

# Osteoarthritis and Cartilage



## A meta-analysis of interleukin-6 promoter polymorphisms on risk of hip and knee osteoarthritis

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### ARTICLE INFO

#### Article history:

Received 28 October 2009

Accepted 21 December 2009

#### Keywords:

Interleukin-6  
Genetic susceptibility  
Meta-analysis

### SUMMARY

**Objective:** Interleukin-6 is a pro-inflammatory cytokine involved in the pathogenesis of osteoarthritis (OA). We investigated the role of two single nucleotide polymorphisms (SNPs) mapping to the promoter of the *IL-6* gene on genetic susceptibility to hip and knee OA.

**Methods:** The  $-174$  G/C (rs1800795) and  $-597$  G/A (rs1800797) SNPs, implicated in the literature in risk of hip and hand OA, were genotyped in 2511 controls, 1101 hip OA cases and 1904 knee OA cases from four cohorts from the UK and Estonia. Data were analysed in conjunction with published data on rs1800797 from the Genetics of OA and Lifestyle study (UK) on 791 controls, 1034 knee and 997 hip OA cases and rs1800795 data on 75 hip OA cases and 96 controls from Italy. Cases included both radiographic OA only and radiographic and symptomatic OA. Fixed and random-effects meta-analysis models were tested.

**Results:** No significant association was found with hip OA or knee OA with either SNP nor with the haplotypes formed by them. For individual SNPs the smallest *P*-value for hip OA was observed using a random-effects model for rs1800795  $OR_{G\text{ allele}} = 1.066$  (95% CI 0.89–1.28)  $P < 0.49$ , and significant heterogeneity between cohorts ( $I^2 = 65\%$ ,  $P < 0.034$ ) was detected. For knee OA the smallest *P*-value was seen for rs1800797  $OR_{A\text{ allele}} = 1.055$  (95%CI 0.98–1.12)  $P < 0.18$ , no significant heterogeneity was observed ( $I^2 = 0\%$ ,  $P < 0.68$ ).

**Conclusions:** Our data do not support a role for the  $-174$  and  $-597$  *IL-6* promoter polymorphisms in genetic susceptibility to knee or hip OA in Caucasian populations.

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### Introduction

Osteoarthritis (OA) is the most common joint disorder and represents a leading musculoskeletal health and socioeconomic burden. OA is not considered to be classically inflammatory because of the absence of neutrophils and minimal levels of other pro-inflammatory cells in the synovial fluid and systemic inflammatory responses<sup>1</sup>. However, over-expression of pro-inflammatory

cytokines is common in OA and there is convincing evidence that chondrocytes contribute to cytokine production leading to cartilage matrix degradation<sup>2</sup>. Moreover, the overproduction of cytokines and growth factors from the inflamed synovium may play a role in the pathophysiology of OA. The low-grade OA synovitis is itself cytokine-driven, although the levels of pro-inflammatory cytokines are lower than in rheumatoid arthritis (RA)<sup>2</sup>.

Interleukin-6 (IL-6) is one of the pro-inflammatory cytokines involved in OA pathogenesis. A significant increase in the level of IL-6 mRNA has been detected in OA affected cartilage, and the IL-6 levels in the serum and synovial fluid have been reported to be elevated among OA patients<sup>3</sup>. Both the mRNA expression and protein production of IL-6, have been found to be increased in osteophytes

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compared to subchondral bone from femoral neck fractures when mechanical stress loads were applied<sup>4</sup> further implicating this cytokine in OA pathogenesis. Increased IL-6 circulating serum levels have also been shown to be associated with radiographic progression of knee OA<sup>5</sup>.

Polymorphisms in the *IL-6* gene, in particular single nucleotide polymorphisms (SNPs) at positions –174 (rs1800795), –597 (rs1800797) and –572 (rs1800796) in the promoter region of the *IL-6* gene, have been reported to be associated with several diseases e.g., the extent of radiographic damage in RA<sup>6</sup>. Haplotypes formed by these three *IL-6* polymorphisms have also been implicated in total hip arthroplasty failure<sup>7</sup>. These variants are involved in the transcriptional regulation of the *IL-6* gene, are associated with plasma levels of IL-6<sup>8</sup> and have been reported to be associated with risk of hand OA<sup>9</sup>. One of these *IL-6* SNPs (rs1800795) has also been implicated in risk of hip OA in an Italian case–control study<sup>10</sup>. Recently, Limer *et al.*<sup>11</sup> attempted to replicate this result using rs1800797 and found no significant association in a large UK case–control study of hip and knee OA (Genetics of OA and Lifestyle or GOAL).

Our scope was to clarify the role of rs1800795 and rs1800797 *IL-6* promoter polymorphisms on risk of knee and hip OA. To do so, we have carried out a meta-analysis of four independent UK cohorts (including the data from the GOAL study), one Estonian cohort and the original Italian study on hip OA, comprising a total of 3423 radiographic controls, 2946 knee OA cases, and 2335 hip OA cases.

## Subjects and methods

### Study subjects

A full detailed description of each study cohort, recruitment, radiographic and clinical assessment is presented in the supplementary methods section. Study subjects from the UK came from two case–control studies in Nottingham labelled as “Nottingham” and “GOAL”, and from population-based cohorts from Chingford (North London) and Hertfordshire. In addition a population-based cohort from Estonia was included as well as data from a published Italian study. All studies were approved by the relevant Ethics Committee and informed consent was obtained from all study participants. In total two hip OA replication sets and four knee OA replication sets were tested totalling 1404 controls and 1101 cases for the hip OA study and 2511 controls and 1904 cases for the knee OA study. The descriptive characteristics of the sample size and diagnosis criteria of each cohort are presented in Table I. A reference where each cohort is described in detail is also included.

### Genotyping data

For the Nottingham, Hertfordshire, Chingford and Estonian study participants, genomic DNA was extracted from peripheral blood leukocytes of affected individuals and controls using standard

**Table I**  
Descriptive characteristics of study subjects by cohort with genotype counts and minor allele frequencies of *IL-6* promoter polymorphisms rs1800795 and rs1800797

Study	New data				Published data		
	Nottingham	Hertfordshire	Estonia	Chingford	Limer <i>et al.</i> (GOAL)	Pola <i>et al.</i>	
Reference	12	13	14	15	10	11	
Type of study	Case–control	Population based	Population based	Population based	Case–control	Case–control	
Origin	UK	UK	Estonia	UK	UK	Italy	
Age mean (SD)	67.7 (9.1)	65.5 (2.7)	47.1 (6.4)	64.1 (6.0)	66.5 (7.9)	71.2 (9.6)	
Range	(40–96)	(60–72)	(32–60)	(53–77)	(45–86)		
BMI mean (SD)	28.0 (4.9)	27.1 (4.3)	28.1 (5.4)	26.7 (4.7)	29.3 (5.3)	28.3 (3.3)	
Range	(15–51)	(16–48)	(15–47)	(17–50)	(17–58)		
F%	59%	49%	69%	100%	49%	53%	
Controls	Definition	Hip and knee ROA- & Sx-	TF K/L <2	TF K/L <2	Knee: TF K/L <2	Hip: JSN-	Hip ROA- & Sx-
	<i>n</i>	736	784	448	543	668	96
rs1800795	CC/CG/GG (C%)	122/373/241 (41.9%)	143/354/287 (40.8%)	102/244/102 (50.0%)	81/262/200 (39.0%)	104/327/237 (40.0%)	33/40/34 (49.5%)
rs1800797	AA/AG/GG (A%)	118/372/245 (41.3%)	135/359/299 (39.6%)	96/247/109 (48.5%)	76/264/206 (38.0%)	105/324/248 (39.4%)	151/352/287 (41.3%)
Knee OA	Definition	TF K/L ≥2 (80% TKR)	TF K/L ≥2	TF K/L ≥2	TF K/L ≥2	JSN ≥2 or osteophyte score >1 (78% TKR)	N/A
	<i>n</i>	1435	145	69	255	1034	
rs1800795	CC/CG/GG (C%)	283/660/492 (42.7%)	24/72/49 (41.3%)	18/40/11 (55.0%)	41/126/88 (40.7%)	N/A	N/A
rs1800797	AA/AG/GG (A%)	273/663/497 (42.1%)	22/75/53 (39.6%)	16/42/11 (53.6%)	44/124/92 (40.7%)	193/493/348 (42.5%)	N/A
Hip OA	Definition	THR	N/A	N/A	Definite JSN	Croft score ≥3 (92% THR)	JSW ≤1.5 mm and K/L ≥3
	<i>n</i>	1024			77	997	75
rs1800795	CC/CG/GG (C%)	196/511/317 (44.0%)	N/A	N/A	13/31/33 (37.0%)	N/A	12/30/33 (36.0%)
rs1800797	AA/AG/GG (A%)	188/505/333 (42.9%)	N/A	N/A	12/33/32 (37.0%)	176/471/350 (41.2%)	N/A
LD between rs1800795 and rs1800797	D'	0.996	0.995	0.996	0.989	N/A	N/A
	r <sup>2</sup>	0.952	0.935	0.935	0.920		
Haplotype frequencies	GG/CA/CG %	56.5/42.4/1.1%	59.0/39.3/1.5%	49.3/49.2/1.4%	59.7/38.4/1.6%	N/A	N/A

ROA- & Sx- = absence of radiographic and symptomatic OA; TF = tibiofemoral K/L = Kellgren and Lawrence grade; TKR = total knee replacement; THR = total hip replacement, JSN = joint space narrowing; JSW = joint space width; JSN- = absence of definite joint space narrowing.

protocols. Genotyping was carried out by Kbioscience Ltd., Hertfordshire UK. SNPs were genotyped using the KASPar chemistry, which is a competitive allele-specific polymerase chain reaction (PCR) SNP genotyping system using fluorescence resonance energy transfer (FRET) quencher cassette oligos. Genotyping accuracy, as determined from the genotype concordance between duplicate samples is 99.6% on average and for this study was 100% (52 samples genotyped in duplicate for each SNP). Both polymorphisms were in Hardy-Weinberg equilibrium in controls ( $P > 0.05$ ). For the genotyping methods used in published studies from Italy and the UK the reader is referred to the original studies by Pola *et al.*<sup>10</sup> and Limer *et al.*<sup>11</sup> respectively.

### Statistical analysis

Allele and genotype odds ratios were calculated by comparing the numbers among cases and controls and the  $P$ -value was computed using a Pearson's chi-square.

In the absence of inter-study heterogeneity within samples we constructed a Mantel–Haenszel meta-analysis of data from the samples to assess the overall evidence of association. The Mantel–Haenszel chi-squared test and the Mantel–Haenszel estimate of the odds ratio (OR)<sup>16</sup> were used to provide a summary test and odds ratio.

**Random-effects meta-analysis:** In the DerSimonian and Laird method, studies are considered as a random sample from a population of studies. The random effect model incorporates the heterogeneity of the studies. The overall treatment effect is estimated by a weighted average of the individual effects with weights inversely proportional to the variance of the observed effects. We tested the assumption of heterogeneity for each planned analysis using the method of DerSimonian and Laird based on work first presented by Cochran<sup>17</sup>. The statistical significance of the combined effect for the DerSimonian–Laird odds ratio was estimated using the  $Z$ -statistic which is the ratio of the point estimate to its standard error.

**Linkage disequilibrium (LD):** The normalized gametic disequilibrium coefficient ( $D'$ ), the squared correlation coefficient between rs1800795 and rs1800797 and the case and control haplotypes

were estimated in the Estonian, Nottingham, Hertfordshire and Chingford samples using Haploview v 4.1 ([www.broad.mit.edu/mpg/haploview/](http://www.broad.mit.edu/mpg/haploview/)).

### Results

Before undertaking the project we assessed the statistical power of our study and given the frequency of the minor allele and the sample sizes available we estimated that we had 80% to find associations with an OR = 1.12 for hip OA and OR = 1.10 for knee OA with  $P < 0.05$ .

The genotype counts for cases and controls in each are presented in Table I and the summary results of hip and knee OA meta-analyses for both SNPs are presented in Table II. No significant association was found with hip OA or knee OA with either SNP. The results between males and females did not differ significantly and no statistically significant association with OA ( $P < 0.20$ ) was seen when genders were analysed separately (not shown).

We did find significant heterogeneity for hip OA when the Italian data were included, excluding the Italian subjects eliminated the heterogeneity but did not result in a smaller  $P$ -value with hip OA (Table II). No heterogeneity was found for either rs1800795 or rs1800797 with knee OA and neither SNP showed a significant association. Although there appears to be not-statistically significant trend ( $P < 0.18$ ) for the A allele to be associated with increased risk it is worth pointing out that this association is exactly in the opposite direction to the one reported with hand OA<sup>9</sup> and with hip OA<sup>10</sup>.

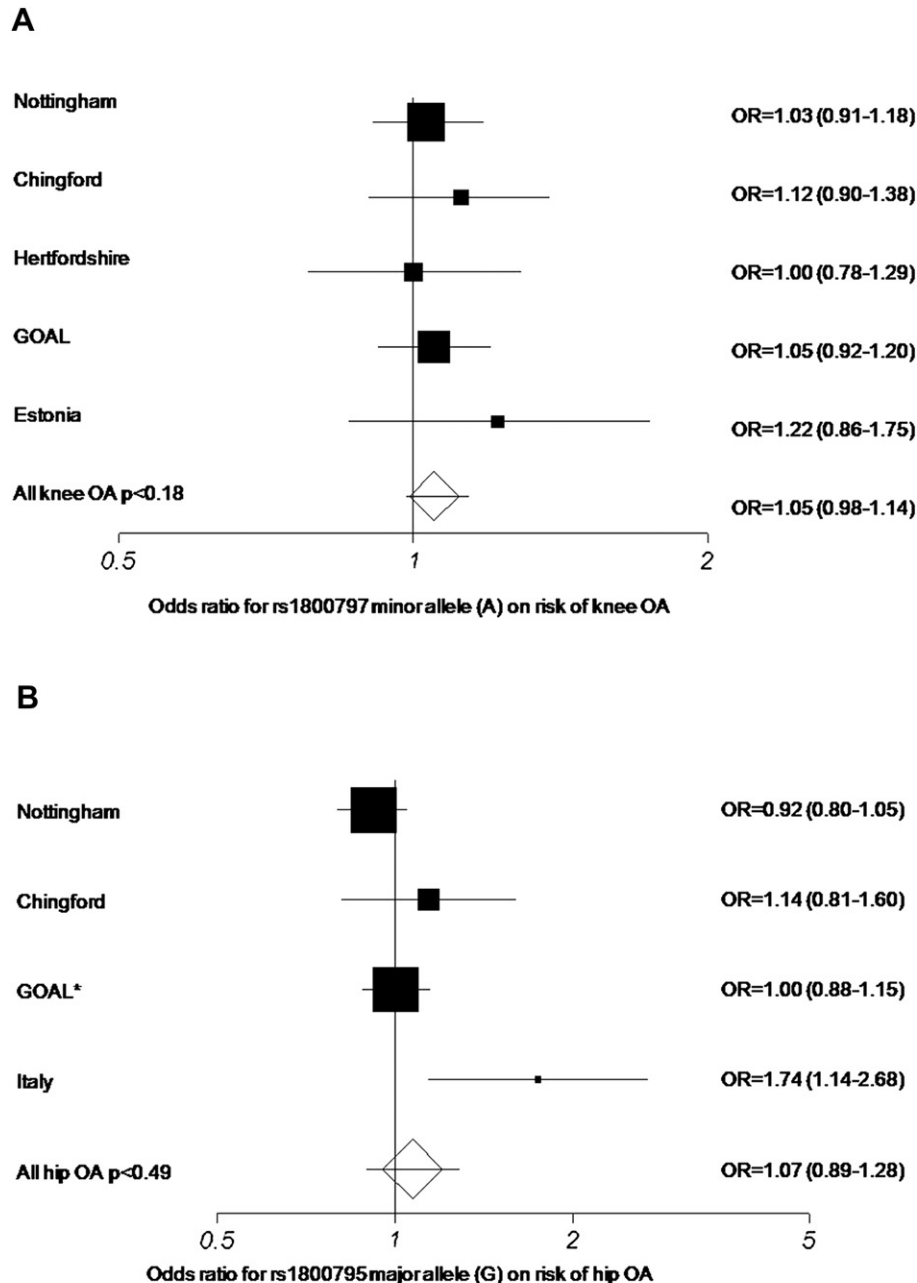
The smallest  $P$ -value for hip OA was observed using a DerSimonian–Laird random-effects model for rs1800795. The odds ratios for individual cohorts along with the summary random-effects odds ratio are shown in Fig. 1(A). For knee OA the smallest  $P$ -value was seen for rs1800797 and the individual cohort odds ratios along with the summary Mantel–Haenszel fixed effects odds ratio is shown in Fig. 1(B).

We also investigated, in the cohorts where both promoter SNPs were genotyped, if there could be a genetic association with the haplotypes defined by these two promoter polymorphisms. Given

**Table II**

Meta-analysis results for association between rs1800795 and rs1800797 alleles and haplotypes and hip and knee OA

Trait	Cohorts included	OR for allele	Fixed effects OR	95% CI	$P$ -value association	$Q$	df	$P$ -value heterogeneity	$I^2$	Random-effects OR	95%CI	$P$ -value association
Knee OA	GOAL, Nottingham, Chingford, Hertfordshire, Estonia	rs1800795C	1.042	0.963–1.127	0.30	1.10	4	0.89	0.00%	1.042	0.963–1.127	0.30
Knee OA	GOAL, Nottingham, Chingford, Hertfordshire, Estonia	rs1800797A	1.055	0.975–1.140	0.18	1.23	4	0.87	0.00%	1.055	0.975–1.140	0.18
Hip OA	GOAL, Nottingham, Chingford, Italy	rs1800795C	1.003	0.916–1.097	0.95	8.69	3	0.034	65.47%	0.938	0.781–1.125	0.49
Hip OA	GOAL, Nottingham, Chingford, Italy	rs1800797A	0.994	0.908–1.087	0.90	7.71	3	0.052	61.07%	0.940	0.792–1.116	0.48
Hip OA	GOAL, Nottingham, Chingford	rs1800797A	1.020	0.930–1.118	0.67	0.76	2	0.68	0.00%	1.020	0.930–1.118	0.67
Trait	Cohorts included	OR for haplotype rs1800795–rs1800797	Fixed effects OR	95% CI	$P$ -value association	$Q$	df	$P$ -value heterogeneity	$I^2$	Random-effects OR	95%CI	$P$ -value association
Knee OA	Nottingham, Chingford, Hertfordshire, Estonia	GG	0.960	0.860–1.072	0.47	2.52	3	0.48	0.00%	0.960	0.860–1.072	0.47
Knee OA	Nottingham, Chingford, Hertfordshire, Estonia	CA	1.029	0.920–1.149	0.62	2.64	3	0.45	0.00%	1.029	0.920–1.149	0.61
Knee OA	Nottingham, Chingford, Hertfordshire, Estonia	CG	1.307	0.824–2.073	0.25	2.98	3	0.39	0.00%	1.357	0.858–2.146	0.19
Hip OA	Nottingham, Chingford	GG	0.926	0.807–1.062	0.27	0.68	1	0.41	0.00%	0.926	0.807–1.062	0.27
Hip OA	Nottingham, Chingford	CA	1.058	0.921–1.214	0.43	0.84	1	0.36	0.00%	1.058	0.921–1.214	0.43
Hip OA	Nottingham, Chingford	CG	1.476	0.776–2.805	0.23	0.04	1	0.84	0.00%	1.467	0.775–2.775	0.24



**Fig. 1.** Forest plot of study-specific estimates and fixed effects summary odds ratio (OR) and 95% confidence intervals for (A) the association between the A (minor) allele at rs1800797 and knee OA. (B) The association between the G (major) allele at rs1800795 and hip OA. \*For the GOAL study, data refer to the odds ratio for the G allele at rs1800797.

the very high LD between the two SNPs only three haplotypes are defined by these SNPs and the frequencies in each cohort are shown in Table I. No significant association with any of these haplotypes was seen with hip or knee OA and no significant heterogeneity between cohorts was observed (Table II). For both hip and knee OA the rare “CG” haplotypes appears to be increased in OA cases relative to controls.

## Discussion

Our data do not support a genetic association between two *IL-6* promoter polymorphisms and knee or hip OA either individually or as haplotypes. Although it is possible that these individual SNPs could be associated with hip or knee OA, the genetic effect would

need to be very small, i.e., an odds ratio under 1.10 which our study was powered to detect. For the rare haplotypes “CG” with a frequency <1.5% the sample size required to find as significant a genetic association of the magnitude observed with 80% statistical power is  $n = 5450$  cases and 5450 controls. Our data cannot exclude an association due to this rare haplotype. Alternatively a role for these variants might apply only to a subset of clinical populations not stratified in our study.

We note some potential study limitations. Our data was derived exclusively from subjects of European descent, therefore it may be that results do not apply to cases from other ethnicities. Another potential limitation is that of the heterogeneity between cohorts and the mixture between radiographic and clinical phenotypes. The data in this study derive from three case–control

studies and three population-based cohorts, yet in terms of the genetic effects we find no evidence of heterogeneity between cohorts for knee OA.

For hip OA, however, we observed significant heterogeneity between the Italian study and the other studies included. The subjects investigated by Pola *et al.*<sup>10</sup> were affected by severe hip OA, radiographically and clinically to require a THR and the UK study cohorts were for the most part also severe hip OA or THR so the heterogeneity appears unlikely to be introduced by the clinical definitions used.

For knee OA all radiographic data refer to tibiofemoral OA, we have not investigated the role of *IL-6* SNPs on patellofemoral OA and hence we cannot exclude an association with OA in a different joint compartment as has been reported for other genes<sup>17</sup>. Further, given the reported associations with hand OA there may be differences between generalized OA cases and non-generalized OA cases which we have not explored in this study.

Studies in animal models suggest that IL-6 may have some protective effects in the joint. IL-6 has been reported to induce the expression of the tissue inhibitor of metalloproteinases and stimulate proteoglycan synthesis when injected into the joints of mice with antigen-induced arthritis<sup>18</sup>. *IL-6* knock-out male mice develop more severe spontaneous OA than wildtype animals<sup>19</sup>. In addition, recent reports have shown that non-steroid anti-inflammatory drugs (NSAIDs) increases IL-6-induced production of MMPs from human chondrocytes, while completely inhibiting the IL-6/sIL-6R-induced production of prostaglandin E2<sup>20</sup>. This dual role of IL-6 in the OA pathogenesis could in part explain the lack of genetic association observed with risk of OA. As only two of the cohorts (Estonia and Chingford) have longitudinal data we did not explore the role on disease progression, but given that serum levels of IL-6 have been found in the Chingford Study to be a significant predictor of knee OA radiographic progression<sup>5</sup> a role for these variants in OA progression cannot be excluded.

Our findings appear to share some similarity with RA. Extensive evidence suggests that dysregulation of IL-6 contributes to both systemic and local RA symptoms<sup>21</sup> as well as OA symptoms<sup>3</sup>. Nevertheless, different genotypes in the *IL-6* promoter polymorphisms do not increase susceptibility to RA<sup>22,23</sup>, confirming that lack of genetic association does not imply a lack of involvement in disease. Further, the *IL-6* promoter polymorphisms appear to influence disease severity in RA<sup>6,22,23</sup>. It is thus possible that, analogously, an association may exist between of *IL-6* SNPs and measures of OA severity, or with specific disease phenotypes such as synovitis in OA.

#### Conflict of interest

All authors declare to have no conflict of interest.

#### Acknowledgements

This work was supported by EC framework 7 programme grant 200800, the Arthritis Research Campaign (UK), the Medical Research Council (UK), the Oxford NIHR Musculoskeletal Biomedical Research Unit, the Estonian Science Foundation grant No 5308, the Estonian Ministry of Social Affairs grants No 9.6-4/2035 and 12.1-5/597 and by AstraZeneca Macclesfield UK.

#### Supplementary material

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.joca.2009.12.012.

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