Chapter 5

Risk of progression from undifferentiated arthritis to rheumatoid arthritis: the effect of the PTPN22 1858T-allele in anti-citrullinated peptide antibody positive patients

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Abstract

Objectives. Anti-citrullinated peptide antibodies (ACPA) and the C1858T missense single-nucleotide polymorphism (SNP) in the PTPN22 gene are both associated with the development of rheumatoid arthritis (RA). We investigated whether the combination of these two biomarkers yielded better test characteristics to predict progression from undifferentiated arthritis (UA) to RA compared to ACPA alone.

Methods. Three-hundred ninety-four individuals with UA from a Dutch population-based inception cohort were included in this study. At baseline ACPA were measured and the PTPN22 C1858T and HLA-DRB1 genotypes determined. Progression to RA was monitored at one year after entry into the cohort.

Results. A priori, UA patients had a 35% (95% CI 30-40%) risk of developing RA, which increased to 66% (95% CI 57-75%) in patients who were ACPA-positive. There was an additional although non-significant (p = 0.34) increase in RA risk to 76% (95%CI 57-90%) when patients were positive for both ACPA and the PTPN22 1858T-allele. The area under the receiver operator characteristic curve increased from 0.68 for ACPA-status alone to 0.70 for the combination of ACPA-status and the PTPN22 C1858T polymorphism. In logistic regression analysis, ACPA predicted RA-development independent of PTPN22, while the PTPN22 polymorphism had no independent effect. In HLA-DRB1 shared epitope positive, ACPA-positive UA patients, ACPA-levels were significantly increased in PTPN22 1858T allele carriers compared to non-1858T carriers.

Conclusions. In this Dutch cohort of UA-patients, the PTPN22 1858T allele does not markedly improve individual decision-making to predict RA-development over ACPA alone, but it is associated with higher ACPA-levels.
Introduction

Anti-citrullinated peptide antibodies (ACPA), which can be detected years before disease onset, are highly specific for rheumatoid arthritis (RA) (1-3). Approximately 72% of patients presenting with an early form of arthritis that cannot be properly classified (undifferentiated arthritis, UA) and who are positive for ACPA develop RA (4). The C1858T missense single-nucleotide polymorphism in the PTPN22 gene is also associated with RA, UA (5-11) and several other autoimmune diseases (for a review see 12). The relevance for ACPA-determination is increasingly accepted in clinical practice (3); however, the role of the PTPN22 1858T-allele in RA prediction has not been studied. Consequently, we investigated whether the combination of these two biomarkers improved prediction of progression from UA to RA compared to ACPA alone. Since the PTPN22 1858T-allele confers risk to ACPA-positive arthritis (10) we hypothesized that the PTPN22 C1858T polymorphism may also influence autoantibody levels. Therefore we examined whether ACPA-levels differed between ACPA-positive UA-patients with and without the PTPN22 T-allele.

Material and Methods

Study population.

The Leiden Early Arthritis Clinic (EAC) is a population-based inception cohort of white Dutch patients with recent-onset arthritis (median symptom duration 112 days, IQR 55-239 days) which, at present, includes 1944 individuals (for a detailed description of this cohort see ref 13). A subset of these patients (n=394), who could not be classified according to the 1987 ACR criteria at baseline and were categorized as having undifferentiated arthritis (UA), were included in this study. At one year of follow-up, subjects were re-evaluated as having RA or not according to the ACR 1987 criteria (14). Patients who during the one year of follow-up cumulatively fulfilled the ACR criteria were diagnosed as RA and all other patients as non-RA.

Laboratory methods.

Blood specimens, obtained at baseline, were typed for anti-CCP2 antibodies (ACPA) using an ELISA (Immunoscan RA Mark 2; Euro-diagnostica, Arnhem, The Netherlands). The cut-off level for ACPA positivity was set at 25 arbitrary units, according to the manufacturer’s instructions. Rheumatoid factor levels were also
Figure 1. Characteristics of the study population. A. Description of the patient cohort. The RA diagnosis is based on the diagnosis after 1 year of inclusion in the cohort. UA=undifferentiated arthritis, n=number of patients in this group. B. Proportional values for RA-development taking into account ACPA status (positive or negative), the PTPN22 C1858T-polymorphism (T-allele positive or negative) or a combination of these two markers. PPV = positive predictive value, NPV = negative predictive value.

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measured at baseline with an ELISA. The *PTPN22* C1858T polymorphism and HLA-DRB1-alleles were genotyped as previously described (5,15).

**Analysis.**

Statistical analyses were performed using SPSS_12.0 Software (Chicago, IL, USA). 95% confidence intervals (95%CI) of the proportional data were calculated with CIA software (16). Predictive values were compared by logistic regression analysis and by determination of the area under the receiver operator characteristic curve (AUC). The Mann-Whitney test and linear regression analysis were used to compare ACPA levels.

**Results**

Of the 394 UA patients, 138 patients (35%) progressed to RA within one year (Figure 1A). Of these 138, 69 (50%) were positive for ACPA at baseline, compared to 36 of the 256 individuals (14%) in the non-RA group. The *PTPN22* 1858T-allele was present in 21% (n=29) of UA-patients who progressed to RA and in 23% (n=58) of the non-RA group. The unadjusted relative risk (RR) for RA-development was 2.75 (95%CI 2.15-3.53) for ACPA-positivity and 0.94 (95%CI 0.67-1.31) for carriage of the *PTPN22* 1858T allele.

Of the 105 ACPA-positive patients, 66% (95%CI 57-75%) developed RA in 1 year, compared to 24% (95%CI 19-29%) of the 289 ACPA-negative patients. When the ACPA-positive and ACPA-negative patient groups were further stratified for *PTPN22* 1858T-allele carriage, 76% (22 of 29, 95%CI 57-90%) of the ACPA-positive, *PTPN22* 1858T-allele positive patients developed RA, compared to 62% (47 of 76, 95%CI 50-73%) of the ACPA-positive *PTPN22* 1858T-allele negative patients. Although these results show an increased predictive value if the *PTPN22* C1858T polymorphism is combined with ACPA status, this difference was not statistically significant (p=0.34). While ACPA-negative patients had a 24% risk of progressing to RA, information on the presence or absence of *PTPN22* 1858T allele did not significantly alter this probability (Figure 1A). A similar analysis examining the effect of the *PTPN22* 1858T allele conditional on baseline levels of rheumatoid factor showed no significant differences in the probabilities for developing RA (data not shown).

To further understand the role of ACPA and the *PTPN22* 1858T allele in RA risk prediction, we used logistic regression analysis to examine how these factors influenced risk for RA-development, mutually adjusting for both the presence of ACPA and the *PTPN22* 1858T allele. Only ACPA-positivity was identified as an
independent predictive variable in this model (OR 6.3, 95% CI 3.9-10.3, p<0.0001), whereas the \( PTPN22 \) 1858T-allele was not (OR=0.7, 95% CI 0.4-1.3, p=0.282). Addition of the \( PTPN22 \) C1858T polymorphism to ACPA status in the regression model increased the AUC marginally (AUC 0.68 for ACPA alone and 0.70 for both).

Because we previously observed a stronger association of the \( PTPN22 \) C1858T polymorphism with ACPA-positive RA than ACPA-negative RA (10), we stratified and compared the ACPA levels of all 96 ACPA-positive subjects according to \( PTPN22 \) 1858T carriage status. As HLA-DRB1 alleles that encode for a common amino acid sequence, the shared epitope (SE), dominantly influence the level of ACPA (4), this analysis was performed after stratification for the presence or absence of SE-alleles (defined as DRB1*0101, 0102, 0401, 0404, 0405, 0408, 0410, 1001 and 1402) and both SE+ and SE- patients were divided in two groups based on the presence or absence of the \( PTPN22 \) 1858T allele. Only the SE+ patients are shown in the graph.

\( n \) = number of patients in each group, AU=arbitrary units. The lines represent the median of the ACPA antibody levels in each group.

**Figure 2.** Influence of \( PTPN22 \)-polymorphism on anti-CCP (ACPA) levels.

Ninety-six of the 105 UA patients who had ACPA were successfully genotyped for the DRB1 Shared Epitope (SE) alleles (defined as DRB1*0101, 0102, 0401, 0404, 0405, 0408, 0410, 1001 and 1402) and both SE+ and SE- patients were divided in two groups based on the presence or absence of the \( PTPN22 \) 1858T allele. Only the SE+ patients are shown in the graph.

\( n \) = number of patients in each group, AU=arbitrary units. The lines represent the median of the ACPA antibody levels in each group.

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(p=0.04) (Figure 2). No difference by PTPN22 1858T allele was observed among the SE-negative patients (SE PTPN22- n= 21, median 346 units, IQR 80-1712 units; SE- PTPN22+, n=4 median 584 units, IQR 43-1848 units); however, the sample sizes were too small to draw a statistically sound conclusion. Linear regression analysis adjusting for HLA-SE confirmed the independent association of the PTPN22 C1858T polymorphism with ACPA levels in ACPA positive patients (B= 452 units, 95%CI 58-847 units, p=0.03).

Discussion

Previous studies showed that the PTPN22 C1858T polymorphism and ACPA-status are both associated with development of RA in the general population (1,2,5-11). Our study demonstrates an independent association of ACPA but not the PTPN22 1858T allele with progression to RA among patients presenting with UA. Johansson et al. observed that the specificity for future onset of RA in a population of healthy blood donors increased from 98.6%, when only ACPA-status was taken into account, to 100% when both ACPA and PTPN22 C1858T polymorphism were analyzed together (17). They did not determine the sensitivity and specificity of subsequently APCA alone and the combination of ACPA and the PTPN22 1858T allele, but compared the patients positive for both ACPA and the PTPN22 1858T allele with all other patients (including patients positive for either ACPA or the PTPN22 1858T allele or negative for both). This resulted in different values for specificity and sensitivity compared to this study. Since the value of a positive test result as for an individual patient in clinical practice is not measured by the sensitivity or specificity, but is reflected by the a priori and post priori probabilities, the current study evaluated the risk of a cohort of patients presenting with UA to progress to RA given their ACPA and PTPN22 1858T status. The risk to develop RA increased significantly in the case of ACPA-positivity. This risk increased marginally for those ACPA-positive patients who also harbored the PTPN22 1858T allele; however, this increase was not significant. We cannot exclude a Type-II error although, given the effect size, approximately 3000 UA-patients, from which approximately 800 are ACPA positive, would be needed to detect a significant difference. In the ACPA-negative group, knowledge of the PTPN22 C1858T polymorphism did not increase the predictive value of RA development. Moreover, only ACPA was identified as a significant independent predictor for RA-development. These data indicate that in clinical practice, testing for
the $PTPN22$ polymorphism in addition to ACPA-status will be of limited relevance for patients with UA.

The notion that for clinical practice testing of the $PTPN22$ C1858T polymorphism has no additive value to ACPA-determination is not in contrast with the findings that the $PTPN22$ C1858T polymorphism is involved in the pathogenesis of RA. The $PTPN22$ C1858T polymorphism is only associated with ACPA-positive RA, indicating that the $PTPN22$ genotype confers risk to a serologically defined subset of RA. Thus, pathophysiologically both factors are on the same pathway, and by measuring ACPA the effect of the $PTPN22$ 1858T-allele when present, is already included.

It should also be noted that, given there is a gradient in the frequency of the $PTPN22$ 1858T allele that increases from the south (2-3% in Italy) to the north (15.5% in Finland) (for a review see 12) of Europe, the utility of the SNP may vary in different populations.

We also showed that the presence of the $PTPN22$ 1858T-allele in shared epitope positive, ACPA positive UA-patients was associated with higher levels of ACPA. This is of interest as a recent study revealed that ACPA-levels were a predictor for RA-development in ACPA-positive UA-patients (18). The observation that ACPA-positive patients who carry the $PTPN22$ disease-associated 1858T-allele have higher ACPA-levels than those who are homozygous for 1858C is interesting and may shed some light on how the $PTPN22$ 1858T polymorphism, which appears to be a “gain of function” allele, mediates susceptibility to multiple autoimmune diseases (5,19,20).

Given the findings of this study we conclude that (i) testing for the $PTPN22$ C1858T-polymorphism in ACPA-positive UA-patients does not significantly improve prediction of RA development and (ii) the $PTPN22$ T-allele is associated with higher ACPA levels.
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