Predictive value of interleukin 1 gene polymorphisms for surgery

Anne Crilly, Nicola Maiden, Hilary A Capell, Rajan Madhok

Abstract

Objectives—To determine the influence of interleukin 1 α (IL1 α), IL1 β , and IL1 receptor antagonist gene polymorphisms on disease outcome as assessed by the need for major joint surgery within 15 years of diagnosis.

Patients and methods—50 patients with rheumatoid arthritis (RA) who required major joint surgery (hip, knee, or shoulder arthroplasty) within a 15 year period of disease diagnosis and 50 patients with RA with disease duration greater than 15 years and no major surgery were recruited together with 66 normal west of Scotland controls. Genomic DNA and polymerase chain reaction were used to determine polymorphisms in the genes for IL1 α , IL1 β , and IL1 receptor antagonist. For all patients with RA recruited to the study, HLA-DR β 1 gene status was recorded as was the erythrocyte sedimentation rate (ESR) at the first ever clinic visit.

Results—No difference in the allele frequencies or genotypes of the IL1 α and IL1 receptor antagonist gene polymorphisms was found between the controls and patients with RA, with or without previous surgery. IL1 β allele 2 was overrepresented in patients with RA who had undergone surgery compared with patients who had not (40% *v* 27%, $\chi^2=4$, 1df, *p*=0.04). ESR at the first ever clinic visit was significantly higher in those carrying allele 2 (36 mm/1st h *v* 22 mm/1st h, *p*=0.04). When patients, with or without previous surgery, who did not carry two disease associated HLA-DR β 1 alleles were compared, an increase in allele 2 was observed in the surgery cohort (42% *v* 25%, $\chi^2=4.8$, 1df, *p*=0.03).

Conclusions—Patients who require major joint surgery were found to carry the IL1 β allele 2 more often than expected. Patients with this allele also had a higher initial ESR. This may be useful in predicting early surgery in patients who do not carry two disease associated HLA-DR β 1 alleles. Although these findings are interesting, further functional and epidemiological studies to confirm these observations are required.

(Ann Rheum Dis 2000;59:695–699)

years, 25% of patients required total joint arthroplasty.¹ The median time interval from diagnosis to surgery was approximately 10 years. Predictors of joint surgery at disease onset currently include duration of morning stiffness, global severity, Health Assessment Questionnaire (HAQ) disability score, grip strength, and erythrocyte sedimentation rate (ESR).¹ What is not clear is which molecular events within the inflamed joint determine severity.

Although RA is a multifactorial process, there is a strong genetic association with the class II major histocompatibility complex (MHC) HLA-DR β 1. HLA-DR β 1*101, 0102, 0401, 0404, 0405 0408, 1001, and 1402 give rise to a conserved amino acid sequence (QKRAA, QRRAA, or RRRRAA) in the third hypervariable region of the class II MHC which predisposes to RA in white subjects.² Inheritance of HLA-DR β 1 alleles, which give rise to a disease associated epitope (DAE), seems to result in a hierarchical disease phenotype, homozygotes having the worst articular outcome with more extra-articular features and heterozygotes having less severe articular disease than those with no DAE.³ In a cohort of patients with RA, based in a west of Scotland hospital, we reported that patients with two DAEs required major joint surgery more commonly than those with one or no DAE (odds ratio (OR) = 5), with the initial ESR having no influence on disease outcome.⁴ In those with no or one DAE the need for surgery could be predicted from the initial ESR. Considering these initial observations we wondered what other markers might be genetic regulators of ESR and, therefore, possible markers for predicting the need for early surgery.

Interleukin 1 (IL1) is a proinflammatory cytokine which is overexpressed in RA serum, synovial fluid, and tissue.⁵ Together with IL6 and tumour necrosis factor α (TNF α), IL1 mediates the acute phase protein response. IL1 is implicated in joint erosion, and plasma levels correlate with ESR.^{6,7} Three members of the IL1 family exist—namely, IL1 α , IL1 β , and IL1 receptor antagonist. Biallelic polymorphisms have been described for the IL1 α gene at position –889⁸ and for the IL1 β gene at position –511,⁹ and a variable number tandem repeat has been identified in intron 2 of the IL1 receptor antagonist gene.¹⁰ We postulated that one of these polymorphisms might explain why ESR is a predictor of early major joint surgery in patients with RA. To examine this we investigated these polymorphisms in patients with RA with and without early major joint surgery.

University
Department of
Medicine, 10
Alexandra Parade,
Glasgow Royal
Infirmary, Glasgow
G31 2ER, UK
A Crilly

Centre for Rheumatic
Diseases, 84 Castle
Street, Glasgow Royal
Infirmary, Glasgow
G4 0SF, UK
N Maiden
H Capell
R Madhok

Correspondence to:
Dr Madhok
Email:
gcl103@clinmed.gla.ac.uk

Accepted for publication
15 February 2000

Total joint arthroplasty is the single most expensive treatment in the management of rheumatoid arthritis (RA). In a survey of 1600 patients with RA from a single centre over 23

Table 1 Summary of polymerase chain reaction (PCR) conditions for IL1 α , IL1 β , and IL1 receptor antagonist polymorphisms

	IL1 α	IL1 β	IL1 receptor antagonist
Primers	5CTCAGCAACACTCTAT3 5TCTTGGTCTGCAGGTAA3	5TGGCATTGATCTGGTTCATC3 5GTTTAGGAATCTTCCCACTT3	5AGGCAATAGGTTTGTAGGGCCAT3 5TCCTCCCTGCTCCGATTCCG3
PCR conditions	94°C 1 min (94°C 1 min) (46°C 1 min) $\times$ 40 (72°C 1 min) 72°C 5 min	95°C 2 min (95°C 1 min) (53°C 1 min) $\times$ 35 (74°C 1 min) 74°C 5 min	96°C 1 min (94°C 1 min) (60°C 1 min) $\times$ 30 (70°C 1 min) 72°C 5 min
PCR product size (after digestion)	Allele 1 = 83bp + 16bp Allele 2 = 99bp	Allele 1 = 190bp + 114bp Allele 2 = 304 bp	Allele 1 = 410bp Allele 2 = 240bp Allele 3 = 500bp Allele 4 = 325bp Allele 5 = 595bp

Patients and methods

SELECTION OF PATIENTS AND CONTROL SUBJECTS

A retrospective case-control study was performed to determine the relation between IL1 gene polymorphisms and the need for joint surgery before 15 years of disease duration. In a previous survey of 100 consecutive patients with RA who had undergone total joint arthroplasty attending the same clinic, the median disease duration before needing surgery was 14.6 years. One hundred white patients with RA, seen by one doctor and who satisfied the study entry criteria between March 1995 and April 1996, were included. They were part of a cohort of 1512 patients attending a hospital based clinic for patients with RA. All study patients fulfilled the 1987 American College of Rheumatology criteria for RA,¹¹ were seropositive for rheumatoid factor, and had erosive disease. Patients were selected if they had required either shoulder, hip, or knee arthroplasty within 15 years of disease (n=50). Patients with a disease duration greater than 15 years who had not required surgery served as controls (n=50). Also included were 66 normal controls.

DNA EXTRACTION

The Genomix DNA extraction kit (Genomix Kit, VH Bio, Newcastle Upon Tyne, UK) was used to extract DNA from 10 ml venous blood collected in EDTA. Genomic DNA served as a template in the polymerase chain reaction (PCR) for specific primers to regions of interest. Table 1 summarises the primers and conditions used for PCR.

IL1 α POLYMORPHISMS

The biallelic polymorphism at position -889 of the IL1 α gene was investigated using specific primers and PCR.⁸ PCR products were then digested using the restriction enzyme *NcoI*.

Subjects carrying allele 1 (cytosine) generate a PCR product with a recognition site for the *NcoI*, whereas those carrying allele 2 (thymine) generate a PCR product with no recognition site (table 1). Digested PCR products were analysed on an 8% acrylamide gel and visualised by ethidium bromide staining.

IL1 β POLYMORPHISMS

A biallelic polymorphism at position -511 of the IL1 β gene was investigated using specific primers and PCR.⁸ After amplification PCR products were digested with the restriction enzyme *AvaI* and analysed on an 8% acrylamide gel and stained with ethidium bromide. Subjects who carry allele 1 (cytosine) generate a PCR product with a recognition site for *AvaI*, whereas those with allele 2 (thymine) generate a product with no recognition site (table 1).

IL1 RECEPTOR ANTAGONIST POLYMORPHISM

A variable number tandem repeat in intron 2 of the IL1 receptor antagonist gene was investigated.⁸ PCR products were analysed on a 2% agarose gel stained with ethidium bromide.

HLA-DR β 1 GENE STATUS

All patients included in the study had been typed for HLA-DR β 1 gene status as previously outlined.³

MEASUREMENT OF ESR

When available, the initial ESR at the first ever visit to the outpatient rheumatology department at Glasgow Royal Infirmary was recorded. The ESR had been measured by the Westergren method.

STATISTICAL ANALYSIS

Medians and interquartile ranges are reported. Continuous data were analysed by the Mann-

Table 2 Demographic characteristics of those patients who had undergone surgery and those who had not

	Surgery (n=50)	Surgery 2 DAE‡ (n=19)	Surgery 0/1 DAE (n=31)	No surgery (n=50)	No surgery 2 DAE (n=5)	No surgery 0/1 DAE (n=45)
Age (years)	58 (52-67)	52* (44-60)	61* (56-68)	62.5 (56-69)	62 (59-74)	63 (55-68)
M:F	12:38	3:16	9:22	8:42	1:4	7:38
Duration (years)	15** (13-17)	15 (14-16)	15 (12-18)	20** (18-24)	18 (15-30)	20 (18-24)
Age at onset	42 (36-52)	37† (28-43)	46† (40-54)	40 (32-48)	41 (31-58)	40 (31-47)
ESR (mm/1st h)	52†† (24-76)	43 (18-86)	52 (31-74)	22†† (15-36)	11 (6-44)	22.5 (15-36)
DMARD‡	3 (2-5)	4 (2-6)	3 (2-5)	2 (1-4)	1 (1-2)	3 (1-4)
HAQ‡	2.5*† (2-2.62)	2.56 (2-2.75)	2.38 (2-2.6)	2.19*† (1.75-2.5)	1.125 (1.125-2)	2.25 (1.75-2.5)
Patient surgery (years)	9 (6-11)	9 (7-11)	9 (7-11)	NA	NA	NA

Median values are reported and interquartile ranges are given in parentheses. Groups compared with each other and which are significantly different (p<0.05) have been indicated by similar symbols (*, †, **, ††, *†).

‡DAE = disease associated epitope; DMARD = disease modifying antirheumatic drug; HAO = Health Assessment Questionnaire.

Table 3 Allele frequencies of IL1 gene polymorphisms in those patients who had undergone surgery and those who had not. Results are shown as number (%)

	Controls	Surgery	Surgery 2 DAE*	Surgery 0/1 DAE	No surgery	No surgery 2 DAE	No surgery 0/1 DAE
IL1 β							
Allele 1	79 (60)	60 (60)	24 (63)	36 (58)	72 (73)	6 (60)	66 (75)
Allele 2	53 (40)	40 (40)	14 (52)	26 (42)	26 (27)	4 (40)	22 (25)
IL1 α							
Allele 1	88 (73)	72 (73)	30 (83)	42 (69)	65 (65)	4 (40)	61 (68)
Allele 2	32 (27)	26 (27)	6 (17)	20 (32)	35 (35)	6 (60)	29 (32)
IL1 receptor antagonist							
Allele 1	105 (82)	88 (88)	34 (89)	54 (87)	91 (91)	8 (80)	83 (92)
Allele 2	21 (16)	10 (10)	4 (11)	6 (10)	8 (8)	2 (20)	6 (7)
Allele 3	2 (2)	2 (2)	0	2 (3)	1 (1)	0	1 (1)

*DAE = disease associated epitope.

Whitney test and discrete data were analysed by χ^2 test using 2×2 contingency tables. p Values <0.05 were taken as significant.

Results

DEMOGRAPHIC CHARACTERISTICS OF PATIENTS

Table 2 shows the clinical characteristics of the cohort. When patients who had undergone surgery were compared with those who had not the initial ESR and HAQ were significantly higher in the surgery cohort whereas disease duration was shorter. Patients carrying two DAEs were significantly younger than those carrying either one or no DAE. They also had an earlier age of disease onset (table 2).

IL1 α POLYMORPHISMS

There was no difference in allele or genotype frequency between patients and controls (tables 3 and 4). Patients with early major surgery (before 15 years, $n=49$) had a similar allele and genotype frequency than patients who had not undergone surgery ($n=50$).

IL1 β POLYMORPHISMS

No significant difference in allele frequency or genotype was seen between controls ($n=66$) and patients with RA ($n=99$) (tables 3 and 5). Patients with RA who had had surgery ($n=50$) had a significantly higher allele 2 frequency than the non-surgery group (40% *v* 27%, $\chi^2=4$, 1df, $p=0.04$); OR=2 (95% confidence interval (CI) 1 to 4). Test sensitivity was 40% and specificity was 73%. There was no significant difference in the IL1 β genotype.

Table 4 IL1 α genotypes. IL1 α genotypes are shown in controls ($n=60$), and patients with rheumatoid arthritis who had received surgery ($n=49$) and those who had not ($n=50$). Results are shown as number (%)

	1*1	1*2	2*2
Controls	33 (55)	22 (37)	5 (8)
Surgery	26 (53)	20 (41)	3 (6)
No surgery	19 (38)	27 (54)	4 (8)

Table 5 IL1 β genotypes. IL1 β genotypes are shown in controls ($n=66$), and patients with rheumatoid arthritis who had received surgery ($n=50$) and those who had not ($n=49$). Results are shown as number (%)

	1*1	1*2	2*2
Controls	25 (38)	29 (44)	12 (18)
Surgery	20 (40)	20 (40)	10 (20)
No surgery	26 (53)	20 (41)	3 (6)

INFLUENCE OF IL1 β POLYMORPHISMS ON INITIAL ESR

Patients with RA carrying allele 2 (that is, 1*2 or 2*2, $n=51$) had a higher ESR than those without allele 2 (that is, 1*1, $n=44$), 36 mm/1st h *v* 22 mm/1st h, $p=0.04$. Patients homozygous for allele 2 ($n=12$) had a higher ESR than those homozygous for allele 1 ($n=44$), 40 mm/1st h *v* 23 mm/1st h, $p=0.04$.

INFLUENCE OF IL1 β POLYMORPHISMS ON SURGERY BASED ON HLA-DR β 1 STATUS

We previously found that patients with RA with two DAEs were more likely to require surgery, and in those with one or no DAE the initial ESR predicted the need for surgery.³ We decided to compare the -511 IL1 β gene polymorphism in patients with or without previous surgery with either one or no DAE. Allele 2 frequency in the surgery cohort compared with the non-surgery group was higher than expected (42% *v* 25%, 1 df, $\chi^2=4.8$, $p=0.03$, table 3), giving an OR of 2 (95% CI 1 to 4), test sensitivity of 70%, and 75% specificity. More surgery patients were homozygous for allele 2 than non-surgery patients (22% *v* 4% respectively, 2df, $\chi^2=5.9$, $p=0.05$, table 6).

IL1 RECEPTOR ANTAGONIST

There was no significant difference in allele frequency or genotype between controls and patients with RA with or without previous surgery (tables 3 and 7).

Table 6 IL1 β genotypes in patients who had undergone surgery ($n=31$) and those who had not ($n=44$) who do not carry two disease associated alleles. Significantly more patients who had undergone surgery were homozygous for allele 2 than those who had not, 22% *v* 4% respectively, 2 df, $\chi^2=5.902$, $p=0.05$. Results are shown as number (%)

	1*1	1*2	2*2
Surgery	12 (39)	12 (39)	7 (22)
No surgery	24 (55)	18 (41)	2 (4)

Table 7 IL1 receptor antagonist genotypes. IL1 receptor antagonist genotypes are shown in controls ($n=64$), and patients with rheumatoid arthritis who had received surgery ($n=50$) and those who had not ($n=50$). Results are shown as number (%)

	1*1	1*2	2*2	1*3	2*3
Controls	50 (78)	4 (6)	8 (12)	1 (2)	1 (2)
Surgery	42 (84)	2 (4)	4 (8)	2 (4)	0
No surgery	45 (90)	0	4 (8)	1 (2)	0

Discussion

No difference, either in allele frequency or genotype in any of the IL1 gene polymorphisms studied, was found between patients with RA and normal controls. There was no difference in allele frequency or genotype for the IL1 α -889 or IL1 receptor antagonist gene polymorphism between those patients with RA who had undergone surgery and those who had not. In contrast, we did find an increase in the frequency of allele 2 of the IL1 β -511 gene polymorphism in patients who had undergone early major joint surgery (OR=2 (95% CI 1 to 4)). There was no difference in allele 2 frequency between patients with RA requiring early major joint surgery with two DAEs and those with one or no DAE. However, those with one or no DAE who required early joint surgery had an increased frequency of IL1 β -511 allele 2 than those who had not required surgery (OR=2 (95% CI 1 to 4)). The test had a sensitivity of 40% and a specificity of 73% in predicting need for surgery in the whole group, but in those with one or no DAE the sensitivity and specificity were 70% and 75% respectively. Previously, we had noted that ESR at the first ever clinic visit was a predictor of early surgery in patients who did not carry two DAEs.⁴ In this study we found that patients who carried allele 2 had a significantly higher initial ESR.

We chose surgery as the end point for several reasons. Conceptually, the need for surgery represents a failure of treatment. Clinically, it can be easily quantified and has none of the observer and patient biases of other measures. It is also the single most expensive treatment cost. The validity of surgery as a useful clinical end point relative to other measures of disease activity has been recently confirmed.¹ Studying an inception cohort would have been the ideal patient population and study design. However, to obtain the numbers required to show the extent of difference confidently would have required a large cohort followed up for at least 20 years. We therefore considered a case-control study a more appropriate study design for the patient group available at our centre.

We chose to look at three polymorphisms within the IL1 gene, though other polymorphisms exist at this loci.¹²⁻¹⁴ The polymorphisms investigated were well characterised when the study began and we wished to assess their usefulness as prognostic markers of disease outcome in RA. Associations with intron 2 IL1 receptor antagonist and other diseases had been previously reported,^{8 15-19} but not in RA.

An association with allele 2 of the IL1 α -889 gene polymorphism has been reported in juvenile rheumatoid arthritis,²⁰ but we are not aware of studies of this polymorphism in RA. Bailly *et al* found no association with IL1 α intron 6 polymorphism and RA.²¹ We know of one study that looked at -511 IL1 β polymorphism in RA,²² but that study by Cantagrel *et al* differed from ours in two major ways.²² Firstly, the cohort consisted of patients with early disease and, secondly, the study end point was development of erosive disease. No association was noted with the -511 polymorphism, but

allele E2 in the IL1 β exon 5 was overrepresented in the patients who developed erosive disease.

The functional significance of the association seen between -511 IL1 β allele 2 and the need for early surgery is not clear. A recent study by Santilla *et al* reported that mononuclear cells from subjects carrying the -511 allele 2 and non-carriers of allele 2 at IL1 β exon 5 have a slight but not significant rise in IL1 β production.²³ Increased IL1 β production was found in those who carried the IL1 receptor antagonist allele 2 regardless of their IL1 β polymorphism. Currently, the functional significance of our findings is unclear and further investigation is required. Future studies focusing on IL1 β production in cells from patients with specific genotypes may provide some of the answers.

The frequency of allele 2 in our patients who had had surgery does not sufficiently explain the need for surgery or fully explain the higher initial ESR. Possibly, there is an extended IL1 gene family haplotype. Alternatively, there may be polymorphisms in other cytokine genes that mediate the acute phase response.

Genetic regulation of ESR and other factors which influence this variable should be considered. Polymorphisms in the genes for TNF α ²⁴ and IL6^{25 26} have already been described. Associations between the TNF α gene polymorphism and RA have been described.^{27 28} In one study severity showed a direct correlation with the microsatellite TNF α 11.²⁸ We are in the process of investigating this association because it may influence severity by interaction with DAE. In juvenile rheumatoid arthritis an association with the cc genotype of the -174 IL6 gene polymorphism has been reported.²⁹ In a preliminary study we have not seen associations in our cohort with the -174 IL6 gene polymorphism (unpublished data).

Our results suggest that patients who require early major joint surgery have an inherited predisposition. In those without two DAEs who require early major joint surgery the need for surgery can be predicted by the initial ESR. We show that allele 2 of the -511 IL1 β polymorphism is found more often than expected in such patients and they have a higher ESR. Further studies to define its role precisely in clinical prediction are necessary in a large inception cohort.

This work was funded by Tenovus, Scotland, and Dr Maiden is a BUPA/Glasgow Royal College of Physicians and Surgeons research fellow.

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