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The genetic architecture of cam morphology
A GWAS meta-analysis of alpha angle suggests cam-type morphology may be a specific feature of hip osteoarthritis in older adults

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Objectives

To examine the genetic architecture of cam morphology, using alpha angle (AA) as a proxy measure, we conducted an AA genome wide association study (GWAS), followed by Mendelian randomisation (MR) to evaluate its causal relationship with hip osteoarthritis (HOA).

Methods

Observational analyses examined associations between AA derived from hip DXA scans in UK Biobank (UKB), and radiographic HOA (rHOA) and subsequent total hip replacement (THR). Afterwards, an AA GWAS meta-analysis was performed (n=44,214), using AA previously derived in the Rotterdam Study (RS). Linkage disequilibrium score regression assessed the genetic correlation between AA and HOA. Genetic associations with $P<5x10^{-8}$ instrumented AA for two-sample MR.

Results

DXA-derived AA showed expected associations between AA and rHOA (OR 1.63 [95% CI 1.58-1.67]) and THR (HR 1.45 [1.33-1.59]) in UKB. The heritability of AA was 10% and AA had a moderate genetic correlation with HOA (r_g=0.26 [0.10-0.43]). Eight independent genetic signals were associated with AA. Two-sample MR provided weak evidence of causal effects of AA on HOA risk (inverse variance weighted (IVW): OR=1.84 [1.14-2.96], P 0.01). In contrast, genetic predisposition for HOA had stronger evidence of a causal effect on increased AA (IVW: β=0.09 [0.04-0.13], P 4.58 x 10^{-05}).

Conclusions

Expected observational associations between AA and related clinical outcomes provided face-validity for the DXA-derived AA measures. Evidence of bidirectional associations between AA and HOA, particularly in the reverse direction, suggests that hip shape modelling secondary to a genetic predisposition to HOA contributes to the well-established relationship between HOA and cam morphology in older adults.

Introduction

Cam morphology describes a non-spherical femoral head and has been associated with hip osteoarthritis (HOA) (1, 2). Longitudinal studies have shown that cam morphology precedes HOA and from this causation has been inferred (2, 3), prompting research into the benefits of surgical correction (4, 5). That said, observational studies showing temporal associations still suffer from confounding hindering causal inferences (1, 6, 7).

Alpha angle (AA), a measure of femoral head sphericity, is widely used to define cam morphology when exceeding a pre-specified limit, which varies between studies (4, 8). Cam morphology may develop in adolescence due to antero-lateral femoral head offset or increased impact as the growth plate fuses leading to greater bone deposition (9, 10). However, a similar morphology may also develop in later life as a consequence of modelling changes occurring as part of the osteoarthritic process (11). The Croft scoring for HOA specifically recognises abnormal hip shape as the most advanced stage of osteoarthritis (OA) (12).

Femoro-acetabular impingement (FAI) has been proposed to explain the causal pathway between cam morphology and HOA (13). FAI syndrome encompasses individuals with hip pain, coexistent with cam morphology on imaging and specific examination findings (14). FAI syndrome is seen predominantly in younger adults before the onset of HOA. Clinical trials of surgical interventions to remove cam lesions in this population have demonstrated an improvement in short-term activities of daily living and hip-related quality of life (4, 5). As well as potentially improving hip pain, these surgical procedures have been suggested to prevent the development or progression of HOA (5).

One method to derive causal inferences from observational data is Mendelian randomisation (MR) which uses genetic loci as instrumental variables and largely removes the effects of confounding and reverse causation (15). The UK Biobank study (UKB), a cohort study of adults aged 40-69 years at inception, provides a large sample size required to study the relationship between hip shape and HOA using MR (16, 17). In this study, we aimed to (i) provide face validity for our novel automated method for deriving AA from hip DXA scans in UKB by confirming expected relationships with HOA, (ii) perform an AA GWAS meta-analysis to establish the genetic architecture of cam morphology, and (iii) use MR analysis based on genetic instruments identified in (ii) to establish whether there is a causal relationship between increased AA and HOA.

Patients & Methods

Alpha Angle

This study included UKB participants, aged 44 to 82 years, with a left hip dual-energy X-ray absorptiometry (DXA) scan (iDXA GE-Lunar, Madison, WI). Outline points were automatically placed around each hip and features of radiographic HOA (rHOA) were measured semi automatically as previously described (16, 18, 19). AA was estimated using the outline points that excluded identified osteophytes (Figure 1) and a previously published Python code (20, 21). This study also included individuals from the Rotterdam Study (RS), aged 45 years and older who had AA measured from anterior-posterior pelvic radiographs using similar methods (see Supplementary Methods) (22). Ethics approval was given by the appropriate body for each study (see Ethics approval statement).

Outcome measures of osteoarthritis and observational associations in UK Biobank

UKB participants were asked whether they had hip pain for >3months via questionnaire on the same day as their DXA scan. Hospital diagnosed HOA was based on hospital episode statistics (HES) data, termed HES OA, as was total hip replacement (THR) (18). Logistic regression was used to examine associations between AA with clinical outcomes, apart from with THR which was examined using Cox proportional hazard modelling. Further sensitivity analyses were performed defining cam morphology as an AA ≥60º (see Supplementary Methods).

Alpha angle genome-wide association study

AA was standardised to create a Z-score (standard deviation (SD)=1, mean =0). Subsequently, a GWAS meta-analysis of AA was conducted between a GWAS in each study (UKB n=38,173), RS1 (n=2,970), RSII (n=1,817) & RSIII (n=1,254)). EasyQC was used to clean and harmonise the data (23), SNPs with a minor allele frequency (MAF) <0.01 and imputation

score <0.4 were removed. Meta-analysis between the studies was performed using fixed-effects inverse variance weighting using the METAL software package (24). A threshold of P-value $<5x10^{-8}$ was used to define genome-wide significance as is done routinely (25). The independent SNPs of interest for AA were identified using the linkage disequilibrium (LD) clumping method (see MR methods section). A sensitivity analysis using GCTA-COJO was used to verify that the independent SNPs selected were also conditionally independent of each other (26). Genetic correlations and heritability were estimated using LD score regression (LDSR) (27). See the Supplementary Methods for further details.

Downstream analyses

To identify genes responsible for genetic associations, colocalisation was used to relate genetic signals found through GWAS to expression quantitative trait loci (eQTL) (28). The latter represent the genetic association between a locus and messenger ribonucleic acid (mRNA) expression of a specific gene. eQTL data were examined from an online database (GTEx) and from human cartilage tissue that is not readily available in GTEx (29). The presence of colocalisation was defined as a posterior probability (PP) of >80% and suggestive if PP >60% (30). In addition, genetic loci were examined to see if they were predicted to be non-coding regulatory regions. Subsequently, immunohistochemistry staining in human knee osteochondral tissue was examined for any gene which colocalised in human cartilage. For further details see the Supplementary Methods.

Mendelian randomisation

MR was used to look for evidence of a causal effect between AA and HOA using genetic instruments as proxies (15). Our AA GWAS meta-analysis provided genetic instruments for AA. The HOA genetic instruments were based on a GWAS of hospital diagnosed HOA in

UKB. Bi-directional two sample MR was conducted using the inverse variance weighted method along with sensitivity analyses. These sensitivity analyses relax the assumption of no horizontal pleiotropy, assuming uncorrelated pleiotropy (MR-Egger), or relax the assumption about the number of invalid instruments (weighted median, simple mode and weighted mode). Single SNP and leave one out analyses were performed to check that results were not driven by individual variants (17). Further, we used causal analysis using summary effect estimates (MR-CAUSE) to examine for evidence of causality whilst considering the effects of both correlated and uncorrelated horizontal pleiotropy (31). The MR-STROBE guidelines, governing MR study reporting, provided a framework for this analysis (32). Further details are in the Supplementary Methods.

Glossary of terms

A glossary of methodological terms is available in the Supplementary Methods.

Results

Observational associations

In order to provide validity for our DXA measurement of AA, the cross-sectional association of AA with clinical HOA outcomes was examined in our UKB population (n=40,337, Supplementary Table 1). The mean AA was 47.8º (31.8-115.0) with a positively skewed distribution (Supplementary Figure 1) similar to a previous study (33). In both unadjusted and adjusted analyses, higher AA was associated with hip pain (adjusted results: OR 1.15 [95% CI 1.11-1.19]), rHOA grade ≥2 (1.63 [1.58-1.67]), HES OA (1.44 [1.35-1.54]) and subsequent THR (HR 1.45 [1.33-1.59]) (Table 1). Similar associations were seen when investigating cam morphology as a binary variable (Supplementary Table 2).

Alpha Angle GWAS

The GWAS meta-analysis comprised 44,214 participants (Supplementary Figure 2 & Supplementary Table 3). Eight statistically independent genome-wide significant signals were observed (see Table 2 for summary, Supplementary Table 4 for GCTA-COJO results, Supplementary Figure 3 for Manhattan plot, and Supplementary Figure 4 for locus zoom plots). The QQ plot showed some genetic inflation (λ 1.08) which was expected given UKB provides most of the sample (Supplementary Figure 5). SNP trait heritability was modest (h^2 0.10). Rs561578905 was the only genome-wide significant hit after meta-analysis that was not present in RS. To mitigate the effects of this, the SNP in highest LD (rs7302982, r^2 0.77) was used instead for meta-analysis in RS. Three SNPs showed weak evidence of heterogeneity (rs7571789: I^2 53, heterogeneity P 0.09; rs10478422 I^2 33, P 0.21; rs561578905 I^2 25, P 0.26) (Supplementary Figure 6). The lead 8 SNPs showed the same direction of effect in a GWAS of cam morphology as a binary variable (AA ≥60º, n=38173 in UKB) albeit with p-values that did not reach our genome-wide significance threshold (Supplementary Table 5). The closest

gene to each independent SNP associated with AA was initially used to label the loci, namely *TGFA, TNFAIP8, TFB1M-TIAM2, LMX1B, GRK5, SOX5, CYP19A1* and *UQCC1*) (Table 2). *TGFA, LMX1B, SOX5, CYP19A1* and *UQCC1-GDF5* loci have previously been associated with OA (34). The *LMX1B* locus shared the same lead SNP for AA and OA whereas *SOX5, TGFA, UQCC1-GDF5* AA SNPs were in moderate-high LD (r^2 0.30, 0.65 & 0.79 respectively) and *CYP19AI* showed only very weak LD (r^2 0.06) with their OA equivalents.

Downstream analysis of alpha angle hits

In eQTL analyses, AA genetic association signals for rs10478422, rs62578126 and rs1048584 respectively co-localised with mRNA expression of *TNFAIP8* (cultured fibroblasts, sample n=504, PP 0.97)*, LMX1B* (adipose tissue, sample n=663, PP 0.96), and *CLDN20*/*RP11-477D19*/*TFB1M* (cultured fibroblasts, sample n=504, PP 0.87/0.90/0.66), suggesting these genes underlie the genetic associations observed (Supplementary Table 6). In further eQTL studies based on human cartilage samples, the AA genetic association signal at the *TNFAIP8* locus colocalised with the *TNFAIP8* mRNA expression in highly degraded human cartilage (sample n=115, PP 0.97, Supplementary Figure 7). No other SNPs showed evidence of colocalisation with eQTL data from less or highly degraded cartilage (Supplementary Table 7). We evaluated the likely effect of identified SNPs on DNA-protein interactions using the RegulomeDB database, which predicts the impact of the base change on DNA binding of transcriptional regulators. Rs7571789 (*TGFA*), rs6595186 (*TNFAIP8)*, rs62578126 (*LMX1B)*, rs561578905 (*SOX5)* and rs246939415 (*CYP19A1)* were all predicted to affect enhancer or promotor activity (Supplementary Table 8), suggesting they may be directly responsible for the genetic association observed.

Given the finding that the AA genetic association signal at the *TNFAIP8* locus colocalised with *TNFAIP8* expression in highly degraded human cartilage, we used immunohistochemistry to further explore *TNFAIP8* expression in human knee cartilage and underlying bone (n=4). TNFAIP8 immunopositivity was localised to chondrocytes and osteocytes within the osteochondral tissue samples (Figure 2-A). An increase in percentage immunopositivity in both chondrocytes ($P=5.1x10^{-3}$) and osteocytes ($P=2.5\ x10^{-3}$) was seen in highly degraded compared to less degraded tissues (Figure 2-B).

Genetic correlations

The inter-study AA genetic correlation was reasonable (rg=0.57 [0.05-1.09] but the estimate was unreliable due to the small size of the RS GWAS (Supplementary Table 9). There was a moderate genetic correlation between AA and HOA (r_g=0.26 [95% CI 0.10-0.43]) and minimum joint space width (r_g= -0.31 [95% CI -0.46- -0.15]) with an inverse relationship with the latter as expected. There was no or very limited evidence of a genetic correlation with hip pain, height, body mass index, bone mineral density or fracture (Supplementary Table 9).

Mendelian randomisation

We subsequently performed an MR analysis of AA versus HOA risk, using the 8 SNPs identified above as our genetic instrument for AA (Table 2). The mean F-statistic for our AA genetic instrument was 31.5, indicating acceptable instrument strength. IVW analysis, the primary MR test statistic, provided weak evidence of an effect of increasing AA on HOA risk (OR per SD change in AA 1.86 [1.09-3.15]) (Table 3). Additional MR sensitivity analyses, including MR Egger, showed no evidence of a causal effect of AA on HOA risk (Figure 3). IVW and MR Egger Q statistics were 56.1 and 54.4 indicating heterogeneity and possible pleiotropy. The individual AA SNP effects for HOA are shown in Supplementary Table 5.

Further sensitivity analyses, in the form of leave one out and single SNP analyses were employed, however similar effect estimates were observed (Supplementary Figures 8&9), suggesting no single SNP was responsible for the heterogeneity.

For MR analyses in the opposite direction, the HOA GWAS (323,948 participants) identified 34 independent SNPs, with a mean F-statistic of 45.0 indicating acceptable instrument strength (Supplementary Table 10). In IVW analyses, there was reasonably strong evidence of a causal effect of increasing HOA risk on AA (β 0.09 [0.04-0.13], where β is SD change in AA per doubling in odds of OA) (Table 3). Sensitivity analyses were broadly in agreement (Figure 3). IVW and MR Egger Q statistics were 97.6 and 94.8 indicating heterogeneity and possible pleiotropy.

MR-CAUSE analyses, which use whole GWAS summary statistics, were performed to examine for causal effects, and for correlated pleiotropy whereby two traits are related to each other as a consequence of shared pathways (31). For AA versus HOA there was only weak evidence the causal model (model 2) performed better than the null model (model 1) (expected log pointwise predictive density (ELPD) -3.80, p=0.07, an ELPD ≤0 suggests model 2 fits the data better than model 1) (Supplementary Table 11). For HOA versus AA, there was stronger evidence that the causal model (model 2) performed better than the null (model 1) (ELPD -7.12, p=0.03), and better than the model assuming correlated pleiotropy (sharing model) (ELPD -3.65, p=0.02).

Discussion

This is the first GWAS meta-analysis of AA, which identified eight loci and indicated a heritable component of 10%. *TGFA, TNFAIP8, CLDN20-RP11-477D19-TFB1M, LMX1B, GRK5, SOX5, CYP19A1* and *UQCC1* were implicated in increasing AA with *TNFAIP8* showing the strongest gene-SNP relationship. Despite strong evidence of observational associations, bi-directional two-sample MR analyses provided limited evidence of a causal association between increasing AA and the development of HOA, but rather showed greater evidence that a genetic predisposition to HOA causes an increase in AA, as measured by DXA in this subject cohort.

Of these eight loci, the AA genetic association signal at the *TNFAIP8* locus colocalised with *TNFAIP8* mRNA expression in chondrocytes obtained from highly degraded, but not healthy, cartilage of the same individual, indicating *TNFAIP8* is the likely effector gene for this GWAS locus. This suggestion that *TNFAIP8* is preferentially expressed in degraded cartilage was further explored by subsequent immunohistochemistry staining which showed greater expression of *TNFAIP8* in chondrocytes and osteocytes from degraded joint tissue. TNFAIP8 is a tumour necrosis factor binding protein forming part of inflammatory, catabolic and neuro-sensitisation pathways during the pathogenesis of OA, and is also involved in cell apoptosis (35). Inflammatory changes are well recognised in HOA (36). Our findings raise the possibility that TNFAIP8 expression in osteocytes/chondrocytes contributes to hip shape modelling in the setting of OA, however further work is required to establish the mechanisms involved. How hip shape and an individual's AA changes over time is not well understood and it could be that the observed shape variation arises in later life as part of the HOA process, as distinct to cam morphology caused by altered shape development in adolescence (9).

AA genetic association signals at the rs62578126 and rs1048584 loci showed evidence of colocalisation with mRNA expression of *LMX1B* and *CLDN20- RP11-477D19 -TFB1M* in adipose tissue and fibroblasts respectively. Although the same colocalisation was not seen with mRNA expression in chondrocytes, this provides some evidence that these genes may be responsible for the genetic associations found with AA. These discrepancies might be explained by adipose and fibroblasts cells being the effector cells in this disease process or that the chondrocyte analysis had less power. *LMX1B* is the gene responsible for Nail-patella syndrome, which features poorly developed nails, patella and multiple limb malformations, and when knocked out in mice is associated with abnormal ventral limb development (37). *TFB1M* is important in preventing oxidative stress in mitochondria in the context of osteoarthritis (38). *CLDN20* is from the claudin family which are known to regulate osteoblast activity (39). *RP11-477D19* was also identified through co-localisation although little is known about its function.

The other SNPs provided no specific evidence of a causal gene through eQTL or colocalisation analyses. Nonetheless, many of the closest genes identified have previously been implicated in limb development and in OA. An intronic variant of *UQCC1* has been associated with developmental dysplasia of the hip (DDH) in a Han Chinese population (40). Interestingly, our *UQCC1* SNP (rs4911180) was in high LD with the lead *GDF5* OA SNP (r^2 0.79) from previous GWAS (2). The *UQCC1-GDF5* locus has been implicated in abnormal limb development and in OA with both genes commonly expressed in chondrocytes (41-43). *TGFA* (rs7571789) is a growth factor that is expressed in developing limbs in chicks (44) and is important in the development of OA (45). *SOX5* (rs561578905) has previously been shown to be critical in joint morphogenesis through its action on growth plate and articular chondrocytes (46). *CYP19A1* (rs146939415) has been associated with large joint osteoarthritis and is thought to act via

aromatase inhibition (47). Finally, *GRK5* is thought to regulate cartilage degradation and might be a possible therapeutic target for OA (48).

The eight independent AA SNPs had acceptable instrument strength, suggesting they were good genetic proxies for AA, when combined in subsequent MR analyses. However, there was only weak evidence of a causal link between increased AA and HOA. Interestingly, our bidirectional MR study provides stronger evidence that a genetic predisposition for HOA causes a higher AA, suggesting that the morphological features identified in this cohort may develop as part of, or in parallel to, the HOA process. Modelling changes are recognised by Croft grading in late-stage HOA (12), however we are not aware of any previous reports describing cam-like morphology as a specific feature of HOA. That said, in the same set of DXA images, we have recently found that hip shape changes suggestive of cam morphology are associated with more severe forms of HOA (11).

Although the evidence for a causal effect of a genetic predisposition to HOA on AA was somewhat stronger than that of AA on HOA, it should be noted that our genetic instrument for HOA was stronger than that for AA, reflecting the greater number of SNPs, so caution needs to be exercised in comparing these effects. Moreover, given that our cohort was aged 44 to 82 years, variation in AA may largely have reflected modelling changes that are part of the HOA disease process as opposed to cam morphology developing in earlier life. Alternatively, rather than bi-directional causal effects, it may be that our findings reflect common genetic pathways involved in the development of AA and HOA causing them to develop in parallel rather than as a consequence of one another. Though MR-CAUSE analysis favoured a causal over a shared model, this was only supported by weak evidence, and given the disparity in instrument strength between the two traits it is difficult to reach any firm conclusions. Nevertheless, to the

extent that a causal effect of a genetic predisposition of HOA on AA and/or shared genetic pathways contribute to associations between a raised AA and HOA, our results suggest that HOA should not necessarily be attributed to cam morphology especially in individuals where they might co-exist. This has implications when considering hip shape remodelling surgery in an older adult population.

In this study, AA as a continuous measure was used as a proxy for cam morphology. There are several limitations to this approach. For example, measuring AA on anterior-posterior images can be partially out of plane to the cam lesion, leading to an underestimation of size. Further, AA in UKB was obtained from DXA images rather than X-ray which might lead to discrepancies between the two. However, our observational analyses suggest we are measuring a clinically relevant shape signal from DXA images despite of this. Moreover, any failure to detect cam lesions that were present would tend to reduce associations to the null rather than produce biased estimates. When measuring AA in a population in later life there is the possibility that AA captures osteophytes or other hip shape features of OA. However, we rigorously excluded osteophytes and if these were included in our measures we might have expected to see a stronger causal relationship between AA and HOA. The newer generation of DXA scanner used in UKB has a similar resolution to plain radiographs, and some authors report comparable ability to detect osteophytes (49). Although we used AA as a continuous measure to optimise statistical power, this method has less clinical relevance than dichotomising into the presence or absence of cam morphology based on a pre-defined cut-off (2, 22). That said, we found similar observational relationship between AA and cam morphology, and HOA outcomes irrespective of whether we used a continuous (AA) or binary (cam) measure. Moreover, sensitivity analyses based on a binary AA variable showed similar but underpowered GWAS results. Further work is needed to recruit hip imaging cohorts that

are closer to UKB in terms of scale and phenotyping to allow for further replication of our results, and to extend our findings to more ethnically diverse populations. Finally, as with any MR study, several assumptions need to be made: the relevance assumption is satisfied by our ample F-statistics but the independence and exclusion restriction assumptions are harder to test (15). Several sensitivity analyses were performed to examine for possible pleiotropy which suggested this was present as has been discussed.

In conclusion, using a novel GWAS meta-analysis of AA, our study suggests that causal relationships between AA and HOA, and particularly a genetic predisposition for HOA and AA, contribute to observational associations between HOA and AA in an older population. Changes in AA as a consequence of HOA development may involve up-regulation of inflammatory/catabolic pathways, given our observation that *TNFAIP8,* one of the top AA-associated loci, was preferentially expressed in degraded human articular cartilage and bone. Further studies are justified to explore the contribution of increased AA to clinical consequence of HOA, and to determine whether targeting the underlying molecular mechanisms might prove useful in ameliorating these.

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Competing interests:

CL has a patent for a image processing apparatus and method for fitting a deformable shape model to an image using random forest regression voting. This is licensed with royalties to Optasia Medical. NH reports consultancy fees and honoraria from UCB and Kyowa Kirin. CLM reports consultancy fees and patent royalties from Flexion Therapeutics.

Ethical approval statement:

The National Information Governance Board for Health and Social Care and Northwest Multi-Centre Research Ethics Committee (11/NW/0382) and UK Biobank Ethics Advisory committee gave ethical approval for all work in this study undertaken with UK Biobank data (UK Biobank application number 17295). The South Yorkshire and North Derbyshire Musculoskeletal Biobank (REC reference: 20/SC/0144) ethics committee gave ethical approval for the immunohistochemistry experiments in this study. The Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG) gave ethical approval for all work undertaken with data from the Rotterdam Study included in this study. The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl) and into the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/ictrp/network/primary/en/) under shared catalogue number NTR6831. All participants provided informed consent for this study.

Data availability statement:

The alpha angle GWAS meta-analysis summary statistics have been uploaded to the GWAS catalog (https://www.ebi.ac.uk/gwas/, GCP000398). The individual level data from this study will be available from UK Biobank in a forthcoming data release. Users must be registered with UK Biobank to access their resources (https://bbams.ndph.ox.ac.uk/ams/).

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Table 1. Cross-sectional and longitudinal associations between standardised alpha angle and osteoarthritis outcomes in UK Biobank.

Cross-sectional analyses outcomes	Standardised Alpha Angle			
	Unadjusted		Adjusted	
	OR [95% CI]	*P*	OR [95% CI]	*P*
Hip Pain	1.05 [1.01-1.08]	6.98×10^{-03}	1.15 [1.11-1.19]	5.52×10^{-14}
rHOA grade ≥2	1.75 [1.70-1.79]	1.00×10^{-271}	1.63 [1.58-1.67]	4.00×10^{-271}
rHOA grade ≥3	2.00 [1.92-2.08]	3.00×10^{-244}	1.91 [1.83-2.00]	1.00×10^{-174}
rHOA grade 4	2.17 [2.00-2.35]	2.10×10^{-80}	2.10 [1.93-2.30]	4.70×10^{-62}
HES OA	1.35 [1.27-1.43]	1.70×10^{-23}	1.44 [1.35-1.54]	2.77×10^{-28}
Longitudinal analyses outcomes	HR [95% CI]	*P*	HR [95% CI]	*P*
THR	1.37 [1.27-1.49]	1.18×10^{-14}	1.45 [1.33-1.59]	2.10×10^{-17}

Logistic regression was used to examine these associations apart from with total hip replacement which was examined using Cox proportional hazard modelling. OR/HR presented are per standard deviation increase in alpha angle. Adjusted model includes age, sex, height, weight. OR – odds ratio, CI – confidence interval, rHOA – radiographic hip osteoarthritis, HR – hazard ratio, P – p-value, HES OA – hip diagnosed hip osteoarthritis, THR – total hip replacement

Table 2. The top independent single nucleotide polymorphisms associated with alpha angle.

RSID	Closest gene	CHR	BP	EA	NEA	EAF	Beta	*P*
rs7571789	*TGFA*	2	70714793	T	C	0.48	0.04	7.52×10^{-09}
rs10478422	*TNFAIP8*	5	118747441	T	C	0.30	0.04	9.64×10^{-10}
rs1048584	*TFB1M*	6	155578599	A	T	0.39	-0.04	7.67×10^{-09}
rs62578126	*LMX1B*	9	129375338	T	C	0.37	-0.04	9.00×10^{-09}
rs10787959	*GRK5*	10	121131313	A	G	0.28	-0.04	1.08×10^{-08}
rs561578905[†]	*SOX5*	12	24206118	A	C	0.27	0.05	3.37×10^{-08}
rs146939415	*CYP19A1*	15	51522210	C	G	0.01	0.17	2.47×10^{-08}
rs4911180	*UQCC1*	20	33972948	A	G	0.63	-0.04	1.25×10^{-11}

Results presented are from the fixed-effects meta-analysis after linkage disequilibrium clumping. Only SNPs with MAF >0.01 and p $<5 \times 10^{-8}$ are listed. UK Biobank GWAS adjusted for age, sex, genetic chip and 20 principal components and Rotterdam Study GWAS adjusted for age, sex and 4 principal components. CHR – chromosome, BP – Base position, EA – effect allele, NEA – non-effect allele, EAF – effect allele frequency, P - p-value. [†]rs561578905 was not available in Rotterdam and rs7302982 (r^2 0.77) was used instead.

Table 3. Bi-directional Mendelian randomisation results comparing the causal effects between alpha angle and hip osteoarthritis.

	Exposure AA, Outcome HOA		Exposure HOA, Outcome AA	
MR Method	OR [95% CI]	*P*	Beta [95% CI]	*P*
Inverse variance weighted	1.84 [1.14-2.96]	0.01	0.09 [0.04-0.13]	4.58×10^{-05}
MR Egger	1.22 [0.18-8.37]$^{+}$	0.84	0.15 [0.01-0.30]†	0.05
Weighted median	1.22 [0.93-1.59]	0.16	0.08 [0.04-0.12]	7.77×10^{-05}
Simple mode	1.33 [0.91-1.93]	0.16	0.10 [0.00-0.19]	0.05
Weighted mode	1.18 [0.92-1.51]	0.27	0.12 [0.02-0.21]	0.02

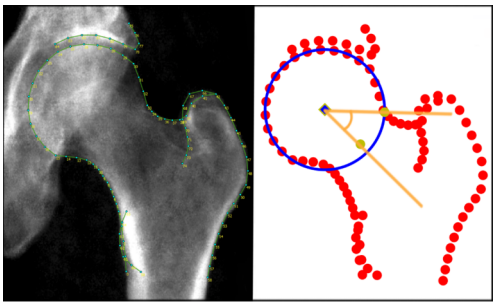
$^{+}$MR Egger intercept 0.02, p-value 0.68. †MR Egger intercept -0.01 p-value 0.34. AA – alpha angle, HOA – UK Biobank GWAS of hospital diagnosed hip osteoarthritis, MR – Mendelian randomisation. OR – odds ratio, per standard deviation change in alpha angle. Beta – per doubling in odds of hip osteoarthritis.

Figure Legends

Figure 1. Calculating alpha angle automatically in UK Biobank.
Left – UK Biobank DXA image with outline points marked and lines connecting the points. Right - the same points are visualised in Python, where a circle of best fit is plotted, and the AA is calculated from the femoral neck mid-point (yellow) and the point at which the femoral neck intersects the circle (yellow). In this individual the AA is 41.7º.

Figure 2. Immunohistochemistry localisation of TNFAIP8 within human osteochondral tissues.
A: TNFAIP8 immunohistochemistry staining was identified in chondrocytes and osteocytes particularly in highly degraded (High Grade) tissues, IgG controls were negative, Middle zone cartilage shown within images. Scale bar = 50μm. (Insert shows zoomed cells) B: Percentage immunopositivity.

Figure 3. Bi-directional Mendelian randomisation results comparing the causal effects between alpha angle and hospital diagnosed hip osteoarthritis in the UK Biobank study.
A – shows the MR analyses using eight genetic instruments for alpha angle (AA) as the exposure and hospital diagnosed hip osteoarthritis (HOA/HES OA) as the outcome. B – shows the MR analyses using thirty-four genetic instruments for HOA as the exposure and AA as the outcome.



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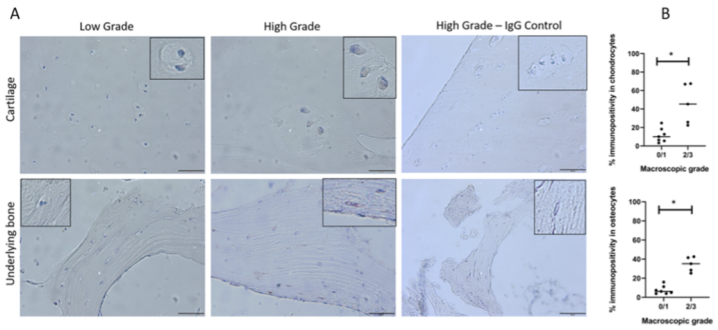

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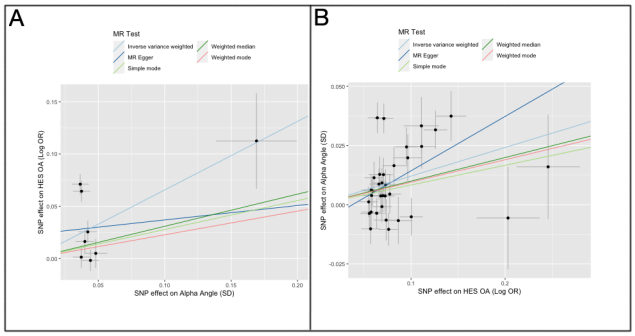

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