CONCISE REPORT

Lack of association between the protein tyrosine phosphatase non-receptor 22 (*PTPN22*)*620W allele and systemic sclerosis in the French Caucasian population

J Wipff, Y Allanore, A Kahan, O Meyer, L Mouthon, L Guillevin, C Pierlot, E Glikmans, T Bardin, C Boileau, F Cornélis, P Dieudé

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The minor allele of the *R620W* missense single-nucleotide polymorphism (SNP; *rs2476601*) in the *PTPN22* (protein tyrosine phosphatase non-receptor 22) gene has been reported to be associated with multiple autoimmune diseases, including type 1 diabetes, systemic lupus erythematosus, rheumatoid arthritis, juvenile idiopathic arthritis, autoimmune thyroiditis and vitiligo. Systemic sclerosis (SSc) is a connective tissue disease with some autoimmune abnormalities. The aim of our study was to test for association of the *PTPN22*620W* allele with SSc in a French Caucasian cohort with a case–control study of 121 patients with SSc and 103 controls. All patients and controls were genotyped for the *PTPN22*R620W* SNP. No association was found between the *PTPN22*620W* allele and SSc (7% *v* 9.2%, p = 0.39). The frequency of genotypes carrying at least one *620W* allele was similar in both groups (13% *v* 17%, p = 0.38). The *PTPN22*620W* allele was also not associated with autoantibody patterns. Thus, the *PTPN22*R620W* polymorphism cannot be regarded as a genetic susceptibility factor for SSc in the French Caucasian population.

The protein tyrosine phosphatase non-receptor 22 (*PTPN22*) gene encodes the lymphoid tyrosine phosphatase that belongs to a family of intracellular tyrosine phosphatases. This molecule interacts with the SH3 domain of an intracellular tyrosine kinase (Csk). This binding leads to the inactivation of Lck, an Src family kinase, which is associated in early T cell signalling. Inhibition of the phosphatase activity of PTPN22 leads to T cell activation. PTPN22 activity is associated with a negative regulatory effect on T cell activation.[1] The *PTPN22*R620W* single-nucleotide polymorphism (SNP), located in the N-terminal SH3-binding domain of the protein, which is necessary for Csk interaction, results in the substitution of an arginine by a tryptophan. The 620W variant disrupts the binding of PTPN22 to Csk.[1] In vitro functional analysis showed that the 620W variant binds less efficiently than the 620R variant to Csk, preventing the down regulation of T cell activation, consistent with greater susceptibility to autoimmunity. The *PTPN22*620W* allele has already been reported to be associated with multiple autoimmune diseases: type 1 diabetes,[2] systemic lupus erythematosus,[3] rheumatoid arthritis,[4] juvenile idiopathic arthritis[5] and autoimmune thyroiditis.[6] These genetic data show that certain susceptibility alleles are common to several autoimmune diseases.

Systemic sclerosis (SSc) is a connective tissue disease characterised by the activation of mononuclear cells (T and B lymphocytes and monocytes), with the production of specific autoantibodies (anti-topoisomerase, anti-centromere) and cytokines. We used a case–control study design to assess the association of the *PTPN22*620W* allele with SSc in the French Caucasian population.

MATERIAL AND METHODS

A case–control study was conducted to investigate the *PTPN22-1858C/T* SNP in SSc for the French Caucasian population. The French Caucasian origin of the patients is defined by the four grandparents being French Caucasian. Patients with SSc were recruited from the French rheumatology and internal medicine departments. The following clinical data were collected: age, sex, disease duration (date of first non-Raynaud symptom), cutaneous SSc subtype according to the definition by LeRoy *et al*,[7] pulmonary involvement, with pulmonary fibrosis on computed tomography and restrictive syndrome with a Forced Vital Capacity <75%, and vascular involvement, with arterial pulmonary hypertension defined by catheterism. The following immunological tests were conducted: anti-centromere antibodies (immunofluorescence on Hep2 cells) and anti-topoisomerase I (counterimmunoelectrophoresis). All patients gave written informed consent and the Ethics Committee of Cochin Hospital, France, approved the study.

Genomic DNA was purified from fresh peripheral blood leucocytes by standard methods. Genotyping of *PTPN22-1858C/T* SNP was carried out by polymerase chain reaction-restriction fragment length polymorphism. Sense and antisense primers were 5′-GATAATGTTGCTTCAACGGAATTT-3′ and 5′-CCATCCCACACTTTATTTTATACT-3′, respectively. The *PTPN22-1858C/T* transition at codon 620 eliminates a restriction site for *Rsa*I in the *1848T* allele. Genotypes of all patients with SSc and of controls were checked with the polymerase chain reaction-restriction fragment length polymorphism using the *Xcm*I enzyme, for which the *1858T* allele creates a restriction site. Each genotype was interpreted independently by two of the author group.

The Hardy–Weinberg equilibrium of the *PTPN22-1858C/T* polymorphism was investigated with a χ^2 test with one degree of freedom. The χ^2 test was also used to compare allele and genotype frequencies between cases and controls, and values of $p<0.05$ were considered to be significant.

Abbreviations: Csk, intracellular tyrosine kinase; *PTPN22*, protein tyrosine phosphatase non-receptor 22 gene; SNP, single-nucleotide polymorphism; SSc, systemic sclerosis

Table 1 Frequency of *PTPN22*R620W* genotypes in all patients with SSc and in patients with SSc with autoantibodies (anti-topoisomerase I and anti-centromere) and in controls

*PTPN22*R620W* genotype	Patients with SSc (n = 121)	Patients with SSc with anti-topoisomerase I antibodies (n = 30)	Patients with SSc with anti-centromere antibodies (n = 20)	Controls (n = 103)	p Value
R/W and *W/W*	16 (13)	4 (13)	0 (0)	18 (17)	0.38*
R/R	105 (87)	26 (87)	20 (100)	85 (83)	0.99†
R/W	15 (12)	4 (13)	0 (0)	17 (16)	0.12‡
W/W	1 (1)	0 (0)	0 (0)	1 (1)	

Values are n (%).
PTPN22, protein tyrosine phosphatase non-receptor 22; SSc, systemic sclerosis.
*PTPN22*R620W* genotyping was carried out by polymerase chain reaction-restriction fragment length polymorphism analysis. Sense and anti-sense primers were 5′-GATATGTTGCTTCAACGGAATTT-3′ and 5′-CCATCCCACACTTTATTTTATACT-3′, respectively. The *PTPN22-1858C/T* transition eliminates an *Rsa*I restriction site. The results were confirmed using *Xcm*I, for which a restriction site is created in the *1858T* allele. Allele and genotype frequencies were compared with the χ^2 test. Differences were considered significant if $p<0.05$.
*Comparison between patients with SSc and controls.
†Comparison between patients with SSc with anti-topoisomerase I antibodies and controls.
‡Comparison between SSc patients with anti-centromere antibodies and controls.

RESULTS

In all, 121 patients with SSc, fulfilling the criteria of LeRoy *et al*,[7] were included from two rheumatology departments and one internal medicine department in France. All patients responded to the Caucasian origin criteria and the characteristics of the patient population were as follows: mean age was 56.9 (SD 12.9) years and mean disease duration 8.6(7.8) years; 42% (n = 51) had the diffuse cutaneous subtype and 58% (n = 70) had the limited cutaneous form; 25% (n = 30) were anti-topoisomerase antibody positive and 17% (n = 20) were anti-centromere antibody positive. The control group consisted of 103 unrelated, matched patients, with French Caucasian origin ascertained by interrogatory confirmation that each control had four grandparents of French Caucasian origin, who have osteoarthritis but not SSc or other autoimmune diseases.

The SNP tested was at Hardy–Weinberg equilibrium in both the patient and control populations. Our sample of 121 patients and 103 controls yielded 90% and 86% power, respectively, at the 5% level (one sided) to detect an absolute difference of 10% between the two groups, on the basis of an expected allele frequency of 8.7% in controls.[4]

We found no marked difference in *PTPN22*620W* allele frequencies between the patients and the controls (7% *v* 9.2%). The frequency of genotypes carrying the *620W* allele (*R/W* and *W/W*) did not differ between patients (13%) and controls (17%; table 1). Moreover, patients whose organs were affected had allele frequency similar to that of the controls (data not shown).

Following previous reports of an association between the *PTPN22*620W* allele and rheumatoid arthritis being restricted to rheumatoid factor-seropositive patients,[4 8] we carried out a second analysis, taking into account the antibody (anti-centromere or anti-topoisomerase) status of the patients with SSc. The frequency of the *620W* allele or of genotypes carrying the suspected allele was not higher in the subsample of patients with SSc testing positive for autoantibodies than in the remaining patients or controls (table 1). We also found no difference in the frequency of the *620W* allele or suspected genotypes when anti-topoisomerase antibody-positive patients with SSc were compared with those who were anti-centromere antibody-positive and anti-topoisomerase antibody-negative (data not shown).

DISCUSSION

This is the first study to investigate the association of the *PTPN22*R620W* SNP in genetic susceptibility to SSc in the French Caucasian population. Our results suggest that this functional SNP, despite its associations with many other autoimmune disorders,[2–6] is not associated with SSc in French Caucasian patients. In SSc, the pattern of autoantibody production is usually specific to the cutaneous form of the disease: anti-topoisomerase I antibodies for the diffuse cutaneous form and anti-centromere antibodies for the limited form. The frequency of the *PTPN22*620W* allele, however, was no higher in patients testing positive for autoantibodies than in those testing negative for such antibodies and also controls.

Autoimmune abnormalities are thought to occur in the pathogenesis of SSc: oligoclonal T lymphocytes have been shown to infiltrate the skin of patients with SSc, to be activated more often in blood, to produce IL4 and to be required for the production of autoantibodies.[9] B lymphocytes also have an important role, as shown by the hyperactivation of CD19 signalling,[10] by the ability of autoantibodies against fibroblasts and against topoisomerase I to bind to the fibroblast surface and for the autoantibodies against fibroblasts to induce a pro-inflammatory phenotype in these cells.[11] *PTPN22*R620W*, which has been reported to be associated with multiple autoimmune diseases,[9] was not associated with SSc in our population. A lack of correlation has also been reported for some other autoimmune disorders (multiple sclerosis,[12] primary Sjögren's syndrome[13] and ankylosing spondylitis[14]). These data suggest that *PTPN22*620W* is a susceptibility allele common to many, but not all, autoimmune phenotypes. One other SNP, however, was recently reported to be associated with rheumatoid arthritis independently of the *620W* allele.[15] The possible association of this new polymorphism in SSc should be investigated before concluding that there is no association between *PTPN22* and SSc. Moreover, patients of other origins should also be tested for the two polymorphisms before ruling out a possible role of *PTPN22* in SSc.

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Authors' affiliations

J Wipff, A Kahan, Rheumatology A Department, Paris 5 Descartes University, Cochin Hospital, Paris, France
Y Allanore, C Boileau, INSERM U781, Paris S Descartes University, Necker Hospital, Paris
O Meyer, Rheumatology Department, Paris 12 University, Bichat Hospital, Paris
L Mouthon, L Guillevin, Internal Medicine Department, Paris 5 Descartes University, Cochin Hospital, Paris
C Pierlot, E Glikmans, F Cornélis, P Dieudé, GenHotel-EA3886, Evry-Genopole, University Evry-Paris 7 Medical School, Paris
T Bardin, Fédération de Rheumatologie—Centre Viggo Petersen, Lariboisiere Hospital, AP-HP, Paris

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Correspondence to: Y Allanore, Service de Rhumatologie A, hôpital Cochin, 27 rue du faubourg St Jacques, 75014 Paris, France; yannick.allanore@cch.ap-hop-paris.fr

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