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A combination of autoantibodies to cyclic citrullinated peptide (CCP) and HLA-DRB1 locus antigens is strongly associated with future onset of rheumatoid arthritis

Ewa Berglin[1], Leonid Padyukov[2], Ulf Sundin[3], Göran Hallmans[4], Hans Stenlund[5], Walther J van Venrooij[6], Lars Klareskog[2] and Solbritt Rantapää Dahlqvist[1]

[1]Department of Public Health and Clinical Medicine, Division of Rheumatology, University Hospital, Umeå, Sweden
[2]Department of Rheumatology, Karolinska Hospital, Stockholm, Sweden
[3]Department of Clinical Immunology, Huddinge University Hospital, Stockholm, Sweden
[4]Department of Nutritional Research, University Hospital, Umeå, Sweden
[5]Department of Epidemiology, University Hospital, Umeå, Sweden
[6]Department of Biochemistry 161, University of Nijmegen, Nijmegen, The Netherlands

Corresponding author: Solbritt Rantapää Dahlqvist, solbritt.rantapaa.dahlqvist@medicin.umu.se

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Abstract

Antibodies against cyclic citrullinated peptide (CCP) and rheumatoid factors (RFs) have been demonstrated to predate the onset of rheumatoid arthritis (RA) by years. A nested case–control study was performed within the Northern Sweden Health and Disease study cohort to analyse the presence of shared epitope (SE) genes, defined as HLA-DRB1*0404 or DRB1*0401, and of anti-CCP antibodies and RFs in individuals who subsequently developed RA. Patients with RA were identified from among blood donors whose samples had been collected years before the onset of symptoms. Controls matched for age, sex, and date of sampling were selected randomly from the same cohort. The SE genes were identified by polymerase chain reaction sequence-specific primers. Anti-CCP2 antibodies and RFs were determined using enzyme immunoassays. Fifty-nine individuals with RA were identified as blood donors, with a median antedating time of 2.0 years (interquartile range 0.9–3.9 years) before presenting with symptoms of RA. The sensitivity for SE as a diagnostic indicator for RA was 60% and the specificity was 64%. The corresponding figures for anti-CCP antibodies were 37% and 98%, and for RFs, 17–42% and 94%, respectively. In a logistic regression analysis, SE (odds ratio [OR] = 2.35), anti-CCP antibodies (OR = 15.9), and IgA-RF (OR = 6.8) significantly predicted RA. In a combination model analysis, anti-CCP antibodies combined with SE had the highest OR (66.8, 95% confidence interval 8.3–539.4) in predicting RA, compared with anti-CCP antibodies without SE (OR = 25.01, 95% confidence interval 2.8–222.2) or SE without anti-CCP antibodies (OR = 1.9, 95% confidence interval 0.9–4.2). This study showed that the presence of anti-CCP antibodies together with SE gene carriage is associated with a very high relative risk for future development of RA.

Keywords: anti-CCP antibodies, rheumatoid arthritis, rheumatoid factor, shared epitope

Introduction

Autoimmune diseases, such as rheumatoid arthritis (RA), are believed to develop as a result of dysregulation of the immune system, leading ultimately, in RA, to the clinical features of inflammation and destruction in several joints [1]. The aetiology of RA has been suggested to be an interaction between genetic and environmental factors. To date, it has not been possible to identify individuals at early stages of this dysregulation, i.e. before presentation with clinically obvious polyarthritis. If methods were available to predict future development of RA, a better understanding of the events triggering the disease would be achieved, thereby creating the possibility of developing and testing preventive measures and of instituting therapy at earlier stages of disease development than is current practice.

Previous studies have demonstrated that the presence of rheumatoid factors (RFs) of IgM, IgG, and IgA class [2,3]

CCP = cyclic citrullinated peptide; CI = confidence interval; IQR = interquartile range; NSHDS = Northern Sweden Health and Disease Study; OR = odds ratio; RA = rheumatoid arthritis; RF = rheumatoid factor; SD = standard deviation; SE = shared epitope.

predict the development of rheumatoid arthritis and in a case–control study we found that antibodies against cyclic citrullinated peptide (CCP), as well as RFs, predated the onset of RA by several years [3]. Both anti-CCP antibodies and IgA-RF predicted the development of RA, with the highest predictive value for anti-CCP antibodies, indicating that citrullination and production of anti-CCP antibodies and RF are early processes in the development of RA [3]. The HLA-DRB1 locus has been shown to be linked to and associated with RA, with an especially high risk in individuals with compound heterozygosity for shared epitope (SE) genes [4]. However, there are no previous reports of studies combining serological and genetic factors in order to optimise the prediction of a future risk of developing RA. In the present study, we have evaluated the significance of the presence of SE genes, defined as DRB1*0404 or DRB1*0401, in relation to anti-CCP antibodies and RFs in individuals who subsequently developed RA.

Materials and methods

Subjects

A nested case–control study was performed within the Northern Sweden Health and Disease Study (NSHDS) and the Maternity cohort of Northern Sweden. All adult individuals of the county of Västerbotten were invited to participate; consequently, the cohorts are population-based and no individual was excluded. The NDHDS cohort consists of three subcohorts, which, together with conditions for recruitment into the cohorts and the collection and storage of blood samples, have previously been described in detail [3]. The registry of patients who fulfilled the American College of Rheumatology classification criteria for RA [1] and who attended the Department of Rheumatology, University Hospital, Umeå (the only medical centre for rheumatology in the county of Västerbotten), and with a known date of onset of symptoms or signs of joint disease, was coanalysed with the registers of the cohorts from the Blood Bank for Västerbotten located in Umeå. At the time of the study, the median duration of disease since the diagnosis of RA was 3.0 years (interquartile range [IQR] 1.8–5.8 years). Eighty-six individuals were identified from the cohorts as having donated blood samples before the onset of symptoms or signs of joint disease. Samples from three individuals were not available. Of the remaining 83 individuals (referred to here as 'prepatients'), blood samples for DNA analysis were available only from the NSHDS cohort, resulting in 59 prepatients (45 women and 14 men); the Maternity cohort did not include collection of samples for DNA analysis. Power calculations showed that two controls per patient would be sufficient, based on pretest probability of our previous results of HLA-DR4 frequencies in patients and controls from this area [5]. Therefore, we selected for genetic analysis two controls (out of the four who were previously analysed for antibody titres [3]) for every prepatient. The controls were randomly selected from the same subcohorts as the original cases within the NSHDS cohort and matched for sex, for age at the time of blood sampling, and for area of residence (rural or urban). The mean age of the prepatients at the time of blood sampling was 53 years (range 31–67 years) and of the controls, 53 years (range 30–67 years). The median sampling time before onset of symptoms of joint disease was 2.0 years (IQR 0.9–3.9 years). The antedating time for the samples was calculated to the onset of any symptoms of RA in all prepatients. Additional samples were collected from the prepatients at their first visit to the early-arthritis clinic ($n = 52$), i.e. when RA was diagnosed. On average, the diagnosis of RA was established 7.1 ± 2.8 (SD) months after the first symptoms of joint disease. The mean age at the onset of disease was 56.6 years, range 34–68 years. The Ethics Committee approved this study at the University Hospital, Umeå, and the blood donors to the Blood Bank had given their written informed consent.

HLA-DRB1 genotyping was performed using polymerase chain reaction sequence-specific primers from DR low-resolution kit and DRB1*04 subtyping kit (Olerup SSP AB, Saltsjöbaden, Sweden). The SE genes were defined as DRB1*0404 and DRB1*0401. Samples for DNA analysis from one prepatient and three controls were not available, and HLA typing of one prepatient and two controls was unsuccessful. Consequently, results of HLA typing were available from 57 prepatients and 112 controls.

The anti-CCP2 (Mark2) antibodies and the RFs were determined using enzyme-linked immunoassays as previously described [3].

Statistical analysis

The chi-square test was used for testing differences in frequencies of categorical data between groups. The sensitivity and specificity of SE gene carriage both separately and in combination with anti-CCP antibodies and RFs were calculated. Logistic regression analyses were used to estimate the odds ratio (OR) for the presence of SE gene carriage separately and in combination with anti-CCP antibodies or RFs as predictors for RA. The OR was calculated with 95% confidence intervals (CI). All P values are two-sided, and P values equal to or less than 0.05 were considered statistically significant. The calculations were performed using the SPSS package for Windows (version 11.0; SPSS, Chicago, IL, USA).

Results

The sensitivity found for the presence of SE genes as a diagnostic indicator for RA in prepatients was 60% (34/57) and the specificity was 64% (Table 1). The respective figures for carriers of two SE genes were 28% (16/57) and 95%. The specificity for the allele B1*0401 (74%) was higher than that for SE given either B1*0401 or B1*0404

Table 1

Sensitivity and specificity as diagnostic indicators for rheumatoid arthritis for antibodies against cyclic citrullinated peptide (anti-CCP Ab) and for rheumatoid factor (RF) of IgA, IgM, and IgG isotypes, in combination with the presence of a shared epitope (SE) allele – HLA-DRB1*0401 or B1*0404 – in 59 'prepatients' whose blood samples antedated the appearance of symptoms of rheumatoid arthritis (median 2.0 years [interquartile range 0.9–3.9]) and in 118 matched controls.

Variables	Sensitivity		Specificity	
	%	95%CI	%	95%CI
Anti-CCP Ab	37	25–51	98	93–100
IgA-RF	42	29–56	94	87–98
IgM-RF	22	12–35	94	87–98
IgG-RF	17	8–30	94	87–98
SE (B1*0404 or 0401)[1]	60	45–72	64	54–73
SE + anti-CCP Ab	28	17–42	99	94–100
SE + IgA-RF	25	14–38	98	93–100
SE + IgM-RF	14	6–27	98	93–100
SE + IgG-RF	11	4–22	99	94–100
SESE (B1*0404 or 0401)[1]	28	17–42	95	88–98
SESE + anti-CCP Ab	14	6–27	99	94–100
SESE + IgA-RF	7	2–18	99	94–100
SESE + IgM-RF	5	1–16	99	94–100
SESE + IgG-RF	4	0–14	100	95–100

[1]Analysed in 57 prepatients and in 112 controls. CI, confidence interval.

(data not shown). The frequencies of the presence of one or both of the SE genes studied in the prepatients were significantly greater than in the controls ($P = 0.003$ and $P = 0.0001$, respectively). Of the prepatients, 37% (22/59) tested positive for anti-CCP-antibodies, with a specificity of 98%. The sensitivity for IgA-RF was 42% (25/59), for IgM-RF 22% (13/59), and for IgG-RF 17% (10/59) (Table 1). The specificity was 94% for all three RF isotypes. The combination of SE gene carriage and anti-CCP antibodies increased the specificity to 99%, as did the combination of SE genes and IgG-RF (Table 1). The presence of double doses of the SE genes studied, in combination with either anti-CCP-antibodies, IgA-RF, or IgM-RF, gave a specificity of 99%, and, in combination with IgG-RF, of 100% (Table 1).

In a univariate logistic regression model, SE gene carriage, and particularly carriage of two SE alleles, significantly predicted RA (OR = 2.66, 95%CI 1.38–5.12 and OR = 6.89, 95%CI 2.52–18.84, respectively). In multivariate models including anti-CCP antibodies and RFs of all isotypes, single or double SE gene carriage significantly predicted RA in addition to our previously described predictive value of anti-CCP antibodies and IgA-RF [3]. The OR for SE gene carriage was 2.35 (95%CI 1.05–5.26) and for double SE gene carriage 7.31 (95%CI 2.26–23.67) (data not shown).

In a univariate logistic regression analysis, the combination of anti-CCP antibodies and SE gene carriage gave an OR of 66.8, while the presence of anti-CCP-antibodies alone gave an OR of 25.1 for the risk of developing RA compared with not having any of these factors (Table 2). The calculation on the SE allele B1*0401 selectively in the same model gave essentially the same results (data not shown). Furthermore, in the same type of analysis, SE gene carriage and IgA-RF showed similar results but at a lower level (Table 2). However, in the analysis including IgM-RF and SE, only SE gene carriage separately or in combination with IgM-RF significantly predicted RA; the same pattern was found for combinations of IgG-RF and SE (Table 2).

Except for a borderline significant association between the SE allele B1*0401 and anti-CCP-antibodies ($P = 0.051$), no significant association between SE gene carriage and the expression of anti-CCP-antibodies or RFs could be demonstrated (data not shown). As previously reported [3], anti-CCP antibodies and RFs were associated (data not shown).

When the prepatients were diagnosed after having developed RA, the sensitivity for anti-CCP antibodies was 71%, for IgG-RF 45%, for IgM-RF 73%, and for IgA-RF 71%. As regards SE, a significant association was found only between the presence of anti-CCP antibodies and B1*0401 ($P = 0.027$), and not between SE and any of the RFs.

Discussion

This study shows a greatly increased OR for the development of RA in individuals with the combination of SE gene carriage and anti-CCP antibodies or an RF of any isotype, in comparison with individuals not having any of the factors or having any one of them separately. In particular, the combination of SE gene carriage and the presence of anti-CCP antibodies appeared to be prognostic for the future development of RA. Previous studies by us [3] and others [2] have demonstrated that an increased production of autoantibodies may precede the development of RA. However, this is the first report in which autoantibody analyses have been combined with genotyping to show a remarkably high predictive value for the future development of RA. The main methodological strength of the current study is that the blood sampling of individuals who later developed RA and their controls was population based.

The results do not support the notion that there is a direct association between SE gene carriage and the occurrence of antibodies directed to CCP (or RFs) leading to the devel-

Table 2

Results of logistic regression analyses of anti-CCP antibodies (anti-CCP Ab) or rheumatoid factor (RF) of IgG, IgM, or IgA isotype and shared epitope (SE) in predicting rheumatoid arthritis, analysed in individuals who later developed the disease and in controls.

Combinations of variables	Patients (no.)	Controls (no.)	OR	95%CI
SE^- and anti-CCP Ab^-	17	71	1.0	
SE^+ and anti-CCP Ab^-	18	39	1.9	0.9–4.2
SE^- and anti-CCP Ab^+	6	1	25.1	2.8–222.2
SE^+ and anti-CCP Ab^+	16	1	66.8	8.3–539.4
SE^- and IgA-RF^-	12	67	1.0	
SE^+ and IgA-RF^-	20	38	2.9	1.3–6.7
SE^- and IgA-RF^+	11	5	12.3	3.6–41.7
SE^+ and IgA-RF^+	14	2	39.2	7.9–193.9
SE^- and IgM-RF^-	18	71	1.0	
SE^+ and IgM-RF^-	26	38	2.6	1.2–5.2
SE^- and IgM-RF^+	5	5	3.7	0.99–14.3
SE^+ and IgM-RF^+	8	2	14.9	2.9–76.3
SE^- and IgG-RF^-	19	67	1.0	
SE^+ and IgG-RF^-	28	39	2.5	1.3–5.1
SE^- and IgG-RF^+	4	5	2.8	0.7–11.6
SE^+ and IgG-RF^+	6	1	21.2	2.4–186.5

CI, confidence interval; OR odds ratio.

opment of RA, but rather suggest that there is a synergistic interaction between these factors. Our knowledge about what triggers the production of anti-CCP antibodies in healthy individuals is limited, as is the role of SE genes in this context. The conversion of arginine to citrulline in HLA-DRB1*0401 transgenic mice has been demonstrated to significantly increase activation of $CD4^+$ T cells [6]. In a previous study of patients with early RA, a significant association between anti-CCP antibodies and expression of B1*0401/0101 was reported [7]. This finding suggests that individuals carrying the SE genes may have more sustained T- and B-cell responses to citrullinated antigens than noncarriers. Taken together, these data suggest that in individuals carrying one or two SE genes, a specific T-cell-dependent immune response to citrullinated peptides may contribute to the occurrence of RA. In the prepatient cohort, there appeared to be a weak association, with borderline statistical significance, between the presence of anti-CCP antibodies and B1*0401. This association was strengthened when the prepatients had developed RA and when the number of individuals with anti-CCP antibodies had increased. However, there does not seem to be an absolute requirement for SE genes to develop anti-CCP antibodies [8]. The ORs for predicting RA were high for anti-CCP antibodies and for RFs but comparatively low for SE gene carriage. The overriding reason for this difference is the relatively high frequency among controls of the SE gene, which is also evident from the relatively low specificity of SE gene carriage in the prepatients.

A quite contradictory suggestion is that HLA antigens do not predispose to the autoimmune disease per se but rather fail to provide protection. Abnormal T-cell regulation associated with certain HLA haplotypes leads to the loss of self-tolerance followed by polyclonal activation of T and B cells and the subsequent production of autoantibodies [9]. This mechanism could be applicable to autoantibodies assessed in this study and would, therefore, explain the findings.

In a recent report on the genetic control of RF production in a rat model of RA, it was shown that the antibody response is controlled by several other genetic regions in addition to the defined arthritis loci [10]. The strength of the genetic association between HLA-DR4 and RA is reported to vary according to disease severity and the population studied [11]. The HLA gene locus has been calculated to contribute to one-third of the genetic risk for developing RA [12,13]. It is, therefore, apparent that other, non-HLA linked genes contribute to the risk of RA [14]. Two recent reports presented numerous single-nucleotide polymorphisms in the peptidyl arginine deiminase enzyme 4, i.e. the enzyme

converting peptidylarginine to peptidylcitrulline, several of which are strongly associated with RA [15,16]. Carriers of the susceptibility haplotype had antibodies against citrullinated proteins significantly more often than noncarriers [15,16].

The limitation of this study is the sample size, which resulted in a relatively low number of individuals in each group when the data were stratified for individuals positive or negative for a given antibody. Furthermore, we emphasise that this is a population-based case–control study, which makes it possible to establish associations between the outcome and the factors studied but precludes calculation of the probability of being a case, given certain values on the analysed factors. In this study, calculations are based on the combination of SE and the analysed autoantibodies. This is because anti-CCP antibodies and RFs are significantly associated, and consequently prediction of RA is only marginally increased when they are considered in combination.

Conclusion

This study has demonstrated that the presence of anti-CCP antibodies together with SE gene carriage is associated with a very high relative risk for future development of RA. This strong association with future development of RA in individuals positive for both SE and anti-CCP antibodies poses important questions relating to ethics and health policy. Thus, we shall need new strategies, both in research intended to understand factors that determine whether an individual with the presently identified risk factors will develop RA, and in clinical practice, where we may now possess a new means for analysing the risk of future development of RA in individuals who will have different needs or wishes to acquire such information.

Competing interests

None declared.

Author contributions

EB was a main investigator, designed the investigation, was involved in all aspects of the study, and contributed to the preparation of the manuscript.

SR-D was a main investigator, designed the investigation, was involved in all aspects of the study, and contributed to the preparation of the manuscript.

LP participated in the discussion on the design of the study, was responsible for the HLA typing, and contributed to the preparation of the manuscript.

LK participated in the discussion on the design of the study, was responsible for the HLA typing, and contributed to the preparation of the manuscript.

US performed the analyses of the rheumatoid factors and contributed to the preparation of the manuscript.

WJvV was responsible for analyses of the anti-CCP antibodies and contributed to the preparation of the manuscript.

GH was involved in the design of the study and is responsible for the Blood Bank in Umeå.

HS assisted in the statistical analyses and discussions.

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