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Research Article

Programmed cell death 1 gene (*PDCD1*) polymorphism and pemphigus foliaceus (fogo selvagem) disease susceptibility

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Abstract

Pemphigus foliaceus, also known as *fogo selvagem*, is an autoimmune disease of the epidermis characterized by superficial blisters and antibodies against desmoglein 1. It is a multifactorial disease and genetic susceptibility is oligogenic or polygenic. Considering the crucial function of the programmed cell death 1 molecule (PD-1) in the immune response, the aim of this study was to verify if variants of the *PDCD1* gene influence susceptibility and resistance to pemphigus foliaceus, in a case - control disease association study. We analyzed patients (n = 154) and unaffected control individuals (n = 325) of the Brazilian population, in respect to the *PD1.3(G,A) PD1.5(C,T)* and *PD1.6(A,G)* single nucleotide polymorphisms (SNPs) and also investigated, for the first time, the exon 5 *PDCD1* microsatellite $(CTG)_n$. The patient and control samples were divided into strata, according to the predominant ancestry of the individuals (African or European). The *PD1.5* genotype distribution in the patients sample was almost indistinguishable from that in the control sample, in both population strata. A possible negative association between pemphigus foliaceus and allele *PD1.3A* was observed in the total African and European ancestry population sample (odds ratio (OR) = 0.55, p = 0.066) and should be investigated in forthcoming studies. The *PD1.6A* allele was over-represented among the patients of predominantly European ancestry due to an increase of both the *G/A* and the *A/A* genotypes (OR = 2.12 and 1.74, respectively; p = 0.035). We conclude that polymorphisms of the *PDCD1* gene may influence susceptibility to pemphigus foliaceus, at least in Brazilians of predominantly European ancestry.

Key words: genetic association, PD-1, *PDCD1*, genetic polymorphism, pemphigus.

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Introduction

Pemphigus foliaceus is an epidermal autoimmune disease, characterized by autoantibodies against a cellular adhesion molecule, desmoglein 1, expressed in keratinocytes (Anhalt *et al.*, 1990). This complex multifactorial disease is endemic in Brazil, with inter-individual differences in susceptibility being due to multiple factors, which include variants of several genes. According to the polygenic model of disease susceptibility, the presence of any of the susceptibility alleles is neither sufficient nor required for the disease to occur. The strongest associations thus far described are those with HLA class II alleles, known since 1989 and well established (Petzl-Erler and Santamaria, 1989; Moraes *et al.*, 1991; Pavoni *et al.*, 2003; VMMS Roxo, personal communication). Other candidate genes have also been analyzed in pemphigus foliaceus association studies, including genes of several cytokines and cytokine receptors (*IL1A, IL1B, IL1R, IL4, IL4R, IL6, IL10, TNF, LTA*) (Pereira *et al.*, 2004; Roxo *et al.*, 2003), *TP53* and *BAX*, whose products participate in apoptosis (Köhler and Petzl-Erler, 2006) and *DSG1*, which encodes desmoglein 1, the major autoantigen in pemphigus foliaceus (Petzl-Erler and Malheiros, 2005).

The programmed cell death 1 (PD-1) molecule belongs to the CD28 family of receptors and is expressed by activated T, B and myeloid cells. PD-1 is postulated to play an important role in induction and/or maintenance of peripheral tolerance. Upon interaction with its ligands (PD-L1 and PD-L2), PD-1 inhibits antigen stimulation of T and B cells (Sharpe and Freeman, 2002). The PD-Ls belong to the B7 family and may be expressed on both lymphoid and non-lymphoid organs. The balance of stimulatory and inhibitory signals provided by cell-surface interactions between lymphocytes and antigen presenting cells or target cells, provided by co-stimulatory and inhibitory molecules, is crucial for maintaining peripheral tolerance, while retaining a protective effect against foreign antigens. Disruption of this delicate balance could lead to breakdown of self-tolerance and might be involved in the pathogenesis of autoimmune diseases (Okazaki and Honjo, 2006).

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The function of PD-1 as a regulator of peripheral tolerance was further supported by the observation that PD-1 deficient mice developed autoimmune diseases, despite having distinct phenotypes on different genetic backgrounds (Nishimura *et al.*,1998; Nishimura *et al.*, 2001). Indeed, PD-1$^{-/-}$ mice on the C57BL/6 background show spontaneous development of a lupus-like syndrome, including glomerulonephritis and destructive arthritis (Nishimura *et al.*, 1999). PD-1$^{-/-}$ mice on a BALB/c background show development of dilated cardiomyopathy (Nishimura *et al.*, 2001), which appears to be mediated by autoantibodies to a cardiac protein and deposition of IgG on the surface of cardiomyocytes (Okazaki *et al.*, 2002). These findings support the notion that PD-1 might suppress immune activation and prevent autoimmune diseases (Okazaki and Honjo, 2006).

The human gene for PD-1, *PDCD1*, is localized on chromosome 2q37. Various single nucleotide polymorphisms (SNPs) in the *PDCD1* gene have been identified (Prokunina *et al.*, 2002) and some associations have been reported with autoimmune disorders such as systemic lupus erythematosus (SLE), rheumatoid arthritis and type I diabetes (Prokunina *et al.*, 2002; Nielsen *et al.*, 2003; Prokunina *et al.*, 2004). An intronic SNP, *PD1.3* (*A*,*T*) has been associated with SLE, with an analysis of familial and sporadic cases of SLE having found consistent over-representation of the *A* allele among American SLE patients of European ancestry and Mexican and Swedish SLE patients. The authors suggested that the associated *A* allele could interfere with the binding site of the RUNX1 transcription factor and thus affect expression of the *PDCD1* gene (Prokunina *et al.*, 2002). In addition, allele *T* of another SNP, named *PD1.5* (*C*,*T*), was observed to be associated with the development of rheumatoid arthritis but not SLE in Chinese patients living in Taiwan (Lin *et al.*, 2004).

To test the hypothesis that polymorphisms of the *PDCD1* gene influence susceptibility to pemphigus foliaceus, we performed a case-control association study. We analyzed three SNPs of the *PDCD1* gene: *PD1.3(G,A)* in nucleotide position 7146 of intron 4; *PD1.5(C,T)* in nucleotide 7785 of exon 5; and *PD1.6(A,G)* in nucleotide 8737 of the 3' untranslated region (UTR). We also investigated the exon 5 $(CTG)_n$ microsatellite polymorphism.

Material and Methods

Population sample

The sample consisted of 154 patients (76 women and 78 men, age range 6 to 88 years) with pemphigus foliaceus and 325 controls (176 women and 149 men, age range 12 to 86 years) without any history of pemphigus foliaceus. Blood samples were collected from patients at Hospital Adventista do Pênfigo, Campo Grande municipality, Mato Grosso do Sul (MS) state, Brazil. Diagnosis of pemphigus foliaceus was based on clinical criteria, histopathology and indirect immunofluorescence. Blood samples were also collected from unaffected control individuals in Campo Grande-MS and Curitiba (Paraná state, Brazil). The control sample was similar to the patient sample in respect to age, birthplace, residence, socio-economic status, sex ratio and occupation. The total population sample (patients plus controls) was of predominantly European and African ancestry and extensively admixed, but not panmictic. Therefore, we considered that the different population strata might differ regarding allelic frequencies. In view of the interest of association analysis in the sub-populations and the need of matching the patient and the control samples for ancestry, individuals were classified according to morphological characteristics, as a proxy to an estimate of the relative contribution of European and African ancestry to each individual's genome. On the basis of facial morphological features, hair type and skin color, individuals were grouped as of mixed African and European ancestry (AF: patients = 46.5%, controls = 53.5%) or of predominantly European ancestry (EU: patients = 26.1%, controls = 73.9%).

This approach to subdividing the population into strata according to ancestry and degree of admixture is validated by population genetics studies (Braun-Prado *et al.*, 2000; Probst *et al.*, 2000). They have shown that the distribution of alleles known to be restricted to populations from one continent follows a gradient among the strata of the Southern Brazilian population classified as in the present study, as expected if the classification discriminated among the population strata according to the relative contributions of the ancestral populations.

To verify the population frequencies of the *PDCD1* $(CTG)_n$ microsatellite, we analyzed a sample of 95 unaffected individuals of four population strata according to ancestry: 40 individuals of predominantly European ancestry (EU), 20 of Eastern Asian ancestry (EA), 20 of Amerindian ancestry (AM) and 15 of predominantly African ancestry (AF). The Amerindians are Guarani and the samples were collected in Rio das Cobras, Laranjeiras do Sul (Paraná state, Brazil) (Petzl-Erler *et al.*,.1993); the samples of the other groups were obtained in Curitiba (Paraná state, Brazil).

Individuals were made aware of the study aims and signed an informed consent document prior to participating in the project. The study has been approved by the Human Subjects Research Ethics Committee of the Federal University of Paraná, according to Brazilian Federal laws.

Genotyping

The three *PDCD1* SNPs have been previously described by Prokunina *et al.* (2002) and we have used the nomenclature suggested by these authors. The intron 4 nucleotide 7146 *PD1.3*(*G*,*A*) SNP was genotyped as described by Prokunina *et al.* (2002). To genotype the exon 5 nucleotide 7785 *PD1.5*(*C*,*T*) SNP and the 3' UTR nucleotide 8737 *PD1.6*(*A*,*G*) SNP we performed the polymerase

chain reaction followed by hybridization of sequence-specific oligonucleotide probes (PCR-SSOP) with the denatured PCR products immobilized on a nylon membrane (dot blot). Primers and probes were designed based on the sequence of the human *PDCD1* gene (GenBank accession *AF363458*) (Table 1). For PCR, a 20 µL reaction mixture was set up to contain 0.06 µg of genomic DNA, 1X PCR buffer, 6 pmoles of each primer, 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, and 0.5 units of Taq DNA polymerase (Invitrogen), and the DNA segment was amplified for 35 cycles, after initial denaturation at 95 °C for 5 min. Each cycle consisted of three steps: denaturation for 15 s at 95 °C, annealing for 30 s at 67 °C for *PD1.5* and 68 °C for *PD1.6*, and primer extension for 15 s at 72 °C. The PCR products (568 bp for *PD1.5* and 727 bp for *PD1.6*) were dot blotted, hybridized with digoxigenin-labeled probes, washed and the hybridized probes were detected using chemiluminescence according to previously described procedures (Bignon and Fernandez-Viña, 1997). Genotyping results for *PD1.5* were confirmed by the PCR-restriction fragment length polymorphism (PCR-RFLP) method using the *Pvu*II restriction enzyme (Fermentas) and the genotype of each individual was detected by fractionating the digest by size in a 4% agarose gel. The size of the resulting fragments were 491 bp and 77 bp for allele *PD1.5T*, with only the larger fragment being visualized in the gel. Microsatellite typing was performed for 95 individuals (15 AF, 20 AM, 40 EU and 20 EA). The PCR was carried out with 6 pmol of each the forward fluorescent (FAM-6) primer 5'ctacagggag ggccagatgca 3' and the reverse primer 5' tgctccaaggccatctc caac 3' in a total volume of 20 µL, containing 30 ng of genomic DNA, 1X PCR buffer, 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, and 0.5 units of Taq DNA polymerase (Invitrogen). The PCR conditions were initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 15 s. The PCR products were denatured for 3 min at 94 °C and separated by size on a MegaBACE 1000 capillary array electrophoresis system (Amersham/GE Healthcare). Electropherograms were generated using the Fragment Profiler software 1.2 (Amersham/GE Healthcare).

Statistical analysis

Genotypic frequencies, allelic frequencies, and allele carrier frequencies (*i.e.*, the frequency of individuals having the allele in either homozygosity or heterozygosity) were estimated by direct counting. Maximum likelihood estimates of haplotype frequencies were obtained using an expectation-maximization (EM) algorithm included in the program package ARLEQUIN 3.0 (Excoffier, Laval and Schneider, 2005). Lewontin's D' (relative linkage disequilibrium coefficient) between the SNPs was calculated using the ARLEQUIN 3.0 package. Hardy-Weinberg equilibrium was assessed using a modified version of the Markov-chain random walk algorithm, described by Guo and Thompson (1992) and included in the ARLEQUIN 3.0 program package.

For disease association analysis, genotypic frequencies, allelic frequencies and allele carrier frequencies were compared between the patient and control groups in the AF and EU population strata, by analysis of contingency tables using an exact test to estimate the probability (p) value via the metropolis algorithm and the RxC computer program (Miller, 1997). Using the same test, allelic frequencies were compared between the AF and EU population strata. If the two strata did not differ significantly, association analysis was also performed for the total population sample. The p value of 0.05 was adopted as the significance limit in all comparisons. Whenever the p value was lower than 0.1, odds ratios (OR) with 95% confidence intervals (CI) were calculated by Woolf's method.

Results

This is the first work investigating the *PDCD1* $(CTG)_n$ microsatellite. To describe the allele frequencies and to evaluate the utility of this microsatellite as a marker

Table 1 - *PDCD1* single nucleotide polymorphisms (SNPs) and typing methods used. The variants of the SNPs detected by the probes are highlighted in boldfaced type in the last column. Probes SND3CT and SND3GA anneal to monomorphic positions and were used as controls for the quality of the PCR and the dot blots.

SNP*		Location	Method and (restriction enzyme)	PCR oligonucleotides (5'-3')	Oligonucleotide-probes (5'-3')
PD1.3	*7146(G,A)*	Intron 4	PCR-RFLP (*Pst* I)‡	Forward: ccccaggcagcaacctcaat Reverse: gaccgcaggcaggcacatat	
PD1.5	*7785(C,T)*	Exon 5	PCR-SSOP and PCR-RFLP (*Pvu* II)	Forward: cctgagcagacggagtatgcc Reverse: tgctccaaggccatctccaac	SND1T: ggggctcagc**t**gacg SND2C: ctcagc**c**gacggccc SND3CT: gcagggtgcaggcca
PD1.6	*8737(G,A)*	3' UTR	PCR-SSOP	Forward: ctacagggagggccagatgca Reverse: agcccggccaacccctttaaa	SND1A: ccccccat**a**tgccca SND2G: ccat**g**tgcccaccc SND3GA: ccaggcagcacctca

*SNP nomenclature to Prokunina *et al.* (2002), DNA sequence accession number *AF363458*.
‡Prokunina *et al.* (2002).
UTR = untranslated region. PCR = polymerase chain reaction. RFLP = restriction fragment length polymorphism. SSOP = sequence-specific oligonucleotide probes.

for the disease association study, we analyzed a sample of the Brazilian population, stratified according to ancestry. The most frequent allele in all four ethnic groups was *197* (Table 2). The degree of polymorphism was low in the Amerindian, African and European strata but in the East Asian group only the *197* allele occurred. Because of the low degree of polymorphism, this marker was not used for the disease association analysis.

The genotypic frequency distributions of the three SNPs analyzed (*PD1.3*, *PD1.5* and *PD1.6*) were in Hardy-Weinberg's equilibrium (Tables 3 and 4).

Comparison of the allele frequencies between the AF and EU population strata revealed significant differences ($p < 0.05$) for the *PD1.6*(*G*,*A*) SNP and homogeneity ($p > 0.05$) for the *PD1.3*(*G*,*A*) and *PD1.5*(*C*,*T*) SNPs. Therefore, association analyses were performed also for the

Table 2 - Allelic frequencies (%) of the *PDCD1* $(CTG)_n$ microsatellite in four Brazilian population strata of different ancestry.

	Ancestry			
Alleles	European (n = 40)	East Asian (n = 20)	Amerindian (n = 20)	African (n = 15)
**176*	0.0	0.0	2.5	0.0
**197*	98.8	100.0	97.5	96.7
**200*	1.2	0.0	0.0	0.0
**206*	0.0	0.0	0.0	3.3

Table 3 - Genotype, allele and allele carrier frequencies (%) and association analysis between pemphigus foliaceus and the *PD1.3 7146(G,A)* and *PD1.5 7785(C,T)* single nucleotide polymorphisms (SNPs) in samples of patients and unaffected control subjects of the Brazilian population.

SNP	Total (European + African ancestry)			Predominant ancestry					
				European			African		
	Patients	Controls	p	Patients	Controls	p	Patients	Controls	p
PD1.3	(n = 138)	(n = 302)		(n = 75)	(n = 244)		(n = 63)	(n = 58)	
Genotype frequencies									
G/*G*	89.1	81.8	0.126	86.7	80.3	0.404	92.1	87.9	0.542
G/*A*	10.2	17.2		12.0	18.5		7.9	12.1	
A/*A*	0.7	1.0		1.3	1.2				
Allele frequencies									
G allele*	94.2	90.4	0.068	92.7	89.6	0.358	96.0	94.0	0.560
A allele†	5.8	9.6		7.3	10.4		4.0	6.0	
Allele carriage frequencies									
G allele positivity	99.3	99.0	1.000	98.7	98.8	1.000	100.0	100.0	1.000
A allele positivity‡	10.9	18.2	0.066	13.3	19.7	0.231	7.9	12.1	0.547
Hardy-Weinberg (p)	0.374	0.744		0.328	0.733		1.000	1.000	
PD1.5	(n = 131)	(n = 288)		(n = 76)	(n = 231)		(n = 55)	(n = 57)	
Genotype frequencies									
C/*C*	39.7	42.7	0.644	39.5	43.7	0.537	40.0	38.6	1.000
C/*T*	48.9	44.1		50.0	42.9		47.3	49.1	
T/*T*	11.4	13.2		10.5	13.4		12.7	12.3	
Allele frequencies									
C allele	64.1	64.8	0.818	64.5	65.2	0.921	63.6	63.2	1.000
T allele	35.9	35.2		35.5	34.8		36.4	36.8	
Allele carriage frequencies									
C allele positivity	88.5	86.8	0.643	89.5	86.6	0.693	87.3	87.7	1.000
T allele positivity	60.3	57.3	0.437	60.5	56.3	0.591	60.0	61.4	1.000
Hardy-Weinberg (p)	0.567	0.610		0.613	0.385		1.000	0.778	

*Odds ratio (OR) and 95% confidence interval (CI) for the total population (European + African ancestry) = 1.73 (0.97-3.06).
†Odds ratio and 95% CI for the total population (European + African ancestry) = 0.58 (0.33-1.03).
‡Odds ratio and 95% CI for the total population (European + African ancestry) = 0.55 (0.30-1.01).
p = probability.

Table 4 - Genotype, allele and allele carrier frequencies (%) and association analysis between pemphigus foliaceus and the *PD1.6 8737(G,A)* single nucleotide polymorphism (SNP) in samples of patients and unaffected control subjects of the Brazilian population.

	Predominant ancestry						
	European				African		
PD1.6	Patients (n = 71)	Controls (n = 191)	p	Odds ratio (95% CI)	Patients (n = 51)	Controls (n = 51)	p
Genotype frequencies							
G/*G*	56.3	72.8	0.035	1	58.8	43.2	0.256
G/*A*	39.5	24.1		2.12 (1.18-3.80)	33.3	43.1	
A/*A*	4.2	3.1		1.74 (0.42-7.26)	7.9	13.7	
Allele frequencies							
G allele	76.1	84.8	0.028	0.57 (0.35-0.92)	75.5	64.7	0.123
A allele	23.9	15.2		1.76 (1.09-2.83)	24.5	35.3	
Allele carriage frequencies							
G allele positivity	95.8	96.9	0.704	0.74 (0.18-3.02)	92.2	86.3	0.530
A allele positivity	43.7	27.2	0.016	2.07 (1.18-3.65)	41.2	56.9	0.164
Hardy-Weinberg (p)	0.745	0.400			0.463	0.763	

CI = confidence interval; p = probability.

total population sample (AF plus EU) for *PD1.3* and *PD1.5* (Table 3) but not for *PD1.6* (Table 4).

Comparisons of the genotypic, allelic, and allele carrier frequencies between the patient and the control groups did not reveal any statistically significant difference for the *PD1.3* and *PD1.5* SNPs (Table 3). In fact, for both the AF and the EU population strata, the *PD1.5* frequencies in the patient group were very similar to those observed in the control group. The decreased frequency of allele *PD1.3A* observed among patients of both population strata approached the significance limit in the total population sample (OR = 0.55, p = 0.066) (Table 3).

Statistical analysis of the *PD1.6* SNP revealed significant differences in the genotypic and allelic frequencies between the patient and control groups in the EU but not the AF population stratum (Table 4). The *PD1.6A* allele was over-represented among the EU patients, due to an increase of both the *G*/*A* and the *A*/*A* genotypes (OR = 2.12 and 1.74 respectively; p = 0.035). These results indicate that the *PD1.6* SNP is associated with susceptibility to pemphigus foliaceus and the disease risk is increased by the presence of the *PD1.6A* allele in the population stratum of predominantly European ancestry.

Association analyses considering the haplotypes of the three polymorphic positions (*PD1.3*, *PD1.5* and *PD1.6*) showed a positive association with the GCA haplotype in the EU stratum (OR = 3.32, p = 0.004) but in the AF stratum a negative association with the GTA haplotype with the p value close to the significance limit was observed (OR = 0.39, p = 0.048) (Table 5). The three SNPs were in linkage disequilibrium (Table 6).

Table 5 - Haplotype frequencies (%) and association analysis between pemphigus foliaceus and *PDCD1* haplotypes, as defined by the *PD1.3*, *PD1.5*, and *PD1.6* single nucleotide polymorphisms (SNPs), in samples of patients and unaffected control subjects of the Brazilian population.

Haplotypes			Predominant ancestry									
			European					African				
*PD1.*3	*PD1.5*	*PD1.6*	Patients (n = 58)	Controls (n = 176)	Odds ratio	95% CI	p	Patients (n = 44)	Controls (n = 46)	Odds ratio	95% CI	p
A	*C*	*G*	8.6	9.8	0.87	0.41-1.81	0.856	3.4	5.4	0.61	0.14-2.65	0.724
G	*C*	*A*	13.2	4.4	3.32	1.59-6.96	0.004	15.9	16.7	0.95	0.43-2.09	1.000
G	*C*	*G*	42.8	49.0	0.78	0.51-1.19	0.261	45.4	38.8	1.31	0.73-2.38	0.451
G	*T*	*A*	11.8	9.2	1.30	0.67-2.54	0.373	8.0	18.1	0.39	0.15-0.99	0.048
G	*T*	*G*	23.6	25.5	0.91	0.55-1.48	0.714	27.3	21.0	1.41	0.71-2.81	0.392
A	*C*	*A*	0	0.7				0	0			
A	*T*	*A*	0	0.7				0	0			
A	*T*	*G*	0	0.7				0	0			

CI = confidence interval; p = probability.

Table 6 - Lewontin's D' relative linkage disequilibrium (LD) coefficient estimation for pairs of the three single nucleotide polymorphisms of the *PDCD1* gene in samples of patients with pemphigus foliaceus and unaffected control subjects of the Brazilian population.

	Polymorphism and predominant ancestry							
	PDCD1 7785 (PD1.5)				*PDCD1 8737 (PD1.6)*			
	European		African		European		African	
Polymorphism	Patients (n = 58)	Controls (n = 176)	Patients (n = 44)	Controls (n = 46)	Patients (n = 58)	Controls (n = 176)	Patients (n = 44)	Controls (n = 46)
PDCD1 7143 (*PD1.3*)	1.0000*	0.7339***	1.0000^{ns}	1.0000^{ns}	1.0000*	0.2093^{ns}	0.3380^{ns}	1.0000^{ns}
PDCD1 7785 (*PD1.5*)					0.2597*	0.4873***	0.0957^{ns}	0.2049*

p values: ^{ns}not significant (p > 0.05); * 0.05 > p > 0.01; ** 0.01 > p > 0.001; ***p < 0.001.

Discussion

Co-inhibitory molecules of the CD28 family are increasingly being recognized as important players in the control of T-cell immunity (Chambers, 2001). Inhibitory signals produced by the PD-1/PD-L pathway are of crucial relevance to the mechanisms maintaining tolerance and to terminate immune response in peripheral tissues, thus limiting organ damage (Freeman *et al.*, 2000; Latchman *et al.*, 2001; Nishimura and Honjo, 2001). The causes of initiation and perpetuation of the auto-aggressive responses in pemphigus foliaceus patients are largely unknown, although there is consensus about the influence of both genetic and environmental factors on susceptibility to this condition (Eaton *et al.*, 1998; Petzl-Erler and Santamaria, 1989). The pathogenic concepts include defects in the maintenance of peripheral immune tolerance towards potential autoantigens (Coyle and Gutierrez-Ramos, 2001).

Our study is the first to consider *PDCD1* a candidate gene for differential susceptibility to pemphigus foliaceus and also the first to analyze the exon 5 microsatellite in a human population. The low degree of polymorphism of this microsatellite may reflect functional constraints resulting from involvement of this region of the *PDCD1* gene in the control of gene expression.

The mechanisms that account for the observed positive association between pemphigus foliaceus and the *PD1.6A* allele remain to be clarified. Although the relative contribution of the *PD1.6A* allele to genetic predisposition to pemphigus foliaceus appears to be small, it is possible that this allele could lead some individuals to cross the threshold of disease manifestation, if these individuals bear susceptibility alleles (polygenes) at other loci and are exposed to the putative non-genetic susceptibility factors. The *PD1.6(G,A)* SNP is located in the 3' untranslated region of the *PDCD1* transcript and could potentially alter the expression of the *PDCD1* gene. A lowered expression of the PD-1 receptor at the cell membrane due to the *PD1.6A* allele might result in faulty control of peripheral tolerance. A functional effect and a direct involvement of this SNP in susceptibility to pemphigus foliaceus should be addressed in future studies. An alternative interpretation is supported by the absence of association in the population stratum of predominantly African ancestry. The association might be secondary, resulting from linkage disequilibrium of the *PD1.6* SNP with other, as yet unanalyzed, polymorphisms of *PDCD1* or other, closely linked, genes.

In the proximity of the *PDCD1* gene is a cluster of homologous genes (*CTLA4, CD28* and *ICOS*) related to immune activation and considered promising candidate genes for susceptibility to autoimmune disease (Holopainen *et al.*, 2004). A more comprehensive analysis of the 2q33-2q37 genomic region might reveal whether variants of one or more of these genes are involved in susceptibility and/or resistance to pemphigus foliaceus.

According to our results, the polymorphic variants of *PD1.5* do not seem to be involved in the pathogenesis of pemphigus foliaceus and do not alter the risk of developing the disease in the population studied. The lack of association leads us to suggest that this single nucleotide polymorphism does not influence susceptibility to pemphigus foliaceus.

We observed a possible negative association between allele *PD1.3A* and pemphigus foliaceus. It has been suggested that this SNP is functional, altering a binding site for the transcription factor RUNX1, and positive associations of allele *PD1.3A* with systemic lupus erythematosus and other autoimmune diseases have been reported. However, results of the autoimmune disease association studies are contradictory (Nielsen *et al.*, 2003; Prokunina *et al.*, 2002, 2004; Ferreiros-Vidal *et al.*, 2004, among others). It should be noticed that the *PD1.3A* allele is not common. Highest frequencies (5%-16%) have been reported for European populations; in other populations the *PD1.3A* allele is uncommon (Mori *et al.*, 2005; Kong *et al.*, 2005, Prokunina *et al.*, 2002; Sanghera *et al.*, 2004). The frequency of the *PD1.3A* allele was lower in our AF than in our EU population sample, in agreement with low frequency or absence of the *PD1.3A* allele in African populations and the known gene flow from Brazilians of European ancestry in the Brazilian population of African ancestry (Braun-Prado *et al.*, 2000; Probst *et al.*, 2000). The difference of allele frequencies between populations might partly account for the conflicting results of disease association studies.

In conclusion, we have shown for the first time that the presence of the *PD1.6A* allele at nucleotide 8737 of the *PDCD1* gene is associated with inter-individual differences in susceptibility to pemphigus foliaceus in the Brazilian population.

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