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Original Article

Association of *IL10*, *IL4*, *IFNG*, and *CTLA4* Gene Polymorphisms with Efavirenz Hypersensitivity Reaction in Patients Infected with Human Immunodeficiency Virus

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SUMMARY: We evaluated interleukin-10 (*IL10*) −592 C/A, *IL4* −589 C/T, interferon gamma (*IFNG*) +874 A/T, cytotoxic T-lymphocyte-associated antigen 4 (*CTLA4*) +49 A/G gene polymorphisms associated with efavirenz hypersensitivity reaction. A total of 63 human immunodeficiency virus-positive patients under treatment at a public hospital were included in the study, of whom 21 presented with efavirenz hypersensitivity. Patients who presented with efavirenz hypersensitivity reaction showed a higher frequency of the *IL10* −592A allele than the controls ($p = 0.028$). The allele A was associated with increased risk of efavirenz hypersensitivity (odds ratio = 2.40). In case of *IL4*, a significant difference in the frequency of the *IL4* −589 (C/T) polymorphism was not observed between patients and controls. A significant inverse correlation was observed when comparing the *CTLA4* +49A/G and *IL4* −589 C/T polymorphisms ($r = -0.650$, $p = 0.001$); that is, the *CTLA4* +49GG genotype, involved with the lowest capacity of inhibition, was inversely correlated *IL4* −589TT genotype, which induces high production of IL-4. With respect to the *CTLA4* +49A/G and *IFNG* +874T/A gene polymorphisms, significant differences in allele and genotype frequencies were not observed between the groups. Therefore, our data suggest that polymorphisms in regulatory regions of cytokine genes could modulate an individual's susceptibility to efavirenz hypersensitivity reaction.

INTRODUCTION

Hypersensitivity drug reactions are about 100 times more frequent in human immunodeficiency virus (HIV)-infected patients than in the general population (1). Efavirenz, nevirapine, delavirdine, etravirine (non-nucleoside reverse transcriptase inhibitors (NNRTI)), abacavir (nucleoside reverse transcriptase inhibitors (NRTI)), and amprenavir (protease inhibitor) are drugs that may cause adverse reactions (2,3).

The Brazilian Ministry of Health recommends two NRTIs (e.g., tenofovir, lamivudine) and one NNRTI (efavirenz) as the first-line therapy for HIV infection. Although efavirenz has a long plasma half-life and allows once-daily dosing, it has a low genetic barrier to the development of drug resistance (4).

Neuropsychiatric disorders, including dizziness, drowsiness, headache, confusion, nightmares, depression are the main adverse events caused by efavirenz. These events are observed in approximately 40% of patients and vary in severity, but diminish after the first month of treatment (3). Rashes may also occur in up to 27% of adults and 45% of children around the second week of treatment (5).

It is hypothesized that the higher rate of drug allergy in HIV-infected patients is due to multiple origins including the use of polypharmacy, recurrent infections, increased vulnerability to oxidative stress, and genetic factors (1,5).

Cytokines are important molecules produced by several types of cells that bind to specific receptors on target cells. They activate a cascade of cellular messengers leading to gene transcription, differentiation, proliferation, effector activity, and generation of memory cells (6).

The role of cytokines in the immune system is quite complex because they act at different stages of cell activation as positive or negative regulators of the immune response. Some examples of the different profiles include Th1 cytokines such as interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and interleukin 2 (IL-2), which have clinical manifestations such as contact dermatitis. The Th2 cytokines such as IL-4, IL-5, and IL-13 can cause immediate hypersensitivity (7, 8). Their increased levels lead

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to different clinical manifestations that are counterbalanced by immunoregulatory cytokines such as IL-10 and TGF-β (8).

In addition to these cytokine-mediated mechanisms, some molecules expressed on the cell surface may also participate in immunoregulatory mechanisms. This is seen in case of the inhibitory CTLA-4 molecule, which binds to CD80 and CD86 molecules with much higher affinity than CD28 and leads to T-cell anergy (9).

Single nucleotide polymorphisms (SNPs) in cytokine and immunoregulatory molecule genes may contribute to the evaluation of individual susceptibility to several diseases including drug allergies (10,11). In our previous study (12), we found a significant association between *IL10* polymorphism (−1082 G > A) and efavirenz hypersensitivity reaction. Thus, our goal here was to evaluate polymorphisms in *IL10* −592 C/A, *IL4* −589 C/T, *IFNG* +874 A/T, and *CTLA4* +49 A/G genes in the same HIV-positive patients with efavirenz hypersensitivity reaction and in control patients, as evaluated before (12).

MATERIALS AND METHODS

Subjects: A case-control study was conducted on 63 HIV-infected adult patients (21 cases and 42 controls) under highly active antiretroviral therapy. The case definition of efavirenz hypersensitivity reaction was clinically determined. The selection was based on a drug change/modification request form (average daily rate reporting form) filled by the physician. The following terms were considered as clinical manifestations: rash, allergy, urticaria, pruritus, drug eruption, and erythema. Moreover, the medical records were searched for any missing data. Drug discontinuation led to a complete remission of the efavirenz hypersensitivity. From 2006 to 2012, all patients whose drug modification was requested were contacted and invited to participate in the study. Twenty-one patients (aged 24 to 71 years) agreed to participate in the study. The control group consisted of 42 HIV-infected patients (aged 20 to 67 years) who used efavirenz for at least 6 months without any evidence of adverse cutaneous reactions to the drug. Patients who complained of neuropsychiatric manifestations due to the use of efavirenz were excluded from the study. The study was approved by the Ethics in Research Committee of the Hospital São Jose de Doenças Infecciosas, Fortaleza, Ceará, Brazil, with process number 025/2011. All the participants signed the informed consent form.

DNA extraction: Genomic DNA was extracted from individual whole blood samples collected in tubes containing ethylenediaminetetraacetic acid (EDTA). DNA extraction was performed using the commercial kit Biopur Extraction Kit Plus Mini Spin-250 (Biopur, Curitiba, Brazil) based on the manufacturer's recommendations.

Polymorphism genotyping of cytokines and CTLA-4: Genetic polymorphisms of *IL10* (−592C/A) *IL4* (−589C/T), *IFNG* (+874 A/T), and *CTLA4* (+49A/G) were analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as described in Table 1.

The PCR reaction was done in a 20 μL reaction volume. The mixture contained 25 ng of DNA, TopTaq PCR Master Mix (Qiagen, GmbH, Germany), 0.01% bovine serum albumin as well as 10 μM primers for *IL10*, 12.5 μM primers for *IL4*, 25 μM primers for *IFNG*, and 10 μM primers for *CTLA4*. The reaction was performed in a thermocycler (Applied Biosystems, Foster City, CA, USA). The annealing step conditions for each PCR reaction are mentioned in Table 1.

The PCR-RFLP assays for SNP genotyping were performed by treating the PCR products with restriction endonucleases (Table 2) for 16 h at 37°C. Analysis of the restriction fragments was done using an electrophoresis

Table 1. Primers used in the polymorphism genotyping of cytokines and regulatory molecule CTLA-4 as well as the annealing temperatures used in the polymerase chain reaction

Polymorphism	Primer	Reference	Annealing temperature
IL10 (− 592 C/A)	F: 5′-CCTAGGTCACAGTGACGTGG-3′ R: 5′-GGTGAGCACTACCTGACTAGC-3′	(14)	60°C, 1 min
IL4 (− 589C/T)	F: 5′-ACTAGGCCTCACCTGATACG-3′ R: 5′-GTTGTAATGCAGTCCTCCTG-3′	(15)	57°C, 1 min
IFNG (+874 A/T)	F: 5′-GATTTTATTCTTACAACACAAAATCAAGAC-3′ R: 5′-GCAAAGCCACCCCACTATAA-3′	(16)	54°C, 1 min
CTLA4 (+49 A/G)	F: 5′-AAGGCTCAGCTGAACCTGGT-3′ R: 5′-CTGCTGAAACAAATGAAACCC-3′	(17)	60°C, 30s

F, Forward; R, Reverse.

Table 2. Restriction fragment length polymorphism technique: PCR products, restriction enzymes, and size of the restriction fragments

Polymorphism	PCR product	Restriction endonuclease	Restriction fragment
IL10 (−592 C/A)	412 bp	*Rsa I*	412 pb (allele C) 236 + 176 (allele A)
IL4 (−589C/T)	252 bp	*BsmFI*	252 (allele T) 192 + 60 (allele C)
IFNG (+874 A/T)	176 bp	*Hinf I*	176 (alleleA) 148 + 28 (allele T)
CTLA4 (+49A/G)	152 bp	*BstEII*	152 (allele G) 130 + 22 (allele A)

F, Forward; R, Reverse.

on 6% polyacrylamide gel after silver staining.

Statistical analysis: Statistical analyses were performed using the commercial software GraphPad Prism version 5.00 for Windows, USA. The Chi-square test or Fisher's exact test was used to determine the significance of the differences in observed frequencies between the efavirenz hypersensitivity reaction group and controls. Moreover, Hardy–Weinberg Equilibrium was tested for each SNP using Michael H. Court's (2005–2008) online calculator (13). The odds ratio (OR) with a confidence interval (CI) of 95% was calculated to identify the risk associated with genotypes (individual or combined), alleles, and haplotypes. We considered $p < 0.05$ to be statistically significant. The correlation between polymorphisms was evaluated by the Spearman correlation test. Genotypes that led to low, intermediate, and high cytokine levels/or CTLA-4 membrane expression were represented by the values "1," "2," and "3," respectively

RESULTS

Genotype distribution and allele frequency of *IL10* −592 C/A, *IL4* −589 C/T, *IFNG* +874 A/T, and *CTLA4* +49 A/G: The genotype distribution and allele frequencies of *IL10* −592 C/A, *IL4* −589 C/T, *IFNG* +874 A/T, and *CTLA4* +49 A/G for patients and controls are shown in Table 3. The distribution of genotypes in both groups was found to be in Hardy-Weinberg equilibrium.

A significant increase in the frequency of −592CA genotype ($p = 0.011$, OR 5.00 [1.486–16.82]) and in the allele −592A ($p = 0.028$; OR of 2.40 [1.088 to 5.294]) was observed in the case group, but not in the control group.

No significant association was observed between cases and controls for genotype and allele frequencies in *IL4* −589 C/T, *IFNG* +874 A/T, and *CTLA4* +49 A/G.

Our previous data on *IL10* −1082 G/A (12) were combined with the present ones (*IL10* −592 C/A) to

Table 3. Genotype and allele frequencies in efavirenz hypersensitivity reaction

Polymorphism	Control *n* (%)	Case *n* (%)	OR (95% CI)	*p*
IL10 (− 592)				
CC	25 (59.52)	5 (23.81)	5.000 (1.486 – 16.82)	0.011*
CA	14 (33.34)	14 (66.67)	3.333 (0.4373 – 25.41)	0.256
AA	3 (7.14)	2 (9.52)	4.706 (1.448 – 15.29)	0.009*
CA + AA	17 (40.48)	16 (76.19)		
Allele C	0.76	0.57		
Allele A	0.24	0.43	2.400 (1.088 – 5.294)	0.028*
IL4 (− 589)				
CC	18 (42.86)	6 (28.57)		
CT	18 (42.86)	11 (52.38)	1.833 (0.5575 – 6.029)	0.384
TT	6 (14.28)	4 (19.05)	2.000 (0.4173 – 9.584)	0.431
CT + TT	24 (57.15)	15 (71.73)	1.875 (0.6074 – 5.788)	0.410
Allele C	0.64	0.55		
Allele T	0.36	0.45	1.487 (0.6995 – 3.161)	0.301
IFNG (+ 874)				
AA	21 (50.00)	10 (47.62)		
AT	19 (45.24)	8 (38.09)	0.8842 (0.2891 – 2.705)	1.000
TT	2 (4.76)	3 (14.29)	3.150 (0.4519 – 21.96)	0.328
AT + TT	21 (50.00)	11 (52.38)	1.100 (0.3854 – 3.139)	1.000
Allele A	0.73	0.67		
Allele T	0.27	0.33	1.326 (0.5951 – 2.955)	0.489
CTLA4 (+ 49)				
AA	22 (52.38)	9 (42.86)		
AG	17 (40.48)	10 (47.62)	1.438 (0.4782 – 4.324)	0.582
GG	3 (7.14)	2 (9.52)	1.630 (0.2317 – 11.46)	0.631
AG + GG	20 (47.62)	12 (57.14)	1.467 (0.5104 – 4.215)	0.595
Allele A	0.73	0.67		
Allele G	0.27	0.33	1.326 (0.5951 – 2.955)	0.489

*: Chi-square test or Fisher test, $p < 0.05$.
CI, confidence interval; OR, odds ratio.

Table 4. Frequency of haplotype *IL10* gene promoter in efavirenz hypersensitivity reaction

Haplotype	Control $2n = 84$ (%)	Case $2n = 42$ (%)	OR (95% CI)	*p*
IL10 (− 1082/ − 592)				
GC	36 (42.86)	9 (21.43)		
AC	28 (33.33)	15 (35.71)	2.143 (0.8181 – 5.613)	0.153
AA	19 (22.62)	18 (42.86)	3.789 (1.430 – 10.04)	0.006[1)]
GA[2)]	1 (1.19)	0 (0.00)	1.281 (0.04819 – 34.04)	1.000

[1)]: Chi-square test or Fisher test, $p < 0.05$.
[2)]: Haplotype rare in Caucasians.
CI, confidence interval; OR, odds ratio. When any cell of the 2 × 2 table was "0", the software performed adjustments to obtain the odds ratio.

Table 5. Distribution of haplotype *IL10* gene promoter in efavirenz hypersensitivity reaction

Haplotype	Control $n = 42$ (%)	Case $n = 21$ (%)	OR (95% CI)	p
IL10 (− 1082/ − 592)				
GC/GC	8 (19.05)	1 (4.76)		
GC/GA	1 (2.38)	0 (0)	1.889 (0.04949 – 72.09)	1.000
GC/AC	12 (28.57)	3 (14.29)	2.000 (0.1753 – 22.81)	1.000
GC/AA	7 (16.67)	4 (19.05)	4.571 (0.4084 – 51.17)	0.319
AC/AC	5 (11.90)	1 (4.76)	1.600 (0.08052 – 31.79)	1.000
AC/AA	6 (14.29)	10 (47.62)	13.33 (1.320 – 134.7)	0.033*
AA/AA	3 (7.14)	2 (9.52)	5.333 (0.3432 – 82.88)	0.506
		Carriage of the haplotype GC		
Haplotype	Control $n = 42$ (%)	Case $n = 21$ (%)	OR (95% CI)	p
IL10 (− 1082/ − 592)				
GC carriage	28 (66.67)	8 (38.10)		
AC/AC; AC/AA; AA/AA	14 (33.33)	13 (61.90)	3.250 (1.093 – 9.665)	0.031*

*Chi-square test or Fisher test, $p < 0.05$.
CI, confidence interval; OR, odds ratio. When any cell of the 2 × 2 table was "0", the software performed adjustments to obtain the odds ratio.

form a haplotype (Table 4). Four possible haplotypes were observed in the population. In the control and case groups, the most common haplotypes were GC and AA, which corresponded to the frequency of 42.86% and 42.86%, respectively. The AA haplotype was significantly associated with efavirenz hypersensitivity reaction ($p = 0.006$; OR = 3.789, 95 [1.430–10.04]).

The possible combinations for the *IL10* haplotypes (−1082/−592) were GC/GC, GC/GA, GC/AC, GC/AA, AC/AC, AC/AA, and AA/AA (Table 5). The case group showed more frequency of AC/AA than the controls ($p = 0.0330$; OR = 13.33[1.320–134.7]). However, a higher frequency of haplotypes without GC was found among the cases compared to the control group ($p = 0.031$; OR = 3.250 [1.093–9.665]). This haplotype is related to higher *IL10* levels.

Table 6 presents the correlation coefficients between *IL10* −1082G/A, *IL10* −592C/A, *IL4* −589C/T and *IFNG* + 874A/T, and *CTLA4*+ 49A/G gene polymorphisms in the groups.

A positive correlation was found between *IL10* −1082 G/A and *IL10* −592 C/A. This event occurred in both groups (cases, r = 0.4332, $p = 0.0042$) and (controls, r = 0.5350, $p = 0.012$), respectively. Moreover, a significant inverse correlation between *CTLA4* +49A/G and *IL4* −589C/T polymorphisms (r = −0.6501, $p = 0.001$) was seen in the case group. No other statistically significant correlations were found when the other polymorphisms were analyzed.

Finally, we evaluated combinations of different polymorphisms (Table 7). The combined presence of the *IL4* −589T allele along with the *IL10* −1082A allele polymorphism was strongly associated with efavirenz hypersensitivity reaction ($p = 0.015$; OR = 15.970 [95% CI = 0.8477–300.9]). Further-more, the combined presence of the *IL4* −589C allele and the allele *IL10*

Table 6. Correlation analysis between gene polymorphisms

Correlation	r	95% CI	p
IL10 (− 1082G/A) versus *IL10* (− 592 C/A)			
Cases	0.4332	0.1397 – 0.6567	0.004*
Controls	0.5350	0.1208 – 0.7905	0.012*
IL10 (− 1082G/A) versus *IL4* (− 589 C/T)			
Cases	− 0.0920	− 0.5139 – 0.3657	0.691
Controls	0.0657	− 0.2518 – 0.3705	0.679
IL10 (− 1082G/A) versus *IFNG* (+874 A/T)			
Cases	0.0400	− 0.4101 – 0.4744	0.863
Controls	0.1874	− 0.1327 – 0.4722	0.235
IL10 (− 592C/A) versus *IL4* (− 589 C/T)			
Cases	− 0.1009	− 0.5205 – 0.3579	0.663
Controls	− 0.1193	− 0.4162 – 0.2006	0.452
IL10 (− 592G/A) versus *IFNG* (+874 A/T)			
Cases	0.2884	− 0.1770 – 0.6484	0.205
Controls	0.2616	− 0.05534 – 0.5306	0.094
CTLA4 (+49A/G) versus *IL4* (− 589 C/T)			
Cases	− 0.6501	− 0.8486 –0.2910	0.001*
Controls	− 0.1874	− 0.4721 – 0.1328	0.235
CTLA4 (+49A/G) versus *IFNG* (+874 A/T)			
Cases	0.1657	− 0.2991 – 0.5669	0.473
Controls	0.2246	− 0.09440 – 0.5018	0.153

*: Statistical significance in Spearman correlation ($p \leq 0.05$).
CI, confidence interval.

Table 7. Combination of cytokine and *CTLA4* gene polymorphisms in efavirenz hypersensitivity reaction

Polymorphism	Control *n* (%)	Case *n* (%)	OR (95%CI)	*p*
IL4 − 589 (CT + TT) combined with				
IL10 − 1082 GG	8 (19.05)	0 (0)		
IL10 − 1082 GA + AA	16 (38.09)	15 (71.43)	15.970 (0.8477 – 300.9)	0.015*
IL4 − 589 (CC) combined with				
IL10 − 1082 GG	1 (2.38)	1 (4.76)		
IL10 − 1082 GA + AA	17 (40.48)	5 (23.81)	0.294 (0.01545 – 5.599)	0.446
IL4 − 589 (CT + CC) combined with				
IL10 − 1082 AA	11 (26.19)	11 (52.38)		
IL10 − 1082 GG + GA	25 (59.52)	6 (28.57)	0.240 (0.0707 – 0.8145)	0.035*
IL4 − 589 (TT) combined with				
IL10 − 1082 AA	3 (7.14)	2 (9.52)		
IL10 − 1082 GG + GA	3 (7.14)	2 (9.52)	1.000 (0.07959 – 12.57)	1.000
IL4 − 589 (CT + TT) combined with				
IL10 − 592 CC	13 (30.95)	3 (14.29)		
IL10 − 592 AA + CA	11 (26.19)	12 (57.14)	4.727 (1.056 – 21.16)	0.048*
IL4 − 589 (CC) combined with				
IL10 − 592 CC	12 (28.57)	2 (9.52)		
IL10 − 592 AA + CA	6 (14.29)	4 (19.05)	4.000 (0.5632 - 28.41)	0.192
IL4 − 589 (CT + TT) combined with				
CTLA4 + 49 AA	12 (28.57)	3 (14.29)		
CTLA4 + 49 AG+GG	12 (28.57)	12 (57.14)	4.000 (0.8949 – 17.88)	0.093
IL4 − 589 (CC) combined with				
CTLA4 + 49 AA	10 (23.81)	6 (28.57)		
CTLA4 + 49 AG+GG	8 (19.05)	0 (0)	0.095 (0.00466 – 1.939)	0.066
IFNG +874 (AT + TT) combined with				
IL10 − 592 CC	15 (35.71)	4 (19.05)		
IL10 − 592 CA + AA	6 (14.29)	7 (33.33)	4.375 (0.9273 – 20.64)	0.072
IFNG +874 (AA) combined with				
IL10 − 592 CC	10 (23.81)	1 (4.76)		
IL10 − 592 CA + AA	11 (26.19)	9 (42.86)	8.182 (0.8736 – 76.62)	0.055
IFNG +874 (AT + TT) combined with				
CTLA4 +49 AA	13 (30.95)	6 (28.57)		
CTLA4 +49 AG+GG	8 (19.05)	5 (23.81)	1.354 (0.3088 – 5.939)	0.721
IFNG 874 (AA) combined with				
CTLA4 +49 AA	9 (21.43)	3 (14.29)		
CTLA4 +49 AG+GG	12 (28.57)	7 (33.33)	1.750 (0.3514 – 8.715)	0.697

*: Chi-square test or Fisher test, $p < 0.05$.
CI, confidence interval; OR, odds ratio. When any cell of the 2 × 2 table was "0", the software performed adjustments to obtain the odds ratio.

−1082G polymorphisms was associated with lower susceptibility to drug hypersensitivity ($p = 0.035$; OR = 0.240 [95% CI = 0.0707–0.8145]). Furthermore, the combined presence of *IL4* −589T allele with the *IL10* −592A allele resulted in almost a 5-fold increase in the risk of efavirenz hypersensitivity reaction ($p = 0.048$, OR = 4.727 [95% CI = 1.056–21.16]).

DISCUSSION

Infections caused by viruses such as Epstein-Barr and HIV may increase the risk for development of adverse drug reactions. In case of HIV, studies have shown that the frequency of drug allergy reaches 3% to 20%, and skin rashes are about 100 times more common in these patients than in the general population (1).

Drug exposure is an important component, but cannot by itself cause hypersensitivity. Susceptibility to drug allergies is multifactorial and includes individual genetic factors (5).

IL-10 is secreted by various immune cells and presents significant immunoregulatory effects such as inhibition of the pro-inflammatory cytokines IL-1, IL-6, IL-12, IL-18, and TNF (18). At the proximal promoter region of the *IL10* gene, 3 SNPs may be found at the −1082 (G/A), −819 (C/T), and −592 (C/A) positions (19). Considering the haplotype, GCC is associated with high levels of IL-10; ACC, to intermediate levels of the cytokine; and ATA, with lower levels of IL-10 (20). The GTA haplotype is frequently found among Chinese individuals, but is rarely found in Caucasians (21).

Qiao et al. (11) found a high frequency of the −1082AA genotype and of the allele −1082A in patients with positive anti-penicillin immunoglobulin E (IgE) versus those in non-allergic controls or in allergic patients with no specific IgE antibodies. Guglielmi et al. (22) found that atopic women carrying the CT/TT (−819) and/or CA/AA (−592) genotypes presented with higher risk of β-lactam antibiotic allergy.

Our group has previously investigated *IL10* (−1082 G/A) gene polymorphism in efavirenz hypersensitivity reaction (12). The frequencies of the −1082AA genotype and of the −1082A allele were higher in the case group than in controls (12). The current data also demonstrated a strong association between *IL10* gene polymorphism (−592 C/A) and hypersensitivity to

efavirenz. Table 3 shows that increased frequencies of the *IL10* −592CA genotype and the *IL10* −592A allele were found in patients with efavirenz hypersensitivity.

An important finding was the high frequency of the haplotype −1082A/−592A in HIV-positive patients with efavirenz hypersensitivity (Table 4). Moreover, individuals carrying the haplotype −1082A−592C/−1082A−592A showed 13.33-fold more risk of developing the efavirenz hypersensitivity reaction (Table 5). The results suggest that *IL10* −1082A and *IL10* −592A polymorphisms led to low IL-10 production. Once IL-10 is involved in immunoregulatory processes, one would expect a lack of homeostatic balance and, therefore, an exacerbation of the Th1, Th2, or Th17 profiles (23).

CTLA-4 is a cell surface glycoprotein that presents a high affinity for the co-stimulatory molecules CD80 and CD86, leading to a negative regulation of T-cell activation (24). The human *CTLA4* gene is located on chromosome 2q33 and contains a dimorphic A/G at position +49 in exon 1 that causes an amino acid change of threonine to alanine at position 17 in the leader sequence of the CTLA-4 protein. Some studies have shown that the presence of allele G at position +49 of exon 1 can mitigate the negative regulation of T cell activation by CTLA-4, making it an important factor in the pathogenesis of autoimmune diseases and other pathologies (25).

For instance, carriers of the A allele (AG/AA), especially Caucasians and the Chinese, present with an increased risk of developing cancer (24). Furthermore, the *CTLA4* +49GG genotype and the G allele were found to be associated with type 1 diabetes mellitus, Graves' disease, rheumatoid arthritis, and severe asthma and airway hyper-responsiveness (26).

We found no difference in genotype distributions and allele frequencies of *CTLA4* (+49A/G) in the patients and controls (Table 3). However, we found a significant negative inverse correlation between *CTLA4* (+49A/G) and *IL4* (−589 C/T) in the patient group ($r = -0.6501$, $p = 0.001$) (Table 6).

IL-4 plays a key role in the differentiation of naive CD4 T cells to the Th2 subset, which is the major cytokine that stimulates IgE production in B cells (27). The gene encoding IL-4 is located on human chromosome 5 between 5q23 and 5q31. A SNP at position −589 (C/T) in the promoter region of the *IL4* gene was identified. The presence of the T allele (−589T) is associated with increased cytokine production, whereas the C allele (−589C) is associated with lower cytokine levels (28, 29).

Micheal et al. (30) have shown that the TT genotype and T allele were more frequent in patients with allergic rhinitis and asthma than in healthy controls.

Our results did not show a significant association of *IL4* polymorphism −589 (C/T) with efavirenz hypersensitivity. Nevertheless, we have observed that the combined presence of the allele *IL10* −1082A (GA + AA) and *IL4* −589T (CT + TT) increased the risk of efavirenz hypersensitivity reaction in approximately 16-fold (Table 7). Therefore, lower IL-10 levels, but increased IL-4 production, would make the subject highly susceptible to drug hypersensitivity. Moreover, the combined presence of *IL10* −592A (CA + AA) with *IL4* −589T (CT + TT) resulted in a higher susceptibility to efavirenz hypersensitivity reaction (Table 7). Skin rashes related to efavirenz are usually mild to moderate maculopapular exanthema (5). Maculopapular exanthema is classified as a subtype IVc of hypersensitivity. In this subtype, cytotoxic CD4 T subpopulation predominates with a mixed Th1/Th2 cytokine profile (31).

IFN-γ plays a critical role in both innate and adaptive immunity, which induces macrophage activation, Th1 subset differentiation, higher expression of MHC class I and II (32).The gene encoding IFN-γ in humans is located on chromosome 12 at 12q14. Some polymorphisms are detected along with the sequence of the *IFNG* gene, particularly in introns 1 and 3. A SNP located in the first intron at position +874 (T/A), which is close to the binding site of the nuclear transcription factor NF-κB, may be associated with differences in the IFN-γ production. The T allele is associated with increased IFN-γ levels, while the A allele is associated with lower cytokine levels (33, 34).

Selma et al. (16) have demonstrated that the AA genotype was significantly associated with active pulmonary tuberculosis. Hussein et al. (35) found that the AA genotype and the A allele frequencies were significantly increased in atopic patients compared with in the control subjects. In the present study, we have found higher frequencies of the TT genotype and T alleles in patients with hypersensitivity to efavirenz than in the control subjects; however, the differences were not statistically significant (Table 3).

Therefore, our data suggest that polymorphisms in regulatory regions of cytokine production may modulate an individual's susceptibility to efavirenz hypersensitivity reaction. Limitations of the study were mostly related to the high rate of patient refusal to participate in the study. Several factors were related to low socioeconomic status, distance from the patient's home to the hospital, inability to understand the purpose of the study, and fear of exposing others to their disease. Furthermore, these limitations also prevented us from performing skin tests for efavirenz hypersensitivity.

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Conflict of interest None to declare.

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