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Original article

Association of *PDCD1* polymorphism to systemic lupus erythematosus and rheumatoid arthritis susceptibility

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ABSTRACT

Objective: This study aims to analyze the relationship of programmed cell death 1 (*PDCD1*) gene polymorphism (*PD1.3G/A* – rs11568821) with features of systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) in a Southern Brazilian population.
Methods: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed in 95 SLE and 87 RA patients and 128 control group individuals from Santa Catarina, Southern Brazil. The Hardy–Weinberg equilibrium (HWE) test, and odds ratio (OR) were analyzed, considering CI 95% and $p \leq 0.05$.
Results: The *PD1.3A* allele frequencies were 0.095 (SLE), 0.115 (RA) and 0.078 (controls). The genotypes of the control group were in HWE, while those of SLE and RA patients were not. However, we found no association between *PD1.3* polymorphism and the SLE or RA susceptibility, nor clinical or epidemiological data.
Conclusion: There was no significant association between *PD1.3* polymorphism and SLE or RA susceptibility in this Southern Brazilian population.

Associação entre o polimorfismo do gene PDCD1 e a susceptibilidade ao lúpus eritematoso sistêmico e à artrite reumatoide

Palavras-chave:
Artrite reumatoide
Lúpus eritematoso sistêmico

RESUMO

Objetivo: Este estudo teve como objetivo analisar a relação entre o polimorfismo do gene *PDCD1* (Programmed cell death 1) (*PD1.3G/A* – rs11568821) com características do lúpus eritematoso sistêmico (LES) e da artrite reumatoide (AR) em uma população do sul do Brasil.

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Autoimunidade
Gene *PDCD1*
Polimorfismo *PD1.3*

Métodos: A técnica de PCR-RFLP (*Polymerase Chain Reaction-Restriction Fragment Lenght Polymorphism*) foi utilizada para analisar amostras de 95 pacientes com LES e 87 com AR e 128 indivíduos do grupo controle de Santa Catarina, sul do Brasil. Foi analisada a probabilidade de equilíbrio de Hardy–Weinberg (EHW) e o odds ratio (OR), considerando um IC 95% e $p \leq 0.05$.

Resultados: As frequências alélicas *PD1.3A* foram de 0,095 (LES), 0,115 (AR) e 0,078 (controles). Os genótipos do grupo controle estavam em EHW, enquanto aqueles dos pacientes com LES e AR não estavam. No entanto, não foi encontrada nenhuma associação entre o polimorfismo *PD1.3* e a susceptibilidade ao LES ou à AR, nem com dados clínicos ou epidemiológicos.

Conclusão: Não foi encontrada associação significativa entre o polimorfismo *PD1.3* e a susceptibilidade ao LES ou à AR nesta população do sul do Brasil.

Introduction

Autoimmune rheumatic diseases share clinical findings and are caused by multiple factors including a complex genetic basis coupled with non-genetic factors, which contribute in different degrees for each affected individual.[1] Genetic polymorphisms of the human genome have been investigated and new evidence of genetic contribution to rheumatic diseases has been discovered. Among autoimmune diseases, systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), have been the main targets of genetic variation studies, once they represent multisystem disorders with a wide spectrum of clinical manifestations.[1,2]

SLE affects mainly women of reproductive age, and its major characteristic is the production of autoantibodies against nuclear antigens, as double stranded DNA (dsDNA), ribonucleoproteins (RNP) and Smith (Sm) antigen; as well as cytoplasmic, and cell-surface antigens.[3] These autoantibodies deposit on several organs causing inflammation and leading to symptoms that could range from subtle to life-threatening. Constitutional manifestations including fever, fatigue and weight loss may occur, as well as joint pain due to arthritis, malar and discoid rashes, photosensitivity, and involvement of the central and peripheral nervous system, kidneys, heart and lungs.[1] Progression of the disease is individual and heterogeneous, so different biomarkers have been sought in order to unveil disease susceptibility and development as well as to guide therapeutic decisions.[4,5]

RA pathogenesis is complex and results in chronic inflammation of joints and, in many patients, systemic complications, such as subcutaneous nodules, pulmonary involvement and early atherosclerosis, that may be challenging regarding treatment.[6] In order to come to better prognosis and outcomes in RA, the development of biomarkers that allow disease sub-categorization are needed.[7] So far, serologic factors such as rheumatoid factor (RF) and anti-citrullinated protein autoantibodies (ACPA); and the acute inflammation marker C-reactive protein (CRP), have helped classifying RA clinical phenotypes.[8–11] Rheumatoid factor is an autoantibody directed against the Fc portion of IgG, and correlates with the severity of the disease[12]; whereas the ACPA are directed against citrullinated proteins, and can also help to predict a more severe and erosive disease.[13]

Although the etiology of SLE and RA are not well established, it is hypothesized that deregulated lymphocyte activation play an important role in the breakdown of immune tolerance, leading to autoreactivity.[2,14] Involved in this processes, co-stimulatory molecules are critical for the balance between T cell activation and inhibition.[15] Among those, the programmed cell death 1 (PD-1) is shown to be an important molecule involved in profound loss of self-tolerance leading to rapid lethality associated with lymphocyte infiltration in many organs.[16] This protein is expressed on the surface of T, B and myeloid cells, and is a member of the CD28 family that belongs to the immunoglobulin superfamily and acts as an inhibitory molecule on T cells, after interacting with its ligands PDL-1 and PDL-2 (programmed cell death 1 ligand 1 and 2).[17] After initial activation of T cell interactions, PD-1-PD-L may limit autoreactive T cell proliferation and cytokine production, whereas stimulated by antigens the PD-1 dampens T cell receptor (TCR) signaling. The amount of expression of PD-1 and the degree of involvement between this protein and its ligands regulate the threshold of T cell activation and the amount of cytokines produced.[18,19] PD-1-deficient mice develop spontaneous autoimmune diseases, indicating an essential function of PD-1 in the mechanisms of tolerance.[20–23]

PD-1 is encoded by the *PDCD1* gene, located at 2q37.3 *locus*. Among the SNPs found within this region, the *PD1.3G/A* (rs11568821) potentially represents a functional polymorphism associated with the transcriptional regulation of PD-1.[24] The *PD1.3A* allele alters the binding site of RUNX1 (or AML1) transcription factor, located on the intron 4 enhancer region, which could lead to aberrant protein expression, suggesting a mechanism for the self-tolerance breakdown.[25,26] Association studies correlated the presence of the allele *PD1.3A* with SLE in Mexican and Scandinavian populations,[25] and with diabetes mellitus 1 and RA in Denmark and Sweden respectively.[26,27] However, some populations in Asia are non-polymorphic for this genomic region, presenting only the *PD1.3G* allele,[28–30] which emphasizes the diversity of allelic frequency among populations, and supports the necessity to study the association of this *PDCD1* polymorphism in other localities. In Brazil, three studies have evaluated the frequencies of *PD1.3* polymorphism. One in patients with pemphigus foliaceus (also an autoimmune disease),[31] in silica-exposed workers,[32] and in a cohort of patients with Chagas disease,[33] demonstrating the presence of both alleles in this population.

Table 1 – Characteristics of systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) patients and controls from Southern Brazil.

	SLE patients *n*	RA patients *n*	Controls *n*
Female (%)	92 (96.84)	75 (86.21)	123 (96.09)
Mean age (SD)	37,35 (±12,16)	54.42 (±13.33)	47,38 (±15.04)

SD, standard deviation.

Hence, through this study we intended to evaluate the frequency of *PD1.3* polymorphism in a Southern Brazilian population and its relationship to SLE and RA susceptibility.

Methods

Altogether, 95 SLE patients, 87 RA patients and 128 control subjects participated on this study, which was approved by the Committee on Ethics of the Federal University of Santa Catarina (UFSC) (CEP/UFSC – case number 172/06), after informed consent was obtained from all patients and controls subjects. Women made up 96.84% of SLE patients, 86.10% of RA patients and 96.09% of controls. The mean age of SLE patients was 37.35 ± 12.16 years, of RA patients was 54.42 ± 13.33 years and of control group was 47.38 ± 15.04 years (Table 1). Patients were admitted at the Hospital Universitário Professor Polydoro Ernani de São Thiago, Florianópolis, Brazil, from 2007 to 2009, and diagnosed according to the 1987 American College of Rheumatology criteria. The control group was composed of healthy volunteers without personal or family history of autoimmune diseases. Familial, epidemiological and clinical data from individuals were obtained by questionnaires and medical records. Regarding clinical data, we evaluated SLE patient's medical chart records of arthritis, photo-sensibility, Raynaud's phenomenon, and nephritis, which were the recurrent clinical manifestation in this group. For RA patients, we considered rheumatoid factor (RF) positivity (>20 IU/ml), and levels of C-reactive protein (CRP) above the reference value (>5 mg/l) as the laboratorial manifestations to be associated with the alleles.

Whole blood samples were obtained from SLE and RA patients and from control subjects. The DNA was extracted using the phenol–chloroform technique.[34] The *PD1.3A* (*PDCD1*) allele was detected by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).[35] The PCR product of 180 bp was digested by *PstI* restriction endonuclease (BioLabs Inc., New England), according to the manufacturer instructions. All experiments were performed with negative and positive internal controls previously genotyped at Laboratório de Genética Molecular Humana – UFPR. The product of digestion was stained with GelRed® solution and subjected to electrophoresis on a 3% agarose gel. DNA was visualized with a photographed gel documentation system (MiniBISPro DNR). The genotype was classified according to the size of the generated fragments (*GG* – 180 bp; *GA* – 180 bp, 150 bp and 30 bp; *AA* – 150 bp and 30 bp).

Hardy–Weinberg equilibrium (HWE) was tested using the χ^2 test. Allele and genotype frequencies were estimated by direct counting. Allele and genotype frequencies were compared between patients and controls by Fisher exact test using SPSS (version 20.0; SPSS Inc., Chicago, IL), which was also used to calculate the *odds ratio* (OR) in order to determine the association of the *PD1.3A* allele and the studied diseases, as well as its association to poor prognosis factors of SLE and RA patients. A *p* value of 0.05 was adopted as the limit of significance for all tests.

Results

The allele and genotype frequencies of *PD1.3* polymorphisms found among the groups are shown in Table 2. The genotype distribution in the control group was in HWE ($\chi^2_{(1)} = 2.24$, $p = 0.13$), but the distributions observed in SLE and RA patients were not ($\chi^2_{(1)} = 6.60$, $p = 0.01$ for SLE and $\chi^2_{(1)} = 9.02$, $p < 0.001$ for RA). Nevertheless, no association was found regarding the alleles or genotypes and both diseases ($p > 0.05$) (Table 2).

Among SLE patients, 49.5% had arthritis, 40.0% complained of photosensitivity, 15.8% experienced the Raynaud's phenomenon and 30.5% presented renal impairment. Association between these clinical factors and *PD1.3* alleles and genotypes were analyzed, but no significant results were found (Table 3).

Less than half of RA patients (46.15%) presented high level of CRP, and 66.67% had RF positivity. However, those factors were not associated with the presence of *PD1.3A* allele either in homozygosis or heterozygosis (Table 3).

Table 2 – Allele and genotype frequencies of *PD1.3* polymorphism observed in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) patients and controls. Hardy–Weinberg equilibrium (HWE) values for genotypic distribution, and association analysis between the diseases and the *PD1.3* (G/A) polymorphism in samples of patients and unaffected control subjects of the Brazilian population were calculated.

	SLE *n* = 95	*p* value	RA *n* = 87	*p* value	Controls *n* = 128
Allele G	0.905	(Ref.)	0.885	(Ref.)	0.922
Allele A	0.095	0.661	0.115	0.364	0.078
GG	0.842	(Ref.)	0.816	(Ref.)	0.859
GA	0.126	0.940	0.138	0.715	0.125
AA	0.032	0.755	0.046	0.206	0.016
HWE (*p* value)	$\chi^2_{(1)} = 6.60$ (0.010)		$\chi^2_{(1)} = 9.02$ (<0.001)		$\chi^2_{(1)} = 2.24$ (0.130)

χ^2 (chi-square value), $p < 0.05$ was not considered in HWE.

Table 3 – Clinical features present in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) patients, and their association to *PD1.3* polymorphism.

	Affected SLE patients *n* (%)	Associated *p* value		
		Allele (A)	AA	AA + AG
Arthritis	47 (49.5)	0.963	0.553	0.752
Photosensitivity	38 (40)	0.312	0.361	0.294
Raynaud's phenomenon	14 (15.8)	0.570	0.827	0.777
Renal impairment	29 (30.5)	0.786	0.440	0.388
	Affected RA patients *n* (%)	Associated *p* value		
		Allele (A)	AA	AA + AG
RF	50 (66.67)	0.122	0.291	0.302
CRP	36 (46.15)	0.910	0.874	0.965

RF, rheumatoid factor; CRP, C-reactive protein.

Discussion

For the first time in Brazil, *PDCD1* gene was considered a candidate for susceptibility to systemic lupus erythematosus (SLE) and to rheumatoid arthritis (RA). Associations between the SNP *PD1.3A* and clinical and laboratorial manifestations were tested, finding no statistically significant results.

Once genotype frequencies of SLE and RA patients were not in Hardy–Weinberg equilibrium (Table 3), diverging from the control group, a putative association with these diseases was investigated. The frequency of *AA* genotype was very low in all groups (0.032 in SLE, 0.046 in RA and 0.016 in controls) as shown by other case–control studies in different populations, where *AA* frequencies were all below 0.05.[31,33,35–44] One Iranian cohort, however, has shown *AA* genotype frequencies of 0.20 in controls and 0.44 in patients with Colorectal Cancer (CCR), revealing as well, an association between this genotype and CCR ($p = 0.0005$).[45] In Brazil, the frequency of the *AA* genotype was also low according to three different cohorts involving patients with Chagas disease (0.03 and 0.01 in controls),[33] pemphigus foliaceus (0.007 and 0.01 in controls),[31] and reaching 0% in a group of silica-exposed workers (0.03 in controls).[32] Interestingly, some populations carry the minor allele, but no homozygous description was found, presenting only *GG* and *AG* individuals. Yet, the analyzed genotypes showed no statistically significant OR value when considering the risk of developing SLE or RA in our study.[35,46–48]

Associations of the *PD1.3A* allele to disease development have been increasingly investigated, not only in SLE and RA patients,[25,26,28,29,35–37,46,49–55] but also in other autoimmune and chronic inflammatory diseases such as Diabetes Mellitus type I (T1D),[27,38,42] Graves and Addison disease,[39] Ankylosing spondylitis,[44] and Myasthenia Gravis,[41] as well as in other conditions as CCR,[45] and silica exposed workers (Table 4).[32] The *PD1.3A* allele frequency highly differs among populations worldwide, three Chinese studies related to RA and Vogt–Koyanagi–Harada Syndrome found no allele variation in their population,[28,30,55] which was also observed in Japan in a similar study.[29] European studies show the presence of the *PD1.3* polymorphism, yet association to diseases varies among studies. An association of *PD1.3A* to SLE was demonstrated by Prokunina et al.[25,26] in European women and Mexicans, and by Ferreiros-Vidal et al.[51] in SLE patients from Germany, Czech Republic and Hungary. Nevertheless, Ferreiros-Vidal et al. have previously shown a reversal of patterns in a Spanish cohort, with decreased risk of SLE development in *PD1.3A* carriers.[50] RA patients were also genotyped for *PDCD1* rs11568821 polymorphism by few groups,[28,29,36,55] and as mentioned previously, Chinese and Japanese cohorts carried solely the *PD1.3G* allele. A Swedish study was the only one to present data on RA and rs11568821 polymorphism, but association between the disease and allele or genotypes was not demonstrated.[36] Nonetheless, the same group showed an association of *PD1.3A* and RA patients negative for RF. In the present study we could not find any association between RF positivity or high levels of CRP and the alleles or genotypes investigated.

SLE patients were also inquired about clinical manifestations as arthritis, photosensitivity, Raynaud's phenomenon, and renal involvement. However, we found no association between *PD1.3* alleles or genotypes and these manifestations. Thorburn et al.[53] evaluated the role of four *PDCD1* SNPs (*PD1.1A*, *PD1.3A*, *PD1.5T* and *PD1.6A*) and SLE nephritis, arthritis, antiphospholipid antibody (APA), and double-stranded DNA positivity, finding no association of *PD1.3A* allele and its associated haplotype and any clinical phenotype. The occurrence of APA in SLE patients was also analyzed by Sanghera et al.[35] unraveling a protection of *PD1.3A* carriers against APA in both SLE (OR = 0.57; 95% CI: 0.32–1.01) and controls (OR = 0.40; 95% CI: 0.19–0.82). Prokunina et al.,[26] Johansson et al.,[37] and Nielsen et al.[49] evaluated renal manifestations in SLE patients. The first and second studies showed an association between the *PD1.3A* allele and renal disorders in patients with SLE from Sweden (OR = 2.6; 95% CI: 1.4–4.8, and OR = 2.62; 95% CI: 1.28–5.35, respectively); and the third one did not find an association of lupus nephropathy and the minor allele of *PD1.3*. As for RA, neither RF nor CRP was associated to *PD1.3A* allele presence either in homozygosis or heterozygosis, as opposed to what was found by Prokunina et al.[26]

Although studies show the *PD1.3* G/A polymorphism associated with RA, SLE and other autoimmune diseases and their clinical manifestations, our study revealed no differences in minor allele carries between RA and SLE patients and controls neither disease manifestations.

Table 4 – *PD1.3A* (rs11568821) allele frequencies found by different studies.

Population	Studied disease	*PD1.3A* frequencies %		OR (95% CI)	Reference
		Controls	Patients		
Swedish	SLE	8.0	11.0	1.44 (0.93–2.23)	Prokunina et al., 2002
Mexican	SLE	2.0	7.0	3.23 (1.46–7.16)[b]	Prokunina et al., 2002
Danish	T1D	6.8	12.2	1.92 (1.1–3.3)[b]	Nielsen et al., 2003
Spanish	SLE	12.9	9.4	0.70 (0.54–0.90)[b]	Ferreiros-Vidal et al., 2004
Danish	SLE	6.8	11.6	1.80 (0.96–3.4)	Nielsen et al., 2004
European (women)	SLE	7.0	11.0	1.60 (1.17–2.18)[b]	Prokunina et al., 2004a
Swedish	RA	7.3	8.5	1.18 (0.99–1.4)	Prokunina et al., 2004b
African American (women)	SLE	10.0	5.0	2.19 (0.54–8.85)	Sanghera et al., 2004
European American (women)	SLE	11.0	13.0	1.23 (0.87–1.73)	Sanghera et al., 2004
North Swedish	SLE	5.6	7.3	1.35 (0.90–2.02)	Johansson et al., 2005
Hong Kong Chinese	RA	0	0	NA	Kong et al., 2005
Finnish	SLE	6.0	3.0	0.50 (0.20–1.26)	Sigurdsson et al., 2005
Swedish	SLE	9.0	9.0	1.00 (0.68–1.45)	Sigurdsson et al., 2005
Mato Grosso do Sul – Brazilian	Pemphigus Foliaceus	9.6	5.8	0.58 (0.33–1.03)	Braun-Prado and Petzel-Erler 2007
CEU[a]	SLE	8.1	17.1	2.35 (1.1–4.9)[b]	Ferreiros-Vidal et al., 2007
Italian[a]	SLE	10.7	18.7	1.92 (0.9–3.9)	Ferreiros-Vidal et al., 2007
Greek	SLE	10.6	12.7	1.22 (0.8–1.8)	Ferreiros-Vidal et al., 2007
Japanese	RA	0	0	NA	Iwamoto et al., 2007
British	Graves' disease	88.4	89.7	1.13 (0.85–1.50)	Sutherland et al., 2007
American	SLE	4.7	7.2	1.59 (0.78–3.23)	Thorburn et al., 2007
Mexican	Childhood-onset SLE	2.0	5.2	2.73 (1.35–5.56)	Velázquez-Cruz et al., 2007
American	Primary biliary cirrhosis	11.7	12.5	1.08 (0.74–1.57)	Juran et al., 2008
Swedish	Autoimmune HMG	8.5	10.8	1.32 (0.74–2.35)	Sakthivel et al., 2008
French	CMV infection after kidney transplantation	12.9	12.8	1.38 (0.90–2.11)	Hoffmann et al., 2009
Han Chinese	Vogt–Koyanagi–Harada syndrome	0	0	NA	Meng et al., 2009
Caucasian Polish	T1D	12.9	13.5	1.05 (0.71–1.55)	Fichna et al., 2010
Iranian	Ankylosing spondylitis	11.2	9.3	0.81 (0.48–1.34)	Soleimanifar et al., 2010
Mexican	Hypersensitivity pneumonitis	3.8	6.1	1.65 (0.59–4.75)	Zúñiga et al., 2010
Caucasian Polish	Chronic urticaria	10.0	10.0	0.97 (0.51–1.86)	Brzoza et al., 2012
Brazilian	Silica-exposed workers	13.3	4.3	0.29 (0.10–0.88)[b]	Rocha et al., 2012
African American	SLE	2.0	2.6	1.31 (0.94–1.81)	Sanchez et al., 2012
Brazilian	Chagas disease	10.0	9.0	0.90 (0.61–1.33)	Dias et al., 2013
German	Liver transplant recipients	12.5	10.0	0.78 (0.46–1.33)	Thude et al., 2013
Iranian	Colorectal cancer	40.5	60.6	2.27 (1.50–3.44)[b]	Yousefi et al., 2013
Han Chinese	RA	0	0	NA	Liu et al., 2014

OR, odds ratio; CI, confidence interval; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; T1D, type 1 diabetes; NA, not available; HMG, human myasthenia gravis.

[a] Collections of samples from Germany, the Czech R. and Hungary were grouped as CEU; collections from Milan, Rome and Naples were grouped as Italy; collections from Greece were considered by their own (as presented by Ferreiros-Vidal et al.[51]).

[b] $p < 0.05$ statistically significant.

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Conflicts of interest

The authors declare no conflicts of interest.

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