Research article

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

Ewa Berglin1, Leonid Paduykov2, Ulf Sundin3, Göran Hallmans4, Hans Stenlund5, Walther J van Venrooij6, Lars Klareskog2 and Solbritt Rantapää Dahlqvist1

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

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

Received: 13 Jan 2004 Revisions requested: 11 Feb 2004 Revisions received: 2 Apr 2004 Accepted: 6 Apr 2004 Published: 11 May 2004

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]...
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, predicted 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 analysed 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.
The specificity was 94% for all three RF isotypes. The shared epitope (SE) allele - HLA-DRB1*0401 or B1*0404 - in 59 tested positive for anti-CCP antibodies, with a specificity of 99%, and, in combination with IgG-RF, of 100% (Table 1). The presence of double SE gene carriage 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 development of RA. However, the presence of double SE gene carriage increased the specificity to 99%, as did the combination of SE gene carriage and IgG-RF.
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.
was involved in all aspects of the study, and contributed to the preparation of the manuscript.

Author contributions

wishes to acquire such information.

This strong association with future development of RA in individuals who will have different needs or wishes to acquire such information.

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.

Acknowledgements

We gratefully acknowledge the technical assistance of Mrs Lisbeth Årlestig, Solveig Linghult, and Margareta Holmgren, Department of Public Health and Clinical Medicine, Rheumatology and Nutritional Research Divisions. We also thank Dr Olle Olenup for providing us with HLA typing kits, Miss Diab Diab for technical assistance with HLA typing, and Mr Ben de Jong for technical assistance with serum analyses. The work was supported by grants from The Swedish Research Council (K2003-74XD-14705-01A, SRD); Konung Gustaf V’s 80-års fund; the Swedish Rheumatism Association; and the Medical Faculty, Umeå University, Umeå, Västerbottens läns landsting (Spjutspets), University Hospital, Umeå, Sweden. The work undertaken in Nijmegen (WJvV) was supported by the Netherlands Organization for Scientific Research in the Medical Sciences and Het Nationaal Reumafonds (Dutch League against Rheumatism) (NWO-MW grant 940-35-037) and by the Council for Chemical Sciences of the Netherlands Organization for Scientific Research (NWO-CW), with financial aid from the Netherlands Technology Foundation (STW grant 549-5077).

References

1. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger TA, Mitchell DM, Neusadler DH, Pinals RS, Schaller JG, Sharp JT, Wilder RL, Hunder GG: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988, 31:315-324.
2. Aho K, Palouso T, Raunio V, Puska P, Aromaa A, Salonen JT: When does rheumatoid disease start? Arthritis Rheum 1985, 28:485-489.
3. Rantapää-Dahlqvist S, de Jong BAW, Berglin E, Hallmans G, Wadell G, Stenlund H, Sundin U, van Venrooij WJ: Antibodies against cyclic citrullinated peptide (CCP) and immunoglobulin-A rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 2003, 10:2741-2749.
4. Jawheer D, Gregersen PK: Rheumatoid arthritis. The genetic components. Rheum Dis Clin North Am 2002, 28:1-15.
5. Rantapää-Dahlqvist S: Genetic markers in rheumatoid arthritis. Scand J Rheumatol 1986, Suppl 58:1-25.
6. Hill JA, Southwood S, Sette A, Jevnikar AM, Bell DA, Carsen Ed: Cutting edge: The conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC class II molecule. J Immunol 2003, 171:538-541.
7. Goldbach-Mansky R, Lee J, McCoy A, Howxworth J, Yarboc C, Smolen JS, Steiner G, Rosen A, Zhang C, Manard HA, Zhou Z, Palouso T, van Venrooij WJ, Wilder RL, Klippe JH, Schumacher HR Jr, El-Gabalawy HS: Rheumatoid arthritis associated autoantibodies in patients with synovitis of recent onset. Arthritis Res 2000, 2:236:243.
8. Bas S, Genevay S, Meyer O, Gabay C: Anti-cyclic citrullinated peptide antibodies, IgM and IgA rheumatoid factors in the diagnosis and prognosis of rheumatoid arthritis. Rheumatology 2003, 42:677-680.
9. Zanelli E, Breedveld FC, Vries de RRP: HLA association with autoimmune disease: a failure to protect? *Rheumatology* 2000, 39:1060-1066.

10. Wernhoff P, Olofsson P, Holmdahl R: The genetic control of rheumatoid factor production in a rat model of rheumatoid arthritis. *Arthritis Rheum* 2003, 48:3584-3596.

11. Gorman JD, Criswell LA: The shared epitope and severity of rheumatoid arthritis. *Rheum Dis Clin North Am* 2002, 28:59-78.

12. Daighton CM, Walker DJ, Griffiths ID, Roberts DF: The contribution of HLA to rheumatoid arthritis. *Clin Genet* 1999, 36:176-182.

13. Rigby AS, Silman AJ, Voolm L, Gregory JC, Ollier WE, Khan MA, Nepom GT, Thomson G: Investigating the HLA component in rheumatoid arthritis: an additive (dominant) mode of inheritance is rejected, a recessive mode is preferred. *Genet Epidemiol* 1991, 8:155-175.

14. Rigby AS, Voolm L, Silman AJ: Epistatic modeling in rheumatoid arthritis: an application of the Risch theory. *Genet Epidemiol* 1993, 10:311-320.

15. Suzuki A, Yamada R, Chang X, Tokuhiro S, Sawada T, Suzuki M, Nagasaka M, Nakayama-Hamada M, Kawaiida R, Ono M, Ohtsuki M, Furukawa H, Yoshino S, Yukioka M, Tohma S, Matsubara T, Wakiyama S, Teshima R, Nishikura Y, Sekine A, Iida A, Takahashi A, Tsunoda T, Nakamura Y, Yamamoto K: Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat Genet* 2003, 34:395-402.

16. Caponi L, Petit-Teixeira E, Sebbag M, Bongiorni F, Moscato S, Preatesi F, Osorio J, Guerrini-Weber M, Cornelis F, Sorre G, Migliorini P: Analysis of the peptidylarginine deiminase V gene in rheumatoid arthritis [abstract]. *Arthritis Res* 2003, Suppl 8:1.