

Supplementary sections.

Study subjects:

1.- The TwinsUK study cohort is a population-based sample of twins from the UK studying the hereditary basis of a wide variety of age-related traits and diseases (<http://www.twinsUK.ac.uk>). These twins, without selecting for particular diseases or traits, were recruited from the general population through a series of national media campaigns in the UK. Overall the TwinsUK cohort comprises both men and women, but only women participated in an X-ray study to investigate the heritability of radiographic OA. From those participating in such study, 520 unrelated women of European descent (only one twin from each pair) with both a knee and a hip Kellgren and Lawrence grade [1](K/L) <2 at both the femoro-acetabular and tibiofemoral compartments were selected as controls for the present study.

2- *Nottingham case-control.* Hip and knee OA cases were recruited from hospital orthopaedic surgery lists (current and for the previous 5 years). All research participants gave written informed consent to take part. Approval for recruitment of knee and hip OA cases was obtained from the research ethics committees of Nottingham City Hospital and North Nottinghamshire, UK. All cases had been referred to hospital with symptomatic, clinically severe hip or knee OA and the majority had undergone unilateral or bilateral total hip replacement (THR) or total knee replacement (TKR) within the previous 5 years. Subjects were excluded from the study if they had other major arthropathy (e.g. rheumatoid arthritis, ankylosing spondylitis); Paget's disease of bone affecting the pelvis or femur; overt childhood hip disease (e.g. Legg-Calve-Perthe's disease, slipped femoral epiphysis, severe acetabular dysplasia), THR due to trauma or avascular necrosis of the femoral head, or terminal illness. We only included patients if they had a diagnostic code of primary osteoarthritis, and we excluded patients aged over 90 or those who had joint replacement surgery when they were under 40 years of age. Cases were further characterized by enquiry, examination and investigation. Height and weight were measured to calculate BMI. Pre-operative knee or pelvis radiographs of knee or hip OA cases were examined to confirm the diagnosis and to grade for changes of OA. [2-3] All pelvis and knee radiographs were scored for individual radiographic features of OA by a single observer and graded 0-3 according to a standard atlas using the K/L grade for the tibiofemoral compartments of each knee and the femoro-acetabular compartment of each hip [1]. Self-reported ethnicity was assessed by

a nurse administered questionnaire and only individuals of European descent were included in the genetic study.

Controls were unaffected sibs of joint replacement probands, free from radiographic OA and over the age of 45. In addition subjects aged 45-85 who had previously undergone intravenous urography (IVU) in the same hospital were assumed to represent the average genetic susceptibility of the general population and recruited as unrelated controls. These subjects and the unaffected sibs of cases were then assessed radiographically for OA and those with no OA and of European origin were included as controls in the present study. Hip OA was defined as K/L ≥ 2 for one or both hips, and knee OA was defined as K/L ≥ 2 for the tibiofemoral compartment [1]. A maximum of one unaffected sib per family was included among the controls and the allele frequencies between unaffected sibs and unrelated controls were compared to ensure that the use of family based controls did not bias the association results. No significant difference in allele frequencies were found between these two groups of controls ($p=0.65$), the frequency for the G allele rs12901499 among the sib controls was 53.1% vs 51.9% in unrelated controls.

3- *The Chingford Study* is a prospective population-based longitudinal cohort of women of European descent (assessed by questionnaire) derived from the age/sex register of a large general practice in North London, representative of the general UK population in terms of weight, height and smoking characteristics. The study design and rationale are described elsewhere in detail [4]. The Guys & St Thomas' Trust and Waltham Forest Trust ethics committees approved the study protocol. After study procedures were explained to participants, they gave written consent. The K/L grade was scored for the tibiofemoral compartment of each knee [1]. Pelvic radiographs with the subject in the supine antero-posterior position were also obtained and films were scored for the following radiographic features: minimum joint space width, the presence of osteophytes, maximum thickness of subchondral sclerosis, and cyst formation. Hip OA was defined as definite joint space narrowing and a K/L score ≥ 2 for one or both hips. Controls were individuals from the population with neither hip OA nor knee OA as defined above for the respective analyses.

4- *The MRC Hertfordshire Cohort Study* is a large population-based study designed to investigate the relationship between growth in infancy and the development of adult disease in

the UK. Details of the study design have been published previously [5-6] . In brief, in the late 1990s, 3000 men and women were recruited to this study which included a home interview and a subgroup (498 men and 468 women) underwent knee X-rays and DXA scans for assessment of BMD. Ethical approval was obtained from East and North Hertfordshire ethical committees and all participants gave written informed consent. Weight bearing anteroposterior and lateral semi-flexed radiographs of both knees were taken at the same hospital using the same radiographic equipment; a standard tube to film distance of 100 cm was used. Subjects who were taking or had previously had bisphosphonate treatment were excluded. Radiographs were graded at the tibio-femoral and patello-femoral joints for osteophytes and scleroses individually using a standard atlas and the K/L score was determined [1]. Two trained readers graded the radiographs; the intra Operator Variability for K/L grading was good with kappas of 1.00 for the presence of OA and 0.52–0.53 for individual K/L grades at the tibiofemoral joint. A K/L grade ≥ 2 was defined as definite OA. For consistency with the other radiographic cohorts in this study, only data on the tibiofemoral compartment has been included here.

5- *The Estonian Study*: The study subject recruitment consisted of two parts: one of which was population based from the Estonian towns of Elva and Võru (421 subjects), A questionnaire on knee problems was sent to subjects from population cohorts aged 32-55 years using primary care lists [7]. In addition 95 consecutive arthroscopy patients were recruited from the clinic. All of them were selected from the age group 32-55 years and both the subjects from the population based studies and the arthroscopy cases underwent the same X-ray tests. All contacted subjects who agreed to participate had standardised weight-bearing antero-posterior radiographs of the tibiofemoral joint. Joint space narrowing (JSN) and presence of osteophytes, were graded independently by two radiologists using a grading system (grades 0 –III) based on a line-drawing atlas [8]. The JSN and osteophytes grades were then used it to derive K/L score. Individuals with a K/L grade ≥ 2 were considered cases, otherwise were classified as controls. The study was approved by the Ethics Committee of the University of Tartu and informed consent was obtained from all subjects. Blood samples were collected and genomic DNA was purified from the samples of peripheral venous blood by salt extraction.

Genome-wide scan genotyping in TwinsUK and SMAD3 SNPs

These individuals had also been genotyped in a genome-wide association scan for a larger sample of the TwinsUK cohort. All samples were typed with the Infinium assay (Illumina, San Diego, USA) with fully compatible SNP arrays, the Hap300 Duo, Hap300, and Hap550. The quality control procedures on the genomewide genotypes are described in detail elsewhere [9]. The study was approved by St Thomas' Hospital Research Ethics Committee and all participants provided informed written consent.

Supplementary Table 1. Contribution of each individual study to the overall genetic association and meta-analysis results excluding one cohort at a time.

Trait	study	%samples of total for trait	Mantel-Haenszel OR (95%CI) excluding this study	p-value excluding this study
Knee OA	Discovery	16.9%	1.187 (1.076-1.309)	6.2 x 10 ^[-4]
	Chingford	14.6%	1.241 (1.126-1.367)	1.3 x 10 ^[-5]
	Nottingham	36.9%	1.244 (1.106-1.399)	2.7 x 10 ^[-4]
	Estonia	10.5%	1.218 (1.112-1.333)	2.1 x 10 ^[-5]
	Hertfordshire	21.0%	1.219 (1.109-1.341)	4.0 x 10 ^[-5]
Hip OA	Discovery	23.4%	1.192 (1.052-1.349)	0.0057
	Chingford	23.0%	1.216 (1.082-1.367)	0.0010
	Nottingham	56.6%	1.279 (1.065-1.536)	0.0084

References for supplementary subjects and methods:

- 1 Altman RD, Hochberg MC, Murphy WA, Wolfe F. Atlas of individual radiographic features in osteoarthritis. *Osteoarthritis Cartilage* 1995;3(suppl A):3–70.
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- 3 Lanyon P, Muir K, Doherty S, Doherty M. Assessment of a genetic contribution to osteoarthritis of the hip: sibling study. *BMJ* 2000;321:1179-1183.
- 4 Hart DJ, Spector TD. The relationship of obesity, fat distribution and osteoarthritis in women in the general population: the Chingford Study. *J Rheumatol* 1993 20(2):331-335
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- 6 Syddall HE, Aihie Sayer A, Dennison EM, Martin HJ, Barker DJ, Cooper C. Cohort profile: the Hertfordshire cohort study. *Int J Epidemiol*. 2005 34(6):1234-42.
- 7 Tamm, A.; Lintrop, M.; Veske, K.; Hansen, Ü.; Tamm, A. Prevalence of patello- and tibiofemoral osteoarthritis in Elva, Southern Estonia. *J Rheumatology*, 2008, 35(3), 543 - 544.
- 8 Nagaosa Y, Mateus M, Hassan B, Lanyon P, Doherty M. Development of logically devised line drawing atlas for grading of knee osteoarthritis. *Ann Rheum. Dis* 2000; 59: 587-95
- 9 Richards JB, Rivadeneira F, Inouye M, Pastinen TM, Soranzo N, Wilson SG, Andrew T, Falchi M, Gwilliam R, Ahmadi KR, et al. Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. *Lancet*. 2008; 371(9623):1505-12.