Concise Report

Association analysis of the interleukin 17A gene in Caucasian rheumatoid arthritis patients from Norway and New Zealand

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Objective. Elevated levels of IL-17A have been detected in the inflamed synovium of RA patients, and murine arthritis models deficient in IL17A have shown reduced inflammation. Our aim was to investigate IL17A as a candidate gene for RA, and to assess correlations between risk variants and disease phenotypes.

Methods. Five single nucleotide polymorphisms (SNPs) were selected to tag the genetic variability of the IL17A region and were genotyped by TaqMan technology on 950 RA cases and 933 random controls from Norway. Associations to progression of radiographic damage and presence of autoantibodies were examined in a 10-yr follow-up cohort of early RA. In addition, 580 RA patients and 504 controls from New Zealand were used as a replication data set.

Results. A weak association between RA and the promoter SNP rs2275913 [odds ratio (OR) = 1.17; 95% CI 1.02, 1.34; P = 0.02] was found in the Norwegian population. The association was also evident at the genotype level where it indicated a recessive model. The allelic association was not replicated in the RA cohort from New Zealand (OR = 0.96; 95% CI 0.81, 1.16; P = 0.69). However, combined analysis suggested a weak recessive association (OR = 1.19; 95% CI 1.02, 1.37; P = 0.02). No significant associations were observed with radiographic progression, anti-cyclic citrullinated peptide or IgM-RF.

Conclusions. Modest evidence of an association with IL17A in Norwegian RA patients was observed. Although, our findings were not replicated in an independent RA material from New Zealand, a significant common risk estimate indicated that IL17A warrants further investigation in RA.

Key words: RA, IL17A, Genetic predisposition.

Introduction

RA is a systemic chronic inflammatory disease which affects ~1% of the population worldwide. Although the aetiology of the disease is not completely understood, RA is believed to result from a complex interaction between genetic and environmental factors that triggers and maintains synovial inflammation and disease in affected individuals. RA patients experience histological changes in the synovium such as angiogenesis, cellular hyperplasia, an influx of mononuclear cells and pannus formation, which together cause the loss of cartilage and bone.

A role for T cells in RA is implied both by the influx of T cells into the synovium and by genetic data showing increased susceptibility for RA in individuals carrying certain human leukocyte antigen (HLA)-DRB1 and protein tyrosine phosphatase non-receptor 22 (PTPN22) alleles [1, 2]. Despite low levels of Th cell type 1 (Th1) cytokines, such as IFN-γ and IL-2, in the inflamed synovium, established RA is considered a Th1 disease [3]. Mice deficient in IFN-γ or IFN-γ receptor have shown development of CIA, indicating involvement of other T cell subsets [4, 5]. Lately, much attention has been given to an IL-17 secreting new lineage of Th cells, named Th17 cells. The differentiation of Th17 cells appears to follow a distinct pathway, where species-specific transcription factors and cytokines are involved [6]. Further experimental support for the involvement of Th17 cells in RA has been obtained from mouse studies, as CIA was suppressed in both IL17A knockout mice and mice treated with anti-IL-17A [7, 8]. Although Th17 cells have been detected in both the synovium and in peripheral blood mononuclear cells of RA patients, the level of these cells was not elevated compared with healthy controls [9]. Irrespective of this, elevated levels of IL-17A have been detected in the inflamed synovium of RA patients [10], thereby indicating a possible functional role during pathogenesis. Th17 cells have, for example, been shown to influence osteoclastogenesis through IL-17A in an arthritic mouse model [11]. One recent genetic study has linked polymorphisms in IL17A to radiographic progression in Japanese RA patients [12]. The aim of this study was to investigate IL17A as a candidate gene for RA with a possible involvement in disease susceptibility, effect on disease progression and autoantibody production.

Materials and methods

Case–control cohorts

A total of 950 Norwegian RA patients classified according to the ACR criteria [13] were included in the study. Clinical data were available for 216 of these patients who were from the European Research on Incapacitating Disease and Social Support (EURIDISS) cohort. These patients have been longitudinally followed with examinations after 1, 2, 5 and 10 yrs. Radiographs of the hand were scored according to the van der Heijde modification of the Sharp method [14]. Controls (n = 933) were recruited through the Norwegian Bone Marrow Donor Registry. Whole genome amplification of DNA was performed prior to genotyping (GenomiPhi DNA Amplification Kit, GE Healthcare, Chalfont St Giles, UK).

The replication data set from New Zealand consisted of 580 RA cases, diagnosed according to the ACR criteria [13]. The New Zealand control group (n = 504) consisted of individuals over
17 yrs of age, recruited from the Auckland and Otago regions, who reported no history of inflammatory disorders.

Clinical and demographic characteristics for the patient and control materials are described in Table 1. Written, informed consent was obtained according to the Declaration of Helsinki from all participating subjects, and the work has been approved by The Regional Committees for Research Ethics in Eastern and Southern Norway and in New Zealand by the MultiRegion (cases) and Otag Ethics Committees (controls).

Single nucleotide polymorphism selection and genotyping

Single nucleotide polymorphism (SNP) selection was done in Haploview v4.0 by pairwise tagging \((r^2 > 0.80)\) and minor allele frequencies \(>0.1\), using Caucasian (CEU) genotype data from HapMap (http://www.hapmap.org/) covering the \(IL17A\) gene and 2 kb upstream (Release 21a/jan07). Six SNPs were selected as tags, but due to assay design criteria, only five of these SNPs, capturing \(\sim86\%\) of the genetic variation, were genotyped by allele discrimination by the TaqMan technology (Applied Biosystems, Foster City, CA, USA) on an ABI7900 instrument: \(rs4711998\) (C_1799586_20), \(rs8193036\) (C_1799585_10), \(rs3819024\) (C_11545877_10), \(rs2275913\) (C_15879983_10) and \(rs7747909\) (C_29315993_10). Only \(rs2275913\) was genotyped in the replication data set. Each 384-well plate consisted of RA cases and healthy controls, as well as positive and negative controls. Genotypes in both data sets were in Hardy–Weinberg equilibrium, and the genotype success rates were \(>96\%\).

Statistical analysis

\(A\) \textit{priori} power calculations (http://pngu.mgh.harvard.edu/~purcell/gpc/) were performed in the two different data sets (at \(P = 0.05\)). For the initial data set, we had 80% power to detect an odds ratio (OR) in the range of 1.4–1.5 assuming an allele frequency between 10% and 50%. For the replication data set, we had 29% power assuming the Norwegian genotype frequency (36%) and risk estimate (OR = 1.28) in a recessive model. Unphased v3.0.10 was used for comparing allele and genotype frequencies between cases and controls \([15]\). The remaining statistical analyses were performed in SPSS v15.0 (SPSS, Chicago, IL, USA). The homogeneity of the ORs from the two populations was tested by the Breslow–Day test, and a common distribution showed an increased frequency for being homozygous for the major G allele among the RA patients compared with controls (42% vs 36%; \(P = 0.04\)), and when analysed in a recessive model, patients carrying the G/G genotype showed an increased risk (OR = 1.28; 95% CI 1.01, 1.54; \(P = 0.01\)). The association was also evident at the allelic level (OR = 1.17; 95% CI 1.02, 1.34; \(P = 0.02\)).

Results

A significant deviation in genotype and allele frequencies was observed in the Norwegian material for one of the SNPs (rs2275913) tagging the \(IL17A\) gene (Table 2). The genotype distribution showed an increased frequency for being homozygous for the major G allele among the RA patients compared with controls (42% vs 36%; \(P = 0.04\)), and when analysed in a recessive model, patients carrying the G/G genotype showed an increased risk (OR = 1.28; 95% CI 1.01, 1.54; \(P = 0.01\)). The association was also evident at the allelic level (OR = 1.17; 95% CI 1.02, 1.34; \(P = 0.02\)).

No significant increase in the annual progression, measured by the van der Heijde modification of the Sharp method, was seen for RA cases homozygous for the G allele [median (IQR) for G/G: 2.4 (5–12)] vs A/G + A/A: 1.8 (5–9); \(P = 0.44\). Neither was any significant association between autoantibody status and the G/G genotype observed for anti-cyclic citrullinated peptide (anti-CCP) (OR = 1.34; 95% CI 0.77, 2.35; \(P = 0.30\)) or RF-IgM (OR = 1.25; 95% CI 0.73, 2.16; \(P = 0.42\)).

We also tested whether the rs2275913 SNP was a risk variant in a New Zealand Caucasian case–control cohort, but no significant differences were observed in the genotype distribution [cases: A/A 83 (14.3%), A/G 251 (43.3%), G/G 246 (42.4%); and controls: A/A 58 (12%), A/G 238 (47%), G/G 208 (41%); \(P = 0.27\)]. No differences in genotype frequencies were seen in a recessive model (OR = 1.05; 95% CI 0.82, 1.34; \(P = 0.70\)). In line with this, no association was observed at the allelic level [G allele; cases 743 (64%) and controls 654 (65%); OR = 0.96; 95% CI 0.81, 1.16; \(P = 0.69\)].

Despite the lack of association in the New Zealand cohort, no significant heterogeneity was observed between the Norwegian and New Zealand cohorts (Breslow–Day test, \(P = 0.20\)), which allowed us to calculate a common OR and \(P\)-value for the two populations. In a recessive model of the combined data set (\(n_{\text{cases}} = 1518\) and \(n_{\text{controls}} = 1424\)), an association with the G/G genotype was evident (OR = 1.19; 95% CI 1.02, 1.37; \(P = 0.02\)).

TABLE 1. Clinical and demographic characteristics of patients and controls from Norway and New Zealand

<table>
<thead>
<tr>
<th>Country</th>
<th>RA total material</th>
<th>Control material</th>
<th>Norway</th>
<th>N = 938</th>
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<tr>
<td></td>
<td>Females, %(^a)</td>
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<td>SE positivity, %</td>
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<td>Age, mean (s.d.), yrs</td>
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<td>Disease duration, mean (s.d.), yrs</td>
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<td>RF-IgM positivity, %</td>
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<td>anti-CCP positivity, %</td>
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<td>Sharp–van der Heijde score, median (IQR)(^c)</td>
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</table>

\(N\): number of individuals for whom data were available; SE: shared epitope. \(^a\)A total of 12 RA patients with unknown gender, \(^b\)SE status available for 837 RA patients and \(^c\)annual progression available from 144 patients in a 10 yr period of time.
**Discussion**

We have investigated the *IL17A* gene by tagging the main genetic variation and found a weak, but significant, association with the promoter SNP, rs2275913, in Norwegian RA patients. The association was not confirmed in a smaller cohort from New Zealand, but a combined data set provided evidence for *IL17A* as a susceptibility locus for RA, with a weak effect (OR = 1.20).

Albeit the observed association was weak, the risk falls within the range of most of the identified non-HLA RA susceptibility loci (OR = 1.1–1.5). Small risk effects require large sample sizes, and power calculations showed that the New Zealand cohort lacked power to confirm the initial finding. Although we cannot exclude that our finding in the well-powered Norwegian RA cohort was false positive, the low-powered replication data set could more likely be a false negative. Furthermore, if the causal genetic variant is not the weakly associated SNP, but an untested SNP located elsewhere in the gene, different linkage disequilibrium (LD) patterns in the two populations could enhance the discrepancy of the results. No statistically significant heterogeneity was detected between the two populations, which allowed us to combine the cohorts, and evidence of a weak association between *IL17A* and RA was revealed. Nevertheless, we cannot fully exclude the presence of population differences, and that the association with *IL17A* rs2275913 is population-specific.

The proximal promoter of *IL17A* contains several binding sites for transcription factors, including binding sites for the transcription factor nuclear factor of activated T cells (NFATC), which is important in regulating *IL17A* expression [16]. Interestingly, rs2275913 maps between two NFTAC sites. No known SNPs are found within the binding site of NFTAC (http://genome.ucsc.edu/; March 2006 assembly). Furthermore, the associated SNP did not, according to the current HapMap information, tag any other SNPs in this region. Nevertheless, yet unidentified SNPs located in these transcription sites or other functionally important positions of the gene may be the causal variant.

Only one other genetic study has addressed *IL17A* polymorphisms in RA. A recent Japanese study reported weak evidence of association between an *IL17A* intronic SNP, rs3804513, and joint destruction (Larsen score) in early RA (P = 0.049), but found no association with the risk of developing RA [12]. The rs3804513 SNP is monomorphic in Caucasian populations (HapMap Data Rel 21a:jan07) and was therefore not included in our study. The association from the Japanese study could represent a distinct causal variant, however the existence of LD (D = 1.0; r² = 0.12) between this intronic SNP (rs3804513) and our associated promoter SNP (rs2275913) in the Japanese population (HapMap Data Rel 21a:jan07) could also imply that the two studies, to some extent, have detected the same effect. In the Norwegian EURIDISS cohort, no significant difference in joint damage, measured by the van der Heijde modification of the Sharp score, according to rs2275913 genotypes was observed, albeit a weak tendency was seen. Previous studies in the same cohort have been able to reveal that both anti-CCP and PTPN22 1858T are independent predictors of radiographic progression [14, 17].

Different genetic background, use of dissimilar scores for joint damage and the sizes of the two data sets (n_Norway = 144 and n_Japan = 72) may explain the deviating results, as well as differences regarding disease duration at time of inclusion (within 1 yr in the Japanese study and < 4 yrs in our study), as increased IL-17A production in early stages of RA has been reported [18]. Interestingly, a correlation between mRNA level of IL-17A and TNF-α synovial membrane expression and joint damage progression in patients with short disease duration of RA has been reported [19].

Much attention to the role of Th17 cells in RA, and a possible involvement of IL-17A during joint destruction, entailed us to investigate *IL17A* as a candidate gene in RA. A promoter SNP showed evidence of a weak association in the Norwegian cohort, but no correlation to autoantibody status or joint damage was observed.

Although our findings were not replicated in an independent, but smaller cohort from New Zealand, a weak association was evident in the combined cohorts. Therefore before any definite conclusions can be drawn, further investigations of the *IL17A* gene in large RA cohorts with clinical data are warranted.

**Rheumatology key message**

- *IL17A* shows weak association with RA in case–control cohorts from Norway and New Zealand.

**Acknowledgements**

The Norwegian Bone Marrow Donor Registry is acknowledged for making the control material available. Marita Olsson was of great assistance with statistical calculations.

**Funding:** This project has been funded by the Southern Norway Regional Health Authority, the Norwegian Women’s Health Association, the New Zealand Health Research Council and Arthritis New Zealand. J.E.H.-M. is supported by a New Zealand National Heart Foundation Postdoctoral Fellowship.

**Disclosure statement:** The authors have declared no conflicts of interest.

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