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Genetic polymorphisms of glutathione *S*-transferases and cytochrome P450 enzymes as susceptibility factors to systemic lupus erythematosus in southern Brazilian patients

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Abstract Systemic lupus erythematosus (SLE) is an autoimmune chronic inflammatory disease that presents several clinical manifestations, affecting multiple organs and systems. Immunological, environmental, hormonal and genetic factors may contribute to disease. Genes and proteins involved in metabolism and detoxification of xenobiotics are often used as susceptibility markers to diseases with environmental risk factors. Cytochrome P450 (CYP) enzymes activate the xenobiotic making it more reactive, while the Glutathione *S*-transferases (GST) enzymes conjugate the reduced glutathione with electrophilic compounds, facilitating the toxic products excretion. *CYP* and *GST* polymorphisms can alter the expression and catalytic activity of enzymes. This study aimed to investigate the role of genetic variants of CYP and GST in susceptibility and clinical expression of SLE, through the analysis of *GSTM1 null*, *GSTT1 null*, *GSTP1*Ile105Val*, *CYP1A1*2C* and *CYP2E1*5B* polymorphisms. 371 SLE patients from Hospital de Clínicas de Porto Alegre and 522 healthy blood donors from southern Brazil were evaluated. *GSTP1* and *CYP* variants were genotyped using PCR–RFLP and *GSTT1* and *GSTM1* variants were analyzed by multiplex PCR. Among European-derived individuals, a lower frequency of *GSTP1*Val* heterozygous genotypes was found in SLE patients when compared to controls ($p = 0.005$). In African-derived SLE patients, the *CYP2E1*5B* allelic frequency was higher in relation to controls ($p = 0.054$). We did not observe any clinical implication of the CYP and GST polymorphisms in patients with SLE. Our data suggest a protective role of the *GSTP1*Ile/Val* heterozygous genotype against the SLE in European-derived and a possible influence of the *CYP2E1*5B* allele in SLE susceptibility among African-derived individuals.

Keywords Glutathione *S*-transferase · Cytochrome P450 · Genetic polymorphism · Systemic lupus erythematosus

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune chronic inflammatory disease that exhibits a wide spectrum of clinical manifestations and the involvement of multiple organs, including kidneys, joints, nervous system and hematopoietic organs [1, 2]. It is characterized by production of autoantibodies, formation and deposition of immune complexes, resulting in chronic inflammation and tissue damage [3, 4]. The disease occurs in all populations, but the prevalence and severity of SLE varies across the world [5]. The etiology of SLE is probably multifactorial, with involvement of genetic, hormonal, immunological and environmental factors [3, 6]. Multiple abnormalities of both the innate and adaptive immune systems have been described in SLE and environmental exposures to sunlight, organic solvents and infections have been related to development or exacerbation of the disease [1, 3]. Several

studies have identified potential genetic markers associated with SLE that participate of many immune pathways, processing and activity of different types of cells [7–9].

Genes and proteins involved in metabolism/detoxification of xenobiotics are commonly used as markers of susceptibility to the development of diseases in which the etiology is related to exposure to environmental factors. Since SLE has possibly an environmental contribution and the impaired metabolic activity may aggravate the course of disease, genes coding for enzymes responsible for activation (phase I reactions) or deactivation (phase II reactions) of xenobiotics have emerged as potential targets in studies with SLE [10]. The activation enzymes, represented by the Cytochrome P450 (CYP) superfamily, activate the xenobiotic, making it more electrophilic and thus more reactive, usually with the introduction of a functional grouping. The detoxification enzymes, such as Glutathione *S*-transferase (GST) superfamily, usually conjugate metabolites with an endogenous substrate. As a result, the metabolites are transformed in hydrophilic substances that can be excreted [11, 12]. Interindividual differences regarding the biotransformation capacity of the endogenous and exogenous substances may be related to polymorphisms in genes of metabolism/detoxification enzymes, because it can lead to change of the enzymes activity involved in this process [11].

The *CYP1A1* gene is located on chromosome 15q24.1 and polymorphisms in this gene have been associated with increased inducibility and/or activity of enzyme. Among them, the variant *CYP1A1*2C* (*rs1048943*) occurs due to the A→G transition at nucleotide 4889 in exon 7 (Ile462-Val) and it shows an almost two-fold higher catalytic enzyme activity than the *Ile* variant [13–15]. It has been reported that the higher catalytic activity of CYP1A1 leads to an increased generation of oxidative stress in cells [16]. The *CYP2E1* gene is located on chromosome 10q24.3-qter [17]. Several polymorphisms have been described throughout this gene. The *CYP2E1*5B* variant (*rs381 3867*), located in the 5′ regulatory, with replacement at position −1,293 (G→C *Pst*I) may affect the gene transcription and it is one of the most important polymorphisms identified [14, 18–20].

The *GSTM1*, *GSTT1*, and *GSTP1* genes are involved in the detoxification of a broad range of toxic substances [21, 22]. *GSTM1* gene is located on chromosome 1p13.3. Interindividual differences regarding the enzyme activity are due to the gene deletion or allelic variation, and individuals with the *GSTM1 null* do not express the protein [14, 22]. The *GSTT1* gene is located on chromosome 22q11.23 and it has also been described the deletion of this gene, *GSTT1 null*, resulting in complete absence of enzyme activity [23]. The *GSTP1* gene, located on chromosome 11q13, displays a polymorphism at codon 105 resulting from an A→G transition at nucleotide +313 (*Ile105Val*), producing an enzyme with both substrate affinity and thermal stability altered [21, 24, 25].

Since many *GST* and *CYP* genes are polymorphic, there has been great interest in determining whether certain allelic variants are associated with the risk of a variety of diseases, such as SLE [22, 26–28]. Metabolism of environmental factors or xenobiotics is regulated by a balance of a number of steps that involve the production and detoxification of reactive oxygen species (ROS). ROS bind covalently to DNA, leading to somatic mutation or disruption of cell cycle. The toxic substances are metabolized to generate ROS by CYP enzymes. On the other hand, GSTs play a critical role in detoxification. Therefore, *CYP* variants that impair this process contribute to the increased generation of ROS and the lack or low GSTs activity lead to the decreased detoxification of these compounds. The combination these effects will result in the increased level of ROS [29], considered an important factor in autoimmunity [30], and a marked characteristic of inflammatory responses [10]. It is believed that ROS increase immunogenicity of DNA, LDL and IgG, generating ligands for which autoantibodies show higher avidity [31]. Evidences suggest that oxidative damage may contribute to the pathogenesis of SLE in several ways, including promotion of apoptosis and consequent exposure of intracellular antigens to the immune system, modification the properties of antibody linked to DNA and cell membrane damage [32].

Considering the crucial role of biotransformation enzymes in activation and detoxification of several metabolites, the possible genetic and environmental contributions to the SLE and the fact that in Brazil there has not been studies linking SLE and polymorphisms of phase I and II enzymes analyzed together, the objectives of this work were investigated the frequency of *GSTM1 null*, *GSTT1 null* and *GSTP1*Ile105Val* polymorphisms of Glutathione *S*-transferases, and the frequency of *CYP1A1*2C* and *CYP2E1*5B* polymorphisms of Cytochrome P450 in SLE patients and healthy controls from southern Brazil, looking for possible associations among these variants and clinical and laboratory expression of the disease.

Materials and methods

Study populations

A total of 371 samples from SLE patients followed at the Division of Rheumatology of Hospital de Clínicas de Porto Alegre (HCPA) was obtained. The mean age of patients was 49.0 ± 14.7 years and the mean age at diagnosis was

Table 1 Demographic, clinical and laboratorial features of SLE patients

Patienťs features	Whole (n = 371) % (N)	European-derived (n = 283) % (N)	African-derived (n = 88) % (N)	*p* value[a]
Females	91.9 % (371)	91.5 % (283)	93.2 % (88)	0.783
Age (years)	49.0 ± 14.7 (370)	49.3 ± 15.0 (282)	48.0 ± 13.6 (88)	0.478
Age of symptoms (years)	30.5 ± 13.5 (367)	30.4 ± 13.5 (279)	31.0 ± 13.6 (88)	0.717
Age at diagnosis (years)	32.4 ± 13.8 (369)	32.1 ± 13.8 (281)	33.5 ± 13.6 (88)	0.399
Malar rash	55.5 % (371)	56.5 % (283)	52.3 % (88)	0.562
Discoid rash	15.4 % (371)	15.2 % (283)	15.9 % (88)	1.000
Photosensitivity	75.7 % (371)	80.6 % (283)	60.2 % (88)	**<0.001***
Oral/nasal ulcers	36.7 % (371)	37.8 % (283)	33.0 % (88)	0.485
Arthritis	82.7 % (371)	83.4 % (283)	80.7 % (88)	0.670
Serositis	31.1 % (370)	29.4 % (282)	36.4 % (88)	0.274
Nephritis	43.7 % (371)	42.4 % (283)	47.7 % (88)	0.449
Neurologic disorders	11.9 % (371)	12.0 % (283)	11.4 % (88)	1.000
Psychosis	7.0 % (371)	6.7 % (283)	8.0 % (88)	0.874
Convulsions	6.5 % (371)	7.1 % (283)	4.5 % (88)	0.554
Hematologic disorders	77.6 % (371)	74.2 % (283)	88.6 % (88)	**0.007***
Hemolytic anemia	29.9 % (371)	30.4 % (283)	28.4 % (88)	0.825
Leukopenia/lymphopenia	62.0 % (371)	58.0 % (283)	75.0 % (88)	**0.006***
Thrombocytopenia	19.1 % (371)	19.1 % (283)	19.3 % (88)	1.000
Immunologic disorders	68.6 % (369)	68.7 % (281)	68.2 % (88)	1.000
Anti-DNA	47.7 % (369)	48.8 % (281)	44.3 % (88)	0.545
Anti-Sm	19.6 % (367)	17.9 % (280)	25.3 % (87)	0.171
Anticardiolipin	28.0 % (368)	28.1 % (281)	27.6 % (87)	1.000
Lupus anticoagulant	7.6 % (369)	8.9 % (281)	3.4 % (88)	0.143
False-positive VDRL	4.6 % (369)	5.3 % (281)	2.3 % (88)	0.381
ANA	99.5 % (370)	99.6 % (282)	98.9 % (88)	0.420
Anti-Ro/SSA	43.3 % (342)	38.1 % (257)	58.8 % (85)	**0.001***
Anti-La/SSB	13.7 % (342)	10.9 % (257)	22.4 % (85)	**0.013***
Anti-RNP	31.6 % (342)	30.7 % (257)	34.1 % (85)	0.655
Anti-Scl 70	3.2 % (342)	3.5 % (257)	2.4 % (85)	1.000
Sjögren	7.7 % (364)	9.0 % (277)	3.4 % (87)	0.141
APS	5.8 % (364)	6.1 % (277)	4.6 % (87)	0.784
SLEDAI[b]	1 (0–4) (318)	1 (0–4) (236)	1 (0–4) (82)	0.857
SLICC[b]	1 (0–2) (354)	1 (0–2) (268)	1 (0–2) (86)	0.959

ANA antinuclear antibody, *APS* antiphospholipid syndrome, *SLEDAI* systemic lupus erythematosus disease activity index, *SLICC* systemic lupus international collaborating clinics, *VDRL* venereal disease research laboratory test

* Significant *p* value <0.05

[a] Chi square test for qualitative variables and *t* test or Mann–Whitney test for quantitative variables

[b] Median (minimum–maximum)

32.4 ± 13.8 years. The control group was comprised of 522 healthy blood donors, with the mean age of 45.8 ± 9.4, from the urban population of Porto Alegre, the capital of the southernmost state of Brazil. Patients and controls were classified as European-derived or African-derived, according to phenotypic characteristics of individuals, as judged by the researcher at the time of blood collection, and data about the ethnicity of parents/grandparents reported by the participants. The issue concerning skin color-based classification criteria that is used in Brazil is well documented and has been already assessed by our group in previous studies [33, 34].

Information regarding demographic, clinical and laboratory features of SLE patients were collected from data contained in medical records filled in the Medical Archive Service and Health Information (Table 1). The clinical and laboratory features evaluated were: presence or absence of malar rash, discoid rash, photosensitivity, oral or nasal ulcers, arthritis, serositis, nephritis, neurological manifestations (psychosis and convulsions), hematologic events (hemolytic anemia, leukopenia, lymphopenia and thrombocytopenia), positive antinuclear antibody (ANA) (titer >1:100) and other auto-antibodies (anti-double-stranded DNA, anti-Sm, anti-Ro/SSA, anti-La/SSB, anti-RNP, anti-Scl 70, anticardiolipin, lupus anticoagulant and false positive VDRL). The definition of each variable followed the description from the classification criteria for SLE, according to American College of Rheumatology [35].

Furthermore, patients were evaluated for the presence of Sjögren's Syndrome and Secondary Antiphospholipid Syndrome, according to the classification criteria proposed for both diseases [36, 37]. SLICC (systemic lupus international collaborating clinics) damage index [38] and SLEDAI (systemic lupus erythematosus disease activity index) [39] were also performed for each patient. All patients and controls participating in this study gave their written informed consent. This study was approved by the Ethics Committee of HCPA.

Sample collection

The DNA used for molecular techniques was obtained from 5 mL peripheral blood samples collected with EDTA and purified through the salting-out method as described by Lahiri e Nurnberger [40]. DNA samples were stored at −20 °C.

CYP1A1 and CYP2E1 genotyping

Molecular identification of Cytochrome P450 polymorphisms was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) assay. The *CYP1A1*2C* polymorphism was genotyped using the primers and PCR conditions previously described by Cascorbi et al. [15]. The amplified fragments (204 bp) were visualized in 1 % agarose gel stained with ethidium bromide. The amplified product was digested with *BsrD*I restriction enzyme, and the fragments of 149 and 55 bp generated were visualized in 3 % agarose gel. The genotyping of *CYP2E1*5B* polymorphism was performed according to Kato et al. [19]. The amplified fragments (410 bp) were checked in 1 % agarose gel and an aliquot of the amplified product was subjected to the *Pst*I restriction enzyme. Genotypes were determined through the visualization of the fragments of 290 and 120 bp in 3 % agarose gel.

GSTM1, GSTT1 and GSTP1 genotyping

The *GSTM1* and *GSTT1* genes polymorphisms of Glutathione *S*-transferase were genotyped using the amplification method by polymerase chain reaction (PCR) in multiplex, with specific primers and PCR–RFLP for *GSTP1* gene as described by Rohr et al. [10]. The primer sequences used were as previously reported [41–43]. An aliquot of amplified product was analyzed by electrophoresis in 3 % agarose gel to verify the presence or absence of *GSTT1* (480 pb) and *GSTM1* (215 pb) genes, and the product of *GSTP1* (176 pb) was used as an internal control in this reaction. A second aliquot of the amplified product of *GSTP1* was digested by the restriction enzyme *Bsma*I as described by Harries et al. [42]. The fragments of 91 and 85 bp formed after the enzymatic digestion were visualized in 8 % polyacrylamide gel stained with silver nitrate.

Statistical analysis

Allelic and genotypic frequencies were estimated by direct counting. The genotypic frequencies of cases and controls were compared to Hardy–Weinberg expectations using Chi Square tests. *CYP* and *GST* allelic frequencies were compared between patients and controls using the Chi square test or Fisher's exact test, if appropriate. The adjusted residuals and odds ratio (OR) estimative were also calculated. Demographic, clinical and laboratorial features were compared between European-derived and African-derived patients through the Chi square test for qualitative variables and *t* test or Mann–Whitney test for quantitative variables. Associations among clinical and laboratory variables of patients and the frequencies of polymorphisms were also performed through the Chi square test (or Fisher's exact test) for qualitative variables and *t* test, Mann–Whitney test and Kruskal–Wallis test for quantitative variables, using Bonferroni correction to the level of statistical significance. All data were analyzed with SPSS software and WinPepi. Significance level was established at $p < 0.05$ (two-tailed).

Results

Published data report that the SLE incidence and the polymorphic variants of *GST* and *CYP* genes frequencies show a high variability in different ethnic populations [44–46]. For this reason, all analyzes were performed subdividing the individuals according to their ethnic origin. Our study included 371 patients diagnosed with SLE, of which 283 (76 %) were classified as European-derived and 88 (24 %) as African-derived, and 522 controls, being 328 (63 %) classified as European-derived and 194 (37 %) as African-derived. Due to the lack of clinical and/or laboratory data from medical records of some patients, a difference between the total number of individuals sampled and the number of individuals analyzed may be observed in some cases.

The frequency of clinical and laboratory features of SLE patients are shown in Table 1. Clinical features more prevalent in this study population were arthritis (82.7 %), hematologic disorders (77.6 %), photosensitivity (75.7 %) and immunologic disorders (68.6 %), highlighting the presence of ANA, which was found in 99.5 % of SLE patients. The presence of photosensitivity was significantly higher in European-derived patients in relation to African-derived group (80.6 vs. 60.2 %, $p < 0.001$). On the other hand, the African-derived SLE patients presented a higher frequency of hematologic disorders (88.6 vs. 74.2 %,

Table 2 Frequencies of *GSTT1* and *GSTM1 null* genotypes among SLE patients and controls

Genotypes	European-derived		African-derived	
	Patients (n = 282)	Controls (n = 241)	Patients (n = 87)	Controls (n = 87)
GSTT1 null	56 (0.20)	44 (0.18)	14 (0.16)	18 (0.21)
GSTT1 non-null	226 (0.80)	197 (0.82)	73 (0.84)	69 (0.79)
	χ^2 with Yates $p = 0.724$		χ^2 with Yates $p = 0.557$	
	OR 1.11 (CI 95 % 0.70–1.77)		OR 0.74 (CI 95 % 0.31–1.7)	
Genotypes	European-derived		African-derived	
	Patients (n = 282)	Controls (n = 241)	Patients (n = 87)	Controls (n = 88)
GSTM1 null	133 (0.47)	129 (0.54)	33 (0.38)	25 (0.28)
GSTM1 non-null	149 (0.53)	112 (0.47)	54 (0.62)	63 (0.72)
	χ^2 with Yates $p = 0.173$		χ^2 with Yates $p = 0.239$	
	OR 0.77 (CI 95 % 0.54–1.11)		OR 1.54 (CI 95 % 0.78–3.06)	
Genotypes	European-derived		African-derived	
	Patients (n = 282)	Controls (n = 241)	Patients (n = 87)	Controls (n = 87)
GSTT1 null and GSTM1 null	18 (0.06)	20 (0.08)	5 (0.06)	4 (0.05)
Other genotypes	264 (0.94)	221 (0.92)	82 (0.94)	83 (0.95)
	χ^2 with Yates $p = 0.501$		Fisher p=1.000	
	OR 0.75 (CI 95 % 0.37–1.54)		OR 1.27 (CI 95% 0.26 – 6.60)	

Absolute frequency and (relative frequency) are shown for genotypes

$p = 0.007$) and leucopenia or lymphopenia (75.0 vs. 58 %, $p = 0.006$). Likewise, a higher proportion of African-derived individuals presenting anti-Ro/SS-A and anti-La/SS-B antibodies was observed when compared to European-derived (58.8 vs. 38.1 % and 22.4 vs. 10.9 %, $p = 0.001$ and $p = 0.013$, respectively). No other statistically significant differences were found between ethnicities with respect to clinical variables.

Table 2 shows the frequencies of *GSTT1 null and GSTM1 null* genotypes, which did not differ significantly between SLE patients and controls in both European-derived ($p = 0.724$ and $p = 0.173$, respectively) and African-derived ($p = 0.557$ and $p = 0.239$, respectively) groups. We also performed a combined analysis, comparing the individuals who had double deletion of both *GSTT1* and *GSTM1* with the rest of the population and no significant difference was found in European-derived ($p = 0.501$) as well as in African-derived ($p = 1.000$).

The allelic and genotypic frequencies of *GSTP1*Ile105-Val* polymorphism were compared between patients and healthy controls (Table 3). The analysis results showed a statistically significant difference in genotypic frequencies among European-derived. Interestingly, a lower frequency of heterozygous for the variant *GSTP1*Val* was observed among European-derived SLE patients when compared to their controls (36 vs. 48 % respectively; $p = 0.005$). The overall OR for heterozygous in relation to wild-type and mutant homozygous was 0.63 (95 % CI 0.43–0.93, $p = 0.044$) and 0.49 (95 % CI 0.26–0.92, $p = 0.051$), respectively. The genotypic frequencies of *GSTP1*Ile105-Val* polymorphism in European-derived SLE patients were not in Hardy–Weinberg equilibrium. For all other groups of cases and controls, genotypic distributions were in concordance from those expected under the Hardy–Weinberg equilibrium. The African-derived group did not present any statistically significant difference for *GSTP1*Ile105Val* polymorphism. The allelic frequencies did not differ significantly between patients and controls in both groups. However, it is important to emphasize that there was a higher frequency of *GSTP1*Val* variant in African-derived SLE patients (40 %) in relation to matched controls (30 %), but without significant difference ($p = 0.061$).

Data resulting from analysis of *CYP* genes polymorphisms, *CYP1A1* and *CYP2E1*, are presented in Tables 4 and 5, respectively. Concerning the *CYP1A1*2C* variant, the allelic and genotypic frequencies observed in SLE patients were compared to frequencies of the healthy controls, including also frequencies of a control group from the same region of Brazil previously reported [45, 47]. Similar allelic and genotypic frequencies for this variant were found in patients and controls of both ethnic groups (Table 4). Analysis performed for the *CYP2E1*5B*

Table 3 Genotypic and allelic frequencies of *GSTP1*Ile105Val* in SLE patients and controls

Genotypes	European-derived		African-derived	
	Patients (n = 282)	Controls (n = 237)	Patients (n = 87)	Controls (n = 88)
GSTP1				
[b]*Ile/Ile*	143 (0.51)	102 (0.43)	33 (0.38)	46 (0.52)
[c]*Ile/Val*	101 (0.36)[a]	114 (0.48)[a]	38 (0.44)	31 (0.35)
[d]*Val/Val*	38 (0.13)	21 (0.09)	16 (0.18)	11 (0.13)
	χ^2 $p = 0.013$*		χ^2 $p = 0.152$	
Alleles	European-derived		African-derived	
	Patients (2n = 564)	Controls (2n = 474)	Patients (2n = 174)	Controls (2n = 176)
*GSTP1*Ile*	387 (0.69)	318 (0.67)	104 (0.60)	123 (0.70)
*GSTP1*Val*	177 (0.31)	156 (0.33)	70 (0.40)	53 (0.30)
	χ^2 with Yates $p = 0.646$		χ^2 with Yates $p = 0.061$	
	OR 0.93 (CI 95 % 0.71–1.22)		OR 1.56 (CI 95 % 0.98–2.49)	

Absolute frequency and (relative frequency) are shown for genotypes and alleles

* Significant p value <0.05

[a] Adjusted residual, **χ^2 $p = 0.005$***

About European-derived:

[c,b] OR 0.63 (CI 95 % 0.43–0.93) $p = 0.044$

[c,d] OR 0.49 (CI 95 % 0.26–0.92) $p = 0.051$

About African-derived:

[c,b] OR 1.71 (CI 95 % 0.85–3.45) $p = 0.317$

[d,b] OR 2.03 (CI 95 % 0.76–5.48) $p = 0.347$

Table 4 Genotypic and allelic frequencies of *CYP1A1*2C* in SLE patients and controls

Genotypes	European-derived		African-derived	
	Patients (n = 283)	Controls[a] (n = 328)	Patients (n = 88)	Controls[b] (n = 194)
CYP1A1 (Ile/Val)				
**1A/*1A*	214 (0.76)	250 (0.76)	56 (0.64)	138 (0.71)
**1A/*2C*	59 (0.21)	71 (0.22)	31 (0.35)	52 (0.27)
**2C/*2C*	10 (0.04)	7 (0.02)	1 (0.01)	4 (0.02)
	χ^2 $p = 0.571$		Fisher $p = 0.354$	
Alleles	European-derived		African-derived	
	Patients (2n = 566)	Controls[a] (2n = 656)	Patients (2n = 176)	Controls[b] (2n = 388)
*CYP1A1*1A*	487 (0.86)	571 (0.87)	143 (0.81)	328 (0.84)
*CYP1A1*2C*	79 (0.14)	85 (0.13)	33 (0.19)	60 (0.16)
	χ^2 with Yates $p = 0.669$		χ^2 with Yates $p = 0.394$	
	OR 1.09 (CI 95 % 0.77–1.54)		OR 1.26 (CI 95 % 0.76–2.06)	

Absolute frequency and (relative frequency) are shown for genotypes and alleles

[a] Included data from Gaspar et al. [47]

[b] Included data from Gaspar et al. [45]

polymorphism (Table 5) showed an allelic frequency of 11 % in African-derived SLE patients against 5 % in healthy individuals. Although this difference was not found to be significant, it is a marked difference that deserves to be highlighted ($p = 0.054$; OR 2.69, 95 % CI 1.00–8.42). In European-derived, the allelic frequencies between patients and controls were very similar to each other. The frequencies of *CYP2E1*1A/*1A,*1A/*5B* and **5B/*5B* genotypes were not significantly different between patients and controls in both ethnic groups. Considering the

Table 5 Genotypic and allelic frequencies of *CYP2E1*5B* in SLE patients and controls

Genotypes	European-derived		African-derived	
	Patients (n = 276)	Controls (n = 236)	Patients (n = 88)	Controls (n = 66)
CYP2E1				
**1A/*1A*	240 (0.87)	207 (0.88)	70 (0.80)	60 (0.91)
**1A/*5B*	35 (0.13)	28 (0.12)	16 (0.18)	6 (0.09)
**5B/*5B*	1 (0.004)	1 (0.004)	2 (0.02)	0 (0.00)
	Fisher $p = 0.895$		Fisher $p = 0.110$	
Alleles	European-derived		African-derived	
	Patients (2n = 552)	Controls (2n = 472)	Patients (2n = 176)	Controls (2n = 132)
*CYP2E1*1A*	515 (0.93)	442 (0.94)	156 (0.89)	126 (0.95)
*CYP2E1*5B*	37 (0.07)	30 (0.06)	20 (0.11)	6 (0.05)
	χ^2 with Yates $p = 0.923$		χ^2 with Yates $p = 0.054$	
	OR 1.06 (CI 95 % 0.62–1.81)		OR 2.69 (CI 95 % 1.00–8.42)	

Absolute frequency and (relative frequency) are shown for genotypes and alleles

Table 6 Frequencies of SLE patients and controls with at least one allelic variant of both *GSTP1*Ile105Val* and *CYP2E1*5B* polymorphisms

Genotypic Combinations	European-derived		African-derived	
	Patients (n = 276)	Controls (n = 233)	Patients (n = 86)	Controls (n = 66)
Other genotypes	256 (0.93)	221 (0.95)	78 (0.91)	64 (0.97)
At least one allelic variant of both polymorphisms	20 (0.07)	12 (0.05)	8 (0.09)	2 (0.03)
	χ^2 with Yates $p = 0.431$		Fisher $p = 0.188$	

Absolute frequency and (relative frequency) are shown for genotypic combinations

importance of investigating the interaction between phase I and II enzymes, we analyzed the frequencies of SLE patients with at least one allelic variant of both *GSTP1*Val* and *CYP2E1*5B* compared to controls, whose frequencies of the variants were more relevant in this work (Table 6). However, no statistically significant difference was found.

Finally, we investigated the clinical and laboratory differences between patients with SLE according to the GST and CYP polymorphisms studied (data not shown). The results of these tests indicated a higher frequency of anti-Ro/SSA antibodies in European-derived patients with *GSTT1 null* genotype ($p = 0.031$) and a higher frequency of deletion of *GSTT1* gene in African-derived patients with secondary antiphospholipid syndrome ($p = 0.013$). Interestingly, the frequency of *GSTM1 null* was lower than the *GSTM1 non-null* in African-derived SLE patients presenting nephritis ($p = 0.012$) and higher in those with IgG and IgM anticardiolipin antibodies ($p = 0.022$) while European SLE patients presenting anti-Scl 70 antibodies showed a statistically lower frequency of *GSTM1 null* ($p = 0.037$). Moreover, a frequency of 85.7 % of African-derived heterozygous for the *CYP1A1*2C* variant presenting psychosis was observed compared to 30.9 % of individuals without psychosis ($p = 0.014$). In European-derived group, heterozygous for *GSTP1*Ile105Val* showed a higher prevalence of false-positive VDRL compared to wild-type and mutant homozygous ($p = 0.047$), and this group also showed a higher frequency of heterozygous for *CYP2E1*5B* among patients with positivity for anti-La/SSB antibodies ($p = 0.025$). Regarding SLICC and SLEDAI, we only found a statistical difference in European-derived group, which showed a higher damage index (SLICC) in patients with the presence of *GSTT1* gene compared with those who have the absence of gene ($p = 0.035$). However, after applying Bonferroni correction, the significant p values were not maintained.

Discussion

In this study, we investigated the possible influence of Glutathione *S*-transferases and Cytochrome P450 genes polymorphisms in the development and clinical progression of SLE in a southern Brazilian population. GST and

CYP enzymes are responsible for the activation and detoxification of many xenobiotics and endobiotics and their involvement in defense against oxidative stress suggests polymorphisms that affect the activity of these enzymes may be significant determinants of individual risk for several diseases, in which the etiology is related to exposure to environmental factors [14, 27, 48]. Considering that SLE is a multifactorial disease, with possible combined genetic and environmental contributions, studies have evaluated polymorphisms of the metabolic enzyme genes in the context of SLE [27, 28, 49, 50], although there are conflicting reports regarding the association of *GST* and *CYP* genes with SLE [8, 28, 29, 50]. Differences among results may be due to ethnic diversity of the populations studied or interaction between different environmental and genetic factors.

It has been reported that the frequencies of *CYP* and *GST* polymorphisms vary in different ethnic groups [19, 44, 51] as well as the prevalence and incidence of SLE [52, 53]. Therefore, our analyses were performed grouping cases and controls according to their ethnic origin. Although the individuals classified as European-derived or African-derived can present a certain degree of admixture, a recent study published by Santos et al. [54] assessed individual interethnic admixture using a 48–insertion-deletion ancestry-informative marker panel and validated our classification criteria. The authors identified a very high level of European contribution (94 %) and fewer Native American (5 %) and African (1 %) genes in a sample of 81 European-derived individuals from southern Brazil. Therefore, the subgrouping of our SLE patients and controls seems to reflect the actual ethnic/genetic background of this human population.

The most frequent clinical involvement presented by this SLE population was arthritis (82.7 %), followed by hematologic disorders (77.6 %), what is in agreement with the frequencies found in a study by Font et al. [55], performed with a Spanish population (83 and 75 %, respectively). The frequency of nephritis in our patients (43.7 %) was very similar to a study from Tunisia (43 %). However, this frequency was higher, as well as the frequency of photosensitivity (75.7 %), in relation to Spanish study (34 and 41 %, respectively). Moreover, it is important to note that, considering the immunologic disorders, almost all our SLE patients presented ANA (99.5 %), in the same way that the patients from Spanish study (99 %).

When we divide the total sample in European-derived and African-derived, we observed a higher prevalence of photosensitivity in European-derived patients ($p < 0.001$) and lower frequencies of anti-Ro/SSA and anti-La/SSB antibodies ($p = 0.001$ and $p = 0.013$, respectively) when compared to African-derived group. These results may be due to difficulty in detecting this manifestation in dark-skinned patients. The African-derived patients presented a higher frequency of hematologic disorders ($p = 0.007$), specially leukopenia and lymphopenia ($p = 0.006$). African-Latin Americans SLE patients studied in a prospective multinational inception cohort also presented a higher prevalence of lymphopenia in relation to white ethic group ($p < 0.0001$) agreeing with our work [56]. A recent retrospective, multicentre, observational study carried out in five countries (France, Germany, Italy, Spain and the UK), evaluating the clinical manifestations of SLE patients, unlike our results, observed higher disease activity in African-derived as compared to European-derived patients [57]. Therefore, since ethnicity influences the clinical manifestations and laboratory features of SLE patients, these findings show the importance of dividing the sample according to the ethnic origin to perform the analyses.

The analysis of *GSTT1 null* and *GSTM1 null* polymorphisms showed similar frequencies between SLE patients and controls in both ethnicities. Unlike our results, one study found a significant difference for the *GSTM1 null* genotype between SLE patients and controls in a Chinese population, suggesting an association between *GSTM1* deletion and the risk of SLE. With respect to the *GSTT1 null* genotype and the double deletion of both *GSTT1* and *GSTM1*, no association was observed [28]. Likewise, Kang et al. [50] investigated Korean SLE patients and suggested that genetic polymorphisms *GSTM1* and *GSTT1* do not influence the risk of SLE, supporting our findings. Our data are also in agreement with the results from another previous study, where the authors observed no association between *GSTM1* deletion and SLE [29]. Interestingly, a recent study showed that female Japanese smokers presenting the *GSTM1* deletion combined with another polymorphism of *CYP1A1* (*rs4646903*) had an increased risk of developing SLE [58]. With respect to other autoimmune diseases, Bekris et al. [59] showed that the presence of the *GSTM1* and not the null genotype may be a predisposition factor to Type 1 diabetes in Americans, suggesting that the autoimmunity may be involved with GST conjugation. Moreover, a meta-analysis suggested that the *GSTM1* and *GSTP1* polymorphisms are not associated to rheumatoid arthritis susceptibility and unlike what was found in the study with SLE female Japanese smokers, no association was observed between smoking and the presence of a *GSTM1 null* genotype [60].

Analyzing the *GSTP1*Ile105Val* polymorphism in European-derived, we found similar allelic frequencies between SLE patients and controls, but a genotypic frequency of 36 % of heterozygous *Ile/Val* in SLE patients compared to 48 % in matched-controls was observed, suggesting an interesting protective role of genotype *Ile/Val* against the SLE. A possible explanation for this result is that individuals with heterozygous genotype would have

a good metabolism of a larger amount of substrates than individuals with mutant or wild-type homozygous genotypes. Depending of homozygous genotype, the detoxification might be effective for certain substances but not for others. Data from literature reported that the codon 105 comprises part of the active site of the GSTP1 enzyme for linking reactive electrophilic substrates, then the substitution of amino acid at position 105 may affect the substrate-specific catalytic activity and thermal stability of the enzyme [24]. Moreover, analysis pointed that enzymes with Ile105 and Val105 differ significantly in their catalytic activity according to the substrate on which the enzymes act [61] and it has been shown that enzymes with Val105 have a sevenfold higher efficiency for the PAH diol epoxides than the enzymes with Ile105. In contrast, enzymes with Val105 are threefold less effective using 1-chloro-2,4-dinitrobenzene [62]. Taken together, these reports provide the basis for our hypothesis. However, the genotypic frequencies of *GSTP1*Val* variant in European-derived SLE group were not in Hardy–Weinberg equilibrium, which can be explained by the possible and relevant effect of this polymorphism in a sample of non-healthy individuals. The African-derived group showed no significant difference in allelic and genotypic frequencies between patients and controls. However, it is worth noting that there was a higher frequency of *GSTP1*Val* allele in SLE patients of this group as compared to their controls, although not statistically significant (40 vs. 30 %). The research with Korean individuals also assessed the *GSTP1*Ile105Val* polymorphism in susceptibility to SLE and, contrary to our findings, no association was observed in that population [50]. It is also interesting to cite a study conducted in London that identified a possible protective role of *GSTP1*Val* allele against polymorphic light eruption, a feature significantly increased in patients with Lupus Erythematosus [63]. In another study, Chinese patients diagnosed with SLE and under use of pulsed Cyclophosphamide, were evaluated according to effects of *GSTM1*, *GSTT1*, and *GSTP1* genetic variants on the severity of myelosuppression, gastrointestinal toxicity and incidence of infections. The authors found that the *GSTP1*Ile105Val* polymorphism, but not the *GSTM1* or *GSTT1* null genotypes, significantly increased the risk of short-term side-effects of pulsed high-dose Cyclophosphamide therapy in SLE patients. This finding shows the importance and the need to optimize the Cyclophosphamide therapy in SLE patients according to these variants [64].

With respect to *CYP1A1* gene analysis, the allele and genotypic frequencies of *CYP1A1*2C* variant in both European-derived and African-derived SLE patients were not different of the frequencies observed in controls. Other studies have also suggested the role of *CYP1A1* polymorphisms in the context of SLE. In Taiwan, *CYP1A1* polymorphisms, in combination with manganese superoxide dismutase gene polymorphisms, were also evaluated in the pathogenesis of SLE, and no significant difference was found in frequencies of this polymorphism between patients and healthy individuals, corroborating our findings [27]. However, this is different than the results of two previously published studies. Investigations from von Schmiedeberg et al. [65] showed a significant increase of the *Val* allele in Germans with SLE when compared to controls, indicating that the enhanced formation of reactive metabolites, resulting from a higher basal and inducible enzyme activity, could alter self-proteins presented to the immune system, stimulating autoreactive T cells which induce autoimmunity. The authors of Chinese study, beyond the *GSTT1* and *GSTM1* polymorphisms, also examined the influence of *CYP1A1* in susceptibility to SLE and, in this analysis, significant difference was observed when comparing the genotypic frequencies between SLE patients and controls, with higher frequency of *CYP1A1*2C* mutant genotype in SLE patients. However, no statistical difference was obtained when comparing the allelic frequencies [28]. Yen et al. [27] suggested that the discrepancy observed among these results may be due to different genetic backgrounds in the different ethnics. Furthermore, it is important to take into account that the Chinese population has over 30 provinces and 1/5 of the worlds total population [28]. Thus, the extrapolation of these results to other populations should be considered with caution.

Genotypic and allelic frequencies of *CYP2E1* were analyzed and no significant difference in the frequencies between European-derived SLE patients and controls was obtained. In African-derived, similar genotypic frequencies between patients and controls were also observed, however a frequency of 11 % for allele *CYP2E1*5B* was observed in patients against 5 % in matched-controls, indicating a possible association of *CYP2E1**5B allele with SLE. Individuals carrying this allele present about threefold higher chance to develop the disease than individuals carrying the wild-type allele. This association may be due to influence of *CYP2E1*5B* allelic variant in the increase of *CYP2E1* gene transcription. In addition to the CYP2E1 ability to metabolize a wide variety of low molecular weight compounds, it is also an effective generator of ROS [49], which cause damage in cell membranes and macromolecules and leads to formation of DNA adducts [18]. The involvement of ROS in the pathogenesis of SLE has been described by many authors [27, 29, 66, 67]. A study revealed that there was an increased production of superoxide by neutrophile in the serum from patients with SLE and oxygen intermediates produced by immune complex-activated neutrophils play an important role in vasculitis, nephritis and other tissue damage [27, 67]. Furthermore,

ROS may modify DNA molecules and increase the immunogenicity of damaged DNA, inducing the formation of pathological anti-DNA antibodies [27]. Among the wide substrate spectrum of CYP2E1, trichloroethylene (TCE) has been implicated in autoimmune disorders in both humans and animal models. TCE is an organic solvent that is primarily used as a degreasing agent for metals. Griffin et al. [68] showed that treatment with TCE promoted expansion of the percentage of $CD4^+$ T cells, with a reduction in the secretion of IL-4 and increased secretion of IFN-γ in autoimmune prone MRL+/+ mice. They also reported that after the mice were treated with diallyl sulfide, a specific inhibitor of CYP2E1, the proliferative capacity of T cells was inhibited and the reduction in IL-4 levels was reversed, showing that the metabolism of TCE by CYP2E1 is required for immunomodulation. Therefore, it is possible that higher expression of CYP2E1 contributes to the SLE development through the production of more ROS during the compounds metabolization with the production of toxic intermediates as, for example, the metabolites of TCE [49].

Since we observed possible influence of *GSTP1*Ile105Val* and *CYP2E1*5B* polymorphisms in SLE susceptibility and because of the importance of investigating the interaction between phase I and II enzymes, the frequencies of SLE patients and controls with the presence of at least one allelic variant of both polymorphisms were analyzed. No significant differences were found.

Besides the relevance of *GST* and *CYP* polymorphisms in the onset of SLE, their role in clinical progression of disease has also attracted the attention of researchers. Fraser et al. [8] observed a higher prevalence of anti-Ro/SSA (+) autoantibodies among Caucasians bearing the *GSTM1 null* genotype although the prevalence of these autoantibodies was weaker in African-Americans SLE patients. Kang et al. [50] also reported association of the *GSTM1 null* genotype with a higher frequency of anti-Ro/SSA (+)/anti-La/SSB (−) autoantibody profile, but with a lower frequency of hematological disorders. Compared to SLE patients with the *GSTT1 non-null* genotype, those with the *GSTT1 null* had a lower frequency of discoid rash and nephritis, suggesting that deletion of *GSTT1* or *GSTM1* may influence the manifestation of SLE but not the risk of SLE. So far, we have found no significant influence of the polymorphisms studied in clinical and laboratory features of SLE patients. The study with Taiwanese individuals is consistent with ours, where associations between the *CYP1A1**2C polymorphisms and clinical manifestations of SLE were not observed [27].

It is important to note that functionally significant polymorphisms in genes of other metabolic enzymes could also contribute to susceptibility and/or affect the hepatic metabolism of drugs in SLE patients. For example, hydroxychloroquine, one of the choice oral therapies for SLE treatment, is metabolized by the enzymes CYP2C8, CYP3A4, and CYP2D6, and therefore patients with certain variants of these genes may respond differently to regimens using such drug [69].

Available studies about the influence of biometabolism genetic polymorphisms in SLE are scarce and have limitations since generally they only include some of the metabolizing enzymes genes. This is the first southern Brazilian study that provides evidence for an association between polymorphic variants of genes related to oxidative metabolism and SLE risk. In conclusion, our results indicate a possible protective role of *GSTP1*Ile105Val* heterozygous genotype in susceptibility of SLE in European-derived and a possible contribution of the *CYP2E1*5B* allele and more weakly of the *GSTP1*Val* allele in the pathogenesis of SLE in African-derived. It can be suggested that the first variant would be facilitating the accumulation of ROS while the second would contribute to an impaired detoxification of ROS in these individuals. Thus, the joint effects of these two polymorphisms, possibly also associated with effects of others gene variants, could lead to inflammation and an autoimmune disorder in African-derived group. No clinical implications of *GST* and *CYP* variants were observed in SLE patients. Although there are indications of the importance of enzymes GST and CYP in the autoimmune process, how this exactly happens is still unclear and our findings do not exclude the possibility that the combination of other biometabolism genes somehow contribute to SLE development. This shows the need for additional analyzes encompassing more genes presumably involved, as well as populations of different ethnic origins.

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Conflict of interest The authors declare no conflict of interest.

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