CONCISE REPORT

Genetic contribution to radiographic severity in osteoarthritis of the knee

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ABSTRACT
Objective Knee osteoarthritis (OA) has a significant genetic component. The authors have assessed the role of three variants reported to influence risk of knee OA with p<5×10−8 in determining patellofemoral and tibiofemoral Kellgren Lawrence (K/L) grade in knee OA cases.

Methods 3474 knee OA cases with sky-line and weight-bearing antero-posterior x-rays of the knee were selected based on the presentation of K/L grade ≥2 at either the tibiofemoral or patellofemoral compartments for one or both knees. Patients belonging to three UK cohorts, were genotyped for rs143383, rs4730250 and rs11842874 mapping to the GDF5, CD05 and MCF2L genes, respectively. The association between tibiofemoral K/L grade and patellofemoral K/L grade was assessed after adjusting for age, gender and body mass index.

Results No significant association was found between the rs4730250 and radiographic severity. The rs11842874 mapping to MCF2L was found to be nominally significantly associated with patellofemoral K/L grade as a quantitative trait (p=0.027) but not as a binary trait. The GDF5 single nucleotide polymorphism rs143383 was associated with tibiofemoral K/L grade (B=0.05 95% CI 0.02 to 0.08 p=0.0011).

Conclusions Our data indicate that within individuals affected by radiographic knee OA, GDF5 has a modest but significant effect on radiographic severity after adjustment for the major risk factors.

INTRODUCTION
Osteoarthritis (OA) is the most common joint disorder1 and has an important genetic component associated with its development.2 Many genes contribute modestly to the risk for developing OA.2,3 To date, variants in three genes have been reproducibly associated in European-descent populations with knee OA with p<5×10−8. These are: a variant in the promoter region of the growth differentiation factor 5 (GDF5) gene,4 a variant mapping to a gene cluster in chromosome 7 including the component of oligomer golgi complex 5 (COG5) gene6 and a variant in the MCF2 cell-line-derived transforming sequence-like (MCF2L) gene.6 The role of GDF5 in the pathogenesis of OA has been widely explored2 but little is known about the biological processes influenced by the other two variants.

The most common definition of OA for genetic studies has been a radiographic one, based on the Kellgren-Lawrence (K/L) system5 using the cut-off value of 2 as a case definition, which corresponds to x-ray-revealed presence of definite osteophytes and possible joint space narrowing. However, the presence of osteophytes is extremely common in the general population,9 and a K/L grade=2 is not necessarily of clinical concern and, in fact, it rarely leads to joint replacement.10–12 An important aspect to investigate is the extent to which genetic variation influences radiographic severity. In three independent study cohorts from the UK, we have investigated the role of the variants so far identified to be reproducibly associated with knee OA in Caucasians. We have assessed their role among individuals with radiographic evidence of knee OA to the extent of radiographic damage, as defined by the K/L grade, at both the tibiofemoral and patellofemoral compartments.

SUBJECTS AND METHODS
Patients
Knee OA patients, taken from two case-control cohorts, Genetics of Osteoarthritis and Lifestyle (GOAL) Study and the Nottingham Case-Control Study, were included. Patients recruited from the community selected from a population-based study, the Herfordshire Cohort Study, were also included. A description of each cohort is detailed in the online supplementary methods.

Genotyping
Genotyping of the rs143383, rs4730250 and rs11842874 single nucleotide polymorphisms (SNPs) was carried out by Kbioscience Ltd, Hertfordshire, UK, using the KASPar chemistry. Further details on quality control in the genotyping are shown in the online supplementary methods.

Statistical analysis
The association between SNPs and radiographic severity of knee OA was first assessed by linear regression using as outcome variables, the K/L grade at the tibiofemoral and patellofemoral compartments, and as independent variables the number of risk alleles (0,1 or 2) at each SNP. Age, sex and body mass index (BMI) were included as covariates. Mean K/L values adjusted for covariates and cohort of origin for each genotype were derived from ANOVA.

Significant results were also tested using logistic regression analyses (adjusting for age, sex and BMI) comparing patients with K/L=2 with patients with K/L≥3. Meta-analyses of data from different cohorts were performed using R V2.13.1 (http://
www.r-project.org/). Random effects models were not used as no heterogeneity was seen in any of the analyses. The statistical power to detect associations with severity of OA, given the number of individuals sampled at an α level = 0.05, was calculated separately for the two SNPs that showed nominal associations with severity of OA based on the risk allele frequency of each marker using a normal approximation, using the Quanto Software V1.2.4.

RESULTS

The descriptive characteristics of the study cohorts, including the distribution of K/L grades at the tibiofemoral and patellofemoral grades, are presented in table 1.

The individual association between the polymorphisms and the measures of severity of knee OA for each cohort adjusting for age, sex and BMI were computed, and the resulting coefficients were meta-analysed (table 2). Of the three variants, the risk allele at the GDF5 SNP is significantly associated with higher tibiofemoral K/L grade even after adjusting for the six tests carried out. The MCF2L variant was only nominally significantly associated with patellofemoral K/L and is not significant if adjusted for multiple tests (table 2).

The test results with K/L grade might be reflecting the already known association with risk of OA. We therefore performed a sub-analyses on tibiofemoral K/L and GDF5 and patellofemoral K/L and MCF2L only among patients affected at the tibiofemoral and patellofemoral compartments, respectively (K/L≥2 at the relevant compartment). There is a clear linear relationship between the mean tibiofemoral K/L grade (adjusted for covariates) and the number of predisposing T alleles carried at rs143383 (figure 1A). The difference observed between genotypes is 0.055 of K/L grade, and the current study has a power of 69% to detect such difference with p<0.05. The rs11842874 homozygote for the protective G allele does not have a lower mean patellofemoral K/L than the heterozygote carrier of the risk allele A. The average difference between genotypes is 0.027 of K/L grade. The study has a power of 10% for such an effect size given the allele frequency (figure 1B). The forest plots illustrating the association coefficients and the overall meta-analysis summary statistic of the individual cohort, are also shown for this sub-analysis in figure 1C for rs143383 and tibiofemoral K/L, achieving a p=0.0011 by meta-analysis; in figure 1D, we show the association between rs11842874 and patellofemoral K/L which achieves a p=0.027 by meta-analysis.

K/L grade is a discrete measure and it is not truly normally distributed. We therefore ran logistic regressions for the two nominally significant results. We assessed the GDF5 effect including knee OA cases with tibiofemoral K/L=2 versus K/L≥3 (figure 1E) and the MCF2L effect comparing patellofemoral K/L=2 versus K/L≥3 (figure 1F). The GDF5 result remains statistically significant (p=0.008), but the association of MCF2L with patellofemoral grade is not statistically significant (p=0.08). Thus, the association between GDF5 and tibiofemoral grade is not a statistical artifact due to the non-normality of the distribution of K/L grades.

DISCUSSION

We have shown that two of the three genes which have, to date, been convincingly implicated in genetic risk of knee OA also correlate with the extent of radiographic damage at the
tibiofemoral or patellofemoral compartments. This observation suggests that the mechanisms by which GDF5 influences risk of OA underlie biological processes involved in joint damage, which may be due to an association with disease progression, or this may be due to an influence of genotype on age of onset so that patients who develop the disease earlier in life had more years at risk for progression.

The rs143383 variant maps to the promoter in the GDF5 gene and has been shown to influence expression levels of the gene product, with the predisposing allele T resulting in lower expression of this chondroprotective growth factor. On the other hand, the MCF2L gene product has been implicated in peripheral nervous system cell migration as modulated by neurotrophins and, hence, it has been hypothesised that its role in OA could be via an effect on nociception. The weak correlation found with patellofemoral radiographic grade, suggests that this gene might play a role in joint damage and indicates the need for studies on the functional relevance of this molecule in the biology of joints. No genetic studies have been published specifically on either of the knee compartments, and the association we report with severity does not reach genome-wide significance. However, our results suggest that genetic variation may...

Figure 1 Association between knee K/L grade and gene variants: (A) mean tibiofemoral (TF) K/L grade ±SE for each GDF5 rs143388 genotype adjusted for cohort, age, sex and BMI. (B) mean patellofemoral (PF) K/L grade ±SE or each MCF2L rs11842874 genotype adjusted for cohort, age, sex and body mass index. (C) Forest plot for association between TF K/L and rs143388 for each study cohort, and fixed-effects meta-analysis summary statistics. Only patients with TF K/L ≥2 are included. (D) Forest plot for association between PF K/L and rs11842874 for each study cohort and fixed-effects meta-analysis summary statistics. Only patients with PF K/L ≥2 are included. (E) Forest plot for association between TF K/L = 2 cases versus TF K/L ≥3 and rs143388 for each study cohort, and fixed-effects meta-analysis summary statistics. (F) Forest plot for association between PF K/L = 2 cases versus PF K/L ≥3 and rs11842874 for each study cohort, and fixed-effects meta-analysis summary statistics. All results are adjusted for age, sex and body mass index.
influence specific joint compartments and, given the differences in biomechanics between tibiofemoral and patellofemoral compartments and the integral functioning of the patella within the quadriceps mechanism, this warrants further research into the genetic contribution to both forms of disease. We observed no association with the COG5 SNP. The role of this variant in OA remains unknown although it has been convincingly associated with risk of knee OA. Given the strong linkage disequilibrium in the region it is not possible, genetically, to determine which of the five genes in this gene cluster is the functional one.6

We note some study limitations. Our study has only tested samples from the UK; thus, our results may not generalise with respect to other ethnic groups. The association between patellofemoral grade and the MCF2L SNP is only nominally significant (p=0.027) using a linear regression approach, is not significant when severity is analysed as a binary trait, and does not stand a correction for multiple tests. Although potentially of interest, this observation needs to be validated in independent cohort studies.

On the other hand, the association between GDF5 and tibiofemoral grade remains significant after adjustment for multiple tests and, moreover, is statistically significant in the population-based cohort alone (Hertfordshire Cohort Study, p<0.006) and in the two case-control cohorts combined (p=0.028). This association remained significant when a binary definition of severity was used indicating that the lack of normality of the distribution of K/L grades was not a statistical artefact. Our data show a continuous effect between gene dosage and, presumably, the extent of joint damage detected by x-ray. In conclusion, we find that GDF5 influences the extent of radiographic damage, but not so the other two genes reported to affect risk of knee OA.

Contributors All authors contributed to the study design, data interpretation and the final manuscript. In addition, AMV analysed and interpreted the data and prepared the manuscript. SD, MW, NA, MD and CC evaluated the study subjects. AMV and MD finalised the manuscript. In addition, AMV prepared the CRF, undertook the genetic analysis, performed the statistical analysis, and interpreted the data. SD, MW, NA and CC evaluated the CRF.

Funding This study was supported by the European Commission framework programme 7.

Competing interests RAM is an employee and owns stock of Astra Zeneca plc. All other authors declare no conflict of interest.

Patient Consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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