Genetics, autoantibodies and clinical features in understanding and predicting rheumatoid arthritis

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Chapter 4

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

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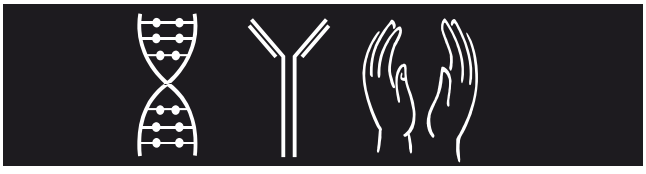

A missense SNP (rs2476601, C1858T) in *PTPN22,* which encodes a tyrosine phosphatase, has been associated with multiple autoimmune diseases including RA (1-3). This SNP results in the substitution of a conserved arginine with tryptophan at codon 620 (R620W) in the protein's SH-3-binding domain. The 1858T risk allele, which results in disruption of the interaction between PTPN22 and the C-Src kinase, Csk, (3) potentially alters these proteins' function as negative regulators of T-cell activation. In this study, we investgate 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 recent onset arthritis described by van Aken et al (4), and fulfilled ACR criteria for RA at one year follow-up. EAC participants who could not be properly classified at one year (n=265) were categorized as UA cases. All patients and unrelated control individuals (described in reference 5) were Dutch whites. Appropriate institutional review boards approved the protocol.

The C1858T SNP and HLA-DRB1 alleles were genotyped as described and genotyping accuracy was > 99.8% (1,6). An individual was considered positive for the shared epitope (SE) if they carried at least one copy of any of the following HLA-DRB1 alleles: 0101, 0102, 0401, 0404, 0405, 0408, 1001 or 1402. X-rays of hands and feet were taken at baseline, six months, one year and annually thereafter. The χ^2 test or Fisher's exact test, unconditional logistic regression and tests for trend were used for statistical analysis. Reported p-values are two-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 to controls (0.119 vs. 0.091; p=0.0257) (Table 1). Genotypic analysis showed an increase in risk of RA associated with having one or two copies of the 1858T allele (OR=1.37; p=0.0336). In agreement with previous reports (1-2), TT homozygotes (OR=1.95) appeared to be at greater RA risk than TC heterozygous individuals (OR=1.34) when compared to CC homozygotes.

Next we investigated this SNP in the patients stratified according to autoantibody status (Table 1). Compared to the controls, the 1858T allele frequency was elevated in both RF positive (p=0.0091) and CCP positive (p=0.0132) RA patients but not in patients negative for these autoantibodies. Genotypic analysis showed that carriers of the 1858T allele were

Table 1. *PTPN22* C1858T SNP case-control analysis[a]

	Frequency		Genotype			CT	TT		CT + TT	
Study Group	(T)	P[b]	CC	CT	TT	OR (95% CI)	OR (95% CI)	P_{Trend}[c]	OR (95% CI)	P[d]
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[f]										
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

[a]SNP = single nucleotide polymorphism; OR = odds ratio; 95% CI = 95% confidence interval; RA = rheumatoid arthritis; CCP = cyclic citrullinated peptide; UA = undifferentiated arthritis.

[b]Test of significant difference in allele frequency compared to control group.

[c]Test of linear trend for genotypic ORs.

[d]Test of significant genotypic OR, 1858T carriers vs. non-carriers.

[e]One year diagnosis.

[f]Data were not available for all cases.

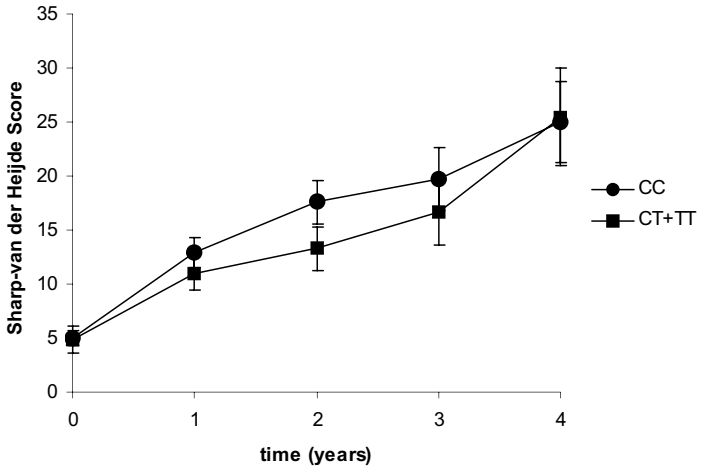

Figure 1. Sharp-van der Heijde scores (mean ± SEM) over time among RA patients, by *PTPN22* 1858T carrier status (CT+TT vs. CC). Radiological data (X-rays of hands and feet) of 220 CC and 68 CT+TT patients were available at baseline. Respectively, data for 254 and 72 patients at year 1, 178 and 56 patients at year 2, 117 and 36 patients at year 3, and 101 and 27 patients at year 4 were analyzed.

at increased risk for RF positive (p=0.0173) and anti-CCP positive RA (0.0237). This was not the case for autoantibody negative patients suggesting an association between the *PTPN22* 1858T risk allele and autoantibody production in RA. Consistent with previous studies (1-2), *PTPN22* C1858T genotype frequencies were similar in HLA-DRB1 shared epitope (SE)-positive and SE-negative cases (0.125 vs. 0.105; p=0.4022), suggesting that the *PTPN22* risk allele acts independently of HLA-DRB1 susceptible alleles to influence RA risk.

Allele frequencies of C1858T were similar in 45 RA patients who achieved remission (defined as absence of arthritis with no use of DMARDs for at least one year) versus 319 patients with persistent inflammation (0.10 vs. 0.118, p=0.707)(7). Mean baseline and yearly Sharp-Van der Heijde scores of X-rays of hand and feet of RA patients with different *PTPN22* genotypes (Figure 1) were also similar in 1858T carriers and non-carriers (8). These data suggest no association of the *PTPN22* risk allele with rate of joint destruction.

The 1858T allele frequency was also elevated in UA cases compared controls (0.126 vs. 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* C*1858*T SNP as a common, HLA-independent, genetic risk factor for RA. Moreover, this study also replicated the dosage effect 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 this 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 the level of RA joint destruction and remission 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 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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