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## Original article

# Fc gamma receptor IIIa polymorphism is not associated with susceptibility to systemic lupus erythematosus in Brazilian patients



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### ABSTRACT

We evaluated the possible association between FCGR3A V/F (158) polymorphism and SLE susceptibility and clinical phenotype in 305 sequentially retrieved SLE patients and 300 healthy controls from the southeastern part of Brazil by allele-specific polymerase chain reaction. Our results showed no association between FCGR3A 158V/F alleles and susceptibility to SLE in this series of patients albeit the heterozygous genotype was strongly associated with the disease.

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### Polimorfismo do receptor Fc gama IIIa não está associado à susceptibilidade ao lúpus eritematoso sistêmico em pacientes brasileiros

### RESUMO

Avaliou-se a possível associação entre o polimorfismo FCGR3A V/F (158) e a suscetibilidade e o fenótipo clínico do lúpus eritematoso sistêmico (LES) em 305 pacientes com LES admitidos sequencialmente e 300 controles saudáveis da Região Sudeste do Brasil por reação em cadeia da polimerase alelo-específica. Os resultados do presente estudo mostraram não haver associação entre os alelos FCGR3A 158V/F e a suscetibilidade ao LES nessa série de pacientes, ainda que o genótipo heterozigoto tenha sido fortemente associado à doença.

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## Introduction

Systemic lupus erythematosus (SLE) is considered the prototype of chronic immune complex-mediated diseases. In physiological conditions, circulating immune complexes (IC) are removed from the peripheral blood and other biological fluids by the mononuclear phagocytic system.<sup>1,2</sup> In SLE, the inadequate clearance of IC may lead to tissue damage due to IC tissue deposition and overload, which results in release of inflammatory mediators and inflammatory cell influx.<sup>2,3</sup> The Fc gamma receptors (FcγR), present in mononuclear phagocytic cells, play an important role in the clearance of IC and apoptotic cells.<sup>4,5</sup> In addition, the internalization of nucleic acid-containing IC via FcγR by plasmacytoid dendritic cells allows the binding of intracellular toll-like receptors (TLR) and consequent activation of downstream cascades that culminate in the synthesis of type I interferon.<sup>6,7</sup> Several single nucleotide polymorphisms (SNPs) have been described in the FcγR encoding genes, and some of them have functional consequences. As expected, some of these polymorphisms have been extensively studied in autoimmune diseases.<sup>5,8</sup>

Human FcγRIII (CD16) receptor is an extensively glycosylated heterogeneous protein with an apparent molecular weight of 50–80 kDa. FCGR3A gene codes for the FcγRIIIa receptor present in macrophages, NK and γδ T cells, with low-affinity for IgG-containing IC.<sup>4,9,10</sup> The FCGR3A gene presents a G559T polymorphism that leads to the substitution of Valine for Phenylalanine at position 158 of the polypeptide chain (158V/F), reducing even more the receptor affinity for IgG subclasses.<sup>9–11</sup> The association between FCGR3A gene polymorphism and SLE has been studied by several investigators. However, conflicting results have been reported concerning this association in different populations.<sup>9,10,12–16</sup> Given this scenario, the present study aimed at investigating the FcγRIIIa 158V/F polymorphism in Brazilian SLE patients that are embedded in a population with mixed ethnic background.

## Material and methods

### Patients and controls

Peripheral blood was obtained from 305 sequentially retrieved SLE patients attending the Rheumatology Outpatient Clinic at Universidade Federal de São Paulo – UNIFESP, Brazil. All patients met at least four of the revised criteria for classification of SLE according to the American College of Rheumatology.<sup>17</sup> Detailed clinical characteristics were not available for all patients and only the charts with consistent data were considered in the analysis of the clinical phenotype of disease. Accordingly, Systemic Lupus International Collaborating Clinics/American College of Rheumatology-Damage Index (SLICC-DI) data was available for 167 patients. The control group comprised 300 healthy blood donors who had no family history of autoimmune diseases as assessed by a detailed questionnaire. The study was approved by the Institutional Ethics Committee (# 2074/07) and all participants provided written informed consent.

### Ethnic classification

Latin American countries in general, and Brazil in particular, present intense ethnic miscegenation, mainly at the expense of African and European elements. We adopted the ethnic classification used by the Grupo Latinoamericano de Estudio del Lupus (GLADEL).<sup>18</sup> According to this classification, the individual sorts himself regarding his own ethnicity as well as his parents and four grandparents. The ethnic groups studied were defined as follows:

- Black ethnicity: individual in the study, parents and grandparents classified as black.
- White ethnicity: individual in the study, parents and grandparents classified as white.
- Mixed ethnicity: existence of at least one disagreement in the lineage of the individual classification by maternal or paternal grandparents.

### Nucleic acid isolation and FCGR3A polymorphism analysis

Genomic DNA was extracted from blood samples by salting out, based on the methodology previously described by Laitinen et al.<sup>19</sup> We examined the single nucleotide polymorphism (SNP)G559T (rs396991) of FCGR3A (<http://www.ncbi.nlm.nih.gov/gene/2214>) by allele-specific PCR according to Wu et al.<sup>9</sup> with minor modifications, using a single sense primer (5' TCA CAT ATT TAC AGA ATG GCA ATG G 3') and two antisense primers: 5' TCT CTG AAG ACA CAT TTC TAC TCC CTA C 3' for G allele; and 5' TCT CTG AAG ACA CAT TTC TAC TCC CTA A 3') for T allele. The 50 μL reaction mixture contained 100 ng DNA, 1.2 mM MgCl<sub>2</sub>, 0.2 m MnTP, 2.5 U Platinum Taq DNA Polymerase and 10 pmol of each allele-specific sense and antisense primers. PCR started with an initial step of 5 min at 95 °C, followed by 35 cycles of 30 s at 94 °C, 45 s at 54 °C, and 20 s at 72 °C, with a final extension step of 8 min at 72 °C. The 138 bp amplicon was analyzed by electrophoresis on a 3% agarose gel in TBE.

### Research for autoantibodies

We identified all subserologies (double-stranded DNA, Sm, RNP, SSA, SSB). We used indirect immunofluorescence for ANA test and ds-DNA and Ouchterlony double diffusion for the autoantibodies Sm, RNP, SSA-Ro and SSB-La.

### Statistical analysis

Chi-square test and the Bonferroni correction for multiple comparisons were used to analyze categorical variables, and the Fisher's exact test was employed when appropriate. Continuous variables were tested for the pattern of distribution by the Kolmogorov–Smirnov test. Variables with normal distribution were analyzed by the Student's t test, and those with non-parametric distribution were analyzed by the Kruskal–Wallis test (three or more groups) followed by the Mann–Whitney test. Significance was established at  $p < 0.05$ . Statistical calculations were performed with SPSS 17.0 and Minitab Statistical 15.0.

**Table 1 – Ethnic and gender distribution of SLE patients and controls.**

Ethnicity	SLE		Controls		<i>p</i> <sup>a</sup>
	<i>n</i>	%	<i>n</i>	%	
Black	12	3.9	16	5.4	0.113
White	111	36.4	130	43.3	
Mixed	182	59.7	154	51.3	
Gender	Female	Male	Female	Male	<i>p</i> <sup>a</sup>
	291	14	178	122	

<sup>a</sup> Chi-square test.

**Table 2 – FCGR3A genotype and allele distribution in SLE patients and controls.**

Genotype	SLE		Controls		<i>p</i> <sup>a</sup>	<i>p</i> <sup>b</sup>
	<i>n</i>	%	<i>n</i>	%		
VV	35	11.5	54	18.0	0.002	0.023
FF	23	7.5	39	13.0		
VF	247	81.0	207	69.0		
Allele	<i>n</i>	%	<i>n</i>	%	<i>p</i> <sup>c</sup>	
	V	317	52.0	315		52.5
F	293	48.0	285	47.5		

<sup>a</sup> Chi-square test.

<sup>b</sup> Bonferroni correction for multiple comparisons method.

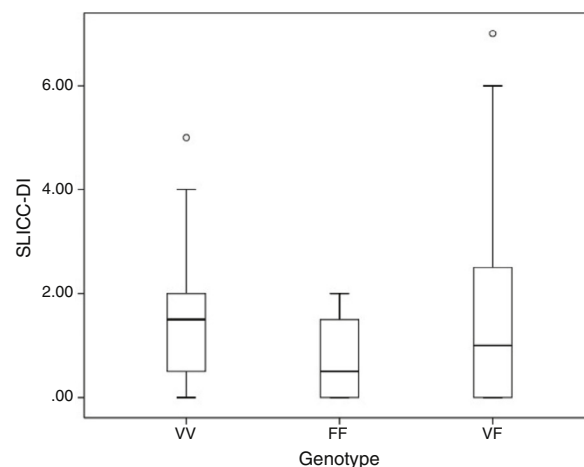
<sup>c</sup> Chi-square test.

## Results

There was no difference between patients and controls regarding ethnic background distribution, but the SLE group had a lower frequency of males as compared to the control group (Table 1). However, the statistical analysis performed within each group confirmed that neither allele distribution (SLE patients  $p=0.863$ ; controls  $p=1.000$ ) nor genotype distribution (SLE patients  $p=0.449$ ; controls  $p=1.000$ ), was gender related (data not shown).

The patient group had a significantly higher proportion of heterozygous individuals (VF) than the control group (Table 2). Accordingly, the control group presented a significantly higher proportion of the homozygous FCGR3A genotypes VV ( $p=0.023$ ) and FF ( $p=0.027$ ). As expected, there was no difference between groups regarding the prevalence of individual alleles ( $p=0.853$ ) (Table 2). The FCGR3A genotype distribution showed deviation from the Hardy Weinberg equilibrium in both, SLE and control groups.

There was no association between most clinical manifestations and genotypes (Table 3) with the exception of a higher frequency of central nervous system (CNS) manifestations, observed in patients with FCGR3A VV genotype (5 patients had psychosis, three had convulsions and one had other manifestation). Among the 167 SLE patients with available SLICC/ACR-DI records, no association was found between this damage score and any FCGR3A genotype ( $p=0.300$ ) (Fig. 1). No association was found between FCGR3A genotypes and the presence of the individual autoantibodies anti-dsDNA,



**Fig. 1 – Boxplot distribution of SLICC/ACR-DI according to the FCGR3A genotypes. No association was found between the damage score and any particular FCGR3A genotype ( $p=0.300$ ).**

anti-Sm, anti-RNP, anti-SS-A/Ro and anti-SS-B/La among the 305 SLE patients (Table 4).

## Discussion

This is the first study analyzing FCGR3A polymorphism in Brazilian SLE patients. Despite the sizable number of patients and normal controls, no difference was found in the

**Table 3 – Clinical manifestations of SLE patients according to FCGR3A genotype.**

Manifestation (n)		VV		FF		VF		p <sup>a</sup>
		n	%	n	%	n	%	
Skin (178)	⊕	16	100.0	6	100.0	147	94.0	0.511
	∅	0	0.0	0	0.0	9	6.0	
Oral ulcers (145)	⊕	1	6.7	0	0.0	28	22.6	0.158
	∅	14	93.3	6	100.0	96	77.4	
Arthritis (178)	⊕	16	100.0	6	100.0	138	88.5	0.244
	∅	0	0.0	0	0.0	18	11.5	
Hematologic (178)	⊕	10	62.5	5	83.3	116	74.3	0.509
	∅	6	37.5	1	16.7	40	25.7	
Renal (178)	⊕	10	62.5	2	33.3	88	56.4	0.463
	∅	6	37.5	4	66.7	68	43.6	
Serositis (178)	⊕	4	25.0	1	16.7	38	24.3	0.909
	∅	12	75.0	5	83.3	118	75.7	
CNS (177)	⊕	9	53.3	1	17.7	30	19.4	0.003
	∅	7	43.8	5	83.3	125	80.6	

n, number of patients with consistent records for each clinical trait; CNS, central nervous system; ⊕, presence genotype; ∅, absence genotype.

<sup>a</sup> Chi-square test.

**Table 4 – Presence of individual autoantibodies in SLE patients according to the FCGR3A genotype.**

Autoantibody	Genotype						p <sup>a</sup>
	VV (n = 35)		FF (n = 23)		VF (n = 247)		
	+	%	+	%	+	%	
Anti-dsDNA	12	34.3	8	34.8	72	29.1	0.832
Anti-Sm	8	22.9	6	26.1	37	15.0	0.231
Anti-RNP	15	42.9	8	34.8	73	29.6	0.267
Anti-SS-A/Ro	8	22.9	10	43.5	74	30.0	0.243
Anti-SS-B/La	0	0	3	13.0	25	10.1	0.122

<sup>a</sup> Chi-square test.

distribution of FCGR3A 158V/F alleles between SLE and healthy controls. Interestingly, SLE patients did show a significantly higher frequency of the heterozygous 158V/F genotype. In addition, there was a higher frequency of the VV homozygous genotype in patients with a history of CNS involvement. Other clinical traits and disease severity were not associated with FCGR3A polymorphism in this series. According to our results, the FCGR3A genotype distribution did not obey the Hardy Weinberg equilibrium. One possible explanation for this observation is the existence of gene copy number variation, a major cause of deviation of Hardy–Weinberg equilibrium.<sup>20</sup> In fact, recent studies have shown that Fc $\gamma$  receptor genes may present copy number variation, including FCGR3A, FCGR3B, and FCGR2C genes.<sup>21,22</sup>

Willcocks and colleagues found an association between low FCGR3B gene copy number and SLE in Caucasians, however this association was not seen in Chinese SLE patients in general or when SLE patients with lupus nephritis were analyzed separately.<sup>23</sup> In another study with the Chinese population, Zhou and colleagues found an association between increased FCGR3A gene copy number (3 or 4) and the presence of anti-glomerular basement membrane antibody.<sup>24</sup>

It is known that the FCGR3A 158V allele encodes for a higher affinity receptor than the 158F allele.<sup>9,10</sup> Consistent with the role played by Fc $\gamma$ RIIIa in immune complex clearance, it is

conceivable that the decreased binding capacity of the 158F allele would be associated with immune complex-mediated diseases. In fact, an increased susceptibility to SLE in FCGR3A 158F/F individuals has been reported in some ethnic groups.<sup>3</sup> However, the literature is controversial regarding this association. Among Japanese, homozygosity for the 158F allele contributed to SLE susceptibility,<sup>15</sup> but in the Thai population, only a tendency to association was found between the FF genotype and SLE susceptibility.<sup>25</sup> No association was found between the 158V/F polymorphism and SLE in Spanish,<sup>14</sup> African-American,<sup>12</sup> and Mexican individuals.<sup>11</sup> In Koreans, according to Salmon et al.,<sup>26</sup> homozygosity for the F allele constituted a risk factor for lupus nephritis, in contrast to the results of Lee and colleagues, who found no association between this polymorphism and SLE in this ethnic group.<sup>27</sup> In the German population, although the Fc $\gamma$ RIIIa polymorphism did not confer susceptibility to SLE, the presence of the F allele was associated with clinical manifestations, prognosis, and course of disease (Table 5).<sup>1</sup>

In our study the heterozygous genotype (VF) was more prevalent in SLE patients, and as expected this caused no difference in the allelic distribution between SLE and control groups. The reason for a higher frequency of the heterozygous 158V/F genotype in SLE patients is not readily clear. It could be related to copy number variation and it may be hypothesized that an intermediate affinity state in the pool of Fc $\gamma$ RIIIa receptors, as provided by the coexistence of high avidity (158V) and low avidity (158F) FCGR3A variants, would set an immune complex-clearance scenario favorable to the development of immune complex-mediated diseases in this ethnic set-up. Experimental data are required to test this hypothesis.

Another important aspect to be considered is that the isolated analysis of FCGR3A polymorphism may be an oversimplification of the problem. It should be remembered that Fc $\gamma$ RIIIa is one of several receptors involved in immune complex clearance. Therefore, the final immune complex clearance capacity should correspond to the overall genetic make-up of the several receptors involved in this process.

**Table 5 – FCGR3A-158V/F polymorphism and SLE in various ethnic groups.**

Ethnicity	Patients (n)	Increased susceptibility	Genotypic association	Phenotype association		Ref	Year publication
				Clinical trait	p <sup>a</sup>		
Korean	148	No	158 F/F	Nephritis	<0.007	20	1999
African-American	77	No	ND	No	ND	12	1999
Japanese	193	Yes	158 F/F	Nephritis	0.03	15	2002
Spanish	276	No	ND	No	ND	14	2002
Korean	145	No	ND	No	ND	21	2002
German	140	No	ND	No	ND	1	2002
Thai	87	Trend	158 F/F	Malar rash	0.03	19	2003
		Trend		Discoid rash	0.04		
Mexican	94	No	ND	No	ND	11	2011

ND, not determined.

<sup>a</sup> Chi-square test.

With respect to SLE clinical manifestations, Wu and colleagues found that there was a strong association between the FF genotype and lupus nephritis in Americans from diverse genetic background,<sup>9</sup> similarly to what was observed in the Korean population by Salmon et al., in 1999.<sup>26</sup> However, even in Americans with varied genetic background the role of FCGR3A polymorphism in SLE phenotype is not clear, since Alarcon and colleagues found that homozygosity for the V allele (FCGR3A\*GG) was a significant predictor of end-stage renal disease among SLE patients with kidney disease.<sup>28</sup> The association of the 158 F allele with lupus nephritis<sup>9,26</sup> and the association of the V allele with end-stage renal disease<sup>28</sup> suggest that other still unknown factors may influence the development and outcome of renal manifestations in SLE. Association between the VV genotype and history of CNS involvement has not been reported by other authors. This original finding is intriguing, but one should keep in mind that, due to the low number of homozygous VV SLE patients in the present series, this association must be regarded as preliminary.

In conclusion, this original analysis of FCGR3A 158V/F polymorphism in Brazilian SLE patients showed no association between any of the alleles and susceptibility to SLE, but disclosed a remarkably higher frequency of the heterozygous 158V/F genotype in SLE patients as compared to healthy controls. In addition, we have not confirmed the previously reported association between the 158 F allele and lupus nephritis in our series, but we did find an intriguing association between the 158V allele and central nervous system involvement. The present findings support the impact of the genetic variability of the Fc $\gamma$  receptors in general and the Fc $\gamma$ RIIIa in particular on the susceptibility and phenotype of systemic lupus erythematosus. Our preliminary results warrant further studies to confirm and investigate the role of the family of Fc $\gamma$  receptors in the pathophysiology of systemic lupus erythematosus.

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## Conflicts of interest

The authors declare no conflicts of interest.

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