Meta-analysis of genome-wide association studies confirms a susceptibility locus for knee osteoarthritis on chromosome 7q22

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ABSTRACT

Objectives Osteoarthritis (OA) is the most prevalent form of arthritis and accounts for substantial morbidity and disability, particularly in older people. It is characterised by changes in joint structure, including degeneration of the articular cartilage, and its aetiology is multifactorial with a strong postulated genetic component.

Methods A meta-analysis was performed of four genome-wide association (GWA) studies of 2371 cases of knee OA and 35 909 controls in Caucasian populations. Replication of the top hits was attempted with data from 10 additional replication datasets.

Results With a cumulative sample size of 6709 cases and 44 439 controls, one genome-wide significant locus was identified on chromosome 7q22 for knee OA (rs4730250, $p=9.2\times10^{-9}$), thereby confirming its role as a susceptibility locus for OA.

Conclusion The associated signal is located within a large (500 kb) linkage disequilibrium block that contains six genes: *PRKAR2B* (protein kinase, cAMP-dependent, regulatory, type II, β), *HPB1* (HMG-box transcription factor 1), *COG5* (component of oligomeric golgi complex 5), *GPR22* (G protein-coupled receptor 22), *DUS4L* (dihydrouridine synthase 4-like) and *BCAP29* (B cell receptor-associated protein 29). Gene expression analyses of the (six) genes in primary cells derived from different joint tissues confirmed expression of all the genes in the joint environment.

INTRODUCTION

Osteoarthritis (OA) is the most prevalent form of chronic joint disease and accounts for substantial morbidity and disability, particularly among older people. It is characterised by loss of joint homeostasis. The articular cartilage cannot maintain its integrity and is progressively damaged, the subchondral bone envelope is thickened changing loads in the bone-cartilage biomechanical unit, the synovium shows signs of inflammation and bony spurs (osteophytes) appear at the edges of the bone. Its aetiology is multifactorial with a significant genetic component as shown by twin and family studies.[1 2]

Many genetic variants have been considered as potential risk factors for OA, but most of the reported associations are inconclusive or not replicated. A recent large-scale meta-analysis found evidence that the *GDF5* locus on chromosome 20 was associated with the increased risk of knee OA in Caucasians.[3–6] Other genome-wide data have reported an association with the *DVWA* gene in Asians but not Caucasians[7] and a *PTGS2* variant that replicated but did not reach genome-wide significance (GWS).[8] Recently, a genome-wide association (GWA) study identified a locus on chromosome 7q22 which has an association with combined knee OA and/or hand OA phenotype.[9]

In this study we have synthesised available data from four GWA studies under the auspices of the

Translational Research in Europe Applied Technologies for Osteoarthritis (TreatOA) consortium (www.treatoa.eu). A total of 2371 cases of knee OA were available for this first stage of the analysis. The most significant signals were further investigated in additional samples of European descent and single nucleotide polymorphisms (SNPs) that reached GWS were further evaluated in Asian samples.

METHODS

Study design

A detailed description of all samples used in this study is provided in the online supplement. A three-stage design was used for the identification of any potential associations between sequence variants and knee OA in populations of European ancestry. We first synthesised the available data from four GWA studies (deCODE, Rotterdam Study, Framingham, Twins UK) using inverse variance fixed effects models. The variants that reached the 2×10^{-5} level of significance were selected for further replication. These SNPs were followed up in eight additional European cohorts (arcOGEN, Greek, Spanish, Finnish, Nottingham, Chingford study, GARP, Estonian and Swedish). The SNPs that replicated in the follow-up samples were genotyped in two additional European samples (deCODE (Icelandic) and Swedish). One cohort provided computer-generated replication from an ongoing GWA study (arcOGEN, 12 SNPs were directly genotyped and 6 were imputed) while de novo replication was performed in the other cohorts. Furthermore, the top hits were followed up in Asian populations (Chinese and Japanese samples). The effect sizes from the meta-analysis of the GWA studies and the effect sizes from the replication effort were all combined to provide an overall estimate. We also synthesised the effect estimates of the European and Asian samples to provide a global summary effect estimate.

Phenotype definitions

Study subjects with a radiographic Kellgren and Lawrence (K/L) grade ≥2[10] or total knee replacement were included as cases in the analysis. When clinical criteria were considered (Greek, Spanish and GARP study groups), the American College of Rheumatology classification criteria were used.[11] Subjects who had no known affected joints among those assessed acted as controls. For example, in a cohort that assesses knee, hip and hand OA, controls were participants with no affected hip or hand joints for the knee OA analysis. Population-based controls were used for the arcOGEN study.

Genotyping and imputation

Samples from the GWA studies were genotyped using the Infinium HumanHap300 (Illumina) for deCODE and Twins UK samples, HumanHap550v3 Genotyping BeadCHip (Illumina) for the Rotterdam Study and the Affymetrix GeneChip Human Mapping 500K for the Framingham cohort. The number of SNPs genotyped ranged from 314 075 to 500 510. Imputations were performed to increase the coverage. All the top SNPs studied had acceptable imputation quality. The genotyped and imputed SNPs that successfully passed the quality control criteria (n=2 335 627) were considered for the analyses. Detailed information on genotyping platform, quality control and imputation methods for each cohort are shown in table S1 in the online supplement.

The replication samples for the Greek, Spanish, Finnish, Chingford and GARP studies were genotyped using the MassArray iPlex Gold from Sequenom. Replication genotyping was carried out by a genotyping contractor (Kbiosciences Ltd, Hertfordshire, UK) using a competitive allele-specific PCR SNP genotyping system for the Nottingham and the Estonian cohort. The additional 622 Icelandic cases and the samples from the Swedish cohort were genotyped by deCODE genetics using the Centaurus (Nanogen) platform.[12] Detailed information on genotyping is provided in the online supplement.

Statistical analysis

Association analysis

Each team performed an association test per gender for knee OA under a per-allele model. The λ inflation factor was calculated per gender-specific effect size using the genomic control method[13] and the standard errors were corrected by the square root of the λ inflation factor was calculated per gender-specific effect size using the genomic control method[13] and the standard errors were corrected by the square root of the λ inflation factor ($SE_{corrected} = SE_{observed} \times \sqrt{\lambda}$). Robust standard errors were estimated to adjust for the family relationships (Framingham and GARP studies)). Robust standard errors were estimated to adjust for the family relationships (Framingham and GARP studies)

Meta-analysis

The effect size for each SNP (OR per copy of minor allele as per HapMap) was calculated using inverse variance fixed effects models,[14] synthesising all the sex-specific effect sizes and the corrected standard errors. Analyses combining men and women were also performed. In family studies the results from men and women combined were used to account for relatedness between women and men within families. Meta-analyses of the GWA studies were performed using the METAL software (www.sph.umich.edu/csq/abecasis/metal). Between-study heterogeneity was tested using the Cochran Q statistic, which is considered significant at $p<0.1$. The extent of inconsistency across studies was quantified using the I^2 metric which ranges from 0 to 100%.[15] Heterogeneity is considered low, moderate, high and very high for 0–24%, 25–49%, 50–74% and >75%, respectively.[16] We also computed the 95% CI for the I^2.[17] The calculation was repeated with random effects models for all SNPs that were further evaluated in replication datasets. Meta-analyses of the 18 top hits were performed using Stata Version 10.1.

Assessment of credibility

In order to assess the credibility of the top hit, we calculated the Bayes factor under a spike and smear prior to using as an alternative an average genetic effect corresponding to an OR of 1.2 and a conservative agnostic prior of 0.0001%.[18]

Functional analysis

Two methodological approaches were used to investigate the functional role of genes identified by GWA studies: (1) by assessing their expression in primary human joint cells (synovial fibroblasts, chondrocytes and meniscal cells) and its change in response to the proinflammatory cytokines tumour necrosis factor α and interleukin 1β as well as comparing their gene expression profiles during chondrocyte dedifferentiation (3D pellet cultures vs monolayer culture); and (2) by assessing their expression dynamics by whole mount in situ hybridisation using zebrafish (*Danio rerio*) embryos aged 6 h (shield), 10 h (bud), 13 h (5–9 somites) and 1, 2, 3 and 4 days to explore their role during embryogenesis.

RESULTS

Meta-analysis of GWA studies and replication of top findings

The descriptive characteristics of the GWA studies used for the meta-analyses are from Iceland (deCODE), the Netherlands (Rotterdam study), USA (Framingham) and the UK (Twins UK). The characteristics of these studies are shown in table 1 and in the online supplement. The four GWA datasets included a total of 2371 cases and 35 909 controls. A quantile-quantile plot comparing the meta-analysis association results of the four studies with those expected by chance showed an excess of SNP associations indicating a likely true association signal (figure 1). Data analysis showed the strongest association on chromosome 7q22 with a p value of 5.06×10^{-8} for rs4730250 localised in dihydrouridine synthase 4-like gene (*DUS4L*) (figure 2). Other associated signals in the 7q22 gene cluster were in high linkage disequilibrium (LD) ($r^2>0.8$) with the top signal (figure 2).

We selected for follow-up in replication samples all SNPs with a p value $<2\times10^{-5}$ in the meta-analysis association results. A total of 18 SNPs from 10 chromosomal loci satisfied this criterion (see table S2 in online supplement). However, as some of those SNPs were fully equivalent in the HapMap-CEU dataset, a total of 11 non-identical SNPs were tested for replication in 3326 cases and 7691 controls from eight European studies (see table 1 and online supplement). Two SNPs (rs4730250 and rs10953541), both located at 7q22, replicated nominally ($p<0.05$) in the combined analysis of the follow-up samples with p values of 6.3×10^{-4} and 8.3×10^{-3}, respectively. The two SNPs rs4730250 and rs10953541 were then further genotyped in two additional replication sets.

Both SNPs reached GWS in a meta-analysis of all European sample sets (GWA datasets and replication cohorts, table 2). A total of 6709 cases of knee OA cases and 44 439 controls were analysed. SNP rs4730250 was genome-wide significant with a per-allele summary OR of 1.17 (95% CI 1.11 to 1.24) and a p value of 9.2×10^{-9}. The minor allele frequency was 0.17 in the combined dataset. Low heterogeneity was observed ($I^2=15\%$, 95% CI 0% to 48%) which was not statistically significant ($p=0.26$ for Cochran Q statistic, figure 3). No gender-specific effects were seen. The summary estimates did not differ significantly in men and women ($p=0.74$, test of homogeneity, figure 3). Analysis of both sexes together in all cohorts did not alter the results (OR 1.17, 95% CI 1.07 to 1.27, $p=4.1\times10^{-8}$). The summary effect sizes of all loci under study are shown in table 2

Table 1 Characteristics of the studies included in the analysis

Team	Knee OA cases/ controls	Platform used	Age mean (range)	BMI mean (range)	Women (%)	Knee OA definition	Control definition
GWA studies							
deCODE	1033/32482	Infinium HapMap 300	69 (19–99)	26 (14–60)	58	TKR	Healthcare records
Framingham	419/1674	Affymetrix GeneChip	64 (29–93)	26 (14–54)	56	Radiographic	Radiographic
Rotterdam	868/1464	Illumina HapMap550v3	67 (55–94)	26 (16–56)	59	Radiographic	Radiographic
TwinsUK	51/289	Infinium HapMap 300	54 (37–76)	25 (15–51)	100	Radiographic	Radiographic
Replication cohorts: stage 1							
arcOGEN	1643/4894	Illumina 610 Quad	NA	NA	71	Radiographic/clinical	General population
Chingford*	64/236	NP	63 (54–77)	26 (17–43)	100	Radiographic	Radiographic
Finnish	112/210	NP	67 (51–74)	29 (20–42)	75	TKR	Population-based
Greek	368/606	NP	61 (20–90)	26 (17–34)	72	Clinical	Clinical
GARP	161/758	NP	60 (30–79)	27 (19–47)	63	Radiographic/clinical	Radiographic/clinical
Spanish	262/294	NP	66 (32–94)	31 (18–53)		TKR/clinical	Clinical
Nottingham*	647/237	NP	66 (40–97)	27 (15–51)	53	TKR	Radiographic and clinical
Estonian	69/456	NP	47 (32–60)	28 (15–47)	69	Radiographic	Radiographic
Replication cohorts: stage 2							
deCODE	622/32482†	Illumina and Centaurus (Nanogen)	77 (40–99)	29 (19–49)	63	TKR	Population-based
Swedish	390/839	NP	62 (46–73)	29 (18–51)	63	TKR+concomitant clinical and radiographic diagnosis of OA	General population without TKR

*Numbers excluding the samples already included in the arcOGEN study.
†Same controls as for discovery cohort.
BMI, body mass index; GWA, genome-wide association; NP, not pertinent; OA, osteoarthritis; TKR, total knee replacement.

Table 2 Summary OR and 95% CI of SNPs in the analysis including all European descent data

SNP rs number	Minor (risk) allele	Chromosome	Position	Gene	MAF	OR (95% CI) fixed effects	p Value	I^2 (95% CI)	Cochran Q
rs4730250	G	7	106994931	*DUS4L*	0.17	1.17 (1.11 to 1.24)	9.17×10^{-9}	15 (0 to 49)	0.26
rs10953541	T	7	107031781	*BCAP29*	0.24	1.17 (1.10 to 1.23)	3.90×10^{-8}	19 (0 to 54)	0.23
rs3749132	A	2	68907001	*ARHGAP25*	0.07	1.17 (1.05 to 1.30)	4.08×10^{-3}	47 (0 to 74)	0.04
rs886827	C	7	42285581	*GLI3*	0.27	1.07 (0.99 to 1.16)	0.089	65 (43 to 80)	0.001
rs1886695	G	20	33643949	*CPNE1*	0.16	0.89 (0.84 to 0.95)	1.76×10^{-4}	42 (2 to 66)	0.02
rs10071956	T	5	173093290	Intergenic	0.38	1.12 (1.06 to 1.19)	5.05×10^{-5}	15 (0 to 53)	0.29
rs6816070	G	4	16089455	*LDB2*	0.42	0.91 (0.86 to 0.95)	1.34×10^{-4}	0 (0 to 54)	0.46
rs661924	T	10	21353562	*NEBL*	0.39	1.11 (1.05 to 1.17)	1.82×10^{-4}	30 (0 to 67)	0.18
rs436354	G	5	783271	*ZDHC11*	0.17	1.19 (1.01 to 1.30)	1.79×10^{-2}	41 (2 to 63)	0.06
rs1994104	T	12	83040643	Intergenic	0.13	0.88 (0.80 to 0.96)	3.13×10^{-3}	46 (2 to 70)	0.02
rs9857056	G	3	181698548	Intergenic	0.12	1.11 (1.02 to 1.20)	1.65×10^{-2}	72 (43 to 87)	0.001

Minor allele is the OR allele.
MAF, minor allele frequency; SNP, single nucleotide polymorphism.

and the results from the random effects analysis for the top hits are shown in table S3 in the online supplement.

The two significant SNPs at 7q22, rs4730250 and rs10953541, are highly correlated (D'=1, r^2=0.63 in HapMap-CEU) and are likely to represent the same underlying association signal as shown by conditional association analysis (see table S4 in online supplement). Age and body mass index are considered to be significant risk factors for the development of knee OA.[19–25] We performed an analysis where the top hit was adjusted for these risk factors in deCODE samples and the Rotterdam study. The association of the top hit remained largely unchanged in analyses adjusted for body mass index and age.

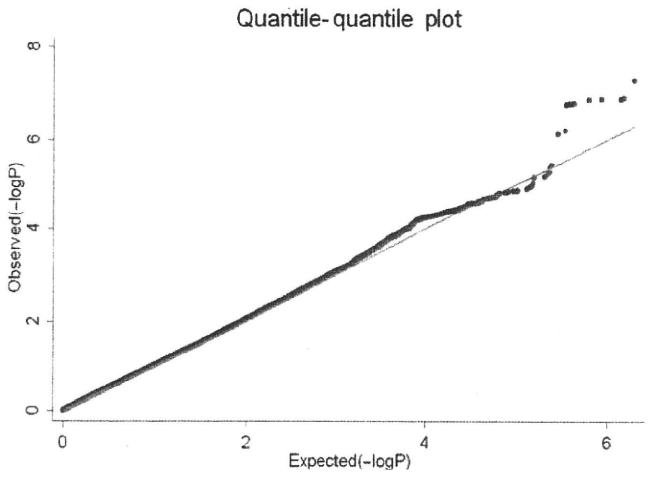

Figure 1 Quantile–quantile plot of the expected versus observed distribution of p values.

In order to assess the credibility of the associations of the two SNPs, we calculated the Bayes factor[18] under a spike and smear prior using an average genetic effect corresponding to an OR of 1.2 and a conservative agnostic prior (assuming no prior knowledge of the association) of 0.0001%. The posterior credibility of these associations was 98% and remained similarly high even with a small alternative effect size of 1.1.

We also tested if the observed signal at the 7q22 region was replicated in East Asian samples (Japanese and Chinese cohorts). The total numbers of cases of knee OA and controls assessed were 1183 and 1245, respectively. rs12535761 was used as a proxy for rs4730250. The two SNPs are in strong LD (r^2=1, D'=1 in HapMap Asian samples). The finding was not replicated in the Asian samples with a summary effect size of 1.03 (95% CI 0.85 to 1.25). A meta-analysis including both European and Asian samples with 7892 cases and 45 684 controls yielded a global summary effect of 1.15 (95% CI 1.10 to 1.22) with a p value of 5.7×10^{-8} for rs4730250 with low heterogeneity (I^2=19%).

Expression patterns of genes in 7q22 cluster

The associated signal at 7q22 is located within a large (500 kb) LD block which contains six genes: *PRKAR2B* (protein kinase, cAMP-dependent, regulatory, type II, β), *HPB1* (HMG-box transcription factor 1), *COG5* (component of oligomeric golgi complex 5), *GPR22* (G protein-coupled receptor 22), *DUS4L* (dihydrouridine synthase 4-like) and *BCAP29* (B cell receptor-associated protein 29).

We performed additional experiments to get more information about the genes in the cluster and their potential role in joint biology and pathology. Analysis of mRNA expression data in a chondrocyte pellet indicates that *BCAP29*, *COG5*, *DUS4L* and *HBP1* expression levels were higher than in monolayer cultures, suggesting that they are expressed in an environment that

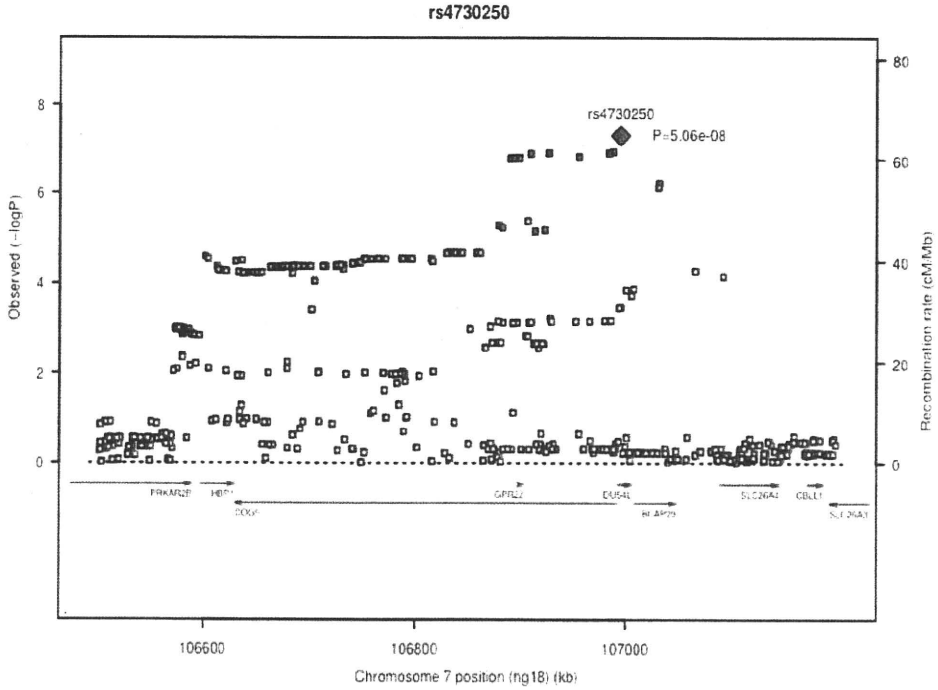

Figure 2 Regional association plot of rs4730250. Statistical significance of the associated SNPs are illustrated on $-\log_{10}$ scale. The p value of the rs4730250 and the other 10 selected SNPs are based on the meta-analysis of all datasets (both genome-wide association (GWA) studies and replication studies); p values for the other SNPs are based on the meta-analysis of the GWA studies. The sentinel single nucleotide polymorphism (SNP) is shown in blue. The correlation of the sentinel SNP is shown on a scale from minimal (gray) to maximal (red). SNPs in red have $r^2 \geq 0.8$ with the sentinel SNP and SNPs in orange have $r^2 \geq 0.5$. Chromosome positions are based on HapMap release 22 build 36.

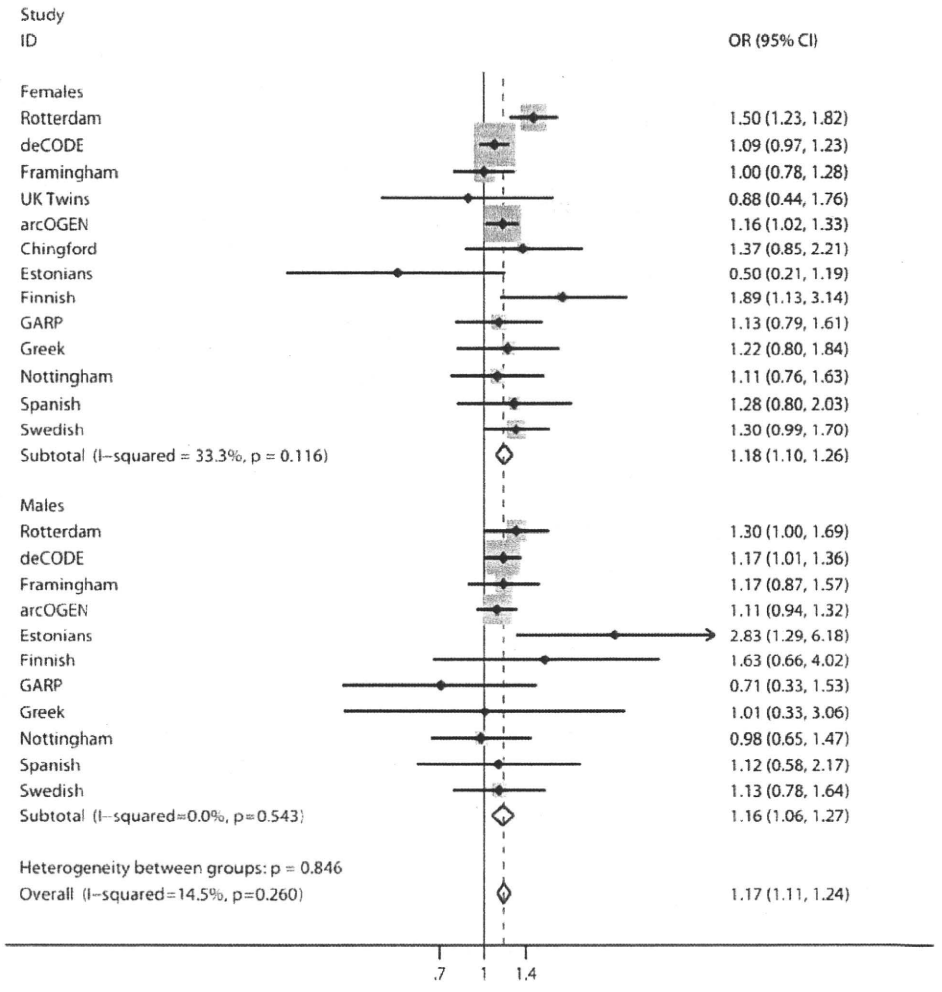

Figure 3 Forest plot of study-specific estimates (black boxes) and summary OR estimates and 95% CIs (diamonds) for the association between the rs4730250 single nucleotide polymorphism and osteoarthritis of the knee.

more accurately recapitulates articular cartilage (see figure S1 in online supplement). In contrast, no difference was seen for GPR22 and PRKAR2B mRNA expression. In a zebrafish model, the expression of all genes was detectable from the shield stage onwards (see detailed results and figures S2 and S3 in the online supplement).

DISCUSSION

This study provides further evidence for a knee OA signal localising to the 7q22 cluster region and associated with knee OA. The statistical credibility and confidence of this evidence is very high, based on the calculations of the Bayes factor. The same locus has been identified and proposed as an OA susceptibility locus from the Rotterdam study for the prevalence and progression of OA.[9] Our study and the earlier Rotterdam study do include overlapping populations. However, our study was specifically targeting the knee OA phenotype. An additional three European cohorts and two Asian populations were used for further replication. Our study uses the largest sample size in the genetics of knee OA research to date with almost 8000 cases of knee OA analysed.

The most significant hits identified by our study are located within a large (500 kb) LD block that contains six genes: *PRKAR2B, HPB1, COG5, GPR22, DUS4L* and *BCAP29*. The top hit rs4730250 is annotated in intron 3 of the *DUS4L* gene. Any of the genes at the 7q22 region may confer risk for knee OA as the LD pattern across the region is high.

The gene expression data support the epidemiological findings but do not exclude any of the six candidate genes. Specifically, the zebrafish experiments show that both *COG5* and *DUS4L* are expressed in developing cartilage, supporting the notion that either of these genes could have a biological function during chondrogenesis. The studies in the dedifferentiation model of human chondrocytes (3D vs 2D culture) show that *BCAP29, COG5, DUS4L* and *HBP1* all have different expression patterns in 3D culture (chondro-like cells) from 2D culture (dedifferentiated cells), suggesting that these four genes may play a role in cartilage metabolism.

A major issue in the field of OA is the definition of the disease phenotypes.[4 26] Different criteria may introduce bias and dilute the effect. The cases in our study were defined either clinically by the presence of a knee replacement or radiographically using the K/L system. The K/L system is, however, far from perfect and can be affected by differences in the position of the knee in which the x-rays were obtained, observer biases, interpretation of grading criteria and random error.[27 28] Similarly, there are no standard criteria for replacing knee joints. This may introduce heterogeneity and move the observed effects towards the unity and so underestimate the true strength of an association. In our study we synthesised data with a standardised definition of the

phenotype; however, small individual locus effects with ORs in the range of 1.1–1.2 as for other chronic diseases may well be plausible for knee OA, explaining the paucity of other significant hits despite the reasonable large-scale effort. These findings highlight that even larger collaborative studies and improved standardisation of the phenotypes are needed to better understand and identify further genetic variants of OA.

Moreover, even though we were able to accumulate a large sample size, the power of the study to detect very small effect sizes in the range of 1.05–1.15 is inadequate. For example, identification of a GWS signal with an effect size of 1.15 and minor allele frequency of 20% with 80% power would require almost 7000 additional cases of knee OA.

Our results confirm that the 7q22 chromosomal region confers risk for knee OA which, along with our functional work, implicates six possible genes. Further in-depth genetic analysis of the locus including deep sequencing of the region and functional work including in vitro assays and animal models will be required to deepen our understanding of the underlying molecular pathways associated with the disease.

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Ethics approval Each study participating in this meta-analysis has obtained approval from respective ethics committe.

Contributors EE, AMV, HJMK, US and IM contributed equally to data analysis and replication. AGU, DTF, JBvM, KS, JPAI and TDS contributed equally to the assembling of the GWA sets included in the manuscript.

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Meta-analysis of genome-wide association studies confirms a susceptibility locus for knee osteoarthritis on chromosome 7q22

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ORIGINAL ARTICLE

Radiographic progression of cervical lesions in patients with rheumatoid arthritis receiving infliximab treatment

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Abstract We evaluated radiographic change in the cervical lesions of 47 RA patients receiving continuous infliximab therapy for at least 1 year. Infliximab treatment had been initiated between November 2003 and December 2007. Patients who were progressive and non-progressive in terms of RA cervical lesions were compared. Matrix metalloproteinase 3 (MMP3) values improved significantly only in non-progressive patients within the 1-year treatment window. Cervical lesion progression was suppressed in 19 of the 23 patients (83%) showing a good response to infliximab treatment and occurred in 16 of the 24 patients (67%) showing a moderate response. This difference was shown to be significant by the Fisher's exact test ($p = 0.002$). In the well-responding patients ($n = 23$) and moderately responding patients ($n = 24$), the respective changes in the cervical lesion parameters within 1 year were: atlanto-dental interval, 0.17 ± 0.49 and 0.54 ± 0.58 mm ($p = 0.013$); spinal cord, -0.17 ± 0.49 and -0.54 ± 0.59 mm ($p = 0.025$); Ranawat value, -0.09 ± 0.29 and -0.42 ± 0.65 mm ($p = 0.032$). Based on these results, we conclude that infliximab treatment can be used to suppress the progression of rheumatoid arthritis (RA) cervical lesions. It is possible that response to infliximab and MMP3 values can be used to predict the progression of these cervical lesions.

Keywords Cervical lesions · Infliximab · Radiographic progression · Rheumatoid arthritis

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Introduction

Cervical lesions (i.e., cervical vertebral lesions) are known to occur at a high frequency as a complication of rheumatoid arthritis (RA). The progression of cervical lesions leads to severe pain in the post-cervical lesion and myelopathy, which in turn result in a deterioration in the daily living activities of RA patients with joint damage in the limbs. Reports of sudden death due to damage to the brain stem and/or upper cervical region have also been reported [1, 2], and a link between the progression of RA cervical lesions and an unfavorable prognosis has been reported in patients who develop myelopathy [3–5].

The recent introduction of biological agents has had a major impact on RA treatment [6]. Anti-cytokine treatments using inhibitors of tumor necrosis factor α (TNF-α) are more clinically effective than those with the disease-modifying antirheumatic drugs (DMARDs) that were in use previously. Specifically, biological agents have been shown to be efficacious in suppressing joint destruction [7]—in contrast to the scarcity of evidence verifying that therapy with any of the DMARDs can suppress joint destruction. As a result, the administration of DMARDs to many patients has been discontinued due to this lack of efficacy in preventing joint destruction [8]. In contrast, the efficacy of TNF-α inhibitors in suppressing joint destruction has been well documented [9–11]. In our clinical practice, we have found TNF-α inhibitors to be effective not only as evidenced by the

various disease indices, such as the disease activity score using 28-joint counts (DAS28) and matrix metalloproteinase 3 (MMP3), but also in terms of radiographic findings, such as those showing the alleviation of subcartilaginous bone destruction and the recovery of joint space.

To date, most clinical studies on the efficacy of biological agents in suppressing joint destruction have concentrated on the small joints in the hands and feet, and their efficacy against cervical lesions has not yet been reported. We have therefore carried out a prospective investigation on patients receiving infliximab with the aim of elucidating its efficacy for inhibiting the progression of RA cervical lesions, as measured by radiography.

Materials and methods

Patients

We prescribed infliximab treatment for 70 Japanese patients with active RA who were undergoing outpatient treatment at the Department of Orthopedic Surgery and Rheumatology, Nagoya University, and who met the diagnostic criteria stipulated by the American College of Rheumatology in 1987 [12]. Treatment with infliximab was initiated between November 2003 and December 2007; the final study cohort of 47 patients had received continuous infliximab treatment for at least 1 year. All patients were concomitantly administered methotrexate and one or more nonsteroidal antiinflammatory drugs (NSAIDs); they were also allowed to take oral doses of steroidal agents up to 10 mg equivalents of prednisolone/day.

Study protocol

The infliximab dose was 3 mg/kg. The first three doses were administered at weeks 0, 2, and 6, and the fourth and subsequent doses were administered at 8-week intervals up to week 54.

Clinical evaluation, which involved the collection of blood and examination of the patients, was carried out at each time point the drug was administered. The clinical endpoints were 28-joint swollen joint and 28-joint tender joint counts, the patient's global assessment of disease activity (using a 100-mm visual analog scale), C-reactive protein (CRP) level, erythrocyte sedimentation rate (ESR), DAS28 (4ESR), and MMP3 level.

X-radiography of the cervical spine was carried out at the initiation of infliximab treatment and at week 54, and the lesions in the cervical spine were evaluated. This evaluation involved assessment of (1) progression of cervical lesions between Week 0 and 54 and (2) risk factors affecting cervical lesion progression.

Radiographic evaluation

The radiographic evaluations of the cervical lesions were made at the initiation of infliximab treatment and at week 54. The atlanto-dental interval (ADI) [13], the space available for the spinal cord (SAC), and the Ranawat value [14] were measured by plain lateral radiographs with the patient in the flexion position, and the anterior atlanto-axial subluxation (AAS) and vertical subluxation of the axis (VS) were evaluated with the patient bending forward. Assessment of these values (in millimeters) was carried out using digital radiography by two rheumatologists (YK and TK) blinded to the results.

Statistical analysis

Statistical analysis was carried out using SPSS, ver. 11.0 for Windows (SPSS, Chicago, IL). The statistical tests used were:

(1) Wilcoxon signed-rank test. This test was used to evaluate correlations between (1) changes that occurred between treatment initiation (week 0) and week 54 in mean ADI, SAC, and Ranawat value (cervical lesion indices) and (2) changes in CRP, DAS28, ESR, and MMP3 between week 0 and 54 in the patient groups classified as progressive and non-progressive, respectively, in terms of cervical lesions.
(2) Mann–Whitney U test. This test was used to compare (1) the mean of each clinical parameter at the initiation of infliximab administration between the cervical-lesion progressive and non-progressive groups and (2) the changes in ADI, SAC, and Ranawat value from week 0 (treatment initiation) to week 54 between patients having different levels of response to infliximab at week 54.
(3) Fisher's exact test. This test was used to evaluate the relationship between cervical lesion progression and alleviation of pain based on the criteria stipulated by the European League Against Rheumatism (EULAR) [15].

In the above tests, p values <0.05 were considered to be significant.

Results

Patients' characteristics

The patients' demographic characteristics are shown in Table 1. The study cohort comprised 11 male and 36 female patients, with a mean age of 53.0 ± 13.4 years,

Table 1 Baseline patient characteristics

Characteristic	Total ($n = 47$ patients)
Demographics	
Age (years)	53.0 ± 13.4
Female, *n* (%)	36 (77)
Disease status	
Disease duration (years)	11.0 ± 10.1
RF positive, *n* (%)	38 (81)
Swollen joint count	7.8 ± 5.6
Tender joint count	8.3 ± 5.0
Patient's global assessment of disease activity (mm)	66.6 ± 20.0
CRP (mg/dL)	3.3 ± 2.6
ESR (mm/h)	49.5 ± 30.2
DAS28 (4ESR)	5.71 ± 1.14
MMP3 (ng/mL)	373.2 ± 331.2
Steinbrocker classification stage (I/II/III/IV)	2/9/22/14
Functional class (1/2/3/4)	7/23/17/0
Drug treatments	
Methotrexate (mg/week)	7.2 ± 2.1
Prednisone use, *n* (%)	30 (64)
Prednisone dose (mg/day)	6.7 ± 4.9

Values are given as the mean ± standard deviation SD unless stated otherwise

RF Rheumatoid factor, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *DAS28* 28-joint disease activity score, *MMP3* matrix metalloproteinase 3

mean disease duration of 11.0 ± 10.1 years, and mean methotrexate dose of 7.2 ± 2.1 mg/week.

Thirty patients (64%) were administered steroids, and the mean dose was 6.7 ± 4.9 mg equivalents prednisolone/day. Clinical findings related to RA were: swollen joint count, 8.3 ± 5.0; tender joint count, 7.8 ± 5.6; patient's global assessment of disease activity, 66.6 ± 20.0 mm; CRP, 3.3 ± 2.6 mg/dL; ESR, 49.5 ± 30.2 mm/h; DAS28 (4ESR), 5.71 ± 1.14; MMP3, 373.2 ± 331.2 ng/mL. Thirty-eight patients (81%) were rheumatoid factor (RF)-positive.

With respect to the Steinbrocker classification, two, nine, 22, and 14 patients were assessed to be at stages I, II, III, and IV, respectively, and seven, 12, and 27 patients were classified into functional classes [16, 17] 1, 2, and 3, respectively. Based on these results, a large proportion of patients (77%; 36) showed radiographic progression at Steinbrocker stage III or higher.

Changes in the ADI, SAC, or Ranawat value from week 0 to 54

The mean ADI changed from 4.1 ± 2.2 mm at week 0 to 4.5 ± 2.3 mm after 1 year of continuous infliximab treatment ($p < 0.001$). During the same period, the mean SAC changed from 17.1 ± 2.5 to 16.7 ± 2.6 mm ($p < 0.001$), and the mean Ranawat value changed from 13.6 ± 2.6 to 13.4 ± 2.7 mm ($p = 0.002$). All of these changes indicate minor but significant disease progression (Fig. 1).

Investigation of cervical-lesion progressive and non-progressive patients

When progression was defined as a ≥1-mm change in one of the radiographic cervical lesion parameters over a 1-year period, 16 (34%), 15 (32%), and 10 (21%) patients showed cervical lesion progression in terms of the ADI, SAC, and Ranewat value, respectively. Twenty patients (43%) showed progression in at least one of these three parameters.

In order to elucidate the factors that affect cervical lesion progression, we compared 27 non-progressive patients and these 20 progressive patients. This comparison revealed

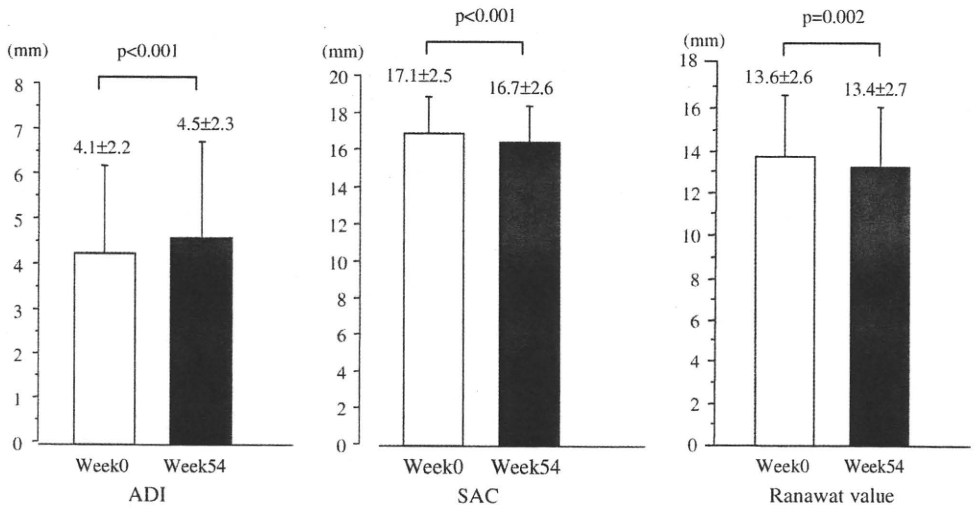

Fig. 1 Changes in the atlanto-dental interval (*ADI*), the space available for the spinal cord (*SAC*), and Ranawat value in rheumatoid arthritis (RA) patients receiving continuous infliximab therapy for a least 1 year. *Week 0* Initiation of treatment, *Week 54* end of study period of continuous infliximab therapy

(non-progressive vs. progressive groups): disease duration, 10.7 ± 10.9 versus 11.4 ± 9.0 years ($p = 0.836$); DAS28 (4ESR) at initiation of infliximab administration, 5.71 ± 1.28 versus 5.72 ± 0.95 ($p = 0.961$); CRP, 3.4 ± 2.7 versus 3.2 ± 2.4 mg/dL ($p = 0.787$); ESR, 48.1 ± 30.6 versus 51.5 ± 30.4 mm/h ($p = 0.705$); MMP3, 423.6 ± 389.4 versus 305.2 ± 222.7 ng/mL ($p = 0.451$). None of these values were significantly different between groups, and no significant differences between groups were observed for Steinbrocker stage or functional class.

The improvement in the clinical parameters of RA disease activity during the 1-year study period was also investigated in both groups. The changes in the CRP, DAS28, and ESR values (non-progressive vs. progressive groups) were: CRP, 3.4 ± 2.7–0.8 ± 1.2 ($p < 0.001$) versus 3.0 ± 2.3 to 1.2 ± 1.2 mg/dL ($p = 0.002$); DAS28, 5.70 ± 1.28 to 3.11 ± 1.27 ($p < 0.001$) versus 5.76 ± 0.94 to 4.18 ± 1.06 ($p < 0.001$); ESR, 48.1 ± 30.6 to 31.9 ± 21.9 ($p = 0.003$) versus 50.9 ± 31.8 to 34.1 ± 28.8 mm/h ($p = 0.008$). These parameters had improved significantly in both groups. In terms of MMP3, the non-progressive group showed marked and significant alleviation in 1 year, from 423.6 ± 389.4 to 165.5 ± 150.8 ng/mL ($p < 0.001$), whereas the change in the progressive group was from 305.2 ± 222.7 to 224.4 ± 109.5 ng/mL ($p = 0.136$), which was not significant (Fig. 2).

Relationship between cervical lesion progression and alleviation according to the EULAR response criteria at week 54

At week 54, the responses to infliximab based on the EULAR response criteria were assessed to be good in 23 patients (49%) and moderate in 24 patients (51%). Whereas cervical lesion progression was suppressed in 19 of 23 patients (83%) showing a good response, progression occurred in 16 of the 24 patients (67%) showing a moderate response. This difference was shown to be significant by the Fisher's exact test ($p = 0.002$).

Comparison between patients responding well and moderately, respectively in terms of changes in ADI, SAC, and the Ranawat value from week 0 to 54

A comparison of patients responding well to infliximab treatment ($n = 23$) with those having a moderate response ($n = 24$) revealed the following changes in cervical lesion parameters within the 1-year treatment window: ADI, 0.17 ± 0.49 (good response) versus 0.54 ± 0.58 mm (moderate response) ($p = 0.013$); SAC, −0.17 ± 0.49 versus −0.54 ± 0.59 mm ($p = 0.025$); Ranawat value, −0.09 ± 0.29 versus −0.42 ± 0.65 mm ($p = 0.032$). All three parameters showed significant progression in the patients with a moderate response to treatment (Fig. 3).

Discussion

Upper cervical lesions, such as the AAS and VS, and middle and lower cervical lesions, such as subaxial subluxation, have long been recognized as complications that occur at a high frequency in RA patients. In general, upper cervical lesions in patients with RA begin with AAS and then progress to VS. Various publications have reported the frequency of AAS and VS in RA patients to be approximately 25% [18–20] and 5–22% [20–22], respectively. VS

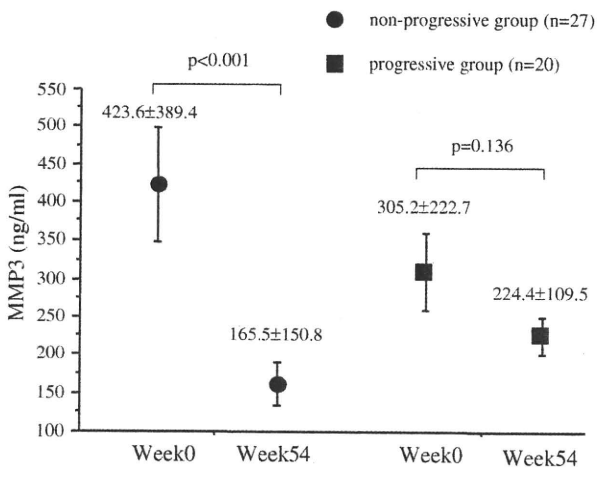

Fig. 2 Comparison of the changes in the matrix metalloproteinase 3 (*MMP3*) value in RA patients of the non-progressive and progressive group, respectively, receiving continuous infliximab therapy for a least 1 year (week 0 to week 54). Data are shown as the mean (*filled squares* or *circles*) ± standard deviation (*bars*)

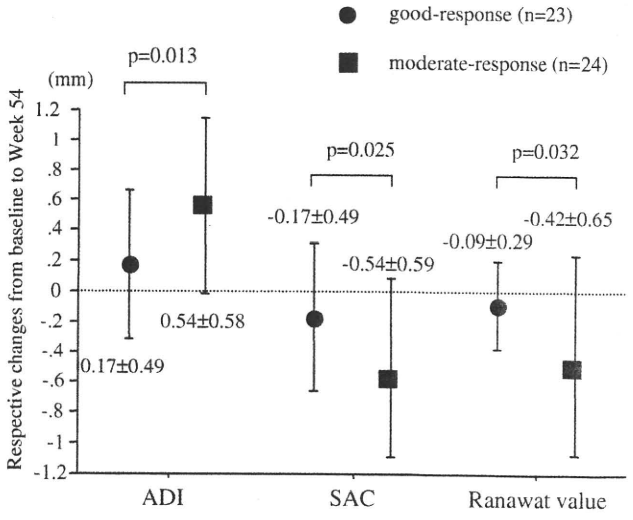

Fig. 3 Respective changes in the ADI, SAC, and Ranawat value in RA patients showing a good response to infliximab therapy between treatment initiation (week 0) and week 54 of treatment and those with a moderate response to treatment

is a particularly serious lesion, and it has been reported to be associated with cases of sudden death [22–26]. Martel and Page [24] reported that the risk of sudden death due to VS is especially high in patients with refractory cervical pain and progressive VS.

Fujiwara et al. reported that, in Japanese RA patients, the frequency of cervical lesions increases with the duration of RA. In their patient cohort, the mean RA duration was 12.3 years, by which time 43% of patients had suffered one or more cervical lesions; this proportion increased to 57% after a duration of 16.5 years and to 60% after 17.5 years. The mean times between RA onset and the appearance of the first lesion have been reported to be 12.7 and 16.5 years for AAS and VS, respectively [27, 28].

The introduction of biological agents has resulted in major changes in RA treatment. Due to the high efficacy of these new agents, clinicians now strongly advise the administration of biological agents from an early disease stage as a means to suppress disease activity and joint damage.

Infliximab is an anti-TNF-α antibody and thus a good representative of a biological agent. Its high clinical efficacy and high efficacy in suppressing joint destruction has been reported in numerous publications [9, 10, 29–32]. In a representative study, the anti-tumor necrosis factor trial in RA with concomitant therapy (ATTRACT), the van der Heijde modification of the Sharp scoring system (vdH Sharp score) [33–37] increased gradually over 1 and 2 years despite the administration of methotrexate at a mean dose of 15 mg/week, whereas joint destruction progression was markedly suppressed in patients receiving infliximab [29]. Further, the vdH Sharp score in patients administered 10 mg/kg infliximab concomitantly with methotrexate showed a negative value [29]. In two other studies, the active controlled study of patients receiving infliximab for treatment (ASPIRE) [10] and the Behandel Strategieen (BeSt) [30], both of which were carried out with patients in the early stages of RA, less than 3 years after onset, infliximab showed excellent efficacy in suppressing joint destruction. However, all infliximab studies published to date have examined its efficacy in suppressing the destruction of the small joints in the hands and feet, and no studies have yet been carried out on its efficacy in suppressing cervical lesion progression.

Robinson et al. [38] reported a case of the clinical effect of infliximab on RA cervical lesions in which symptomatic relief was achieved by 6 weeks in a patient who complained of severe cervical pain and occipital headaches without having AAS. A comparison of the magnetic resonance imaging (MRI) scans of the cervical vertebrae at treatment initiation with those taken 4 months later revealed a reduction in periodontoid rheumatoid pannus formation. Based on these results, these authors propose the necessity of early anti-TNF therapy to prevent the progression of RA cervical lesions. However, this report is a case report, and not a prospective study with many patients having RA cervical lesions.

All of the patients in our study cohort showed significant, albeit minor, progression in the ADI, SAC, and Ranawat value during 1 year of continuous treatment with infliximab. Two probable (partial) explanations for this progression is that our study included numerous patients who had long disease durations and showed radiographic progression and the infliximab and methotrexate doses were low. We compared patients who were progressive and non-progressive in terms of RA cervical lesions and found that CRP levels, DAS28 score, and ESR values had improved significantly in both groups 1 year after the initiation of infliximab treatment, whereas the MMP3 values had improved significantly only in the non-progressive patients. These findings suggest that there is a possibility that MMP3 improvement is an index that predicts the progression of RA cervical lesions. There was no significant difference between the MMP3 values of the two groups at baseline, but these values are still considerably different. It is possible that the significant difference in the non-progressive group that arose between week 0 and week 54 due to high MMP3 values and that a significant difference would be observed in the progressive group when a larger number of patients are available. Therefore, further investigation is needed.

We compared the patients who showed good and moderate responses, respectively, in the EULAR response criteria at week 54 and found a significantly higher suppression of cervical lesion progression in the good responders. In addition, 1 year after treatment initiation, significantly more suppression of the indices of cervical lesion progression (ADI, SAC, and Ranawat value) was observed in the good responders compared to the moderate responders.

On the basis of our results, we suggest that improvement in the MMP3 value is an appropriate index for predicting the progression of RA cervical lesions and hand and foot joint lesions. The high efficacy of infliximab in suppressing joint destruction when it is used to treat early RA suggests that the administration of infliximab at an early stage of the disease will be successful in suppressing the progression of both RA cervical lesions and foot and joint lesions.

Investigations on joint damage in RA generally focus on the hand and foot joints. However, our results suggest that rheumatologists should also pay attention to preventing the progression of RA cervical lesions since such a progression negatively impacts on both the prognosis and daily living activities of RA patients.

This study is the first to investigate the efficacy of infliximab treatment in suppressing the progression of RA cervical lesions in a relatively large patient cohort.

Conclusions

Infliximab treatment can be used to suppress the progression of both RA cervical lesions and lesions of the hand and foot joints. It is possible that response to infliximab and MMP3 improvement can be used to predict the progression of RA cervical lesions.

Conflict of interest statement N. Ishiguro has received speaking fees (less than $10,000) from Mitsubishi Tanabe Pharma Corporation Osaka, Japan. The other authors have declared no conflict of interest.

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ORIGINAL ARTICLE

Influences of anti-tumour necrosis factor agents on postoperative recovery in patients with rheumatoid arthritis

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Abstract The aim of this study is to investigate the influences of the anti-tumour necrosis factor (TNF) agents infliximab and etanercept on the postoperative recovery of patients with rheumatoid arthritis (RA). We also investigated the effects of biologics on wound healing. Patients with RA were split into a TNF group (n=39) that underwent 39 operations and were treated with anti-TNF agents, and a non-TNF group (n=74) that underwent 74 operations and were treated only with conventional disease-modifying antirheumatic drugs. Operations included ankle arthrodesis and total arthroplasty of the hip, knee, elbow, shoulder and ankle. Adverse events (AEs) of surgical wounds, time for complete wound healing, febrile period after operation and recovery parameters after operation (%recovery of haemoglobin (Hb), total protein and albumin at 4 weeks after operation compared with pre-operation levels) were investigated. AEs of surgical wounds occurred in two operations (5.1%) in the TNF group and in five operations (6.8%) in the non-TNF group, but this difference was not statistically significant. There were also no significant differences in the time for complete wound healing and in the length of the febrile period between the two groups. Percentage recovery of Hb was significantly better in the TNF group than in the non-TNF group (96.3% vs. 90.1%, respectively; $p<0.05$). These results suggest that the use of anti-TNF agents does not cause specific AEs on surgical wounds after elective orthopaedic operations in RA patients and might improve the percentage recovery of Hb due to its prompt anti-TNF effects.

Keywords Anti-tumour necrosis factor agent · Operation · Postoperative recovery · Rheumatoid arthritis · Surgical wound

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Introduction

Anti-tumour necrosis factor (TNF) therapy provides great benefit to patients with rheumatoid arthritis (RA) by inhibiting joint destruction and suppressing inflammation [1, 2]. TNF-α is a proinflammatory cytokine that has numerous effects on both the physiology and the pathology of humans. It plays a key role in the joint pathology of RA patients [3], as well as in the healing of wounds [4–6], and in host defences against bacterial [7–9], viral [10] and mycobacterium infections [11]. According to these reports, anti-TNF therapy might cause a delay in the healing of surgical wounds, increasing the number of postoperative infections. As TNF-α affects general condition of the host, such as general fatigue or febrile condition, Anti-TNF usage may affects postoperative general condition, especially febrile condition, in the patient. However, the clinical influences of anti-TNF agents on wound healing and RA patient postoperative recovery are not well known.

Anti-TNF therapy could be expected to decrease the number of orthopaedic operations, such as total arthroplasty, required for RA patients. However, as many RA patients

have long-term disease and advanced joint destruction, the percentage of patients taking anti-TNF agents and undergoing surgery seems to be increasing. den Broeder et al. reported a threefold increase from 1997 to 22% in 2004 [12]. In our institute (Nagoya University Hospital, Japan), we recorded an increase from 11.5% in 2005 to 52.9% in 2008 in patients treated with anti-TNF agents undergoing orthopaedic operations on large joints.

It is important to understand both the advantages and the disadvantages of anti-TNF therapy for surgical procedures. In this study, we therefore investigated the influences of anti-TNF agents on the postoperative recovery in patients with RA. We also investigated the effects of biologics on wound healing.

Materials and methods

One hundred and thirteen patients with RA who underwent surgical procedures between April 2004 and July 2007 in either Nagoya University Hospital (Nagoya, Japan) or Nagoya Medical Centre (Nagoya, Japan) were included in this study. Only the first surgery for each patient was included in this study. Patients were divided into a TNF group (n=39) that underwent 39 operations and were treated with anti-TNF agents, and a non-TNF group (n=74) that underwent 74 operations and were treated with conventional disease-modifying antirheumatic drugs (DMARDs). The TNF group comprised patients from both institutes, whereas the non-TNF group comprised patients only from Nagoya University Hospital. The anti-TNF agents used were either infliximab (INF) or etanercept (ETA) as adalimumab was not available in Japan during the study period.

The operations included in this study were primary total hip arthroplasty (THA), total knee arthroplasty (TKA), total elbow arthroplasty, total ankle arthroplasty, total shoulder arthroplasty and ankle arthrodesis. Revision surgery and other minor operations, such as foot operations and wrist operations, were excluded.

We investigated the following: (1) the occurrence rate of adverse events (AEs) of surgical wounds, such as wound dehiscence, continuation of discharge and infection. Wound dehiscence is defined wound which is not completely healed in 14 days after operation or wound which needs secondary suture. Infection is defined that culture examination is positive; (2) the period of time taken for complete wound healing, from the date of operation to the removal of surgical staples; (3) postoperative febrile periods (body temperature >37.5°C); and (4) comparison of preoperative and postoperative parameters including haemoglobin (Hb), serum total protein (TP) and serum albumin (Alb). The %recovery of Hb (%Hb), %recovery of TP (%TP) and %recovery of Alb (%Alb) were defined as follows: (4-week postoperative level/preoperative level) × 100%. Additionally, we analysed the results in the THA subgroup or TKA subgroup.

Data were collected from medical records and results were compared between the TNF and non-TNF groups. Anti-TNF agent administration was stopped prior to the operations and restarted after surgical wounds were completely healed. Our protocol of preoperative cessation of anti-TNF agents is 3-4 weeks before operation in case of INF and 1-2 weeks before operation in case of ETA. Mean periods from last administration of anti-TNF agents to operation were 29.8 days for INF and 9.6 days for ETA.

Data were expressed as the mean value ± standard deviation. The Mann-Whitney U test was used to evaluate the significance of differences in continuous variables because not all data were normally distributed. Fisher's exact test was used to evaluate the significance of differences in proportions. $P<0.05$ was considered statistically significant.

Results

The baseline characteristics of patients at the time of the operations are shown in Table 1. The total numbers of operations in the TNF group and the non-TNF group were 39 and 74, on 39 and 74 patients, respectively. Only the first operation for each patient was included. No patients belonged to both groups. The difference in mean age at the time of operation was statistically significant: 58.9± 9.0 years in the TNF group and 62.6±9.1 years in the non-TNF group ($P<0.05$). More patients of stage IV were included in TNF group than non-TNF group ($P<0.05$). Methotrexate was more frequently used in TNF group than non-TNF group ($P<0.05$). The operation performed is shown in Table 2.

AEs of surgical wounds occurred after two operations in the TNF group (5.1%) and after five operations in the non-TNF group (6.8%), which was not a statistically significant difference by Fisher's exact test (P=1.0000). Odds ratio was 0.7459 (95% confidence interval; 0.1380-4.0336). Although most AEs of surgical wounds were wound dehiscence and continuation of discharge that were healed by conservative treatment, postoperative infection occurred after one TKA operation in the TNF group. The patient was a 68-year-old female who received preoperative treatment of INF (200 mg/infusion), methotrexate (6 mg/week) and oral predonisolone (5 mg/day). The last administration of INF was 21 days before the operation. The patient had a high fever immediately after the operation date, and a discharge continued from the wound although no bacteria were detected by culture examinations. Finally, surgical debridement without removal of the implant was performed

Table 1 Baseline characteristics of patients

Characteristics		TNF group (n=39)	Non-TNF group (n=74)	P value[a]
Gender	Male	7	9	
	Female	32	65	
	%female	82.1%	87.8%	0.4087
Age at operation, year (range)		58.9±9.0 (31-73)	62.6±9.1 (30-77)	0.0308
RA duration, year (range)		13.5±7.8 (4-32)	16.5±11.7 (1-51)	0.5720
Stage (Steinbrocker)				
%stage III		18.8%	52.7%	0.0249
%stage IV		81.2%	47.3%	
Class (Steinbrocker)				
%class 2		18.8%	20.3%	1.0000
%class 3		81.2%	68.9%	
%class 4		0	10.8%	
%RF positivity		69.2%	67.1%	1.0000
Mean CRP (mg/dl)		1.56±1.49	1.99±2.41	0.6424
Mean ESR (mm/hour)		41.1±34.7	41.9±24.2	0.5982
%MTX use		92.3%	50.0%	0.0001
Mean PSL usage (mg/day)		4.3±3.6	2.8±3.0	0.0871
Anti-TNF usage				
Infliximab		24		
Etanercept		15		

[a] Mann-Whitney U test was used in case of continuous variables. Fisher's exact test was used in case of dichotomous variables

TNF tumour necrosis factor, *RA* rheumatoid arthritis, *RF* rheumatoid factor, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate, *MTX* methotrexate, *PSL* predonisolone

and *Capnocytophaga* species was detected from a synovia specimen. Ampicillin/sulbactam at 6 g/day was administrated for 4 weeks and the infection settled down well.

The average time from the date of operation to the removal of surgical staples was 10.9±1.2 days in the TNF group and 10.8±1.3 days in the non-TNF group, which was not a statistically significant difference. The postoperative febrile periods (body temperature ≥37.5°C) were 2.6± 2.2 days in the TNF group and 2.9±1.7 days in the non-TNF group, which was also not a statistically significant difference.

The results for postoperative anaemia and recovery are shown in Fig. 1. The preoperative levels of serum Hb, serum TP and serum Alb in the TNF group were 11.2± 2.0 g/dl, 7.3±0.8 g/dl and 3.8±0.4 g/dl, respectively. The preoperative levels of serum Hb, serum TP and serum Alb in the non-TNF group were 11.6±1.8 g/dl, 7.1±0.6 g/dl and 3.8±0.5 g/dl, respectively. There were no statistically significant differences in the three parameters between the TNF and non-TNF groups. There were also no statistically significant differences in %TP and %Alb between the TNF and non-TNF groups, with %TP values of 100.8±9.5% and 100.8±9.2% and %Alb values of 98.9±13.5% and 98.0± 11.3%, respectively. However, the %Hb was significantly

Table 2 Performed operations

operation	TNF group	Non-TNF group
Total number	39	74
TKA	14	51
THA	13	17
TEA	8	4
AD	3	1
TSA	1	0
TAA	0	1

TNF tumour necrosis factor, *TKA* total knee arthroplasty, *THA* total hip arthroplasty, *TEA* total elbow arthroplasty, *AD* ankle arthrodesis, *TSA* total shoulder arthroplasty, *TAA* total ankle arthroplasty

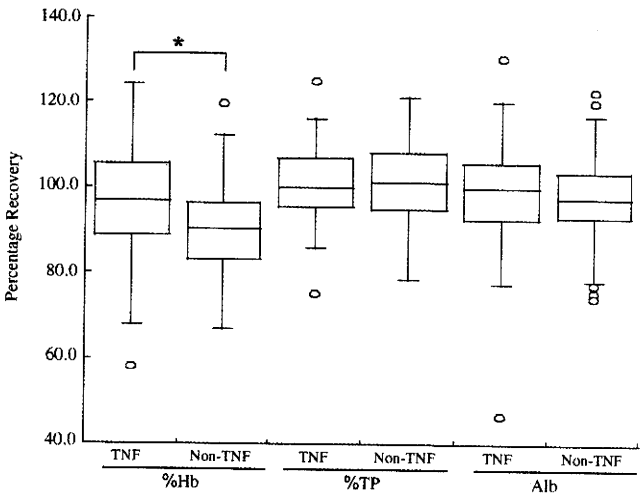

Fig. 1 %recovery of serum Hb, serum TP and serum Alb. Percentage recovery was defined as follows: (4-week postoperative value/ preoperative value) × 100 (%). *Asterisk* statistically significant differences in %Hb (P<0.05, Mann-Whitney U test)

higher in the TNF group (96.3±14.3%) than in the non-TNF group (90.1±11.5%; P=0.0156).

We analysed the influence of anti-TNF agents on the healing of surgical wounds and postoperative recovery in the THA subgroup (30 operations in total, 13 in the TNF group and 17 in the non-TNF group) and the TKA subgroup (65 operations in total, 14 in the TNF group and 51 in the non-TNF group; Table 2). The AE occurrence rates of surgical wounds were 0.0% in the TNF group and 0.0% in the non-TNF group in the THA subgroup. The AE occurrence rates of surgical wounds were 7.1% (one operation) in the TNF group and 7.8% (four operations) in the non-TNF group in the TKA subgroup. There was no statistically significant difference between the two groups. Odds ratio was 0.9038 (95% confidence interval; 0.0928-8.7992).

The febrile period after the operation was 3.5±2.0 days vs. 3.1±1.9 days in the THA subgroup and 2.6±2.5 days vs. 2.9±1.7 days in the TKA subgroup, comparing the TNF and non-TNF groups, respectively. There were no statistically significant differences between the two groups in either subgroup. The %Hb, %TP and %Alb were 101.0± 14.4% vs. 83.8±10.0%, 103.5±10.5% vs. 100.3±9.3% and 100.3±10.1% vs. 94.7±11.7% in the THA subgroup, comparing the TNF and non-TNF groups, respectively (Fig. 2a). There was a statistically significant difference between the %Hb of the TNF and non-TNF groups (P= 0.0016), but not between the other two parameters. The %Hb, %TP and %Alb were 92.6±16.9% vs. 92.0±11.5%, 101.1±10.7% vs. 101.0±9.6% and 98.8±19.4% vs. 99.3± 11.7% in the TKA subgroup, comparing the TNF and non-TNF groups, respectively (Fig. 2b). There were no statistically significant differences in any parameter between the TNF and non-TNF groups.

Discussion

In this study, we showed that anti-TNF agents could be beneficial in post-operative recovery during orthopaedic procedures such as total joint arthroplasty. To our knowledge, this is the first report to study the influences of anti-TNF agents on the postoperative recovery of patients with RA.

Anaemia of chronic disease (ACD) is one of the most common comorbidities of RA [13]. Inflammatory cytokines such as TNF-α and interleukin 6 play an important role in the pathogenesis of ACD [14], with increased levels of TNF-α inducing apoptosis in erythroblasts, thus decreasing their numbers. Administration of anti-TNF antibodies has been found to decrease apoptosis of erythroid cells [15]. The present study suggests that anti-TNF agents could prevent these processes occurring in active RA patients undergoing joint surgery, and might lead to improved recovery of Hb levels. Indeed, anti-TNF agents could have more beneficial effects on post-operative recovery than conventional DMARDs.

Influences of TNF-α on wound healing have been documented in several in vitro studies. Mooney et al. reported that the application of recombinant TNF-α improved wound

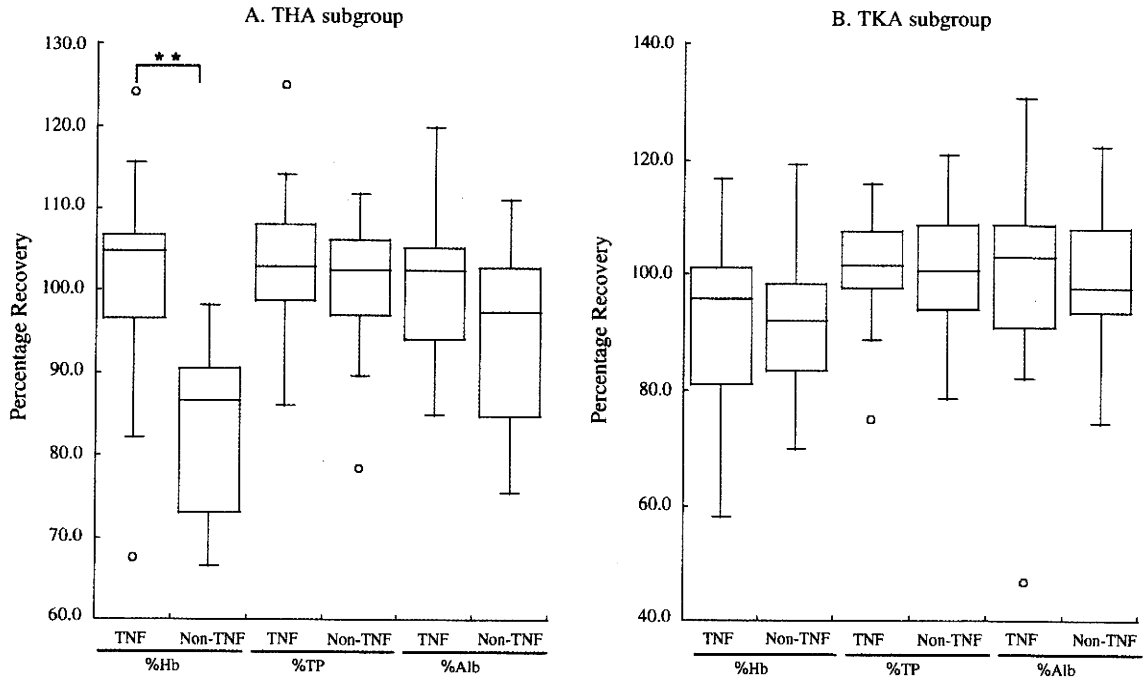

Fig. 2 Comparison of %recovery in THA (A) and TKA (B) subgroups. *Double asterisk* statistically significant differences in %Hb in THA subgroup (P<0.01, Mann-Whitney U test)

healing of normal and adriamycin-impaired mice [4]. By contrast, Salomon et al. found that local TNF application impaired both wound healing and collagen gene expression in rats [5], while Mori et al. showed that TNF receptor p55-mediated signals negatively affected wound healing by reducing angiogenesis and collagen accumulation in TNF receptor p55-deficient mice [6]. Although the influences of TNF-α on wound healing are therefore controversial, these results suggest that modulation of TNF-α affects the collagen synthesis necessary for wound repair.

Animal model studies have shown that TNF-α is involved in the host defence mechanism against bacterial infection [7–9]. This suggests that excessive decreases in the effects of TNF-α might increase the number of bacterial infections, and that it might therefore be safe to stop the administration of anti-TNF agents for an appropriate period before an operation. The American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic DMARDs in RA suggest that biologic agents should not be used during the perioperative period for at least 1 week before and after surgery [16]. The report also recommends that a period of discontinuation should be tempered by the half-lives of the agent used. As INF has a longer half-life than ETA (7.7–9.5 days vs. 102±30 h), it is reasonable that a longer time period is given from the last administration of INF before surgery than that with ETA. The Japanese guidelines advise that surgery should be delayed until a sufficient time has elapsed from the last administration of anti-TNF agents [17]. Although no definite time is stated in these recommendations, the biological half-lives of the drugs should be taken into consideration. In the present study, the mean time periods from the last administration of anti-TNF agents before surgery were 29.8 days for INF and 9.6 days for ETA. Although Talwalkar et al. reported that the continuation of anti-TNF agents in the perioperative period did not increase either the rate of infection or the complication rate [18], we decided to use a safe and appropriate period for stopping agent administration.

In clinical studies, the influences of anti-TNF agents on the healing of surgical wounds or the manifestation of infection are controversial. Bibbo et al. reported that the use of anti-TNF agents might be safely undertaken in the perioperative period without increasing healing risks or infectious complications in RA patients undergoing elective foot and ankle surgery [19]. den Broeder et al. investigated postoperative surgical site infections in 1,219 operations carried out in 768 patients, and found that the crude infection risks were 4.0%, 5.8% and 8.7% in patients who did not use anti-TNF agents, patients who did but then stopped and patients who continued anti-TNF preoperatively, respectively [12]. Although an increase in infection rate was associated with anti-TNF agent use, the authors concluded that anti-TNF agents were not an important risk factor for surgical site infection. Wendling et al. reported that an interrupted use of anti-TNF treatment in patients with RA undergoing orthopaedic and non-orthopaedic surgery did not increase frequency of adverse events [20]. These studies, along with our own, suggest that the use of anti-TNF agents is not disadvantageous in terms of AEs such as infection. By contrast, Giles et al. reported that anti-TNF agents increased the rate of infection in elective orthopaedic operations [21].

There are some limitations of the present study. At first, the method used is a retrospective cohort study which can lead to information bias, as not all consequences have been measured in a prospective and standardised way. Although future studies should be of a prospective design, we think that a retrospective study can show the results in the real-world clinical setting and show useful information. Second limitation is that sample size is small. A prospective study based on a larger sample size to ascertain whether the use of anti-TNF agents is wholly advantageous. Third limitation is confounding by indication. Patients are RA patients that either use anti-TNF or are non anti-TNF users. This can lead to confounding by indication, as patients that use anti-TNF can have a different risk of infection and wound healing problems by virtue of the severity of the RA itself, not due to the difference of the treatment. A study which includes random cessation of anti-TNF treatment is necessary in the future. Forth limitation is the problems of cessation of anti-TNF agents before operation. Because cessation of anti-TNF agents, 3-4 weeks in case of INF and 1-2 weeks in case of ETA, cause the reduction of effects of anti-TNF agents at the operation date, the results of this study may not show the accurate influence of anti-TNF agents on surgical wounds and postoperative recovery. This is also a limitation from a retrospective study in the real-world clinical setting.

In conclusion, the present findings suggest that the controlled use of anti-TNF agents causes no specific AEs on surgical wounds after elective orthopaedic operations in RA patients and might improve the recovery from post-operative anaemic conditions due to anti-TNF effects on bone marrow.

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Disclosures None

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