Advances in Rheumatology

RESEARCH

Open Access

TP53 and *p21* (*CDKN1A*) polymorphisms and the risk of systemic lupus erythematosus

Jacyara Maria Brito Macedo[1], Amanda Lima Silva[1], Amanda Chaves Pinto[1], Leandro Ferreira Lopes Landeira[1], Elyzabeth Avvad Portari[2,3], Cintia Barros Santos-Rebouças[2] and Evandro Mendes Klumb[4*]

Abstract

Background The p53 and p21 proteins are important regulators of cell cycle and apoptosis and may contribute to autoimmune diseases, such as systemic lupus erythematosus (SLE). As genetic polymorphisms may cause changes in protein levels and functions, we investigated associations of *TP53* and *p21* (*CDKN1A*) polymorphisms (*p53* 72 G > C—rs1042522; *p53* PIN3—rs17878362; *p21* 31 C > A—rs1801270; *p21* 70 C > T—rs1059234) with the development of systemic lupus erythematosus (SLE) in a Southeastern Brazilian population.

Methods Genotyping of 353 female volunteers (cases, n = 145; controls, n = 208) was performed by polymerase chain reaction, restriction fragment length polymorphism and/or DNA sequencing. Associations between *TP53* and *p21* polymorphisms and SLE susceptibility and clinical manifestations of SLE patients were assessed by logistic regression analysis.

Results Protective effect was observed for the genotype combinations *p53* PIN3 A1/A1-*p21* 31 C/A, in the total study population (OR 0.45), and *p53* PIN3 A1/A2-*p21* 31 C/C, in non-white women (OR 0.28). In Whites, *p53* 72 C-containing (OR 3.06) and *p53* PIN3 A2-containing (OR 6.93) genotypes were associated with SLE risk, and higher OR value was observed for the combined genotype *p53* 72 G/C-*p53* PIN3 A1/A2 (OR 9.00). Further, *p53* PIN3 A1/A2 genotype was associated with serositis (OR 2.82), while *p53* PIN3 A2/A2 and *p53* 72 C/C genotypes were associated with neurological disorders (OR 4.69 and OR 3.34, respectively).

Conclusions Our findings showed that the *TP53* and *p21* polymorphisms included in this study may have potential to emerge as SLE susceptibility markers for specific groups of patients. Significant interactions of the *TP53* polymorphisms with serositis and neurological disorders were also observed in SLE patients.

Highlights

- The polymorphisms *TP53* rs1042522 (G > C) and *TP53* rs17878362 (16 bp Del/Ins) were associated with SLE risk in whites.
- In whites, the combined genotype *TP53* rs1042522 GC- *TP53* rs17878362 A1A2 and the haplotype *TP53* rs1042522 C-rs17878362 A2 represented higher SLE risk.

*Correspondence:
Evandro Mendes Klumb
klumb@uol.com.br
Full list of author information is available at the end of the article

- Combination of *TP53* rs17878362 (16 bp Del/Ins) and *p21* rs1801270 (C > A) protected against SLE in non-white women.
- *TP53* and *p21* (*CDKN1A*) polymorphisms may be SLE susceptibility markers for specific groups.

Keywords Systemic lupus erythematosus, Genetic polymorphisms, *TP53*, *p21*

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by a breakdown in self-tolerance, dysregulation of lymphocytes number and subsets in association with an increased autoantibody production [1, 2]. Although the interaction of genetic, immunological and environmental factors is widely accepted, this complex pathophysiological process is not fully understood [3]. There is a central role for an excess of autoantigens derived mainly from altered programmed cell-death mechanisms like NETosis, pyroptosis and apoptosis [4] in association with a deficient clearance of extracellular vesicles associated autoantigens [5, 6].

It has already been suggested that apoptosis plays an important role in the elimination of autoreactive lymphocytes in SLE patients. Cell cycle control, in its turn, is responsible for maintaining lymphocyte homeostasis as it participates in lymphocyte differentiation, effector function, memory development, tolerance induction, and apoptosis [5]. Therefore, defects in the regulation of apoptotic process and cell cycle may cause loss of tolerance, generating autoimmune responses [1].

One of the major regulators of cell cycle and apoptosis is the p53 tumor suppressor protein. The protein p53 acts as a transcription factor and regulates the expression of genes involved in several cellular processes, including cell cycle arrest, DNA repair and activation of apoptosis [7]. In SLE patients, it has been observed high levels of p53 expression and anti-p53 antibodies, with a significant correlation between p53 levels and SLE activity index [8].

The *TP53* gene, which encodes the p53 protein, is located on chromosome 17 (17p13.1), contains 11 exons, and its expression is controlled by two promoter regions giving rise to 12 different p53 protein isoforms [9]. This gene has numerous polymorphisms, and some of them are known to alter protein function [10]. Two *TP53* polymorphisms have been more widely studied. The single nucleotide polymorphism (SNP) rs1042522 is characterized by substitution of guanine to cytosine at the second position of the codon 72, which is located in exon 4 of the TP53 gene [NM_000546.5(TP53):c.215C > G (p.Pro72Arg)]. Experimental evidence suggests that the p53Arg (CGC codon) variant is more effective in activating the apoptosis pathway in altered cells than the p53Pro (CCC codon) variant, which, in turn, showed to be more efficient in the activation of cell cycle arrest [11]. The second polymorphism, rs17878362, is a 16 base pairs duplication in intron 3 of the *TP53* gene (NM_000546.5(TP53):c.96 + 25_96 + 40ACCTGGAGGGCTGGGG(1_2)—intron variant) [12]. The 16 bp duplication allele is associated with lower levels of p53 transcript, which might be due to modification of mRNA processing [13].

One of the mechanisms by which p53 acts on cell cycle arrest is through the transcriptional activation of the gene encoding the p21 protein, a kinase dependent cyclin inhibitor (CDKI). In DNA damaged cells, the p21 protein acts on different cyclin/CDK complexes, promoting cell cycle arrest at the G1/S and G2/M checkpoints [12]. p21 inhibits DNA synthesis by binding to proliferating cell nuclear antigen, and also affects key molecules of the apoptotic process, such as p53, and contributes to cell senescence [14]. In addition, the p21 protein seems to be associated with susceptibility to autoimmune diseases, especially to SLE [15].

The p21 protein is encoded by the *p21* (*WAF1*, *CIP*, *CDKN1A*) gene, which is located in the chromosomal region 6p21.2 [16]. Changes in the *p21* (*CDKN1A*) gene sequence may result in p21 inactivation, which in turn, may lead to an increased activation and proliferation of self-reactive T cells, and apoptosis [17]. More than 40 polymorphisms have already been identified in the *p21* gene. One of the most studied polymorphism is rs1801270, characterized by a C to A substitution at the third base of codon 31 of the *p21* (*CDKN1A*) gene (NM_000389.5(CDKN1A):c.93C > A (p.Ser31Arg)). This SNP results in the substitution of serine (AGC codon) by arginine (AGA codon) in a conserved region of the p21 protein [18]. The second most studied polymorphism, rs1059234, is a C to T substitution at 20 nucleotides after the stop codon at exon 3 in the 3' untranslated region of the gene (NM_000389.5(CDKN1A):c.*20C > T). By altering mRNA stability and inducing its faster degradation, this SNP leads to a change in the p21 protein level [18].

In recent years, associations of multiple genes and susceptibility to SLE have been analyzed, and among these candidate genes are *p21* (*CDKN1A*) and *TP53* [15, 19]. The number of studies published so far is still small, and there are no reports of studies evaluating *TP53* and *p21* (*CDKN1A*) polymorphisms in Brazilian SLE patients. Taking all this into consideration, the objective of this work was to evaluate possible associations of *TP53* rs1042522 (G > C), *TP53* rs17878362 (16 bp Del/Ins), *p21* rs1801270 (C > A) and *p21* rs1059234 (C > T) polymorphisms with risk of systemic lupus erythematosus, some SLE clinical manifestations and/or age at onset of symptoms.

Material and methods

Study population

The population included in the group of cases was composed of women with SLE (n = 145) according to the American College of Rheumatology (ACR) criteria [20] who were selected sequentially during routine outpatient consultations at the Rheumatology Department of the State University of Rio de Janeiro. The control group included healthy women without any complain (n = 208) who attended for a routine outpatient clinic at the same institution. Sociodemographic data and clinical manifestations were obtained with a standardized questionnaire and medical records review. The presence of any autoimmune disease was excluded after anamnesis and physical examination performed by an experienced rheumatologist. Skin color/ethnicity was established by self-classification as phenotypic proxy for ancestry previously proved to have a high concordance in Southeastern Brazil [21]. All participants signed an informed consent previously approved by the University Hospital Ethics Committee (#321 and #909).

DNA extraction and genotyping of polymorphisms in the TP53 and p21 (CDKN1A) genes

A total of 5 mL of peripheral blood from all participants was collected in Vacutainer tubes containing ethylenediamine tetraacetic acid (EDTA) and transported on ice to the Molecular Biology Laboratory, Department of Biochemistry/IBRAG/UERJ, where molecular analyses were performed.

DNA was extracted from mononuclear cells following a salting out method described by Vargas-Torres et al. [22].

The polymorphisms rs1042522 and rs17878362 in the *TP53* gene were analyzed by the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques. A primer pair (Forward: 5′GAGACCTGTGGGGAAGCGAA-3′ and Reverse: 5′GGAAGCCAGCCCCTCAG-3′) was specifically designed for amplification of the genomic region containing both *TP53* gene polymorphisms and generated a 476 or 492 bp fragment depending, respectively, on the absence or presence of 16 bp duplication in the intron 3 region.

The PCR reaction mixture was composed of 1× PCR Buffer (BIOTOOLS), 2 mM $MgCl_2$ (BIOTOOLS), 15 pmol of each oligonucleotide (P5334R and P5334F), 65 µM dNTPs (dATP, dCTP, dGTP, dTTP-PHARMACIA) 0.15 U *Taq* DNA polymerase (BIOTOOLS) and about 200 ηg genomic DNA in a final volume of 30 µL adjusted with sterile deionized water.

The amplification reaction was performed in a thermocycler (model MJ96 + Biocycler) using the following program: an initial denaturation step at 94 °C for 5 min; 30 cycles of denaturation at 94 °C for 45 s, primer pairing at 61 °C for 45 s and extension at 72 °C for 45 s; and a final step extension at 72 °C for 10 min. The PCR products were analyzed by electrophoresis on 6% polyacrylamide gels followed by ethidium bromide staining, which allowed the identification of the three *TP53* rs17878362 (16 bp Del/Ins) genotypes composed of alleles without (A1) and with (A2) 16 bp duplication.

The *TP53* rs1042522 (G > C) polymorphism was analyzed by digestion of PCR products (7 µL) with 2 U of the B*stU*I enzyme (BioLabs) in a final volume of 12 µL adjusted with sterile deionized water. Samples were incubated at 60 °C for approximately 16 h and then electrophoresed on 2% agarose gels stained with ethidium bromide.

The two *p21* (*CDKN1A*) gene polymorphisms were analyzed by PCR–RFLP techniques using the endonucleases *Alw*26I (Thermo Scientific), for rs1801270 (C > A), and *Pst*I (INVITROGEN), for rs1059234 (C > T), as previously described by Vargas-Torres et al. (2016) [23].

Genotyping identification was performed by two independent researchers. In order to validate the genotyping results, 10% of the total samples were reanalyzed using PCR or PCR–RFLP techniques. In addition, representative samples of each genotype were confirmed by direct sequencing of PCR products using an ABI 3130 sequencing platform (Applied Biosystems), following the manufacturer's instructions.

Statistical analysis

Deviation from the Hardy–Weinberg equilibrium was estimated within the study groups (cases and controls) by using the chi-square (χ^2) test.

Associations between *TP53* and *p21* (*CDKN1A*) polymorphisms and SLE susceptibility in the whole population and in skin color/ethnicity-stratified subgroups

were assessed by logistic regression analysis using four genetic models, codominant, dominant, recessive, and overdominant. Combinatorial interactions of the four studied polymorphisms, linkage disequilibrium, haplotype frequency estimation (Expectation maximization algorithm), and haplotype association analyses were also performed. The magnitude of association between *TP53* and *p21* (*CDKN1A*) polymorphisms and systemic lupus erythematosus was estimated by calculating odds ratio (OR) and 95% confidence interval (CI). Interaction analysis between the *TP53* and *p21* polymorphisms and some clinical manifestations of SLE and age at onset of symptoms were performed. Reference categories were the most frequent allele for each polymorphism, the homozygous genotypes for these alleles [24], the combination of these homozygous genotypes, and haplotypes formed by the most frequent variants. Adjusted OR was calculated controlling for age and/or skin color/ethnicity.

Statistical analyses were performed using the software GraphPad Prism version 6.05 (GraphPad Software, Inc., San Diego, CA) and the SNPStats program (Institut Català d'Oncologia, Barcelona, Spain) [25], a webtool for genetic association analysis. A p value below 0.05 was considered statistically significant.

Results

Characteristics of study population

The mean ± standard deviation and median age of patients (n = 145) and controls (n = 208) at the time they were included in the study were 39.7 ± 11.2 [Median (25–75%): 39.0 (31.0–46.5)] and 37.6 ± 10.5 years [Median (25–75%): 40.0 (29.0–45.5)], respectively. The study population was composed of 76% Afro descendant (cases: n = 95; controls: n = 171) and 34% Whites (cases: n = 50; controls: n = 35), mostly of European ancestry. The most frequent clinical manifestations in patients with SLE were polyarthritis (87.4%), malar rash (75.5%), photosensitivity (73.9%), hematological abnormalities (76.1%) and glomerulonephritis (73.1%). The mean ± standard deviation age at the onset of symptoms was 27.1 ± 10.6 years.

Association of TP53 and p21 (CDKN1A) polymorphisms with SLE risk

All samples were genotyped with respect to at least one of the four polymorphisms, *TP53* rs1042522 (G > C), *TP53* rs17878362 (16 bp Del/Ins), *p21* rs1801270 (C > A), and *p21* rs1059234 (C > T). PCR or PCR–RFLP patterns of genotypes corresponding to the four analyzed polymorphisms and their confirmation by DNA sequencing can be seen in Fig. 1. Genotype distribution of the four polymorphisms was in Hardy–Weinberg equilibrium in both study groups, cases and controls ($p > 0.05$) (Table 1).

No significant results were observed in comparative analyzes of genotype and allele distributions in the groups of cases and controls regardless the genetic model used (Table 1).

Concerning skin color/ethnicity-classification there was no significant difference for the *p21* polymorphisms (data not shown). However, both polymorphisms in the *TP53* gene showed significant results in the subgroup of white women (Table 2). Statistically relevant differences were observed in the distribution of *TP53* rs1042522 (G > C) polymorphism between cases and controls, in both codominant (GC vs. GG: adjusted OR 2.73; 95% CI 1.02–7.34; $p = 0.041$) and dominant (GC + CC *vs*. GG: adjusted OR 3.06; 95% CI 1.17–8.04; $p = 0.021$) models. Regarding the *TP53* rs17878362 (16 bp Del/Ins), the A1A2 genotype (codominant model: adjusted OR 5.22; 95% CI 1.33–20.47; $p = 0.003$) and A2-containing genotypes (dominant model: adjusted OR 6.93; 95% CI 1.81–26.51; $p = 0.001$ and overdominant: adjusted OR 4.47; 95% CI 1.15–17.33; $p = 0.018$) were more prevalent among cases in comparison with controls. Still among whites, the alleles rs1042522 C and rs17878362 A2 were more frequent in the case group than in controls (OR 2.23; 95% CI 1.10–4.54; $p = 0.037$ and OR 7.00; 95% CI 1.99–24.66; $p < 0.001$, respectively).

(See figure on next page.)

Fig. 1 Molecular analysis of polymorphisms in the *TP53* and *p21* (*CDKN1A*) genes. *Left*: **A** *TP53* rs1042522 (G > C). Photography of an ethidium bromide-stained agarose gel (2%) showing PCR–*Bst*UI RFLP patterns. Lane 1: 50 bp ladder (INVITROGEN); lanes 2, 3 and 4: genotypes rs1042522 CC (one fragment of 492 or 476 bp), rs1042522 GC (three fragments of 492 or 476, 305 or 284 and 187 bp) and rs1042522 GG (two fragments of 305 or 284 bp and 187 bp), respectively. **B** *TP53* rs17878362 (16 bp Del/Ins). Photography of an ethidium bromide-stained polyacrylamide gel (8%) showing PCR patterns. Lane 1: 50 bp ladder (INVITROGEN); lanes 2, 3 and 4: genotypes rs17878362 A1A1 (one fragment of 492 bp), rs17878362 A1A2 (two fragments of 492 and 476 bp) and rs17878362 A2A2 (one fragment of 476 bp), respectively. **C** *p21* rs1801270 (C > A). Photography of an ethidium bromide-stained polyacrylamide gel (8%) showing PCR–*Alw26*I RFLP patterns. Lane 1: 50 bp ladder (INVITROGEN); lanes 2, 3 and 4: genotypes rs1801270 CC (two fragments of 105 and 74 bp), rs1801270 CA (three fragments of 179, 105 and 74 bp) and rs1801270 AA (one fragment of 179 bp), respectively. **D** *p21* rs1059234 (C > T) polymorphism. Photography of an ethidium bromide-stained polyacrylamide gel (8%) showing PCR–*Pst*I RFLP patterns. Lane 1: 50 bp ladder (INVITROGEN); lanes 2, 3 and 4: genotypes rs1059234 CC (two fragments of 115 and 68 bp), rs1059234 CT (three fragments of 183, 115 and 68 bp) and rs1059234 TT (one fragment of 183 bp). *Right*: **A**–**D** DNA sequencing—electropherograms corresponding to different genotypes. The polymorphic sites are indicated

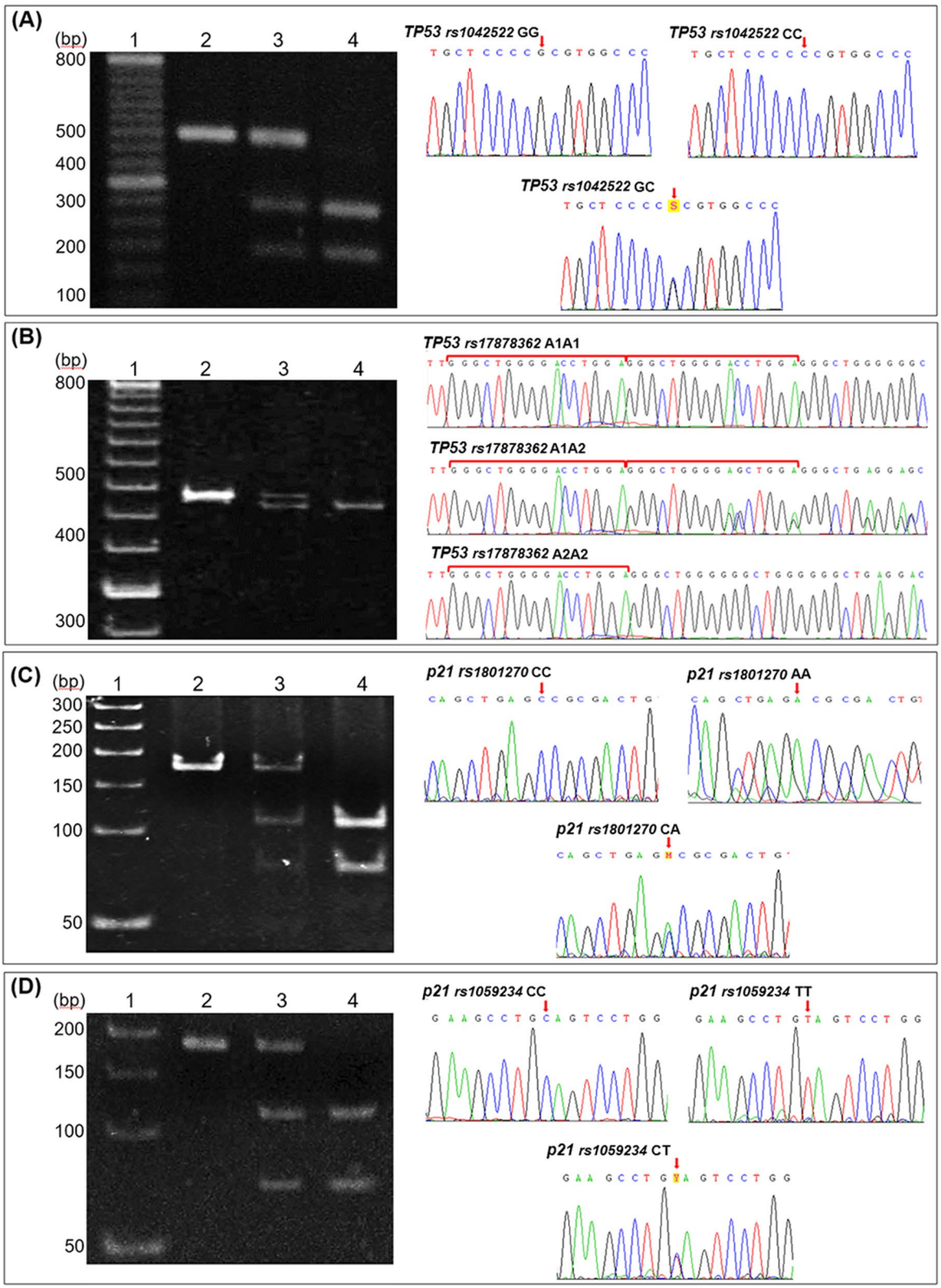

Fig. 1 (See legend on previous page.)

Table 1 Comparative analysis of genotype and allele distributions of the four polymorphisms, *TP53* rs1042522 (G > C), *TP53* rs17878362 (16 bp Del/Ins), *p21* rs1801270 (C > A) and *p21* rs1059234 (C > T), between the groups of cases and controls using different genetic models

Polymorphism	Genotype/Allele	Controls[a] n (%) or n (*f*)	Cases[b] n (%) or n (*f*)	*p* value [c]	Genetic model		Adjusted OR[d] (95% CI)
TP53 rs1042522 G > C	GG	64 (34.0)	37 (31.6)	0.6424	Codominant	GC vs. GG	1.21 (0.71–2.08)
	GC	88 (46.8)	61 (52.1)			CC vs. GG	1.09 (0.53–2.22)
	CC	36 (19.1)	19 (16.2)		Dominant	GC + CC vs. GG	1.18 (0.71–1.97)
	G	216 (0.57)	135 (0.58)	1.0000	Recessive	CC vs. GG + GC	0.97 (0.51–1.81)
	C	160 (0.43)	99 (0.42)		Overdominant	GC vs. GG + CC	1.18 (0.73–1.90)
TP53 rs17878362 16 bp Del/Ins	A1A1	136 (69.4)	77 (65.8)	0.7349	Codominant	A1A2 vs. A1A1	1.21 (0.70–2.07)
	A1A2	50 (25.5)	32 (27.4)			A2A2 vs. A1A1	1.44 (0.53–3.92)
	A2A2	10 (5.1)	8 (6.8)		Dominant	A1A2 + A2A2 vs. A1A1	1.25 (0.75–2.06)
	A1	322 (0.82)	186 (0.79)	0.4598	Recessive	A2A2 vs. A1A1 + A1A2	1.37 (0.51–3.67)
	A2	70 (0.18)	48 (0.21)		Overdominant	A1A2 vs. A1A1 + A2A2	1.17 (0.69–1.99)
p21 rs1801270 C > A	CC	103 (57.5)	83 (64.3)	0.3696	Codominant	CA vs. AA	0.86 (0.52–1.41)
	CA	66 (36.9)	42 (32.6)			AA vs. AA	0.54 (0.16–1.83)
	A/A	10 (5.6)	4 (3.1)		Dominant	CA + AA vs. CC	0.81 (0.50–1.32)
	C	272 (0.76)	208 (0.81)	0.2005	Recessive	AA vs. CC + CA	0.58 (0.17–1.91)
	A	86 (0.24)	50 (0.19)		Overdominant	CA vs. CC + AA	0.89 (0.55–1.46)
p21 rs1059234 C > T	CC	104 (61.5)	84 (65.6)	0.7948	Codominant	CT vs. CC	0.88 (0.52–1.47)
	CT	55 (32.5)	37 (28.9)			TT vs. CC	0.89 (0.32–2.46)
	TT	10 (5.9)	7 (5.5)		Dominant	CT + TT vs. CC	0.88 (0.54–1.43)
	C	263 (0.78)	205 (0.80)	0.5437	Recessive	TT vs. CC + CT	0.92 (0.34–2.54)
	T	75 (0.22)	51 (0.20)		Overdominant	CT vs. CC + TT	0.88 (0.53–1.48)

n number of volunteers, *f* allele frequency

[a] Controls—Hardy–Weinberg equilibrium test: *TP53* rs1042522 (G > C) ($p = 0.559$); *TP53* rs17878362 (16 bp Del/Ins) ($p = 0.068$); *p21* rs1801270 (C > A) ($p = 0.893$); *p21* rs1059234 (C > T) ($p = 0.454$)

[b] Cases—Hardy–Weinberg equilibrium test: *TP53* rs1042522 (G > C) ($p = 0.462$); *TP53* rs17878362 (16 bp Del/Ins) ($p = 0.081$); *p21* rs1801270 (C > A) ($p = 0.634$); *p21* rs1059234 (C > T) ($p = 0.287$)

[c] χ^2 test or Fisher test.

[d] Adjusted for age and skin color/ethnicity. *p* values > 0.05

Association of combined genotypes and haplotypes regarding the TP53 and p21 (CDKN1A) polymorphisms with SLE risk

Results of genotype combination analysis are shown in Table 3. Considering the entire study population, the *TP53* rs17878362 A1A1-*p21* rs1801270 CA combined genotype was less frequent in the case group than in controls (OR 0.45; 95% CI 0.23–0.87; $p = 0.018$). In non-white individuals, the frequency of the genotype combination rs17878362 A1A2-rs1801270 CC was lower among SLE patients (OR 0.28; 95% CI 0.10–0.80; $p = 0.015$). In contrast, among Whites the prevalence of the *TP53* rs1042522 GC- *TP53* rs17878362 A1A2 combined genotype was significantly higher in SLE patients as compared with controls (OR 9.00; 95% CI 1.70–47.62; $p = 0.008$).

Linkage disequilibrium was observed between the two *TP53* (rs1042522 and rs17878362) polymorphisms ($D = 0.1011$, $D' = 0.9246$, $r = 0.5215$, $p < 0.001$), and the two SNPs (rs1801270 and rs1059234) in the *p21* gene ($D = 0.1403$, $D' = 0.8518$, $r = 0.825$, $p < 0.001$). No statistically significant difference was observed between haplotype frequencies of cases and controls in the entire

Table 2 Analysis of interaction between the *TP53* rs1042522 (G > C) and *TP53* rs17878362 (16 bp Del/Ins) polymorphisms, skin color/ethnicity and development of SLE

Polymorphism	Skin color/ethnicity	Genetic model	Controls n	Cases n	Adjusted OR (95% CI)[c]
TP53 rs1042522 (G > C)[a]	White	GG	18	12	1.00
		GC	14	25	**2.73 (1.02–7.34)[d]**
		CC	1	5	7.88 (0.80–77.25)
		GG	18	12	1.00
		GC + CC	15	30	**3.06 (1.17–8.04)[e]**
		GG + GC	32	37	1.00
		CC	1	5	4.43 (0.49–40.17)
		GG + CC	19	17	1.00
		GC	14	25	2.01 (0.80–5.080)
	Non-white	GG	45	25	1.00
		GC	74	36	0.85 (0.56–1.60)
		CC	35	14	0.72 (0.32–1.59)
		GG	45	25	1.00
		GC + CC	109	50	0.81 (0.45–1.47)
		GG + GC	119	61	1.00
		CC	35	14	0.79 (0.39–1.59)
		GG + CC	80	39	1.00
		GC	74	36	0.97 (0.55–69)
TP53 rs17878362 (16 bp Del/Ins)[b]	White	A1A1	30	25	1.00
		A1A2	3	13	**5.22 (1.33–20.47)[f]**
		A2A2	0	4	NA
		A1A1	30	25	1.00
		A1A2 + A2A2	3	17	**6.93 (1.81–26.51)[g]**
		A1A1 + A1A2	33	38	1.00
		A2A2	0	4	NA
		A1A1 + A2A2	30	29	**1.00**
		A1A2	3	13	**4.47 (1.15–17.33)[h]**
	Non-white	A1A1	104	52	1.00
		A1A2	47	19	0.81 (0.43–1.52)
		A2A2	10	4	0.79 (0.24–2.64)
		A1A1	104	52	1.00
		A1A2 + A2A2	57	23	0.80 (0.45–1.45)
		A1A1 + A1A2	151	71	1.00
		A2A2	10	4	0.84 (0.25–2.77)
		A1A1 + A2A2	114	56	1.00
		A1A2	47	19	0.82 (0.44–1.54)

n number of volunteers, *NA* not applicable

[a] *TP53* rs1042522 (G > C)—Allele frequencies: *Whites*—C versus G: OR 2.23; 95% CI 1.10–4.54; $p = 0.037$. *Non-whites*—C versus G: OR 0.85; 95% CI 0.57–1.26; $p > 0.050$

[b] *TP53* rs17878362 (16 bp Del/Ins)—Allele frequencies: *Whites*—A2 versus A1: OR 7.00; 95% CI 1.99–24.66; $p < 0.001$. *Non-whites*—A2 versus A1: OR 0.83; 95% CI 0.51–1.37; $p > 0.050$

[c] Adjusted for age

Statistically significant results are in bold. [d]$p = 0.041$; [e]$p = 0.021$; [f]$p = 0.003$; [g]$p = 0.001$; [h]$p = 0.018$

Table 3 Genotype interactions of the *TP53* rs1042522 (G>C), *TP53* rs17878362 (16 bp Del/Ins), *p21* rs1801270 (C>A) and *p21* rs1059234 (C>T) polymorphisms between the group and subgroups of cases and controls stratified by skin color/ethnicity

Genotype combination[a]				Total group			Whites			Non-whites		
TP53 **rs104252253 G>C**	***TP53*** **rs17878362 16 bp Del/Ins**	***p21*** **rs1801270 C>A**	***p21*** **rs1059234 C>T**	**Controls n**	**Cases n**	**OR (95% CI)**	**Controls n**	**Cases n**	**OR (95% CI)**	**Controls n**	**Cases n**	**OR (95% CI)**
GG	A1A1			63	36	1.00	18	12	1.00	44	24	1.00
GC	A1A1			55	39	1.24 (0.70–2.22)	11	13	1.77 (0.60–5.25)	44	26	1.08 (0.54–2.17)
GC	A1A2			30	22	1.28 (0.65–2.55)	2	12	**9.00 (1.70–47.62)[b]**	28	10	0.65 (0.27–1.57)
GG		CC		36	22	1.00	9	10	1.00	27	12	1.00
GC		CC		44	38	1.41 (0.71–2.80)	9	15	1.50 (0.44–5.10)	35	23	1.37 (0.57–3.26)
GC		CA		30	16	0.87 (0.39–1.95)	3	7	2.10 (0.41–10.67)	27	9	0.75 (0.27–2.07)
CC		CA		15	11	1.20 (0.47–3.07)	1	3	2.70 (0.24–30.86)	14	8	1.29 (0.43–3.88)
GG			CC	32	21	1.00	7	9	1.00	25	12	1.00
GG			CT	17	8	0.72 (0.26–1.96)	6	1	0.13 (0.01–1.34)	10	7	1.46 (0.44–4.78)
GC			CC	45	38	1.29 (0.64–2.59)	9	15	1.30 (0.36–4.70)	36	23	1.33 (0.56–3.16)
GC			CT	25	17	1.04 (0.45–2.37)	2	7	2.72 (0.42–17.43)	23	10	0.91 (0.33–2.49)
CC			CT	5	10	3.05 (0.91–10.19)	1	1	0.78 (0.04–14.76)	9	4	0.93 (0.24–3.62)
	A1A1	CC		66	52	1.00	16	19	1.00	50	33	1.00
	A1A1	CA		48	17	**0.45 (0.23–0.87)[c]**	10	3	0.25 (0.06–1.08)	36	14	0.59 (0.28–1.26)
	A1A2	CC		30	11	0.46 (0.21–1.02)	3	6	1.68 (0.36–7.84)	27	5	**0.28 (0.10–0.80)[d]**
	A1A1		CC	62	46	1.00	14	17	1.00	48	29	1.00
	A1A1		CT	40	19	0.64 (0.33–1.25)	9	5	0.46 (0.12–1.68)	29	14	0.80 (0.36–1.76)
	A1A1		TT	6	5	1.12 (0.32–3.91)	2	2	0.82 (0.10–6.62)	4	3	1.24 (0.26–5.95)
	A1A2		CC	30	19	0.85 (0.43–1.70)	3	7	1.92 (0.42–8.84)	27	12	0.74 (0.32–1.67)
		CC	CC	89	68	1.00	16	25	1.00	73	43	1.00
		CC	CT	3	5	2.18 (0.50–9.45)	1	2	1.28 (0.11–15.31)	2	3	2.55 (0.41–15.86)
		CA	CT	49	29	0.77 (0.44–1.35)	8	9	0.72 (0.23–2.25)	39	20	0.87 (0.45–1.68)

n number of volunteers

[a] Genotype combinations not represented in at least one group or subgroup are not shown

Statistically significant results are in bold. [b]$p = 0.008$; [c]$p = 0.018$; [d]$p = 0.015$

Table 4 Haplotype frequency estimation and haplotype association analysis, with respect to the *TP53* polymorphisms, rs1042522 (G > C) and rs17878362 (16 bp Del/Ins), and the SNPs in the *p21* (*CDKN1A*) gene, rs1801270 (C > A) and rs1059234 (C > T), in the entire study sample and in the groups stratified according skin color/ethnicity

Haplotype	Total group			Whites			Non-whites		
	Controls (*f*)	Cases (*f*)	OR (95% CI)	Controls (*f*)	Cases (*f*)	OR (95% CI)	Controls (*f*)	Cases (*f*)	OR (95% CI)
TP53 rs17878362-*rs1042522*									
A1–G	0.565	0.572	1.00	0.766	0.583	1.00	0.520	0.565	1.00
A1–C	0.255	0.223	0.85 (0.56–1.30)	0.203	0.167	1.40 (0.52–3.75)	0.268	0.255	0.88 (0.54–1.43)
A2–C	0.170	0.200	1.14 (0.75–1.71)	0.031	0.250	**9.67 (2.02–46.28)[a]**	0.200	0.172	0.81 (0.49–1.35)
A2–G	0.010	0.005	0.62 (0.09–4.22)	0	0	NA	0.013	0.008	0.69 (0.10–4.64)
Global haplotype association *p* value		0.69			**< 0.001**			0.83	
p21 rs1801270-*rs1059234*									
C–C	0.743	0.766	1.00	0.759	0.787	1.00	0.744	0.755	1.00
A–T	0.202	0.167	0.80 (0.51–1.24)	0.167	0.162	0.98 (0.39–2.49)	0.206	0.170	0.80 (0.47–1.35)
A–C	0.035	0.036	0.97 (0.37–2.52)	0	0.026	NA	0.042	0.041	0.94 (0.33–2.69)
C–T	0.019	0.031	1.49 (0.52–4.27)	0.074	0.026	0.42 (0.09–2.09)	0.008	0.034	4.46 (0.84–23.74)
Global haplotype association *p* value		0.65			0.34			0.23	

f frequency

Statistically significant results are in bold. [a]*p* value: 0.006

study population. However, haplotype frequency estimation considering skin color/ethnicity-stratified subgroups revealed that in Whites the haplotype rs17878362 A2-rs1042522 C was more frequent among SLE patients in comparison with controls (OR 9.67, 95% CI 2.02–46.28; $p = 0.006$) (Table 4).

Association of genotypes of TP53 and p21 (CDKN1A) polymorphisms with clinical manifestations of SLE and age at onset of symptoms

Interaction analysis between the *TP53* and *p21* (*CDKN1A*) polymorphisms and some clinical manifestations of SLE patients was also investigated. Statistically significant results were not found with respect to cutaneous-articular manifestations, hematological and immunological disorders and nephritis (data not shown). Notably, serosistis was more prevalent among SLE patients carrying the *TP53* rs17878362 A1A2 genotype (overdominant model: OR 2.82; 95% CI 1.18–6.74; $p = 0.021$) (Table 5). Neuropsychiatric disorders (seizures and psychosis) were more frequent in SLE patients carrying the *TP53* rs17878362 A2A2 genotype (codominant model: OR 4.69; 95% CI 1.04–21.24; $p = 0.054$) and in the *TP53* rs1042522 C/C genotype carriers (codominant model: OR 5.82; 95% CI 1.46–23.17; $p = 0.015$ and recessive model: OR 3.34; 95% CI 1.17–9.55; $p = 0.031$) (Table 5). The *TP53* alleles rs17878362A2 and *p53* rs1042522 C were more frequent in SLE patients with neuropsychiatric manifestations (OR 2.15; 95% CI 1.06–4.35; $p = 0.048$ and OR 2.23; 95% CI 1.18–4.29; $p = 0.015$, respectively). No association of *TP53* and *p21* polymorphisms with age at onset of symptoms was observed (data not shown).

Table 5 Interaction analysis between the polymorphisms *TP53* rs17878362 (16 bp Del/Ins) and *TP53* rs1042522 (G > C) and clinical manifestations (serositis and neuropsychiatric disorders) of SLE patients

Polymorphism	Genetic model	Serositis			Neuropsychiatric disorders		
		Yes n	No n	OR (CI 95%)	Yes n	No n	OR (CI 95%)
TP53 rs17878362 16 bp Del/Ins	A1A1	35	39	1.00	13	61	1.00
	A1A2	21	10	2.34 (0.97–5.64)	8	24	1.56 (0.58–4.25)
	A2A2	0	8	NA	4	4	**4.69 (1.04–21.24)[b]**
	A1A1	35	39	1.00	13	61	1.00
	A1A2 + A2A2	21	18	1.30 (0.60–2.83)	12	28	2.01 (0.81–4.96)
	A2A2	0	8	1.00	4	4	1.00
	A1A1 + A1A2	56	49	NA	21	85	4.05 (0.93–17.53)
	A1A2	21	10	1.00	8	24	1.00
	A1A1 + A2A2	35	47	**2.82 (1.18–6.74)[a]**	17	65	1.27 (0.49–3.34)
	A1	91	88	1.00	34	146	1.00
	A2	21	26	0.78 (0.41–1.49)	16	32	**2.15 (1.06–4.35)[c]**
TP53 rs1042522 G > C	GG	19	18	1.00	4	32	1.00
	GC	31	26	1.13 (0.49–2.59)	13	46	2.26 (0.68–7.57)
	CC	6	13	0.44 (0.14–1.40)	8	11	**5.82 (1.46–23.17)[d]**
	GG	19	18	1.00	4	32	1.00
	GC + CC	37	39	0.90 (0.41–1.97)	21	57	2.95 (0.93–9.34)
	GG + GC	50	44	1.00	17	78	1.00
	CC	6	13	0.41 (0.14–1.16)	8	11	**3.34 (1.17–9.55)[e]**
	GG + CC	25	31	1.00	12	43	1.00
	GC	31	26	1.48 (0.70–3.10)	13	46	1.01 (0.42–2.46)
	G	69	62	1.00	21	110	1.00
	C	43	52	0.74 (0.44–1.26)	29	68	**2.23 (1.18–4.29)[f]**

n number of volunteers, *NA* not applicable

Statistically significant results are in bold: [a]$p = 0.021$; [b]$p = 0.054$; [c]$p = 0.048$; [d]$p = 0.015$; [e]$p = 0.031$; [f]$p = 0.015$

Discussion

In the present work, the *TP53* rs1042522 (G > C) and *TP53* rs17878362 (16 bp Del/Ins) polymorphisms were associated with risk of SLE in the subgroup of white women (Table 2). The rs1042522 C allele carriers and rs17878362 A2 allele carriers presented two-, and seven-fold higher risk of developing SLE ($p = 0.037$ and $p < 0.001$, respectively).

To date, we could not find any association studies regarding the *TP53* rs17878362 (16 bp Del/Ins) polymorphism and SLE or the *TP53* rs1042522 (G > C), *p21* rs1801270 (C > A) and *p21* rs1059234 (C > T) polymorphisms and the development of SLE in Brazilian populations. Moreover, the few published studies related to the importance of *TP53* and *p21* polymorphisms for the development of SLE present discordant results [15, 19, 26–29]. A meta-analysis on association between *TP53* polymorphisms and SLE showed significant results only after stratification by ethnicity, confirming the rs1042522 C allele as risk factor for SLE development in Asians (Koreans) [30]. We are aware that some studies describing Brazilian genetic structure have reported divergences between self-identified skin color and genetic ancestry [31, 32]. However, most clinical and genetic studies all around the world employ self-declaration of skin color [33], and this criterion previously proved to have a high concordance in the Southeastern region of Brazil [21].

The divergence of susceptibility genes found among various ethnicities may be related to the influence of local environmental factors, which may interfere with epigenetic modifications [3, 28, 34, 35]. Besides, allele distributions of the above four polymorphisms vary widely among different populations and may have different impact on susceptibility for SLE [36, 37]. Particularly, Brazilian population presents a mixture of genetic ancestry, mostly European and African, which may partially explain the present results [32].

Even though a single polymorphism may represent a valuable biomarker of a complex disease, such as SLE, it is believed that multiple small-effect genetic variants influence disease susceptibility [38]. Therefore, genotype combination and haplotype association analyses were also performed to assess the combined effect of the four polymorphisms in the *TP53* and *p21* (*CDKN1A*) genes on the risk of SLE.

Some genotype combinations were absent in some groups or subgroups stratified according to skin color/ethnicity (Table 3). These findings are not unexpected, since some individual genotypes were found at a very low frequency in our study sample (Tables 1 and 2). Noteworthy, a combined effect of both *TP53* polymorphisms seems to exist among Whites, since the rs1042522 GC- rs17878362 A1A2 combined genotype were ninefold more frequent in cases than in controls ($p=0.008$) (Table 3). Although a significant difference does exist, due to the limited sample size when the total study population was stratified according to skin color/ethnicity, the statistical power is not strong enough to establish the true association with SLE development.

It has been previously shown that the p53 Arg (rs1042522 G allele) variant has a greater ability to activate apoptosis pathways, while the p53Pro (rs1042522 C allele) variant demonstrates greater efficacy in promoting cell cycle arrest [11], a mechanism that may be associated with SLE development [1, 2]. Besides, removal of self-reactive B and T cells in SLE patients and mice with lupus-like syndrome could be impaired by defective apoptotic processes [39, 40]. This might support the hypothesis that the p53 Pro variant may reduce the clearance of auto-reactive lymphocytes in patients with SLE by reducing the efficiency of apoptosis [41]. On the other hand, the rs17878362 A2 allele is associated with lower levels of p53 transcription [42]. Thus, the suggested combined effect of *TP53* polymorphisms could be explained by a lower expression of the p53 protein and the presence of the p53 Pro variant, which is less effective in apoptosis process.

On the other hand, *TP53* rs17878362 A1A1-*p21* rs1801270 CA (OR 0.45) and *TP53* rs17878362 A1A2-*p21* rs1801270 CC (OR 0.28) combined genotypes showed protective effects on SLE development in the entire study sample and in the subgroup of non-white women, respectively. Of note, the allele *TP53* rs17878362 A2 has been associated with lower constitutive levels of p53 mRNA in lymphoblast cell lines, suggesting that this polymorphism may interfere in mRNA splicing, thus influencing transcript stability [13]. A consequence of that includes changes in the p53 protein activity, which may negatively alter its response to apoptosis activation, cell cycle arrest, and DNA repair, all recognized as important mechanisms for the development of SLE. Regarding the *p21* rs1801270 (C>A) polymorphism, previous studies reported that both p21 variants exhibit similar activity in relation to CDK inhibition and tumor suppressor activity, although A allele (Arg) seems to be associated with a decrease in mRNA expression [43]. In addition, *p21* deficiency proved to be associated with loss of immune tolerance in mice [44]. Taken together, the protection against SLE attributed to these genotype combinations cannot be explained by the combined influence of the individual genotypes on transcript levels, and/or levels and functions of proteins. However, it is important to emphasize that both p53 and p21 proteins may play different roles, with p21 not always acting as a downstream signaling component of p53 [45] and are involved in several pathways [46] that could be responsible for the observed effect.

In this study, a statistically significant difference was also observed for distribution of the *TP53* haplotypes between white SLE patients and controls, with the haplotype rs17878362 A2–rs1042522 C being associated with risk of SLE development (OR 9.67) (Table 4). This estimated risk effect is higher than the effects observed for the individual alleles rs1042522 C (OR 2.23) and rs17878362 (OR 7.00) and corroborate the risk of SLE associated with the rs1042522 GC/rs17878362 A1A2 combined genotype. To determine if the polymorphism are *in* cis or *in* trans other methods, such as NGS (Next Generation Sequencing) or genotyping of mother–father–child trios followed by robust bioinformatics tools have to be used [47, 48]. We believe that our findings may pave the way for further research in this area.

In this work, the relationship between the studied polymorphisms and clinical characteristics of SLE patients was also observed. The SNP *TP53* rs1042522 (G>C) was associated with risk for both serositis and neuropsychiatric manifestations, while the *TP53* rs17878362 (16 bp Del/Ins) polymorphism was associated only with neuropsychiatric disorders. Serositis is a common manifestation related to lupus, and its pathogenesis is still not well described. It has been proposed that the appearance of serositis may be associated with an inflammatory response resulting from an abnormal influx of Ca^{2+} [49]. Although increasing number of genetic associations with pathways involved in innate and adaptive immunity systems has been observed [50], more genetic studies concerning neuropsychiatric disorders are needed.

In conclusion, we found associations of the *TP53* (rs1042522 and rs17878362) and *p21* (rs1801270) polymorphisms, individually or in combination, with SLE development, particularly in skin color/ethnicity-stratified subgroups. In addition, interaction analysis revealed that *TP53* polymorphisms might be associated with serositis and neuropsychiatric disorders in

SLE patients. Our results suggest that ethnic variations of these polymorphisms should be considered in association studies for complex diseases, such as SLE, as widely shown by different authors [30, 31, 35, 51]. To our knowledge, this is the first study to investigate associations of *TP53* and *p21* gene polymorphisms with the development of SLE in a Brazilian population. Our findings may provide the basis for further studies on association of *TP53* and *p21* polymorphisms with the development of SLE in different and larger populations, since these polymorphisms may have potential to emerge as SLE susceptibility markers for specific groups of patients.

Acknowledgements
Not applicable.

Author contributions
JMBM conceived the study design, acquired financial support for the project, supervised the laboratory work and the statistical analysis, discussed the results, and participated in the manuscript preparation and revision. ALS carried out the analysis of p21 polymorphism, performed the statistical analysis, and participated in the discussion of results and manuscript preparation. ACP carried out the DNA extraction from peripheral blood samples and DNA sequencing and participated in the manuscript preparation. LFLL carried out the analysis of TP53 polymorphisms. EAP carried out the analysis of TP53 and p21 polymorphisms. CBS-R conceived the study design, carried out the analysis of TP53 and p21 polymorphisms and participated in the manuscript preparation and revision. EMK acquired financial support for the project, participated in the clinical patient selection and clinical data analysis and participated in the manuscript preparation and revision. All authors read and approved the final manuscript.

Funding
CNPq—National Council for Scientific and Technological Development (# 476116/2009-0). FAPERJ—Carlos Chagas Filho Foundation for Research Support of the State of Rio de Janeiro (# E-26/170.922/2004 and # E-26/111.334/2011).

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
The Research Ethics Committee of the Pedro Ernesto University Hospital approved the projects that involved the selection of members of both groups (#321 and #909). All participants signed an informed consent form agreeing to participate in the study.

Consent for publication
All participants signed an informed consent form, and they were told that the results would be used for education and publishing purposes, but their names would not be revealed.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Biochemistry, State University of Rio de Janeiro - UERJ, Rio de Janeiro, Brazil. [2]Department of Pathological Anatomy, State University of Rio de Janeiro - UERJ, Rio de Janeiro, Brazil. [3]Department of Pathology, Fernandes Figueira Institute - FIOCRUZ, Rio de Janeiro, Brazil. [4]Department of Rheumatology, Pedro Ernesto University Hospital, State University of Rio de Janeiro - UERJ, Boulevard 28 de Setembro, 87, Vila Isabel, Rio de Janeiro, RJ CEP 20551-030, Brazil.

Received: 8 March 2023 Accepted: 3 August 2023
Published online: 21 August 2023

References

1. Balomenos D. Cell cycle regulation and systemic lupus erythematosus. In: Lahita RG, editor. Systemic lupus erythematosus. 5th ed. London: Academic Press; 2011. p. 191–8. https://doi.org/10.1016/B978-0-12-374994-9.10011-7.
2. Sharabi A, Tsokos GC. T cell metabolism: new insights in systemic lupus erythematosus pathogenesis and therapy. Nat Rev Rheumatol. 2020;16:100–12. https://doi.org/10.1038/s41584-019-0356-x.
3. Pan L, Lu MP, Wang JH, Xu M, Yang SR. Immunological pathogenesis and treatment of systemic lupus erythematosus. World J Pediatr. 2020;16:19–30. https://doi.org/10.1007/s12519-019-00229-3.
4. Gupta S, Kaplan MJ. The role of neutrophils and NETosis in autoimmune and renal diseases. Nat Rev Nephrol. 2016;12:402–13. https://doi.org/10.1038/nrneph.2016.71.
5. Gaipl US, Munoz LE, Grossmayer G, Lauber K, Franz S, Sarter K, et al. Clearance deficiency and systemic lupus erythematosus (SLE). J Autoimmun. 2007;28:114–21. https://doi.org/10.1016/j.jaut.2007.02.005.
6. Zhao Y, Wei W, Liu ML. Extracellular vesicles and lupus nephritis—new insights into pathophysiology and clinical implications. J Autoimmun. 2020;115:102540. https://doi.org/10.1016/j.jaut.2020.102540.
7. Takatori H, Kawashima H, Suzuki K, Nakajima H. Role of p53 in systemic autoimmune diseases. Crit Rev Immunol. 2014;34:509–16. https://doi.org/10.1615/CritRevImmunol.2014012193.
8. Kovacs B, Patel A, Hershey JN, Dennis GJ, Kirschfink M, Tsokos GC. Antibodies against p53 in sera from patients with systemic lupus erythematosus and other rheumatic diseases. Arthritis Rheum. 1997;40:980–2. https://doi.org/10.1002/art.1780400531.
9. Joruiz SM, Bourdon JC. p53 isoforms: key regulators of the cell fate decision. Cold Spring Harb Perspect Med. 2016;6:a026039. https://doi.org/10.1101/cshperspect.a026039.
10. Bouaoun L, Sonkin D, Ardin M, Hollstein M, Byrnes G, Zavadil J, et al. TP53 variations in human cancers: new lessons from the IARC TP53 database and genomics data. Hum Mutat. 2016;37:865–76. https://doi.org/10.1002/humu.23035.
11. Thomas M, Kalita A, Labrecque S, Pim D, Banks L, Matlashewski G. Two polymorphic variants of wild-type p53 differ biochemically and biologically. Mol Cell Biol. 1999;19:1092–100. https://doi.org/10.1128/mcb.19.2.1092.
12. Senturk E, Manfredi JJ. p53 and cell cycle effects after DNA damage. Methods Mol Biol. 2013;962:49–61. https://doi.org/10.1007/978-1-62703-236-0_4.
13. Gemignani F, Moreno V, Landi S, Moullan N, Chabrier A, Gutiérrez-Enríquez S, et al. A TP53 polymorphism is associated with increased risk of colorectal cancer and with reduced levels of TP53 mRNA. Oncogene. 2004;23:1954–6. https://doi.org/10.1038/sj.onc.1207305.
14. Lawson BR, Baccala R, Song J, Croft M, Kono DH, Theofilopoulos AN. Deficiency of the cyclin kinase inhibitor p21(WAF-1/CIP-1) promotes apoptosis of activated/memory T cells and inhibits spontaneous systemic autoimmunity. J Exp Med. 2004;199:547–57. https://doi.org/10.1084/jem.20031685.
15. Kong EK, Chong WP, Wong WH, Lau CS, Chan TM, Ng PK, et al. p21 gene polymorphisms in systemic lupus erythematosus. Rheumatology (Oxford). 2007;46:220–6. https://doi.org/10.1093/rheumatology/kel210.
16. El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, et al. WAF1, a potential mediator of p53 tumor suppresion. Cell. 1993;75:817–25. https://doi.org/10.1016/0092-8674(93)90500-P.
17. Santiago-Raber ML, Lawson BR, Dummer W, Barnhouse M, Koundouris S, Wilson CB, et al. Role of cyclin kinase inhibitor p21 in systemic autoimmunity. J Immunol. 2001;67:4067–74. https://doi.org/10.4049/jimmunol.167.7.4067.
18. Li G, Liu Z, Sturgis EM, Shi Q, Chamberlain RM, Spitz MR, Wei Q. Genetic polymorphisms of p21 are associated with risk of squamous cell carcinoma of the head and neck. Carcinogenesis. 2005;26:1596–602. https://doi.org/10.1093/carcin/bgi105.
19. Yang J, Zhu JM, Wu S, Li J, Wang MR, Wang TT, Lu YW. Association study between the TP53 rs1042522G/C polymorphism and susceptibility to

systemic lupus erythematosus in a Chinese Han population. Rheumatol Int. 2017;37:523–9. https://doi.org/10.1007/s00296-017-3662-0.
20. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40:1725. https://doi.org/10.1002/art.1780400928.
21. Pena SD, Di Pietro G, Fuchshuber-Moraes M, Genro JP, Hutz MH, Kehdy FS, et al. The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. PLoS ONE. 2011;6:e17063. https://doi.org/10.1371/journal.pone.0017063.
22. Vargas-Torres SL, Portari EA, Klumb EM, Guillobel HCR, Camargo MJ, Russomano FB, et al. Association of CDKN2A polymorphisms with the severity of cervical neoplasia in a Brazilian population. Biomarkers. 2014;19:121–7. https://doi.org/10.3109/1354750X.2014.881419.
23. Vargas-Torres SL, Portari EA, Silva AL, Klumb EM, Guillobel HCR, Camargo MJ, et al. Roles of CDKN1A gene polymorphisms (rs1801270 and rs1059234) in the development of cervical neoplasia. Tumour Biol. 2016;37:10469–78. https://doi.org/10.1007/s13277-016-4850-3.
24. Iniesta R, Guinó E, Moreno V. Análisis estadístico de polimorfismos genéticos en estudios epidemiológicos. Gac Sanit. 2005;19:333–41. https://doi.org/10.1157/13078029.
25. Solé X, Guino E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. Bioinformatics. 2006;22:1928–9. https://doi.org/10.1093/bioinformatics/btl26.
26. Onel KB, Huo D, Hastings D, Fryer-Biggs J, Crow MK, Onel K. Lack of Association of the TP53 Arg72Pro SNP and the MDM2 SNP309 with systemic lupus erythematosus in Caucasian, African American, and Asian children and adults. Lupus. 2009;18:61–6. https://doi.org/10.1177/0961203308094558.
27. Lee YH, Rho YH, Choi SJ, Ji JD, Song GG. The functional p53 codon 72 polymorphism is associated with systemic lupus erythematosus. Lupus. 2005;14:842–5. https://doi.org/10.1191/0961203305lu2224oa.
28. Sánchez E, Sabio JM, Callejas JL, Ramón E, Haro M, Jiménez-Alonso J, et al. Study of a functional polymorphism in the p53 gene in systemic lupus erythematosus: lack of replication in a Spanish population. Lupus. 2006;15:658–61. https://doi.org/10.1177/0961203306070986.
29. Piotrowski P, Lianeri M, Mostowska M, Wudarski M, Chwalinska-Sadowska H, Jagodzinski PP. Contribution of polymorphism in codon 72 of p53 gene to systemic lupus erythematosus in Poland. Lupus. 2008;17:148–51. https://doi.org/10.1177/0961203307084722.
30. Lee Y, Bae SC, Choi SJ, Ji JD, Song GG. Associations between the p53 codon 72 polymorphisms and susceptibility to systemic lupus erythematosus and rheumatoid arthritis: a meta-analysis. Lupus. 2012;21:430–7. https://doi.org/10.1177/0961203311434941.
31. Barbosa FB, Cagnin NF, Simioni M, Farias AA, Torres FR, Molck MC, et al. Ancestry informative marker panel to estimate population stratification using genome-wide human array. Ann Hum Genet. 2017;81:225–33. https://doi.org/10.1111/ahg.12208.
32. Pereira FDSCF, Guimarães RM, Lucidi AR, Brum DG, Paiva CLA, Alvarenga RMP. A systematic literature review on the European, African and Amerindian genetic ancestry components on Brazilian health outcomes. Sci Rep. 2019;9:8874. https://doi.org/10.1038/s41598-019-45081-7.
33. Braun-Prado K, Petzl-Erler ML. Programmed cell death 1 gene (PDCD1) polymorphism and pemphigus foliaceus (fogo selvagem) disease susceptibility. Genet Mol Biol. 2007;30:314–21. https://doi.org/10.1590/S1415-47572007000300003.
34. Mazzone R, Zwergel C, Artico M, Taurone S, Ralli M, Greco A, Mai A. The emerging role of epigenetics in human autoimmune disorders. Clin Epigenet. 2019;11:34. https://doi.org/10.1186/s13148-019-0632-2.
35. Surace AEA, Hedrich CM. The role of epigenetics in autoimmune/inflammatory disease. Front Immunol. 2019;10:1525. https://doi.org/10.3389/fimmu.2019.01525.
36. Hu Z, Li X, Qu X, He Y, Ring BZ, Song E, Su L. Intron 3 16 bp duplication polymorphism of TP53 contributes to cancer susceptibility: a meta-analysis. Carcinogenesis. 2010;31:643–7. https://doi.org/10.1093/carcin/bgq018.
37. Sucheston L, Witonsky DB, Hastings D, Yildiz O, Clark VJ, Rienzo A, et al. Natural selection and functional genetic variation in the p53 pathway. Hum Mol Genet. 2011;20:1502–8. https://doi.org/10.1093/hmg/ddr028.
38. Liu N, Zhang K, Zhao H. Haplotype-association analysis. Adv Genet. 2008;60:335–405. https://doi.org/10.1016/S0065-2660(07)00414-2.
39. Chae BS, Shin TY. Immunoregulatory abnormalities of T cells and hyperreactivity of B cells in the in vitro immune response in pristane-induced lupus mice. Arch Pharm Res. 2007;30:191–8. https://doi.org/10.1007/BF02977694.
40. Tiller T, Tsuiji M, Yurasov S, Velinzon K, Nussenzweig MC, Wardemann H. Autoreactivity in human IgG memory B cells. Immunity. 2007;26:205–13. https://doi.org/10.1016/j.immuni.2007.01.009.
41. Sakamuro D, Sabbatini P, White E, Prendergast GC. The polyproline region of p53 is required to activate apoptosis but not growth arrest. Oncogene. 1997;15:887–98. https://doi.org/10.1038/sj.onc.1201263.
42. Park JS, Lim MA, Cho ML, Ryu JG, Moon YM, Jhun JY, et al. p53 controls autoimmune arthritis via STAT-mediated regulation of the Th17 cell/Treg cell balance in mice. Arthritis Rheum. 2013;65:949–59. https://doi.org/10.1002/art.37841.
43. Su L, Sai Y, Fan R, Thurston SW, Miller DP, Zhou W, et al. p53 (codon 72) and p21 (codon 31) polymorphisms alter in vivo mRNA expression of p21. Lung Cancer. 2003;40:259–66. https://doi.org/10.1016/S0169-5002(03)00081-3.
44. Arias CF, Ballesteros-Tato A, García MI, Martín-Caballero J, Flores JM, Martínez-A C, Balomenos D. p21 CIP1/WAF1 controls proliferation of activated/memory T cells and affects homeostasis and memory T cell responses. J Immunol. 2007;178:2296–306. https://doi.org/10.4049/jimmunol.178.4.2296.
45. Kim J, Bae S, An S, Park JK, Kim EM, Hwang SG, Kim WJ, Um HD. Cooperative actions of p21WAF1 and p53 induce slug protein degradation and suppress cell invasion. EMBO Rep. 2014;15:1062–8. https://doi.org/10.15252/embr.201438587.
46. Engeland K. Cell cycle regulation: p53–p21-RB signaling. Cell Death Differ. 2022;29:946–60. https://doi.org/10.1038/s41418-022-00988-z.
47. Dilernia D, Amin P, Flores J, Stecenko A, Sorscher E. Mutation profiling of the c.1521_1523delCTT (p.Phe508del, F508del) cystic fibrosis transmembrane conductance regulator allele using haplotype-resolved long-read next generation sequencing. Hum Mutat. 2022;43:595–603. https://doi.org/10.1002/humu.24352.
48. Wakita S, Hara M, Kitabatake Y, Kwatani K, Kurahashi, Hashizume R. Experimental method for haplotype phasing across the entire length of chromosome 21 in trisomy 21 cells using a chromosome elimination technique. J Hum Genet. 2022;67:565–72. https://doi.org/10.1038/s10038-022-01049-6.
49. Andrews BS, Arora NS, Shadforth MF, Goldberg SK, Davis JS 4th. The role of immune complexes in the pathogenesis of pleural effusions. Am Rev Respir Dis. 1981;124:115–20. https://doi.org/10.1164/arrd.1981.124.2.115.
50. Bentham J, Morris DL, Graham DSC, Pinder CL, Tombleson P, Behrens TW. Genetic association analyses implicate aberrant regulation of innate and adaptive immunity genes in the pathogenesis of systemic lupus erythematosus. Nat Genet. 2015;47:1457–64. https://doi.org/10.1038/ng.3434.
51. Sebastiani GD, Galeazzi M. Immunogenetic studies on systemic lupus erythematosus. Lupus. 2009;18:878–83. https://doi.org/10.1177/0961203309106918.

Publisher's Note