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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Supported by a grant No 502-01-01124182-07474 Poznan University of Medical Sciences.

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Received on May 30, 2007; accepted in revised form on November 22, 2007.

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Key words: PDCD1, systemic lupus erythematosus, polymorphism.

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 anti-nuclear 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 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 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

Competing interests: none declared.


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

<table>
<thead>
<tr>
<th>PDCD1 Gene Polymorphism</th>
<th>dbSNP ID</th>
<th>Primer Forward (5’→3’)</th>
<th>Primer Reverse (5’→3’)</th>
<th>Fragment length (bp)</th>
<th>Annealing temp. (°C)</th>
<th>Restriction enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>5708 C&gt;T</td>
<td>rs7421861</td>
<td>CCCACCCAGACGTTTACAC</td>
<td>GTGCCCTCTCCTGACACCA</td>
<td>471</td>
<td>62</td>
<td>HhaI</td>
</tr>
<tr>
<td>6438 G&gt;A</td>
<td>rs34819809</td>
<td>GGTGGGAGGAGAGGAGGG</td>
<td>CTTGGGGGAGGAGGAGGG</td>
<td>273</td>
<td>60</td>
<td>MspI</td>
</tr>
<tr>
<td>7146 C&gt;T</td>
<td>rs11568821</td>
<td>GAGAGGGTACCACTTTTCC</td>
<td>GTTTTGTTTTTCTTCTTCC</td>
<td>301</td>
<td>60</td>
<td>Pal</td>
</tr>
<tr>
<td>7209 C&gt;T</td>
<td>-</td>
<td>CCACCTCTCTCTCTCT</td>
<td>GTACGAGAGAGAGAGGC</td>
<td>355</td>
<td>59</td>
<td>BstUI</td>
</tr>
<tr>
<td>8737 G&gt;A</td>
<td>rs10204525</td>
<td>TGCACCTGAGTACCGCTGG</td>
<td>GTAGGTGGGGAGAAGGAGG</td>
<td>375</td>
<td>62</td>
<td>NlaIII</td>
</tr>
</tbody>
</table>

*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 | n | CC | CT | TT | Allele absolute number (frequency) | Odds ratio (95% CI) | p value*
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls Total 140</td>
<td>n</td>
<td>G1</td>
<td>G2</td>
<td>A</td>
<td>n</td>
<td>G</td>
<td>A</td>
</tr>
<tr>
<td>8737G&gt;A</td>
<td>GCACGAGGCTACACATTTTCC</td>
<td>GTACGAGAGAGAGGC</td>
<td>GTAGGTGGGGAGAAGGAGG</td>
<td>375</td>
<td>62</td>
<td>NlaIII</td>
<td></td>
</tr>
<tr>
<td>SLE Total 102</td>
<td>18 (0.18)</td>
<td>56 (0.55)</td>
<td>28 (0.27)</td>
<td>204</td>
<td>92 (0.45)</td>
<td>112 (0.55)</td>
<td>0.778 (0.388 – 1.559)</td>
</tr>
<tr>
<td>6438G&gt;A</td>
<td>GCACGAGGCTACACATTTTCC</td>
<td>GTACGAGAGAGAGGC</td>
<td>GTAGGTGGGGAGAAGGAGG</td>
<td>375</td>
<td>62</td>
<td>NlaIII</td>
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<tr>
<td>7146G&gt;A</td>
<td>GCACGAGGCTACACATTTTCC</td>
<td>GTACGAGAGAGAGGC</td>
<td>GTAGGTGGGGAGAAGGAGG</td>
<td>375</td>
<td>62</td>
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</tbody>
</table>

*The odds ratio was calculated for patients homozygous carrying risk allele vs. homozygous or heterozygous, homozygous or heterozygous carrying risk allele vs. homozygous, Fisher exact test.*

### 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 from Hardy-Weinberg equilibrium in any group. The frequency of the PDCD1 7209 TT genotype was 3.0-fold times higher in patients with SLE.
Association of PDCD1 polymorphisms with SLE / M. Mostowska et al.

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<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 389 C>T polymorphisms associated with SLE were shown in Table III. We found that the allele and genotype frequencies of 7209 C>T polymorphism were significantly different 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 binding 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.

Acknowledgements

We would like to thank Margarita Lianeri for her editorial assistance.

References


20. OKAZAKI T, MAEDA A, NISHIMURA H, KUROSAKI T, HONJO T: PDCD1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine.


