Contribution of PTPN22 1858T, TNFRII 196R and HLA-shared epitope alleles with rheumatoid factor and anti-citrullinated protein antibodies to very early rheumatoid arthritis diagnosis

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Objectives. To evaluate the predictive value of TNFRII 196R, PTPN22 1858T and HLA-shared epitope (SE) alleles, RFs and anti-citrullinated protein antibodies (ACPAs) for RA diagnosis in a cohort of patients with very early arthritis.

Methods. We followed up 284 patients who had swelling of at least two joints that had persisted for longer than 4 weeks but had been evolving for <6 months. At 2 yrs, patients were classified as having RA or non-RA rheumatic diseases according to the ACR criteria. Patients were genotyped with respect to TNFRII 196R and PTPN22 1858C/T polymorphisms and HLA-SE. The presence of IgA, IgG and IgM RF isotypes and ACPA was sought in sera collected at disease onset.

Results. HLA-SE alleles alone, concomitant presence of TNFRII 196R and PTPN22 1858T alleles, IgA, IgG and IgM RF alone and ACPA were found to be significantly associated with RA diagnosis. Using logistic regression analysis, the concomitant presence of RF and ACPA at disease onset was the best association to predict RA diagnosis. In patients (n = 34) who did not fulfil the ACR criteria for RA at inclusion but who progressed to ACR positivity, the study of the genetic risk markers did not contribute to predict RA diagnosis at 2 yrs.

Conclusions. PTPN22 1858T, TNFRII 196R and HLA-SE alleles do not improve the predictive value of RF and ACPA for RA diagnosis in our cohort, and do not contribute to an earlier diagnosis in undifferentiated patients initially negative for RF and ACPA.

Key words: Rheumatoid arthritis, TNFRII, PTPN22, HLA-DRB1, Diagnosis, Autoantibodies, Anti-citrullinated protein antibodies, Rheumatoid factor.

Introduction

Rheumatoid arthritis (RA), affecting between 0.3% and 1.0% of the population in developed countries, can lead to progressive joint destruction and severe disability. Early diagnosis of RA is crucial in order to initiate DMARDs at the convenient period to prevent joint damage and optimize the functional outcome [1]. Identification of markers that would allow one to establish a diagnosis of RA at the early onset of the disease process is a crucial goal for clinicians. Genetic markers have the advantages of being present from the onset of the disease and of remaining unchanged under therapy. To date, two RA genetic susceptibility factors have been identified: HLA-DRBI-SE [2] and PTPN22 620W alleles [3]. The predictive value of the SE alleles for diagnosis of RA was previously investigated in cohorts of patients with early unclassified arthritis that showed restrained association between RA and the HLA-DRBI-SE [4]. The contribution of HLA to the overall genetic risk has been estimated to range from 30% to 50% [5]. These data suggest that non-HLA genes are involved in RA susceptibility and could represent a helpful tool for diagnosis. In a previous study, we have reported that the TNFRII 196R allele, previously identified as a genetic susceptibility factor in a French Caucasian population [6], was a risk factor for development of RA in a cohort of patients with very early arthritis (VErA cohort) [7]. Recently, a genetic association involving the functional polymorphism of the protein tyrosine phosphatase non-receptor type 22 (PTPN22) gene was reported to be associated with multiple autoimmune diseases such as RA [8–12]. The PTPN22 gene encodes for the intracellular lymphocyte tyrosine phosphatase that acts as a negative regulator of early T-cell activation [13, 14]. The PTPN22 single nucleotide polymorphism (SNP) rs2476601 (I858C/T) results of an amino acid substitution of arginine for tryptophan at position 620 (R620W), in the P1 proline-rich domain. Functional significance of R620W remains unclear since both gain and loss of function have been suggested [15, 16]. ACPAs (anti-citrullinated protein antibodies) have been reported to be specific for RA [17], and thus contribute to the diagnosis of RA even if they lack sensitivity. On the contrary, RF has a stronger sensitivity but a lower specificity for RA. IgM and IgG RF isotypes may respectively have the highest sensitivity and specificity for RA [18]. However, although concomitant positivity of ACPA and IgA-RF has been shown to be highly predictive of RA [19], it has a low sensitivity (<50%) [20, 21].

Taking those data into account, the aim of this study was to assess the contribution of several genetic markers—PTPN22 I858T, TNFRII 196R and HLA-SE alleles—alone or in combination with IgA, IgG and IgM RF isotypes and ACPA, in predicting RA diagnosis in a community-based VErA cohort.

Patients and methods

Patients

Patients studied are those of the VErA cohort that comprises 310 patients with very early arthritis who were prospectively recruited between October 1998 and January 2002 [7]. Briefly, patients were required to have swelling of at least two joints that had persisted for longer than 4 weeks but had been evolving for <6 months (median: 4.2 months, range: 0.9–6.0 months), and who had not received DMARDs and/or steroid therapy before inclusion. The mean (± s.d.) age of the 310 VErA patients was 52.0 yrs (range

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19–84 yrs) and the female/male ratio was 2.17. All were European Caucasians. No information was available concerning the past history of RA in first-degree and second-degree relatives. At 2 yrs of follow-up, VERA patients were evaluated and classified using the ACR 1987 criteria for RA [22]. IgA, IgG and IgM RF isotypes and ACPA (see subsequently) were collected and HLA-SE typing, TNRFII 196R [7] and PTPN22 1858C/T polymorphisms genotyping were performed. Before entry into the protocol, each patient gave written consent after receiving verbal and written information regarding the nature, duration and purpose of the study. The protocol was approved by the Committee for Protection of Persons Participating in Biomedical Research of Rouen (French law 88–1138; 20 December 1988).

Autoantibodies detection

ELISA determination of IgM, IgG and IgA RF isotypes were performed in the Immunology Laboratory of Rouen [23]. ACPA was detected using second generation commercially available kits (EuroImmun, GMBH, GroB Gröna, Germany). ACPA levels ≥10 arbitrary units (AU) were considered as positive.

PTPN22 1858T, TNRFII 196R and HLA-SE alleles genotyping

Genomic DNA used for genotyping was extracted from EDTA anti-coagulated peripheral blood leukocytes using standard methods. Genotypes of all available DNA patients from the VERA cohort were assessed with the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using (i) the XcmI enzyme, for which the PTPN22 1858T allele creates a restriction site and (ii) the NlaIII enzyme for which the substitution at codon 196 [i.e. ATG (methionine) → AGG (arginine)] of TNRFII eliminated the NlaIII restriction site. Each genotype was interpreted independently by two individuals who were unaware of the underlying disease process. The HLA-DRB1 subtyping was performed by PCR using specific primers and hybridization with sequence specific oligonucleotides. Individuals who carried at least one copy of the DRB1 alleles—HLA-DRB1*0101, *0102, *0401, *0404, *0405, *0408, *0410, *1001 and *1402—were classified as SE positive.

Statistical analysis

Fischer’s exact test was performed to look for an association between RA diagnosis and the different genetic and immunological markers measured at baseline, alone or in combination. Student’s t-test or Mann-Whitney U-test were performed to compare autoimmune titres according to PTPN22 1858T and HLA-SE presence. Logistic regression analysis assessed the contribution of the different markers in predicting RA diagnosis (i) for the whole of the patients and (ii) specifically for patients with undifferentiated arthritis (UA) that did not fulfill the ACR criteria at baseline and among them, in a subgroup of patients negative for both RF and ACPA at inclusion. For all tests, \( P < 0.05 \) was considered statistically significant.

Results

Characteristics of the VERA cohort patients included in the study

From the VERA cohort, 284 patients were studied. According to ACR criteria, 161 patients were classified as having RA and 123 as having non-RA disease, including well-defined \( (n = 77) \) and undifferentiated \( (n = 46) \) arthritis after 2 yrs of follow-up. The baseline characteristics of the 284 patients are summarized in Table 1. Over the 2-2 yrs follow-up period, 34 patients who did not fulfill the ACR criteria for RA at inclusion progressed to ACR positivity. Among them, 28 were negative for both RF and ACPA at inclusion.

PTPN22 1858C/T genotypes, 1858T allele and RA diagnosis

A total of 246 patients were genotyped for the PTPN22 1858C/T polymorphism. DNA material was not available for the whole of the patients either because of patient refusal to participate in the genomic study or delayed inclusion of the last patients. Moreover, several genotypes were uninterpretable. Patients carrying the PTPN22 1858 C/C, C/T and T/T genotypes were, respectively, 107, 29 and 5 in the RA subgroup and 83, 22 and 0 in the non-RA subgroup. The PTPN22 1858T/T genotype as well as the PTPN22 1858T allele were not found to be associated with RA diagnosis \( (P = 0.17 \) and 0.64, respectively). PTPN22 1858T allele was not found to be associated with RA patient’s gender \( (P = 0.68) \). In both women and men subgroups, no association was found between the PTPN22 1858T allele and RA (data not shown).

HLA-SEs and RA diagnosis

Among the whole VERA cohort, 280 patients were genotyped for the HLA-SE allele. They were 64 RA and 35 non-RA patients carrying only one copy of HLA-SE allele and eight RA and three non-RA patients carrying two copies of HLA-SE alleles. Carrying two copies of HLA-SE alleles was not associated with RA diagnosis \( (P = 0.08) \). Carrying at least one copy of HLA-SE allele was found to be associated with RA diagnosis \( (P = 0.03); \) odds ratio \( (OR) 1.72; 95\% CI 1.02, 2.92).\n
TNRFII 196R allele and RA diagnosis

We have previously observed in the VERA cohort patients [7] that the TNRFII 196R allele was associated with diagnosis of RA \( [P = 0.002]; \) positive predictive value (PPV) 66.6%; negative predictive value (NPV) 51.9%; OR 2.158; 95% CI 1.284, 3.641).

PTPN22 1858T, TNRFII 196R and HLA-SE alleles

We have investigated the combination of each of these alleles with RA diagnosis (data not shown). The concomitant presence of the PTPN22 1858T and TNRFII 196R alleles was the only combination significantly associated with RA \( (P = 0.04); \) OR 2.97; 95% CI 1.01, 10.6).

RFs and ACPA

Positivity of IgA, IgG and/or IgM isotypes of RF and ACPA, alone or in combination, was found to be associated with RA diagnosis. An infinite OR was found for the concomitant positivity of RF, whatever their isotype and ACPA (Table 2).
Influence of PTPN22 1858T and HLA-SE alleles upon autoantibodies production

Table 3 shows mean titres of IgM, IgG and IgA RF isotypes and ACPA according to the PTPN22 1858 C/T polymorphism and the presence of HLA-SE alleles. RA patients carrying the PTPN22 1858C/T and T/T genotypes had significantly higher ACPA levels (P = 0.03) than those carrying the PTPN22 1858C/C genotype. A trend for a higher IgM RF production in PTPN22 1858T carriers was also observed (P = 0.09). RA patients carrying at least one copy of HLA-SE were more likely to produce IgM (P = 0.01) and IgG (P = 4.2 × 10^{-3}) RF and particularly ACPA (P = 1.4 × 10^{-6}) than other RA patients.

Logistic regression analysis

The question as to whether presence of PTPN22 1858T, TNFRII 196R and/or HLA-SE alleles and/or RF isotypes and ACPA contribute to early diagnosis of RA was investigated with logistic regression analysis (i) for the whole of the patients and (ii) for those classified as having undifferentiated arthritis at the inclusion. In this logistic regression analysis, RA diagnosis was entered as the dependent variable and antibodies and genetic markers were possible explanatory variables. This analysis revealed that the best independent variables were IgA RF and ACPA. The combination of the different autoantibody populations was the best tool to predict RA diagnosis. Thus, using only autoimmune markers, 68% of the whole of the patients were well classified (Sensitivity: 0.54, Specificity: 0.86, PPV: 0.84, NPV: 0.58) (Table 4). The use of genetic markers did not provide additional information to predict RA diagnosis in patients with very early arthritis, even in the sub-groups of RA patients (ACR criteria after 2 yrs of follow-up) who did not fulfill the ACR criteria for RA at inclusion (n = 34) and were also negative for RF and ACPA (n = 28) (data not shown).

Discussion

This study was conducted in a French Caucasian VErA cohort to evaluate the association between several genetic (PTPN22 1858T, TNFRII 196R and/or HLA-SE alleles) and immunological (RF and ACPA) markers and RA diagnosis. The results of this prospective longitudinal study show in RA patients an association between two genetic markers (PTPN22 1858T and HLA-SE alleles) and the production of ACPA, and to a lesser degree that of RF. However, while concomitant presence of RF and ACPA strongly contribute to RA diagnosis, the presence of genetic risk markers do not improve the accuracy of these autoantibodies for the prediction of RA diagnosis in our cohort of patients. The failure to find a diagnostic contribution of the genetic markers might be due to the main weakness of the study that is the limited size of the population studied. Nevertheless, this study has some strengths [well-defined population based cohort, simultaneous analysis of the three genetic markers known to be associated with RA, their contribution compared with gold standard (RF and ACPA)] and most results are in accordance with those observed in previous reports.

The observed PTPN22 1858 CC, CT and TT genotypes frequencies were concordant with the previously reported frequencies in the French [12] and UK [24] Caucasian RA populations. Our study did not detect any effect arising for the homozygous PTPN22 1858TT genotype. However, this could be
secondary to a lack of power as a small number of patients were carrying the homozygous TT genotype \((n = 5)\). The \textit{PTPN22 1858T} allele does not appear to be significantly associated with RA diagnosis and to be a risk factor of progression from undifferentiated arthritis to RA. However, our findings indicate that RA \textit{PTPN22 1858T} allele carriers were likely to produce more ACPA than non-carriers did. This is in accordance with the conclusions of Feitasma \textit{et al.} \cite{25} who have shown that the \textit{PTPN22 1858T} allele does not improve individual decision making to predict RA development over ACPA alone, but that it is associated with higher ACPA levels. In this regard, the \textit{PTPN22 1858T} allele is associated with type 1 diabetes \cite{26} and autoimmune disorders that have a prominent humoral component. This allele may influence thresholds for T-cell receptor signalling and conduct to a higher production of autoantibodies by a more effective B-cell co-stimulation \cite{27}.

Pierer \textit{et al.} \cite{28} have found an increased frequency of the \textit{PTPN22 1858T} allele in male RA patients and suggested that the genetic contribution of this allele to disease pathogenesis might be more prominent in men. On the contrary, we have shown that there was no association of the \textit{PTPN22 1858T} allele with RA patient’s gender and with RA diagnosis in both women and men subgroups. Further studies are needed to assess the contribution of the \textit{PTPN22 1858T} allele in male RA.

Our study’s contribution is also a comparison of the \textit{HLA-SE} alleles frequencies between community-recruited RA and non-RA patients with very early arthritis and similar clinical manifestations at inclusion. Indeed, this study assessed the diagnostic value of the \textit{HLA-SE} alleles for RA. In accordance with previous reports \cite{29, 30}, we noticed a significant association between \textit{HLA-SE} alleles, even for patients carrying only one copy, and RA diagnosis.

Taking into account a single genetic marker for RA diagnosis and/or prognosis could lead to weak performances. In the case of the \textit{TNFRII 196R} allele \cite{6–7}, the relative risk observed was under 3, suggesting the involvement of other genetic markers. Thus, we have tested here for the hypothesis that the \textit{PTPN22 1858T} allele could be part of a diagnostic combination including \textit{HLA-SE} and \textit{TNFRII 196R} alleles. However, the concomitant presence of these three risk alleles was not found to be associated with RA diagnosis in the VErA cohort.

One goal of our study was to determine whether genetic markers identified patients with RA more accurately than RF and ACPA autoantibodies did. Using logistic regression analysis, it appeared that these autoimmune markers were the best parameters to be taken into account to predict the diagnosis of RA in a VErA cohort. The concomitant presence of RF (IgA, IgG and/or IgM isotypes) and ACPA was strongly associated with RA diagnosis and these antibodies were shown to have an higher predictive value for the development of RA than genetic markers had. Our results are in accordance with those of Van der Helm-van Mil \textit{et al.} \cite{31} who observed that the \textit{HLA-SE} alleles do not independently contribute to the progression to RA from undifferentiated arthritis but rather contribute to the development of ACPA. In this study, \textit{HLA-SE} alleles were shown to be associated with an higher production of IgG and IgM RF and notably of ACPA in RA patients. In this respect, the fact that \textit{PTPN22 1858T} and \textit{HLA-SE} alleles are associated with autoantibody production might explain that they could not contribute independently to RA diagnosis. Orozco \textit{et al.} \cite{32} have recently assessed the relationship between the presence of RF, ACPA, \textit{HLA-DRB1} alleles and \textit{PTPN22 1858 C/T} polymorphism and tested the value of their combination as susceptibility markers for RA. Our results are in accordance with theirs since they have shown that (i) the \textit{SE} alleles predispose to the presence of ACPA and (ii) only RF and ACPA were, among all variables included in their logistic regression analysis, the two independent parameters able to predict RA diagnosis, with an OR of 22.4 and 9.8, respectively. However, we cannot conclude like Orozco \textit{et al.} \cite{32} that the combination of the \textit{1858T} variant of \textit{PTPN22} and ACPA gave a higher specificity for RA than ACPA alone since, in our study, presence of this allele was associated with the production of ACPA.

While the contribution of the three genetic markers is modest, that of autoantibodies is particularly pertinent to predict the progression from UA to RA. First, the present study confirms the interest of the concomitant positivity of RF and ACPA as previously shown in other studies. Interestingly, testing for the different isotypes of RF might be of great importance. Indeed, Jonsson \textit{et al.} \cite{33} have shown that combined elevation of IgM and IgA RF isotypes had high diagnostic specificity for RA and concluded to the superiority of isotype-specific RF assays to agglutination tests for RA diagnosis. Bas \textit{et al.} \cite{34} have described the diagnostic value of these RF isotypes and ACPA in discriminating between RA and other rheumatic diseases. Our study also confirms the diagnostic interest of assessing the different RF isotypes, which might be particularly helpful early in the course of RA, especially when the disease is not fully differentiated. Indeed, in the present study, multivariate analysis has shown that detection of all isotypes of RF in addition to ACPA enhance the diagnostic accuracy of autoantibodies with a correct classification of patients in 68% of cases, while concomitant positivity of a single RF isotype with ACPA has usually a sensitivity lower than 50% \cite{20–21}.

Given the findings of this study, we conclude that testing for the \textit{PTPN22 1858T}, \textit{TNFRII 196R} and \textit{HLA-SE} alleles in a VErA cohort does not significantly improve the predictive value of IgA, IgG and IgM RF isotypes and ACPA for RA diagnosis.

### Rheumatology key message

- \textit{PTPN22 1858T}, \textit{TNFRII 196R} and \textit{HLA-SE} alleles do not improve the predictive value of RF and ACPA for RA diagnosis in the VErA cohort.

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### Disclosure statement

The authors have declared no conflicts of interest.

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