Chapter 9

Association of variants in *IL2RA* with progression of joint destruction in Rheumatoid Arthritis

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ABSTRACT

Background

Heritability studies have suggested an important role of genetic predisposition to progression of joint destruction in Rheumatoid Arthritis (RA); the heritability is estimated at 45-58%. Several SNPs have been identified to associate with RA susceptibility. We studied the association of several of these loci with progression of joint destruction.

Method

In total 1,750 RA-patients with 4,732 Sharp-van der Heijde scored X-rays of four independent data-sets were studied. Thirteen susceptibility SNPs that were not associated with joint destruction before were tested in 596 Dutch RA-patients. Subsequently, significant SNPs were studied in RA data-sets from North-America and Iceland. Data were summarized in inverse weighted variance meta-analyses. Further, the association with circulating protein levels was studied and the associated region was fine-mapped.

Results

In stage-1, three loci (AFF3, IL2RA and BLK) were significantly associated with rate of joint destruction and were further analyzed in the additional data-sets. In the combined meta-analyses, the minor allele of IL2RA-rs2104286(C) was associated with less progression of joint destruction ($P=7.2x10^{-4}$). Furthermore, the IL2RA-rs2104286 protective genotype was associated with lower circulating levels of soluble IL-2RA (0.85 95%CI 0.77-0.93, $P=1.4x10^{-3}$). Additionally, lower sIL-2RA levels were associated with a lower rate of joint destruction ($P=4.2x10^{-3}$). The association of IL2RA with rate of joint destruction was further focused to a region of 40kb encompassing the IL2RA intron 1 and the 5′ region of IL2RA and RBM17.

Conclusion

Present genetic and serologic data suggest that inherited altered genetic constitution at IL2RA locus may predispose to a less destructive course of RA.

INTRODUCTION

Rheumatoid Arthritis (RA) is an autoimmune disorder that affects approximately 1% of the population and is characterized by inflammation and subsequent destruction of joints.[1] The disease is associated with significant morbidity, disability and costs for society.[2] The severity of RA progression is objectively measured by radiographic joint destruction scores of the hand and foot joints,[3] which is also reflective of the cumulative burden of inflammation.[4] In the past decades, genetic studies have identified many loci involved in disease pathogenesis of autoimmune diseases; several of these have led to the identification of new pathways and targets for therapy.[5] For RA susceptibility, 32 risk factors have been identified. Since RA is a chronic disease, interrogation of the genome with disease severity as an outcome is attractive since this could increase the understanding of processes that are fundamental to RA progression and it may provide new therapy opportunities. Heritability studies have recently suggested an important role of genetic predisposition to progression of joint destruction in RA; the heritability of the radiologic progression rate is estimated at 45-58%.[6] However, thus far, only a few genetic variants have been reported to associate with joint destruction in RA.[7-10] To date, 20 of the 32 loci that are known to associate with RA susceptibility[11-24] have been tested for an association with joint destruction.[7-9,25,26] In the current study, we investigated the remaining 12 susceptibility loci for their association with rate of joint destruction (AFF3, ANKRD55, BLK, CCL21, CTLA4, IL21, IL2RA, IL2RB, IRF5, REL, SPRED2 and STAT4, Table 1).

For this, a multistage approach was conducted to detect an association between the selected SNPs and the rate of joint destruction in four data-sets RA-patients with longitudinal radiological data on joint destruction. All data-sets included patients that were diagnosed in a period when treatment strategies were less aggressive and less controlled than today. These conservative treatment strategies made these data-sets suitable for the present study as the natural course was less inhibited.

We will show an association of IL2RA-rs2104286 with the severity of joint damage progression. We further explored this association by fine mapping of this IL2RA region and evaluating the IL2RA serum levels in relation to both genotypes and the radiologic progression rate.

PATIENTS AND METHODS

Study population

Four data-sets consisting of adult European RA-patients were studied (Table 2). RA was defined according to the 1987 ACR criteria in all data-sets. To obtain high-quality phenotypic radiological data, all X-rays studied in the current project were scored by experienced

Table 1: Schematic overview of the SNPs selection for the analyses with rate of joint destruction.

							[14]AFF3
						[12]REL	**[14]ANKRD55/IL6ST**
						[12]BLK	[14]C5orf30[e]
						[13]TAGAP[e]	[14]CCR6[e]
				[3,4]TNFAIP3[b]	[11]CD40[d]	[13]CD28[e]	**[14]IRF5**
				[5]STAT4	[11]CCL21[d]	[13]TRAF6	[14]PXK[e]
				[6,7]C5-Traf1[c]	[11]MMEL[d]	[13]PTPC[e]	[14]RBJP[e]
	Shared epitope			**[9]IL2RA**	[9,11]PRKCQ[d]	[13]FCGR2A[e]	**[14]SPRED2**
	hypothesis			**[8,9]IL2RB**	[9,11]KIF5[d]	[13]PRDM1[e]	**[14]CCL21**
HLA-DR1		[1]PTPN22[a]	**[2]CTLA4**	**[10]IL21**	[11]CDK6[d]	[13]CD2/CD58[e]	**[14]IL2RA**
1978	1987	2004	2005	2007	2008	2009	2010

References discovered susceptibility SNPs in white RA-patients

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12. Gregersen, Nat Genet. 2009 Jul;41:820-3
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References tested susceptibility SNPs with rate of joint destruction

a. De Nies, Ann Rheum Dis. 2010 Sep;69(9):1730-1
b. Sherer, Ann Rheum Dis. 2010;69:567-70
c. Kurreeman, PLoS Med. 2007 Sep;4:e278
d. Van der Linden, Arthritis Rheum. 2009;60:2242-7
e. Teare, EULAR 2011-2739 (abstract)

Bold SNPs were tested in the current manuscript

readers from one center (LUMC) who were blinded for the clinical and genetic data using the Sharp- van der Heijde score (SHS).[3] All patients gave their informed consent and approval was obtained from the local Ethical Committee of each study.

Table 2: Characteristics of the individual data-sets.

Cohort	N patients	Total no. of X-ray sets	Year of diagnosis	Year of X-ray	Anti-CCP+ n (%)
Leiden-EAC	596	3,136	1993-2006	1993-2006	302 (51)
Iceland	285	285	1942-2008	1989-2010	148 (52)
Wichita	113	555	1963-1999	1976-2006	110 (97)
NBD	756	756	1980-1999	1989-2006	490 (72)
Total	1,750	4,732			

Leiden-RA cohort (Leiden-EAC)

This cohort concerned 596 early RA-patients from the western part of the Netherlands, who were included in the Leiden Early Arthritis Clinic between 1993 and 2006.[28] Patients were included at time of diagnosis and followed yearly-. X-rays were taken at baseline and on yearly follow-up visits during 7-years. In total, 3,136 sets of hands and feet X-rays were available. All X-rays were chronologically scored with SHS by one experienced reader who was unaware of genetic or clinical data. 499 randomly selected X-rays were scored twice. The correlation coefficient (ICC) within the reader was 0.91. The treatment of these patients could be divided into three treatment periods. Patients included in 1993-1995 were initially treated with NSAIDs, patients included in 1996-1998 were initially treated with chloroquine or sulphasalazine and patients included after 1999 were promptly treated with methotrexate or sulphasalazine.

Iceland data-set

325 RA patients that were referred to Landspítali hospital or the private clinic of Reykjavik with X-rays available of both hands and both feet at one similar time-point were studied. 285 patients had also genotypes available. Joint destruction was determined using SHS by two trained readers. Twelve percent of the X-rays were scored by both readers and each scorer rescored 15% of their own scored X-rays. The ICCs between and within readers were all >0.95.

Wichita

These patients from one practice in Wichita (Kansas, USA) were recruited between 1973 and 1993.[28] Because of the rather low minor allele frequency in some variants and the low number of X-rays at a longer disease duration of these patients, the follow-up duration was restricted to a maximum of 10 years. Collection of laboratory and radiographic data was not protocollized but obtained when needed for clinical care. In total, 461 sets of hands X-rays were available of 90 patients. All hand X-rays were chronologically scored with SHS by one experienced reader, the within reader ICC was 0.98.

NDB patients

These patients are included in the National Databank for Rheumatic diseases, a databank that consists of patients with rheumatic diseases from the USA and Canada.[29] 756 patients had a single time-point X-ray of the hands available and scored using SHS by one the same reader who scored the Wichita X-rays.

SNP selection and genotyping

Of the 32 regions that are known to associate with RA susceptibility in patients of European origin, 12 loci have thus far not been tested for an association with joint destruction

and were investigated in the current study; AFF3, ANKRD55, BLK, CCL21, CTLA4, IL21, IL2RA, IL2RB, IRF5, REL, SPRED2 and STAT4. The thirteen reported susceptibility SNPs within these twelve loci were studied (Table 1). These twelve loci were not in LD with each other.

Leiden, Wichita and NDB SNPs were typed with the Immunochip, Illumina Infinium High-Density array (Illumina Iscan Platform), which has recently been designed to densely genotype immune- mediated disease loci identified by GWAS of common variants using data.[30] Genotypic data was accepted after quality control, requiring MAF>0.0001, HWE p>0.001 and genotyping success rate (% of samples that were successful) >0.98. Genetic outliers and relatives (both defined by principal component analysis) and patients with a gender mismatch between data-file and DNA were excluded. In Wichita and NDB, BLK had a genotyping success rate of 0.977 and therefore did not pass the initial quality control. After manually checking the genotyping quality, it was concluded that the SNP data could be used for analyses

The Icelandic chip-typed samples were assayed with the Illumina Human Hap300, Hap CNV370, Hap 610, 1M or Omni-1 Quad bead chips at deCODE genetics. Only the 317,503 SNPs from the Human Hap300 chip were used in the long range phasing and the subsequent SNP imputations.[31] SNPs were excluded if they had (i) yield lower than 95%, (ii) minor allele frequency less than 1% in the population or (iii) significant deviation from Hardy-Weinberg equilibrium in the controls ($P < 0.001$), (iv) if they produced an excessive inheritance error rate (over 0.001), (v) if there was substantial difference in allele frequency between chip types (from just a single chip if that resolved all differences, but from all chips otherwise). All samples with a success rate below 97% were excluded from the analysis.

Soluble IL-2RA

Soluble IL-2RA (sIL-2RA) serum levels were evaluated of 159 Leiden RA- patients. Patients were selected based on their SHS progression over 1 year, to include 1/3 without SHS progression, 1/3 with mild SHS progression (1-5 SHS) and 1/3 with severe SHS progression (>5 SHS). Samples were taken several years after diagnosis of RA (mean disease duration 4.2±2.2, range 1-9 years). Standard sandwich ELISA for sIL-2RA was performed according to the manufacturer's recommendations (BD Biosciences).

Fine-mapping

The IL2RA region was fine-mapped to seek stronger associations with rate of joint destruction than the initial susceptibility variant rs2104286. Patients included in the discovery cohort (Leiden-EAC) and in the North-American replication data-sets (NDB and Wichita) were genotyped with the Immunochip which densely types 186 auto-immune loci among which IL2RA. Previous sequencing by Lowe et al. has demonstrated that the flanking

gene downstream of IL2RA, IL15RA, is clearly genetically separated from IL2RA and that there is no clear breakdown in LD in the 21kb region between IL2RA and RBM17, the upstream flanking gene (see Entrez Gene URLs).[32] Therefore, data of genetic variants from 23kb downstream IL2RA to 11kb downstream RBM17, including the haplotypes at the end of IL2RA and RBM17 (Chr10:6070000-6210000) were retrieved. In this way, 495 variants were obtained, 473 of which were polymorphic. These variants were analyzed in the discovery data-set for their association with joint destruction in a manner similar as the SNP analyses. Genetic variants that were stronger associated with rate of joint destruction than rs2104286 in the discovery data-set were subsequently analyzed in the North-American samples.

Statistical analyses

Associations between genotypes and radiographic joint destruction were analyzed, applying a multiple stage approach. First, all thirteen SNPs were analyzed in 596 Leiden RA-patients with 3,136 sets of X-rays. Genotypes were tested additively assuming a greater impact on rate of joint destruction for two minor variants than for one. Since this concerned a discovery stage no correction for multiple testing was applied and SNPs with a p-value =0.05 were studied in stage-2. For the second stage three data-sets were used, one from Iceland and two from the USA (Wichita and the National Databank (NDB)), with a total of 1,154 patients and 1,596 X-rays. In all data-sets the radiological scores were log-transformed to approximate a normal distribution. For the analyses in the cohorts with multiple measurements per patient (Leiden-EAC and Wichita) a multivariate normal regression analysis was used with radiological damage as response variable. This method analyzes all repeated measurements at once and takes advantage of the correlation between these measurements. This model is similar to a linear mixed model, only no random effects is added.[33] To model the correlation over time, the heterogeneous first-order autoregressive (ARH1) matrix was used. It assumes a stronger correlation for measurements taken in a shorter period than taken over a longer period in time. The effect of time was entered as factor in the model, to properly capture a mean response profile over time. For each tested SNP an analysis with the SNP and its interaction with time in the model were conducted, thereby testing the progression of joint destruction, which was the outcome of interest. The effect of time in the interaction term was linear. Since the analyses were performed on the log-scale, the resulting effect estimate were back transformed to the original scale (βorg). The βorg indicates how much higher the rate of joint destruction is per year for each minor allele compared to the reference genotype; the βorg changes for each follow-up year by the power of the number of years.

For the data-sets in which patients were radiographed once during follow-up (Iceland and NDB), the estimated yearly progression rate (dividing the total SHS by the number of

disease years at time of X-ray) was calculated in order make the scores comparable to the other data-sets. Then the SNP association was tested in a linear regression analysis with log-transformed estimated yearly progression rate as outcome variable. Also here, the resulted estimate reflects how many fold the rate of joint destruction increases per year in the presence of a minor allele compared to the absence of this allele.

In all data-sets adjustments for age and sex were made. In the analyses on the discovery data- sets, consisting of the most recently diagnosed patients, an additional adjustment for the described treatment periods was made.

Due to the differences in study designs, the separate data-sets could not be combined in one analysis directly. To test the overall association, the effect sizes and standard deviations of the individual analyses were combined in an inverse-weighting meta-analyses. This method weights the results with a low standard deviation stronger than the results with a higher standard deviation, hence preventing an overrepresentation of less precise data on the outcome. The analysis was conducted in STATA, version 10.1.[34] In this stage, correction for multiple testing was performed using the Bonferroni method.

Haplotype analysis was performed on the top fourteen significant SNPs in an attempt to further differentiate between the significant findings. When a haplotype has a stronger association with joint destruction a combined effect of the SNPs on joint destruction is suggested. Haplotype blocks were defined by Gabriels method. Haplotypes were assigned to each individual using PLINK 1.06. Analyses of the haplotypes were performed with methods similar to those used for the analyses of the individual SNPs, now testing the once or twice presence of a haplotype compared to the absence of the haplotype.

The association between IL2RA-rs2104286 and sIL-2RA was tested with a linear regression analysis with $\log_e$(sIL-2RA+1) as outcome variable and rs2104286 tested additively as independent variable. Adjustments were made for covariates that were associated with the response variable in simple analysis, which were age at diagnosis and disease duration at the time of serum collection.

All analyses were performed in SPSS 17.0 (see URLs) unless stated otherwise.

RESULTS

In the discovery stage, the analysis of the 13 susceptibility SNPs (at 12 loci) in the discovery data-set resulted in three significant findings: AFF3 (rs11676922) βorg=1.02 (95%CI 1.002-1.03, $P=2.8x10^{-2}$; BLK (rs13277113) βorg=0.98 (95%CI 0.97-1.00) $P=3.1x10^{-2}$; IL2RA (rs2104286) βorg=0.97 (95%CI 0.96-0.99) $P=4.4x10^{-3}$.

In stage-2, these three associations were further examined in one Icelandic and two USA datasets (NDB and Wichita). Each of these cohorts contained fewer X-rays per patient

compared to the discovery cohort, influencing the precision with which an individual patient's progression rate is estimated. The results of the individual cohorts as well as of the meta-analyses are presented in Figure 1. BLK was not significant in any of the

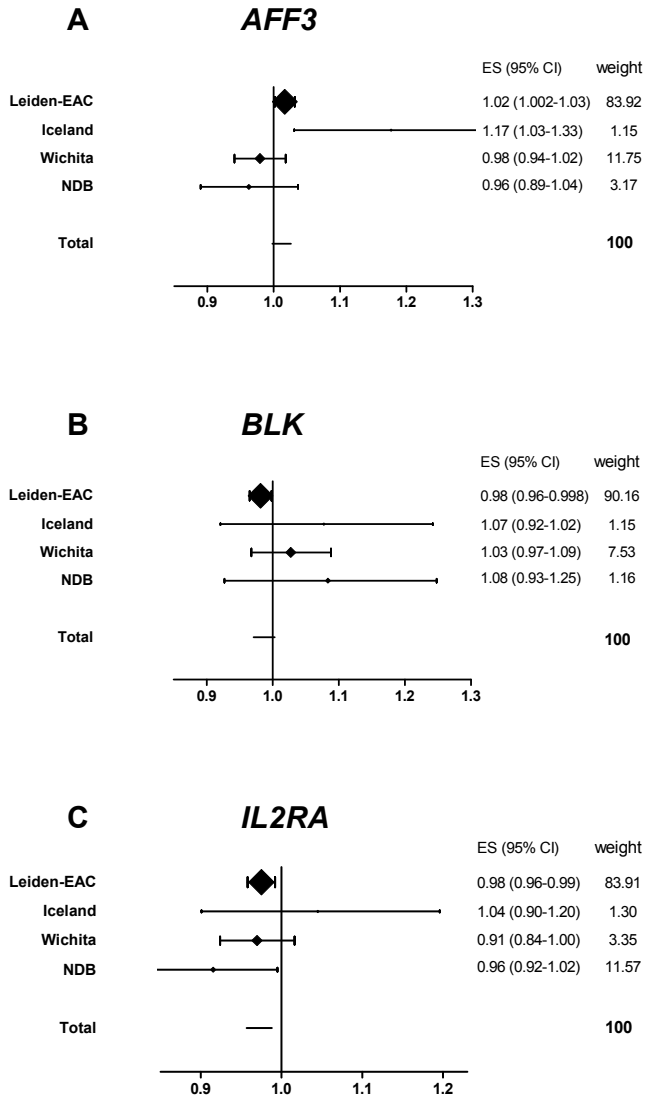


Figure 1 Results for the three SNPs associated with joint destruction in the discovery cohort.
Three susceptibility SNPs were associated with rate of joint destruction in the discovery cohort: A) AFF3-rs11676922, B) BLK-rs2736340 and C) IL2RA-rs2104286. The estimates are the coefficients back-transformed onto the original scale (β_{org}). The meta-analyses are based on a fixed effect model, which is applied to genetic studies to test whether there is a statistically significant effect but which may yield effect size estimates that do not generalize well. The effect size of the meta-analyses should thus be interpreted with caution and is therefore depicted in grey.

replication data-sets or in the meta-analysis (P=0.11). AFF3 was independently significant in Iceland (P=0.015), but was not significant in the meta- analysis (P=0.08). The result of IL2RA-rs2104286 was supported by a significant finding in NDB (P=0.045), an a suggestive association when the three data-sets of stage-2 were combined (P=0.056), and a significant association in the meta-analysis of all four data-sets ($P=7.2x10^{-4}$, Figure 1). This association was strong enough to survive the conservative Bonferroni correction.

sIL-2RA

Supported by these findings, we studied whether IL2RA-rs2104286 was associated with sIL-2RA concentrations in RA-patients. The minor variant of rs2104286 (C), which is associated with lower rate of joint destruction, was associated with 0.85 (95%CI 0.77-0.93) fold sIL-2RA levels ($P=1.4x10^{-3}$, Figure 2C). Accordingly, we determined whether sIL-2RA was associated with the rate of joint destruction. A decrease of 1,000 pg/mL sIL-2RA was associated with a 0.57 (95%CI 0.39-0.83) fold rate of joint destruction per year ($P=3.4x10^{-3}$, Figure 2D). When the association of both IL2RA-rs2104286 and sIL-2RA were tested in one analysis, only sIL-2RA was significant ($P=8.0x10^{-3}$). This suggests that the association between IL2RA-rs2104286 and joint destruction is mediated by a mechanism affecting sIL-2RA concentrations.

Fine-mapping

To further localize the association of IL2RA with rate of joint destruction, the IL2RA region was fine-mapped and stronger associations with rate of joint destruction than rs2104286 were sought.

In the Leiden-EAC cohort, fifteen variants had a statistically stronger association with rate of joint destruction than rs2104286 (Figure 3). Thirteen of these top hits were situated in one LD block of 40kb ($1.3x10^{-4}>p<4.4x10^{-3}$, $0.94=\beta org=.97$), in which rs2104286 is also situated. This region encompasses IL2RA intron 1 and the 5′ region of IL2RA and RBM17 (Chr10:6118559-6158117). Two variants were outside this region; seq-Novel-50 (Chr10:6074516) ($\beta org=1.79$ (95%CI 1.40-2.28), $P=3.2x10^{-6}$), which lies 19kb downstream of IL2RA, and rs41295367 in RBM17 ($\beta org=1.22$ (95%CI 1.09-1.36), $P=3.4x10^{-4}$). These two variants both had an effect opposite to that of rs2104286. To assess whether these effects were independent, the genetic analysis was repeated including the three SNPs, rs2104286, rs41295367 and seq-Novel-50, in one analysis. In this analysis, all three SNPs were independently significant (data not shown).

We further focused on the rs2104286 region by studying the fourteen top hits of this region (rs2104286 and the thirteen additional variants in the IL2RA region) in the two USA data-sets (NDB and Wichita). Six SNPs were significantly associated with progression of joint destruction in the USA data-sets. In the meta-analysis combining the Dutch and USA data- sets (Figure 4A), the association of all fourteen SNPs were significant and

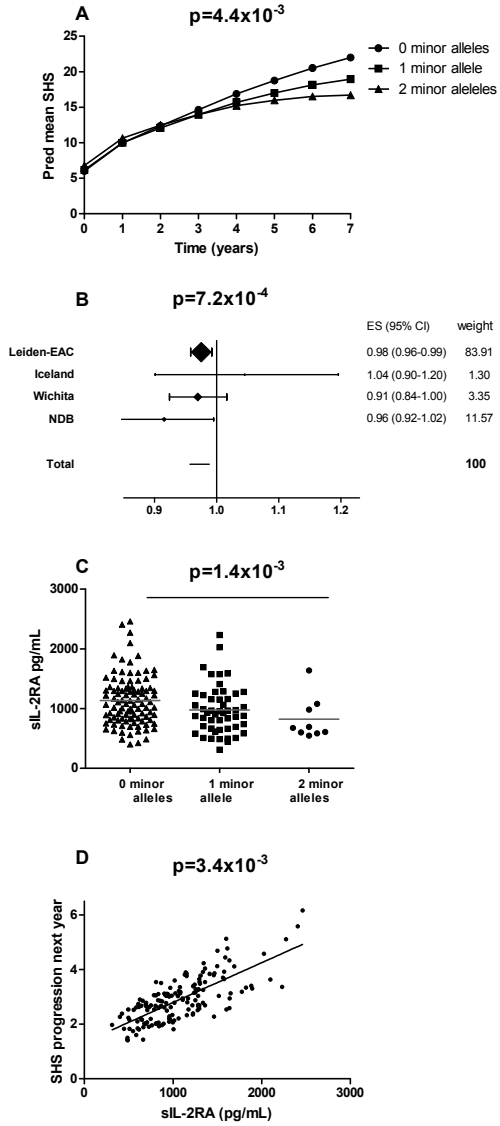


Figure 2 IL-2RA-rs2104286 is associated with rate of joint destruction and sIL-2RA serum levels.
(A) A significant association was found for the IL2RA (rs2104286) genotype with rate of joint destruction in the discovery cohort, the Leiden-EAC (β_{org}=0.97 (0.96-0.99), P=4.4x10^{-3}), the minor variant or rs2104286 was associated with 0.97 fold lower rate of joint destruction per year, corresponding to 0.65 fold lower rate of joint destruction over 7 years in the presence of 2 minor alleles (0.97^7^2=0.65). The plotted data are the result of the multivariate normal regression model adjusted for age, sex and treatment. (B) The association was in the meta-analyses of 1,750 RA-patients with 4,732 X-rays (P=7.2x10^{-4}). The depicted effect sizes (ES) indicate that the rate of joint destruction is lower in the presence of a minor allele compared to the common genotype. (C) The minor variant of rs2104286 was associated with lower sIL-2RA levels in 159 Leiden-RA patients. The green line indicates the mean. (D) Lower sIL-2RA levels are associated with less progression of joint destruction in the next year (P=7.2x10^{-4}). Plotted data are the results of the linear regression analysis adjusted for disease duration at time of serum sample and for age at inclusion.

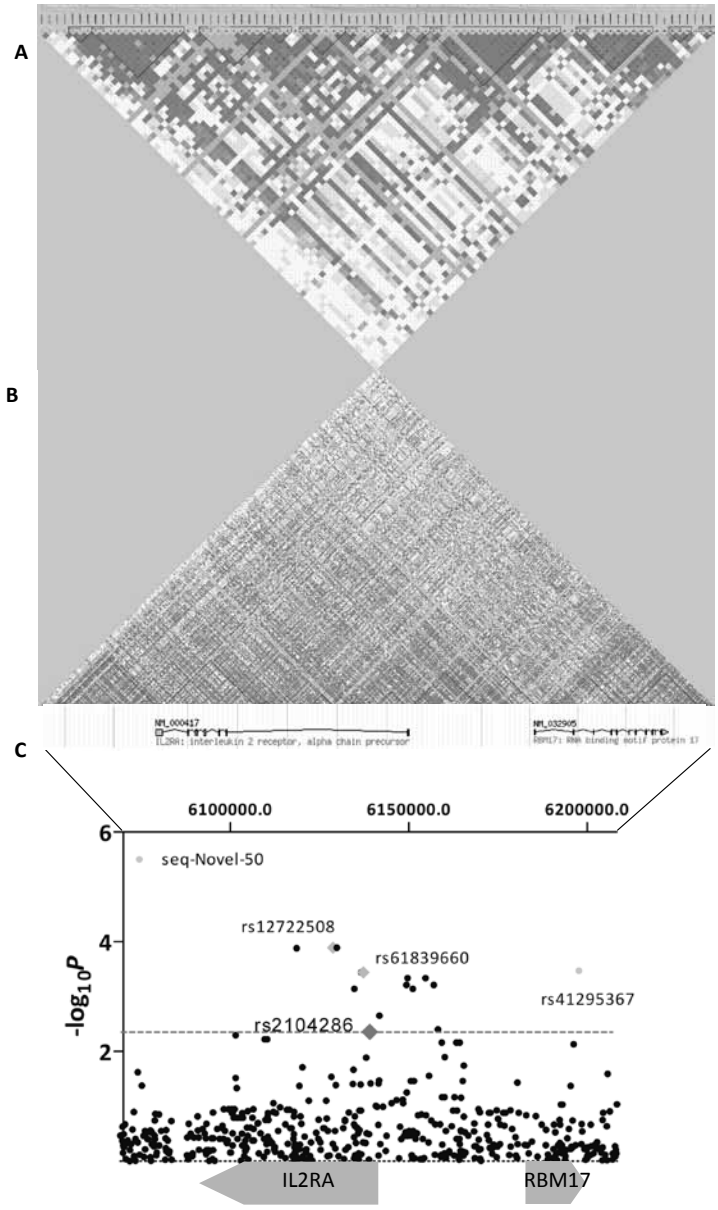
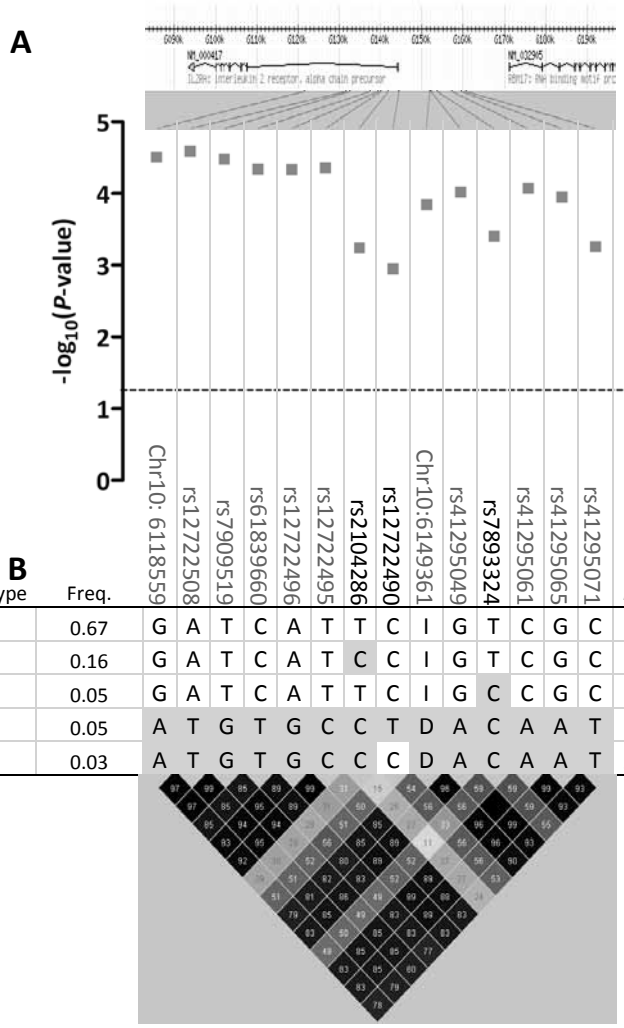


Figure 3 LD plots of the IL2RA-RBM17 region

(A) Total region in hapmap CEU patients and (B) fine-mapped in Leiden-EAC. (C) Results of the multivariate normal regression analysis for 495 genetic variants in the IL2RA-RBM17 region in Leiden-EAC. The colors reflect the D′ between the SNPs. Coordinates relate to NCBI36 hg18 release 2006.

the strongest association was found for rs12722508 ($P=2.6x10^{-5}$). This SNP was highly correlated (r2>0.80, Figure 4B) to ten other top hits in the region, making it difficult to distinguish which of these variants is of most importance.



B

haplotype	Freq.	Chr10: 6118559	rs12722508	rs7909519	rs61839660	rs12722496	rs12722495	rs2104286	rs12722490	Chr10:6149361	rs41295049	rs7893324	rs41295061	rs41295065	rs41295071	*P*-value
I	0.67	G	A	T	C	A	T	T	C	I	G	T	C	G	C	$1.0x10^{-3}$
II	0.16	G	A	T	C	A	T	C	C	I	G	T	C	G	C	0.15
III	0.05	G	A	T	C	A	T	T	C	I	G	C	C	G	C	0.23
IV	0.05	A	T	G	T	G	C	C	T	D	A	C	A	A	T	$1.0x10^{-3}$
V	0.03	A	T	G	T	G	C	C	C	D	A	C	A	A	T	$3.9x10^{-2}$

Figure 4 Exploration of the IL2RA region

The results presented here are the combined results of the Leiden and the USA samples (1,465 patients with 4,447 X-rays). (A) In total fourteen variants (including rs2104286) in a 40kb region were strongly associated with rate of joint destruction (dotted line presents the cut-of for significance at 0.05) (B) Haplotype analysis for these fourteen SNPs. Three haplotypes were significantly associated with joint destruction, the most common with risk and two minor haplotypes with protection for joint destruction. Minor alleles are highlighted in grey. SNPs with an r^2>0.8 with each other are depicted in the same color (orange, green, purple and blue). The LD between the strong variants in RA-patients is depicted with HaploView (see URLS). The color and the numbers in the graph represent the r^2 between the genetic variants.

Since the presented results are from the fixed effect meta-analysis only p-values are given.

Subsequently haplotype analyses were performed in an attempt to further differentiate between the fourteen genetic variants. To enlarge power, the data of the Leiden-EAC and the North-American samples were combined in inverse weighting meta-analyses

The haplotype that associated most strongly with rate of joint destruction (haplotype IV, P=1.0x10^{-3}, Figure 4B) was characterized by one SNP, rs12722490. When the haplotypes were analyzed in the Dutch and the North-American samples separately, similar results were observed (data not shown).

Four SNPs together tag the fourteen top SNPs in the 40kb region; rs12722508 (the strongest associating SNP), rs2104286 (the initial SNP), rs12722490 (the haplotype-tagging SNP) and rs7893324 (in low LD with all other SNPs in 40kb region) (see Figure 4B). The correlation between these four SNPs was modest (r2 0.15-0.50).

DISCUSSION

The variance in joint destruction between patients is considerable and the mechanisms driving these differences are thus far scarcely understood. In the past decades, many genetic variants have been identified to associate with the susceptibility of RA. We hypothesized that similar variants could be involved in disease severity as well. Therefore, a candidate gene study was performed to investigate the association of identified RA-susceptibility SNPs with rate of joint destruction. We observed that RA-patients with the minor genotype of IL2RA-rs2104286 had lower rate of radiographic joint destruction than patients with the common genotype. This association was further supported by the observations that the minor genotype of rs2104286 were associated with lower circulating soluble IL-2RA levels and that lower sIL-2RA levels was associated with lower rate of joint destruction. Finally, the association was localized to a region of 40kb encompassing the IL2RA intron 1 and the 5′ region of IL2RA and RBM17.

The present study uniquely combines data-sets of patients who started treatment in a time when treatment was not as aggressive as nowadays. Hence, the radiologic progression rate of the patients studied here are more reflective of the natural course of RA than that of recently treated patients. Replication data-sets are ideally larger than the discovery data-set, since at a replication stage effects sizes are generally smaller. Unfortunately, relatively few large prospective data-sets exists with both X-rays and DNA available in conventionally treated patients. The number of patients and the number of X-rays of each separate data-set were insufficient to expect well powered analyses. However, the combination of the data in an inverse variance weighting analysis supported the association of IL2RA-rs2104286 with rate of joint destruction in the discovery data-set. In addition, independent replication of the association of rs2104286 was obtained in the NDB. Furthermore six hits in the 40 kb IL2RA region were significantly associated with progression of joint destruction in the Dutch and the North-American RA-patients.

IL2RA (CD25) encodes the high affinity alpha chain of the IL-2 receptor. It is expressed by numerous immune cell types, including several subsets of CD4+ T-cells, and it is

upregulated in inflammatory conditions.[35,36] Besides the known association with RA susceptibility,[20,24] IL2RA is also associated with the risk on other auto-immune diseases such as Juvenile Idiopathic Arthritis[37], Type-I-Diabetes (T1D)[38,39], Multiple Sclerosis (MS)[40], Systemic Lupus Erythematosus[41] and Grave's disease[42]. In most of these associations, the minor allele rs2104286 was associated with a protective effect on disease susceptibility. Closer studies on the IL2RA region in T1D and MS have demonstrated that other genetic variants in the region of rs2104286 play a supplementary role, which is sometimes in the opposite direction of rs2104286.[38,43,44] Some studies in T1D, in MS and in healthy individuals also revealed that carrying the rs2104286 minor allele is associated with lower circulating levels of soluble IL-2RA (sIL-2RA)[43,44] sIL-2RA is produced by the proteolytic cleavage of membrane-bound IL-2RA after the induction of growth and cell division.43,45-473 and hence considered as a marker for T-cell proliferation.

The association of IL2RA-rs2104286 with susceptibility to RA was initially discovered by Barton et al.[20] and afterwards supported by Stahl et al.[24] In the latter study another variant, rs706778, was more strongly associated with RA susceptibility. The association was strongest for the haplotype defined by rs706778-rs2104286-rs11594656. In the current study evaluating the severity of RA, rs760778 was analyzed in the discovery cohort; no association of rs760778 with rate of joint destruction was observed. Also rs11594656 was not significantly associated in the analyses on the fine-mapped data.

The RA susceptibility SNPs analyzed in this study were identified in previous GWAs studies. GWAs platforms are designed to type SNPs throughout the genome but genes are not fine-mapped, making it possible that other, non-typed variants that are linked to the observed variant, are the cause of phenotypic variation. To further localize the association of IL2RA with rate of joint destruction, we fine-mapped the IL2RA region and sought stronger associations with rate of joint destruction. Here we found SNPs that had a stronger association with rate of joint destruction than rs2104286 and several were independently replicated in the North-American samples.

In our study, fourteen SNPs in a 40 kb region of IL2RA associated with progression of joint destruction. It cannot be concluded which one of these are the most important. Fine-mapping of IL2RA in case-control studies revealed an association of the same set of linked SNPs (linked to rs12722508) found in studies of T1D and MS.

Thus far, no studies have investigated genetic variants in IL2RA with long-term disease outcome. The present study provides evidence for a role of IL2RA in disease progression in RA. Dendrou et al. observed in healthy individuals that presence of the rs2104286 minor allele reduced the probability of CD4+ T-cells expressing CD25, thereby reducing the likelihood of CD4+ T cell activation.[36] Notably, an effect of the IL2RA genotypes on the expression of CD25+ in T- regulatory cells was not observed.[36] Those results are consistent with our observation of lower sIL-2RA and lower rate of joint destruction in the presence of the rs2104286 minor allele. Possibly, the protective IL2RA genotypes mediate a reduced

expression of CD25 and less activation of CD4+ T cells, preventing severe inflammation and reducing long term joint destruction.

Hampering the expansion of T cells with daclizumab, an antibody against IL-2RA, has shown to be beneficial as treatment for MS.[48] The effects of daclizumab on specific immune cells are not completely characterized. Since T cell activation is very probably involved in disease development and progression in RA, it would be interesting to assess the efficacy of daclizumab in RA.

The present study is one of the few large scale studies evaluating genetics in relation to the severity of RA. The associations of candidate gene loci with progression of joint destruction were studied using longitudinal data-sets with high quality phenotypic data. The genetic and serologic data provide evidence for a role of IL2RA in progression of RA, making it an interesting target for future therapeutic studies.

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WEB RESOURCES

The URLs for data presented here are as follows: SPSS version 17.0, www.spss.com
Stata version 10.0, www.stata.com
Entrez Gene, http://www.ncbi.nlm.nih.gov/gene http://genome.ucsc.edu/cgi-bin/hgGateway
Plink version 1.07, http://pngu.mgh.harvard.edu/~purcell/plink/ Haploview version 4.2, www.broad.mit.edu/mpg/haploview/

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