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The programmed cell death 1 gene 7209 C>T polymorphism is associated with the risk of systemic lupus erythematosus in the Polish population

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ABSTRACT

Numerous investigations indicated that the programmed cell death 1 (PDCD1) gene polymorphisms contribute to the development of systemic lupus erythematosus (SLE). However, their association with SLE has been found to be controversial. Therefore, in patients with SLE (n=102) and controls (n=140) we examined the association of six polymorphisms of this gene with susceptibility to SLE in the Polish population. We found that PDCD1 7209 CT or 7209 TT genotype exhibited 3.282-fold increased risk of SLE (95% CI=1.553 – 6.935; p=0.0017). The allele and genotype frequencies of the remaining polymorphisms: 5708 C>T, 6438 G>A, 7146 G>A and 8737 G>A did not exhibit statistical differences between SLE patients and controls. Our results confirmed the association of 7209 C>T polymorphism of PDCD1 gene with SLE that was previously observed in the Taiwanese population.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease of unclear etiology that involved multiple organs and system (1). Occupational exposure, drugs, chemicals, food, viruses and other infectious factors might result in significant changes in the immune system (2-4). Mechanisms responsible for initiation and promotion of SLE include increased amounts of nuclear autoantigens, abnormal presentation of them, T-cell-dependent stimulation of B cells for the biosynthesis of antinuclear antibodies and damages of tissues mediated by anti-DNA antibodies and immune complexes (5-8). The role of susceptibility genes in SLE etiology has also been firmly established. Numerous genes that encode proteins significant for the immune system or proteins contributing to SLE manifestations have been considered as candidate susceptibility genes (9-13).

Reduced apoptosis may be responsible for insufficient clearance of autoreactive lymphocytes in patients with SLE (7). It has been found that deficiency of *programmed cell death 1 (PDCD1)* gene expression may result in insufficient removal of autoreactive lymphocytes and breakdown of self-tolerance leading to onset of SLE, type 1 diabetes and other autoimmune disorders in mice (8, 14-17). PDCD1 was identified during induction of apoptosis in thymic T-cell line (18). This molecule is present on surface of activated T-cells, B-cells, and myeloid cells (19). PDCD1 is 55-kDa transmembrane protein composed of extra-cellular IgV-like fragment and cytoplasmic domain including one immunotyrosine switch motif and one immunotyrosine inhibitory motif (ITIM) (20). PDCD1 interacts with programmed death-1 ligands that transduces negative signals resulting in cell cycle arrest in G0/G1 phase but does not increase cell death (21). Many polymorphisms have been found in the *PDCD1* gene, which is located on 2q37 SLE susceptible locus (22, 23). Moreover, numerous investigations have indicated that the *PDCD1* gene polymorphisms contribute to the development of SLE (24, 25), rheumatoid arthritis (26, 27, 28), type 1 diabetes (29) and progression of multiple sclerosis (30).

Prokunina *et al*. reported that *PDCD1* 7146 G>A polymorphism located in intron 4 was overrepresented in patients with SLE disease (31). Furthermore, Wang *et al*. observed in SLE patients a higher distribution of another variant, 7209 C>T, also located in intron 4 of this gene (32). However, contribution of these polymorphisms in SLE development has been found to be controversial (24). Therefore, we decided to examine the association of *PDCD1* 5708 C>T, 6438 G>A, 7146 G>A, 7209 C>T, and 8737 G>A polymorphic variants with susceptibility to SLE in the Polish population.

Materials and methods

Patients and controls

One hundred and two patients (women only) fulfilling the American College of Rheumatology Classification (ACRC) criteria for systemic lupus erythematosus (33, 34) were chosen for investigation at Institute of Rheumatology Warsaw, Poland. In addition, 140 healthy women were recruited as controls. The protocol of the study was approved by the Local Ethical Committee of Poznan University of Medical Sciences. Written

Table I. Conditions for the identification of *PDCD1* polymorphisms.

PDCD1 Gene Polymorphism[1]	dbSNP ID	Primer		Fragment length (bp)	Annealing temp. (°C)	Restriction enzyme
		Forward (5'→3')	Reverse (5'→3')			
5708 C>T	rs7421861	CCCACCCAGACCAGTTACAC	TGTCCCCTTCGGTCACCAC	471	62	*HhaI*
6438 G>A	rs34819629	GGTCCTGGGGTGGGTGTC	CTGGGTGAGGGGCTGGGG	273	60	*MspI*
7146 G>A	rs11568821	GCAGGACTCACATTCTATTATA	CAATGTAAGATAAGAAATGACC	301	60	*PstI*
7209 C>T	-	TCCACTGTGCCTTCCTTCC	GATAAGAAATGACCAAGCCC	355	59	*BstUI*
8737 G>A	rs10204525	TGAGGCAGTAAGCGGGCAG	GTGTGTGGATGTGAGGAGTG	375	62	*NlaIII*

[1]Single nucleotide polymorphisms (SNPs) numbered in relation to the transcription start site (NCBI, AF363458).

Table II. Allele frequencies of *PDCD1* polymorphisms in SLE patients and controls.

		Genotype distribution absolute number (frequency)			Allele absolute number (frequency)			Odds ratio (95% CI)	*p* value[c]
5708C>T	n	CC	CT	TT	n	C	T		
	Controls Total 140	20 (0.14)	73 (0.52)	47 (0.34)	280	113 (0.40)	167 (0.60)	0.749 (0.428 – 1.309)[a]	0.3277
	SLE Total 102	18 (0.18)	56 (0.55)	28 (0.27)	204	92 (0.45)	112 (0.55)	0.778 (0.388 – 1.559)[b]	0.4807
6438G>A	n	GG	GA	AA	n	G	A		
	Controls Total 140	135 (0.96)	5 (0.04)	0 (0.00)	280	275 (0.98)	5 (0.02)	4.153 (0.167 – 103.06)[a]	0.4215
	SLE Total 102	96 (0.94)	5 (0.05)	1 (0.01)	204	197 (0.97)	7 (0.03)	1.688 (0.500 – 5.691)[b]	0.5342
7146G>A	n	GG	GA	AA	n	G	A		
	Controls Total 140	107 (0.76)	28 (0.20)	5 (0.04)	280	242 (0.86)	38 (0.14)	0.818 (0.191 – 3.506)[a]	1
	SLE Total 102	82 (0.80)	17 (0.17)	3 (0.03)	204	181 (0.89)	23 (0.11)	0.791 (0.423 – 1.478)[b]	0.5300
7209C>T	n	CC	CT	TT	n	C	T		
	Controls Total 140	128 (0.91)	11 (0.08)	1 (0.01)	280	267 (0.95)	13 (0.05)	4.212 (0.432 – 41.113)[a]	0.3129
	SLE Total 102	78 (0.76)	21 (0.21)	3 (0.03)	204	177 (0.87)	27 (0.13)	3.282 (1.553 – 6.935)[b]	0.0017
8737G>A	n	GG	GA	AA	n	G	A		
	Controls Total 140	106 (0.76)	29 (0.21)	5 (0.03)	280	241 (0.86)	39 (0.14)	1.102 (0.288 – 4.211)[a]	1
	SLE Total 102	76 (0.74)	22 (0.22)	4 (0.04)	204	174 (0.85)	30 (0.15)	1.067 (0.591 – 1.923)[b]	0.8806

The odds ratio was calculated for patients [a]homozygous carying risk allele *vs*. homozygous or heterozygous, [b]homozygous or heterozygous carying risk allele *vs*. homozygous. [c]Fisher exact test.

consent was obtained from all participating subjects.

Both patients and control groups were of Polish Caucasian origin. The mean age of SLE patients at diagnosis was 36±12 years and for controls 37±12 years. Clinical manifestation of SLE in the patient group includes central nervous system (18%), vascular (13%), renal (53%), musculoskeletal (60%), serosal (17%), dermal (50%), immunologic (25%), constitutional (fever) (9%), and hematologic (37%) components.

Genotyping

DNA was isolated from peripheral blood lymphocytes by salt extraction. All analyzed polymorphic variants 5708 C>T (intron 1), 6438 G>A (intron 2), 7146 G>A (intron 4), 7209 C>T (intron 4) and 8737 G >A (3'UTR) were identified using PCR, followed by appropriate restriction enzyme digestion (PCR-RFLP; Table I) (32). DNA fragments were separated by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining.

Statistical analysis

The distribution of genotypes in all groups was tested for deviation from Hardy-Weinberg. The Fisher's exact test was applied to examine differences in the genotypic and allelic distribution between patients and controls. Moreover, the Odds Ratio (OR) and 95% Confidence Intervals were calculated. A p-value <0.05 was considered statistically significant. Power analysis was performed using uncorrected chi-square test available from an on-line internet service, http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize.

Results

PDCD1 7209 C>T transition is associated with SLE development in the Polish population.

Genotype analysis of all investigated polymorphisms revealed no significant deviation form Hardy-Weinberg equilibrium in any group. The frequency of the *PDCD1* 7209TT genotype was 3.0-fold times higher in patients with SLE

compared to the controls, and was 0.03 and 0.01 in those groups, respectively (Table II). The *PDCD1* 7209CT heterozygous prevalence in patients was higher than in controls and amounted to 0.08 and 0.21 respectively. The frequency of the T allele was 2.6-fold times higher in the patients with SLE compared to the controls, and was 0.13 and 0.05 in those groups, respectively (Table II). The calculated odds ratio (OR) for SLE patients having the T allele (CT or TT genotype) was 3.282 (95% CI=1.553 – 6.935; p=0.0017; Table II) and the p-value remained statistically significant after Bonferroni correction as well (p_{corr} = 0.0085). The statistical power of this study amounted to 89% for 7209CT or 7209TT genotypes.

The allele and genotype frequencies of 5708 C>T, 6438 G>A, 7146 G>A and 8737 G>A gene variants did not exhibit statistical differences between SLE patients and controls. We also did not find significant association between clinical manifestations of SLE and distribution of investigated polymorphic variants of *PDCD1*.

Discussion

The PDCD1 protein suppresses autoimmune response and maintains self-tolerance. PDCD1 shortcoming might result in the breakdown of peripheral tolerance and the onset of autoimmune diseases (19). It has been shown that polymorphisms located in *PDCD1* may impact on the expression level of this gene. One of them *PDCD1* 7146 G>A transition placed in an enhancer-like structure is located in the binding site for transcription factor RUNX1 (31). This G>A nucleotide substitution may inhibit the biding of RUNX1 to this site leading to reduction of the *PDCD1* expression and initiation of the breakdown of self-tolerance. The *PDCD1* 7209 C>T polymorphism is also located in the intronic enhancer, in the neighborhood of the binding sites of transcription factors NFkB and RUNX1 (31, 32).

We found that *PDCD1* 7209 C>T polymorphism contribute to the risk of SLE in the Polish population (Table II). This transition may reduce the binding affinity and transcription effect of NFkB and RUNX1 (31, 32). Therefore, the contribution of *PDCD1* 7209 C>T polymorphism to SLE development might result from lowering of *PDCD1* expression. The association of 7209 C>T transition with SLE patients was also demonstrated in the Taiwan population (32).

Moreover, we did not observe the contribution of *PDCD1* 7146 G>A polymorphism to SLE in the same group of patients (Table II). Our negative results are consistent with the findings in Taiwan and northern Sweden populations, where this gene variant also exhibits similar distribution in both SLE patients and controls (25, 32). However, the association of 7146G>A transition with SLE have been demonstrated in other studies. Prokunina *et al*. showed that 7146 A allele contributed to the development of SLE in Europeans and Mexicans (31). Also Ferreiros-Vidal *et al*. found in a large Spanish cohort that the 7146 G>A transition might be a risk factor for SLE, but interestingly the allele that was associated with SLE susceptibility was the allele G (24). The described discrepancies might be a result of population differences and genetic heterogeneity (31, 35, 36).

Furthermore, Prokunina and Johansson found association between PDCD1 7146 G>A polymorphism and lupus nephritis (25, 37). However, we did not observe any correlation between SLE clinical symptoms and *PDCD1* 7209 C>T or 7146 G>A polymorphisms (results not shown).

In summary, our findings in the Polish population confirmed that only the *PDCD1* 7209 C>T polymorphism is associated with susceptibility to SLE. However, to establish more precisely the contribution of *PDCD1* gene variants to SLE, their further studies performed in other populations are still needed.

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