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

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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*620W 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*620W polymorphism cannot be regarded as a genetic susceptibility factor for SSc in the French Caucasian population.

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., 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'-GATAATTTGCTTCACACCGAAATTTT-3' and 5'-CATCCACACCTTTATTTATATCT-3', respectively. The PTPN22-1858C/T transition at codon 620 eliminates a restriction site for Rsal in the 1858T allele. Genotypes of all patients with SSc and of controls were checked with the polymerase chain reaction-restriction fragment length polymorphism using the XcmI enzyme, for which the 1858T allele creates a restriction site. Each genotype was interpreted independently by both of the author group.

The Hardy–Weinberg equilibrium of the PTPN22-1858C/T polymorphism was investigated with a χ² test with one degree of freedom. The χ² 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.
RESULTS

In all, 121 patients with SSc, fulfilling the criteria of LeRoy et al., 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 87 (n = 121) patients with SSc and 17% (table 1). Moreover, patients whose organs (9.2%). The frequency of genotypes carrying the suspected allele was not higher in the subsample of patients with SSc 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. B lymphocytes also have an important role, as shown by the hyperactivation of CD19 signalling, 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. PTPN22 R620W, which has been reported to be associated with multiple autoimmune diseases, was not associated with SSc in our population. A lack of correlation has also been reported for some other autoimmune disorders (multiple sclerosis, primary Sjögren’s syndrome and ankylosing spondylitis). These data suggest that PTPN22 R620W 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. 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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