

No Association Between Systemic Sclerosis and C77G Polymorphism in the Human PTPRC (CD45) Gene

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ABSTRACT. *Objective.* The functional variant C77G (rs17612648) of PTPRC (CD45) was described to confer risk for systemic sclerosis (SSc) in German Caucasians. We analyzed this association in an independent, larger German cohort.

Methods. We genotyped 171 cases and 179 controls. Cases were subgrouped according to sex, autoantibody profiles, or clinical subsets.

Results. No association of SSc with C77G was detected in the whole dataset, in subgroups, or in combined analyses with a previous study.

Conclusion. The results do not confirm PTPRC C77G as a general and independent risk factor for development of SSc. (First Release July 15 2008; J Rheumatol 2008;35:1817–9)

Key Indexing Terms:

SCLERODERMA SYSTEMIC SCLEROSIS LEUKOCYTE COMMON ANTIGEN
CD45 ANTIGENS AUTOIMMUNITY SINGLE-NUCLEOTIDE POLYMORPHISM

The CD45 molecule (leukocyte common antigen) is a large transmembrane protein-tyrosine phosphatase receptor-type C (PTPRC) expressed by all hematopoietic cells except erythrocytes¹. Different isoforms can be generated by alternative splicing. These isoforms appear in various combinations on the surface of individual cells. Expression of exons 4, 5, and 6 can be detected by antibodies specific for domains RA, RB, and RC, respectively. The functions of individual isoforms are not understood in detail, but expression patterns are cell-type-specific and vary in relation to changes in cell differentiation and activation. The presence of CD45 is

required for efficient development of the immune system, especially functional lymphocytes, where it is involved in antigen receptor signal transduction^{1,2}.

The rare synonymous polymorphism C77G (rs17612648) affects the expression of exon 4. The exchange of 77C to 77G decreases splicing of exon 4 pre-mRNA, leading to increased presence of exon 4 in mature transcripts. On the protein level, heterozygous individuals (77C/G) express isoforms including the RA domain in addition to their normal set of CD45 isoforms¹⁻³. Concerning T lymphocytes, such changes were shown to have functional consequences: naive T cells usually express the RA domain (CD45RA+) but lose it during activation and become CD45RA-/CD45R0+. In 77C/G individuals, naive T cells are CD45RA+/CD45R0+ instead. These T cells show an increased intensity of T cell signaling, observed as enhanced proliferation and interleukin 2 production after receptor-mediated stimulation^{2,4,5}. This functional evidence demands investigation of various diseases for association with the C77G polymorphism. The frequency of the 77G allele showed a wide variation in healthy individuals and was increased in several autoimmune diseases, including systemic sclerosis (SSc) in German Caucasians^{1,6}. As the first small study at this locus for systemic sclerosis (SSc), with 67 patients, was not confirmed or contradicted by any other published study, we sought to improve evidence and analyze clinical subgroups in an independent larger Caucasian cohort.

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MATERIALS AND METHODS

We included 171 patients (139 female, 32 male) seen in the Department of Rheumatology, Freiburg, and Department of Dermatology, Cologne. We classified patients according to LeRoy, *et al*⁷. Patient sera were analyzed for presence of autoantibodies to topoisomerase I and centromere as described⁸, with modifications. Controls were 179 healthy blood donors matched for sex (143 female, 36 male) and age (mean age was 50 ± 7 yrs

and 56 ± 14 yrs in patients and controls, respectively). Ethnicity of cases and controls was ascertained by excluding individuals with obvious indications of non-European ancestry. In addition, anonymous blood donors were analyzed for evidence of individuals with non-European ancestry using additional genetic information (data not shown).

Genotyping in DNA from blood isolated by standard procedures was done by applying single-base extension and MALDI-TOF mass spectrometry (GenoSNIP; Bruker Daltonics, Billerica, MA, USA) as described⁹, PCR forward primer, reverse primer, and the genotyping primer were 5'ACG TTG GAT GAT TAA CAG GAT TGA CTA CAG CA 3', 5'ACG TTG GAT GAT TGT CTG GAC TAA GAG AAG TTG T3, and 5'bioATT TTC TCT TTL AAA GGT GCT TGC 3', respectively ("L" indicates a photocleavable linker and "bio" a biotin residue).

Power to detect an association and power to detect the same trend were calculated as described^{10,11}. These calculations were based on the allelic odds ratio⁶ and allele frequencies in our controls. Fisher's exact test was applied to test for allelic association between patients with SSc and controls for carriage of the 77G allele at the 5% test level.

RESULTS

PTPRC C77G genotypes of cases and controls were consistent with Hardy-Weinberg equilibrium. No 77G/G homozygotes were observed. Quantitative analysis of genotyping data did not indicate the presence of copy number variation (data not shown). Our study had a power of > 89% to detect an association of the 77G allele as reported⁶ and more than 99% power to detect at least the same trend. Unexpectedly, the frequencies of heterozygotes and the 77G allele were decreased in this SSc cohort compared to the healthy controls (Table 1). This was found for both female and male patients. As usually observed in SSc patients¹², the subgroups of diffuse and limited SSc (dSSc, lSSc) correlated with the presence of autoantibodies to topoisomerase I and centromere, respectively (p < 0.001 for both comparisons). When the analysis was stratified for these 4 groups, again no significant differences were observed between patients and controls (Table 1). Interestingly, within the small group of patients with overlap syndrome, the frequency of the 77G allele was nominally, but not significantly, increased (Table 1).

Data for individuals from the earlier study⁶ with patients

from the Departments of Dermatology, Cologne, and Clinical Immunology, Hannover, were available (sex, genotype, diagnosis, autoantibody profile). All patients were classified according to LeRoy, *et al*⁷ and were of European Caucasian origin. Both cohorts were comparable, with exception of a significant difference in the frequency of 77G (p = 0.02) in patients. Comparability of both studies was further supported by the observation that the frequency of 77G did not differ significantly between the controls in both studies. This allowed a combined analysis of 238 SSc patients and 384 controls. This combined case-control analysis again did not reveal an association between SSc and 77G (Table 2). Median unbiased estimation to account for possible differences of study cohorts¹³ led to the same conclusion (data not shown). Analysis with SSc patients from both studies, stratified for sex and carriage of autoantibodies (only including patients described previously⁶ with available subgroup information), also did not show an association (Table 2).

DISCUSSION

There is compelling evidence for functional implications of the PTPRC 77G variant. However, its general epidemiological influence on autoimmune diseases remains controversial. Seven association studies reported an association, 16 others did not^{1,14}.

Epidemiological analyses of PTPRC C77G are generally challenged by the low allele frequency of the G allele. Our study was sufficiently powered to replicate the reported association for SSc. An increase of the 77G allele in cases had to be detected in our population if the 77G allele had an odds ratio > 1.4 (based on a power level of 80%). Since we did not observe an increase of the 77G allele, the effect size of the 77G allele, if existent, would have to be lower than 1.4 in our population. This is considerably less than the initially described allelic odds ratio of 5.3⁶.

The results of our study and of the combined analysis do not confirm PTPRC C77G as a general and independent risk

Table 1. Distribution of PTPRC C77G variant in 171 SSc patients and 179 healthy controls.

Group	Individuals Tested, n	77C/G Heterozygous Individuals, n (%)	77G Allele Frequency, n (%)	Allelic Odds Ratio (95% CI)	p
Healthy controls	179	5 (2.8)	5 (1.4)		
Female	143	4 (2.8)	4 (1.4)		
Male	36	1 (2.8)	1 (1.4)		
SSc patients	171	2 (1.2)	2 (0.6)	0.4 (0.1–2.2)	0.45
Female	139	2 (1.4)	2 (0.7)	0.5 (0.1–2.8)	0.69
Male	32	0 (0)	0 (0)	—	1.00
ATA+ SSc patients	68	0 (0)	0 (0)	—	0.33
ACA+ SSc patients	73	2 (2.7)	2 (1.4)	1 (0.2–5.1)	1.00
Diffuse cutaneous SSc	61	0 (0)	0 (0)	—	0.34
Limited cutaneous SSc	85	2 (2.4)	2 (1.2)	0.8 (0.2–4.4)	1.00
SSc with overlap syndrome	17	2 (11.8)	2 (5.9)	4.4 (0.8–23.7)	0.12

ATA: anti-topoisomerase I antibodies; ACA: anticentromere antibodies.

Table 2. Distribution of *PTPRC* C77G variant in 238 SSc patients and 384 healthy controls (combined data from our present and the previous study⁶).

Group	Individuals Tested, n	77C/G Heterozygous Individuals, n (%)	77G Allele Frequency, n (%)	Allelic Odds Ratio (95% CI)	p
Healthy controls	384	8 (2.1)	8 (1.0)		
SSc patients	238	7 (2.9)	7 (1.5)	1.4 (0.5–3.9)	0.60
Female	169	6 (3.6)	6 (1.8)	1.7 (0.6–5.0)*	0.38*
Male	37	1 (2.7)	1 (1.4)	1.3 (0.2–10.6)*	0.56*
ATA+ SSc patients	77	2 (2.6)	2 (1.3)	1.3 (0.3–5.9)	0.68
ACA+ SSc patients	85	4 (4.7)	4 (2.4)	2.3 (0.7–7.7)	0.25

* Calculated using all controls (N = 384). ATA: anti-topoisomerase I antibodies; ACA: anticentromere antibodies.

factor for development of SSc. Nevertheless, this polymorphism may contribute to SSc with a small effect, possibly in the sense of a modifier gene, or in small subgroups of patients¹⁵.

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