

Lack of Association of Programmed Cell Death 1 Gene (*PDCD1*) Polymorphisms With Susceptibility to Chronic Urticaria in Patients With Positive Autologous Serum Skin Test

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■ Abstract

Background: Autoimmune mechanisms play an important role in the pathophysiology of chronic urticaria (CU), and the autologous serum skin test (ASST) helps to identify patients with autoreactive CU. One of the factors involved in autoreactive mechanisms is the cell surface receptor programmed death-1 which is encoded by the programmed cell death 1 gene (*PDCD1*).

Objective: To investigate whether *PDCD1* polymorphisms influence susceptibility to CU.

Methods: We enrolled 93 ASST-positive patients with CU and a control group consisting of 105 healthy volunteers. In all individuals, PD1.3 (7146 A/G; rs 11568821) and PD1.5 (7785 C/T; rs 2227981) polymorphisms were analyzed.

Results: No statistically significant differences were found between CU patients and controls for allele or genotype distribution. We also did not observe any association between *PDCD1* genotypes and severity of urticaria or age of disease onset.

Conclusions: PD1.3 and PD1.5 polymorphisms were not proven to be implicated in susceptibility to ASST-positive CU in the Polish population. A more comprehensive analysis of the 2q33-2q37 genomic region might reveal whether variants of 1 or more of the genes in this region are involved in susceptibility to CU.

Key words: Chronic urticaria. Polymorphism. Autologous serum skin test. ASST. *PDCD1*.

■ Resumen

Antecedentes: Los mecanismos autoinmunes desempeñan un papel importante en la fisiopatología de la urticaria crónica (UC). La prueba cutánea con suero autólogo (PCSA) ayuda a discriminar a los pacientes con una causa autorreactiva en su enfermedad. Uno de los factores implicados en los mecanismos autorreactivos es el receptor de superficie de la muerte celular programada tipo 1 codificado por el gen *PDCD1*.

Objetivo: El objetivo de nuestro estudio fue probar la hipótesis de que los polimorfismos del gen *PDCD1* influyen en la susceptibilidad a desarrollar UC.

Métodos: Se estudió un grupo formado por 93 pacientes con UC y PCSA positiva. El grupo de control estaba formado por 105 voluntarios sanos. En todos los sujetos se estudiaron los polimorfismos: PD1.3 (7146 A / G; rs 11568821) y PD1.5 (7785 C / T; rs 2227981).

Resultados: No hubo diferencias estadísticamente significativas en la distribución de los alelos y los genotipos entre los pacientes de UC y los controles. No se observó ninguna dependencia entre los genotipos *PDCD1* y la gravedad de la urticaria, ni relación con la edad de inicio de la enfermedad.

Conclusiones: No se demostró que los polimorfismos PD1.3 y PD1.5 pudieran estar implicados en la susceptibilidad a la UC en una población polaca con resultados positivos en la PCSA. Un análisis más exhaustivo de la región genómica 2q33-2q37 podría revelar si las variantes de uno o más de estos genes están implicados en la susceptibilidad a la UC.

Palabras clave: Urticaria crónica. Polimorfismo. Prueba cutánea con suero autólogo (ASST). *PDCD1*.

Introduction

Autoimmune mechanisms play an important role in the pathophysiology of chronic urticaria (CU) [1-3]. The autoimmune origin of urticaria is related to immunoglobulin (Ig) G anti-IgE antibodies or antibodies directed against the high-affinity IgE receptor (FcεR1) [4]. The autologous serum skin test (ASST) helps to point to a possible autoreactive cause of disease [5,6].

CU is associated with other autoimmune abnormalities [7-10]. Furthermore, in CU patients with the presence of antithyroid antibodies, a high incidence of positive antinuclear antibodies with a speckled pattern can be observed [1]. As genetic susceptibility to autoimmune disorders is well proven, the question about the role of genetics in CU has been raised.

The genetic background of CU has not been deeply explored. Autoimmune CU has been shown to be related to the major histocompatibility complex (MHC) class allele as well as to the HLA II allele [11]. Autoimmune urticaria would therefore appear to be associated with HLA alleles related to autoimmune diseases. In a recent study on genetic susceptibility to CU, the *PTPN22* (1858C>T) polymorphism was not shown to contribute to CU pathogenesis [12].

Autoimmune defects can result from insufficient suppression of the autoreactive response. One of the factors involved in autoreactive mechanisms is cell surface receptor programmed death-1 (PD-1), which is expressed on T and B cells and encoded by the programmed cell death 1 gene (*PDCD1*) located on chromosome 2q37.3. PD-1 is a member of the immunoglobulin superfamily B7-CD28 [13,14]. T-cell receptor mediated proliferation and cytokine production in activated T lymphocytes are inhibited by PD-1 ligands, ie, PDL-1 (B7-H1) and PDL-2 (B7-DC) [15].

The role of *PDCD1* is the suppression of autoreactive response [16]. *PDCD1* has 2 tyrosine domains in the cytoplasmic tail, ie, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). ITIM tyrosine residue phosphorylation during cell activation is an effect of the interaction of *PDCD1* with ligands PD-L1 and PD-L2, which results in the deactivation of signaling molecules and the subsequent inhibition of cytokine production. These processes result in the inhibition of the cell cycle [17,18].

PDCD1 was identified to have several single nucleotide polymorphisms. Decreased expression of *PDCD1* is an effect of a polymorphism in intron 4 (PD1.3) that affects the binding of the runt-related transcription factor 1 (RUNX1) [19]. This deregulates self-tolerance and contributes to lymphocyte hyperactivity [20]. In a Danish group, the A allele of the PD1.3 polymorphism was shown to be related to type 1 diabetes mellitus (T1DM) [21], and in a study of Swedish patients, the same allele was shown to be associated with lupus nephritis [22]. Moreover, PD1.3A may also be a risk factor for systemic lupus erythematosus (SLE) in non-Spanish European populations [17].

Another *PDCD1* polymorphism, PD1.5, has been shown to be associated with susceptibility to rheumatoid arthritis, but not SLE [23]. In the Korean population, the PD1.5C allele was significantly associated with ankylosing spondylitis [24].

Furthermore, PD1.5 was found to be significantly associated with the onset of T1DM in the Japanese population [25], contrasting with results from a Swedish study, where such a correlation was not observed [26].

The aim of the present study was to investigate whether *PDCD1* polymorphisms might influence susceptibility to CU.

Material and Methods

Characteristics of Study Sample

The study group comprised 93 unrelated CU patients (65 females and 28 males with a mean age of 38.5 years [range, 21-58 years]) with positive ASST results. The diagnosis of CU was established by careful history taking and physical examination. Mean (SD) disease duration was 47.1 (9.64) months. We also included a control group consisting of 105 unrelated healthy volunteers (71 females and 34 males with a mean age of 46.1 years [range, 19-59]). All the participants were white and from Poland.

Disease severity was assessed using the Urticaria Activity Score (UAS). This symptom assessment tool is based on the analysis of wheals and pruritus, and is recommended for use in clinical practice, trials, and scientific studies [27,28]. As it is recommended that patients' self-perception scores are assessed over several days, we performed a 7-day assessment (UAS7). Our observation yielded total scores ranging from 0 to 42. Furthermore, in all CU patients, age of disease onset was analyzed.

Genomic DNA Isolation and PDCD1 Genotyping

Blood samples were obtained from all participants, and genomic DNA from peripheral blood leukocytes was extracted using the MasterPure DNA Purification Kit (Epicentre Technologies) in accordance with the manufacturer's instructions. Subsequently, the PD1.3 (7146 A/G; rs 11568821) and PD1.5 (7785 C/T; rs 2227981) polymorphisms were analyzed. We performed genotyping by allelic discrimination using Custom TaqMan SNP Genotyping Assays and the 7300 Real-time PCR System (Applied Biosystems).

The study was approved by the ethics committee of the Medical University of Silesia.

Statistical Analysis

The differences in the allele and genotype frequencies between respective groups were analyzed with the Pearson χ^2 test with Yates' continuity correction. Furthermore, odds ratios (OR) with 95% CIs were calculated. Uncertain haplotype frequencies were estimated using the Package 'BayHap' (R version 2.7.0 from The R Foundation for Statistical Computing, <http://cran.r-project.org>). For UAS7 and disease onset age comparisons among different genotype distribution subgroups the unpaired *t* test and analysis of variance test were used. All statistical calculations were performed using Statistica 8.0 PL (Statsoft INC).

Results

The Hardy-Weinberg equilibrium analysis revealed no deviations in either of the groups analyzed. The genotype and allele frequencies for the *PDCD1* polymorphisms PD1.3 and PD1.5 are presented in Table 1. In PD1.3 the AA genotype was not found, whereas the AG genotype was found in 20% of our CU population. The corresponding figures for the CC and CT

genotypes of the PD1.5 polymorphism were 33% and 50%. In both polymorphisms no statistically significant differences in the allele or genotype distribution between CU patients and controls were found. Additionally, there were no differences in haplotype frequencies (Table 2).

On analyzing urticaria severity and age of disease in patients with different genotype distributions, we assumed no associations between *PDCD1* genotypes and these parameters (Tables 3 and 4).

Table 1. *PDCD1* Genotype and Allele Distribution in Patients With Chronic Urticaria (CU) and Healthy Controls

SNP	Group	Genotype Distribution, No. (%)			Allele, No. (%)		OR ^a (95% CI)	P
		AA	AG	GG	A	G		
PD1.3 (A/G)	CU	0 (0)	19 (20)	74 (80)	19 (10)	167 (90)	0.97 (0.46-2.04)	.93
	Control	0 (0)	22 (21)	83 (79)	22 (10)	188 (90)		
PD1.5 (C/T)	CU	31 (33)	46 (50)	16 (17)	108 (58)	78 (42)	0.87 (0.38-1.99)	.85
	Control	30 (29)	59 (56)	16 (15)	119 (57)	91 (43)		

Abbreviations: OR, odds ratio; SNP, single nucleotide polymorphism.

^aThe odds ratio was calculated for patients homozygous or heterozygous for risk allele vs homozygous.

Table 2. *PDCD1* Haplotype Distribution in Patients With Chronic Urticaria (CU) and Healthy Controls

Haplotypes		Haplotype Frequencies		P
PD1.3 Allele	PD1.5 Allele	CU	Controls	
G	T	0.41	0.41	NS
A	C	0.09	0.08	NS
G	C	0.49	0.49	NS
A	T	0.01	0.02	NS

Abbreviation: NS, not statistically significant.

Table 3. UAS7 and Age of Disease Onset in Patients With Chronic Urticaria With Different PD1.3 Genotypes

	Genotype Distribution		P
	AG	GG	
Mean (SD) UAS7 score	19.8 (8.79)	21.2 (7.58)	NS

Abbreviations: NS, not statistically significant; UAS7, Urticaria Activity Score (1-Week Assessment).

Table 4. UAS7 and Age of Disease Onset in Patients With Chronic Urticaria and Different PD1.5 Genotypes

	Genotype Distribution			P
	CC	CT	TT	
Mean (SD) UAS7 score	20.7 (7.68)	19.6 (9.36)	21.1 (8.43)	NS
Mean (SD) age of disease onset, y	33.1 (6.92)	34.4 (7.72)	36.1 (7.52)	NS

Abbreviations: NS, not statistically significant; UAS7, Urticaria Activity Score (1-Week Assessment).

Discussion

An autoreactive mechanism is detected in approximately 35% to 50% of CU patients. Autoimmune urticaria is assumed to be clinically and immunologically related to other autoreactive disorders. In patients with rheumatoid arthritis, SLE, systemic sclerosis, and dermatomyositis immunoreactive non-histamine-releasing anti FcεRIα autoantibodies have been found [29,30]. Furthermore, in some rheumatoid arthritis patients anti-IgE autoantibodies have been found [29,30]. The association between *PDCD1* and autoimmune disorders is relatively well established [20,21]. Moreover, the role of PD-1 gene polymorphisms was taken into consideration in the pathogenesis of anti-neutrophil cytoplasmic antibody-associated vasculitis, and ankylosing spondylitis [24,31].

Considering the close relationship between autoimmune urticaria and autoreactive disorders, we speculated on the genetic background of CU. Our study is the first to consider *PDCD1* as a candidate gene for susceptibility to CU. We selected ASST-positive patients as ASST serves as a marker of the presence of circulating functional autoantibodies and indicates a potential role for autoreactivity in CU [6]. In both of our analyzed polymorphisms the distribution of the genotype and allele in CU and the control group was similar. Moreover, in the case of PD1.3, it was similar to the results obtained in another study of the Polish population [32]. Therefore PD1.3 and PD1.5 do not appear to be implicated in susceptibility to ASST-positive CU in the Polish population.

The role of PD1.5 in relation to autoimmune processes has been moderately investigated. It has been shown to be associated with rheumatoid arthritis but not SLE [23]. The results of studies concerning T1DM, however, are contradictory [25,26]. To the best of our knowledge, the present study is the first to examine this polymorphism in the Polish population.

The role of PD1.3 in autoimmunity is better understood. In previous studies, the PD1.3A allele has been shown to be associated with susceptibility to SLE [17,20], although contradictory results have also been published [33]. PD1.3 has been implicated in susceptibility to SLE in Swedish, European, American, and Mexican families, as well as in sporadic cases, and the PD1.3A allele has been associated with SLE with renal manifestations in Swedish patients [20,22]. On the other hand, no associations have been found between PD1.3 and either SLE or T1DM in the Polish population [16,32]. Finally, a Spanish study showed the PD1.3G allele to be associated with

susceptibility to SLE [33]. These discrepancies may be due to ethnic differences but this remains to be elucidated in future studies of *PDCD1* polymorphisms in CU patients from other populations [34].

The mechanisms of initiation of the autoreactive process in CU are largely unknown. There is some suspicion about the influence of both genetic and environmental factors on susceptibility to this condition. In our opinion, the present study is valuable as a pilot study for further investigations regarding *PDCD1* and CU. 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 to CU.

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