Table 3. Various parameters classified by Kellgren-Lawrence grade

KL grade	Proportion of knees (%)	Medial mJSW (mm)	Lateral mJSW (mm)	Medial JSA (mm^2)	Lateral JSA (mm^2)	OPA (mm^2)	FTA (°)
Men							
KL0	24.4	3.70 (0.77)	4.77 (1.01)	125.0 (27.1)	140.0 (33.6)	0	176.1 (2.6)
KL1	38.4	3.40 (0.76)[a]	4.50 (0.93)[a]	109.8 (23.5)[a]	128.9 (29.0)[a]	0.48 (2.24)	176.6 (2.7)
KL2	28.5	3.02 (0.78)[ab]	4.38 (1.02)[a]	99.3 (22.5)[ab]	125.1 (29.8)[a]	1.08 (3.25)[ab]	177.5 (3.1)[ab]
KL3	6.3	2.10 (1.00)[abc]	4.06 (1.40)[abc]	84.1 (31.3)[abc]	129.5 (38.2)[a]	5.37 (8.70)[abc]	178.1 (4.5)[ab]
KL4	2.4	0.79 (0.84)[abcd]	4.04 (1.12)[ab]	44.7 (32.7)[abcd]	137.3 (39.5)	12.05 (10.36)[abcd]	184.2 (6.2)[abcd]
Total	100.0	3.22 (0.96)	4.48 (1.02)	107.3 (29.1)	130.8 (31.8)	1.12 (4.08)	177.0 (3.3)
Women							
KL0	13.9	3.26 (0.65)	4.22 (1.08)	100.9 (23.7)	111.0 (29.4)	0	174.9 (2.9)
KL1	30.6	2.95 (0.73)[a]	3.95 (0.99)[a]	89.7 (24.3)[a]	101.3 (26.0)[a]	0.68 (2.26)	175.6 (3.0)[a]
KL2	38.3	2.66 (0.73)[ab]	3.93 (0.96)[a]	84.5 (23.5)[ab]	100.3 (25.5)[a]	3.39 (6.67)[ab]	176.6 (3.3)[ab]
KL3	13.1	1.85 (0.92)[abc]	3.91 (1.20)[a]	73.3 (27.4)[abc]	106.5 (30.2)[bc]	11.15 (17.54)[abc]	178.7 (4.8)[abc]
KL4	4.1	0.67 (1.02)[abcd]	3.83 (1.68)[a]	34.6 (34.8)[abcd]	112.1 (43.7)[bc]	19.70 (20.65)[abcd]	183.8 (7.1)[abcd]
Total	100.0	2.65 (0.94)[#]	3.97 (1.06)[#]	84.9 (27.9)[#]	103.4 (28.1)[#]	3.76 (9.90)[#]	176.6 (4.1)[#]

Results are the mean (SD)
[a] Significantly different from those of KL0 ($P < 0.05$)
[b] Significantly different from those of KL1 ($P < 0.05$)
[c] Significantly different from those of KL2 ($P < 0.05$)
[d] Significantly different from those of KL3 ($P < 0.05$)
[#] Significantly different from those of men ($P < 0.05$)

medial JSA in both sexes tended to be smaller with increasing KL grade ($p < 0.05$). Values for OPA and FTA in both sexes were significantly larger in the KL 2–4 group than in the KL 0–1 group ($P < 0.05$). Age differences in values of lateral mJSW and JSA were smaller than those for medial mJSW and JSA.

We performed ROC curve analysis to identify threshold values of these indices to determine the knee OA defined by KL ≥ 2 and KL ≥ 3. ROC curve analysis provided threshold values of KL ≥ 2 and KL ≥ 3 in OA parameters for the two knees (Table 4). Threshold values of KOACAD parameters for KL ≥ 2 with AUC > 0.7 were medial mJSW 2.8 mm and medial JSA 107.3 mm^2 in men and medial mJSW 2.7 mm in women. Those for KL ≥ 3 were medial mJSW 2.1 mm, medial JSA 81.1 mm^2, OPA 2.4 mm^2, and FTA 179.6° in men; and they were medial mJSW 2.1 mm, medial JSA 66.6 mm^2, OPA 2.5 mm^2, and FTA 178.1° in women. In contrast, the AUC of the lateral mJSW and lateral JSA for KL ≥ 2 and KL ≥ 3 in OA parameters was near 0.5, meaning that the capacity of these parameters to distinguish diseased knees from normal knees was low.

In addition, we provided threshold values for parameters for both the medial and lateral knee OA using ROC curve analysis (Table 4). Medial OA comprised 97.8% of total OA cases, with the lateral type making up the remaining 2.2%. Although most threshold values for medial OA were similar to those for total OA, the AUC values for parameters of medial OA (e.g., medial mJSW, medial JSA) were higher than for total OA. In contrast, for lateral OA, the AUC values for lateral mJSW and lateral JSA for KL ≥ 2 and KL ≥ 3 in OA parameters were higher than those for total OA, which were near 0.99, meaning that the capacity of these parameters to distinguish disease states from the normal population was high.

Discussion

We have reported elsewhere the automated computer-assisted program KOACAD, which can accurately measure values of mJSW, JSA, OPA, and FTA.[9] In the previous report,[9] we clarified that KOACAD allows accurate, easy assessment of the structural severity of knee OA without any manual operation. The present study applied this system to baseline data from the ROAD study, obtaining normal and threshold values of the above-mentioned indices for objective diagnosis of knee OA.

In the present study, we first established normal values for mJSW, JSA, OPA, and FTA using mean values of these parameters for knees with KL grade 0. The mean values were medial mJSW 3.70 mm, lateral mJSW 4.77 mm, medial JSA 125.0 mm^2, lateral JSA 140.0 mm^2, OPA 0, and FTA 176.1° in men; and medial mJSW 3.26 mm, lateral mJSW 4.22 mm, medial JSA 100.9 mm^2, lateral JSA 111.0 mm^2, OPA 0, and FTA 174.9° in women. All these indices except OPA were significantly lower in women than in men, suggesting that the values are influenced by differences in stature. We concluded that normal and threshold values for knee OA should be established for each sex.

The JSW has been recommended as a candidate index for progression of knee OA,[12] but few data

Table 4. Threshold values of various parameters, by Kellgren-Lawrence grades 2 and 3

Parameter	Threshold value	AUC	Sensitivity	Specificity (%)
Total				
KL ≥ 2				
Men				
Medial mJSW (mm)	2.8	0.726	58.4	76.8
Lateral mJSW(mm)	4.3	0.566	52.3	59.0
Medial JSA (mm^2)	107.3	0.715	71.0	60.3
Lateral JSA (mm^2)	115.5	0.551	39.5	68.2
OPA (mm^2)	1.0	0.599	23.9	95.5
FTA (°)	178.5	0.633	42.7	79.3
Women				
Medial mJSW (mm)	2.7	0.730	63.7	72.5
Lateral mJSW(mm)	4.3	0.521	66.4	38.5
Medial JSA (mm^2)	85.9	0.654	64.5	59.9
Lateral JSA (mm^2)	79.2	0.509	19.8	83.4
OPA (mm^2)	1.0	0.691	44.3	92.4
FTA (°)	177.4	0.664	48.6	77.0
KL ≥ 3				
Men				
Medial mJSW (mm)	2.1	0.875	73.6	92.1
Lateral mJSW(mm)	4.3	0.608	65.2	54.3
Medial JSA (mm^2)	81.1	0.800	58.4	88.9
Lateral JSA (mm^2)	135.7	0.522	50.0	60.1
OPA (mm^2)	2.4	0.739	52.8	93.5
FTA (°)	179.6	0.702	52.5	85.5
Women				
Medial mJSW (mm)	2.1	0.842	65.3	92.0
Lateral mJSW(mm)	2.5	0.507	15.7	93.0
Medial JSA (mm^2)	66.6	0.717	48.7	83.2
Lateral JSA (mm^2)	116.4	0.562	38.8	73.0
OPA (mm^2)	2.5	0.768	66.1	82.2
FTA (°)	178.1	0.744	64.6	76.3
Medial OA				
KL ≥ 2				
Men				
Medial mJSW (mm)	2.8	0.728	58.5	76.8
Lateral mJSW(mm)	4.3	0.560	51.7	59.0
Medial JSA (mm^2)	107.3	0.717	71.3	60.3
Lateral JSA (mm^2)	115.5	0.545	38.8	68.2
OPA (mm^2)	1.2	0.599	23.9	95.5
FTA (°)	178.5	0.639	43.2	79.3
Women				
Medial mJSW (mm)	2.7	0.732	63.9	72.5
Lateral mJSW(mm)	5.4	0.505	92.9	10.9
Medial JSA (mm^2)	85.9	0.655	64.7	59.9
Lateral JSA (mm^2)	97.9	0.505	56.1	46.3
OPA (mm^2)	1.0	0.693	44.7	92.4
FTA (°)	177.4	0.677	49.9	77.0
KL ≥ 3				
Men				
Medial mJSW (mm)	2.1	0.888	76.3	90.4
Lateral mJSW(mm)	4.3	0.598	64.2	54.4
Medial JSA (mm^2)	81.1	0.809	59.0	89.0
Lateral JSA (mm^2)	135.3	0.536	52.6	59.7
OPA (mm^2)	2.4	0.741	53.2	93.4
FTA (°)	179.6	0.719	54.0	85.5
Women				
Medial mJSW (mm)	2.1	0.854	66.6	92.2
Lateral mJSW(mm)	4.6	0.512	29.7	75.8
Medial JSA (mm^2)	66.6	0.727	49.4	83.4
Lateral JSA (mm^2)	116.5	0.587	40.8	72.8
OPA (mm^2)	2.5	0.774	67.3	82.1
FTA (°)	178.1	0.771	67.9	76.0

Table 4. *Continued*

Parameter	Threshold value	AUC	Sensitivity	Specificity (%)
Lateral OA				
KL ≥ 2				
Men and women				
Medial mJSW (mm)	2.1	0.683	43.1	92.4
Lateral mJSW(mm)	2.2	0.995	100.0	98.1
Medial JSA (mm^2)	75.7	0.664	50.0	84.2
Lateral JSA (mm^2)	69.7	0.990	100.0	95.4
OPA (mm^2)	1.2	0.626	30.6	93.8
FTA (°)	173.3	0.795	65.3	81.5
KL ≥ 3				
Men and women				
Medial mJSW (mm)	2.1	0.680	46.0	92.0
Lateral mJSW(mm)	2.2	0.992	100.0	97.0
Medial JSA (mm^2)	75.1	0.638	48.7	84.5
Lateral JSA (mm^2)	69.1	0.987	100.0	95.6
OPA (mm^2)	4.8	0.706	43.2	96.5
FTA (°)	173.3	0.805	64.9	80.8

AUC, area under the curve

regarding normal values have been accumulated.[13] Gensburger et al. showed that the mean medial and lateral JSW in women were 5.1 mm and 6.0 mm, respectively,[13] suggesting that those values in Caucasian populations may be larger than our results in women; no normal values for men were available. In addition, although evaluations of knee alignment are known to be useful for diagnosing arthritic conditions affecting the knee joint and also serve as a guide for conservative management and surgical planning,[14,15] few reports have shown normal values of FTA along with JSA and OPA.

Koshino measured the FTA of 85 knees in men and 97 knees in women aged 25–35 years and reported normal FTA values of 178° in men and 176° in women.[16] These results seem broadly consistent with our results, although no sex differences were apparent in our study, with values of 176° for both men and women. In any case, this represents the first report of reference values for the above-mentioned parameters using a population-based cohort. The results may thus be useful for diagnosing knee OA. Furthermore, by a longitudinal follow-up of the present cohort, these parameters would be expected to predict the progress of knee OA.

We then determined the threshold values for knee OA using ROC curve analysis. In this analysis, we regarded parameters with AUC > 0.7 as good indices for features of knee OA according to a previous report.[17] For KL ≥ 2, threshold values of KOACAD parameters with AUC > 0.7 were only the mJSW in men and women and the medial JSA in men. AUCs > 0.7 on ROC curve analysis means that the threshold of parameters might show good capacity for accurate diagnosis of the disorder in question. In contrast, AUCs of threshold values of parameters regarding the lateral region (i.e., KL ≥ 2; 0.566 for lateral mJSW 4.3 mm, 0.551 for lateral JSA 115.5 mm^2 in men; 0.521 for lateral mJSW 4.3 mm, 0.509 for lateral JSA 79.2 mm^2 in women) seem insufficient as indicators for knee OA. In contrast, for KL ≥ 3, OPA and FTA seem to represent good predictors with satisfactory AUCs. These results suggest that such parameters are more useful in severe knee OA than in mild knee OA.

We also tried to determine threshold values for medial knee OA and lateral knee OA. Because most cases of knee OA were medial knee OA (97.8%), the above-mentioned threshold values were considered applicable for medial OA. Conversely, in the diagnosis for lateral OA, for both KL ≥ 2 and KL ≥ 3, threshold values for medial mJSW and medial JSA were no longer parameters with good predictive capacity. By contrast, AUCs of threshold values for parameters of the lateral region (KL ≥ 2: 0.995 for lateral mJSW 2.2 mm, 0.990 for lateral JSA 69.7 mm^2; KL ≥ 3: 0.992 for lateral mJSW 2.2 mm, 0.987 for lateral JSA 69.1 mm^2) were preferable as good predictors. Similar to medial knee OA, for KL ≥ 3 the OPA and FTA seem to represent good predictors with satisfactory AUC. These results suggest that parameters at the medial side are useful in medial knee OA, and parameters at the lateral side are useful in lateral knee OA. However, evaluation of lateral OA was performed in only 65 participants (2.2%), so we could not analyze data for men and women separately. Regarding lateral OA and threshold KOACAD parameters, further investigation is warranted.

On the other hand, discrepancies between continuous values obtained from the KOACAD system and categorical scales such as the KL scale might add to the limitations of the KL grading scale. Most previous

studies have been performed in patients with knee OA defined by a KL score; but utilizing this categorical scale at the diagnosis of OA seems to result in the loss of a considerable amount of information, as the contribution of joint space narrowing and osteophytes is relatively small. Even though these indices are linear and constant in number, joint space narrowing is simply categorized as mild or severe and osteophytes as slight, definite, or large. In addition, the optimal method for handling joints with severe joint space narrowing but no osteophyte formation is unclear.

One solution to such problems might be found in a radiographic atlas of individual features published by the Osteoarthritis Research Society International (OARSI).[18] OARSI proposed a new grading scale in which joint space narrowing and osteophyte formation at the medial and lateral tibiofemoral compartments on radiographs should be evaluated separately. Several studies have evaluated the severity of joint space narrowing and osteophytes in the osteoarthritic knee utilizing the OARSI scale,[19] although these studies did not assess distinct features of knee OA such as joint space narrowing, osteophyte formation, or joint angulation in one sitting. To the best of our knowledge, no quantitative assessment systems for osteophytes have been described other than in the KOACAD,[9] so the present study is the first to assess threshold values for knee OA in a population-based cohort.

Unlike categorical methods for grading the severity of knee OA (e.g., KL or OARSI scales), KOACAD enables measurement of independent parameters for knee OA. We have already confirmed that low medial mJSW and high FTA are associated with the presence of knee pain, unlike lateral mJSW or an osteophyte area.[9] These accurate and continuous parameters obtained by KOACAD might be candidates for predictors of rapid progress from mild knee OA. These parameters might also be helpful for assessing risk factors for the occurrence of OA. We assumed that 25.3 million people (8.6 million men, 16.7 million women) ≥40 years would be affected by radiographic knee OA, and 7.8 million people (2.2 million men, 5.6 million women) ≥40 years would be affected by knee OA with knee pain.[10] Preventive strategies for OA are certainly in urgent demand. At the planning stage for the strategies against knee OA, the provision of accurate, objective, quantitative indices to measure outcomes seems highly important.

However, some limitation might apply to automated systems for all knee OA. First, as we stated, the number of cases with lateral knee OA was small for accurate determination of thresholds. Second, with radiographs of cases showing severe flexion contracture of the knee (>20°), the KOACAD system failed to measure parameters automatically. However, the system includes a manual mode, and in such cases orthopedic specialists can obtain values by manual measurement.

We believe this system may not only be useful for objective evaluation of knee OA in daily clinical practice or population-based epidemiological studies, it also acts as a proper surrogate measure for the development of disease-modifying drugs for OA. We hope in the future that this system will be applied worldwide to develop international criteria and for the diagnosis and treatment of knee OA.

Conclusion

We have established normal and threshold values of parameters for knee OA using an automated computer-assisted program, KOACAD, on plain radiographs.

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No conflict of interest has been declared by the authors.

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Editorial

Human genetic studies on osteoarthritis from clinicians' viewpoints

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Despite high prevalence and social impact, osteoarthritis (OA) is far behind other skeletal diseases like osteoporosis in the development of disease-modifying treatments. This is mainly because little is known about the underlying molecular mechanism that could be the therapeutic target. Since OA is a multifactorial disease caused by complex interplay between environmental and genetic factors with estimates of around 50% heritability depending on the site[1], numerous efforts and great expense have been spent on human genetic studies on OA worldwide. Although linkage studies have shown large areas of chromosomes associated with the disease, they have failed to detect the susceptible genes. Candidate gene studies have proposed over 100 genes as being responsible; however, most of them have not later been reproduced in larger meta-analysis studies. Recently, while genome-wide association studies (GWAS) have led to the discovery of over 600 gene loci in over 50 common multifactorial diseases, most of the gene variants are of only minimal individual effect. Even though the identified genes with such small effect sizes could possibly be therapeutic targets or at least prognostic markers, it is questionable whether or not these conventional OA genetic studies are worthy of such enormous investment. Aiming at a well-powered approach for this highly polygenic disease with multiple risk loci conferring small effects, consortium studies have been developed to enlarge the sample size. Considering the disease characteristics and prevalence, however, it is our opinion that not only the quantity but also the quality of studies is critical for identification of the genetic architecture. In this sense, the conventional OA genetic studies do not seem to us who are clinicians, although not genetic experts, to have been performed with sufficient scientific strictness, even as compared to those on other common diseases.

In this issue of *Osteoarthritis and Cartilage*, Kerkhof *et al.* clearly indicate that inconsistent and ambiguous definition of OA is a critical limitation of conventional genetic studies[2]. In addition to the stringency of disease definition raised by them, here we propose two other capital issues in the conventional studies: selection of appropriate controls and adjustment for environmental/clinical factors, from a clinician's point of view.

Stringency of disease definition

Kerkhof *et al.* show that there are five different definitions of knee OA in 28 studies involved in the Treat-OA consortium, and the definitions significantly influence the prevalence and association results[2]. Although most conventional genetic studies determine OA on radiographs as Kellgren–Lawrence (KL) score = 2 or higher (Table I)[3–7], the KL grading is limited in reproducibility and sensitivity due to the subjective judgment of observers and the categorical classification into only a five-grade scale[8]. In the ROAD (Research on Osteoarthritis Against Disability) study with a high-quality population-based cohort database of detailed environmental and genetic information of more than 3,000 participants[9], we delete the middle and ambiguous KL = 2 subgroup for the case-control analysis to increase the detection power. For example, our recent association analysis of the *EPAS1* gene which was identified to be crucial for OA development in mice was able to detect a significant difference of the minor allelic frequency (mAF) of a single nucleotide polymorphism (SNP) in the gene between KL = 3 & 4 (case; mAF = 11.1%) and KL = 0 & 1 (control; mAF = 15.2%)[10]. The mAF of the omitted KL = 2 subgroup was 12.3%, confirming an inverse relationship between mAF of the SNP and KL scores. This clearly indicates that inclusion of the KL = 2 subjects in the case group had caused a decrease in the detection power. In fact, this association was not reproduced by conventional Japanese and Chinese studies that include KL = 2 in the case group[11]. Considering that prevalence of the KL = 2 subgroup is shown to be fairly high in representative epidemiologic studies (17.3–41.3%; difference between KL ≥ 2 and KL ≥ 3 in Table II), removal of this subgroup may inevitably cause a decrease in the total sample size. However, we agree with the Kerkhof's opinion that improvement of the definition stringency may compensate a moderate decrease of the sample size to achieve a high detection power[2].

Generally, a lack of objective and quantitative measure for the disease definition remains a fatal limitation of clinical OA studies. The ROAD study has recently established the fully automatic program KOACAD (knee OA computer-aided diagnosis) to quantify the major OA parameters (joint space, osteophyte, *etc.*) on plain radiographs[8]. We believe that the KOACAD system as well as magnetic resonance image systems[12] will serve as optimal measures for the definition of OA in the near future, just as bone mineral density does in osteoporosis.

Selection of appropriate controls

In genetic studies on common diseases with a high prevalence, selection of disease-free controls is essential to avoid the potential bias due to contamination of affected subjects in the control. In representative epidemiologic studies worldwide, the prevalence of radiographic knee OA (KL ≥ 2) in the elderly was ≥ 30% in all

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Table I
Source of subjects, definition, and adjustment for confounders in representative knee OA genetic studies

Gene	GDF5[3]	PTGS2[4]	DVWA[5]	Chromosome 7q22[6]	HLA Class II/III[7]
	Candidate	GWAS	GWAS	GWAS	GWAS
Discovery population	**Case**	**Case**	**Case**	**Case**	**Case**
Source (N)	PBC+HP (718)	HP (243); PBC (114)	HP (740)	PBC (698)	HP (899)
Definition	KL≥2 or CL	CL; KL≥2	CL	KL≥2	CL
Mean age, %female	72y, 83%	NA, 100%; NA, 100%	72y, 90%; 72y, 82%	NA, NA	72y, 84%
	Control	**Control**	**Control**	**Control**	**Control**
	PBC+HP (861)	PBC (196); HS (89)	HP (1,289)	PBC (1,893)	HP+HS (3,396)
	KL≤1 or OR	KL≤1	OR	KL≤1	OD or NA
	49y, 54%	NA, 100%; NA, 87%	49y, 44%; 54y, 46%	NA, NA	53y, 44%
Replication population	**Case**	**Case**	**Case**	**Case**	**Case**
Source (N)	HP (313)	PBC (647); HP (530)	HP (417); PBC (242)	HP (3,142); PBC (741)	HP (813); PBC(167)
Definition	CL	KL≥2; CL	CL; KL≥2	CL, TKR; KL≥2	TKR; KL≥2
Mean age, %female	59y, 66%	NA, 100%; NA, 100%	71y, 75%; 60y, 70%	NA, NA; NA, NA	74y, 74%; 68y, 81%; 66y, 82%
	Control	**Control**	**Control**	**Control**	**Control**
	HS (485)	PBC (1,712); HS (660)	PBC (485); HS (413)	HS (33,825); PBC (2,718); HP (294)	HS (1,071); PBC (347)
	NS	KL≤1; ND	KL≤1; NS	NS; KL≤1 or KL=0; NS	NS; KL≤1 or KL=0
	57y, 65%	NA, 100%; NA, 100%	68y, 63%; 56y, 74%	NA, NA; NA, NA; NA, NA	66y, 64%; 68y, 39%; 60y, 65%
Adjustment	Population	Gender, population	Population	Gender, population	Population

GDF5: growth differentiation factor 5, PTGS2: prostaglandin-endoperoxide synthase 2, DVWA: double von Willebrand factor A domains, HLA: human leukocyte antigen. PBC: population-based cohort, HP: hospital patients, HS: healthy subjects, CL: clinical diagnosis, TKR: total knee replacement, OR: orthopaedic disease or injury, OD: other disease than OA, ND: not diagnosed for OA, NS: no sign of OA, NA: not available.

populations and >60% in Asian populations like Japan (ROAD study) and China (Shanghai) (Table II)[13]. Furthermore, the prevalence of asymptomatic knee OA was 24–36% in all populations. Hence, if so-called healthy subjects without knee symptoms were collected as controls, a considerable number of OA subjects would be included in the control group. Even in a series of genetic studies in Japan with a high OA prevalence[13], the control subjects are miscellaneous mixtures of various populations including considerable numbers of so-called healthy subjects and other disease patients without radiographic diagnosis (Table I)[3,5,7], indicating that a substantial percentage in the control groups are affected subjects. A recent analysis of the effect of controls selected with different levels of stringency on the association of known knee OA susceptibility genes demonstrates that a control with poor selection or without selection cannot be compensated by increase of the sample size[14]. Hence, selection of appropriate controls confirmed to be disease-free may be crucial to achieve a high detection power.

Adjustment for confounding environmental/clinical factors

Lastly, we should again note that OA is a multifactorial disease with environmental and genetic backgrounds and that the genetic contribution is less than half in knee OA[1]. A recent report by Takahashi *et al.* constructed knee OA prediction models based on genotype (combination of three risk alleles of asporin, GDF5 and DVWA) and environmental/clinical information (age, gender and body mass index), and evaluated the predictive power by area under the curve (AUC; range, 0.5 [worst] to 1 [best]) on a receiver operating characteristic (ROC) curve in a case-control association study[15]. The result was that the power by the genotype information was very small (AUC = 0.554), implicating uselessness of the three famous genotypes as a prognostic marker. Contrarily, the environmental/clinical information was a much better predictor (AUC = 0.678), but was little improved by the combination with the genotype information (AUC = 0.685), again confirming its uselessness. Hence, to achieve a high detection power for the susceptibility gene, all efforts should be made to exclude the influence of environmental/clinical factors. Surprisingly, however, there are big differences in age and gender between case and control groups in previous representative studies (Table I). Even a sole difference in age of about 20 years between case and control groups that is seen in the Japanese studies[3,5,7] is calculated to cause an increase of odds ratio for OA to 2.65 (=1.05^{20}), according to the authors' own estimation (1.05/year)[15]. Indeed, we are not opposed to recent activities of OA consortiums to pool subjects worldwide; however, we should note that the pooled subjects are miscellaneous mixtures of various populations with different backgrounds. Selection of case and control subjects with similar backgrounds is essential to minimize selection bias which strongly influences the results in genetic studies with small effect sizes of the risk alleles. Hence, at least for the initial screening, case and control groups should be selected from a single population-based

Table II
Prevalence of radiographic knee OA in representative population-based cohorts

Cohort	ROAD	Framingham	Zoetermeer	Johnston county	Beijing	Shanghai	NHANES III
Ethnicity	Japan	White in USA	Netherlands	Black & whites in USA	China	China	Black & whites in USA
Age	≥60	≥63	≥60	≥65	≥60	60–69	≥60
Total number	2,282	1,420	1,123	1,175	1,781	700	2,415
Radiographic knee OA (%)							
KL ≥ 2	61.9	33.0	30.0	40.6	38.8	64.1	37.4
KL ≥ 2 (symptomatic)	26.1	9.5		13.6	12.0		12.1
KL ≥ 2 (asymptomatic)	35.8	23.5		27.0	26.8		25.3
KL ≥ 3	20.6	15.7	10.2	13.6			10.2

KL ≥ 2 (asymptomatic) was defined as KL ≥ 2 (radiographic) but KL ≥ 2 (symptomatic). NHANES: National Health and Nutrition Examination Survey.
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cohort to adjust the living environment and stratified by confounding environmental/clinical factors which have been identified in preparatory epidemiologic analysis in the cohort. The reproducibility may then be examined in other replication cohorts of the worldwide consortiums, after adjustment for the specific confounding factors in the respective cohorts.

Taken together, conventional OA genetic studies appear to compare a case group containing a substantial number of subjects with ambiguous definition vs a control group containing a substantial number of affected subjects, plus without adjustment for confounding environmental/clinical factors. Contrary to the genetic studies, studies of clinical trial and observational epidemiology are performed under a sound scientific rigidity in compliance with very strict rules to examine the accurate effect sizes of interventions and environmental/clinical factors, respectively. Although genetic studies also examine the effect sizes of genes, they seem to have their fling in the lawless zone. Introduction of strict regulation in the genetic field, just like Consolidated Standards of Reporting Trials (CONSORT) guidelines in the clinical trial field[16], might improve the scientific rigidity. Otherwise, genetic studies seem to be unable to reach a genuine therapeutic target or even a prognostic marker of OA despite numerous efforts and great expense.

Author contributions

All authors contributed to writing and editing of the manuscript, and approved the final submitted manuscript.

Conflicts of interest

One author (HK) is an associate editor for Osteoarthritis and Cartilage. We declare no conflict of interest.

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ORIGINAL ARTICLE

Capacity of endogenous sex steroids to predict bone loss in Japanese men: 10-year follow-up of the Taiji Cohort Study

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Abstract This prospective cohort study aimed to evaluate the capacity of endogenous sex steroids to predict male osteoporosis (OP) among community-dwelling inhabitants. Among 1,028 male residents aged 40–79 years, 50 men belonging to each age stratum (200 in total) were randomly selected from a resident registration list. In the years 1993, 1996, 2000, and 2003, bone mineral density (BMD) of the lumbar spine and proximal femur was measured by dual-energy X-ray absorptiometry. Serum total estradiol (E_2) and free testosterone (FT) were measured using samples extracted in 1993. Among the 200 participants at baseline, 153 subjects completed 10-year follow-ups. Mean values of serum E_2 and FT were 22.4 and 9.4 pg/ml, respectively. Rates of change for BMD at the lumbar spine and femoral neck were 0.8% and 0.5% during the first 3 years, 0.0% and 0.5% during 7 years, and 0.8% and –0.3% over 10 years, respectively. According to multivariate regression analysis after adjusting for age and body mass index, mean values of FT were significantly related to the rate of change of BMD at the femoral neck at 3 years (beta = 0.21; $r^2 = 0.05$; $P < 0.01$), but not at 7 or 10 years. Serum FT level could offer a useful predictor of bone loss within 3 years.

Keywords Testosterone · Estrogen · Bone loss · Male osteoporosis · Population-based cohort study

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Introduction

Osteoporosis (OP) is associated with impairment of activities of daily living (ADL) and quality of life (QOL), leading to increased morbidity and mortality in the elderly [1, 2]. As the proportion of the elderly population is rapidly increasing, an urgent need exists for the development of methods to prevent OP. The estimated number of patients with OP in Japan is about 10 million [3], and cases of hip fracture, as the most severe complication of OP and a key cause of bedridden status, are increasing annually, according to the results of a national survey [4].

Although OP is widely considered as a disorder that mainly affects women, 13% of cases of lumbar spine OP and 24% of cases of femoral neck OP involve men [3]. Up to 20% of hip fractures occur in men, and the number of men with fractures has been rising in Japan [3, 4]. In addition, several studies have shown higher mortality rates after hip fracture in men than in women [5–8], suggesting that male OP warrants urgent attention.

Estrogen is a well-known determinant of low bone mass, bone loss, and osteoporotic fracture in women [9–12]. Reports from the study of osteoporotic fracture suggest that in elderly women, undetectable levels of estradiol, which occur in about one-third of the population, are strongly associated with low bone mineral density (BMD), rapid

bone loss, and increased fracture risk [13–15]. In addition, lower androgen concentrations are reportedly weakly associated with lower BMD and rapid bone loss at some skeletal sites [13].

By contrast, less epidemiological evidence has been gathered regarding the influence of serum sex hormone levels on bone loss, OP, and osteoporotic fracture in men. Some studies of BMD in men have reported positive associations with endogenous androgen levels [16–19], but others have found no significant association [20, 21]. The influence of endogenous sex hormone concentrations on bone loss in men thus remains controversial.

In the present study, to clarify the age distribution of serum levels of endogenous sex steroids and to explore the predictive capacity of these levels for bone loss in men for the early detection of male OP, we measured baseline concentrations of endogenous sex steroids in male subjects randomly selected from a rural population in Japan and conducted follow-up for 10 years.

Materials and methods

Establishment of baseline cohort

This survey was performed in the Japanese town of Taiji. The Taiji cohort has been profiled in detail elsewhere [22–24] and so is summarized here only briefly. Taiji is located in the southern coastal area of Wakayama Prefecture. A list of all inhabitants born in 1913–1952, and therefore aged between 40 and 79 years old in 1993, was compiled based on resident registrations as of the end of 1992. A cohort of 2,261 inhabitants (1,028 men, 1,233 women) was identified, and all members of the cohort completed a self-administered, 125-item questionnaire addressing topics such as dietary habits, smoking habits, alcohol consumption, and physical exercise (whole cohort).

From the whole cohort, 50 men in each of four age groups between 40 and 79 years by decade of birth year (1913–1922, 1923–1932, 1933–1942, and 1943–1952), for a total of 200 participants, were randomly selected. BMD was measured for these 200 participants in 1993. At this time, blood samples of all participants were taken. An interviewer administered a second questionnaire to these 200 participants covering items of past medical history, including questions related to osteoporotic fractures and falls, family history, calcium intake, dietary habits, physical exercise, occupational activities, sun exposure, and, for women, additional questions about reproductive variables (baseline study).

Measurements of endogenous sex steroids

At the baseline study in 1993, blood samples were taken from all participants. After centrifugation of blood samples, sera were immediately placed in dry ice, transferred to a freezer within 24 h, and kept at −80°C until assayed. Serum levels of total estradiol (E_2) and free testosterone (FT) were measured using an immunoradiometric assay (DPC-free estradiol kit and DPC-free testosterone kit, respectively; Mitsubishi Kagaku, Tokyo, Japan). The lowest measurable levels of E_2 and FT were 10 and 0.4 pg/ml, respectively, and percent of coefficient of variation (CV%) for E2 and FT were both less than 15% (unpublished data).

BMD measurements

Baseline BMD was measured in 1993 using dual-energy X-ray absorptiometry (DXA) (QDR 1000; Hologic, Bedford, MA, USA), providing anteroposterior images of lumbar vertebrae L2–L4 and the proximal femur (femoral neck, Ward's triangle, trochanter). These measurements were repeated on the same participants after 3, 7, and 10 years (1996, 2000, and 2003).

To control for the precision of DXA, the equipment was checked at every examination in 1993, 1996, 2000, and 2003 using the same phantom, and BMD of the phantom was regulated to 1.030 ± 0.016 g/cm^2 (1.5%) during all examinations. All BMD measurements were performed by the same medical doctor (N.Y.). Intraobserver variability for DXA scans by this investigator was 0.35% using the phantom, as reported previously [25].

Annual rates of change for BMD during 3-, 7-, and 10-year observations were calculated as follows:

$$\text{Annual rate (\%/year)} = [(\text{BMD follow-up} - \text{BMD baseline})/\text{BMD baseline/follow-up years}] \times 100$$

All examinations were performed with the full consent of the participants. These studies were approved by the ethics committees of both Wakayama Medical University and the University of Tokyo.

Statistical analysis

All statistical analyses were performed using STATA statistical software (STATA, College Station, TX, USA). Differences were tested for significance using analysis of variance for comparisons among multiple groups, and Scheffe's least significant difference (LSD) test for pairs of groups. Significant items were selected, and multiple regression analysis was performed with adjustment of suitable variables.

Results

Eligible participants and baseline characteristics

Background data including physical characteristics for all male participants at baseline are shown in Table 1. Mean weight and height in their fifties, sixties, and seventies, and mean body mass index (BMI) in their seventies were significantly lower than those in their forties ($P < 0.05$).

Among the 200 male participants at baseline, 1 man in his sixties declined to undergo blood and urinary examinations for endogenous hormones. Examinations at baseline were thus performed on 199 men. The second visit, aimed at evaluating changes in BMDs over 3 years, obtained measurements for 181 of the 200 initially recruited participants (90.5%). The following reasons were given for the loss of 19 participants at the 3-year follow-up: 8 men had died, 1 man had moved, 1 man was ill, 4 men declined to participate, and 2 men were away from the area at the time of follow-up. The third visit, aimed at evaluating changes in BMDs over 7 years, evaluated 170 of the 200 initially recruited participants (85%). Loss of 30 participants at the 7-year follow-up was explained as follows: 14 men had died, 3 men had moved, 6 men were ill, 5 men declined to participate, and 2 men were away from the area at the time of follow-up. Among the 200 male participants initially recruited, 153 men participated in the fourth visit held in 2003 (76.5%). Loss of 47 participants at the 10-year follow-up was explained as follows: 33 men had died, 6 men had moved, 4 men were ill, 2 men declined to participate, and 2 men were away from the area at the time of follow-up.

Mean levels of serum concentration of sex steroids at baseline

Age distributions of mean E_2 and FT levels at the initial survey are also shown in Table 1. Because data below the measurable range were excluded from analysis, E_2 and FT data could be obtained for 178 and 198 participants, respectively. Mean serum levels of E_2 and FT were 22.4 and 9.4 pg/ml, respectively. Although no significant age-related trends were seen for E_2, a significant trend toward low values of FT was noted according to age ($P < 0.001$). In addition, mean serum FT was significantly higher for men in their forties than for men in their sixties and seventies ($P < 0.05$).

Predictive capacity of endogenous sex steroids for bone change

Initial mean values and rates of change in L2–L4 BMD over the 3-, 7-, and 10-year periods, classified by age stratum, are shown in Table 2. BMD values at L2–L4 for men had increased slightly by the 10-year follow-up in their fifties and sixties but had decreased a little in the forties and seventies. BMD values at the femoral neck over 10 years had decreased for men in their forties and fifties and had increased considerably in their seventies.

According to multivariate regression analysis using each rate of change for BMD at the lumbar spine over 3, 7, and 10 years as an objective factor and serum levels of E_2 as an explanatory factor after adjusting for age and BMI, beta values for the rate of change for BMD for the first 3, 7, and 10 years were 0.02, 0.04, and –0.02, respectively. Similarly, on multivariate regression analysis using each rate of change for BMD at the femoral neck over 3, 7, and 10 years as an objective factor and serum levels of E_2 as an explanatory factor after adjusting for age and BMI, beta values for the rate of change for BMD for the first 3, 7, and 10 years were –0.07, 0.09, and –0.01, respectively. Total E_2 values could not predict bone change at the lumbar spine or femoral neck at 3, 7, or 10 years.

Again, using the results of multivariate regression analysis to clarify associations between serum FT and

Table 1 Summary characteristics for male participants at baseline classified by age

Birth cohort	Age-group (years)	*n*	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	E2 (pg/mL)		FT (pg/mL)	
			Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)
1943–1952	40–49	50	44.2 (2.6)	168.8 (5.2)	69.0 (10.4)	24.2 (3.2)	46	22.1 (7.4)	50	10.9 (2.8)
1933–1942	50–59	50	54.8 (2.7)	165.6 (5.0)[a]	63.5 (9.4)[a]	23.1 (2.9)	43	22.2 (7.0)	50	9.8 (2.6)
1923–1932	60–69	50	64.6 (2.5)	163.0 (4.8)[a]	62.9 (9.6)[a]	23.6 (3.2)	46	23.1 (8.5)	49	8.8 (2.6)[a]
1913–1922	70–79	50	74.0 (2.7)	160.7 (5.4)[a,b]	57.5 (8.3)[a,b,c]	22.2 (2.8)[a]	43	22.3 (7.7)	49	8.2 (3.1)[a]
1913–1952	40–79	200	59.4 (11.4)	164.5 (5.9)	63.2 (10.2)	23.3 (3.1)	178	22.4 (7.6)	198	9.4 (2.9)

BMI body mass index, *E2* total estradiol, *FT* free testosterone, *n* number of participants, *SD* standard deviation

[a] Significantly different ($P < 0.05$) from values of participants in their forties

[b] Significantly different ($P < 0.05$) from values of participants in their fifties

[c] Significantly different ($P < 0.05$) from values of participants in their sixties

Table 2 Mean values (SD) of bone mineral density (g/cm^2) and change rate (%) at lumbar spine L2–L4 and femoral neck over 3, 7, and 10 years, classified by age and gender

Birth cohort	Age-group (years)	L2–L4								Femoral neck			
		Baseline		2nd visit (3-year follow-up)		3rd visit (7-year follow-up)		4th visit (10-year follow-up)		Baseline	2nd visit	3rd visit	4th visit
		n	BMD (g/cm^2)	*n*	Change rate (%/3 years)	*n*	Change rate (%/7 years)	*n*	Change rate (%/10 years)	BMD (g/cm^2)	Change rate (%/3 years)	Change rate (%/7 years)	Change rate (%/10 years)
1943–1952	40–49	50	1.05 (0.15)	48	0.6 (3.8)	46	−0.6 (5.1)	43	−0.2 (5.8)	0.86 (0.09)	0.3 (4.6)	−1.8 (4.8)	−1.5 (10.9)
1933–1942	50–59	50	0.98 (0.17)	47	1.0 (3.3)	46	−0.0 (6.3)	46	1.6 (8.0)	0.80 (0.13)[a]	−0.2 (4.9)	0.7 (10.0)	−3.0 (6.8)
1923–1932	60–69	50	1.04 (0.21)	49	1.3 (3.6)	47	1.4 (7.1)	41	2.3 (9.4)	0.77 (0.11)[a]	1.0 (7.0)	−0.1 (9.3)	0.3 (12.5)
1913–1922	70–79	50	0.97 (0.19)	37	0.1 (5.3)	31	−1.2 (7.9)	23	−1.5 (9.2)	0.71 (0.08)[a,b,c]	0.9 (6.3)	4.6 (10.2)[a]	6.6 (16.2)[b]
1913–1952	40–79	200	1.01 (0.18)	181	0.8 (4.0)	170	0.0 (6.6)	153	0.8 (8.1)	0.79 (0.12)	0.5 (5.7)	0.5 (8.9)	−0.3 (11.7)

SD standard deviation, *BMD* bone mineral density, *n* number of participants

[a] Significantly different ($P < 0.05$) from values of subjects in their forties

[b] Significantly different ($P < 0.05$) from values of subjects in their fifties

[c] Significantly different ($P < 0.05$) from values of subjects in their sixties

BMD changes at the lumbar spine and femoral neck, beta values of FT for the rate of change for BMD at the lumbar spine at the first 3, 7, and 10 years were 0.08, 0.08, and 0.03, respectively, and those at the femoral neck were 0.21, 0.14, and 0.06, respectively. Mean FT levels were significantly related to the rate of change for BMD at the femoral neck during the first 3 years ($R^2 = 0.05$, $P < 0.01$), but could not predict bone change at any site at 7 or 10 years.

Discussion

The present study examined endogenous hormone levels among men in Japan, measuring changes in BMD over spans of 3, 7, and 10 years. The present study clarified the age distribution of endogenous sex steroids, and a significant trend was seen toward low FT levels with age. FT tended to be significantly lower in the sixties and older when compared with levels in the forties in the present study. Our results support the findings of other reports. Orwoll et al. [26] showed that testosterone levels, particularly FT levels, for 2,623 men 65 years or older were associated with increasing age. Similar findings have been described in other cross-sectional and longitudinal studies [27–29]. Based on these results, we concluded that older men tended to show lower testosterone levels than younger men, similar to the situation with E_2 in women. Some men might display testosterone insufficiency, as seen in women with E_2 insufficiency. However, we do not yet have enough evidence regarding normal ranges in young men and thresholds for testosterone insufficiency. In addition, levels of testosterone may vary among individuals and be influenced by body composition such as adipose tissue, muscle, and bone.

In contrast to testosterone, no significant age-related trend in E_2 was found in the present study. Little information is available regarding E_2 levels in older men. Orwoll et al. [26] reported that E_2 concentrations decreased as age increased, and similar findings have been described in various reports [30–33]. However, other studies have noted stable [34–36] or rising [37] E_2 levels with increasing age. Although the reasons for these discrepancies are unclear, E_2 levels may vary among individuals and may be influenced by body composition such as adipose tissue, muscle, and bone, as well as testosterone.

Regarding the ethnic variations in serum sex steroid levels, as most previous reports have been based on studies of Caucasian men, ethnic variations in FT levels among men remain unclear. To the best of our knowledge, the Osteoporotic Fractures in Men Study (MrOS) is the only study in which a sufficient number of Asian men have participated [26]. For reasons of differences in measurement methods, direct comparison of the present results and

those from the MrOS study is inappropriate, but FT levels among Japanese men tended to be lower than those in MrOS participants, although no significant difference in E_2 levels was apparent. Orwoll et al. [26] analyzed ethnic differences in the MrOS study and stated that FT levels were lower in Asian men than in other races such as Caucasian, African-American, and Hispanic subjects, but no such differences were seen for E_2. The present results support these findings.

The present study found that serum levels of FT could offer a useful predictor of bone loss at the femoral neck within 3 years, but this effect was diluted with longer observation. Regarding the effects of testosterone on bone loss at the hip, Cauley et al. [38] reported, in an epidemiological study of 1,327 men ≥65 years old, that men in the lowest FT category experienced greater hip bone loss over 1.8 years. In addition, Ensrud et al. [39] reported that among men with weight loss, the rate of decline in total hip BMD showed a stepwise increase in magnitude with greater decreases in bioavailable testosterone from baseline. In the present study, the effect of FT levels on bone loss within the relatively short term up to 3 years was observed at the femoral neck, independent of age and BMI, supporting previous reports. Although reasons for site-specific differences in the predictive capacity of FT remain uncertain, we have already reported that bone loss rate differs depending on the site involved in another cohort study [40]. We have also reported that characteristics differ between fast bone losers at the lumbar spine and femoral neck [41]. One reason for site-specific differences might be because degenerative changes that increase BMD, such as osteophytosis or sclerotic change, are observed more frequently at the lumbar spine than at the femoral neck. These results suggest that the predictive capacity of FT might differ according to the sites involved.

A recent study showed that older men with total testosterone or E_2 deficiency were more likely to be osteoporotic [19], but no report evaluated the capacity of serum sex steroids to predict occurrence of OP. Regarding the relationship between testosterone and fracture risk, Mellstrom et al. [42] reported that FT within the normal range was independently associated with the presence, but not occurrence, of osteoporotic fracture in elderly men. In contrast, an analysis from the Rotterdam Study failed to confirm any association between testosterone and fracture risk [43]. Data from the Framingham study indicated that men with low serum testosterone and E_2 levels were at increased risk for incident hip fractures [44]. A recent report from the Dubbo osteoporosis epidemiology study revealed that in men older than 60 years, serum testosterone is independently associated with the risk of osteoporotic fracture [45]. We also tried to evaluate the predictive capacity of serum levels of sex steroids and occurrence of OP based on WHO criteria [46] and osteoporotic fractures, but only identified 7 cases of OP and 10 cases of osteoporotic fractures including 1 vertebral fracture, 1 hip fracture, 2 wrist fractures, 3 costal fractures, 2 ankle fractures, and 1 finger fracture. After analysis using Cox proportional hazards models adjusted for age and BMI, serum levels of FT were significantly related to incidence of OP (hazard ratio, 0.42; 95% confidence interval, 0.19–0.90), but not to incidence of osteoporotic fractures. This analysis suggests the possibility of serum FT as a predictor for OP occurrence over 10 years. However, the number of occurrences of OP seems to be too small to reach any conclusion regarding the presence or absence of associations between sex steroids and OP or osteoporotic fractures.

There are several limitations in the present study. First, the small sample size seemed to be the most severe weakness. In fact, as already noted, only 7 cases of OP and 10 cases of osteoporotic fractures were accumulated during the 10 years of the study. Longer observation in the present cohort might be required to confirm the association between sex steroids and OP or osteoporotic fracture. Second, the dropout rate over 10 years for patients in their seventies was considerably high (54.0%). This high dropout rate might cause bias. In fact, the tendency toward an increase in BMD at the femoral neck for patients in their seventies was skewed by withdrawal bias. On the basis of this hypothesis, we reanalyzed the multivariate regression analysis to assess the change rate of BMD at the femoral neck and serum FT with exclusion of subjects in their seventies. However, the results were similar, with serum levels of FT predicting bone loss at the femoral neck within 3 years (beta = 0.17, $P = 0.05$), but diluted effects with longer observation (7 years: beta = 0.8, $P = 0.38$; 10 years: beta = 0.03, $P = 0.77$). Third, all serum samples were extracted between 0900 and 1500, not at a fixed time in the morning, although samples for measurement of FT are recommended to be collected in the morning. Serum levels of testosterone tend to increase toward night, peaking in the early morning, then decreasing rapidly and reaching a nadir between 1300 and 2300. We collected samples when FT levels would probably have been decreasing toward the nadir. The present study might thus have underestimated FT values compared to collection at a fixed time in the morning.

Conversely, the study design shows several notable strengths. In this population-based cohort study, subjects were selected randomly from the resident registration list. BMD was carefully measured by a single observer (N.Y.), and measurements were repeated 3, 7, and 10 years later with high participation rate by the same device and same observer. Another strength was that the effect of serum levels of sex steroids on changes in BMD could be estimated directly.

In conclusion, we clarified that serum levels of FT could predict bone loss within 3 years, but not longer. Further observations are required to confirm the relationship between FT, E_2, and spinal OP and osteoporotic fractures. Other environmental and genetic factors should also be evaluated to develop strategies for the early prevention of OP.

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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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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.

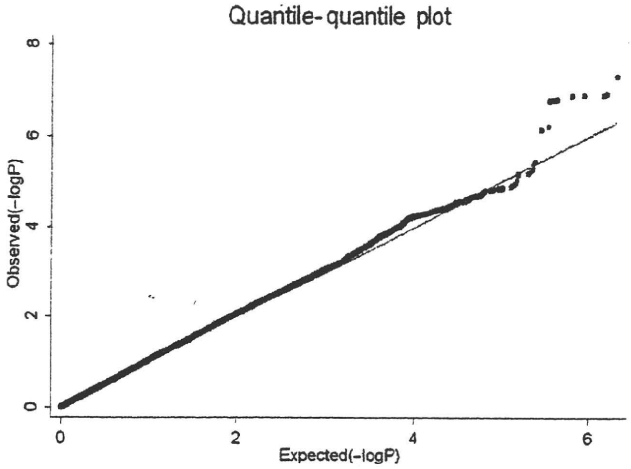

Figure 1 Quantile–quantile plot of the expected versus observed distribution of p values.

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

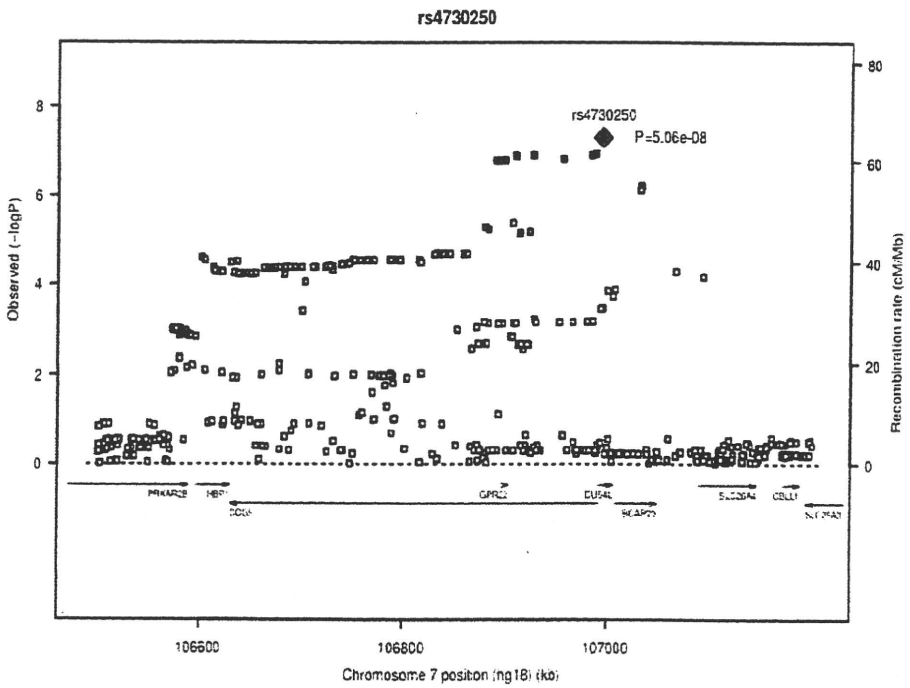

Figure 2 Regional association plot of rs4730250. Statistical significance of the associated SNPs are illustrated on $-\log_{10}$ scale. The p value of the rs4730250 and the other 10 selected SNPs are based on the meta-analysis of all datasets (both genome-wide association (GWA) studies and replication 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 $r^2\geq0.8$ with the sentinel SNP and SNPs in orange have $r^2\geq0.5$. Chromosome positions are based on HapMap release 22 build 36.

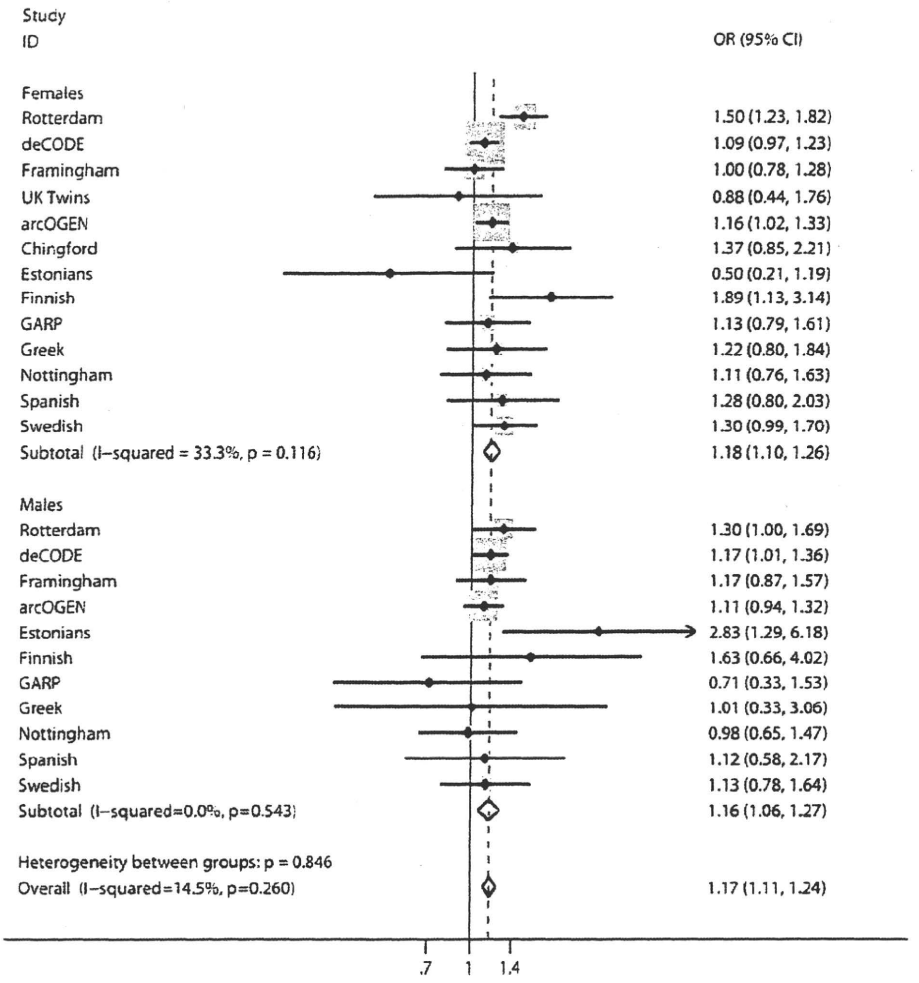

Figure 3 Forest plot of study-specific estimates (black boxes) and summary OR estimates and 95% CIs (diamonds) for the association between the rs4730250 single nucleotide polymorphism and osteoarthritis of the knee.

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