# Genetic studies in rheumatoid arthritis

Kurreeman, F.

# Chapter 9

## Genetic Variants at the Interleukin 10 locus do not associate with Rheumatoid Arthritis Disease Outcomes

F.A.S. Kurreeman

D. van der Woude

M. Thabet

K. Verpoort

J. Houwing-Duistermaat

A.H.M. van der Helm-van Mil

R.E.M. Toes

T.W.J. Huizinga

Department of Rheumatology, Leiden University Medical Center, Leiden, Netherlands
Department of Medical Statistics and Bioinformatics, Leiden University Medical Centre, Leiden, The Netherlands

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**Abstract**

***Objective***

Interleukin 10 has been previously identified as a genetic risk factor involved in the severity of joint destruction in Rheumatoid Arthritis (RA). However many studies provide conflicting results when investigating RA phenotypes such as radiographic damage or clinical remission. The aim of this study was to determine whether tagging SNPs in the *IL10* locus are associated with RA disease outcomes in a well-defined clinical cohort.

***Methods***

RA patients enrolled in the Leiden Early Arthritis Clinic were genotyped for six tagging SNPs in the *IL10* gene (n=594). Yearly radiographs were scored using the Sharp-van der Heijde method. Sustained DMARD-free remission (defined as no swollen joints for at least 1 year after discontinuing all DMARDs) was assessed in all patients. The extent of joint destruction between genotype as well as haplotype groups was compared using Kruskal-Wallis tests at each time point for a follow-up period of 5 years after inclusion. The association between genotype, haplotype groups and remission was investigated using Cox regression analysis.

***Results***

Patients did not show significant differences in the rate of joint damage and remission with respect to the genotypes and haplotypes they harbour for any of the six tag SNPs. No significant differences were also observed when stratifying for the presence or absence of anti-citrullinated protein antibodies (ACPA).

***Conclusion***

*IL10* polymorphisms most likely do not predispose to a higher rate of joint damage or clinical drug free remission in RA patients irrespective of their ACPA status. These data suggest that *IL10* polymorphisms do not play a major role in RA disease outcomes.

## Introduction

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases, affecting ~1% of the population worldwide. Environmental as well as genetic factors are thought to play an important role in both the onset and the progression of the disease (2-5). Recent years have seen large progress in the identification of genetic risk factors involved in RA. To date, fifteen loci identified by genome-wide as well as candidate gene approaches have been implicated in RA. These loci include the HLA region with specific alleles conferring the largest effect size, PTPN22 now widely replicated as an autoimmune locus and many other loci with modest effect sizes(6;7).

Such progress is however as yet unmet in dissecting genetic loci involved in mediating either clinical remission or inversely joint destruction in these patients. Genetic markers associated with these disease-classifying phenotypes are clearly of interest not only to generate more insight into the disease process but possibly also to serve as surrogate predictors of disease course. The sole genetic locus consistently correlated to a more severe radiographic damage is the HLA-shared epitope alleles in the presence of autoantibodies (8). While other loci have been reported, unequivocal replication in large well characterised datasets remains to be obtained.

The *interleukin-10* (*IL10*) locus on chromosome 1q32 has been previously suggested to be associated with RA as well as with a higher rate of joint damage in RA patients(9-12). Since 50-60% of the variation in the level of this protein is likely to be attributed to genetic risk factors, *IL10* promoter polymorphisms have been the prime candidates investigated(13-15). These polymorphisms as well as *IL10* haplotypes have been widely correlated to IL10 mRNA and protein levels(16;17). Very recently, a novel inducible transcript with an extended 5'UTR has been described following lipopolysaccharide (LPS) stimulation of mononuclear cells(18). However extensive analysis of all available genetic variation has so far not been performed in most studies.

Given the important role of this cytokine in mediating immunosuppressive as well as immunostimulatory effects, it remains an important candidate to examine in RA(19). However, the well-powered genome-wide scan performed by the Wellcome Trust Case-Control Consortium consisting of 2000 patients and 3000 healthy individuals has not reported the association of this region with RA. We observed only 54% coverage of this region using SNPs in a 200kb region around *IL10* using a pairwise $R^2$ ≥0.8 and a coverage of 70% using a pairwise $R^2$ ≥0.5)(20). More recently a meta-analysis of all published genome-wide association studies also did not identify the *IL10* region as a susceptibility locus for RA(7). These data suggest that the *IL10* region is unlikely to play a major role in the predisposition to RA as such. However, it remains likely that genetic variants at this locus play a role in the various phenotypes of RA characterized by either joint damage or remission. The identification of genetic markers correlating with disease phenotypes remains a crucial question that may not only result in a better understanding of the disease process but also in improving treatment strategies for patients with a worse disease prognosis.

In this study, we have aimed to investigate the correlation of *IL10* polymorphisms to the rate of joint damage and remission in RA patients by performing a comprehensive effort to capture most of the informative markers in the *IL10* region known to date. Combined with the availability of a large number of RA patients and longstanding follow-up, we have determined the association of these SNPs with the progression of radiological joint destruction and remission in a prospective cohort of Early Arthritis patients (EAC).

**Patients & Methods**

594 RA patients from the population-based arthritis inception cohort (EAC)(21) were included in this study. All RA patients used fulfilled the American College of Rheumatology 1987 revised classification criteria for RA and were selected from a large prospective Early Arthritis Clinic (EAC) cohort that was started in 1993 at the Department of Rheumatology of the Leiden University Medical Center(22). Patients were referred to the EAC by general practitioners in the western part of The Netherlands when arthritis was suspected. The Leiden EAC is the only referral center in an area of ~400,000 inhabitants. Inclusion took place when arthritis was confirmed at physical examination and the symptom duration was less than 2 years. Written informed consent was obtained from all participants. The study and its protocols were approved by the appropriate local institutional review boards. All patients included in the study were of Caucasian origin based on self-reported ethnicity. Five hundred ninety four RA patients who were consecutively included and had DNA available were genotyped and the patient characteristics are summarized in Table 1. The number of patients with available radiographs varied per time point.



At inclusion, blood samples were taken from every patient for routine diagnostic laboratory screening and stored to determine ACPA at a later time point. ACPA were measured by enzyme-linked immunosorbent assay (ELISA) (Immunoscan RA Mark 2; Euro-Diagnostica, Arnhem, The Netherlands). The cutoff level for positivity was set at 25 arbitrary units, according to the manufacturer's instructions. Follow-up visits were performed on a yearly basis and included radiographs of hands and feet. Since the start of the EAC the treatment strategies have changed and four different strategies were applied depending on the inclusion period. Patients included between 1993 and 1995 were treated initially with analgesics and subsequently with chloroquine or salazopyrine if they had persistent active disease (delayed treatment)(23). From 1996 to 1998 RA patients were promptly treated with either chloroquine or salazopyrine (early treatment) (22;23). From 1998 to 2002 patients were promptly treated with either salazopyrine or methotrexate (early treatment) and patients included in 2002 or later were promptly treated with either salazopyrine or methotrexate combined with treatment adjustments based on the disease activity (early and disease activity based treatment).

***Genotyping of IL10 polymorphisms and Haplotype block determination***

43 SNPs spanning the region around the *IL10* gene encompassing 226kb were genotyped in 57 unrelated healthy Caucasian individuals. Out of the 43 SNPs genotyped, 2 SNPs were not polymorphic (rs2945417 and rs17015763) and one SNP had a frequency ≤1% (rs4845140). Additional details are further described in Table S1. Haploview was used to determine the graphical representation of LD(24). 6 Tagging SNPs were selected and further genotyped in DNA from 594 RA patients. These 6 tagging SNPs captured 86% of all the alleles within the 17kb block with an $R^2$ threshold of 0.8. Rs3024505 was not tagged using either pairwise LD measures or 2 to 3 marker haplotypes.

Standard quality control for genotyping was carried out. 10% of all genotypes were repeated and <1% errors detected. The success rate greatly exceeded 95% and varied per SNP. More specifically the success rate was 96% for rs6667202, 98% for rs6676671, 100% for rs6703630, 98% for rs6693899, 99.7% for rs1800896 and 99% for rs1800871. All SNP genotypes were in Hardy-Weinberg equilibrium.

Haplotypes per individual were inferred using plink(25). The most likely haplotypes were included in the analysis. The number of individuals with haplotype posterior probability <80% is 26 indicating that uncertainty is minimal for the larger subset of RA patients.

***Radiographs***

Radiographs of hands and feet were taken on consecutive years starting at baseline and were scored according to the Sharp-van der Heijde method(26). Compared to another frequently used scoring method, the Sharp-van der Heijde method is the most sensitive on the individual patient level in early RA(27). To encompass a reliable sample size during follow-up, radiographic follow-up data were restricted to a maximum of 5 years. The number of available radiographs varied per time-point and was 509 at baseline which declined to 440 after 1 year of follow-up, 401, 340, 283, and 260 after 2 to 5 years of follow-up respectively. Due to the study design (an inception cohort) not all patients achieved a similar duration of follow-up. The cut-off after 5 years of follow-up was applied according to the criteria that at least 10 patients should be available per genotype group. All radiographs were scored by one experienced scorer 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(28). From the total number of scored radiographs, 499 radiographs were rescored by the same reader, consisting of 149 baseline radiographs and 350 radiographs during follow-up belonging to 60 randomly selected RA-patients. Reliability of radiograph scoring was calculated and 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 (van der Linden *et al*, Manuscript in press, A&R).

***Remission***

Sustained DMARD-free remission was defined according to the following three criteria: 1) no current use of DMARDs, 2) no swollen joints and 3) classification as DMARD-free remission by the patient's rheumatologist (Van der Woude *et al*, Manuscript in press at A&R). Corticosteroids were considered to be equivalent to DMARDs for the present study, while NSAIDs did not qualify as DMARDs. Patients had to fulfill all three criteria in order to be diagnosed with remission. To ensure that remission was not temporary, but rather sustained and long-lasting, the absence of swollen joints had to have been observed by a rheumatologist for at least one year after discontinuation of when DMARD-therapy. Patients with remission were discharged from the outpatient clinic if the absence of joint swelling had been observed for at least one year after the discontinuation of DMARDs. Most patients who achieved remission were followed-up longer than the minimum requirement of one year; the median time of observation after discontinuation of DMARDs in the absence of swollen joints was 2.5 years. Patients who had a recurrence of their arthritis after discharge, could easily return to the Leiden University Medical Center, the only referral center for Rheumatology in a health care region of approximately 400.000 inhabitants. The frequency of relapse was recorded and patients with relapse (n=6) were included in the non-remission group. In contrast to previous analyses in which patients who presented to the EAC after 2003 were excluded, the current analysis contained all RA patients for whom *IL10*-genotyping information was available (n=594). This resulted in a slightly lower prevalence of remission (13.2%) than described previously (15%) (Van der Woude et al, manuscript in press at A&R).



***Statistical Analysis***

The association between genotypes and radiographic scoring data was analyzed using SPSS version 16.0 (SPSS Inc., Chicago, IL). As radiographic data were not normally distributed, the raw data on the Sharp-van der Heijde scores are presented using medians. The Kruskal-Wallis nonparametric test was used to compare median scores across genotype groups. Since six SNPs were evaluated a Bonferroni correction for multiple testing was applied and the p-value for significance was set at $p<0.004$.

Summary statistics were generated to investigate the prevalence of the different *IL10* genotypes and haplotypes in remission and non-remission patients. To avoid skewing of the results due to the difference in follow-up time, the present analysis used data from the first 10 years of follow-up for all patients. To take into account the difference in follow-up times among patients, analyses were performed by Cox regression analysis, after verification that the proportional hazards assumption was satisfied. In Cox regression models the dependent variable is the "time-to-event", which consisted of the time to remission for the remission patients, and the time to last follow-up (with a maximum of 10 years) for the non-remission patients. The time of remission was defined as the date at which DMARDs were discontinued due to remission. The analysis was also performed with a later date defined as the time of remission (date described above plus one year), which led to similar results. This indicates that the results were stable regardless of the exact date used to define remission.

## Results

### *Determination of LD in the IL10 locus*

In order to determine the extent of LD in the *IL10* locus, 40 SNPs in the *IL10* region spanning 226kb were genotyped in 57 healthy unrelated Dutch Caucasian individuals. Under the algorithm of Gabriel *et al*, one haplotype block was inferred (Figure 1)(1). This block is restricted to 17 kb encompassing the coding region of the *IL10* gene (5kb) as well as 5'and 3' untranslated regions. Since we are primarily interested in identifying cis-variants predisposing to increased joint damage in RA patients, this block represented the most interesting region to look into. We used Tagger as implemented in Haploview to determine the tagging SNPs that provided the most information with regards to the haplotype variation(29). 6 tagging SNPs were chosen on the basis of pairwise comparisons with $R^2 \leq 0.8$ and a multi-marker LOD threshold of 3. These SNPs captured 93% of all the alleles in the 17kb block. This block was comparable to the haplotype block in HapMap CEU (Figure S1).

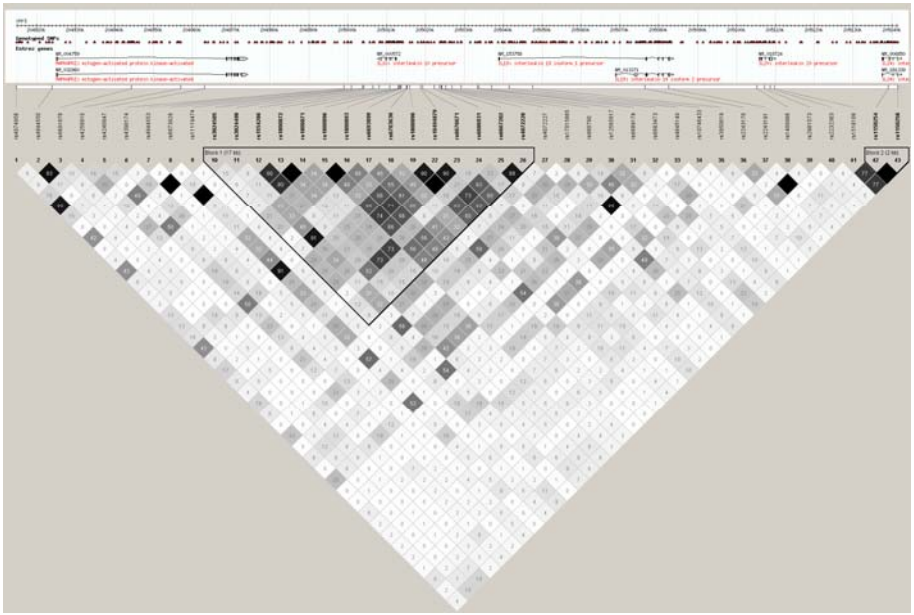


**Figure 1.** 17 kb LD Block around IL10 according to Gabriel et al(1). Paiwise linkage disequilibrium (LD) values ($R^2$) are indicated. Strength of LD is positively correlated to color intensity.

***Association of tagging SNPs and haplotypes with radiological joint damage in RA***

The study cohort consisted of 594 patients, whose characteristics at baseline are detailed in Table 1. None of the genotype distributions deviated from Hardy Weinberg equilibrium. Because Sharp-van der Heijde scores did not fit a normal distribution, we compared the median scores across the three genotypes per time point for all six SNPs using the Kruskall-Wallis non-parametric test.

**Table 1.** Patient characteristics from the Leiden Early arthritis cohort.

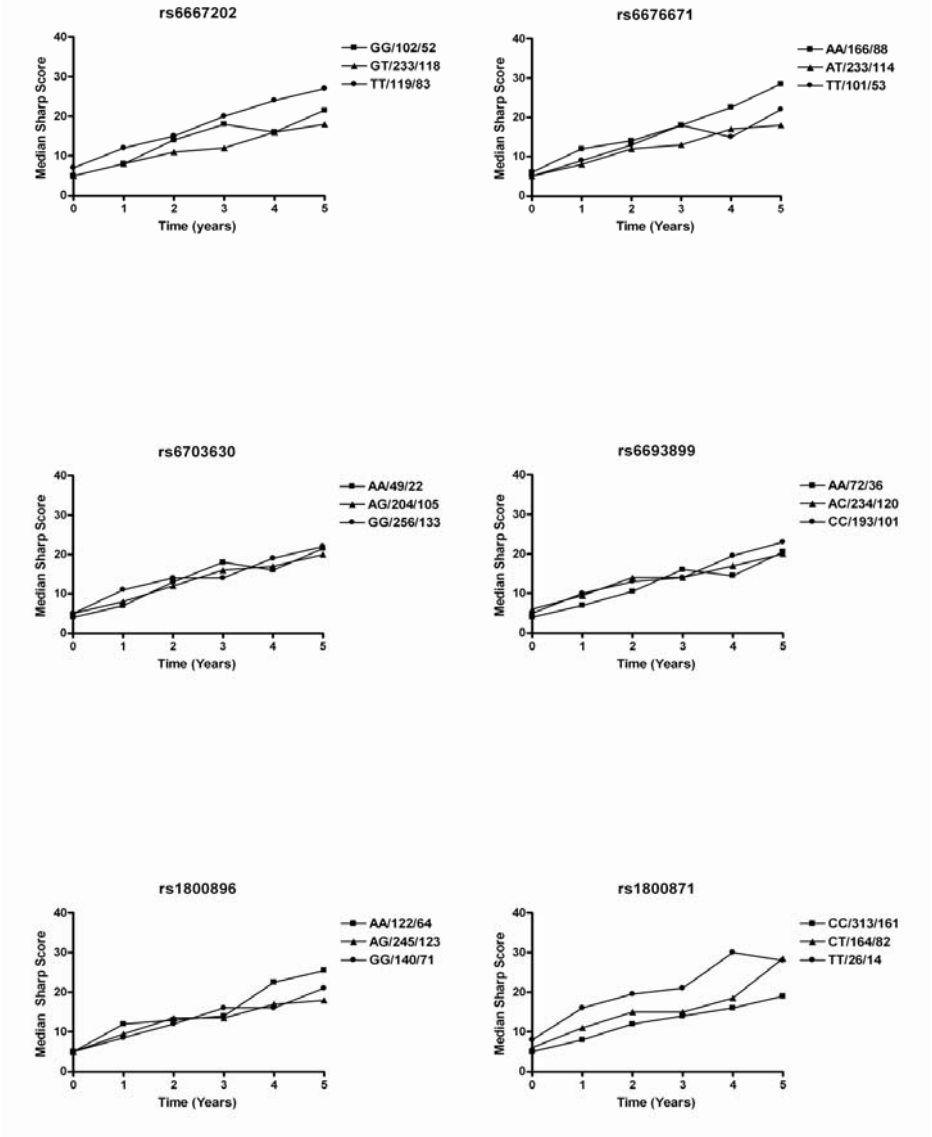
| Patient Characterisitcs | N=594 |
|---|---|
| Age at inclusion, mean years (± SD) | 56.6 (±15.5) |
| Female N (%) | 401 (67.7) |
| ACPA positive | 266 (56.1) |
| IgM-RF positive | 341 (58.3) |

Age and gender data were available for all RA patients. IgM-RF (rheumatoid factor) data was available from 585 RA patients. ACPA (anti-citrullinated protein antibodies) data was available from 474 RA patients



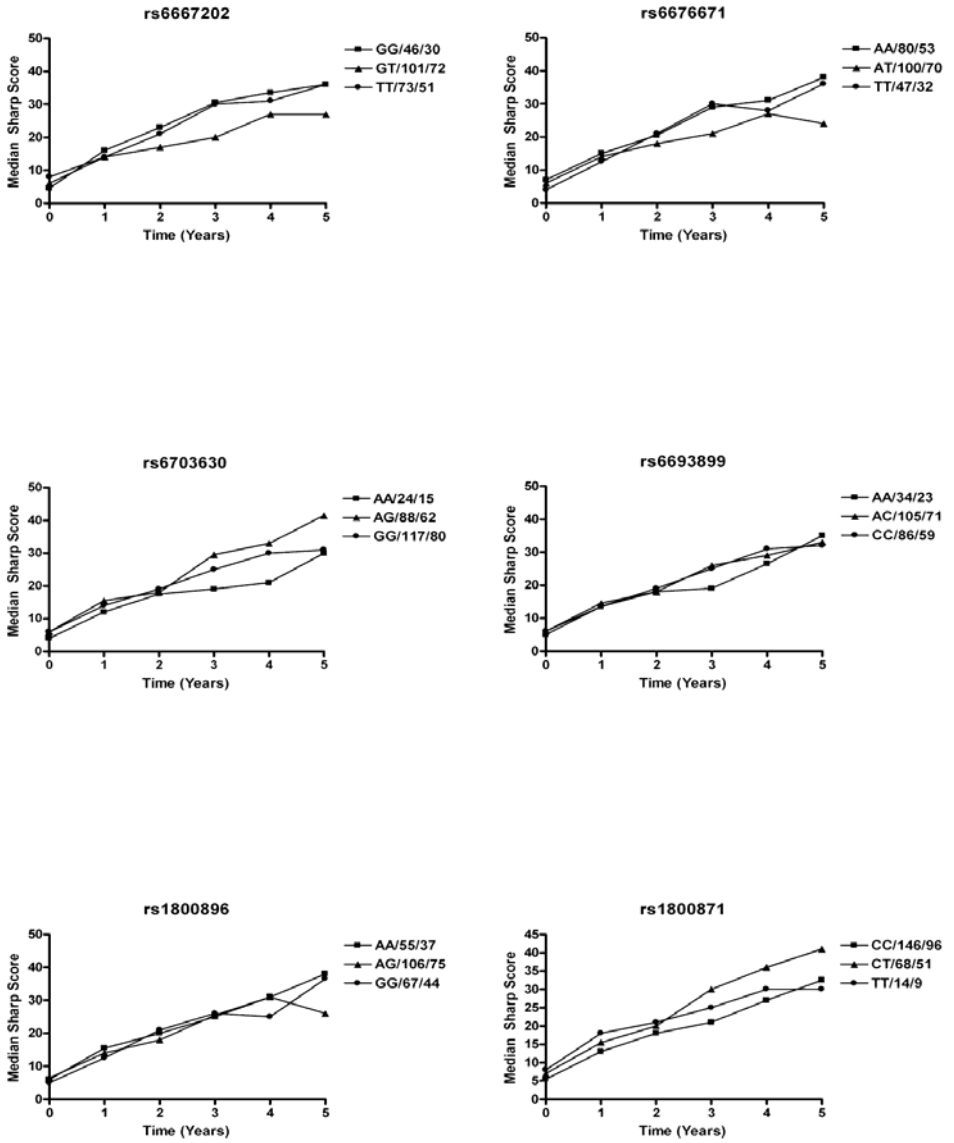
None of the *IL10* tagging SNPs showed a correlation with joint damage in time (Figure 2). Since more severe radiographic damage correlates with the presence of rheumatoid factor and anti-citrullinated protein antibodies (ACPA), we have stratified our data according to the presence or absence of ACPA. No correlation was observed between genotype and joint damage in either stratum (Figure 3 and 4). While the data indicate that patients without ACPA and with rs1800871 TT genotype may have a higher rate of joint destruction, the medians are based solely on 7 individuals at baseline and 4 individuals after 5 years of follow-up.

Figure 2. Median Sharp-van der Heijde Scores for six IL10 tagging SNPs per genotype in all RA patients



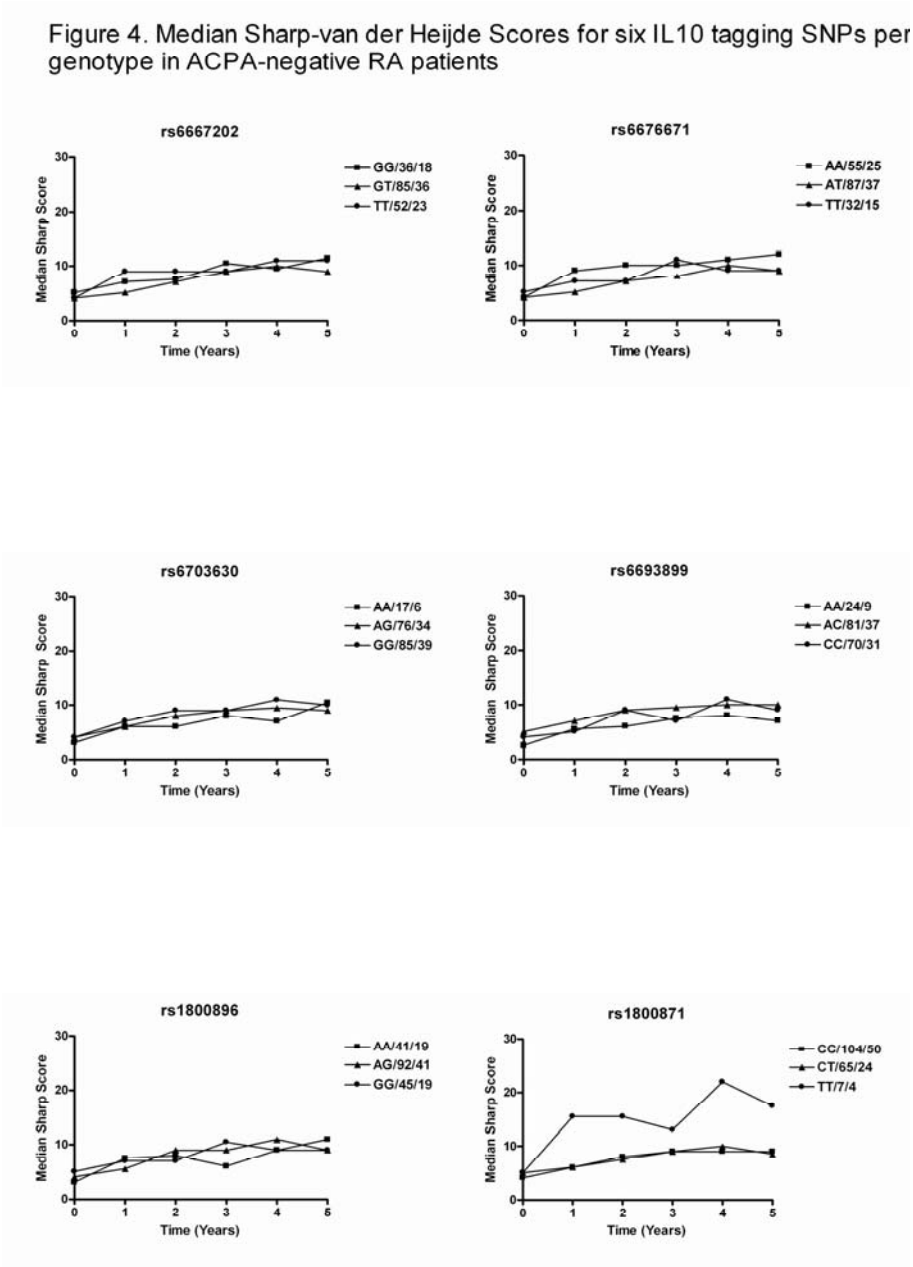

Overview of the raw Sharp-van der Heijde scores, expressed as medians, of all 6 SNPs per genotype for the total patient population. The number of individuals at inclusion (year=0) and after five years of follow-up (years=5) is given in the legend. For example the median sharp van der heijde scores originated from 102 GG, 233GT and 119 TT patients at inclusion and declined to 52 GG, 118 GT and 83 TT after 5 years of follow-up.

Figure 3. Median Sharp-van der Heijde Scores for six IL10 tagging SNPs per genotype in ACPA-positive RA patients




Overview of the raw Sharp-van der Heijde scores, expressed as medians, of all 6 SNPs per genotype for ACPA positive RA patients. The number of individuals at inclusion (year=0) and after five years of follow-up (years=5) is given in the legend. For example the median sharp van der heijde scores originated from 46 GG, 101GT and 73 TT patients at inclusion and declined to 30 GG, 72 GT and 51 TT after 5 years of follow-up.

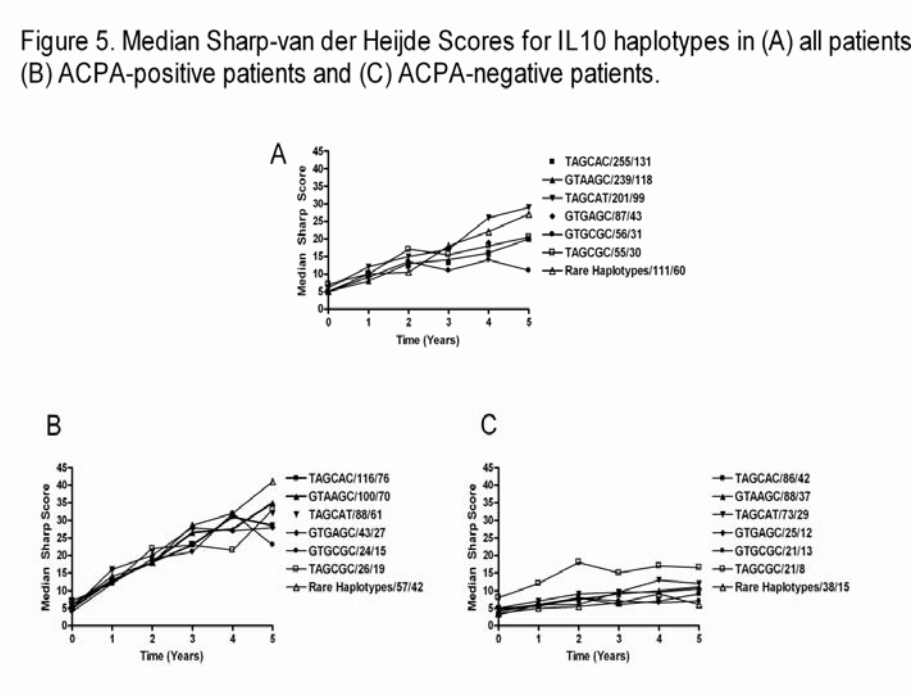
Figure 4. Median Sharp-van der Heijde Scores for six IL10 tagging SNPs per genotype in ACPA-negative RA patients




Overview of the raw Sharp-van der Heijde scores, expressed as medians, of all 6 SNPs per genotype for ACPA negative RA patients. The number of individuals at inclusion (year=0) and after five years of follow-up (years=5) is given in the legend. For example the median sharp van der heijde scores originated from 36 GG, 85 GT and 52 TT patients at inclusion and declined to 18 GG, 36 GT and 23 TT after 5 years of follow-up.

Haplotypes were inferred for 586 individuals from whom genotype data was available in >50% of the SNPs. Haplotypes with a frequency lower than or equal to 2% were pooled into one category termed the rare haplotypes. Six common haplotypes (frequency >2%) capturing 88% of all haplotypes were detected in the total RA population. None of the haplotypes showed a correlation with joint damage in RA patients (Figure 5A). Stratifying RA patients according to ACPA status also revealed no significant correlation (Figure 5 B and C).

Figure 5. Median Sharp-van der Heijde Scores for IL10 haplotypes in (A) all patients (B) ACPA-positive patients and (C) ACPA-negative patients.




Overview of the raw Sharp-van der Heijde scores, expressed as medians, of all 6 SNPs haplotypes for (A) all RA patients (B) ACPA-positive RA patients and (C) ACPA-negative RA patients. The number of haplotypes at inclusion (year=0) and after five years of follow-up (years=5) is given in the legend.



***Association of tagging SNPs and haplotypes with remission in RA***

Sustained DMARD-free remission was achieved by 13.3% of patients (N=79). Despite a trend towards association between some of the SNPs and sustained DMARD-free remission, none of these associations reached statistical significance (Table 2). Stratification of the RA patients by ACPA status also did not yield any statistically significant associations (Tables 3 and 4). Although there was a possible trend for association between the *IL10* haplotypes and ACPA-negative RA (Table 5), this association was not significant after correction for multiple testing. However, our data is largely underpowered to detect modest effects in this dataset.

**Table 2.** Association of IL10 SNPs with remission in RA patients ‡.

| *Genotype* | | *Remission n=79* | *No remission n=515* | *Hazard Ratio* | *95% CI* | *P* |
|---|---|---|---|---|---|---|
| rs1800871 | CC | 50 (71%) | 269 (60%) | | | |
| | CT | 18 (26% | 153 (34%) | | | |
| | TT | 2 (3%) | 26 (6%) | 0.66 | 0.42-1.04 | 0.075 |
| rs1800896 | AA | 13 (18%) | 119 (26%) | | | |
| | AG | 37 (51%) | 223 (48%) | | | |
| | GG | 22 (31%) | 123 (27%) | 1.20 | 0.91-1.57 | 0.28 |
| rs6693899 | AA | 11 (16%) | 62 (14%) | | | |
| | AC | 33 (47%) | 201 (45%) | | | |
| | CC | 26 (37%) | 180 (41%) | 0.92 | 0.70-1.22 | 0.64 |
| rs6703530* | AA | 8 (11%) | 45 (9.7%) | | | |
| | AG | 35 (49%) | 177 (38%) | | | |
| | GG | 29 (40%) | 241 (52%) | 0.62 | 0.41-0.94 | 0.059 |
| rs6676671** | AA | 18 (26%) | 155 (36%) | | | |
| | AT | 38 (54%) | 193 (44%) | | | |
| | TT | 14 (20%) | 89 (20%) | | | |
| rs6667202 | GG | 18 (27%) | 82 (19%) | | | |
| | GT | 34 (51%) | 209 (48%) | | | |
| | TT | 15 (22%) | 145 (33%) | 0.73 | 0.55-0.97 | 0.064 |

‡ The number and the percentage of patients are listed, relative to the total number of patients for whom information about the SNP under investigation was available. * Proportional hazards assumption not fulfilled because the small number of patients with genotype AA have an aberrant survival curve. HR therefore only applies to individuals with genotypes AG and GG. ** Proportional hazards assumption not fulfilled, therefore no HR was calculated.

**Table 3.** Association of IL10 SNPs with remission in ACPA-positive RA patients ‡.

| *Genotype* | | *Remission n=9* | *No remission n=264* | *Hazard Ratio* | *95% CI* | *P* |
|---|---|---|---|---|---|---|
| rs1800871 | CC | 7 (87%) | 152 (62%) | | | |
| | CT | 1 (13%) | 80 (32%) | | | |
| | TT | 0 | 15 (6.1%) | 0.50 | 0.03-1.84 | 0.172 |
| rs1800896 | AA | 1 (11%) | 60 (24%) | | | |
| | AG | 6 (67%) | 122 (48%) | | | |
| | GG | 2 (22%) | 70 (28%) | 1.11 | 0.52-2.40 | 0.82 |
| rs6693899 | AA | 2 (25%) | 34 (14%) | | | |
| | AC | 4 (50%) | 112 (46%) | | | |
| | CC | 2 (25%) | 98 (40%) | 0.61 | 0.27-1.37 | 0.31 |
| rs6703530* | AA | 1 (11%) | 25 (9.9%) | | | |
| | AG | 5 (56%) | 96 (38%) | | | |
| | GG | 3 (33%) | 131 (52%) | 0.46 | 0.14-1.53 | 0.29 |
| rs6676671** | AA | 2 (25%) | 84 (34%) | | | |
| | AT | 4 (50%) | 110 (45%) | | | |
| | TT | 2 (25%) | 51 (21%) | | | |
| rs6667202 | GG | 2 (22%) | 46 (19%) | | | |
| | GT | 4 (44%) | 117 (49%) | | | |
| | TT | 3 (33%) | 77 (32%) | 0.99 | 0.46-2.14 | 0.98 |

‡ The number and the percentage of patients are listed, relative to the total number of patients for whom information about the SNP under investigation was available.* Proportional hazards assumption not fulfilled because the small number of patients with genotype AA have an aberrant survival curve. HR therefore only applies to individuals with genotypes AG and GG. ** Proportional hazards assumption not fulfilled, therefore no HR was calculated.

**Table 4.** Association of IL10 SNPs with remission in ACPA-positive RA patients ‡.

| ***Genotype*** | | ***Remission n=61*** | ***No remission n=146*** | ***Hazard Ratio*** | ***95% CI*** | ***P*** |
|---|---|---|---|---|---|---|
| rs1800871 | CC | 40 (72%) | 72 (54%) | | | |
| | CT | 14 (26%) | 55 (41%) | | | |
| | TT | 1 (2%) | 7 (5.2%) | 0.58 | 0.33-0.99 | 0.045 |
| rs1800896 | AA | 10 (18%) | 36 (26%) | | | |
| | AG | 27 (48%) | 71 (51%) | | | |
| | GG | 19 (34%) | 32 (23%) | 1.29 | 0.95-1.77 | 0.18 |
| rs6693899 | AA | 9 (16%) | 17 (13%) | | | |
| | AC | 24 (44%) | 63 (47%) | | | |
| | CC | 22 (40%) | 53 (40%) | 0.97 | 0.71-1.34 | 0.89 |
| rs6703530* | AA | 7 (13%) | 11 (8.1%) | | | |
| | AG | 26 (46%) | 52 (39%) | | | |
| | GG | 23 (41%) | 72 (53%) | 0.66 | 0.41-1.05 | 0.14 |
| rs6676671** | AA | 14 (25%) | 47 (37%) | | | |
| | AT | 29 (53%) | 57 (45%) | | | |
| | TT | 12 (22%) | 22 (18%) | | | |
| rs6667202 | GG | 16 (31%) | 19 (15%) | | | |
| | GT | 24 (47%) | 64 (52%) | | | |
| | TT | 11 (22%) | 41 (33%) | 0.68 | 0.49-0.94 | 0.050 |

‡ The number and the percentage of patients are listed, relative to the total number of patients for whom information about the SNP under investigation was available.* Proportional hazards assumption not fulfilled because the small number of patients with genotype AA have an aberrant survival curve. HR therefore only applies to individuals with genotypes AG and GG. ** Proportional hazards assumption not fulfilled, therefore no HR was calculated.

**Table 5.** Association of IL10 haplotypes with remission in all RA patients, ACPA-positive as well as ACPA-negative RA patients.

| Haplotype | All Patients | | ACPA-Positive Patients | | ACPA-Negative Patients | |
|---|---|---|---|---|---|---|
| | No Remission | Remission | No Remission | Remission | No Remission | Remission |
| TAGCAC | 224 (88&) | 31 (12%) | 122 (96%) | 5 (4%) | 64 (73%) | 24 (27%) |
| GTAAGC | 193 (83%) | 41 (17%) | 101 (94%) | 6 (6%) | 58 (65%) | 31 (35%) |
| TAGCAT | 187 (90)% | 20 (10%) | 99 (99%) | 1 (1%) | 62 (82%) | 14 (18%) |
| GTGAGC | 80 (90%) | 7 (8%) | 45 (98%) | 1 (2%) | 23 (82%) | 5 (18%) |
| GTGCGC | 44 (77%) | 13 (23%) | 30 (97%) | 1 (3%) | 9 (43%) | 12 (57%) |
| TAGCGC | 52 (85%) | 9 (15%) | 27 (96%) | 1 (4%) | 18 (72%) | 7 (28%) |
| Rate (<2%) | 100 (84%) | 19 (16%) | 66 (98.5%) | 1 (1.5%) | 24 (58.5%) | 17 (41.5%) |
| P (LogRank Test)* | | 0.07 | | 0.59 | | 0.02 |

*The log Rank Test was performed since the proportional Hazards Assumption was not met. The log Rank Test assesses whether there is a significant difference in the survival curves estimated by the time to remission.



## Discussion

IL10 is an important immunoregulatory cytokine with diverse effects on the immune system. The cytokine is produced by a range of immune cells, including B cells and regulatory T cells(19). Stimulation of human blood cultures with bacterial lipopolysaccharide (LPS) showed large interindividual variation in IL10 secretion, which has been shown to have a genetic component of 50-70%(13). Given the fact that IL10 is produced by a variety of different cells and recent evidence which suggests that the correlation of *IL10 cis* variants with IL10 protein levels can be cell and stimuli specific, it is highly plausible that its regulation is both at the *cis* (caused by variations with the gene region itself) and the *trans* (caused by other variations at other gene loci) level.

Administration of IL10 to animals with collagen-induced arthritis reduces several parameters of the disease and would therefore suggest that high levels of IL10 are likely to result in less progressive disease(30). However, the precise role of IL10 is not clear, even in mouse models. One group recently highlighted this complexity. Hansson and colleagues showed that IL10-deficient mice immunized with collagen type II (CII) develop a more severe disease than their heterozygous littermates(31).

Previous findings in human genetic studies show that the presence of the G allele at *IL10*-2849 (rs6703630) is correlated with enhanced IL10 protein levels in healthy individuals as well as increased levels of autoantibodies and more severe joint damage in RA patients(9). Marinou and colleagues have also recently suggested the involvement of *IL10* polymorphisms in severity of RA(10). The authors suggest that in the absence of autoantibodies (RF or ACPA), patients with a *IL10*-592 ($R^2$=1 with rs1800871) CC genotype have more severe joint damage in a Polish

population as measured by Larsen's score. However, both studies were conducted in a cross-sectional fashion with data available at one specific time point. The major strengths of the present study are the use of radiological data during long-term follow-up of five years. However, inherent to the design of an inception cohort, not all patients had achieved five years of follow-up, so the number of missing data increased with longer follow-up.

Our data suggests that six *IL10* tagging polymorphisms do not influence radiographic joint damage in RA patients irrespective of the presence or absence of autoantibodies. In addition, haplotypes at this locus also do not show any correlation with severity of disease indicating that a large effect of known *IL10* polymorphisms on joint destruction is unlikely. We have also investigated the role of the *IL10* locus in relation to patients who achieved sustained DMARD-free remission. Our data suggest that individuals with specific *IL10* genotypes or haplotypes do not have a higher rate of remission, irrespective of their ACPA status. However, we cannot exclude that this locus may have a more modest effect on this phenotype. Large well-characterised RA cohorts will be required to investigate the role of modest effect loci in relation to specific phenotypes in RA.

## References


(1) Firestein GS. Evolving concepts of rheumatoid arthritis. Nature 2003; 423(6937):356-61.

(2) MacGregor AJ, Snieder H, Rigby AS, Koskenvuo M, Kaprio J, Aho K et al. Characterizing the quantitative

(3) genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheum 2000; 43(1):30-7.

(4) Seldin MF, Amos CI, Ward R, Gregersen PK. The genetics revolution and the assault on rheumatoid arthritis. Arthritis Rheum 1999; 42(6):1071-9.

(5) van der Helm-van Mil AH, Kern M, Gregersen PK, Huizinga TW. Variation in radiologic joint destruction in rheumatoid arthritis differs between monozygotic and dizygotic twins and pairs of unrelated patients. Arthritis Rheum 2006; 54(6):2028-30.

(6) Bowes J, Barton A. Recent advances in the genetics of RA susceptibility. Rheumatology (Oxford) 2008; 47(4):399-402.

(7) Raychaudhuri S, Remmers EF, Lee AT, Hackett R, Guiducci C, Burtt NP et al. Common variants at CD40 and other loci confer risk of rheumatoid arthritis. Nat Genet 2008; 40(10):1216-23.

(8) Huizinga TW, Amos CI, van der Helm-van Mil AH, Chen W, van Gaalen FA, Jawaheer D et al. Refining the complex rheumatoid arthritis phenotype based on specificity of the HLA-DRB1 shared epitope for antibodies to citrullinated proteins. Arthritis Rheum 2005; 52(11):3433-8.

(9) 8.Lard LR, van Gaalen FA, Schonkeren JJ, Pieterman EJ, Stoeken G, Vos K et al. Association of the -2849 interleukin-10 promoter polymorphism with autoantibody production and joint destruction in rheumatoid arthritis. Arthritis Rheum 2003; 48(7):1841-8.

(10) Marinou I, Healy J, Mewar D, Moore DJ, Dickson MC, Binks MH et al. Association of interleukin-6 and interleukin-10 genotypes with radiographic damage in rheumatoid arthritis is dependent on autoantibody status. Arthritis Rheum 2007; 56(8):2549-56.

(11) Eskdale J, McNicholl J, Wordsworth P, Jonas B, Huizinga T, Field M et al. Interleukin-10 microsatellite polymorphisms and IL-10 locus alleles in rheumatoid arthritis susceptibility. Lancet 1998; 352(9136):1282-3.

(12) Katsikis PD, Chu CQ, Brennan FM, Maini RN, Feldmann M. Immunoregulatory role of interleukin 10 in rheumatoid arthritis. J Exp Med 1994; 179(5):1517-27.

(13) Westendorp RG, Langermans JA, Huizinga TW, Verweij CL, Sturk A. Genetic influence on cytokine production in meningococcal disease. Lancet 1997; 349(9069):1912-3.

(14) Reuss E, Fimmers R, Kruger A, Becker C, Rittner C, Hohler T. Differential regulation of interleukin-10 production by genetic and environmental factors--a twin study. Genes Immun 2002; 3(7):407-13.

(15) Eskdale J, Keijsers V, Huizinga T, Gallagher G. Microsatellite alleles and single nucleotide polymorphisms (SNP) combine to form four major haplotype families at the human interleukin-10 (IL-10) locus. Genes Immun 1999; 1(2):151-5.

(16) Kurreeman FA, Schonkeren JJ, Heijmans BT, Toes RE, Huizinga TW. Transcription of the IL10 gene reveals allele-specific regulation at the mRNA level. Hum Mol Genet 2004; 13(16):1755-62.

(17) Eskdale J, Gallagher G, Verweij CL, Keijsers V, Westendorp RG, Huizinga TW. Interleukin 10 secretion in relation to human IL-10 locus haplotypes. Proc Natl Acad Sci U S A 1998; 95(16):9465-70.

(18) Forte GI, Scola L, Bellavia D, Vaccarino L, Sanacore M, Sisino G et al. Characterization of two alternative Interleukin(IL)-10 5'UTR mRNA sequences, induced by lipopolysaccharide (LPS) stimulation of peripheral blood mononuclear cells. Mol Immunol 2009.

(19) Mosser DM, Zhang X. Interleukin-10: new perspectives on an old cytokine. Immunol Rev 2008; 226:20518.

(20) Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007; 447(7145):661-78.

(21) van Aken J, van Bilsen JHM, Allaart CF, Huizinga TWJ, Breedveld FC. The Leiden Early Arthritis Clinic. Clinical and Experimental Rheumatology 2003; 21(5):S100-S105.

(22) van Aken J, van Bilsen JH, Allaart CF, Huizinga TW, Breedveld FC. The Leiden Early Arthritis Clinic. Clin Exp Rheumatol 2003; 21(5 Suppl 31):S100-S105.

(23) Lard LR, Boers M, Verhoeven A, Vos K, Visser H, Hazes JM et al. Early and aggressive treatment of rheumatoid arthritis patients affects the association of HLA class II antigens with progression of joint damage. Arthritis Rheum 2002; 46(4):899-905.

(24) Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21(2):263-5.

(25) Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81(3):559-75.

(26) van der Heijde D. How to read radiographs according to the Sharp/van der Heijde method. J Rheumatol 2000; 27(1):261-3.

(27) Bruynesteyn K, van der HD, Boers M, van der LS, Lassere M, van d, V. The Sharp/van der Heijde method out-performed the Larsen/Scott method on the individual patient level in assessing radiographs in early rheumatoid arthritis. J Clin Epidemiol 2004; 57(5):502-12.

(28) van der Heijde D, Boonen A, Boers M, Kostense P, van der Linden S. Reading radiographs in chronological order, in pairs or as single films has important implications for the discriminative power of rheumatoid arthritis clinical trials. Rheumatology (Oxford) 1999; 38(12):1213-20.

(29) Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B et al. The structure of haplotype blocks in the human genome. Science 2002; 296(5576):2225-9.

(30) de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. Nat Genet 2005; 37(11):1217-23.

(31) Trachsel E, Bootz F, Silacci M, Kaspar M, Kosmehl H, Neri D. Antibody-mediated delivery of IL-10 inhibits the progression of established collagen-induced arthritis. Arthritis Res Ther 2007; 9(1):R9.

(32) Hansson AS, Johansson AC, Holmdahl R. Critical role of the major histocompatibility complex and IL-10 in matrilin-1-induced relapsing polychondritis in mice. Arthritis Res Ther 2004; 6(5):R484-R491.