EXTENDED REPORT

The effect of FTO variation on increased osteoarthritis risk is mediated through body mass index: a mendelian randomisation study

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ABSTRACT

Objective Variation in the fat mass and obesity-associated (FTO) gene influences susceptibility to obesity. A variant in the FTO gene has been implicated in genetic risk to osteoarthritis (OA). We examined the role of the FTO polymorphism rs8044769 in risk of knee and hip OA in cases and controls incorporating body mass index (BMI) information.

Methods 5409 knee OA patients, 4355 hip OA patients and up to 5362 healthy controls from 7 independent cohorts from the UK and Australia were genotyped for rs8044769. The association of the FTO variant with OA was investigated in case/control analyses with and without BMI adjustment and in analyses matched for BMI category. A mendelian randomisation approach was employed using the FTO variant as the instrumental variable to evaluate the role of overweight on OA.

Results In the meta-analysis of all overweight (BMI≥25) samples versus normal-weight controls irrespective of OA status the association of rs8044769 with overweight is highly significant (OR[CIs] for allele G=1.14 [1.08 to 1.19], p=7.5×10⁻⁷). A significant association with knee OA is present in the analysis without BMI adjustment (OR[CIs]=1.08[1.02 to 1.14], p=0.009) but the signal fully attenuates after BMI adjustment (OR[CIs]=0.99[0.93 to 1.05], p=0.666). We observe no evidence for association in the BMI-matched analysis without BMI adjustment (OR[CIs]=1.08[1.02 to 1.14], p=0.666). We observe no evidence for association in the BMI-matched analysis matched for BMI category. Mendelian randomisation approaches confirm the causal role of overweight on OA.

Conclusions Our data highlight the contribution of genetic risk to overweight in defining risk to OA but the association is exclusively mediated by the effect on BMI. This is consistent with what is known of the biology of the FTO gene and supports the causative role of high BMI in OA.

INTRODUCTION

Osteoarthritis (OA) is the most common articular disease in the developed world and a leading cause of chronic disability, mostly as a consequence of knee OA and/or hip OA. A number of studies have shown that obesity represents one of the most important risk factors and it is also a predictor for progression of OA, especially of the knee joint and less of the hip joint. There is a strong and highly significant relationship between body mass index (BMI) and OA of the knee. The relationship with hip OA is less striking but is still highly statistically significant and obesity is one of the strongest prognostic factors for large joint OA.

Genome-wide association studies (GWAS), which test the correlation between single-nucleotide polymorphisms (SNPs) across the entire genome and trait variation in a sample of individuals, have succeeded in identifying variants associated reproducibly with complex traits. The association between FTO SNPs and BMI and the risk of being overweight or obese has been confirmed in multiple populations. The effect of FTO SNPs on BMI is modest, with those individuals homoyzogous for the risk allele weighing, on average, 3 kg more than those homoyzogous for the protective allele.

The protein encoded by FTO has been described as a Fe(II)-oxoglutarate-dependent oxygenase and 2-oxoglutarate-dependent oxygenase that might operate as a DNA demethylase. The human FTO gene has been implicated in genetic risk to T2D detected strong association with BMI.6 Experimental animal studies provide direct functional evidence that FTO underlies obesity. Two studies have demonstrated that FTO gene expression in the arcuate nucleus of the hypothalamus is regulated by fasting suggesting that FTO may be important to the control of energy homeostasis.

A recent GWAS on hip and/or knee OA has identified a variant in the FTO gene, rs8044769, as being strongly associated with risk of OA.11 Because of the study design, the authors were not able to test thoroughly whether the association between FTO and OA was mediated by obesity or not. GWAS for type 2 diabetes (T2D) detected strong association between common SNPs in the FTO region and risk of T2D.6 12 13 However, subsequent analyses showed that the association between FTO SNPs and T2D was mediated by an association with BMI.6

There has been a lot of debate in the literature recently about the role of FTO in OA pathogenesis and specifically about the direction of causation between obesity and OA.14 15 Mendelian randomisation, a form of instrumental variable analysis, is a
method of using measured variation in genes of known function to examine the causal effect of a modifiable exposure on disease in non-experimental studies.\textsuperscript{16} The main elements for it to work (having removed the effect of confounders) are that the genetic variant should be reliably associated with the exposure, in this case overweight status, and that there should be no direct effect of genotype on disease or any other mediated effect other than through the exposure of interest. In our study we find that these conditions apply to the relationship between FTO, overweight and OA.

The aim of the present study is to elucidate the role of rs8044769 in genetic risk of OA by testing this SNP in seven independent study cohorts from the UK and Australia with BMI classiﬁcation data from total 5409 knee OA patients, 4355 hip OA patients and 5362 healthy individuals. We explore the causality and direction of the relationship between overweight status and OA using the FTO polymorphism as the instrumental variable in a mendelian randomization analysis.

PATIENTS AND METHODS

We examined genotypes for rs8044769 in knee or hip OA cases and controls with BMI information coming from seven independent studies (table 1 and see online supplementary methods). These comprised: a subset of knee or hip OA cases from the arcOGEN GWAS versus disease-free controls from TwinsUK, and individuals from the Chingford Study, the Hertfordshire Cohort Study (HCS), the Nottingham Case-Control Study, the Genetics of Osteoarthritis and Lifestyle Study, the Scottish National Health Survey for the (TASOAC) study.\textsuperscript{11, 17-22} Cases had either radiographic evidence of the disease with a Kellgren-Lawrence (KL) grade ≥2 or clinical evidence of the disease to a level requiring total joint replacement. Controls were disease-free individuals with KL<2. Individuals from the arcOGEN, Chingford, TwinsUK and the Nottingham cohort with full GWAS quality control have been subjected to standard GWAS analysis including removal of ethnic outliers. The HCS includes only individuals of self-reported Caucasian origin from within the Hertfordshire county, and TASOAC individuals included in this study are all of self-reported British origin and of white ethnicity (see online supplementary methods). Ethical approval for each study was obtained from the relevant ethical committees and all participants gave written informed consent.

We carried out case/control logistic regression analyses for rs8044769 under the multiplicative model (adjusting for gender and BMI were applicable by including them in the model as covariates) and combined summary statistics in a meta-analysis framework (see online supplementary methods). To evaluate the association of the FTO variant with risk of overweight/obese we classified all overweight/obese samples as cases and normal weight subjects as controls in each cohort (irrespective of OA status). For the BMI-matched analyses, we stratified the OA hip or knee cases and the controls into three categories for each cohort according to BMI: normal weight (BMI<25), overweight and obese (BMI ≥25) and obese only (BMI >30).

RESULTS

We analysed genotypes for rs8044769 across a total of 936 normal weight, 2092 overweight and 2381 obese knee OA patients versus 2501 normal weight, 1984 overweight and 877 obese controls and of 1201 normal weight, 1758 overweight and 848 obese healthy controls from seven independent cohorts from the UK and Australia (table 1). First, we investigated the association of the FTO variant with risk of obesity in a case/control analysis on all overweight/obese samples (n=10 538) versus normal weight controls (n=4598) irrespective of OA status and found that to be highly significant. Allele G at rs8044769 was associated with risk of overweight/obesity (BMI ≥25) with an OR[CIs]=1.14 [1.08 to 1.19], p=7.5×10\textsuperscript{-5}. There was no heterogeneity was observed between studies (heterogeneity index, I\textsuperscript{2}=0). We then examined the strength of association of rs8044769 with knee or hip OA across all OA cases versus controls in each cohort (adjusted for gender) and repeated the analyses adjusting for BMI. A significant association of the FTO variant with knee OA only was detected in the meta-analysis without BMI adjustment (OR[CIs]=1.08[1.02 to 1.14], p=0.009) (table 2). The effect of this variant on knee OA is slightly larger and more significant in the analysis of the two genders combined but it appears to be mainly driven by females (see online supplementary table S1) with OR in females=1.07

<table>
<thead>
<tr>
<th>Study</th>
<th>Country of origin</th>
<th>OA status</th>
<th>Definition</th>
<th>N</th>
<th>F (%)</th>
<th>BMI±SD [kg/m\textsuperscript{2}]</th>
<th>Age±SD [yrs]</th>
<th>Controls (OA unaffected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>arcOGEN</td>
<td>UK</td>
<td>Hip</td>
<td>KL≥2 or THR</td>
<td>1310</td>
<td>100</td>
<td>28.4±5.8</td>
<td>64.7±9.1</td>
<td>KL&lt;2</td>
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<td>arcOGEN</td>
<td>UK</td>
<td>Knee</td>
<td>KL≥2 or TRK</td>
<td>1209</td>
<td>100</td>
<td>30.6±6.3</td>
<td>60.0±8.7</td>
<td>KL&lt;2</td>
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<tr>
<td>Chingford</td>
<td>UK</td>
<td>Hip</td>
<td>KL≥2 or THR</td>
<td>97</td>
<td>100</td>
<td>25.0±3.9</td>
<td>60.0±5.6</td>
<td>KL&lt;2</td>
</tr>
<tr>
<td>Chingford</td>
<td>UK</td>
<td>Knee</td>
<td>KL≥2 or TRK</td>
<td>266</td>
<td>100</td>
<td>27.1±4.6</td>
<td>65.7±5.6</td>
<td>KL&lt;2</td>
</tr>
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<td>GOAL</td>
<td>UK</td>
<td>Hip</td>
<td>KL≥2 or TRK</td>
<td>1291</td>
<td>100</td>
<td>29.4±5.3</td>
<td>67.8±7.2</td>
<td>KL&lt;2</td>
</tr>
<tr>
<td>GOAL</td>
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<td>Knee</td>
<td>KL≥2 or TRK</td>
<td>1619</td>
<td>100</td>
<td>30.7±5.4</td>
<td>68.5±7.1</td>
<td>KL&lt;2</td>
</tr>
<tr>
<td>HSC</td>
<td>UK</td>
<td>Knee</td>
<td>KL≥2 or TRK</td>
<td>148</td>
<td>100</td>
<td>29.4±5.1</td>
<td>65.2±2.6</td>
<td>KL&lt;2</td>
</tr>
<tr>
<td>Nottingham</td>
<td>UK</td>
<td>Hip</td>
<td>KL≥2 or THR</td>
<td>1525</td>
<td>63.2</td>
<td>27.7±4.7</td>
<td>68.5±9.2</td>
<td>KL&lt;2</td>
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<td>Nottingham</td>
<td>UK</td>
<td>Knee</td>
<td>KL≥2 or TRK</td>
<td>1780</td>
<td>55.3</td>
<td>29.7±5.3</td>
<td>69.0±8.8</td>
<td>KL&lt;2</td>
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<tr>
<td>TASOAC</td>
<td>Australia</td>
<td>Hip</td>
<td>KL&gt;2</td>
<td>44</td>
<td>100</td>
<td>65.1±9.4</td>
<td>65.4±7.5</td>
<td>KL&lt;2</td>
</tr>
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<td>TASOAC</td>
<td>Australia</td>
<td>Knee</td>
<td>KL&gt;2</td>
<td>239</td>
<td>100</td>
<td>29.3±5.2</td>
<td>67.3±7.3</td>
<td>KL&lt;2</td>
</tr>
<tr>
<td>TwinsUK</td>
<td>UK</td>
<td>Hip</td>
<td>KL≥2 or THR</td>
<td>88</td>
<td>100</td>
<td>26.0±4.6</td>
<td>57.4±8.0</td>
<td>KL&lt;2</td>
</tr>
<tr>
<td>TwinsUK</td>
<td>UK</td>
<td>Knee</td>
<td>KL≥2 or TRK</td>
<td>148</td>
<td>100</td>
<td>28.1±5.3</td>
<td>58.8±7.5</td>
<td>KL&lt;2</td>
</tr>
</tbody>
</table>

GOAL, Genetics of Osteoarthritis and Lifestyle; TASOAC, Tasmanian Older Adult Cohort.
[1.00–1.15], p = 0.049, I² = 0.54 versus OR in males = 1.04[0.94–1.14], p = 0.47, I² = 0, which is in accordance with the finding reported in the arcOGEN GWAS.\(^{11}\) After excluding 2323 knee OA cases and 1671 controls that were part of the arcOGEN GWAS discovery study\(^{14}\) we observe that the effect size is smaller in the replication studies (OR[CI]=1.04[0.97 to 1.12]) and not significant (p = 0.24).

The association signal was fully attenuated after BMI adjustment (table 2) (OR[CI]=0.99[0.93 to 1.05], p = 0.666) in accordance with the finding reported in the full arcOGEN GWAS.\(^{11}\) Adjusting for age as well as BMI did not make a noticeable difference in these results (table 2). We further investigated the \(FTO\) association by performing a large-scale meta-analysis across all case/control datasets matched for BMI but observed no evidence for association in any of the BMI strata studied (table 3). The power of our meta-analyses to detect an association between the \(FTO\) variant \(rs8044769\) and OA is sufficient (>90%) for the knee and hip strata (table 2) but ranges from 0.34 to 0.80 for the hip and knee BMI-stratified analyses (table 3).

We further investigated the direction of causation between overweight status and OA using a mendelian randomisation approach and we computed the summary effect of \(rs8044769\) on overweight status, which results in an OR = 1.13[1.07–1.19], (p = 1×10\(^{-6}\)), and of overweight status on risk of knee OA and hip OA (table 2). Overweight status in the current study samples results in an OR = 3.30[3.02–3.62] for knee OA and OR = 2.00[1.82–2.21] for hip OA. We then estimated the expected effect of the \(G\) allele on risk of knee OA if the effect on OA is due to the effect of \(rs8044769\) on overweight, which would result in OR = 1.16. This value is higher than the upper CI for the observed effect (OR = 1.08[1.02–1.14]). The observed effect for hip OA (OR = 1.04[0.98–1.11]) is similar to the expected effect (OR = 1.09) of \(rs8044769\).

**DISCUSSION**

Variation in the \(FTO\) gene is associated with obesity although the exact mechanism by which \(FTO\) functions in obesity has not been elucidated. A recent study by the arcOGEN Consortium established an association of \(rs8044769\) at the BMI-associated \(FTO\) and knee and/or hip OA reaching almost genome-wide significance in the female stratum. \(FTO\) demonstrated expression within OA joint tissues (cartilage, tendon, ligament, meniscus, synovium, fat pad and osteophyte) and control fracture neck-of-femur joint tissues but it is unclear whether its expression is modulated by OA.\(^{11}\) The discovery and replication studies did not match cases and controls for BMI as the discovery dataset employed population-based controls lacking BMI information. The authors investigated whether the association with this variant was attenuated after adjustment for BMI using a subset of arcOGEN cases and disease-free TwinsUK control data with BMI information and found a substantial attenuation of the association suggesting that the \(FTO\) gene exerts its effect on OA through obesity. However, due to limited power, no attempt was performed to stratify these analyses by either OA site or BMI category.\(^{11}\)

Another concomitant report that evaluated the genetic overlap between OA and BMI using fully overlapping samples to the arcOGEN GWAS reported the same conclusion for a variant in the \(FTO\) locus, \(rs12149832\), which is 3773 kb away and strongly correlated with \(rs8044769\) (\(r^2=0.7\)) and is thus likely to represent the same signal.\(^{23}\) Since it is not clear which causal variant(s) underlie the association between BMI and SNPs in \(FTO\)\(^3\) and OA and SNPs in \(FTO\)\(^{11}\)\(^{23}\) we decided to

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**Table 2** Meta-analysis summary statistics for the association of allele G at \(rs8044769\) with hip or knee OA

<table>
<thead>
<tr>
<th>OA site</th>
<th>Covariates</th>
<th>N cases/controls</th>
<th>*F.E. OR (95% CIs)</th>
<th>F.E. p value</th>
<th>t.R.E. OR (95% CIs)</th>
<th>t.R.E. p value</th>
<th>tq p value</th>
<th>f²</th>
<th>†Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hip</td>
<td>Sex</td>
<td>4355/4967</td>
<td>1.04 (0.98 to 1.11)</td>
<td>0.170</td>
<td>1.04 (0.97 to 1.12)</td>
<td>0.287</td>
<td>0.328</td>
<td>0.13</td>
<td>0.90</td>
</tr>
<tr>
<td>Hip</td>
<td>Sex, BMI</td>
<td>4355/4967</td>
<td>1.00 (0.94 to 1.08)</td>
<td>0.936</td>
<td>0.98 (0.90 to 1.08)</td>
<td>0.749</td>
<td>0.166</td>
<td>0.36</td>
<td>0.90</td>
</tr>
<tr>
<td>Hip</td>
<td>Sex, BMI, age</td>
<td>4258/4788</td>
<td>1.00 (0.94 to 1.08)</td>
<td>0.97</td>
<td>1.00 (0.93 to 1.08)</td>
<td>0.929</td>
<td>0.312</td>
<td>0.16</td>
<td>0.89</td>
</tr>
<tr>
<td>Knee</td>
<td>Sex</td>
<td>5409/5362</td>
<td>1.08 (1.02 to 1.14)</td>
<td>0.009</td>
<td>1.08 (1.00 to 1.16)</td>
<td>0.056</td>
<td>0.138</td>
<td>0.38</td>
<td>0.94</td>
</tr>
<tr>
<td>Knee</td>
<td>Sex, BMI</td>
<td>5409/5362</td>
<td>0.99 (0.93 to 1.05)</td>
<td>0.666</td>
<td>0.99 (0.93 to 1.05)</td>
<td>0.704</td>
<td>0.338</td>
<td>0.12</td>
<td>0.94</td>
</tr>
<tr>
<td>Knee</td>
<td>Sex, BMI, age</td>
<td>5228/5183</td>
<td>0.96 (0.90 to 1.03)</td>
<td>0.298</td>
<td>0.97 (0.90 to 1.05)</td>
<td>0.484</td>
<td>0.227</td>
<td>0.26</td>
<td>0.93</td>
</tr>
</tbody>
</table>

*F.E. Fixed effects.
†Power has been calculated for α = 0.05, risk allele frequency=0.5 and effect size=1.1, as estimated in the arcOGEN replication only GWAS.\(^{11}\)
Follow-up the association of the variant that showed the strongest evidence for association with OA in the arcOGEN GWAS.11

In this study, we have investigated whether rs8044769 is associated with hip or knee OA independently of BMI by performing a large-scale meta-analysis across seven cohorts enabling us to increase the sample size by 55% knee OA cases, 57% hip OA cases and 69% of controls over the discovery study. This gave us sufficient power to perform analyses stratified by joint, joint and gender, and by joint and BMI category, unlike the original report, which examined BMI adjustment in females only. We find no evidence for association between this variant and OA consistent with what is known about the role of the FTO gene product, namely, that it is likely to be important to the control of energy homeostasis. As such, it is difficult to envisage a direct influence on the development of a joint pathology like OA except through its role on body mass.

The data presented here can be interpreted in the context of mendelian randomisation supporting the causal role of overweight on OA. Although the association between BMI and OA is well known, it could be a comorbidity that accompanies the disease.14 15 The genetic results shown by our study indicate that, on the one hand, overweight appears to be indeed causative of OA as we find an association between OA and FTO, which is fully accounted for by the role of FTO on overweight and disappears once we adjust for BMI or for BMI stratum. Furthermore, the observed and expected associations between FTO and hip OA are very similar. On the other hand, we observe a larger correlation between overweight on knee OA than what can be explained merely if overweight is causative of knee OA suggesting that there may be synergistic effects between overweight and knee OA due to, for example, lifestyle factors or lack of mobility. Hence, although overweight may be a cause of OA, the comorbidity and lack of mobility that results from knee OA may be resulting in further risk of overweight.

We note some study limitations. First, the statistical power of the current study to detect association of the FTO SNP and OA is sufficient in the overweight stratum (73%–80%) but is modest for the normal weight and obese strata (34%–47%). Thus, we cannot exclude the possibility that a very modest association may be present among normal weight individuals, which our study failed to detect. On the other hand, overweight individuals constitute the majority of OA cases and represent over 70% of the total joint replacement cases.2 The fact that our study finds no evidence of association with FTO in the overweight stratum despite being sufficiently-powered, suggests no direct implication of the FTO gene in susceptibility to OA. We also note that our study has combined both OA cases from population-based cohorts and severe OA cases from case/control studies recruited via secondary care (see online supplementary methods). However, we believe that this does not present a limitation of the current study because in the arcOGEN GWAS the FTO variant was more strongly associated when all cases (ascertained either by radiography or by total joint replacement) were included in the analysis.11

In summary, unlike the original report of the FTO association with OA, which was able to adjust for BMI only on a modest subset of female cases and controls, the present study gives a definitive answer showing that the effect of this variant on OA is solely due to its effect on BMI. Moreover, having tested the association in the context of mendelian randomisation, the results in this report indicate that overweight is on the causal pathway to OA rather than the inverse, although OA-induced inactivity may also be having an adverse effect on knee OA.

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Contributors All authors contributed to the study design, data interpretation and the final manuscript. In addition GJ, LLL, SAD, MD, TS, NKA and MW evaluated study subjects. AMV and KP analysed and interpreted the data and prepared the manuscript. AMV supervised the study.

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Ethics approval Each of the participating studies obtained approval from the appropriate ethics committee.

Provenance and peer review Not commissioned; externally peer reviewed.

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