

Table 2. Frequency of the *SCL22A4* and *RUNX1* genotypes in patients and controls*

Genotype, slc2F1	runx1						slc2F1/runx1, OR (95% CI)		
	Patients (n = 865)			Controls (n = 939)					
	11	12	22	11	12	22	11	12	22
11	138	180	60	156	197	72	referent	1.03 (0.76–1.40)	0.94 (0.52–1.42)
12	129	199	56	134	211	76	1.09 (0.78–1.52)	1.07 (0.79–1.44)	0.83 (0.55–1.26)
22	34	56	13	23	51	19	1.67 (0.94–2.97)	1.24 (0.80–1.93)	0.77 (0.37–1.62)

* OR = odds ratio; 95% CI = 95% confidence interval.

this dissimilar allele distribution and the failure to confirm the association. The *SLC22A4* gene may possibly play a role in disease susceptibility only in the presence of ethnic-specific environmental or genetic factors. However, because subjects in the present study had the same ethnic background as those in the original study, population-specific differences in environment or LD between our samples and the original samples seem unlikely to have caused this discrepancy.

The lack of confirmation of the previous association by more powerful population studies, irrespective of ethnic background, casts doubt on the previously identified association between the *SLC22A4* polymorphism and RA. However, the importance of the original findings warrants further study of the role of these genes in disease susceptibility.

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Association of the *PTPN22* C1858T single-nucleotide polymorphism with rheumatoid arthritis phenotypes in an inception cohort

A missense single-nucleotide polymorphism (SNP) (rs2476601, C1858T) in *PTPN22*, which encodes a tyrosine phosphatase, has been associated with multiple autoimmune diseases including rheumatoid arthritis (RA) (1–3). This SNP results in the substitution of a conserved arginine with tryptophan at codon 620 (R620W) in the protein's SH3-binding domain. The 1858T risk allele, which disrupts the interaction between *PTPN22* and the C-Src kinase CSK (3), potentially alters these proteins' function as negative regulators of T cell activation. In the present study, we investigated the association of the *PTPN22* C1858T SNP with RA, undifferentiated arthritis (UA), and both quantitative (rate of joint destruction) and qualitative (autoantibody status and remission or progression) RA characteristics.

All RA cases (n = 416) were participants in the Leiden Early Arthritis Clinic (EAC), a population-based inception cohort of patients with recent onset arthritis described by van Aken et al (4), and fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) criteria for RA (5) at 1 year followup. EAC participants who could not be properly classified at 1 year (n = 265) were categorized as UA cases. All of the patients and unrelated control individuals (6) were Dutch Caucasians. The appropriate institutional review boards approved the protocol.

The C1858T SNP and HLA-DRB1 alleles were genotyped as previously described (1,7), and genotyping accuracy was >99.8%. Individuals were considered positive for the shared epitope (SE) if they carried at least 1 copy of any of the following HLA-DRB1 alleles: 0101, 0102, 0401, 0404, 0405, 0408, 1001, or 1402. Radiography of hands and feet was performed at baseline, 6 months, 1 year, and annually thereafter. The chi-square test or Fisher's exact test, unconditional logistic regression, and tests for trend were used for statistical analysis. Reported *P* values are 2-tailed; values less than 0.05 were considered significant.

PTPN22 C1858T genotypes were generated for 416 RA cases, 265 UA cases, and 891 controls, and were in

Hardy-Weinberg equilibrium in all groups. The frequency of the *PTPN22* risk allele in our control group was similar to previously reported allele frequencies in US white controls (0.091 and 0.087, respectively) (1). We observed a higher frequency of the 1858T risk allele in RA cases compared with controls (0.119 versus 0.091; $P = 0.0257$) (Table 1). Genotypic analysis showed an increased risk of RA associated with the presence of 1 or 2 copies of the 1858T allele (odds ratio [OR] 1.37, $P = 0.0336$). Consistent with previous reports (1,2), TT homozygotes (OR 1.95) appeared to be at greater RA risk than CT heterozygous individuals (OR 1.34) when compared with CC homozygotes.

Next, we investigated this SNP in the patients stratified according to autoantibody status (Table 1). Compared with the allele frequency in the controls, the 1858T allele frequency was elevated in both rheumatoid factor (RF)-positive ($P = 0.0091$) and cyclic citrullinated peptide (CCP)-positive ($P = 0.0132$) RA patients, but not in patients who were negative for these autoantibodies. Genotypic analysis revealed that carriers of the 1858T allele were at an increased risk for RF-positive RA ($P = 0.0173$) and anti-CCP-positive RA ($P = 0.0237$). This was not the case for autoantibody-negative patients, which suggests that there is an association between the *PTPN22* 1858T risk allele and autoantibody production in RA. Consistent with findings in previous studies (1,2), *PTPN22* C1858T genotype frequencies were similar in HLA-DRB1 SE-positive and SE-negative cases (0.125 versus 0.105; $P = 0.4022$). This suggests that the *PTPN22* risk allele acts independently of HLA-DRB1 susceptibility alleles to influence RA risk.

Allele frequencies of C1858T were similar in 45 RA patients whose disease entered remission (defined as absence of arthritis without use of disease-modifying antirheumatic drugs or nonsteroidal antiinflammatory drugs) versus 319 patients with persistent inflammation (0.10 versus 0.118; $P = 0.707$) (8). Mean baseline and yearly Sharp-van der Heijde scores (9) of radiographs of the hands and feet of RA patients with different *PTPN22* genotypes (Figure 1) were also similar in 1858T carriers and noncarriers. These data suggest that there is no association of the *PTPN22* risk allele with the rate of joint destruction.

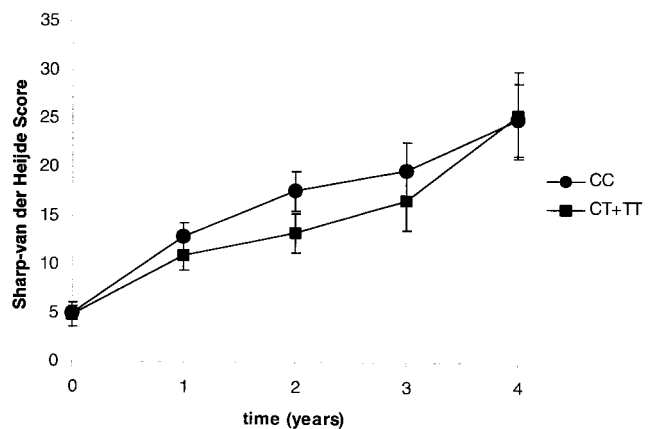


Figure 1. Sharp-van der Heijde scores over time among rheumatoid arthritis patients, by *PTPN22* 1858T carrier status (CT + TT versus CC). Radiographs of the hands and feet of 220 CC and 68 CT + TT patients were available at baseline. Data were analyzed for 254 CC and 72 CT + TT patients at year 1, 178 and 56 at year 2, 117 and 36 at year 3, and 101 and 27 at year 4, respectively. Values are the mean ± SEM.

The 1858T allele frequency was also elevated in UA cases compared controls (0.126 versus 0.091; $P = 0.0163$) (Table 1). Genotypic analysis indicated that carriers of the 1858T allele were at significantly higher risk of UA (OR 1.51).

These results support the role of the *PTPN22* C1858T SNP as a common, HLA-independent, genetic risk factor for RA. Moreover, this study also replicated the dosage effect that occurs when comparing CT and TT risk genotypes (1–3). We confirm the predominant *PTPN22* association with RF-positive RA (1,2) and provide the first evidence that this SNP is also associated with anti-CCP-positive RA. These findings support the hypothesis that the *PTPN22*-1858T variant may predispose individuals to autoimmunity by facilitating the production of certain disease-associated autoantibodies (1,2). Although no association between the *PTPN22* C1858T SNP and rate of RA

Table 1. *PTPN22* C1858T SNP case-control analysis*

Study group	T		Genotype			CT, OR (95% CI)	TT, OR (95% CI)	$P_{\text{trend}}^{\ddagger}$	CT + TT	
	Frequency	P^{\dagger}	CC	CT	TT				OR (95% CI)	P^{\S}
Controls (n = 891)	0.091		736	148	7					
RA cases (n = 416) [¶]	0.119	0.0257	323	87	6	1.34 (1.00–1.80)	1.95 (0.65–5.86)	0.0838	1.37 (1.03–1.82)	0.0336
Rheumatoid factor										
Positive (n = 249)	0.131	0.0091	189	55	5	1.45 (1.02–2.05)	2.78 (0.87–8.86)	0.0319	1.51 (1.08–2.11)	0.0173
Negative (n = 167)	0.102	0.5288	134	32	1	1.19 (0.78–1.82)	0.79 (0.10–6.43)	0.7058	1.17 (0.77–1.78)	0.4636
Anti-CCP [#]										
Positive (n = 197)	0.132	0.0132	149	44	4	1.47 (1.00–2.15)	2.82 (0.82–9.76)	0.0447	1.53 (1.06–2.21)	0.0237
Negative (n = 153)	0.092	0.9734	125	28	0	1.11 (0.71–1.74)		0.8936	1.06 (0.68–1.66)	0.7858
UA (n = 265) [¶]	0.126	0.0163	201	61	3	1.51 (1.08–2.11)	1.57 (0.40–6.12)	0.0492	1.51 (1.09–2.10)	0.0141

* SNP = single nucleotide polymorphism; 95% CI = 95% confidence interval; RA = rheumatoid arthritis; anti-CCP = anti-cyclic citrullinated peptide; UA = undifferentiated arthritis.

[†] Significance of the difference in allele frequency compared with control group.

[‡] Test of linear trend for genotypic odds ratios (ORs).

[§] Significance of the genotypic OR, 1858T carriers versus noncarriers.

[¶] One-year diagnosis.

[#] Data were not available for all cases.

joint destruction, remission, and progression was observed in this study, the sample size is too small to rule out the possibility of a Type II error. Our results also indicate that the 1858T allele is a risk factor for UA. In conclusion, our data suggest that the *PTPN22* 1858T variant acts as a susceptibility allele for autoantibody-positive RA, but does not appear to influence RA severity.

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Soluble complement receptor 1 (CD35) bound to immune complexes in sera of patients with systemic lupus erythematosus

Human complement receptor 1 (CR1, CD35, C3b/C4b receptor) is a membrane glycoprotein found on erythrocytes

and most white blood cells. Reduced CR1 expression has been reported in patients with systemic lupus erythematosus (SLE) (1,2). The origin of soluble CR1 (sCR1) might be direct secretion or proteolytic cleavage at the cell surface. Microvesiculation does occur, suggesting that vesiculation can also be the reason for CR1 loss (3). Elevated serum levels of CR1 have been observed in patients with different diseases but not in those with SLE (4). The aim of our study was to define whether a part of sCR1 is bound to the complement component opsonized circulating immune complexes (CICs) in sera from patients with SLE.

A heterogeneous population of 61 patients with SLE and 30 healthy donors was investigated. Patients fulfilled at least 4 criteria for a diagnosis of SLE as defined by the American College of Rheumatology (5) but were chosen irrespective of their disease duration, disease activity, and the therapy used. Three groups of patients were formed, based on the level of renal involvement. Patients in group I (60.6%) had urinary abnormalities classified as World Health Organization (WHO) class I, 19.7% of patients (group II) were designated as WHO class II and III, and 19.7% of the patients (group III) had diffuse proliferative glomerulonephritis and were designated as WHO class IV. Informed consent was obtained from both patients and healthy controls.

In order to quantitate serum sCR1 CICs, a novel enzyme-linked immunosorbent assay (ELISA) was developed in which anti-CR1 mouse monoclonal antibody HB8592 (a generous gift from R. P. Taylor) as capture antibody and mouse IgG1 as control were fixed on flat-bottomed microtiter plates (Greiner, Frickenhausen, Germany). Serum samples obtained from patients and controls were added (1:40 dilution) to phosphate buffered saline, and the plates were incubated for 1.5 hours at 37°C and washed. The IgG components of CICs were recognized by an anti-human IgG conjugated with horseradish peroxidase (Dako, Glostrup, Denmark). The color was developed by *o*-phenylenediamine–H₂O₂, and absorbances were measured with an ELISA reader (Labsystems Multiscan MS, Helsinki, Finland) at 492 nm. The mean background binding of mouse IgG1 was subtracted from the optical density measured for the samples of patients, and the results were expressed as nanograms per milliliter of equivalent human IgG on the basis of an IgG calibration curve. The level of sCR1 in the same sera was determined by a capture ELISA (4). The immune complex levels in the same sera were determined with a commercial enzyme immunoassay (C1q-IC; Hycor Biomedical, Penicuik, Scotland, UK), according to the manufacturer's instructions.

The analytic properties of the novel assay were evaluated, and the intraassay and interassay coefficients of variation were 9.3% and 10.6%, respectively. The mean \pm SD of the sCR1 CIC concentration was significantly higher ($P < 0.0001$) in sera from 61 patients with SLE than that in sera from 30 controls (Figure 1). The average value of sCR1 CIC in group II ($n = 12$) and group III ($n = 12$) was also significantly increased ($P = 0.001$) compared with group I ($n = 37$), indicating that sCR1 CIC levels are elevated in groups having severe histologic lesions. The sCR1 CIC and C1q immune complex levels in patients, as determined by these ELISAs, were compared by Pearson's regression analysis. A strong correlation ($r = 0.844$, $P < 0.0001$) was observed between the C1q-immune complex and sCR1 CIC concentrations in sera from patients with SLE. The levels of sCR1 found in sera from patients with SLE were not different from those observed in