Association of the 6q23 region with the rate of joint destruction in rheumatoid arthritis

Hans Ulrich Scherer,1,2 Michael P M van der Linden,1 Fina A S Kurreeman,1 Gerrie Stoecken-Rijsbergen,1 Saskia le Cessie,3 Tom W J Huizinga,1 Annette H van der Helm-van Mil,1 René E M Toes1

ABSTRACT
Background Two novel genetic polymorphisms on chromosome 6q23 are associated with susceptibility to rheumatoid arthritis (RA). Both polymorphisms (rs6920220 and rs10499194) reside in a region close to the gene encoding tumour necrosis factor α-induced protein 3 (TNFAIP3). TNFAIP3 is a negative regulator of NF-κB and is involved in inhibiting TNF-receptor-mediated signalling effects. Interestingly, the initial associations were detected in patients with longstanding RA. However, no association was found for rs10499194 in a Swedish cohort with early arthritis. This might be caused by over-representation of patients with severe disease in cohorts with longstanding RA.

Methods Five single nucleotide polymorphisms in 6q23 were genotyped in 324 Dutch patients with early RA. Genotypes were correlated with progression of radiographic joint damage for a follow-up time of 5 years.

Results Two polymorphisms (rs675520 and rs9376293) were associated with severity of radiographic joint damage in patients positive for anti-citrullinated protein/peptide antibodies (ACPA). Importantly, the effects were present after correction for confounding factors such as secular trends in treatment.

Conclusions These data associate the 6q23 region with the rate of joint destruction in ACPA+ RA.

INTRODUCTION
Recent whole-genome association scans have disclosed novel genetic polymorphisms associated with susceptibility to anti-citrullinated protein/peptide antibody-positive (ACPA+) rheumatoid arthritis (RA).1,2 Among those, two single nucleotide polymorphisms (SNPs), rs6920220 (A allele) and rs10499194 (C allele), were found to be independently associated with ACPA+ disease. Both SNPs map to a single linkage disequilibrium block spanning ~60 kb in a region on chromosome 6q23 that lacks known genes or transcripts. The closest genes map to a single linkage disequilibrium block spanning ~60 kb in a region on chromosome 6q23 that lacks known genes or transcripts. The closest genes

PATIENTS AND METHODS
Patients
The Leiden EAC is a population-based inception cohort that includes patients with self-reported symptom duration of ≤2 years. The 6q23 region lacks known genes or transcripts. The closest genes map to a single linkage disequilibrium block spanning ~60 kb in a region on chromosome 6q23 that lacks known genes or transcripts. The closest genes map to a single linkage disequilibrium block spanning ~60 kb in a region on chromosome 6q23 that lacks known genes or transcripts. The closest genes map to a single linkage disequilibrium block spanning ~60 kb in a region on chromosome 6q23 that lacks known genes or transcripts. The closest genes

SNP selection and genotyping
Five SNPs (rs1878658, rs675520, rs9376293, rs10499194 and rs6920220) were selected based on a haplotype analysis across the 6q23 locus published previously. All SNPs are in imperfect linkage disequilibrium with one another (supplementary table 1). Genotyping was performed using predesigned TaqMan allelic discrimination probes (Applied Biosystems, Foster City, California, USA). Each 584-well plate contained 10 ng sample DNA per well and at least eight negative and six positive controls. Genotype calls and clusters were further replicated in an extended UK-based case-control study.4 rs10499194 was initially identified in North American ACPA+ patients (the Brigham Rheumatoid Arthritis Sequential Study, BRASS; minor allele OR=0.67).2 Replication was successful in two additional US cohorts selected from the North American Rheumatoid Arthritis Consortium (NARAC). Replication failed, however, in ACPA+ patients of a Swedish population-based inception cohort (the Epidemiological Investigation of Rheumatoid Arthritis cohort, EIRA).2 This last finding is of interest, as BRASS and NARAC are cohorts of patients with longstanding RA (mean disease duration BRASS: 15.4±12.8 years; NARAC: 14.3±11.1 years). The EIRA study, however, was designed to identify incident cases of RA as soon as possible after disease onset, resulting in an estimated mean disease duration at inclusion of only 10 months.7

Association of a genetic polymorphism in cohorts of patients with longstanding disease but absence of this association in an early arthritis cohort led us to hypothesise that the 6q23 region would be associated with disease severity in ACPA+ patients. Very little information is currently available on the effects of genetic variation on outcome measures in RA.8 Therefore, we genotyped five SNPs in a Dutch early arthritis cohort (the Leiden Early Arthritis Clinic, EAC) and correlated genotyping data to progression of radiographic joint damage for a maximum follow-up of 5 years.
Concise report

Table 1  Median Sharp van der Heijde scores (M) and interquartile ranges (IQR; 25%–75% centiles) per genotype for patients with ACPA + RA (n = number of patients). Genotypes were combined for rs1878658 (G), rs10499194 (T) and rs6920220 (A), as the frequency of patients homozygous for the respective minor allele was ≤5% for these single nucleotide polymorphisms.

Manually checked for discrepancies and doubtful calls were rejected. No SNP deviated from Hardy–Weinberg equilibrium. Genotyping call rates were 96.5% (rs1878658), 98% (rs675520), 95% (rs9376293), 94% (rs10499194) and 98.1% (rs6920220).

Serology and radiographs

Serum samples were tested for citrulline-specific IgG antibodies using a commercially available ELISA kit (Immunoscan Mark2; Eurodiagnostica, The Netherlands). Radiographs were scored according to the Sharp van der Heijde method10 with known time order by one blinded, independent trained reader (supplementary file 1).

Statistical analysis

Association between genotypes and radiographic scoring data was analysed using SPSS version 16.0 (SPSS, Chicago, Illinois, USA), p Values <0.05 were considered significant. All p values reported are two-sided.

Two approaches were chosen for statistical analysis. First, the average increase in Sharp van der Heijde scores during the follow-up period was estimated for each person by regression analysis. Subsequently, the average increase (slope) of scores for each genotype was compared non-parametrically using the Mann–Whitney rank-sum test.

We observed an influence of the time of inclusion (1993–2003) on the progression of radiographic joint damage, probably reflecting an improvement of treatment intensity during this 10-year time period. To account for this effect, we performed, as a second approach, a mixed model analysis described in detail in supplementary file 1.

RESULTS

Radiographic scores of 324 Dutch patients with RA (181 ACPA+, 143 ACPA−) were available for analysis. At least five radiographic follow-up observations were available in 57% of patients. A dominant model was chosen for analysis, as the frequency of patients homozygous for the minor allele of rs1878658 (G), rs10499194 (T) and rs6920220 (A) was ≤5%. Figure 1 depicts the influence of genotypes on radiographic joint damage. ACPA+ and ACPA− subgroups were analysed separately. Median scores and interquartile ranges (IQR) are provided for ACPA+ patients in table 1 (for ACPA− patients, see supplementary table 2).

No influence of genotypes on radiographic joint damage was seen in ACPA− patients (fig 1). In ACPA+ patients, however, two polymorphisms showed reproducible association with disease progression over time. Presence of the G allele of rs675520 was found to be associated with increased Sharp van der Heijde scores, as a significant difference was observed when the average increase (slope) in radiographic scores over time was compared with G as the dominant allele (median slope AG/GG=4.6, AA=2.3; Mann–Whitney p=0.007). In order to account for an effect of improving treatment strategies on radiographic progression during the 10-year period in which patients were included in the study, we next performed a mixed-model analysis. This analysis identified the year of inclusion as a significant variable influencing the extent of radiographic joint damage (p=0.005). After correcting for the year of inclusion, however, we still observed a significant influence of the G allele of rs675520 (AG/GG vs AA, p=0.026).

Similar to the G allele of rs675520, we noted an influence of the C allele of rs9376293 on progression of radiographic joint damage (fig 1). The average increase (slope) in Sharp van der Heijde scores over time was significantly higher for C allele carriers than for T homozygotes (median slope CC/CT=4.5, median slope TT=3.0, Mann–Whitney p=0.021). After correcting for the year of inclusion as described above a trend effect of the C allele remained (p=0.097).

For rs1878658, rs10499194 and rs6920220, no significant influence of individual genotypes on radiographic joint damage was noted.
but not in an early arthritis cohort.\(^2\) This indicated a potential patients with RA in three cohorts with longstanding disease, found to be associated with disease susceptibility in ACPA+ cohorts with differing disease duration. The major allele (C) was rs10499194, a SNP on chromosome 6q23 close to TNFAIP3/A20 and the 6q23 region interesting can- 

The 6q23 region has recently been associated with disease sus-

We were intrigued by recent differential fi ndings for rs10499194, a SNP on chromosome 6q23 close to TNFAIP3, in cohorts with differing disease duration. The major allele (C) was found to be associated with disease susceptibility in ACPA+ patients with RA in three cohorts with longstanding disease, but not in an early arthritis cohort.\(^2\) This indicated a potential impact of the 6q23 region on disease severity. In order to test for such an impact, five SNPs were genotyped in a cohort of Dutch patients with early RA. These SNPs had previously been shown to identify common haplotypes in 6q23.\(^4\) We identifi ed two SNPs for which the presence of alleles was associated with increased joint destruction in ACPA+ patients. Carriers of the G allele of rs675520 developed increased Sharp van der Heijde scores over time. A similar effect, although weaker, was found for the C allele of rs9376293. Interestingly, no association was found for any of the SNPs in ACPA− subjects. Although this does not exclude the possibility of a contribution of the 6q23 region to disease severity in ACPA− disease, the latter observ-

No effect on disease severity was observed for rs10499194 and rs6920220. Based on our data we cannot rule out the possi-

Data linking newly identified genetic polymorphisms to dis-

DISCUSSION

The 6q23 region has recently been associated with disease sus-

rs10499194; p=0.76 (rs6920220)).

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Competing interests None.

Ethics approval This study was conducted with the approval of the Institutional Review Board of Leiden University Medical Centre, Leiden, The Netherlands.

Contributors HUS designed the study, interpreted data and wrote the manuscript; MPVdH performed x-ray scoring; GSR and REMT designed the study, interpreted data and critically drafted and revised the manuscript. All authors approved the submitted version.

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