PCR product was digested at 37°C with *Nco*I (New England Labs, Ipswich, MA), and the restriction pattern was visualized by electrophoresis through a 4% agarose gel. *Nco*I digestion yields 2 fragments of 16 bp and 83 bp in subjects homozygous for allele 1 and a single fragment of 99 bp in subjects homozygous for allele 2. All 3 fragments are present in heterozygous individuals.

IL-1B (+3954) Genotyping

Primers used for IL-1B (+3954) genotyping were IL-1Bf (5′-CTC AGG TGT CCT CGA AGA AAT CAA A–3′) and IL-1Br (5′–GCT TTT TTG CTG TGA GTC CCG–3′)(23). PCR amplifications were performed in 50 μL of reaction mixture containing 5 μL of 10 × PCR buffer (Fermentas), 1 μmol/L concentration of each primer, 2 U of *Taq* DNA polymerase (Fermentas), 2.5 mmol/L $MgCl_2$, and 0.2 mmol/L of each deoxyribonucleoside triphosphate (Biotools). Thermocycling conditions were as follows: 2 cycles of 95°C/2 min, 67.5°C/1 min, and 74°C/1 min followed by 35 cycles of 95°C/1 min, 67.5°C/1 min, and 74°C/1 min. Afterwards, 3 cycles of 95°C/1 min, 67.5°C/1 mm, and 74°C/5 min completed the profile. To confirm amplification, 8 μL of each PCR product was electrophoresed on a 2% agarose gel. PCR products were then digested with *Taq*I (New England Labs) at 65°C and visualized by electrophoresis through a 4% agarose gel. *Taq*I digestion yields 2 fragments of 85 bp and 97 bp in subjects homozygous for allele 1 and a single fragment of 182 bp in subjects homozygous for allele 2. The 3 fragments are observed in heterozygous individuals.

PCR Controls

To ascertain the availability of human DNA for analysis, aliquots of 5 μL of the DNA extracts from clinical samples were subjected to PCR by using primers directed to the T-cell receptor Vα22 gene (V22f:5′-GAT TCA GTG ACC CAG ATG GAA GGG–3′ and V22r:5′-AGC ACA GAA GTA CAC CGC TGA GTC–3′), which amplify a fragment of 270 bp (35). PCR amplifications were performed in 50 μL of reaction mixture containing 5 μL of 10 × PCR buffer (Fermentas), 1 μmol/L concentration of each primer, 1.25 U of *Taq* DNA polymerase (Fermentas), 3 mmol/L $MgCl_2$, and 0.2 mmol/L of each deoxyribonucleoside triphosphate (Biotools). PCR conditions included 94°C/5 min followed by 45 cycles of 94°C/45 s, 60°C/30 s, and 72°C/1.5 min, and a final extension step at 72°C/10 min.

One negative control consisting of sterile ultrapure water instead of the sample was included for every 10 samples in all batches of samples analyzed for each gene tested. To confirm the validity of the methods, 10 samples were genotyped twice for each target gene. The results were identical. Samples exhibiting negative results after PCR were subjected to reamplification. Samples yielding 3 consecutive negative PCR results for a given gene were excluded from the respective analysis.

Statistical Analysis

The data concerning distributions for alleles and genotypes between disease (treated teeth with apical periodontitis) and control (healthy/healing treated teeth) groups were statistically analyzed by means of the Pearson χ^2 test (3 × 2 contingency tables for specific genotype frequencies and 2 × 2 contingency tables for allele frequencies) or the Fisher two-tailed exact test; the latter was used when at least 1 cell of the 2 × 2 contingency table had a value less than 5. Composite IL-1 or FcγR genotypes were also evaluated as to the association with disease. All possible combinations involving alleles and genotypes of IL-1 or FcγR genes were evaluated. Associations of different characteristics of the study population with disease or different genetic patterns were also evaluated by the χ^2 test or the Fisher exact test. Age was checked for association with treatment outcome by means of the Student *t* test. Significance level was set at $P < .05$.

Results

Study Population Characteristics

Table 1 depicts the characteristics of the study individuals and their relationship with health or disease. Diabetes (4 individuals) was the only systemic condition and smoking (10 individuals) the only acquired habit reported by the patients that were judged to be potential disease modifiers and were thereby included as covariates. Both factors were significantly associated with disease, but none of them showed a positive relationship with any genotype or allele carriage rate. Sex, age, and race had no significant influence on treatment outcome.

FcγReceptor Genotypes

The results of genotyping of the FcγRIIA and FcγRIIIB polymorphisms for the individuals with post-treatment apical periodontitis and controls are displayed in Table 2. Overall, the most prevalent alleles in the study population were FcγRIIA-H131 (51.6%) and FcγRIIIB-NA2 (84.5%). The genotypes H/H131 and NA2/NA2 were the most common among the population, occurring in 39.3% and 77.6% of the

TABLE 1. Characteristics of Study Subjects with Treated Root Canals Associated or Not with Apical Periodontitis

Characteristic	Patients with disease n (%)	Patients with no disease n (%)	*P* value
Gender			
Female	9 (24.3)	28 (75.7)	.479*
Male	9 (36.0)	16 (64.0)	
Race			
White	13 (27.7)	34 (72.3)	.924*
African-Brazilian	5 (33.3)	10 (66.7)	
Systemic condition			
Diabetic	4 (100)	0 (0)	.005**
Nondiabetic	14 (24.1)	44 (75.9)	
Habits			
Smoker	7 (70)	3 (30)	.004**
Nonsmoker	11 (21.2)	41 (78.4)	
Age (y)#	44.9 (±14.1)	40.8 (±18.4)	.394***

*χ^2.

**Fisher exact test.

***Student *t* test for independent samples.

#Mean (± standard deviation).

individuals, respectively. No significant associations were found between any specific genotype of FcγRIIA (P = .12) or FcγRIIIB (P = .31) and post-treatment disease. However, individuals carrying the allele FcγRIIA-H131 (R/H131 or H/H131) were significantly more frequent in the disease group (83%) than in the control group (56%) (P = .04, Fisher exact test). Although frequencies for allele FcγRIIIB-NA2 carriage were higher in patients (90.6%) than in controls (82.1%), this difference was not significant (P = .4, Fisher exact test). No other significant associations were found for carriage of the other alleles from either FcγRIIA or FcγRIIIB (P > .05).

Table 3 presents the data on distribution of FcγRIIA and FCγRIIIB genotype combinations in patients and controls. All possible genotype combinations disclosed no significant association with disease (P = .2). However, individuals carrying both allele H131 and allele NA2 were highly associated with disease (87.5% of patients and 49% of controls; P < .01).

IL-1 Genotypes

Table 4 presents the allele carriage rates and genotype frequencies for IL-1A and IL-1B genes in individuals with post-treatment apical periodontitis and healthy controls. The N-alleles (allele 1) for both genes were the most frequent, ie, IL-1A-allele 1 was found in 69.3%, and IL-1B-allele 1 was detected in 74.1% of the population. For both genes, the carriage rate of allele 1 was always higher in diseased than in healthy/healing individuals, but significance was not observed (P = .71 for IL-1A; P = 1 for IL-1B, Fisher exact test).

The homozygous for allele 1 was the most frequently observed genotype for both IL-1A (56.1%) and IL-1B (60.7%). No significant associations were found between the different genotypes of IL-1A (P = .36) or IL-1B (P = .33) and post-treatment apical periodontitis. No possible genotype combinations of IL-1A and IL-1B exhibited significant association with disease (P = .43). Individuals presenting the composite genotype comprising allele 2 of the IL-1A –889 polymorphism and allele 2 of the IL-1B +3954 polymorphism were not linked to disease (P = .47) (Table 5).

TABLE 2. Distribution of FcγRIIA and FCγRIIIB Genotypes and Alleles in Subjects with Treated Root Canals Associated or Not with Apical Periodontitis

	Patients with disease		Patients with no disease		Total	
Genotype	N	%	N	%	N	%
FcγRIIA	18		43		61	
R/R	3	16.7	19	44.2	22	36.1
R/H	6	33.3	9	20.9	15	24.6
H/H	9	50	15	34.9	24	39.3
Allele	36		86		122	
R131	12	33.3	47	54.7	59	48.4
H131	24	66.7	39	45.3	63	51.6
FCγRIIIB	16		42		58	
NA1/NA1	0	0	5	11.9	5	8.6
NA1/NA2	3	18.8	5	11.9	8	13.8
NA2/NA2	13	81.3	32	76.2	45	77.6
Allele	32		84		116	
NA1	3	9.4	15	17.9	18	15.5
NA2	29	90.6	69	82.1	98	84.5

TABLE 3. Distribution of FcγRIIA and FCγRIIIB Genotype Combinations in Subjects with Treated Root Canals Associated or Not with Apical Periodontitis

	Patients with disease			Patients with no disease		
	R/R	R/H	H/H	R/R	R/H	H/H
NA1/NA1	0	0	0	3	2	0
NA1/NA2	0	1	2	3	0	2
NA2/NA2	2	5	6	13	7	11

Discussion

A myriad of genes are undoubtedly involved in host defenses against infection, and a challenging task would be to define which ones display specific variations that predispose to disease and/or influence disease severity and response to treatment. In the present study, we evaluated gene polymorphisms that have been regarded as susceptibility/severity factors associated with several human diseases. Individuals with apparently well-treated root canals associated with periradicular bone destruction (patients) or healthy/healing periradicular tissues (controls) were genotyped for Fcγ receptor and IL-1 genes in an attempt to verify the influence of polymorphisms in these host defense–related genes on treatment outcome.

The most prevalent alleles detected in this Brazilian subpopulation were FcγRIIA-H131 (51.6%), FcγRIIIB-NA2 (84.5%), and allele 1

TABLE 4. Distribution of IL-1A–889 and IL-1B + 3954 Genotypes and Alleles in Subjects with Treated Root Canals Associated or Not with Apical Periodontitis

	Patients with disease		Patients with no disease		Total	
Genotype	N	%	N	%	N	%
IL-1A –889	17		40		57	
1-1	12	70.6	20	50	32	56.1
1-2	3	17.6	12	30	15	26.3
2-2	2	11.8	8	20	10	17.5
Allele	34		80		114	
1	27	79.4	52	65	79	69.3
2	7	20.6	28	35	35	30.7
IL-1B +3954	16		40		56	
1-1	12	75	22	55	34	60.7
1-2	2	12.5	13	32.5	15	26.8
2-2	2	12.5	5	12.5	7	12.5
Allele	32		80		112	
1	26	81.3	57	71.3	83	74.1
2	6	18.8	23	28.8	29	25.9

TABLE 5. Frequency of Composite IL-1A2/IL-1B2 Genotype in Patients with Treated Root Canals Associated or Not with Apical Periodontitis

	Patients with disease		Patients with no disease		Total	
Genotype	N	%	N	%	N	%
Positive*	2	13.3	10	27.8	12	23.5
Negative**	13	86.7	26	72.2	39	76.5

*Carrying both alleles 2 of IL-1A and IL-1B.

**Not carrying both alleles 2 of IL-1A and IL-1B.

from both IL-1A (69.3%) and IL-1B (74.1%). This is in agreement with reports for other populations (14, 35–38), although prevalence values and most frequent genotypes might vary significantly according to geographic location (3). Indeed, the prevalence of Fcγ receptor and IL-1 genotypes might vary significantly among individuals from different ethnic backgrounds, which makes comparisons among studies very difficult. Curiously, the homozygous for these alleles were also the most frequent genotypes in the overall population. This was also true when individuals were separated into patients (diseased) and controls (healthy/healing), except for the fact that homozygous for the genotype FcγRIIA-R131 were more prevalent in controls.

Regardless of the target gene polymorphism, none of the 3 specific genotypes (homozygous for the N-allele or the R-allele and heterozygous) were associated with post-treatment disease. In fact, only 2 situations were found to be associated with post-treatment apical periodontitis: carriage of the allele H131 of the FcγRIIa gene and a composite genotype consisting of FcγRIIA-H131 and FcγRIIIB-NA2.

Antibodies play an important role in promoting bacterial elimination, which involves specific binding on the bacterial cell surface through the antibody Fab portion and then binding to surface receptors on phagocytes through the antibody Fc portion. How well the antibody binds to bacteria and receptors on phagocytes will determine its efficacy and the resulting host response to infection (39). A number of studies have associated polymorphisms in Fcγ receptor genes with a variety of inflammatory and infectious diseases, such as meningococcal and pneumococcal infections, rheumatoid arthritis, systemic lupus erythematosus, and periodontal diseases (9, 15, 40–42). In the present study, we found an association between individuals carrying the FcγRIIA-H131 allele and post-treatment apical periodontitis. These findings relatively agree with others from the periodontal literature (13, 14). Loos et al (13) reported that the N-allele (H131) carriage rate was higher in aggressive periodontitis individuals than in controls, and that periodontitis patients (aggressive and chronic periodontitis) homozygous for the N-allele (H/H131 genotype) have more periodontal bone loss than individuals carrying 1 or 2 R-alleles. The increased carriage rate of the allele FcγRIIA-H131 and the increased prevalence of the FcγRIIA-H/H131 genotype in aggressive periodontitis suggest a role for this allele as putative susceptibility/severity factor for this disease. Similarly, Yamamoto et al (14) reported that individuals exhibiting the FcγRIIA-H/H131 genotype were more prevalent in the periodontitis group than in healthy controls.

These results related to both marginal and apical periodontitis are quite interesting, because FcγRIIA-H131 is the normal (or high affinity) allele for this gene. For the most part, the rare allele is the one usually considered as "poor" polymorphism and, as such, is expected to be associated with disease (36). Polymorphonuclear leukocytes with the H/H131 genotype bind efficiently to IgG2 and exhibit higher phagocytic competence than polymorphonuclear leukocytes expressing the R/R131 genotype (9, 40). Actually, it has been proposed that the high affinity of FcγRIIA-H131 might contribute to a strong proinflammatory cytokine release by monocytes/macrophages on interaction with IgG, possibly leading to an increased risk for chronic marginal periodontitis (14). The same theory might be applicable to post-treatment apical periodontitis.

Although some studies have found the FcγRIIIB-NA2 allele associated with some forms of marginal periodontitis (11, 15, 42), this was not observed in the present study for post-treatment apical periodontitis. Because FcγRIIa and FcγRIIIb receptors have been reported to act synergistically in triggering neutrophils (40), we decided to investigate the association of combinations of genotypes and alleles of the 2 receptors with disease. Our findings revealed that the combined carriage of alleles FcγRIIA-H131 and FcγRIIIB-NA2 was highly associated with post-treatment apical periodontitis. Other studies in the periodontal literature have also reported that some composite genotypes of Fcγ receptor genes might be severity factors for marginal periodontitis (12, 15). The combined carriage of FcγRIIIB-NA2 allele with FcγRIIIA-V158 allele was strongly associated with severe periodontitis (12). A significantly higher frequency of the FcγRIIA-H/H131 plus FcγRIIIB-NA2/NA2 composite in generalized aggressive periodontitis patients than in healthy controls has also been reported (15). The latter findings are somewhat in agreement with ours and suggest that composite genotypes involving alleles H131 from the FcγRIIa gene and NA2 from the FcγRIIIb gene might have biologic importance to the point of influencing disease susceptibility, severity, or response to treatment.

In the present study, the allele carriage rates and genotype frequencies for IL-1A and IL-1B were not different between diseased and healthy/healing groups. The same was true for the IL-1 composite genotype, characterized by combined carriage of the R-alleles of IL-1A –889 and IL-1B +3954, which has been associated with the severity of marginal periodontitis (23). A series of studies published so far only as abstracts revealed that IL-1B polymorphism might influence the response to endodontic treatment, because individuals homozygous for the IL-1B-allele 2 were significantly associated with post-treatment disease (28, 30). The same was not observed for tumor necrosis factor–β and IL-6 (29, 30). Several studies have also reported on the association of IL-1 gene polymorphism with a higher severity of periodontal diseases (23, 25, 26, 43), but there are also studies that failed to confirm such association (37, 44–46). Similarly, our findings do not confirm those from the early post-treatment apical periodontitis studies (28, 30), although deeper comparisons cannot be performed because those are available only as abstracts and only IL-1B polymorphism was evaluated. Even so, differences might be expected when comparisons are made between studies with small sample size in different ethnic groups from different geographic locations. Further studies are needed to address this issue.

Because of the need to include only teeth with root canal treatments that followed acceptable standards, studies evaluating the causative agents and susceptibility/severity factors of post-treatment apical periodontitis are usually plagued by a small sample size. In this regard, the present study was not an exception. Moreover, the fact that samples were taken per individual and not per tooth contributed still more to difficulties in selecting an adequate representative population sample. This is because a given individual with opposite periradicular conditions in his/her mouth (eg, one adequate treatment associated with health and another with disease) had to be excluded from the study. Therefore, although the inclusion criteria related to quality of treatment and periradicular status were rigid enough to increase the reliability of our results, the tradeoff was a resultant low sample size, decreasing statistics power. Another limitation of the present study was the utilization of radiographs to establish absence of disease, although radiographs at the time of treatment were available, and in the worst case

scenario teeth classified as healthy might have been healing. This was not expected to affect the analysis significantly because both healthy and healing individuals were included in the same group (control).

All the individuals participating in the present study pertained to the same geographic area (Brazil southeastern region) and socioeconomic status (low or middle low). Age was relatively well-balanced between diseased patients and healthy/healing controls and along with gender and race had neither significant influence on the outcome nor significant association with any individual genetic pattern. Although ethnicity is an important variable in studies of this nature, it must be realized that the great difficulties in classifying Brazilian individuals by color and race as a result of a high racial miscegenation of the population (47) make any comparisons in this regard unreliable. Diabetes could be a confounding factor, because it has also been suggested to be a disease modifier (5, 6). However, diabetic individuals were not excluded from the study because they were in reduced numbers (4 individuals, all in the disease group). Although they were positively associated with disease, in spite of being in very low numbers for a reliable statistic conclusion, diabetics were not found to influence the results significantly because they were not associated with any specific genotype or allele. Similar results and the same arguments are applicable to smokers, for whom previous reports on association with disease have been conflicting (48–51). Maintenance of diabetics and smokers in the study was then justified by the difficulties to select individuals who met our rigid inclusion criteria and because these conditions were not significantly associated with any specific genetic pattern.

Although the influence of genetic polymorphism on treatment outcome might be suggested, it would appear too simplistic to attribute the cause of post-treatment apical periodontitis to an immunologic failure. Apical periodontitis is apparently a complex disease, in which microorganisms play a major role as causative factors. Genetic polymorphisms, possibly involving multiple genes, can be regarded as disease modifiers, which can influence severity and/or response to treatment, but not probably to the point of being responsible for treatment failure. Because this is a very incipient area in endodontic research, further cross-sectional or case-control studies are required to confirm or refute our findings and evaluate the association of other gene polymorphisms with post-treatment diseases. Afterwards, as putative susceptibility/severity factors are identified, their role as genetic markers influencing the treatment outcome can then be confirmed by longitudinal studies. Because the carriage rate of polymorphic alleles might vary substantially between different ethnic groups, validity of certain genetic markers as predictors of treatment outcome might need to be tested for different populations.

In conclusion, the current study of relatively small sample size suggests that the FcγRIIA-H131 allele carriage and a combination of this allele with the FcγRIIIB-NA2 allele might be putative susceptibility or severity factors influencing the outcome of the endodontic treatment in Brazilians. No other associations were observed for FcγR and IL-1 genotypes or alleles and post-treatment apical periodontitis. Further studies are needed involving a larger sample size from different geographic populations to corroborate the present findings. If they are confirmed, future studies focusing on deciphering the role of the Fcγ receptors in the pathogenesis of post-treatment apical periodontitis are required.

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References

1. Siqueira JF Jr. Aetiology of root canal treatment failure: why well-treated teeth can fail. Int Endod J 2001;34:1–10.
2. Siqueira JF Jr, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod 2008;34:1291–301, e3.
3. Loos BG, John RP, Laine ML. Identification of genetic risk factors for periodontitis and possible mechanisms of action. J Clin Periodontol 2005;32(Suppl 6): 159–79.
4. Kornman KS. Genetics and periodontal diseases. In: Wilson TG Jr, Kornman KS, eds. Fundamentals of periodontics. 2nd ed. Chicago: Quintessence Publishing Co; 2003: 171–84.
5. Fouad AF, Burleson J. The effect of diabetes mellitus on endodontic treatment outcome: data from an electronic patient record. J Am Dent Assoc 2003;134:43–51.
6. Armada-Dias L, Breda J, Provenzano JC, et al. Development of periradicular lesions in normal and diabetic rats. J Appl Oral Sci 2006;14:371–5.
7. Fouad AF, Huang GT. Inflammation and immunological responses. In: Ingle J, Bakland LK, Baumgartner JC, eds. Inglés endodontics Hamilton. 6th ed. Ontario: Canada; BC Decker Inc, 2008:343–75.
8. Schork NJ, Fallin D, Lanchbury JS. Single nucleotide polymorphisms and the future of genetic epidemiology. Clin Genet 2000;58:250–64.
9. van Sorge NM, van der Pol WL, van de Winkel JG. FcgammaR polymorphisms: implications for function, disease susceptibility and immunotherapy. Tissue Antigens 2003;61:189–202.
10. Warmerdam PA, van de Winkel JG, Vlug A, Westerdaal NA, Capel PJ. A single amino acid in the second Ig-like domain of the human Fc gamma receptor II is critical for human IgG2 binding. J Immunol 1991;147:1338–43.
11. Kobayashi T, Westerdaal NA, Miyazaki A, et al. Relevance of immunoglobulin G Fc receptor polymorphism to recurrence of adult periodontitis in Japanese patients. Infect Immun 1997;65:3556–60.
12. Kobayashi T, Yamamoto K, Sugita N, et al. The Fc gamma receptor genotype as a severity factor for chronic periodontitis in Japanese patients. J Periodontol 2001;72:1324–31.
13. Loos BG, Leppers-Van de Straat FG, Van de Winkel JG, Van der Velden U. Fcgamma receptor polymorphisms in relation to periodontitis. J Clin Periodontol 2003;30: 595–602.
14. Yamamoto K, Kobayashi T, Grossi S, et al. Association of Fcgamma receptor IIa genotype with chronic periodontitis in Caucasians. J Periodontol 2004;75: 517–22.
15. de Souza RC, Colombo AP. Distribution of FcgammaRIIa and FcgammaRIIIb genotypes in patients with generalized aggressive periodontitis. J Periodontol 2006;77: 1120–8.
16. Stashenko P, Dewhirst FE, Peros WJ, Kent RL, Ago JM. Synergistic interactions between interleukin 1, tumor necrosis factor, and lymphotoxin in bone resorption. J Immunol 1987;138:1464–8.
17. Lim GC, Torabinejad M, Kettering J, Linkhardt TA, Finkelman RD. Interleukin 1-beta in symptomatic and asymptomatic human periradicular lesions. J Endod 1994;20: 225–7.
18. Barkhordar RA, Hussain MZ, Hayashi C. Detection of interleukin-1 beta in human periapical lesions. Oral Surg Oral Med Oral Pathol 1992;73:334–6.
19. Martinez ZR, Naruishi K, Yamashiro K, et al. Gene profiles during root canal treatment in experimental rat periapical lesions. J Endod 2007;33:936–43.
20. Wang CY, Stashenko P. The role of interleukin-1 alpha in the pathogenesis of periapical bone destruction in a rat model system. Oral Microbiol Immunol 1993;8: 50–6.
21. Pociot F, Molvig J, Wogensen L, Worsaae H, Nerup J. A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro. Eur J Clin Invest 1992;22:396–402.
22. Shirodaria S, Smith J, McKay IJ, Kennett CN, Hughes FJ. Polymorphisms in the IL-1A gene are correlated with levels of interleukin-1alpha protein in gingival crevicular fluid of teeth with severe periodontal disease. J Dent Res 2000;79:1864–9.
23. Kornman KS, Crane A, Wang HY, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol 1997;24:72–7.
24. McDevitt MJ, Wang HY, Knobelman C, et al. Interleukin-1 genetic association with periodontitis in clinical practice. J Periodontol 2000;71:156–63.
25. Papapanou PN, Neiderud AM, Sandros J, Dahlen G. Interleukin-1 gene polymorphism and periodontal status: a case-control study. J Clin Periodontol 2001;28: 389–96.
26. Lopez NJ, Jara L, Valenzuela CY. Association of interleukin-1 polymorphisms with periodontal disease. J Periodontol 2005;76:234–43.
27. de Sa AR, Moreira PR, Xavier GM, et al. Association of CD14, IL1B, IL6, IL10 and TNFA functional gene polymorphisms with symptomatic dental abscesses. Int Endod J 2007;40:563–72.
28. Morsani JM, Mickel AK, Chogle S, et al. Genetic predisposition to persistent apical periodontitis. J Endod 2006;32:258.

29. Kafri D, Mickel AK, Chogle S, Jones JJ, Han Y, Liu H. Role of polymorphism in cytokine overexpression leading to persistent apical periodontitis. J Endod 2007;33:352.
30. Peritore VA, Mickel AK, Chogle SM, Jones JJ, Han Y. Possible interaction of cytokine polymorphisms in persistent apical periodontitis. J Endod 2008;34:364.
31. Siqueira JF Jr, Rôças IN, Riche FN, Provenzano JC. Clinical outcome of the endodontic treatment of teeth with apical periodontitis using an antimicrobial protocol. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106:757–62.
32. Laine ML, Farre MA, Crusius JB, van Winkelhoff AJ, Pena AS. The mouthwash: a non-invasive sampling method to study cytokine gene polymorphisms. J Periodontol 2000;71:1315–8.
33. Carlsson LE, Santoso S, Baurichter G, et al. Heparin-induced thrombocytopenia: new insights into the impact of the FcgammaRIIa-R-H131 polymorphism. Blood 1998; 92:1526–31.
34. Haas M, Kleijer M, van Zwieten R, Roos D, von dem Borne AE. Neutrophil Fc gamma RIIIb deficiency, nature, and clinical consequences: a study of 21 individuals from 14 families. Blood 1995;86:2403–13.
35. Van Den Berg L, Myhr KM, Kluge B, Vedeler CA. Fcgamma receptor polymorphisms in populations in Ethiopia and Norway. Immunology 2001;104:87–91.
36. Wolf DL, Neiderud AM, Hinckley K, Dahlen G, van de Winkel JG, Papapanou PN. Fcgamma receptor polymorphisms and periodontal status: a prospective follow-up study. J Clin Periodontol 2006;33:691–8.
37. Hodge PJ, Riggio MP, Kinane DF. Failure to detect an association with IL1 genotypes in European Caucasians with generalised early onset periodontitis. J Clin Periodontol 2001;28:430–6.
38. Quappe L, Jara L, Lopez NJ. Association of interleukin-1 polymorphisms with aggressive periodontitis. J Periodontol 2004;75:1509–15.
39. Abbas AK, Lichtman AH, Pillai S. Cellular and molecular immunology. 6th ed. Philadelphia: Saunders/Elsevier; 2007.
40. van der Pol W, van de Winkel JG. IgG receptor polymorphisms: risk factors for disease. Immunogenetics 1998;48:222–32.
41. Kobayashi T, Ito S, Yamamoto K, et al. Risk of periodontitis in systemic lupus erythematosus is associated with Fcgamma receptor polymorphisms. J Periodontol 2003; 74:378–84.
42. Kobayashi T, Sugita N, van der Pol WL, et al. The Fcgamma receptor genotype as a risk factor for generalized early-onset periodontitis in Japanese patients. J Periodontol 2000;71:1425–32.
43. Laine ML, Farre MA, Gonzalez G, et al. Polymorphisms of the interleukin-1 gene family, oral microbial pathogens, and smoking in adult periodontitis. J Dent Res 2001;80:1695–9.
44. Sakellari D, Katsares V, Georgiadou M, Kouvatsi A, Arsenakis M, Konstantinidis A. No correlation of five gene polymorphisms with periodontal conditions in a Greek population. J Clin Periodontol 2006;33:765–70.
45. Gonzales JR, Michel J, Rodriguez EL, Herrmann JM, Bodeker RH, Meyle J. Comparison of interleukin-1 genotypes in two populations with aggressive periodontitis. Eur J Oral Sci 2003;111:395–9.
46. Sakellari D, Koukoudetsos S, Arsenakis M, Konstantinidis A. Prevalence of IL-1A and IL-1B polymorphisms in a Greek population. J Clin Periodontol 2003;30: 35–41.
47. Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SD. Color and genomic ancestry in Brazilians. Proc Natl Acad Sci U S A 2003;100:177–82.
48. Segura-Egea JJ, Jimenez-Pinzon A, Rios-Santos JV, Velasco-Ortega E, Cisneros-Cabello R, Poyato-Ferrera MM. High prevalence of apical periodontitis amongst smokers in a sample of Spanish adults. Int Endod J 2008;41:310–6.
49. Bergstrom J, Babcan J, Eliasson S. Tobacco smoking and dental periapical condition. Eur J Oral Sci 2004;112:115–20.
50. Kirkevang LL, Wenzel A. Risk indicators for apical periodontitis. Community Dent Oral Epidemiol 2003;31:59–67.
51. Marending M, Peters OA, Zehnder M. Factors affecting the outcome of orthograde root canal therapy in a general dentistry hospital practice. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;99:119–24.