Predictive factors for the development and disease course of rheumatoid arthritis

Krabben, A.

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Association of Genetic Variants in the *IL4* and *IL4R* Genes With the Severity of Joint Damage in Rheumatoid Arthritis: A Study in Seven Cohorts

A. Krabben, A. G. Wilson, D. P. C. de Rooy, A. Zhernakova, E. Brouwer, E. Lindqvist, T. Saxne, G. Stoeken, J. A. B. van Nies, R. Knevel, T. W. J. Huizinga, R. E. M. Toes, P. K. Gregersen, A. H. M. van der Helm-van Mil

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ABSTRACT

Objective

The progression of joint destruction in rheumatoid arthritis (RA) is determined by genetic factors. Changes in *IL4* and *IL4R* genes have been associated with RA severity, but this finding has not been replicated. This study was undertaken to investigate the association between *IL4*- and *IL4R*-tagging single-nucleotide polymorphisms (SNPs) and the progression rate of joint damage in RA in a multi-cohort candidate gene study.

Methods

IL4- and *IL4R*-tagging SNPs (n = 8 and 39, respectively) were genotyped in 600 RA patients for whom 2,846 sets of radiographs of the hands and feet were obtained during 7 years of follow-up. Subsequently, SNPs significantly associated with the progression of joint damage were genotyped and studied in relation to 3,415 radiographs of 1,953 RA patients; these included data sets from Groningen (The Netherlands), Lund (Sweden), Sheffield (UK), the North American Rheumatoid Arthritis Consortium (US), Wichita (US), and the National Data Bank (US). The relative increase in progression rate per year in the presence of a genotype was determined in each cohort. An inverse variance weighting meta-analysis was performed on the 6 data sets that together formed the replication phase.

Results

In the discovery phase, none of the *IL4* SNPs and 7 of the *IL4R* SNPs were significantly associated with the joint damage progression rate. In the replication phase, 2 SNPs in the *IL4R* gene were significantly associated with the joint damage progression rate (rs1805011 [P = 0.02] and rs1119132 [P = 0.001]).

Conclusion

Genetic variants in *IL4R* were identified, and their association with the progression rate of joint damage in RA was independently replicated.

INTRODUCTION

In the last decade it has been recognized that rheumatoid arthritis (RA) needs to be diagnosed early and treated promptly with disease-modifying anti-rheumatic drugs (DMARDs) in order to successfully interfere with the disease process and the progression to joint damage and disability. The progression of joint destruction is highly variable; only a minority of patients develop rapidly progressive disease. To achieve individualized treatment, the severity of the disease outcome needs to be estimated adequately. Ideally, the biologic processes underlying the interindividual differences should be understood. Clinical and serologic risk factors explain only about one-third of the total variance in joint destruction.[1] Genetic variants are estimated to make a major contribution, consisting of 50–60% of the total phenotypic variation.[2] Further studies of individual risk factors are needed to increase understanding of the processes underlying the progression of joint destruction in RA.

We performed a candidate gene study of the association of *IL4* and *IL4R* with the rate of joint destruction in RA. It has been hypothesized that there is an imbalance between Th1 cells and Th2 cells in RA, with different levels of tumor necrosis factor, interleukin-1 (IL-1), IL-6, IL-4, and IL-13.[3] IL-4 mainly promotes differentiation of T cells toward Th2 cells.[4] The role of IL-4 is underscored by observations that the concentration of IL-4 is increased in early arthritis but decreased or absent in synovial fluid from patients with established RA.[4,5] Furthermore, in fibroblast-like synoviocytes, IL-4 was shown to suppress RANKL expression and increase osteoprotegerin expression, and IL-4–knockout mice are characterized by extensive joint destruction,[6] suggesting that IL-4 not only has anti-inflammatory effects, but also has antiosteoclastogenic effects. The effect of IL-4 is mainly mediated by the IL-4 receptor α chain (IL-4Rα).

Several genetic studies of the association of susceptibility to, and the severity of, RA with *IL4* and *IL4R* genes have already been performed. The *IL4* variable-number tandem repeat (VNTR) in the third intron was reported to be associated with lower radiographic damage.[7] Prots et al reported an association between *IL4R* I50V (rs1805010) and the presence of bone erosions in patients with a disease duration of >2 years.[8] Marinou et al, however, found no association of the I50V variant with joint destruction.[9] Furthermore, the *IL4R* Q551R variant (rs1801275) has been studied and found not to be associated with susceptibility to or the severity of RA, but to be associated with the presence of RA nodules.[7-10]

Because of the proposed role of *IL4* and its receptor genes and the observation that the progression of joint damage is in part heritable, we performed a candidate gene study. The genetic variants tagging *IL4* and *IL4R* were determined in 600 patients for whom 2,846 radiographs were available. Furthermore, 3,415 radiographs of 1,953 patients in 6 additional cohorts were subsequently studied for replication.

PATIENTS AND METHODS

Study population

Seven data sets consisting of adult patients diagnosed as having RA according to the American College of Rheumatology 1987 criteria[11] were studied (Table 1). Informed consent was obtained from all patients, and approval was obtained from the local ethics committees.

Discovery phase cohort

Six hundred patients with early RA who were included in the Leiden Early Arthritis Clinic (EAC) from 1993 to 2006 were studied. Radiographs of the hands and feet were obtained at baseline and at yearly follow-up visits for 7 years.[1] A total of 2,846 sets of radiographs of the hands and feet were available. All radiographs were scored by one experienced reader using the modified Sharp/van der Heijde scoring method (SHS).[12] The intra class correlation coefficient (ICC) was 0.91. The treatment received by these patients differed according to 3 treatment periods, as previously described.[1]

Replication phase cohorts

Six data sets were studied (Table 1).[1,13-16] These included 280 patients with 872 radiographs from Groningen, The Netherlands,[1] 391 patients with 391 radiographs from Sheffield, UK,[1,15] 147 patients with 781 radiographs from Lund, Sweden,[1,13] 385 patients with 385 radiographs from the North American Rheumatoid Arthritis Consortium (NARAC) (14), 101 patients with 337 radiographs from Wichita, and 649 patients with 649 radiographs from the National Data Bank (NDB; US).[16]

Single-nucleotide polymorphism (SNP) selection

IL4 and *IL4R* were captured by haploblocks, using the Haploview algorithm, covering the whole gene and 3 kb upstream and downstream of the coding region. Pairwise tagging SNPs were selected from the Utah residents with ancestry from northern and western Europe (CEPH/CEU) HapMap data set (phase II, release 23a/March 2008) using Haploview software (minor allele frequency [MAF] >0.05, pairwise r^2 >0.8). There were no known amino acid–changing SNPs in *IL4* with an MAF of 2:5%. In total, 8 SNPs in *IL4* were tagged with Haploview, without forcing any SNPs.

In *IL4R*, there were 7 known amino acid–changing SNPs. Forty-three SNPs tagged the *IL4R* gene region, with forcing these 7 SNPs. Four SNPs located on *IL4R* failed typing and, therefore, 39 *IL4R* SNPs were successfully tested. The final SNP selection and linkage disequilibrium information are available from the corresponding author upon request.

Table 1 Characteristics of the different cohorts studied

Cohort	No. of RA-patients	No. of X-ray sets	Year of diagnosis	Follow-up, years†	Mean disease duration ±sd, years†	Method of scoring	ICC	ACPA+, n (%)	Age, mean ±sd	Female gender, n (%)
Discovery phase										
Leiden-EAC	600	2,846	1993-2006	7		SHS	0.91	324 (55)	56.3±15.7	419 (69)
Replication phase										
Groningen	280	872	1945-2001	14		SHS	>0.90 /0.96*	163 (80)	49.3±12.6	197 (70)
Lund	147	781	1985-1990	5		Larsen	0.94*	114 (80)	50.7±11.5	98 (67)
Sheffield	391	391	1999-2006		15.4±10.8	Larsen	0.83#	299 (79)	46.0±13.4	285 (73)
NARAC	385	385	1953-2002		13.8±10.5	SHS	0.99	385 (100)	40.8±11.9	281 (73)
Wichita	101	337	1963-1999	15		SHS	0.99	97 (97)	49.0±11.7	70 (69)
NDB	649	649	1972-1999		11.7±6.4	SHS	0.99	523 (81)	47.7±12.7	506 (78)
Total	1,953	3,415								

In Leiden, Groningen, Lund and Sheffield hands and feet X-rays were taken. In the NARAC, Wichita and NDB cohorts hand X-rays were taken.

†For the studies with cross-sectional data (one radiograph in time) the mean disease duration (±sd) at time of the radiograph was reported. The studies with longitudinal data (more than one radiograph in time) the maximum follow-up duration was reported.

SHS Sharp van der Heijde score

ACPA+ anti-citrullinated peptide antibody positivity

All ICC scores represent the correlation coefficient within the reader, accept for the Sheffield cohort#, where a weighted kappa score was provided.

*Furthermore the radiographs in Groningen and Lund were scored by one of two readers. In the Groningen cohort the within reader ICC was >0.90 and the between reader ICC was 0.96. In Lund the between reader ICC was 0.94.

More information on the cohorts is provided in the Supplementary Methods and in references (1;11-14).

SNP genotyping

In all cohorts, DNA was extracted from whole blood using standard methods. In the discovery cohort, genotyping was performed using multiplex SNP arrays designed using an Illumina GoldenGate platform, according to the protocols recommended by the manufacturer. In the cohorts from Groningen, Lund, and Sheffield, SNPs were typed by multiplex SNP arrays designed with a Sequenom iPlex system, according to the protocols recommended by the manufacturer. In the NARAC, genome-wide SNP typing was performed using Infinium HumanHap550, version 1.0 (Illumina). In the Wichita and NDB cohorts, SNPs were typed with an Immunochip, Illumina Infinium High-Density array (Illumina iScan Platform), which was recently designed to densely genotype immune-mediated disease loci identified by genome-wide association studies of common variants using data. The SNPs identified in the discovery phase were retrieved from the genetic databases of the NARAC, Wichita, and NDB cohorts. Four of the SNPs identified were not available in the genetic databases for the Wichita and NDB cohorts.

Statistical analysis

In all data sets, 1 was added to all radiologic scores and then the scores were log-transformed to obtain a normal distribution. Two phases were carried out. First, an explorative analysis was performed in the Leiden EAC cohort, testing the tagged SNPs both additively and recessively. A multivariate normal regression model for longitudinal data was used with repeated radiologic score as a response variable. This model makes use of repeated radiologic measurements by taking advantage of within-patient correlation, yielding more precise estimates of the progression rates and therefore increasing the power to detect differences. This model uses a covariance matrix, allowing the inclusion of patients who had missing radiographs at some point during follow-up. The model fit was tested by residual analyses.[1,17] Adjustment variables were entered based on their single variable association with joint destruction. Adjustments were made for age, sex, and the treatment periods described previously.[1]

In the second, replication, phase only the model (recessive or additive) that fit best in the discovery phase was tested. Each of the individual replication cohorts had fewer radiographs and hence less power than the discovery cohort. Because of differences in study designs, the data from the separate replication cohorts could not be combined into one analysis directly. Therefore, we decided to test the SNPs in each data set separately, taking advantage of the specific data set characteristics, and to subsequently perform a meta-analysis. Since the beta values obtained from the analyses of the different cohorts all reflected the relative increase in radiologic progression rate per year in patients with a certain genotype compared to patients with the common genotype, the estimates could be pooled in a meta-analysis.

In the Groningen, Lund, and Wichita cohorts, multiple radiographs per individual were available, and a multivariate normal regression analysis was used, which was similar to the analysis applied in the Leiden EAC cohort. Adjustment variables were entered based on their single variable association with joint destruction. The Groningen data set was adjusted for age and inclusion before or after 1990, as a proxy for DMARD therapy. The analysis of the Lund cohort was adjusted for age only, since sex and treatment were not associated with joint destruction in this data set. The analysis of the Wichita cohort was adjusted for age and sex.

In the Sheffield, NARAC, and NDB data sets, radiographs were obtained from each patient at one time point. To derive estimates of the radiologic progression, the estimated yearly progression rate was calculated by dividing the total SHS by the number of years of disease duration at the time the radiograph was obtained. Also in this analysis, 1 was added to the estimated yearly progression rate data before log-transformation. Subsequently, the SNP association was tested in a linear regression analysis with log-transformed estimated yearly progression rate as the outcome variable. The resulting estimate reflected how many fold the rate of joint destruction increases per year in patients with a certain genotype compared to patients with the common genotype. No adjustments were applied to the analysis of the Sheffield and NARAC data sets, since none of the variables tested were significantly associated with joint destruction. Analyses of the NDB data set were adjusted for age and sex. SPSS version 17.0 was used.

Since all of the beta values obtained reflected the relative increase in radiologic progression rate (a relative measure without units), the beta values and standard errors could be combined in a fixed-effects meta-analysis with inverse variance weighting.[18] The standard errors differed between the data sets because the number of radiographs per patient differed between the cohorts, resulting in more precise estimates of the relative progression rates and smaller standard errors in data sets with serial measurements. The meta-analysis was performed in Stata, version 10.1.

Multiple testing increases the possibility of incorrectly rejecting the null hypothesis to >5%. Therefore, Bonferroni correction for multiple testing was applied in phase 2.

Haplotype analysis

Haplotypes of *IL4R* were studied. Haplotype blocks were defined using the model described by Gabriel et al.[19] Haplotypes were assigned to each individual using Plink version 1.07. Analyses of the haplotypes were performed with methods similar to those used for the analyses of the individual SNPs by additive testing of a haplotype.

RESULTS

Discovery phase

A complete overview of the results of all SNPs studied in the discovery phase is available from the corresponding author upon request. None of the 8 SNPs tagging *IL4* were significantly associated with the progression of joint destruction in the Leiden EAC cohort.

Analysis of the 39 SNPs in the *IL4R* gene region revealed 7 SNPs that were significantly associated with the progression of joint destruction (Table 2). For 4 SNPs, the recessive analysis showed the strongest association (rs4787423 [*P* = 0.03], rs7191188 [*P* < 0.01], rs6498016 [*P* = 0.01], and rs1119132 [*P* = 0.04]), and for 3 SNPs, the additive analysis showed the strongest association (rs1805011 [*P* = 0.01], rs1805015 [*P* = 0.04], and

Table 2 Results of the SNPs in *IL-4R* with a significant association with the radiological progression rate in the discovery phase.

		Discovery phase						Replication phase (meta-analysis)	
SNP	Coordinate	MAF EAC	Tested model	β	95%CI		P	No. cohorts	P
rs4787423	27274835	0.14	ADD	0.99	0.96	1.01	0.35		
			REC	0.90	0.83	0.99	0.03	4	0.81
rs1805011	27281373	0.11	ADD	0.96	0.93	0.99	0.01	4	0.02
			REC	0.93	0.81	1.07	0.34		
rs1805015	27281681	0.16	ADD	0.98	0.95	1.00	0.04	4	0.08
			REC	0.99	0.91	1.07	0.76		
rs1801275	27281901	0.20	ADD	0.97	0.95	0.99	0.01	4	0.21
			REC	0.99	0.93	1.05	0.67		
rs7191188	27296912	0.25	ADD	1.02	1.01	1.04	0.01		
			REC	1.10	1.05	1.15	<0.01	6	0.83
rs6498016	27299289	0.21	ADD	1.01	1.00	1.03	0.16		
			REC	1.08	1.02	1.14	0.01	6	0.88
rs1119132	27310970	0.13	ADD	1.02	1.00	1.04	0.15		
			REC	1.09	1.00	1.18	0.04	6	0.001

The β indicates the fold difference in progression rate in the presence of the risk allele or risk genotype. The β from the additive model indicates the fold difference in progression rate for each additive minor allele present. The β from the recessive model indicates the fold difference in progression rate in patients homozygous for the minor allele versus the other patients. For instance patients carrying one minor allele of rs1805011 had a 0.96-fold higher rate of joint destruction compared to patients without a minor allele. This corresponds with a 24% (0.961^7=0.76) lower rate of joint destruction over 7 years.
In the replication phase, analyses were only performed for the model with the highest significance level in the discovery phase. rs4787423, rs1805011, rs1805015 and rs1801275 were not available in the genetic database of Wichita and NDB. An inverse weighted meta-analysis was performed on 4 or 6 independent cohorts. In the NARAC, Wichita and NDB cohort a proxy SNP for rs1119132 was analyzed; rs1859308 (r^2=0.92). In the NARAC cohort a proxy for rs4787423 was analyzed; rs3024660 (r^2=1).

rs1801275 [$P = 0.01$]) (Table 2). In this exploratory phase, no corrections for multiple testing were made, and these 7 variants were studied in the second phase.

Replication phase

A total of 1,953 patients and 3,415 scored radiographs were evaluated. The SNPs rs7191188, rs6498016, and rs1119132 were studied in all 6 cohorts (Groningen, Lund, Sheffield, NARAC, Wichita, and NDB). For rs1805011, rs4787423, rs1805015, and rs1801275, no genotyping data were available for the Wichita and NDB cohorts; hence, these SNPs were studied in 4 cohorts (Groningen, Lund, Sheffield, and NARAC). In addition, proxy SNPs were analyzed for 2 SNPs in some cohorts. In the NARAC, Wichita, and NDB cohorts, a proxy SNP for rs1119132 was analyzed: rs1859308 ($r^2 = 0.92$). In the NARAC cohort, a proxy SNP for rs4787423 was analyzed: rs3024660 ($r^2 = 1$). Since all replication cohorts had fewer available radiographs than the discovery cohort, the power to find significant differences in each of these cohorts was limited, and data were combined in a meta-analysis. The analyses were performed by testing either a recessive or an additive association, depending on the results obtained in the discovery phase. Of the 7 SNPs studied in this phase, 2 were significantly associated with joint progression. These were rs1805011 ($P = 0.02$) and rs1119132 ($P = 0.001$) (Table 2 and Figure 1). These SNPs are in low linkage disequilibrium ($r^2 = 0.01$). In a conditional analysis including both SNPs, both remained significant (rs1805011 [$P = 0.006$] and rs1119132 [$P = 0.041$]), indicating that their effect is likely to be independent. After Bonferroni correction for multiple testing for 7 SNPs in the replication phase, rs1119132 remained significantly associated with the progression of joint damage ($P_{corrected} = 0.007$).

Findings of haplotype analysis

To attempt to further elucidate the associations found, a haplotype analysis was performed using the data for the *IL4R* gene in the Leiden EAC cohort. This resulted in 9 haplotype blocks with a minor haplotype frequency of >0.01 (results available from the corresponding author upon request). All haplotypes with a prevalence of >0.1 were tested for an association with joint destruction in the EAC cohort. Because of this cutoff, no haplotypes that included rs119132 were evaluated. Two haplotypes of one haplotype block showed a better association than the independent SNPs (AAA and CGG, consisting of the following SNPs: rs1805011, rs1805015, and rs1801275, respectively). These haplotypes were analyzed in the replication phase, and no association with the progression of joint damage was observed.

Finally, rs1119132 and rs1805011 were studied in anti–citrullinated protein antibody (ACPA)–positive and ACPA-negative patients separately. This yielded comparable effect sizes in both subgroups (data not shown).

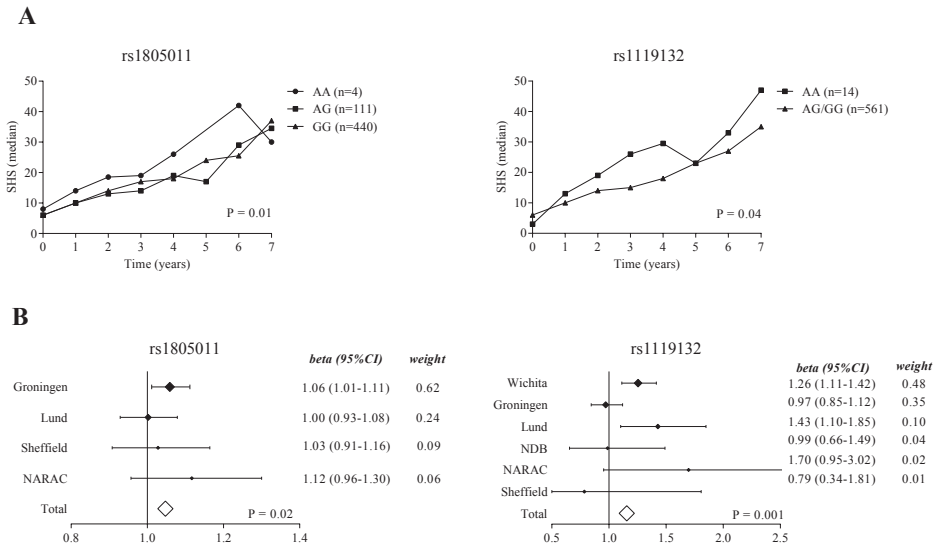

Figure 1 Results of rs1805011 and rs1119132 in the discovery phase (A) and replication phase (B).
A. Presented are the median Sharp van der Heijde scores (SHS) over 7-years of follow-up, per genotype in RA-patients of the EAC. The 'bump' in the line at year five is caused by missing radiographs of part of the patients with rs1119132 genotype AA.
B. Presented is an inverse variance weighted meta-analysis in four cohorts (rs1805011) and six cohorts (rs1119132). In the NARAC, Wichita and NDB cohort a proxy SNP for rs1119132 was analyzed; rs1859308 (r^2=0.92). Genotyping data of rs1805011 were not available for the Wichita and NDB cohort.

DISCUSSION

The severity of RA is reflected by the severity of radiologic joint destruction. It is highly variable between patients, and part of this variance is explained by genetic factors. Several studies of IL-4 at the protein level have suggested that this interleukin is relevant in RA.[3-6] In addition, several genetic studies of *IL4* and *IL4R* and joint damage have been performed,[7-10] though none of the factors identified have been replicated. This prompted us to perform the present multi-cohort candidate gene study. We observed that patients carrying 2 minor alleles of rs1119132 in *IL4R* had more severe joint damage progression. Although a minority of RA patients may have this genetic variant, individual independent replication was found in some of the replication cohorts as well as in the meta-analysis of the 6 replication cohorts.

In addition to the results for rs1119132, another SNP in *IL4R*, rs1805011, showed an association with joint destruction. In a conditional analysis including both SNPs, both remained significantly associated with the progression of joint damage. These SNPs were in low linkage disequilibrium. Since, after applying the conservative Bonferroni correction for

multiple testing only rs1119132 remained significant, we did not draw a definite conclusion regarding rs1805011.

SNP I50V (rs1805010) in *IL4R* was previously found to be associated with joint destruction in RA.[8] Despite studies of the potential functional relevance of this SNP,[8,20] the association of this variant with joint damage was not observed in the study by Marinou et al[9] or in our study. Another coding variant on *IL4R*, Q551R (rs1801275), was not associated with joint destruction in prior studies.[7-10] In the present study, *IL4R* Q551R was significantly associated with joint destruction in the discovery cohort ($P = 0.01$) but not in the replication phase ($P = 0.21$).

Despite previous in vitro studies and mouse studies showing that IL-4 plays a role in suppressing arthritis severity, in the present study no association between SNPs in *IL4* and joint destruction were observed. An association between *IL4* VNTR has been reported previously.[7] This variant was not included in our study.

We used the classic candidate gene approach, including immune response factors that had previously been shown to be involved in RA pathogenesis. This method has a larger a priori chance of finding a true association between SNPs and disease severity. However, this approach may also result in false-positive or false-negative findings. We studied 6 replication cohorts in order to reduce the chance of false-positive findings.

In conclusion, we identified and replicated a genetic variant in *IL4R* predisposing to joint damage progression in RA. Further studies of IL-4R at the protein level are needed to increase insight on the role of this variant in the pathogenesis of RA progression.

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SUPPLEMENTARY METHODS

Brief description of replication cohorts

Groningen 280 RA-patients from the Northern part of The Netherlands, diagnosed between 1945 and 2001 were studied. Over a follow-up duration of at most 14 years the mean number of X-ray sets (hands and feet) per patient was 3.1 (with a maximum of eight X-rays per patient). The total number of sets of X-rays was 872. The X-rays were scored by one of two readers using SHS. ICCs within readers were >0.90 and between readers 0.96. Patients included in the 90's were treated with DMARD therapy in contrast to patients included before the 1990.

Lund 147 early RA-patients from Sweden, recruited from primary care units in the area of Lund during the years 1985-1990 were studied. They were prospectively followed yearly during 5 years. In total 781 sets of X-rays were available and scored according to Larsen by one of two readers. The ICC between the readers was 0.94. In the inclusion period, immediate DMARD therapy was not common and only half of the patients used any DMARD at 5 years follow-up.

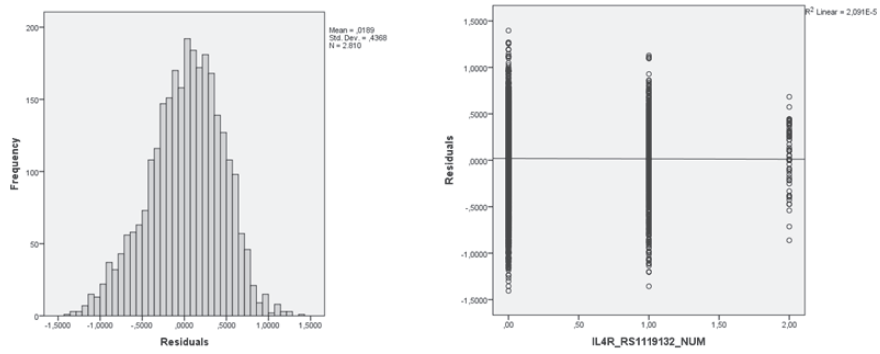
Sheffield 391 RA-patients from the area of Sheffield (UK) recruited between 1999 and 2006 were evaluated. RA-patients were assessed once during their disease course. The mean (±SD) disease duration at assessment was 15±11 years (range 3-65 years). X-rays of hands and feet were scored by one reader using a modification to Larsen's score. The intra-observer variation by a weighted kappa score was 0.83.

NARAC 385 ACPA-positive RA-patients from the North American Rheumatoid Arthritis Consortium who were radiographed between 1953 and 2002, with cross-sectional radiological measurements of hands, were studied. The mean (±sd) disease duration at assessment was 14±11 years. The radiographs were scored according to SHS by a single reader, with an ICC of 0.99.

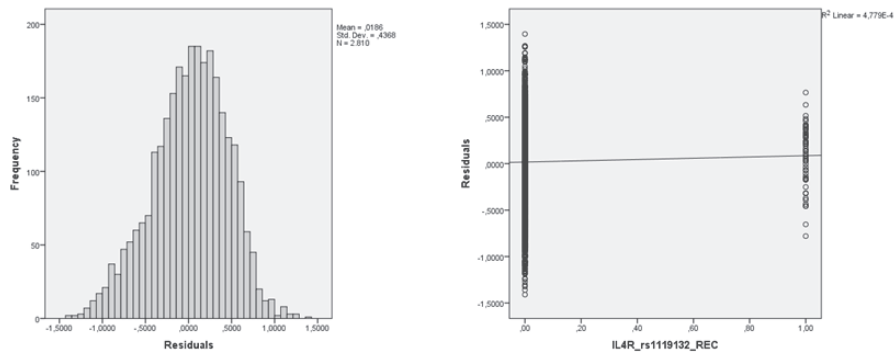
Wichita 101 patients from one practice in Wichita (Kansas, USA), recruited between 1963 and 1999 were studied. Patients were followed for a maximum of 15 years. Radiographic data were obtained when needed for clinical care. In total, 337 sets of hands X-rays were available. All X-rays were scored with SHS by one reader, the within reader ICC was 0.99.

NDB 649 patients included in the National Databank for Rheumatic diseases between 1972 and 1999, a databank that consists of patients with rheumatic diseases from the USA and Canada, were studied. A single time-point X-ray of the hands was available over a follow-up duration of at most 25 years with a mean (±sd) of 12±6 years. All X-rays were scored according to the SHS by the same reader who scored the Wichita X-rays.

Additive model:

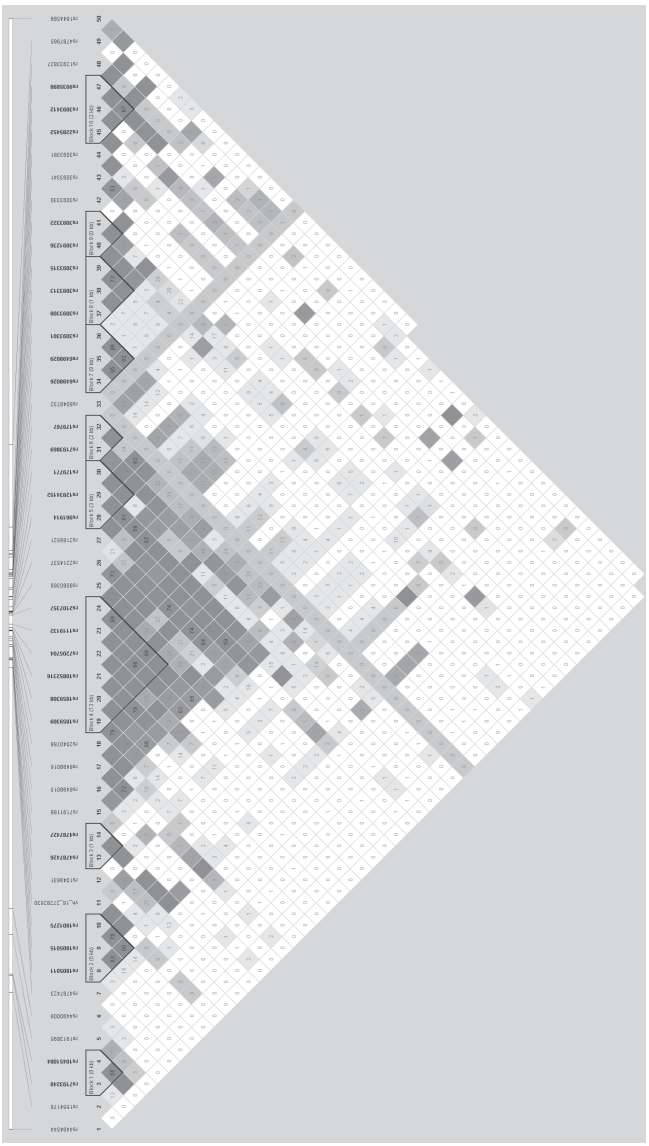


Recessive model:



Supplementary Figure 1 Residual analyses of the model used in the discovery cohort, including all covariates and rs1119132.

These graphs demonstrate the normal distribution of the residuals and that there is no correlation between genotype and residuals, meaning that the model fits the data.



Supplementary Figure 2 LD structure between the 43 SNPs in *IL4-R* in the Leiden-EAC
Presented are the nine haploblocks composed of 43 SNPs in the *IL-4R* gene and with a minor haplotype frequency (MHF) > 0.01. All haplotypes with a prevalence >0.1 were tested with joint destruction. Two haplotypes (AAA and CGG; rs1805011, rs1805015 and rs1801275, respectively) of one haploblock (depicted as block 5) showed a better association with joint destruction then the independent SNPs. Analysis of the additional presence of the haplotype in the discovery cohort, resulted in the following results for AAA β=1.02, 95%CI=1.00-1.05, p=0.02 and for CGG β=0.97, 95%CI=0.94-1.00 p=0.02. AAA and CGG had a frequency of 0.80 and 0.11 in the EAC (discovery cohort).
The frequencies of the AAA and CGG haplotypes were similar in the four additional datasets (frequency 0.75-0.79 and 0.10-0.15). Meta-analysis of the haplotypes of all datasets resulted in not significant results. For additive testing of the haplotypes it resulted in p=0.31 for AAA and p=0.64 for CGG.

Supplementary table 1 Overview of the results of all SNPs studied in the discovery phase

gene	rs number	coordinate	model	p
IL-4R	rs3024530	27258188	ADD	n.s.
			REC	n.s.
	rs4787426	27292232	ADD	n.s.
			REC	n.s.
	rs1805011	27281373	ADD	0.006
			REC	0.335
	rs11074852	27208097	ADD	n.s.
			REC	n.s.
	rs4547335	27204191	ADD	n.s.
			REC	n.s.
	rs1805010	27263704	ADD	n.s.
			REC	n.s.
	rs7205704	27308394	ADD	n.s.
			REC	n.s.
	rs10852316	27306056	ADD	n.s.
			REC	n.s.
	rs1029489	27283718	ADD	n.s.
			REC	n.s.
	rs4787423	27274835	ADD	0.354
			REC	0.028
	rs1801275	27281901	ADD	0.011
			REC	0.672
	rs1805015	27281681	ADD	0.037
			REC	0.764
	rs4787956	27285750	ADD	n.s.
			REC	n.s.
	rs3024560	27264168	ADD	n.s.
			REC	n.s.
	rs2040788	27300443	ADD	n.s.
			REC	n.s.
	rs9944340	27301092	ADD	n.s.
			REC	n.s.
	rs1805012	27281465	ADD	n.s.
			REC	n.s.
	rs1119132	27310970	ADD	0.147
			REC	0.038
	rs7191188	27296912	ADD	0.008
			REC	<0.001

Supplementary table 1 Overview of the results of all SNPs studied in the discovery phase (continued)

gene	rs number	coordinate	model	p
	rs6498016	27299289	ADD	0.164
			REC	0.006
	rs12102586	27285554	ADD	n.s.
			REC	n.s.
	rs11648218	27317583	ADD	n.s.
			REC	n.s.
	rs16976728	27289213	ADD	n.s.
			REC	n.s.
	rs8044444	27236044	ADD	n.s.
			REC	n.s.
	rs2234895	27265428	ADD	n.s.
			REC	n.s.
	rs8050048	27208885	ADD	n.s.
			REC	n.s.
	rs8052962	27234343	ADD	n.s.
			REC	n.s.
	rs4787427	27293895	ADD	n.s.
			REC	n.s.
	rs2074570	27282658	ADD	n.s.
			REC	n.s.
	rs4787948	27248560	ADD	n.s.
			REC	n.s.
	rs3024622	27272954	ADD	n.s.
			REC	n.s.
	rs7204874	27210788	ADD	n.s.
			REC	n.s.
	rs1049631	27283043	ADD	n.s.
			REC	n.s.
	rs2239347	27266522	ADD	n.s.
			REC	n.s.
	rs6498015	27299125	ADD	n.s.
			REC	n.s.
	rs3024613	27271754	ADD	n.s.
			REC	n.s.
	rs4238954	27221612	ADD	n.s.
			REC	n.s.
	rs4787947	27226266	ADD	n.s.
			REC	n.s.

Supplementary table 1 Overview of the results of all SNPs studied in the discovery phase (continued)

gene	rs number	coordinate	model	p
	rs8056488	27210230	ADD	n.s.
			REC	n.s.
IL-4	rs2243248	132036543	ADD	n.s.
			REC	n.s.
	rs6864565	132075870	ADD	n.s.
			REC	n.s.
	rs2243263	132041198	ADD	n.s.
			REC	n.s.
	rs17691077	132071250	ADD	n.s.
			REC	n.s.
	rs3756752	132101772	ADD	n.s.
			REC	n.s.
	rs6883504	132061021	ADD	n.s.
			REC	n.s.
	rs17623617	132060397	ADD	n.s.
			REC	n.s.
	rs1080001	132077015	ADD	n.s.
			REC	n.s.

*p value >0.05 was assumed not significant (n.s.). ADD = additive model and REC= recessive model.