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The role of interleukin 10 promoter polymorphisms in the susceptibility of distal interphalangeal osteoarthritis

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Abstract

Objectives

The IL-10 single nucleotide promoter polymorphism (SNP) –2849A is associated with decreased IL-10 production as measured by lipopolysaccharide (LPS) stimulated whole blood cultures. A low innate production of IL-10 using the same assay, is associated with an increased risk of familial osteoarthritis (OA). Therefore, we aimed to investigate the association of seven novel SNPs located downstream of the IL-10 transcription start site: –2849, –2763, –1330, –1082, –819 and –592, constituting the four ancient haplotypes, with distal interphalangeal (DIP) OA.

Methods

The study population consisted of consecutive patients with and without radiological DIP OA (Kellgren-Lawrence score of ≥ 2 in one joint) aged 40–70 years from a cohort of subjects with different types of arthritis in an early stage referred to an “Early Arthritis Clinic” (EAC). DNA typing for IL-10 SNPs as well as X-rays of the hands were performed at the time of enrolment in the EAC. Patients with RA, SLE, spondylarthropathies and psoriatic arthritis were excluded for the purpose of this study. The distribution of DIP OA and IL-10 SNPs were compared and shown to be comparable to representative samples of the Dutch population.

Results

In the cohort of 172 subjects, 57 had DIP OA (33%) and 115 (67%) had no DIP OA. No significant association was found between DIP OA and IL-10 SNPs and the four common haplotypes IL10.1, IL10.2, IL10.3 and IL10.4.

Conclusions

Our data suggests that IL-10 SNPs, including –2849 associated with differential production do not play a major role in the susceptibility of DIP OA.

Key words

osteoarthritis, hand, promoter polymorphisms, cytokines, IL-10

Introduction

Distal interphalangeal (DIP) osteoarthritis (OA) is one of the most frequent subtypes of OA, causing pain and loss of function in the hands. Familial aggregation and twin studies have shown DIP OA to have a strong familial component (1-3). Despite studies focusing on the genetics of DIP OA, the genes involved have not been identified. Several chromosomal regions are reported to be associated with DIP OA, namely quantitative trait loci (QTL) on chromosomes 2q, 7p and 11q implicated by linkage studies (4), HLA-DR2 by candidate gene analysis in several populations (5, 6) and further, a locus on chromosome 2 containing the matrilin-3 gene, by a genome-wide scan in an Icelandic population (7).

OA is hallmarked by a loss of articular cartilage and changes in the subchondral bone and joint margins. An increased matrix catabolism characterized by an upregulation of metalloproteinases (MMPs) and the depletion of structural macromolecules like proteoglycans contributes to the OA disease process (8). Increasing data support the role of cytokines in these processes. Although pro-inflammatory cytokines have been shown to play a pivotal role in the initiation and development of OA, a shift in the balance between the pro- and anti-inflammatory cytokines is believed to contribute to the loss of integrity of the articular cartilage (9).

Among the anti-inflammatory cytokines, IL-10 appears to be a crucial factor in inflammatory processes (10). In a mouse model of arthritis, cartilage destruction is prevented by IL-10 administration by reducing IL-1 β and TNF- α mRNA expression in articular chondrocytes, whilst neutralizing anti-IL-10 antibodies accelerate the onset and enhance the severity of arthritis (11).

The human IL-10 gene is highly polymorphic. Gibson et al. (12) identified 7 novel single nucleotide polymorphisms (SNPs) in the distal region of the IL-10 promoter and found that certain haplotypes are significantly associated with high or low IL-10 production. Studies have shown that there are striking differences between healthy individuals in their ability to produce IL-10 following lipopolysaccharide (LPS) stimulation of whole blood cultures *in vivo*. Moreover it has been demonstrated that IL-10 haplotypes dictate IL-10 production as measured by this assay (13). Of the IL-10 promoter polymorphisms, -2849A, encoded on haplotype IL-10.01, has been shown to correlate best with protein production (13). Individuals harboring the AA genotype have a reduced IL-10 production in comparison to AG and GG individuals.

Several studies have reported the clinical relevance of IL-10 gene polymorphism in autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) (12, 14) and in infectious diseases (15). In an earlier study we found an increased risk of familial OA at multiple sites in subjects with a low innate production of IL-10, as measured by the same *ex vivo* whole blood assay using LPS stimulation (16) as used in all previous studies (12, 13). This finding implicating IL-10 in cartilage destruction led us to investigate whether genetic variation in IL-10 contributes to the susceptibility of DIP OA. The aim of the present study was to investigate whether IL-10 SNPs: -592, -819, -1082, -2763, -2849 and -3575 that constitute the four ancient IL-10 haplotypes are a risk factor for DIP OA. For this purpose a cohort of consecutive hospital patients were studied with and without OA in the DIP joints.

Patients and methods

Study population

The study population consists of subjects from the outpatient clinic of the Department of Rheumatology between the ages of 40 to 70 years old with and without radiological DIP OA. This population obtained between 1993 and 2000 is part of an ongoing project, the Early Arthritis Clinic (EAC). Consecutive patients with arthritis in at least one joint with a short history of complaints are admitted to this clinic by general practitioners. Each patient subsequently undergoes full clinical, biochemical and radiographic assessment. Diagnoses in the EAC are made according to international classification and if necessary revised up to 1 year of follow-up (17). For the purpose of this study, patients with a definitive EAC diagnosis after one year of follow-up are included. Patients with RA, SLE and spondylarthropathies were excluded because these diseases have been associated with IL-10. Patients with psoriatic arthritis were also excluded since DIP involvement is common in psoriatic arthritis and could thus interfere with our readings.

Reference populations

The prevalence of radiological DIP OA and the distribution of the IL-10 promoter polymorphisms in the study population were compared to two reference populations representative of the general Dutch population. As a reference for radiological DIP OA data were used from the Zoetermeer population, a population survey consisting of 3109 men and 3476 women (18) as described in a previous study (5). A comparison between the frequency of radiological DIP OA in the present population in reference to the Zoetermeer population gave an observed to expected ratio of 0.96 (0.7-1.2). The distribution of the IL-10 promoter polymorphisms was compared to its distribution in a random panel of the southwest region of the Netherlands (n=321) (19).

Radiographs and radiographic scoring

Plain dorsovolar hand radiographs were taken routinely in each patient during the period of the first visit to the EAC. Only radiographs obtained at a maximum of 3 months before till 3 months after the first visit were included. For the purpose of this study each of the radiographs was independently graded for DIP OA by two out of three observers using the Kellgren-Lawrence scale. Furthermore the radiographs are scored for the presence of DIP OA blinded for the underlying EAC diagnosis. This overall score distinguishes five degrees of severity of OA according to the presence of the radiological features: osteophytes, joint space narrowing (JSN), subchondral sclerosis, cysts and deformity. A patient was diagnosed with DIP OA if a Kellgren score of two or more was observed in at least one DIP joint. The inter-rater agreement for the presence or absence of DIP OA was 0.7; Cohen's kappa. In case of disagreement radiographs were re-evaluated until consensus was reached.

Determination of IL-10 promoter polymorphisms

Peripheral blood cells were SDS lysed and treated with proteinase-K. DNA was isolated by phenol-chloroform extraction. A Perkin-Elmer thermal cycler Gene Amp 9600 (PE-Cetus, Norwalk, CA USA)

was used to amplify the IL-10 promoter region by a polymerase chain reaction. The primer combination and methods used have previously been described (20).

Statistical analysis

The means were compared using an independent sample Student t-test. Odds ratios (OR) with 95% confidence intervals (CI95) were calculated in order to determine whether the distribution of IL-10 promoter polymorphisms is comparable to the random panel by comparing the distribution of the minor allele grouped with the heterozygote [11 + 12] versus the major allele [22] in both sets of populations. OR were also used to assess the association between DIP OA and the different genotypes of IL-10 promoter polymorphisms. Multiple-locus haplotype frequencies (SNPHAP- <http://www-gene.cimr.cam.ac.uk/clayton/software/>) and the measures of pair wise LD were determined using the HAPLO program (21). This program implements a fairly standard method for estimating haplotype frequencies using data from unrelated individuals. It uses an expectation-maximization algorithm to calculate maximum-likelihood estimates of haplotype frequencies, given genotype measurements. The estimator of linkage disequilibrium D (where $D = h_{pq} - pq$) indicates the difference in the observed (h_{pq}) and expected (pq) frequencies of haplotypes. Its maximum value depends on the allele frequencies and whether the rare alleles are associated together on a haplotype (positive value of D) or whether the common allele is associated with the rare allele (negative value of D). $|D^f|$ (Lewontins') is the fraction of D of its maximum ($D_{\max} = p - pq$) or minimum ($D_{\min} = -pq$) possible value. Power analysis was based on achieving 5% significance in order to detect a difference if IL-10 promoter polymorphisms or IL-10 haplotypes were associated with an almost three fold increased risk of DIP OA (OR=2.7). This analysis was based on the frequency of the IL-10 A carriage rate in the SNP -2849 associated with differential IL-10 production.

Results

Six hundred and one consecutive patients aged between 40 to 70 years visited the EAC. Sixty-nine patients were excluded because no definitive diagnosis was made within one year, 281 patients were excluded with RA, systemic diseases, spondylarthropathies and psoriatic arthritis and 79 patients were excluded due to missing data resulting in 172 patients included in the present study. Of the 172 patients, 3 were not genotyped for the -3575, -2763, -1082 and -819 SNPs due to poor DNA quality and an additional 18 samples were missing at the time of genotyping the -3575 SNP.

Patient characteristics

The patients in this study were included in the EAC with a broad variety of diagnoses independent of the presence or absence of DIP OA. Fifty-seven (33%) patients of the study population had DIP OA. As expected, the average age of patients with DIP OA 58(43-70) was higher than patients with no DIP OA 51(40-70), mean difference: 6.8 (4.6-9.1). The clinical characteristics of the study population are summarized in Table 1.

Table 1. Clinical characteristics of the study population: patients with and patients without distal interphalangeal arthritis (DIP) osteoarthritis (OA)

	DIP OA (N=57)	No DIP OA (N=115)
Mean age (range)	58 (43-70)	51 (40-70)
Women, no. (%)	33 (58)	55 (47)
EAC diagnoses (no.)		
septic arthritis	1	2
reactive arthritis	3	7
crystal arthropathy	8	18
post-traumatic	1	4
osteoarthritis	17	10
unclassified arthritis	23	51
para malignant	1	5
sarcoidosis	1	5
other	2	11
unknown	0	2

Table 2. The distribution of the minor allele grouped with the heterozygote (11 + 12 versus the major allele (22) in single nucleotide promoter polymorphisms (SNP) of the interleukin (IL)- 10 gene in the study population in comparison to a random panel in the south-west region in the Netherlands expressed as odds ratio's (OR) with 95% confidence intervals (CI95)

SNP ID	11+12	22	OR (CI95)
IL-10 A-3575T			
study population	0.71	0.29	1.4 (0.7-2.7)
random panel	0.64	0.36	
IL-10 A-2849G			
study population	0.55	0.45	1.6 (0.9-3.0)
random panel	0.43	0.57	
IL-10 A-2763C			
study population	0.65	0.35	1.3 (0.7-2.5)
random panel	0.58	0.42	
IL-10 G-1082A			
study population	0.75	0.25	1.0 (0.5-1.9)
random panel	0.76	0.24	
IL-10 T-819C			
study population	0.40	0.60	1.0 (0.6-1.8)
random panel	0.40	0.60	

Table 3. Summary of the association between DIP OA (n=57) and the genotypes of the IL-10 promoter polymorphisms within the study population (n=172) expressed as odds ratios with 95% confidence intervals OR (CI95)

	Genotype distribution			11 versus 12 + 22	11+12 versus 22
IL-10 A-3575T¹	11	12	22	OR (CI95)	OR (CI95)
DIP OA	9	27	14	1.5 (0.5-4.1)	1.1 (0.5-2.5)
No DIP OA	13	57	30		
IL-10 A-2849G					
DIP OA	4	30	23	1.0 (0.2-3.9)	1.4 (0.7-2.7)
No DIP OA	8	52	55		
IL-10 A-2763C²					
DIP OA	7	33	16	1.3 (0.4-3.8)	1.5 (0.7-3.3)
No DIP OA	12	58	43		
IL-10 G-1082A²					
DIP OA	16	26	15	1.6 (0.7-3.6)	0.93 (0.4-2.1)
No DIP OA	22	62	28		
IL-10 T-819C²					
DIP OA	3	18	36	0.98 (0.2-4.7)	0.81 (0.4-1.6)
No DIP OA	6	41	65		

¹Genotypable samples in 150 patients

²Genotypable samples in 169 patients

The distribution of IL-10 promoter polymorphisms in comparison to controls

In Table 2, the distribution of the minor allele grouped with the heterozygote is shown versus the major allele of the IL-10 promoter polymorphisms -3575, -2849, -2763, -1082 and -819 in the study population and in the random panel. No difference was observed in the distribution of these alleles in the two groups. The IL-10 promoter polymorphism -1330 and -592 are in complete linkage disequilibrium with -1082 and -819 respectively, therefore, these variables were excluded from further analyses of single promoter polymorphisms. Genotypes did not show deviations from the Hardy-Weinberg equilibrium (data not shown) (22).

The association of IL-10 promoter polymorphisms and haplotypes with DIP OA

The association between DIP OA and the genotypes of IL-10 SNPs -3575, -2849, -2763, -1082 and -819 is depicted in Table 3. No association was found between these polymorphisms and DIP OA. Since the interaction of two or more SNPs in haplotypes may be more informative than single polymorphisms, a haplotype analysis was done. No difference was observed in the distribution of the four extended haplotypes, IL-10.1, IL-10.2, IL-10.3 and IL-10.4 in patients with and without DIP OA ($p=0.67$). Data are presented in Table 4. In a separate analysis of the distal haplotype frequency

(A-3575T, A-2849G and A-2763C) and the proximal haplotype frequency (A-1330G, -G1082 A, T-819 C and A-592 C) no difference was seen in the distribution in patients with and without DIP OA (data not shown).

Table 4. The haplotype distribution in patients with distal interphalangeal arthritis (DIP) osteoarthritis (OA) (n=57) and patients without DIP OA (n=116)

IL10 HAP	-3575	-2849	-2763	-1330	-1082	-819	-592	DIP OA (%)	No DIP OA (%)
IL10.1	A	A	A	A	G	C	C	0.30	0.23
IL10.2	T	G	C	G	A	C	C	0.28	0.27
IL10.3	A	G	A	A	G	C	C	0.19	0.23
IL10.4	T	G	C	G	A	T	A	0.07	0.09

Discussion

This report is to our knowledge the first to investigate the relationship between a highly genetic form of OA, namely DIP OA and promoter polymorphisms located downstream of the IL-10 transcription start site: -2849, -2763, -1330, -1082, -819 and -592 constituting the four ancient IL-10 haplotypes. These SNPs as well as the four haplotypes were not associated with a higher risk of radiological DIP OA.

The association of DIP OA with IL-10 promoter polymorphisms in the current investigation was studied in patients with a variety of underlying forms of arthritis. This consecutive patient population was collected in a prospective manner. Because the study population consisted of patients included in an EAC, the existing correlation between certain rheumatic diseases and the genetic variables under study were taken into consideration while selecting the patients for the present study. All patients were excluded with diseases that have been reported in the literature to be associated with the polymorphisms under study. Furthermore, diseases which could lead to radiological damage of the DIP joints, such as psoriatic arthritis were also excluded. However, radiological damage would not have been very likely since patients are included in the EAC in a very early stage. In order to ensure that the population at hand was not a highly selected one with respect to the variables under study, the frequency of these variables was compared and shown to be comparable to reference populations assumed to represent the general Dutch population.

Among the IL-10 promoter polymorphisms under study, the SNP -2849A has been associated with low IL-10 production. In the present study we found an OR of one in comparison of the -2849 AA versus -2849AG and -2849GG. These data strongly suggest that the association is absent, although, a type II error may be present given the power of the current study. In summary we conclude that it is unlikely that the SNP -2849A has a large effect in the genetic susceptibility for radiological DIP OA.

Although the anti-inflammatory role of IL-10 is recognized in arthritis, its role in OA is still undefined. Based on its biologic activity it is, however, conceivable that low IL-10 production may contribute to a catabolic state in OA. IL-10 is an important immunoregulatory cytokine in man (10) and plays a crucial role in inflammation and tissue destruction. In an arthritis model, mice lacking the gene for IL-10, experienced higher rates of clinical signs and more severe knee and paw injury as compared to IL-10 wild-type controls. Furthermore, plasma levels of TNF- α , IL-1 β and IL-6 were also enhanced in the knockout mice compared to wild-typed mice (23).

IL-10 has been reported *in vitro* to be synthesized in increased amounts either spontaneously by synovial membrane and cartilage (24) or after stimulation of chondrocytes with IL-1 β or TNF- α (8). IL-10 contributes to cartilage homeostasis through several pathways. In experiments on joint tissue, it has been shown that a lack of IL-10 can lead to joint destruction as a result of an increased expression of metalloproteinases (25). Upregulation of IL-1 receptor antagonist production by isolated monocytes has been found for IL-10 by human monocytes and neutrophils (26). Besides exerting anti-inflammatory activity, IL-10 has been shown to directly stimulate proteoglycan synthesis by human chondrocytes *in vitro* (27).

In an earlier study (16), we have observed that a low innate production of IL-10 using LPS production was associated with an increased risk of familial OA at multiple sites defined as multiple sites in the hands or at two or more joint sites including the hands, spine, knees and hips. The low innate IL-10 production in these patients is assumed to be partly caused by genetic variation at the IL-10 locus (13, 15). The results of the present study indicate that IL-10 promoter polymorphisms constituting the four ancient haplotypes are not implicated in the susceptibility of DIP OA. The discrepancy between the findings in the earlier study and the present study may be due to different OA phenotypes: familial OA at multiple joint sites versus radiographically defined OA in the DIP joints. Alternatively, not all of the genetic variation in IL-10 production is dictated by IL-10 haplotypes (13). It may be that the genetic factors that dictate interindividual IL-10 production differences that are not located in the IL-10 locus, are those relevant for DIP OA. In summary our current work suggests that the currently known IL-10 SNPs do not exhibit a major effect in the genetic susceptibility of DIP OA.

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