Predictive factors for outcome of rheumatoid arthritis

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CHAPTER 6

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

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ABSTRACT

Objective

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 tumor necrosis factor α-induced protein 3 (*TNFAIP3*). *TNFAIP3* is a negative regulator of NFκB and as such involved in inhibiting TNF-Receptor mediated signalling effects. Interestingly, the initial associations were detected in patients with long-standing RA. However, no association was found for rs10499194 in a Swedish early arthritis cohort. As this could be caused by overrepresentation of patients with severe disease in cohorts with long-standing RA, we analyzed the effect of the 6q23 region on the rate of joint destruction.

Methods

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

Results

Two polymorphisms (rs675520 and rs9376293) associated with severity of radiographic joint damage in ACPA+ patients. Importantly, the effects were present after correction for confounding factors such as secular trends in treatment.

Conclusions

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

INTRODUCTION

Recent whole genome association scans have revealed novel genetic polymorphisms associated with susceptibility to ACPA+ RA.[1,2] Among those, two single nucleotide polymorphisms (SNPs), rs6920220 (A allele) and rs10499194 (C allele), were found to independently associate 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 are oligodendrocyte lineage transcription factor 3 (*OLIG3*) and tumor necrosis factor α-induced protein 3 (*TNFAIP3*). The latter is of potential importance to RA pathogenesis, as the protein TNFAIP3 acts as a negative regulator of NF-κB.[3] So far, however, functional relevance of the reported polymorphisms is unknown.

Rs6920220 was initially identified in ACPA+ RA patients (minor allele OR 1.38) originating from the United Kingdom (UK).[1] It was 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 latter finding is of interest, as both BRASS and NARAC are cohorts of patients with long-standing RA (mean disease duration BRASS: 15.4 ± 12.8 years;[5] NARAC: 14.3 ± 11.1 years).[6] 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 hypothesize that the 6q23 region would associate 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.

PATIENTS AND METHODS

Patients

The Leiden EAC is a population-based inception cohort that includes patients with self-reported symptom duration of ≤2 years.[9] DNA samples of 324 patients consecutively included between 1993 and 2003 were used for analysis. For further details see supplementary file 1.

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.[2] All SNPs are in imperfect linkage disequilibrium to one another (supplementary table 1). Genotyping was performed using pre-designed Taqman allelic discrimination probes (Applied Biosystems). Each 384 well plate contained 10 ng sample DNA per well and at least 8 negative and 6 positive controls. Genotype calls and clusters were 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 method[10] with known time order by one blinded, independent trained reader (supplementary file 1).

Statistical analysis

Association between genotypes and radiographic scoring data was analyzed using SPSS version 16.0 (SPSS Inc., Chicago, IL). 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 per person by regression analysis. Subsequently, the average increase (slope) of scores per 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 reflecting most likely an improvement of treatment intensity during this 10 year time period. In order 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 RA patients (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 analyzed separately. Median scores and interquartile ranges (IQR) are provided for ACPA+ patients in table 1 (for ACPA- patients see supplementary table 2).

Table 1: Median Sharp van der Heijde scores (M) and interquartile ranges (IQR; 25 - 75% percentiles) per genotype for ACPA+ RA patients (# = 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 SNPs

	Year																	
	0			1			2			3			4			5		
ACPA+	M	IQR	#	M	IQR	#	M	IQR	#	M	IQR	#	M	IQR	#	M	IQR	#
rs1878658																		
AA	6	2 - 13	126	14	6 - 24	111	18	10 - 32	113	22.5	11 - 42	98	26.5	14 - 51.5	86	32	14 - 51.5	89
AG/GG	3	1 - 7	40	12	5.5 - 20.5	40	17	6 - 32	39	30	11.5 - 56.5	34	32	16 - 69	31	27	17 - 74	31
rs675520																		
AA	9	4.5 - 13	33	14	6.5 - 21	32	15	8 - 27.5	33	19	9 - 34	27	22	13.5 - 39.5	24	20	12.5 - 47.5	21
AG	5	1 - 10	83	12	5 - 21	73	18	9.5 - 32.5	78	25	11.5 - 54	68	32.5	14 - 61	58	33	17 - 62.5	66
GG	5	2 - 11	51	14	7 - 27	48	18	8 - 37	43	26	14 - 48	39	28	16 - 63	37	32	20 - 64	35
AG/GG	5	2 - 10	134	13	6 - 24.5	121	18	9.5 - 33	121	26	12 - 53	107	32	15 - 61	95	32	18.5 - 61	101
rs9376293																		
CC	6.5	3 - 13.5	28	15	9 - 28	27	27	12 - 49.5	25	33	15.5 - 58	24	35	16.5 - 62	24	35	16.5 - 56	21
CT	4	1 - 9	84	12	5 - 21	79	17	7 - 32.5	78	21	10.5 - 54	66	32	14 - 68	57	36	20 - 68	61
TT	8	3 - 13	54	14	6 - 21	46	16	10.5 - 25.5	50	21	12 - 30.5	44	23	13.5 - 38	38	23	13 - 47	40
CC/CT	4	2 - 10	112	13	6 - 26.5	106	18	8 - 36	103	28	11 - 54.5	90	34	15 - 63	81	36	19 - 67.5	82
rs10499194																		
CC	7	2 - 12.5	90	14	6 - 21.5	77	18	9.5 - 28	82	21	12 - 35	71	25.5	14 - 45	62	29	14 - 48	62
CT/TT	4	2 - 7.5	65	12	6 - 24.5	64	17	7 - 37	61	31	11 - 55	55	29.5	14.5 - 67	50	32	15 - 68	51
rs6920220																		
AA/AG	7	2 - 12	58	14	6 - 21	53	20	10 - 32.5	57	24	12 - 48	47	32	18 - 56	35	35.5	18 - 65	42
GG	4.5	2 - 10.5	108	13	6 - 25	99	16.5	8 - 30.5	96	22	11 - 48	86	25	14 - 61	84	24	14 - 51	79

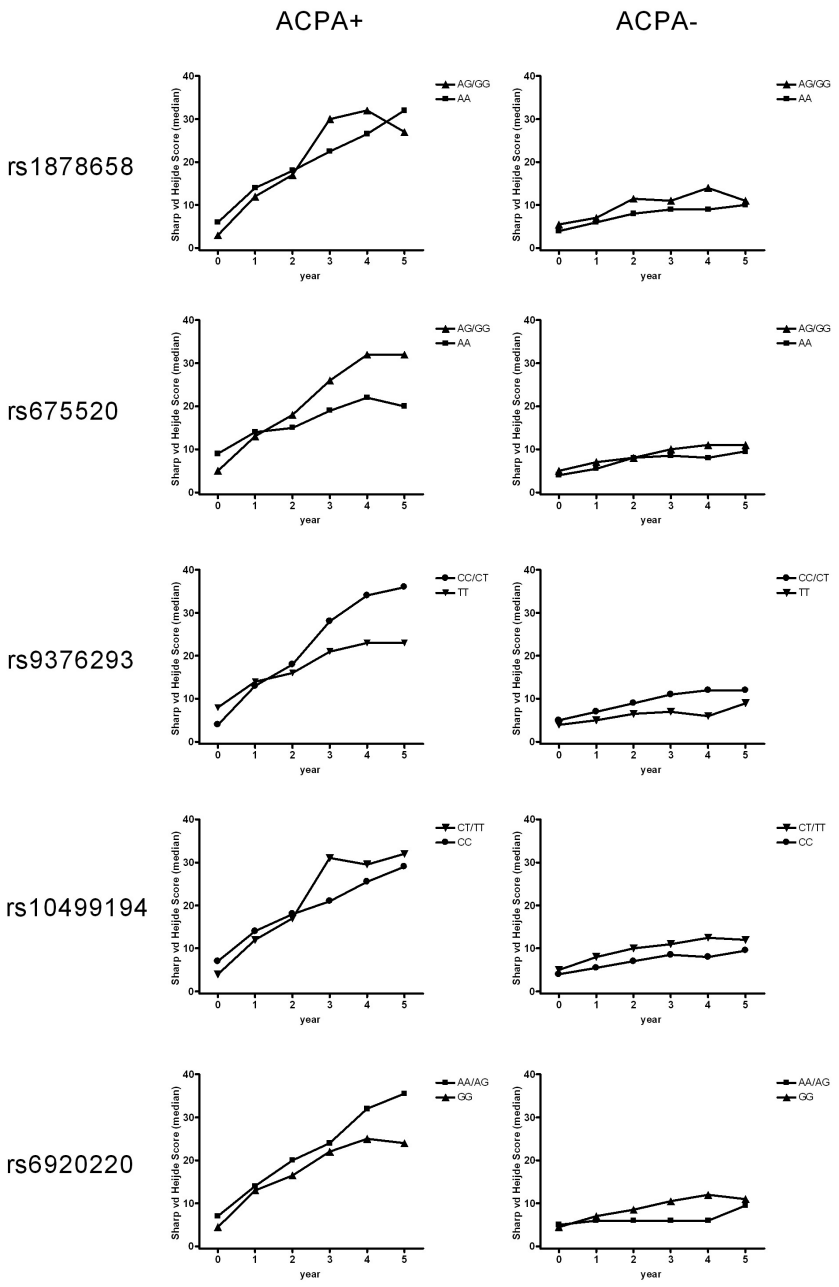

Figure 1. Development of median Sharp van der Heijde scores plotted according to genotype/allele in ACPA+ (left column) and ACPA- (right column) RA patients. Year 0 equals baseline values. Regression analysis was performed in order to estimate the average increase (slope) in Sharp van der Heijde scores over time. Slopes were subsequently compared using the nonparametric Mann-Whitney test (for the ACPA+ subgroup: p = 0.37 (rs1878658); p = 0.007 (rs675520); p = 0.021 (rs9376293); p = 0.05 (rs10499194); p = 0.76 (rs6920220))

No influence of genotypes on radiographic joint damage was observed in ACPA- patients (Figure 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 associate 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 into 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 (Figure 1). The average increase (slope) in Sharp/van der Heijde scores over time was significantly higher for C allele carriers as compared to 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.

DISCUSSION

The 6q23 region has recently been associated with disease susceptibility in RA. This region contains no known transcripts. The closest genes with known function are *OLIG3* and *TNFAIP3*. *TNFAIP3* encodes protein A20, a TNF-α induced negative regulator of NF-κB.[3,11] Decreased levels of A20 lead to uncontrolled NFκB-activity, resulting in increased inflammation. This observation makes *TNFAIP3*/A20 and the 6q23 region interesting candidates that could modulate inflammation also in RA.

We were intrigued by recent differential findings for rs10499194, a SNP on chromosome 6q23 close to *TNFAIP3*, in cohorts with differing disease duration. The major allele (C) was found to associate with disease susceptibility in ACPA+ RA patients in three cohorts with long-standing 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.[2] We identified two SNPs for which 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- individuals. Although this does not exclude a contribution of the 6q23 region to disease severity

in ACPA- disease, the latter observation is in line with recent reports detecting an association of the 6q23 region with disease susceptibility in ACPA+ patients only.[4] No effect on disease severity was observed for rs10499194 and rs6920220. Based on our data we cannot rule out the possibility that either SNP exerts a weak effect that requires larger sample numbers for detection or that cannot be observed during the first years of disease. Interestingly, we observed nominally higher scores for the riskconferring A allele of rs6920220 without reaching statistical significance. The discrepancy between SNPs associating with susceptibility and radiographic progression also indicates that the causal variant at this locus has not yet been identified. Given the large area of linkage disequilibrium surrounding these SNPs, further fine-mapping and functional characterization will have to be performed.

Data linking newly identified genetic polymorphisms to disease outcome in RA are only beginning to emerge. Our data are unique, as they cover a long period of radiographic follow-up and have been scrutinized for artefacts such as secular trends in treatment intensity. Albeit based on relatively low patient numbers, our data indicate a contribution of the 6q23 region to the rate of joint destruction in ACPA+ RA, thereby further refining our understanding of the effects exerted by this locus. Replication of our findings in other cohorts is needed. Nonetheless, this is the first study demonstrating such an effect for genetic polymorphisms located outside the HLA-region in ACPA+ RA patients.

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SUPPLEMENTARY INFORMATION ON PATIENTS AND METHODS

Patients

All patients met the American College of Rheumatology 1987 revised classification criteria for RA and were of Caucasian origin based on self-reported ethnicity. Written informed consent was obtained from all participants, and the study was approved by the local institutional review board.

The Leiden Early Arthritis Clinic (EAC) is a population-based inception cohort that includes patients with self-reported symptom duration of ≤2 years. Follow-up visits are performed and radiographs of hands and feet are taken on a yearly basis. DNA samples of 324 patients (67.6% female; mean age 56.3 ± 15.4 years) consecutively included between 1993 and 2003 for whom radiographic scoring data and ACPA status were available were used for analysis. 2003 as the latest year of inclusion was chosen in order to allow a five year followup period for all patients.

Radiographic scoring

The number of patients with available radiographs varied per time-point (for ACPA+ patients: n = 168 at baseline and n = 153, 154, 134, 119, and 122 at year 1 to 5, respectively; for ACPA- patients: n = 135 at baseline and n = 121, 109, 93, 81, and 65 at year 1 to 5, respectively). In total, radiographs of 324 patients (181 ACPA+, 143 ACPA-) were used for analysis. All radiographs were scored by one experienced reader who was blinded with respect to the patient's autoantibody status, treatment, clinical outcome and genotyping results. Scoring was performed with known time order, which is more sensitive to change compared to scoring with unknown time sequence.[1] For quality control, radiographs of 60 randomly selected RA patients were rescored by the same reader. This selection comprised 499 radiographs, consisting of 149 baseline radiographs and 350 radiographs during followup. Reliability of radiographic scoring was calculated. Intraclass-observer correlation coefficients (ICC) were 0.91 for all scored radiographs, 0.84 for baseline radiographs and 0.97 for the radiographic progression rate.

Statistical analysis

Four different treatment strategies were applied to patients included in the EAC depending on the year of inclusion. Patients included between 1993 and 1995 were treated initially with analgesics and subsequently with chloroquine or sulphasalazine if they had persistent active disease (delayed treatment).[2] From 1996 to 1998 patients were promptly treated with either chloroquine or sulphasalazine (early treatment).[2,3] From 1998 to 2002 patients were promptly treated with either sulphasalazine or methotrexate (early treatment) and patients included in 2002 or later were promptly treated with either sulphasalazine or methotrexate combined with treatment adjustments based on disease activity (early and disease activity based treatment).

To take advantage of the prospective character of the EAC, consisting of repeated measurements, and to avoid multiple testing by performing statistical tests for each time point, a linear mixed model for longitudinal data was used, with the log transformed sharp score as response variable, to compare the radiological progression between genotype groups. We explored different correlation structures between the repeated measurements, and based on the Akaike's information criterion, an autoregressive correlation structure with heterogeneous variances was chosen. This model takes missing observations into account, assuming that the missing is at random. Differences in progression rates between the different genotypes were tested by considering the significance of the interaction between genotype and time with time as linear covariate. The year of inclusion into the study was entered into the model to correct for possible confounding effects. Inclusion period is a proxy for treatment modalities, because treatment strategies improved over time and an influence of the treatment strategy on the progression of radiographic joint damage was observed previously.[2] The interaction between treatment strategy (i.e. inclusion year) and time was significant in all five analyses of the present study ($p<0.05$).

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Supplementary table 1. Comparison of the LD-parameters obtained from HapMap (CEU population in rel. 24 Phase II Nov 08) and the Leiden dataset

		HapMap CEU		Leiden Dataset	
SNP1	SNP2	D'	r^2	D'	r^2
rs1878658	rs675520	1.0	0.112	1.0	0.155
rs1878658	rs9376293	1.0	0.274	1.0	0.263
rs1878658	rs10499194	1.0	0.623	0.98	0.5
rs1878658	rs6920220	1.0	0.028	0.929	0.04
rs675520	rs9376293	0.931	0.356	0.893	0.482
rs675520	rs10499194	1.0	0.191	0.98	0.289
rs675520	rs6920220	1.0	0.209	0.987	0.289
rs9376293	rs10499194	1.0	0.441	0.982	0.478
rs9376293	rs6920220	1.0	0.102	0.985	0.172
rs10499194	rs6920220	1.0	0.045	0.988	0.087

Supplementary table 2. Median Sharp van der Heijde scores (M) and interquartile ranges (IQR; 25 - 75% percentiles) per genotype for ACPA- RA patients (# = 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 SNPs.

	Year																	
	0			1			2			3			4			5		
ACPA-	M	IQR	#	M	IQR	#	M	IQR	#	M	IQR	#	M	IQR	#	M	IQR	#
rs1878658																		
AA	4	2 - 10	103	6	3 - 12	95	8	3 - 18	79	9	5 - 17.5	69	9	3 - 19.5	57	10	5 - 22	47
AG/GG	5.5	1 - 12	30	7	4 - 15	25	11.5	4 - 21.5	28	11	4 - 21	23	14	4 - 24	23	11	4 - 27	18
rs675520																		
AA	4	2 - 7	28	5.5	1.5 - 16	26	8	5 - 21	21	8.5	4 - 14	20	8	2.5 - 19	16	9.5	5.5 - 19.5	16
AG	5	1 - 10	71	6	3 - 12	64	7.5	3 - 14.5	56	8	5 - 19	49	10	3 - 20	42	10	4.5 - 20.5	33
GG	5.5	1 - 11	36	8	2 - 21	31	9.5	4 - 21.5	32	12	7 - 22.5	24	14	5 - 26	23	14.5	6 - 31.5	16
AG/GG	5	1 - 10	107	7	3 - 12	95	8	3 - 16.5	88	10	5 - 20.5	73	11	4.5 - 23.5	65	11	5 - 27	49
rs9376293																		
CC	4.5	0.5 - 11	22	5	1 - 10	19	9	1 - 12	19	12	5 - 19.5	17	12	2.5 - 20	13	9	3.5 - 44.5	8
CT	5	2 - 11	69	7	4 - 17.5	61	8	4 - 20.5	57	11	5 - 21.5	44	12	5 - 25	43	12.5	6 - 27	34
TT	4	2 - 7	43	5	2 - 10	41	6.5	3 - 18.5	32	7	3 - 11	31	6	3 - 10.5	24	9	4 - 14	23
CC/CT	5	1 - 11	91	7	4 - 15	80	9	3.5 - 17.5	76	11	5 - 21.5	61	12	5 - 23.5	56	12	5 - 27	42
rs10499194																		
CC	4	1.5 - 7	76	5.5	2.5 - 11.5	70	7	3 - 16	56	8.5	5 - 16.5	52	8	3 - 17.5	44	9.5	5.5 - 20.5	36
CT/TT	5	1 - 12	53	8	3 - 17.5	46	10	3.5 - 21.5	49	11	4.5 - 22	38	12.5	4.5 - 24.5	36	12	4 - 27	28
rs6920220																		
AA/AG	5	2 - 8.5	45	6	3 - 12	39	6	3 - 21	31	6	3 - 14	29	6	3 - 12	23	9.5	2.5 - 17.5	18
GG	4.5	1 - 10	88	7	2.5 - 13.5	81	8.5	4 - 18	76	10.5	6 - 20.5	62	12	5 - 22.5	57	11	5 - 27	46