

Inducible T-cell costimulator (ICOS) and CD28 polymorphisms possibly play a role in the pathogenesis of chronic autoreactive urticaria

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Summary

Background. Clinical experience emphasizes the coexistence of chronic spontaneous urticaria (CSU) and autoimmune disturbances. In chromosome 2q33-34, there is a cluster of homologous genes that are considered promising candidate genes for susceptibility to autoimmune diseases.

Aim. To examine the possible role of polymorphisms in the genes for CD28 and inducible T-cell costimulator (ICOS) in the background of CSU.

Methods. In total, 149 patients with CSU with positive autologous serum skin test were enrolled in the study. The healthy control (HC) group consisted of 100 healthy volunteers. In all subjects, the CD28 rs2140148 and rs3116496 and the ICOS rs6726035 polymorphisms were analysed. Disease severity was assessed by means of Urticaria Activity Score.

Results. We found a statistically significantly lower prevalence of the ICOS rs6726035 TT genotype among patients with CSU compared with HCs. Furthermore, the haplotype rs2140148A, rs3116496T and rs6726035C presented a possible association with CSU. We did not find any association between the examined polymorphisms and either urticaria severity or age of disease onset.

Conclusions. Our results underline the role of autoimmune components in the pathogenesis of chronic autoreactive urticaria, and indicate it as a potentially genetically related disorder.

Introduction

Chronic spontaneous urticaria (CSU) is a debilitating dermatosis of unknown pathogenesis. Several pathogenic factors have been identified, including an autoimmune origin. There is a close association between autoimmune CSU and major histocompatibility complex, indicating a genetic background in the

pathogenesis of urticaria and pointing at a connection with other autoimmune diseases. Clinical evidence shows the coexistence of CSU with autoimmunity, e.g. autoimmune thyroid diseases (AITDs). In some previous studies, a genetic association was shown between CSU and autoimmune-related polymorphisms such as *PTPN22*, *CCR2* and *CCR5*, and this warrants further analysis.^{1,2}

In chromosome 2q33–34, there is a cluster of homologous genes (*CTLA4*, *ICOS* and *CD28*) related to immune activation, which are considered promising candidate genes for susceptibility to autoimmune diseases. *CTLA4* (cytotoxic T-lymphocyte-associated protein 4) is a regulatory molecule expressed on the surface of activated T lymphocytes, which provides a negative signal to the T cell, limiting immune

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responses.^{3–5} The human *CTLA4* gene is suspected to be associated with AITDs when there is an adenine to guanine transition at position 49 of exon 1 (A49G).⁶ ICOS (inducible T-cell costimulator) is a receptor known to regulate T-lymphocyte activation. It enhances T-cell function for effective antigen-specific immune responses. CD28 (cluster of differentiation 28) influences signals stimulating T-lymphocyte differentiation and proliferation, and induces antibody production.^{7–9} It has been reported that deficiencies in both CD28 and ICOS result in complete T-lymphocyte tolerance.^{10,11}

In previous studies it was shown that *CTLA-4* gene polymorphisms do not play a role in the pathogenesis of chronic urticaria.¹² In the present study, we aimed to test the possible role of *CD28* and *ICOS* genes in susceptibility to CSU.

Methods

The study was approved by the bioethics committee of Medical University of Silesia, Katowice, Poland, and all participants provided written informed consent.

Study sample characteristics

In total, 149 patients (91 women, 58 men; mean age 38.6 years, range 19–60 years), who had positive autologous serum skin test (ASST) results and no concomitant diseases were enrolled in the study. The diagnosis of CSU was established after careful medical history-taking and physical examination. The healthy control (HC) group consisted of 100 healthy volunteers (71 females and 29 males, mean age 43.6 years, range 18–58 years). All studied subjects were white and came from the Polish population.

Disease severity was assessed by means of the Urticaria Activity Score (UAS), which is a symptom analysis tool calculating number of weals and intensity of pruritus.^{13,14} In accordance with general recommendations, we assessed the patients for seven consecutive days (UAS7) and achieved a total score of 0–42. We also analysed the age of disease onset.

Genomic DNA isolation and genotyping

We extracted genomic DNA from peripheral blood leucocytes, using a commercial kit (MasterPure™ DNA Purification Kit; Epicentre Technologies, Madison, WI, USA), in accordance with the manufacturer's instructions. Subsequently, we analysed the *CD28* rs2140148 and rs3116496 and *ICOS* rs6726035

polymorphisms in all examined subjects. We performed genotyping by allelic discrimination (Custom TaqMan SNP Genotyping Assay and 7300 Real-time PCR System; both Applied Biosystems, Foster City, CA, USA).

Statistical analysis

The χ^2 test was used for the analysis of the differences in allele and genotype frequencies, and OR and 95% CI were calculated. We used Bonferroni correction for multiple comparisons. Kruskal–Wallis test was used for the comparisons of UAS7 and disease onset age between the various genotype distribution subgroups, using Statistica software (v8.0 PL; StatSoft Inc., Tulsa, OK, USA). Haplotype frequency was assessed using the 'BayHap' package and linkage disequilibrium between the analysed polymorphisms was analysed by means of the 'genetics' package of R software (v2.11.1; R Project for Statistical Computing; <https://www.r-project.org/foundation/>).

Results

For the *ICOS* polymorphism, Hardy–Weinberg equilibrium assay revealed no deviation in the control group, with a borderline statistically significant difference in the study group. The allele and genotype distributions for the studied polymorphisms are presented in Table 1. We found a statistically significantly lower prevalence of the *ICOS* rs6726035 TT genotype among patients with CSU compared with HCs. No statistically significant differences in allele or genotype frequencies were found between the compared groups for any of the other analysed polymorphisms. The explored polymorphisms were in linkage disequilibrium. Haplotype construction resulted in eight haplotypes, of which *CD28* rs2140148A/rs3116496T and *ICOS* rs6726035C presented a possible association with CSU (Table 2). No association between *CD28* and *ICOS* polymorphisms, urticaria severity or age of disease onset was found when different genotype distribution subgroups were analysed.

Discussion

The causes of CSU remain under debate and the association of CSU with autoimmunity is still controversial although poorly investigated. As the genetic background of autoimmune disorders is now established, we hypothesized that CSU also has a genetic background. In previous studies, we found an

Table 1 CD28 and ICOS genotype and allele distribution in patients with CSU and in HCs.

SNP	Group	Genotype distribution total number (%)			Allele total number (%)		OR (95% CI)*	<i>P</i> †	<i>P</i> ‡
CD28									
rs2140148	CSU	AA: 113 (76)	AC: 33 (22)	CC: 3 (2)	A: 259 (87)	C: 39 (13)	1.54 (0.88–2.81)	0.15	0.21
	HC	AA: 67 (67)	AC: 29 (29)	CC: 4 (4)	A: 163 (82)	C: 37 (18)			
rs3116496	CSU	TT: 110 (74)	TC: 35 (23)	CC: 4 (3)	T: 255 (86)	C: 43 (14)	1.65 (0.92–2.96)	0.10	0.39
	HC	TT: 63 (63)	TC: 35 (35)	CC: 2 (2)	T: 171 (86)	C: 29 (14)			
ICOS									
rs6726035	CSU	CC: 74 (50)	CT: 67 (45)	TT: 8 (5)	C: 215 (72)	T: 83 (28)	3.10 (1.17–8.81)	0.01 (0.04§)	0.27
	HC	CC: 50 (50)	CT: 35 (35)	TT: 15 (15)	C: 135 (68)	T: 65 (32)			

CSU, chronic spontaneous urticaria; HC, healthy control. *Odds ratio was calculated for patients homozygous or heterozygous carrying risk allele vs. homozygous; †statistical significance; ‡allele frequency statistical significance; §corrected *p* value.

Table 2 CD28 and ICOS haplotype distribution in patients with CSU and in HCs.

Haplotypes*	Frequency, % (95% CI)		<i>P</i>
	CSU (<i>n</i> = 298 haplotypes)	HC (<i>n</i> = 200 haplotypes)	
CTT	0.8 (0.1–2.1)	0 (0)	0.47
ACC	0.7 (0–2.7)	3 (1.3–5.9)	0.61
CCT	0.9 (0–2.7)	4.5 (1.8–8.9)	0.36
ATC	59.8 (54.6–64.9)	50.5 (43.4–58.0)	0.04 (0.32†)
CTC	0 (0)	1.9 (0.3–4.4)	0.32
ACT	1.3 (0.1–3.1)	0.2 (0–0.6)	0.48
CCC	11.5 (8.1–15.5)	11.9 (7.3–17.5)	0.59
ATT	25 (20.5–29.9)	28.0 (21.6–34.4)	0.29

CSU, chronic spontaneous urticaria; HC, healthy control. *CD28 rs2140148/rs3116496 and ICOS rs6726035; †corrected *p* value.

association between CSU and *PTPN22*, *CCR2* and *CCR5* polymorphisms with no relation to *PDCD1* and *CTLA-4*.^{1,2,12,15,16} In our present study, we selected ASST-positive patients with CSU, because of the role of ASST as a marker of circulating functional autoantibodies.

The *CTLA-4*, *CD28* and *ICOS* genes are located close to *PDCD1*, and are considered to be candidate genes for susceptibility to autoimmune diseases.¹⁷ *PDCD1* is a member of the immunoglobulin family and is thought to be associated with systemic lupus erythematosus, type 1 diabetes and rheumatoid arthritis (RA).^{18–20} The co-stimulatory receptor gene cluster located on chromosome 2q33 encodes the positive T-cell regulators CD28 and ICOS, as well as the negative regulator CTLA4. Polymorphisms in the *CD28* gene have been shown to be related to autoimmune diseases, such as RA and Behçet disease.^{21,22} In general, CTLA4 counterbalances CD28-mediated signals,²³ while ICOS influences T-cell cytokine

response, i.e. production of interleukin (IL)-4, IL-5, IL-10, interferon (IFN)- α and IFN- γ , but not IL-2.^{10,24} Interaction between ICOS and its ligands induces T-cell proliferation, cytokine secretion and upregulation of cell surface molecules.²⁵ ICOS enhances effective immune responses, whereas CTLA4 prevents lymphoid system overstimulation.²³ In previous studies, *ICOS* gene polymorphisms appeared to be related to multiple sclerosis and coeliac disease,^{24,26} and *ICOS* knockout in mice resulted in an increased risk of experimental autoimmune encephalomyelitis.²⁵ Our findings are similar to those of Kim *et al.*,²⁷ who found an association between ICOS and RA; we found a similar association between *ICOS* polymorphism and susceptibility to CSU.

Conclusion

In summary, we have found an association between CSU and polymorphisms in the *ICOS* gene. To our knowledge, this report is the first to suggest and initially indicate a possible association of the *ICOS* rs6726035 TT genotype with CSU pathogenesis. Our results emphasize the role of autoimmune components in CSU pathogenesis and indicate CSU as a genetically related disorder. These facts should be considered in studies on the pathogenesis of urticaria, so that further genetic studies include this locus area. We intend to continue this analysis in further studies, comparing CSU with other forms of urticaria, and relating these polymorphisms to disease symptoms and comorbidities.

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What's already known about this topic?

- There is a close association between chronic urticaria and major histocompatibility complex, indicating a genetic involvement in the pathogenesis of urticaria.

What does this study add?

- We aimed to test the possible role of the CD28 and ICOS genes in the susceptibility to chronic autoreactive urticaria.
- We are the first to suggest and initially indicate a possible association of the ICOS rs6726035 TT genotype with chronic autoreactive urticaria pathogenesis.

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