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## Original article

# Polymorphisms in NAT2 (N-acetyltransferase 2) gene in patients with systemic lupus erythematosus



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### ABSTRACT

**Objective:** To investigate potential associations of four substitutions in NAT2 gene and of acetylator phenotype of NAT2 with systemic lupus erythematosus (SLE) and clinical phenotypes.

**Methods:** Molecular analysis of 481C>T, 590G>A, 857G>A, and 191G>A substitutions in the NAT2 gene was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, from DNA extracted from peripheral blood samples obtained from patients with SLE (n = 91) and controls (n = 97).

**Results and conclusions:** The 857GA genotype was more prevalent among nonwhite SLE patients (OR = 4.01, 95% CI = 1.18–13.59). The 481T allele showed a positive association with hematological disorders that involve autoimmune mechanisms, specifically autoimmune hemolytic anemia or autoimmune thrombocytopenia (OR = 1.97; 95% CI = 1.01–3.81).

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### Polimorfismos no gene NAT2 (N-acetiltransferase 2) em pacientes com lúpus eritematoso sistêmico

### RESUMO

**Objetivo:** Investigar potenciais associações de quatro substituições do gene NAT2 (N-acetiltransferase 2) e do fenótipo acetilador de NAT2 com o lúpus eritematoso sistêmico (LES) e os fenótipos clínicos.

**Métodos:** A análise molecular das substituições 481C>T, 590G>A, 857G>A e 191G>A do gene NAT2 foi feita com a técnica de PCR-RFLP, usando DNA extraído de amostras de sangue periférico obtidas de pacientes com LES (n = 91) e controles (n = 97).

#### Palavras-chave:

Lúpus eritematoso sistêmico

N-acetiltransferase 2

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**Resultados e conclusões:** O genótipo 857GA foi mais prevalente entre pacientes com LES não brancas (OR = 4,01, IC 95% = 1,18-13,59). O alelo 481 T apresentou associação positiva com as alterações hematológicas que envolvem mecanismos autoimunes, especificamente anemia hemolítica autoimune ou trombocitopenia autoimune (OR = 1,97; IC 95% = 1,01-3,81).

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## Introduction

Systemic lupus erythematosus (SLE) is an autoimmune, chronic and systemic syndrome. The involvement of various tissues and organs is the result of chronic inflammatory processes generated by the deposition of immune complexes, resulting from an increased production of autoantibodies. Although the etiology of SLE is not fully understood, studies point to a complex interplay between genetic and environmental determinants.<sup>1</sup> Ultraviolet radiation, drugs, infectious agents, smoking, and various chemical agents have been described as potential risk factors for this disease.<sup>2</sup> Studies in different populations have shown that smoking habits have a positive association with SLE, with odds ratios between 1.6 and 6.6.<sup>2-7</sup> In addition to influencing the development of SLE, the smoking habit has also been linked to the maintenance of disease activity and to a lower response to treatment with antimalarial drugs.<sup>4,8</sup>

The association between genetic factors and lupus, especially hydralazine-induced lupus, was first assessed in 1970. A relationship between N-acetyltransferase 2 enzyme (NAT2), capable of acetylating the drug, and lupus was observed, with a greater proportion of slow acetylator phenotype among patients with the disease.<sup>9</sup> A lower cumulative dose of procainamide was also able to induce lupus in patients with slow acetylator phenotype when compared to patients with fast acetylator phenotype.<sup>10</sup> In another study, in which the smoking habit and the phenotype of NAT2 enzyme, involved in the acetylation of cigarette smoke components, were considered, the higher risk (2.26 times) developing of SLE attributed to smokers versus nonsmokers was even higher (6.44 times) when the slow phenotype of NAT2 enzyme was observed.<sup>2</sup>

The N-acetyltransferase enzymes (NATs) participate in the second phase of metabolism of xenobiotics, and the acetylation reaction can occur by two distinct pathways: by O-acetylation, after the action of the first-phase enzyme P450 (CYP450), and by N-acetylation, when the enzyme acts on the compound in a previous step to the action of CYP450. Both pathways may result in the formation of a DNA adduct.<sup>11</sup>

There are two isoforms of human N-acetyltransferase enzymes, NAT1 and NAT2, which are differently expressed according to the tissue and have specificity for different substrates.<sup>11-13</sup> Acetylation of aromatic compounds and heterocyclic amines, such as 4-aminobiphenyl, present in cigarette smoke,<sup>14</sup> through the transfer of acetyl group of a molecule of acetyl-CoA to the free-amino group of the compound, is preferably promoted by NAT2.<sup>14</sup>

The NAT2 isoform is encoded by the NAT2 gene, which is located on chromosome 8 (8p22), together with the NAT1 gene and the pseudogene NATP. The NAT2 gene is highly

polymorphic and the diversity of the described alleles results from the combination of point mutations of selected bases (SNPs – single nucleotide polymorphisms).<sup>15</sup> The phenotype of the enzyme can be determined by existing SNPs in the coding region of the gene, which influences the affinity for the substrate, the catalytic activity and/or stability of the resulting protein.<sup>16</sup> Among the nucleotide changes commonly used in order to infer the acetylator phenotype of NAT2, 481C>T (rs1799929), 590G>A (rs1799930), 857G>A (rs1799931), and 191G>A (rs1801279) stand out.<sup>2,17,18</sup>

Due to the importance of the topic in question and the lack of Brazilian research, in this study we performed an evaluation of the presence of four substitutions in the NAT2 gene (481C>T, 590G>A, 857G>A and 191G>A) and the phenotype of NAT2 enzyme in patients with systemic lupus erythematosus residents in the city of Rio de Janeiro. Our study population was stratified according to smoking status and ethnic characteristics. Some clinical features of the disease were also taken into account in our analysis.

## Materials and methods

### Study population

The study population was selected from a group of women participating in a previous study, which sequentially included SLE patients (according to the American College of Rheumatology classification criteria), regularly followed in the Rheumatology Unit, UERJ, and women without lupus who had attended for routine gynecological care in the same university (UERJ).<sup>19,20</sup>

Clinical and socio-demographic aspects, including information on race/ethnicity of the female participant and of their parents, by self-declaration, and smoking habits were obtained by applying a semi-structured questionnaire. Patients and controls were classified as white-colored subjects when the individual and his parents were white-colored by self-declaration, and as nonwhite-colored if the individual and/or at least one of her parents was brown- or black-colored by self-declaration. Specific clinical data were obtained from medical chart review.

The project was approved by the Research Ethics Committee of HUPE/UERJ (HUPE/UERJ #321 and #909), and all patients were included only after signing an informed consent form previously approved.

### Molecular analysis of polymorphisms in NAT2

Genomic DNA was extracted from peripheral blood samples, according to Vargas-Torres et al.<sup>21</sup> Analysis of the substitutions

481C>T, 590G>A, 857G>A and 191G>A in the NAT2 gene was carried out with the use of PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) technique, according to Huang et al.,<sup>17</sup> with some modifications. Amplification of genomic DNA was performed using the following primers: 5'-GGAACAAATTGGACTTGG-3' and 5'-TCTAGCATGAATCACTCTGC-3' (Life Technologies®). After amplification, aliquots of PCR product were digested separately with the restriction enzymes KpnI (481C>T) and BamHI (590G>A), followed by electrophoretic analysis in agarose gel, and with MspI (857G>A) and TaqI (191G>A), followed by analysis by polyacrylamide gel electrophoresis. After electrophoresis, the gels were stained with ethidium bromide and the products of digestion were visualized under UV light. To evaluate the reproducibility of the technique, 10% of samples were randomly selected and reevaluated.

### Phenotypic classification

In this study, the bimodal classification,<sup>17</sup> in which intermediate and fast phenotypes are grouped into a single category, was used. Thus, the presence and absence of the WT allele (absence of the four NAT2 substitutions, 481C>T, 590G>A, 857G>A, and 191G>A) define the fast and slow phenotypes, respectively. In practice, samples lacking one of the four changes, or samples heterozygous for only one of the substitutions, were considered as having fast acetylator phenotypes. On the other hand, the homozygous samples in only one of the substitutions were classified as slow acetylator phenotype. The definition of phenotypes of samples having two or more changes has been made considering all possible combinations of alleles and verifying their frequency in our population, according to the haplotypes' estimation analysis carried out based on the frequencies of individual substitutions, as described later. Combinations containing at least one rare haplotype with an estimated frequency <0.01 were considered unlikely and were discarded.

The samples without a result for one of the four substitutions analyzed whose missing genotype would not interfere in the phenotype defined by the other three substitutions were included in the study.

### Statistical analysis

Student's t-test was used to analyze the differences between means and standard deviations and was performed using the GraphPad Prism software version 6.05 (GraphPad Software, Inc., San Diego, CA).

The study population was tested for deviations from Hardy-Weinberg using the Chi-squared test ( $\chi^2$ ), assuming a degree of freedom = 1, with the use of SNPStats software (Institut Català d'Oncologia, Barcelona, Spain).<sup>22</sup>

The differences between genotype and allele frequencies of groups of cases and controls were evaluated by the  $\chi^2$  test or Fisher's exact test. The odds ratios (OR) and confidence intervals (95% CI) were determined in order to estimate the magnitude of the association between NAT2 substitutions with the presence of systemic lupus erythematosus, using, where possible, co-dominant, dominant, and recessive genetic models. The most frequent allele and the homozygous

genotype of this allele were considered as references. These tests were performed using GraphPad Prism version 6.05 software (GraphPad Software, Inc., San Diego, CA).

The analyses of interactions between genotypes or phenotypes of NAT2, smoking status, ethnic characteristics, and diagnosis of SLE, potential associations between genotypes or phenotypes of NAT2, and several clinical features of SLE, as well as the estimation of haplotypes from the frequencies of genotypes corresponding to the four substitutions, were performed using SNPStats software (Institut Català d'Oncologia, Barcelona, Spain).<sup>22</sup> p-Values <0.05 were considered significant.

## Results

Our study population consisted of 188 participants, 91 SLE patients (the case group) and 97 women without SLE (the control group). The mean age ( $\pm$ standard deviation) of SLE patients (40.6  $\pm$  11.1 years; 20-69 years) and of controls (36.9  $\pm$  10.8 years; 17-66 years) at the time of inclusion in the study was significantly different ( $p=0.0214$ ). Although the distributions by age in both groups also have been shown to be different ( $p=0.0168$ ), approximately 60% of women belonging to the two groups were aged between 30 and 49 years. The percentages of non-white women were different between the two study groups, with a marginal p-value ( $p=0.0539$ ). The most important clinical features observed in patients with SLE are shown in Table 1.

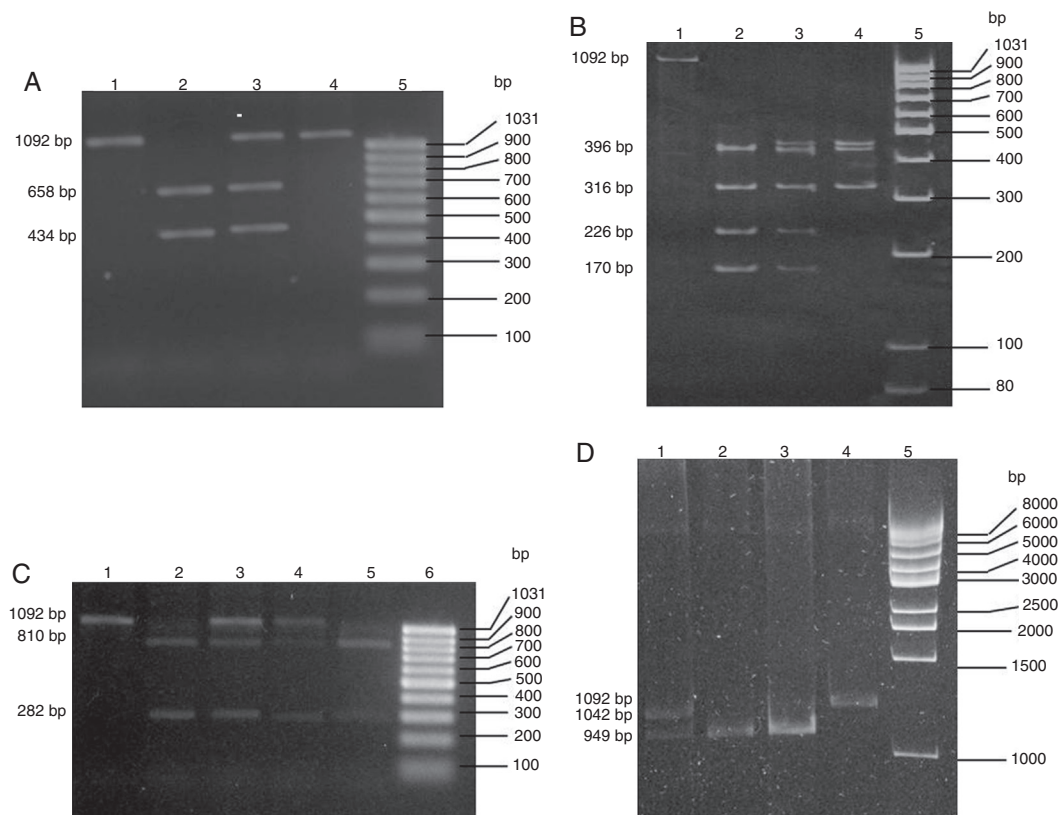
A total of 188 samples of DNA, corresponding to our study population, were assessed with respect to the four NAT2 substitutions. The digestion profiles representative of each analysis can be seen in Fig. 1.

The control group followed the Hardy-Weinberg principle with respect to the analyzed substitutions (Table 2). The genotype and allele distributions corresponding to the four

**Table 1 – Clinical characteristics of patients with systemic lupus erythematosus included in this study.**

<i>Clinical data</i>	
Age at onset of symptoms (mean $\pm$ SD) (years)	27.8 $\pm$ 10.9
Elapsed time between onset of symptoms and diagnosis of SLE (mean $\pm$ SD) (months)	20.0 $\pm$ 28.3
<i>American College of Rheumatology (ACR) criteria (%)</i>	
Malar rash	78.0
Discoid lesions	15.4
Photosensitivity	72.5
Oral ulcers	53.8
Arthritis	84.6
Pleuritis	33.0
Pericarditis	28.6
Hematological abnormalities <sup>a</sup>	39.6
Hemolytic anemia	44.6
Psychosis	14.3
Seizures	5.5
Renal impairment (proteinuria)	78.0
ANA, positive	60.4

<sup>a</sup> Reductions of platelets, lymphocytes and leukocytes were grouped as hematological changes.



**Fig. 1 – Molecular analysis of four substitutions in the NAT2 gene. (A)** Photograph of a 2.0% agarose gel stained with ethidium bromide for analysis of the substitution 481C>T. Lane 1 – negative control of the digestion reaction (intact amplicon); Lane 2 – representative sample of the NAT2 genotype 481CC (two alleles with *KpnI* restriction site); Lane 3 – representative sample of the NAT2 genotype 481CT (only one of the alleles with *KpnI* restriction site); Lane 4 – representative sample of the NAT2 genotype 481TT (two alleles without *KpnI* restriction site); Lane 5 – molecular weight pattern (GeneRuler™ 100 bp DNA Ladder, ready to use – Fermentas). **(B)** Photograph of a 6.0% polyacrylamide gel stained with ethidium bromide for analysis of the 590G>A substitution. Lane 1 – negative reaction control (intact amplicon); Lane 2 – representative sample of the NAT2 genotype 590GG (two alleles with an additional *TaqI* restriction site); Lane 3 – representative sample of the NAT2 genotype 590GA (only one of the alleles with an additional *TaqI* restriction site); Lane 4 – representative sample of the NAT2 genotype 590AA (two alleles without an additional *TaqI* restriction site); Lane 5 – molecular weight pattern (GeneRuler™ 100 bp DNA Ladder, ready to use – Fermentas). **(C)** Photograph of a 1% agarose gel stained with ethidium bromide for analysis of the 857G>A substitution. Lane 1 – negative control of digestion reaction (intact amplicon); Lanes 2 and 5 – representative samples of the NAT2 genotype 857GG (two alleles with an additional *BamHI* restriction site); Lanes 3 and 4 – representative samples of the NAT2 genotype 857GA (only one allele with an additional *BamHI* restriction site); Lane 6 – molecular weight pattern (GeneRuler™ 100 bp DNA ladder, ready to use – Fermentas). **(D)** Photograph of a 5% polyacrylamide gel stained with ethidium bromide for analysis of the 191G>A substitution. Lane 1 – representative sample of the NAT2 genotype 191GA (only one of the alleles with a *MspI* restriction site); Lanes 2 and 3 – representative samples of the NAT2 genotype 191GG (two alleles of an additional *MspI* restriction site); Lane 4 – negative control for digestion reaction (intact amplicon); Lane 5 – molecular weight pattern (GeneRuler™ 1 kp DNA Ladder, ready to use – Fermentas).

substitutions of the NAT2 gene, as well as the phenotype distributions of NAT2 (Table 2) and haplotype frequencies (Table 3) showed no statistically significant differences between cases and controls.

The study population was divided according to ethnic characteristics in two subgroups, white and nonwhite subjects. There was no significant difference in the analysis related to 481C>T, 590G>A, and 191G>A substitutions, or to the phenotype of NAT2. However, with regard to the 857G>A substitution, the GA genotype was more prevalent in patients with SLE

compared to controls, but only among non-white participants (OR = 4.01, 95% CI = 1.18–13.59;  $p = 0.023$ ) (Table 4).

The study population was further stratified according to smoking status (smokers or former smokers, and nonsmokers), and no association of this variable was found, along with NAT2 genotypes or the acetylator phenotype of the enzyme, with the diagnosis of disease (data not shown).

The analyses above mentioned were performed only in patients with lupus, considering the following clinical characteristics: the presence of malar rash, discoid



**Table 2 – Genotype and allele distributions for each of the four substitutions of the NAT2 gene and acetylator phenotype in case- (patients with SLE) and control groups.**

Substitution/acetylator phenotype	Genotype/allele/phenotype	Cases (n = 91) n (%) or (f)	Controls <sup>a</sup> (n = 97) n (%) or (f)	Cases vs. controls OR (95% CI)
NAT2 481C>T	CC	40 (44)	34 (35)	1.00
	CT	39 (43)	46 (48)	0.72 (0.39–1.35)
	TT	12 (13)	16 (17)	0.64 (0.27–1.53)
	CT+TT	51 (56)	62 (65)	0.70 (0.39–1.26)
	C	119 (0.65)	114 (0.59)	1.00
	T	63 (0.35)	78 (0.41)	0.77 (0.51–1.18)
NAT2 590G>A	GG	53 (59)	66 (68)	1.00
	GA	32 (36)	27 (28)	1.48 (0.79–2.76)
	AA	5 (6)	4 (4)	1.56 (0.40–6.09)
	GA+AA	37 (41)	31 (32)	1.49 (0.82–2.71)
	G	138 (0.77)	159 (0.82)	1.00
	A	42 (0.23)	35 (0.18)	1.38 (0.84–2.29)
NAT2 857G>A	GG	70 (84)	88 (93)	1.00
	GA	13 (16)	7 (7)	2.34 (0.88–6.17)
	AA	0	0	N/A
	GA+AA	13 (16)	7 (7)	2.34 (0.88–6.17)
	G	153 (0.92)	183 (0.96)	1.00
	A	13 (0.08)	7 (0.04)	2.22 (0.86–5.71)
NAT2 191G>A	GG	87 (96)	81 (91)	1.00
	GA	4 (4)	7 (8)	0.53 (0.15–1.89)
	AA	0	1 (1)	N/A
	GA+AA	4 (4)	8 (9)	0.47 (0.14–1.61)
	G	178 (0.98)	169 (0.95)	1.00
	A	4 (0.02)	9 (0.05)	0.42 (0.13–1.40)
Phenotype	Fast	45 (53)	48 (53)	1.00
	Slow	40 (47)	43 (47)	1.00 (0.56–1.82)

n, number of patients; f, allele frequency; N/A, not applicable.

The total samples with data relating to the four NAT2 polymorphisms and to the acetylator phenotype may differ from the total of analyzed samples, due to the existence of unsatisfactory results.

<sup>a</sup> Hardy-Weinberg equilibrium test: NAT2 481C>T ( $p = 1.00$ ); NAT2 590G>A ( $p = 0.50$ ); NAT2 857G>A ( $p = 1.00$ ); NAT2 191G>A ( $p = 0.19$ ) (SNPStats).

**Table 3 – Distributions of haplotypes of the NAT2 gene in groups of cases (lupus SLE patients) and controls.**

Haplotype of NAT2				Cases (f)	Controls (f)	Cases vs. controls OR (95% CI)
481C>T	590G>A	857G>A	191G>A			
T	G	G	G	0.3231	0.3990	1.00
C	G	G	G	0.3595	0.3405	1.25 (0.76–2.06)
C	A	G	G	0.2170	0.1653	1.67 (0.94–2.95)
C	G	A	G	0.0476	0.0360	1.89 (0.61–5.88)
C	G	G	A	0.0139	0.0435	0.63 (0.18–2.12)
T	G	A	G	0.0231	0	N/A

f, haplotype frequency; N/A, not applicable.

Haplotypes with frequency <0.01 were not considered.

lesions, photosensitivity, oral ulcers, arthritis, pleuritis, pericarditis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, psychosis, seizures, proteinuria, and positive ANA. The 481T allele was more prevalent in patients with hematological disorders (autoimmune hemolytic anemia or autoimmune thrombocytopenia), when compared to those who did not show this feature (OR=1.97; 95% CI=1.02–3.82;  $p=0.0477$ ). There was no association between the other NAT2 substitutions, or NAT2 phenotype, and clinical characteristics of the patients (data not shown).

In the analysis that included the smoking habit, the group of SLE patients was subdivided into current smokers and non-smokers, including ex-smokers. In isolation, the smoking habit was associated with lupus discoid lesions (OR=8.62, 95% CI=2.40–30.96,  $p=0.0011$ ) and proteinuria (OR=0.17, CI 95%=0.05–0.59;  $p=0.0056$ ). However, the joint analysis of NAT2 genotypes, or of acetylator phenotype, and smoking habit showed no association with the clinical characteristics of patients with SLE (data not shown).

**Table 4 – Analysis of interaction between genotypes related to substitutions in the NAT2 gene or NAT2 phenotype and ethnic origin, in case- (SLE patients) and control groups.**

Substitution/acetylator phenotype	Skin color/ethnicity	Genotype/phenotype	Cases n (%)	Controls n (%)	Cases vs. controls OR (95% CI)
NAT2 481C>T	White	C/C	12 (36)	6 (27)	1.00
		C/T	19 (58)	11 (50)	0.86 (0.25–2.95)
		T/T	2 (6)	5 (23)	0.20 (0.03–1.35)
	Nonwhite	C/C	28 (48)	28 (38)	1.00
		C/T	20 (35)	35 (47)	0.57 (0.27–1.22)
		T/T	10 (17)	11 (15)	0.91 (0.33–2.48)
NAT2 590G>A	White	G/G	18 (55)	17 (77)	1.00
		G/A	14 (42)	4 (18)	3.31 (0.91–12.06)
		A/A	1 (3)	1 (5)	0.94 (0.05–16.34)
	Nonwhite	G/G	35 (61)	49 (65)	1.00
		G/A	18 (32)	23 (31)	1.10 (0.52–2.33)
		A/A	4 (7)	3 (4)	1.87 (0.39–8.87)
NAT2 857G>A	White	G/G	27 (90)	19 (86)	1.00
		G/A	3 (10)	3 (14)	0.70 (0.13–3.87)
		A/A	0	0	N/A
	Nonwhite	G/G	43 (81)	69 (95)	1.00
		G/A	10 (19)	4 (5)	<b>4.01 (1.18–13.59)<sup>a</sup></b>
		A/A	0	0	N/A
NAT2 191G>A	White	G/G	32 (97)	20 (95)	1.00
		G/A	1 (3)	1 (5)	0.62 (0.04–10.57)
		A/A	0	0	NA
	Nonwhite	G/G	55 (95)	61 (90)	1.00
		G/A	3 (5)	6 (9)	0.55 (0.13–2.32)
		A/A	0	1 (1)	N/A
Phenotype	White	Fast	17 (55)	11 (52)	1.00
		Slow	14 (45)	11 (48)	1.21 (0.41–3.63)
	Nonwhite	Fast	28 (52)	37 (54)	1.00
		Slow	26 (48)	32 (46)	0.93 (0.46–1.90)

White, individual and the parents white-colored by self-declaration; Nonwhite, an individual and/or at least one of the parents brown or black-colored by self-declaration; n, number of patients; N/A, not applicable.

Significant result is in bold letters.

<sup>a</sup> Fisher's test:  $p = 0.0230$ .

## Discussion and conclusion

N-acetyltransferase 2 (NAT2) variants have been widely studied in order to assess its distribution among different ethnic groups, which can also be related to the exposure to different environmental factors; and its association with a wide range of diseases, including those of autoimmune origin. In this paper, we evaluate the possible existence of an association between the 481C>T, 590G>A, 857G>A and 191G>A polymorphisms in the NAT2 gene and systemic lupus erythematosus.

The studies of an association between NAT2 and SLE described in the literature, in general, consider the acetylator phenotype and not NAT2 polymorphisms in isolation. Taking into account that the substitutions here discussed may interfere with the levels of gene expression through post-transcriptional regulation mechanisms (481C>T), change the stability of the enzyme (590G>A and 191G>A), or modify the selectivity and catalytic activity (857G>A),<sup>16</sup> an association analysis was also performed with each of the substitutions in isolation. However, our results showed an absence of an association between the four NAT2 substitutions, or the NAT2 acetylator phenotype and the disease (Table 1). The estimate of

haplotypes also showed no association with SLE (Table 2), even though aiding in the phenotypic classification of the samples, as mentioned above. Recent studies have also used this feature to infer NAT2 phenotypes based on genotyping data.<sup>23,24</sup>

The polymorphisms analyzed in NAT2 gene have distinct allelic distributions among different populations. Sabbagh et al. conducted a worldwide survey of the frequency of several NAT2 polymorphisms and also of their phenotype.<sup>25</sup> The worldwide frequency distribution of polymorphisms in this study is found in Table 5. The observed frequencies in the control group are similar to those found on several continents. The allele NAT2 191A showed a frequency of 0.02, equal to that observed in North America, while the frequency of the allele 590A was similar to that observed in Africa (0.23 and 0.25, respectively). On the other hand, the alleles NAT2 481T (0.35) and NAT2 857A (0.08) had intermediate frequencies compared to those found in Africa and North America. The population of Rio de Janeiro, as well as the Brazilian population in general, results from the mixture of different ethnic groups, particularly African, European and Amerindians. Studies have shown that the population of Rio de Janeiro has approximately 31% of ancestry markers characteristic of Africans, 55% of Europeans and 14% of Amerindians.<sup>26</sup> In

**Table 5 – Frequencies of less frequent alleles of NAT2 (481T, 590A, 857A, 191A) observed in various populations.**

Populations	Substitutions of NAT2			
	857G>A	481C>T	590G>A	191G>A
Africa <sup>a</sup>	0.04	0.30	0.25	0.09
Europe <sup>a</sup>	0.03	0.44	0.27	0.01
Asia <sup>a</sup>	0.12	0.18	0.27	0
America <sup>a</sup>	0.20	0.25	0.11	0.02
Oceania <sup>a</sup>	0.04	0.04	0.32	0
This study <sup>b</sup>	0.08	0.35	0.23	0.02

<sup>a</sup> Sabbagh et al., 2011. Control data specific for each of disease models included in the case group.

<sup>b</sup> Patients from control group.

this same population, it was observed that white individuals, through self-declaration, had 86% of European ancestry and 13% of African or Amerindian ancestry. On the other hand, the patients self-declared as nonwhites, of brown or black color, had 24% and 49% of African ancestry and 67% and 43% of European ancestry, respectively.<sup>27</sup> These data reveal the greater degree of miscegenation present in those self-declared as of brown and black color. The high degree of ethnic heterogeneity of our study population could explain the close, but not identical, values to those observed in other populations.

In the literature, there are reports on the association of NAT2 polymorphisms with various diseases, such as different types of cancer,<sup>28</sup> periodontitis,<sup>29</sup> and autoimmune diseases,<sup>3,30,31</sup> who adopt the smoking habit as an environmental risk factor.<sup>29,32</sup> An association between the acetylator phenotype of NAT2, smoking and SLE was already observed.<sup>2</sup> However, in the Brazilian population, there is no study on the association of polymorphisms in NAT2 gene and/or of acetylator phenotype with this disease, especially in smokers. Although several authors consider that the exposure to tobacco components may constitute a “trigger” for the development of SLE,<sup>2-7</sup> we had access to information on smoking habit during the collection of biological samples, and not at the time of disease onset. Therefore, our study population was subdivided into two groups, according to the smoking habit, as smokers or ex-smokers and nonsmokers. Our results showed no association between these variables and the disease (data not shown). It is worth noting that, at the time a patient is diagnosed, she receives guidance to abstain from smoking, which may have created a bias in the analysis. In fact, fewer smokers and former smokers were observed in the group of cases, a fact that was not related to a protection of smoking habits in relation to disease development. It should be noted that additional smoking data, including exposure time and the number of packs of cigarettes/day, at the first indication of disease manifestation, could contribute to the analysis carried out, taking into account the possibility of the differential metabolism of various components of cigarette smoke, depending on the acetylator phenotype. However, the time of onset of the disease is a complex aspect to be established. It is frequent the occurrence of a period of several months between the onset of symptoms and disease diagnosis.

Besides the difference in the frequency of NAT2 polymorphisms in different populations, the incidence of SLE is higher in ethnically mixed patients.<sup>5</sup> Thus, in this study, we evaluated whether there was an association between the distribution of

NAT2 polymorphisms and SLE patients with this ethnic feature. With this in mind, self-declarations and information on the parent (father and mother) skin color were used to define the ethnic origin of the patients, as reported in the literature,<sup>21</sup> but we acknowledge that other methods could be more appropriate to this end. Significant results were observed only in relation to the NAT2 857G>A polymorphism, with the genotype 857GA representing a potential risk for the disease in non-white women (Table 4). The NAT2 857G>A substitution has as a consequence, in the protein, the change of the amino acid glycine by glutamate (G286E). This change modifies the access to the active site of the enzyme, which results in reduced selectivity and catalytic capacity.<sup>16</sup> Therefore, the NAT2 857G>A polymorphism is related to the reduction in the metabolism of aromatic and heterocyclic amines.<sup>16</sup> The substitution 857G>A is present in 11 NAT2 alleles, two of which have a slow acetylator phenotype, and the other ones have not yet had their phenotypes defined.<sup>33</sup> The slow acetylator phenotype could confer an increased risk for SLE, since the body would remain exposed for a longer time, for example, to components of cigarette smoke. It is worth noting that the variables ethnic origin and NAT2 857G>A genotypes were not individually associated with SLE. Nevertheless, the literature points to a higher incidence of SLE in ethnically mixed individuals,<sup>1</sup> and, in a population of the city of Ilheus (Bahia/Brazil), the 857GA genotype was more prevalent in African-Brazilian subjects, compared to whites and Amerindians.<sup>34</sup> The ancestry of the Rio de Janeiro population is similar to those estimated values for the population of Brazilian Northeast.<sup>26,35</sup> However, it has been suggested that large urban centers, like Rio de Janeiro, present more diverse patterns of ethnic mixture.<sup>26</sup>

The organs affected and the intensity of clinical manifestations in SLE patients characterize the severity of the disease and somehow guide the therapeutic scheme selection. The only significant result in the analysis of the interaction of the NAT2 polymorphisms or of the acetylator phenotype with clinical features of SLE was the greater prevalence of the allele 481T in patients with hematologic diseases, specifically autoimmune hemolytic anemia or autoimmune thrombocytopenia (data not shown). The reduction of these cell types may be associated with the immunosuppression caused by the severity of the disease or by the use of immunomodulatory drugs.<sup>36</sup> So far, there is no evidence as to the way the genotypes containing the allele NAT2 481T can influence the reduction of these blood cells, and this undoubtedly requires further investigation.

However, when considering only the smoking habit, we found a correlation with the presence of discoid lupus (OR=8.62, 95% CI 2.40-30.96;  $p=0.0011$ ) and proteinuria (OR=0.17, 95% CI 0.05-0.59;  $p=0.0056$ ). In several studies, dermatologic alterations present in patients with SLE were associated with smoking and, as previously mentioned, the treatment of these lesions in smokers seems to require higher doses of antimalarials, compared to nonsmokers.<sup>37-39</sup> In our study, the small number of patients with SLE and smokers ( $n=7$ ) did not allow us to infer if smoking habit really had an impact on the pharmacological approach. The smoking habit is also associated with renal disorders, such as proteinuria and nephropathy<sup>40</sup>; however, in this study, from 71 patients with proteinuria, only 7 are current smokers, 13 are former smokers, and most were lifetime nonsmokers.

In the Brazilian population, there are few studies on genotype and allele distributions of NAT2, and there are no reports about the association of NAT2 polymorphisms and SLE. We believe that our work has a leading role in this area, and we understand it as a formulator of the hypothesis of a potential role of NAT2 polymorphisms as a factor associated with the disease in our country, and of a possible association with specific clinical phenotypes. However, further studies involving larger populations are needed, to confirm the association between the NAT2 857G>A polymorphism and systemic lupus erythematosus in self-declared non-white Brazilian women, ideally including ethnic profiling with the use of ancestry markers.

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## Conflicts of interest

The authors declare no conflicts of interest.

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