GENETIC MECHANISMS OF KNEE OSTEOARTHRITIS: A POPULATION BASED CASE–CONTROL STUDY

G Jones, C Ding, F Scott, F Cicuttini

Objective: To compare subjects who had at least one parent with a total knee replacement for severe primary knee osteoarthritis with age and sex matched controls who had no family history of knee osteoarthritis.

Design: Population based case–control study of 188 matched pairs (mean age 45 years, range 26 to 60).

Methods: Articular cartilage volume and bone size were determined at the patella and at the medial tibial and lateral tibial compartments by processing images acquired using T1 weighted, fat saturated magnetic resonance imaging. Radiographic osteoarthritis (ROA) was assessed from a standing semiflexed radiograph scored for joint space narrowing and osteophytosis. Knee pain was assessed by questionnaire. Height, weight, body mass index (BMI), lower limb muscle strength, and endurance fitness were measured by standard protocols.

Results: Compared with the controls, index offspring had higher BMI (27.8 v 26.0 kg/m², p = 0.02), weaker lower limb muscles (127 v 135 kg, p = 0.006), more knee pain (47% v 22%, p < 0.001), and greater medial tibial bone area (17.6 v 17.1 cm², p = 0.01). With the exception of BMI, these differences persisted in multivariate analysis. There was a non-significant trend to higher cartilage volume at tibial sites and increased ROA in the offspring in the total and subgroup analyses, but no difference in height and endurance fitness.

Conclusions: BMI, muscle strength, knee pain, and medial tibial bone area, but not cartilage volume, appear to play a role in the genetic regulation and development of knee osteoarthritis.
Weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky clothing removed), using a single pair of electronic scales (Seca Delta Model 707). These were calibrated with known weights at the beginning of each clinic. Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. Body mass index (BMI; kg/m^2) was calculated. Knee pain was assessed by the questionnaire and was defined as pain for more than 24 hours in the last 12 months, or daily pain on more than 30 days in the last year.

Objective measures of physical activity included measurement of muscle strength by dynamometry at the lower limb (involving both legs simultaneously). The subject was instructed to perform each technique at maximum force. Testing and measurement was done twice. A repeatability estimate (Cronbach’s \( \alpha \)) was 0.91. The devices were calibrated by suspending known weights at regular intervals. Physical capacity was also assessed by a bicycle ergometer. Subjects were asked to cycle at a constant 60 rpm for three minutes at each of three successively increasing but submaximal workloads. Heart rate was recorded at one minute intervals at each workload using an electronic heart rate monitor. Work capacity at 170 beats/min (PWC170) was then assessed by linear regression with extrapolation of the line of best fit to a heart rate of 170 beats/min. The PWC170 was not considered a technically adequate measure unless subjects had spent a minimum of two minutes at each workload and the pulse rate increased by at least 5 beats/min with increasing workloads. Repeatability was not assessed in our subjects but has previously been reported as 0.92. A standing anteroposterior semiflexed x ray of the right knee was taken in all subjects. The angle was kept to 10–15° by a purpose built goniometer. The tube to film and tube to tibial plateau angle was 90°. Daily quality assurance was performed on the equipment. Radiographs were then assessed using the OARSI atlas. Each of the following was assessed: medial joint space narrowing (0–3), lateral joint space narrowing (0–3), medial osteophytes (femoral and tibial combined) (0–3), and lateral osteophytes (femoral and tibial combined) (0–3). Each score was arrived at by consensus between two readers (GJ, FS) who assessed the radiograph simultaneously with immediate reference to the atlas. Reproducibility was determined in 50 radiographs, taken over two weeks apart, and yielded an intraclass correlation coefficient (ICC) of 0.99 for osteophytes and 0.98 for joint space narrowing. This may represent an overestimate of the actual agreement because of the high proportion of normal radiographs. However, in one of our previous studies this method also had very high reproducibility for radiographic osteoarthritis of the hands, with ICCs of 0.94 to 0.98.

MRI of the right knee was also undertaken. Knee cartilage volume was determined by image processing on an independent workstation using the software program Osiris (University of Geneva), as previously described. To transform the images to the axial plane, the Analyse Software package developed by the Mayo Clinic was employed. Medial and lateral tibial plateau bone area was determined by creating an isotropic volume from the three input images closest to the knee joint. The bone area of the medial and lateral tibial plateau was then directly measured from the reformatted axial images. The area of patellar bone was determined individually by manually drawing contours around the target patella boundaries on a slice-by-slice basis on sagittal views. The volume of the patella bone was then determined by summing all the pertinent voxels within the resultant binary volume. Total volume was calculated for the patellar bone because its irregular shape made it difficult to identify a simpler representative measure of patellar size. The CVs for these measures in our hands are 2.2–2.6%.

### Statistical methods

A combination of paired \( t \) tests and conditional logistic regression was used for the analysis of this matched dataset. Subgroup analyses were also carried out for hypothesis generation with regard to the sex of the parent, the age of the parent at the time of knee replacement, and the age of the offspring. To check whether relatedness of the subjects was biasing the overall results, a stratified analysis restricted to one randomly selected offspring per family and their matched control was also undertaken. To further check the robustness of the standard errors, random effects models were used. A probability (p) value less than 0.05 (two tailed) or a 95% confidence interval (CI) not including the null point were regarded as statistically significant. All statistical analyses were carried out on SPSS version 10.0 for Windows (SPSS Inc, Chicago, Illinois, USA).

### RESULTS

There were 376 subjects (214 female, 162 male) comprising 188 offspring and 188 age and sex matched controls. The response rate was 71% for the offspring and 40% for the controls. This was a young sample with an average age of 45 years (range 26 to 61). Characteristics of the group are presented in table 1. Offspring and controls were well matched for age, height, injury history, and fitness. However, offspring weighed more and had a higher BMI and a higher prevalence of knee pain (all \( p < 0.05 \)) or a 95% confidence interval (CI) not including the null point were regarded as statistically significant. All offspring per family subgroup.

Table 2 documents the differences in cartilage volume and bone area between offspring and controls. No significant differences were observed in cartilage volume and this persisted in all subgroup analyses (table 3). In contrast, medial tibial plateau bone area was significantly higher in offspring than in controls (\( p = 0.01 \)). This did not apply to the
other bone sites, with the exception of lateral tibial area in the
one offspring per family subgroup. In subgroup analysis,
there were increases in medial tibial bone area in all offspring
subgroups, which reached statistical significance in women,
in the older groups (both offspring and parents), in those
with a female parent who had a total knee replacement, and
in the single offspring per family subgroup.

Table 4 details the multivariate analysis. In the whole
sample, after adjustment for ROA and the other significant
univariate variables all factors remained statistically signifi-
cant except BMI, suggesting that they were independently
associated with offspring status. Results were generally more
marked in the single offspring per family subgroup, where all
factors (including BMI) remained significant in multivariate
analysis. Results were largely unchanged if random effect
models were used (data not shown). If the analysis was
restricted to those over 45 years, there was a non-significant
trend in increased ROA in the offspring (28% vs 20% for
prevalence and 0.55 vs 0.33 for severity, p = 0.13 to 0.17). Past
knee injury was not associated with knee pain or structural
change in this sample, and adjusting for this factor did not
change the results (data not shown).

DISCUSSION

This is the first study to examine differences in knee
structural components between offspring of individuals with
severe knee osteoarthritis in later life and controls. It does not
support the hypothesis that cartilage volume is reduced in the
offspring group. However, offspring weighed more and had
weaker lower limb muscles, more knee pain, and a greater
medial tibial plateau area than controls.

Cartilage loss is the hallmark of established osteoarthritis,\(^{27}\) but it is uncertain whether cartilage or bone is most involved
in the pathogenesis of this disorder.\(^{28}\) Recent twin and sibling
studies have indicated that the heritability of cartilage
volume is very high.\(^{14,17}\) In addition, our original report
suggested that cartilage development might be one explana-
tion for the sex and site differences in knee osteoarthritis in
later life.\(^{16}\) As a longitudinal study to answer this question
would require many years of follow up, the current study was
designed to test this hypothesis indirectly by comparing
offspring of individuals with severe knee osteoarthritis with
controls.

Our study can confidently reject the hypothesis that
cartilage volume is lower in the offspring and does not
support a role for cartilage morphology in the pathogenesis
of knee osteoarthritis. Indeed, there was a trend to greater
cartilage volume in the offspring. This did not reach
statistical significance but may indicate that cartilage volume
increases in some subjects in the early stages of osteoarthritis
because of loss of aggrecan from the matrix and increased
water content.\(^{28}\) Our MRI technique accurately and repro-
ducibly assesses cartilage morphology but cannot measure
cartilage quality. If cartilage volume is increasing because of
swelling then further longitudinal studies may document a
higher rate of loss, which will be less likely if cartilage
homeostasis is intact. However, in contrast to the lack of
difference in cartilage volume between offspring and
controls, significant differences were observed in body mass
index, knee pain, muscle strength, and tibial plateau area.
The latter three were independent factors in multivariate
analysis. This suggests that all these factors may play a role in
the initiation of knee osteoarthritis, and that the association
with BMI is primarily through its association with these
factors, BMI being correlated with knee pain and bone size
but not with muscle strength in this sample. While BMI is
clearly a strong risk factor for knee osteoarthritis, these
results imply that the genetic contribution to the relation
between BMI and osteoarthritis is small, which is consistent
with a recent report suggesting that the heritability of obesity

Table 2 Comparison of cartilage volume, bone area, and bone volume between offspring and controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Whole sample</th>
<th>One offspring per family</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Offspring</td>
<td>Controls</td>
</tr>
<tr>
<td>Cartilage variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial tibial volume (ml)</td>
<td>2.32 (0.56)</td>
<td>2.26 (0.60)</td>
</tr>
<tr>
<td>Lateral tibial volume (ml)</td>
<td>2.65 (0.68)</td>
<td>2.57 (0.69)</td>
</tr>
<tr>
<td>Patellar cartilage volume (ml)</td>
<td>3.46 (0.97)</td>
<td>3.47 (0.99)</td>
</tr>
<tr>
<td>Bone variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial tibial area (cm²)</td>
<td>17.6 (2.6)</td>
<td>17.1 (2.8)</td>
</tr>
<tr>
<td>Lateral tibial area (cm²)</td>
<td>12.0 (2.0)</td>
<td>11.9 (2.0)</td>
</tr>
<tr>
<td>Patellar bone volume (ml)</td>
<td>13.6 (3.2)</td>
<td>14.0 (3.5)</td>
</tr>
</tbody>
</table>

Values are mean (SD).
is largely independent of the heritability of knee osteoarthritis.29

The underlying mechanism of knee pain remains elusive. Body weight and osteophytes have been linked to this symptom.26,30 However, in our study the difference between cases and controls was independent of these factors and was substantial, with 47% of the offspring having knee pain compared with 22% of the controls. Our definition of knee pain was somewhat more liberal than in previous studies, but the high prevalence was surprising nonetheless, as we expected a much lower prevalence when planning the study, even in the absence of other population based data on knee pain from Australia. Knee pain had high heritability in a smaller sibling pair study in this sample,14 confirming a significant genetic contribution. This suggests that genetic factors leading to knee pain may mediate the genetic risk of knee osteoarthritis, and that knee pain could be considered to represent a pre-osteoarthritis state. However, further longitudinal studies are required to determine the relation between unexplained knee pain and subsequent osteoarthritis, as knee pain may be subject to recall bias if there is a positive family history. Similar comments apply to muscle strength. This was 8 kg lower in the offspring, again suggesting a direct role in pathogenesis and consistent with prospective studies in older populations.31 While this was also under strong genetic control in the sibling pairs within this study,14 it is clear that strength can also be influenced by the environment; thus exercise programs in younger life may be protective of knee osteoarthritis.

In this study, medial tibial plateau area was modestly but significantly higher in offspring. This association was consistent in subgroup analysis, being positive in all groups but especially in the older groups (both offspring and parents) and in women (both offspring and those with a female parent). This implies a role for local bone size in the pathogenesis of knee osteoarthritis. A previous study has suggested a role for bone density as predictor of knee osteoarthritis19 and as a mediator of the genetic effect in a study of similar design to the current one.26 Subchondral bone also may have role to play. It is unclear whether it has role in initiation, but it is suggested that it influences progression, based on the Bristol OA500 study.31 Tibial bone area is highly heritable44 but most of the heritability is mediated through body size. Tibial bone area is markedly increased in individuals with early osteoarthritis.26 In this sample, bone area increased with age, BMI, and pain (C Ding et al, unpublished data) and was predictive of knee replacement (A Wluka et al, unpublished data). The difference in bone size persisted after adjustment for these other factors and ROA, suggesting that the bone change is independent of these factors and ROA, and possibly precedes detectable radiographic change. These observations are consistent with bone size having an initiating role in knee osteoarthritis in adult life.

Our bone area measure has potential limitations. It was originally derived to adjust for bone size and has not been validated against cadaveric specimens. However, its measurement is highly reproducible, has face validity, and the current study provides support for content validity. However, it remains to be seen whether bone area is associated with environmental factors and would thus be modifiable.

Our study has other possible limitations. First, there was no significant increase in ROA (either prevalence or severity) in the offspring, apart from in the one offspring per family subgroup. This is most likely to reflect the young age of the cohort and the relative rarity of ROA at this age. However, the

<table>
<thead>
<tr>
<th></th>
<th>Medial tibial difference (95% CI)</th>
<th>Lateral tibial difference (95% CI)</th>
<th>Patello difference (95% CI)</th>
<th>Medial tibial bone area difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (188 pairs)</td>
<td>+54 (-36 to +144)</td>
<td>+80 (-21 to +180)</td>
<td>-7 (-151 to +137)</td>
<td>+53 (-13 to +93)</td>
</tr>
<tr>
<td>Female (107 pairs)</td>
<td>+39 (-63 to +140)</td>
<td>+44 (-74 to +163)</td>
<td>+55 (-119 to +229)</td>
<td>+75 (-27 to +124)</td>
</tr>
<tr>
<td>Male (81 pairs)</td>
<td>+74 (-90 to +238)</td>
<td>+126 (-50 to +301)</td>
<td>-88 (-333 to +156)</td>
<td>+20 (-49 to +91)</td>
</tr>
<tr>
<td>Offspring &lt; 45 years (84 pairs)</td>
<td>+36 (-100 to +173)</td>
<td>+87 (-49 to +222)</td>
<td>+33 (-169 to +263)</td>
<td>+52 (-10 to +115)</td>
</tr>
<tr>
<td>Offspring &gt; 45 years (104 pairs)</td>
<td>+68 (-55 to +191)</td>
<td>+73 (-74 to +221)</td>
<td>-39 (-244 to +165)</td>
<td>+55 (-2 to +107)</td>
</tr>
<tr>
<td>Female parent had TKR (117 pairs)</td>
<td>+83 (-36 to +202)</td>
<td>+34 (-90 to +159)</td>
<td>+19 (-169 to +206)</td>
<td>+73 (-20 to +125)</td>
</tr>
<tr>
<td>Male parent had TKR (71 pairs)</td>
<td>+6 (-133 to +146)</td>
<td>+154 (-19 to +326)</td>
<td>-49 (-277 to +179)</td>
<td>+22 (-41 to +84)</td>
</tr>
<tr>
<td>Parent &lt; 75 years at time of TKR (87 pairs)</td>
<td>+58 (-72 to +188)</td>
<td>+71 (-63 to +206)</td>
<td>-46 (-259 to +166)</td>
<td>+41 (-25 to +106)</td>
</tr>
<tr>
<td>Parent &gt; 75 years at time of TKR (91 pairs)</td>
<td>+52 (-86 to +190)</td>
<td>+111 (-46 to +268)</td>
<td>-3 (-219 to -213)</td>
<td>+66 (-15 to +118)</td>
</tr>
<tr>
<td>One offspring per family (111 pairs)</td>
<td>+69 (-48 to +186)</td>
<td>+124 (-15 to +263)</td>
<td>-16 (-211 to +179)</td>
<td>+81 (-32 to +130)</td>
</tr>
</tbody>
</table>

Significant differences in bold.

CI, confidence interval; TKR, total knee replacement.

Table 4  Multivariate conditional logistic regression analysis of factors discriminating subjects with a parent with total knee replacement for osteoarthritis from controls

<table>
<thead>
<tr>
<th></th>
<th>Univariate OR (95% CI)</th>
<th>Multivariate* OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects (188 pairs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee pain (yes v no)</td>
<td>3.10 (1.92 to 5.01)</td>
<td>2.85 (1.70 to 4.78)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>1.03 (0.99 to 1.07)</td>
<td>1.01 (0.97 to 1.05)</td>
</tr>
<tr>
<td>Lower limb muscle strength</td>
<td>0.59 (0.41 to 0.86)</td>
<td>0.61 (0.40 to 0.93)</td>
</tr>
<tr>
<td>Medial tibial bone area</td>
<td>1.44 (1.06 to 1.96)</td>
<td>1.53 (1.02 to 2.29)</td>
</tr>
<tr>
<td>One offspring per family (111 pairs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee pain (yes v no)</td>
<td>2.86 (1.56 to 5.25)</td>
<td>2.94 (1.36 to 6.40)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>1.13 (1.05 to 1.23)</td>
<td>1.11 (1.01 to 1.22)</td>
</tr>
<tr>
<td>Lower limb muscle strength</td>
<td>0.59 (0.35 to 0.99)</td>
<td>0.43 (0.22 to 0.85)</td>
</tr>
<tr>
<td>Medial tibial bone area</td>
<td>2.09 (1.23 to 3.51)</td>
<td>2.59 (1.36 to 4.96)</td>
</tr>
</tbody>
</table>

*Adjusted for factors in the table as well as for radiographic osteoarthritis (which was not a significant variable in either model). The coefficients are per unit for BMI and per SD for muscle strength and bone area. Significant values in bold.

BMI, body mass index; OR, odds ratio.
link between total knee replacement and family risk is relatively weak, at 1.5 times. This has only been documented for siblings and not offspring; thus it is possible that the offspring risk is weaker than this. However, one would then have expected no differences in any factors between offspring and controls.

Second, while the response rate was acceptable at 71% in the offspring, it was 40% in the controls. We have limited data to assess the reasons for non-response; but selection bias may still be present and would tend to operate in two ways. The first is that subjects with a direct interest in the results may be more likely to take part (for example, those with knee pain and past injury). The second is that those who are more interested in their health may be more likely to take part. These biases imply a lower rate of knee pain, a tendency to higher body weight, and lower muscle strength in the general population than observed in our control population. This bias is unlikely to have an effect on knee bone size, as subjects will be unaware of this factor. Similarly, the high response rate in the offspring suggests that knee pain will be higher than the controls even if no non-responders had knee pain. Overall, this indicates that some caution is necessary in interpreting the results. While the pain and bone size associations appear robust, the muscle strength and body weight observations require confirmation in other populations, preferably in longitudinal studies. Finally, some of the offspring were related (128 from 51 families). This violates the assumption of independence in hypothesis testing. However, the results did not change if random effects models were used to allow for this. Furthermore, if the analysis is restricted to one offspring per family, the strength of the associations increases, implying that this potential bias is not of major concern.

Conclusions
Our study suggests that body mass index, muscle strength, knee pain, and medial tibial bone area, but not cartilage volume, all play a role in the genetic regulation and development of knee osteoarthritis. Further confirmation of these results in longitudinal studies is required.

ACKNOWLEDGEMENTS
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