**FEMORAL NECK WIDTH GENETIC RISK SCORE IS A NOVEL INDEPENDENT RISK FACTOR FOR HIP FRACTURES**

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**ABSTRACT**

Femoral neck width (FNW) derived from dual energy X-ray absorptiometry (DXA) scans may provide a useful adjunct to hip fracture prediction, by providing depth information missing from bone mineral density (BMD) measurements. Therefore, we investigated whether FNW is related to hip fracture risk independently of FN-BMD, using a genetic approach. FNW was derived from points automatically placed on the proximal femur using hip DXA scans from 38,150 individuals (mean age 63.8 years, 48.0% males) in UK Biobank (UKB). GWAS identified 71 independent genome-wide significant FNW SNPs, comprising genes involved in cartilage differentiation, hedgehog, skeletal development, in contrast to SNPs identified by FN-BMD GWAS which primarily comprised runx1/Wnt signalling genes (MAGMA gene set analyses). FNW and FN-BMD SNPs were used to generate genetic instruments for multivariable Mendelian randomisation (MVMR). Greater genetically determined FNW increased risk of all hip fractures (OR 1.53; 95% CI 1.29-1.82 per SD increase) and femoral neck fractures (OR 1.58;1.30-1.92), but not trochanteric or forearm fractures. In contrast, greater genetically determined FN-BMD decreased fracture risk at all four sites. FNW and FN-BMD SNPs were also used to generate genetic risk scores (GRSs), which were examined in relation to incident hip fracture in UKB (excluding the FNW GWAS population; n=338742, 3222 cases) using a Cox proportional hazards model. FNW GRS was associated with increased risk of all incident hip fractures (HR 1.08;1.05-1.12) and femoral neck fractures (HR 1.10;1.06-1.15), but not trochanteric fractures, whereas FN-BMD GRS was associated with reduced risk of all hip fracture types. We conclude that the underlying biology regulating FNW and FN-BMD differs, and that DXA-derived FNW is causally related to hip fractures independently of FN-BMD, adding information beyond FN-BMD for hip fracture prediction. Hence, FNW derived from DXA analyses or a FNW GRS may contribute clinically useful information beyond FN-BMD for hip fracture prediction.

**Key words**

Dual energy X-ray absorptiometry (DXA); bone mineral density (BMD); hip geometry; genome-wide association study (GWAS)

**INTRODUCTION**

Hip fractures account for the greatest impact of osteoporosis in terms of mortality, morbidity, and health economic impact 1. DXA-derived BMD is widely used to evaluate hip fracture risk, for which femoral neck (FN) BMD has greater predictive value compared with measurements at other sites 2. X-ray attenuation, used by DXA to estimate BMD, reflects both bone density and depth of bone. Given the lack of correction for depth, DXA-derived BMD is expressed as g/cm2 and referred to as an “areal” bone density. Alternative methods for fully correcting BMD for bone size have been proposed. For example, re-calibrating lumbar spine BMD to estimate true “volumetric” bone density by dividing lumbar spine BMC by bone area (BA) raised to the power of 1.5 (based on the assumption that a vertebra represents a cuboid) corrected ethnic differences in lumbar spine BMD due to size differences3. However, the same geometric model does not apply to the hip.

Current methods for deriving BMD are well established, and there is a case for retaining these but combining with a separate measure of bone size. Since the height of the FN region of interest (ROI) on DXA scans is fixed, FN-BA solely depends on average femoral neck width (FNW). As the cross section of the femoral neck approximates to a circle, FNW may provide a reasonable estimate of depth within the FN ROI. Hip structural analysis (HSA), developed over 25 years ago, provides an automated means of deriving FNW from hip DXA scans, as well as other geometric indices and estimates of hip strength 4. Using this method, Rivadeneira et al found that as well as a lower FN-BMD, hip fracture cases had greater FNW 5, consistent with the expected reciprocal relationship between FNW and “volumetric” FN-BMD. This raises the possibility that prediction of hip fracture by “areal” FN-BMD might be preferentially enhanced by the addition of FNW, through the provision of missing depth information at the FN ROI. Since HSA or equivalent software is widely available, if confirmed, such understanding could be readily applied to improve hip fracture prediction by DXA through combination of FN-BMD and FNW results.

In the present study, we examined whether FNW contributes to hip fracture prediction independently of FN-BMD, using a genetic approach. First, we aimed to perform a genome wide association study (GWAS) of FNW, derived from automated annotation of hip DXA scans obtained in 38,150 individuals in UK Biobank (UKB) from which minimum FNW could be calculated (Fig S1). Results were then used to provide genetic instruments in Mendelian randomisation (MR) analyses to determine if FNW is causally related to hip fracture risk, including multivariable MR (MVMR) to establish if any such effect is independent of FN-BMD. Finally, given the recent development of genetic risk scores (GRS) for BMD to predict fractures 6, we examined whether combining a FN-BMD GRS with a FNW GRS provides greater prediction of hip fractures compared with use of a single GRS alone.

**METHODS**

**Study population**

The UKB is a prospective cohort study which recruited 500,000 adults from the United Kingdom, aged between 37 and 73 years at a baseline visit which took place between 2006-2010 7. The participants have undergone comprehensive genetic and physical phenotyping (see website for comprehensive catalogue of variables available http://biobank.ctsu.ox.ac.uk/crystal/). The study is overseen by the Ethics Advisory Committee and received approval from the National Information Governance Board for Health and Social Care and Northwest Multi-Centre Research Ethics Committee (11/NW/0382), all participants provided informed consent for this study. As part of the UKB extended imaging study, which commenced in 2014, a number of imaging modalities, including dual-energy X-ray (DXA) are being collected 8. Information on hip fracture was obtained from linkage to the hospital episode statistics (HES) database. All UKB participants were linked, both prospectively and retrospectively at baseline. HES records began on 1.4.97, and end date for data capture for the present study was 30.9.21. The maximum duration of follow-up for hip fracture from the baseline visit was 15.5 years.

**FNW measurement**

We used left hip DXA scans to train an 85-point Statistical Shape Model based machine-learning system (Figure S1A) to outline the proximal femur and acetabulum in all available images as of April 2021 9. A custom Python 3.0 script was developed to calculate the minimum FNW in the FN region (accessible online 10). The pixel dimension data stored in DXA DICOM images was converted to millimetres (mm). FNW was defined as the shortest distance measured between the superior and inferior femoral neck. The inferior side of the FN was mapped with points 6-12, and the superior side with points 32-38. A line-segment approach was used to automatically calculate the narrowest distance between these points (Figure S1A & B). A description of this approach has been published previously 11.

**Genetic analyses**

***Preparation and Quality control of genetic data in UKB***

Genotyping, imputation and quality control (QC) were performed by UKB as previously described 7 (see supplementary methods).

***FNW GWAS***

To test the association between genetic variants and FNW, FNW was stratified by sex and adjusted for age, genotyping chip and the first 20 ancestry principal components. Residuals resulting from female and male analyses were standardised (mean=0, SD=1), and then combined into a single outcome for GWAS. We used a linear mixed model assuming an additive allelic effect implemented in BOLT-LMM (v2.3) to account for cryptic population structure and relatedness. GWAS involved high quality genome-wide imputed v3 genetic data (~10 million SNPs, INFO > 0.3, MAF >= 1%), SNPs reaching genome-wide significance (5×10-8) were taken forward for conditional association analysis. The same methods were also used to generate a GWAS for hip axis length (HAL), derived from hip outline points as previously described 11.

***GCTA-COJO: multi-SNP-based conditional & joint association analysis***

In order to detect multiple independent association signals at each of the genome-wide significant loci, we applied approximate conditional and joint genome-wide association analysis 12 in conjunction with a UKB reference panel using GCTA v1.93 software. Conditionally independent variants at GWAS significance level were annotated with the closest gene using bedtools 13 v2.3.0 and the Hg19 Gene list available from <https://www.cog-genomics.org/plink2/>.

***Look ups, MAGMA gene-set analysis and Gene-set enrichment analysis in FUMA***

Independent FNW signals were looked up in publicly available FN-BMD summary statistics 14 (Tables 2A and 2B & Figure S3A).To gain an overview of which biological pathways are involved, FNW and FN-BMD 14 GWAS summary statistics were uploaded to FUMA web-based platform 15 to perform gene-set analysis with MAGMA v1.06 (Table 3, Table S4 and S5). Gene sets were obtained from MsigDB v7.0. A total of 15496 gene sets, including curated gene sets (5500) and GO terms (9996), were available for testing. Curated gene sets consist of 9 data resources including KEGG, Reactome and BioCarta (<http://software.broadinstitute.org/gsea/msigdb/collection_details.jsp#C2> for details). GO terms consists of three categories, biological processes (bp), cellular components (cc) and molecular functions (mf). All parameters were set as default. The output of gene-set analysis contains all genes in each significant gene set (Table 3, Table S4 and S5).

Based on the genes at the identified loci, we also performed a gene-set enrichment analysis as implemented by the FUMA SNP2GENE function (Table S6 and S7). We also looked up the independent FNW signals in the GWAS catalogue (Table S8).

***Genetic Correlation***

To estimate the genetic correlation between FNW and related traits, we used (cross-trait) linkage disequilibrium score regression (LDSR) 16 as implemented in the LD score tool LDSC available on github 17. This method uses the cross-products of summary test statistics from two GWASs and regresses them against a measure of how much variation each SNP tags (its LD score). The LDSR analyses were restricted to HapMap3 SNPs with MAF > 5% in the 1000 Genomes European reference population. We used pre-calculated LD scores from the same reference population (https://data.broadinstitute.org/alkesgroup/LDSCORE/). We estimated the genetic correlation between FNW and three related traits: hip fracture 18, fracture at any bone site 19 and FN-BMD 20, using public available GWAS summary statistics. We accounted for multiple testing by using a conservative Bonferroni correction for 3 tests (p = 0.05/3 = 0.017).

***Mendelian Randomization***

To assess the effects of FNW on the risk of fractures at different bone sites, we performed two-sample Mendelian randomization (MR) analyses. We used a multivariable MR (MVMR) approach to estimate the independent causal associations for genetically determined FNW and FN-BMD with risk of fractures at different bone sites. Genetic instrument variables for the FNW exposure were derived from the present GWAS on FNW while genetic instruments for FN-BMD were derived from previous GWAS on FN-BMD 20,21. The highest available number of FN-BMD signals (n=49) was identified in the FN-BMD GWAS by Estrada et al 21. However, HapMap imputation was used in that early GWAS meta-analysis, hindering ability to examine FN-BMD associations for FNW signals identified in the more detailed imputation panel used by UKB. We therefore selected the genome-wide significant (GWS) FN-BMD signals from Estrada et al as instruments for FN-BMD, but used effect estimates from the later FN-BMD GWAS study by Zheng et al 20 (the imputation panel in the latter included the majority of FNW GWAS SNPs identified here, making MVMR feasible).

We only used variants that were available in both the present FNW GWAS and the FN-BMD GWAS by Zheng et al. The variants were required to have a MAF > 1% and to be associated with either FNW or FN-BMD at a GWS level (p<5×10-8). We selected instruments with a pairwise r2<0.01 (based on the European populations in LDlink) 22 to ensure that there was low correlation between instruments. One SNP associated with FNW was strongly correlated with one SNP associated with FN-BMD (r2=0.73). For this pair, we first selected the SNP that is most strongly associated with FN-BMD in the GWAS. In sensitivity analyses we selected the other SNP, that is most strongly associated with FNW, revealing virtually identical results 23. Two palindromic FNW SNPs with non-clear strand were removed from the analyses. After quality filtering 40 FNW SNPs and 40 FN-BMD SNPs remained. Using Steiger filtering, no more FNW SNPs were suggested to be removed. We estimated the F-statistic as a measure of instrument strength 24.

The outcome fracture associations (logistic regression adjusted for age,and sex) used in the 2-sample MR were derived from summary statistics from a previous GWAS on hip fractures (including 11.515 hip fracture cases25) or newly performed association analyses in UKB, excluding the FNW GWAS data set (Table S1). As the primary MR analyses, we used combined weighted estimates by IVW using fixed or random effects depending on Cochran’s Q statistic test of heterogeneity. We then used Weighted median MR as a sensitivity analysis and MR-Egger regression to test for possible directional horizontal pleiotropy. To reduce the possible impact of heterogeneity, we also performed sensitivity analyses excluding outliers of genetic instruments using MR-LASSO. The MR analyses were conducted using the R-package MendelianRandomization.

***Weighted Genetic Risk Scores (GRS)***

We defined a weighted GRS for FNW (FNW GRS) based on the 71 conditionally independent (COJO) significant SNPs identified in the present study. We also defined a GRS for FN-BMD (FN-BMD GRS) based on 49 SNPs previously identified to be associated with FN-BMD at GWS level 21. For each individual, the GRSs were defined as the weighted sum of SNP dosages, where SNP effects from the corresponding BMD GWAS were used as weights. The GRSs were standardized to have a mean of zero and SD of 1. The separate and combined associations for FNW GRS and FN-BMD GRS with incident fracture were calculated using Cox-regression in UKB samples excluding the samples used for the FNW GWAS. The effects are given as hazard ratios (HR) per standard deviation (SD) increase in GRS. The base model included sex and baseline age as covariates.

**RESULTS**

**Genome-wide association study**

The discovery set comprised 38,150 UKB participants with available FNW analyses, mean 63.8 years, of whom 48% were male (Table 1). We identified 71 independent signals at 61 loci passing GWS level (p<5×10−8; Tables 2A and 2B; Table S2 and Fig S2), of which 8 were low-frequency (MAF≤5% but >1%) and 63 were common (MAF>5%). These 71 signals explained 7.6% of the variance of FNW (Table S2) and showed limited associations with FN-BMD as evaluated in a previous FN-BMD GWAS data set 20 (Table S3, Fig S3A). Conversely, the previously identified 49 GWS FN-BMD signals showed limited associations with FNW in the present GWAS dataset (Fig S3B)

MAGMA gene set analyses of the FNW GWAS data identified different gene sets (representing cartilage differentiation, hedgehog signalling, skeletal development) compared with FN-BMD GWAS (runx1/Wnt signalling), demonstrating distinct underlying biology for the regulation of FNW and FN-BMD (Table 3, Table S4 and S5). This notion was further supported by clear differences in FUMA gene set enrichment for FNW and FN-BMD signals (Table S6 and Table S7), with the FNW GWAS comprising signals for hip dimensions, fat distribution and height, while the FN-BMD GWAS included signals for fracture risk and BMD at other sites.

Look-up in the GWAS catalogue of the 71 conditionally independent FNW signals including linked signals (r2>0.8) identified multiple signals for osteoarthritis, hip circumference and hip bone size (Table S7). We observed a strong genetic correlation for FNW with hip fractures (rg = 0.48) but not with fractures at any bone site (rg = 0.07) (Table S9). A modest inverse genetic correlation was observed for FNW with FN-BMD (rg = -0.20).A second parameter of hip geometry, HAL, could be derived from our automated annotation of hip shape, for which GWAS summary statistics were also obtained. Although HAL was strongly correlated genetically with FNW (rg = 0.61), this showed a considerably weaker genetic correlation with hip fracture (rg = 0.24).

**Mendelian randomization**

SNPs selected from the current FNW GWAS, and previous FN-BMD GWAS 26, provided well powered genetic instruments for subsequent MR analyses (F-statistic 40.6 and 31.0 respectively (Table S10)). We evaluated the causal associations for genetically determined FNW and FN-BMD with hip fractures using a hip fracture GWAS meta-analysis for the outcome analyses 18 (Table S11 and Fig S4). Univariate MR revealed a significant effect of both genetically determined FNW (OR=1.55, 95% CI=1.35-1.78, per SD increase) and genetically determined FN-BMD (OR=0.44, 95% CI=0.37-0.53) on hip fracture risk. Similar results were observed in MVMR including both genetically determined FNW (FNW OR=1.35, 95% CI=1.16-1.59) and genetically determined FN-BMD (FN-BMD OR=0.48, 95% CI=0.40-0.57; Table S11 and Fig S4) as exposures.

We next evaluated the effects of genetically determined FNW and FN-BMD on fractures at different bone sites in the UKB data set excluding subjects included in the FNW GWAS (Figure 1, Table S12, Fig S5-S8). MVMR revealed that high genetically determined FN-BMD was causally associated with reduced risk of all hip, femoral neck, trochanteric and forearm fractures (Figure 1, Table S12, Fig S5-S8). In contrast, high genetically determined FNW was causally associated with increased risk of all hip fractures (OR=1.53, 95% CI=1.29-1.82), and femoral neck fractures (OR=1.58, 95% CI=1.30-1.92), but not trochanteric (OR=1.18, 95% CI=0.86-1.61) or forearm (OR=0.99, 95% CI=0.88-1.11) fractures (Figure 1, Table S12, Fig S5-S8). Similar results were observed in univariate and multivariate MR analyses (Table S12).

As an alternative exposure in the MR analyses, we used a weighted GRS for FNW (FNW GRS, 71 SNPs). Using this FNW GRS as exposure, MR confirmed that FNW is causally associated with all hip (OR=1.64, 95% CI=1.34-2.02, per SD increase) and femur neck (OR=1.85, 95% CI=1.45-2.36) but not trochanteric (OR=1.22, 95% CI=0.81-1.83) fractures. In sex stratified analyses, the causal effects of FNW on all hip fractures and femoral neck fractures were slightly greater in women compared to men (Table S13). Collectively, these data demonstrate that genetically determined FNW is causally associated with risk of hip fractures, specifically that of femoral neck fractures.

**FNW GRS and FN-BMD GRS add independent information for prediction of incident hip fractures.**

We next determined whether the FNW GRS and/or FN-BMD GRS predict incident hip fractures**.** In separate models, a high FN-BMD GRS was associated with reduced risk of incident hip fracture at any site (HR 0.83; 0.80-0.86 per SD increase), whereas a high FNW GRS was associated with increased hip fracture risk (HR 1.09; 1.05-1.13; Fig 2, Table S14). The associations between the FNW GRS and hip fracture risk were more pronounced for femoral neck (HR 1.11; 1.06-1.16) compared with trochanteric (HR 1.03; 0.97-1.11) fractures. Similar results were observed in combined analyses (including both FNW GRS and FN-BMD GRS) and in models additionally adjusted for BMI, however the association between FNW GRS and hip fracture risk was attenuated by approximately 25% following separate adjustment for height and weight to account for effects of body size (Table S14). An age interaction (P=1.9×10-3 for the age/FNW GRS interaction term) was observed for the association between the FNW GRS and femoral neck fracture risk, reflected by a higher HR for younger (age≤71; 1.14; 1.08-1.21) compared with older (age>71; 1.06; 1.00-1.13) individuals (Fig 2, Table S15). Similar associations for the FNW GRS with incident hip fractures were observed in both men and women (Fig 2, Table S15). Additionally, we observed an interaction between the FN-BMD GRS and the FNW GRS for the prediction of hip fracture risk (p = 0.04 for the interaction term), where individuals with genetically determined low FN-BMD had a more pronounced increased risk from genetically determined high FNW.

Finally, we examined additive associations for binarized high risk FNW GRS and, binarized high risk FN-BMD GRS with hip fractures risk. Participants in UKB were classified as high risk (yes/no) for high FNW based on their FNW GRS and at high risk (yes/no) for low FN-BMD based on their FN-BMD GRS. Participants were divided into four different groups (group 1 = no/no; group 2 = yes/no; group 3 = no/yes; and group 4 = yes/yes) based on their binarized FNW GRS and binarized FN-BMD GRS. We used the lowest risk group (group 1 = no/no) as reference to study the possible additive associations for the two different GRSs. We used three different cut-off limits as definitions of high risk for the two GRS (50%, 25% and 10 %; Fig 3). A high binarized FNW GRS was associated with high hip fracture risk while a low binarized FN-BMD GRS was associated with high hip fracture risk. Using the 10% cut-off for high risk, subjects in the high-risk GRS group for both FNW GRS and FN-BMD GRS (i.e. group 4 = yes/yes) had a more than two-fold increased risk of hip fractures compared with those in the low-risk group for both binarized GRSs (i.e. group 1 = no/no). Binarized FNW GRS and FN-BMD GRS contributed independently to hip fracture prediction. For example, on comparing groups 3 and 4, the high risk FNW GRS added information beyond the high risk FN-BMD GRS (Fig3, Table S16).

**DISCUSSION**

We investigated whether FNW contributes to hip fracture risk independently of FN-BMD, using a genetic approach. Having performed a GWAS of FNW derived from hip DXA scans in over 38,000 individuals in UKB, we identified 71 conditionally independent signals in 61 different loci explaining 7.6% of the variance of FNW, of which 70 signals represented novel genetic associations with femoral neck bone size (the FN-area signal at the HHP locus was previously reported by Styrkarsdottir et al27). The genetic architecture of FNW appeared to be distinct to that of FN-BMD, suggesting these two traits are in large part independent. Less than 20% of FNW genetic signals were nominally associated with FN-BMD. FNW showed a relatively weak genetic correlation with FN-BMD, and MAGMA gene set analysis revealed involvement of FNW and FN-BMD SNPs in distinct biological pathways.

Although FNW only showed weak genetic correlation with FN-BMD, it was correlated relatively strongly with hip fractures. Given the suggestion that FNW is largely independent of FN-BMD, we investigated whether FNW is causally related to risk of hip fracture, independently of FN-BMD. MVMR revealed that greater FNW, or a highly correlated hip shape parameter, increases the risk of any hip fractures, and that of femoral neck fractures specifically, despite adjustment for FN-BMD. In contrast to FNW which was only related to risk of hip fracture/femoral neck fracture, FN-BMD was also related to risk of trochanteric and forearm fractures.

A GRS based on genetic associations with FN-BMD has previously been proposed as an adjunct in clinical fracture prediction, either in isolation or in combination with clinical risk factors such as those included in FRAX 6,28. Therefore, we examined whether a GRS based on FNW might also have utility in hip fracture prediction. We found that both FNW and FN-BMD GRSs were independently related to risk of hip /femoral neck fracture, and that these exerted additive effects on hip fracture risk. For example, an individual with a GRS in the highest 10% risk category for both FNW and FN-BMD has an approximately two-fold increased risk of hip/femoral neck fracture, compared with a 25-35% increase based on either parameter alone. As well as improving use of GRSs to predict fractures by combining two independent scores, a FNW GRS may also prove useful due to its presumed independence to DXA BMD. This would represent an important advantage over a BMD GRS, which provides little additional predictive value if BMD is already known 29.

Though the present study focused on additive effects of GRSs for FNW and BMD on risk of hip fracture, it may be possible to use an equivalent approach based on measured parameters. In the present study, FNW was derived using a bespoke method based on points annotated as part of a separate study on hip shape 30. However, an equivalent measure, obtained using HSA software 4, is widely available (these were strongly correlated (r2=0.97) in a subset of 1744 DXA images where FNW was obtained using both methods). An alternative method would be to combine BMD with femoral neck bone area, which is provided routinely during hip DXA measurements, and also correlated strongly with FNW (r2=0.93).

When used alone, the FNW GRS had a clear independent relationship with hip fracture risk, in the opposite direction to that of FN-BMD and when combining both GRSs, a marked improvement in predictive ability was observed. One explanation for these findings is that the input of additional information about bone size, provided by FNW, enables a more accurate estimate of ‘volumetric’ BMD than that provided by ‘areal’ BMD, by providing missing information about depth. As discussed in the introduction, several approaches have been attempted to more fully account for bone size when evaluating BMD by DXA. Our results suggest that this concept can also be applied to GRSs used for fracture prediction. Rather than providing missing depth information, it may be that greater FNW per se has a negative effect on bone strength and fracture risk. For example, for a given cortical thickness, greater FNW is inversely related to resistance to buckling as reflected by buckling ratio 5 . On the other hand, FNW is positively related to bending strength as reflected by cross sectional moment of inertia 31; to the extent that both types of forces contribute to risk of hip fracture, whether FNW has any net direct effect on hip fracture risk is currently unclear. A further explanation is that, rather than a direct association, FNW is related to hip fracture risk through co-association with height, which is also positively related to risk of hip fracture 32, possibly because height is a proxy for leg length33.

Though the overall genetic correlation between FNW and FN-BMD was relatively weak, for those SNPs related to both traits, an inverse relationship between these two traits was generally observed. However, the *LRP5* locus was an exception, since this was positively related to both FN-BMD and FNW, suggesting that in contrast to other loci, the *LRP5* locus has a positive effect on both bone size and BMD. In-keeping with this suggestion, individuals with high bone mass as a consequence of a mutation in *LRP5* have been found to have both increased BMD and bone size, as reflected by tibial and radial cortical area and thickness 34. Although the *LRP5* locus appears to affect bone size of the skeleton as a whole, genetically determined FNW only influenced risk of femoral neck/hip fracture, suggesting fractures at the latter site are particularly influenced by bone geometry. This contrasts with genetically determined FN-BMD GRS, which influenced fracture risk at multiple sites, suggesting FN-BMD signals influence BMD throughout the skeleton. As well as being restricted to prediction of femoral neck/hip fractures, the FNW-GRS was more strongly related to risk of hip fracture in younger individuals. One possible explanation for this finding is the greater contribution to hip fracture of risk factors unrelated to skeletal fragility in older individuals, such as factors related to fall risk.

This paper reports the first FNW GWAS, which provided the basis for a novel FNW GRS which may have clinical utility as a BMD independent risk factor for hip fracture. In terms of limitations, though many novel loci related to FNW were identified, functional genomic studies intended to characterise genetic mechanisms contributing FNW were not undertaken as part of the current study. That said, MAGMA gene set analysis was used to characterise the biological pathways identified by our GWAS. These primarily comprised genes representing cartilage differentiation, hedgehog signalling, and skeletal development, consistent with determination of overall skeletal size. This contrasted sharply with findings for FN-BMD, which primarily identified Wnt signalling genes, consistent with the important role of Wnt signalling in the regulation of bone mass 35.

In terms of other limitations, UKB on which this GWAS was based is primarily comprised of Caucasians, and further studies are required to investigate whether the GRS has equivalent predictive value in other ethnic groups. To establish the clinical utility of our FNW GRS, further studies are required to confirm that this GRS predicts hip fracture independently of BMD, as well as established clinical risk factors. Furthermore, it should be acknowledged that although the FNW GRS may have clinical utility for predicting fractures, unlike BMD, this appears to be limited to hip fractures. Finally, although we have described relationships between genetically predicted FNW and BMD, and hip fracture, associations between hip fracture and directly measured FNW and BMD were not presented. The latter analyses are restricted to the sub-group with DXA data, and given this smaller sample and the shorter follow-up period, there were relatively few hip fractures on which to base analyses, limiting statistical power.The number of participants undergoing DXA scans in UK biobank, as well as the duration of follow up, is increasing substantially with time, and we plan to re-examine these relationships once more hip fracture cases are available.

In conclusion, our FNW GWAS demonstrates that the biology underlying this trait differs from that of FN-BMD. Consequently, whereas FNW or a highly correlated hip shape parameter is causally related to hip fractures, this is independent of FN-BMD, and DXA-derived FNW adds information beyond FN-BMD for hip fracture prediction. Based on the genetic evidence presented herein, we propose that FNW derived from DXA analyses or a FNW GRS may add clinically useful information beyond FN-BMD for hip fracture prediction.

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*Author contributions*

Contribution to study conception and design: JHT, MF, ML, CO

Contribution to acquisition of data: JHT, MF, BGF, SVH, RE, TC, CL, FRS, JSG, NCH

Contribution to analysis and interpretation of data: JHT, MF, JK, RMA, ML, CO

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All authors approved the final version

*Conflict of interest statement*

The authors have no conflicts to disclose

**FIGURE LEGENDS**

**Figure 1 Independent effects of genetically determined FNW and FN-BMD on fracture risk in UK-Biobank:** Multivariable Mendelian Randomization analyses to estimate the effect of genetically determined femoral neck width (FNW) and femoral neck bone mineral density (FN-BMD) on the risk of hip fracture, femoral neck fractures, trochanteric fractures, and forearm fractures in UK-Biobank. Both prevalent and incident fractures were included. Odds ratios (OR) and 95% confidence intervals are given. Blue = FNW and red = FN-BMD.

**Figure 2 Associations for femoral neck width (FNW) genetic risk score (GRS) and femoral neck bone mineral density (FN-BMD) GRS with incident fractures in UK-Biobank** The effects are given as hazard ratios (HR) per standard deviation (SD) increase in GRS. All models were adjusted for sex and baseline age, except the sex stratified models where sex was not included as a covariate. Age stratification was based on the median age at hip fracture (71.7 yrs.). Blue = FNW and red = FN-BMD.

\*Significant age interaction (p = 1.3×10-4) for FNW GRS

\*\*Significant age interaction (p = 1.9×10-3) for FNW GRS

**Figure 3 Additive associations for binarized high risk FNW GRS and, binarized high risk FN-BMD GRS with fractures risk.** Participants in UK Biobank were divided into four different groups (no/no, yes/no, no/yes and yes/yes) based on their binarized FNW GRS and binarized FN-BMD GRS, using three different cut-off limits as definitions of high risk for the two GRS (50%, 25% and 10 %).

**Table 1 Characteristics of UK Biobank Study participants in the femoral neck width GWAS**

|  |  |  |  |
| --- | --- | --- | --- |
|   | **All** | **Males** | **Females** |
|   | (N= 38150) |  (N= 18314) |  (N=19836) |
| Age (years) | 63.8 (7.5) | 64.5 (7.6) | 63.1 (7.4) |
| Weight (kg) | 75.4 (15.1) | 83.3 (13.4) | 68.2 (12.8) |
| Height (cm) | 170.2 (9.4) | 177.3 (6.6) | 163.7 (6.3) |
| FNW (mm) | 31.7 (3.5) | 34.6 (2.4) | 29.0 (2.0) |

Population characteristics of the UK-Biobank participants in the femoral neck width (FNW) GWAS with complete FNW and covariate data.

**Table 2A Conditionally independent genome wide significant variants for femur neck width (chromosomes 1-9)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|   |   |   |   |   |  | **FNW GWAS** |
| **SNP** | **Position** | **Closest gene** | **Distance to gene** | **EA** | **OA** | **EAF** | **Beta** | **SE** | **P** |
| rs2284747 | chr1:17306596 | MFAP2 | 0 | T | C | 0.52 | 0.04 | 0.01 | 1.4E-08 |
| rs2807365 | chr1:22485467 | WNT4 | 15005 | G | A | 0.32 | 0.04 | 0.01 | 2.5E-08 |
| rs143778922 | chr1:51037660 | FAF1 | 0 | GT | G | 0.09 | 0.09 | 0.01 | 4.0E-12 |
| rs2820449 | chr1:219714999 | SLC30A10 | 143771 | G | A | 0.66 | 0.05 | 0.01 | 7.5E-13 |
| rs767282530 | chr2:19727294 | OSR1 | 168880 | A | AGATT | 0.07 | 0.08 | 0.01 | 9.2E-10 |
| rs7591141 | chr2:42353031 | EML4 | 43460 | T | C | 0.23 | 0.05 | 0.01 | 3.3E-08 |
| rs57121650 | chr2:71944847 | DYSF | 30949 | T | C | 0.52 | 0.04 | 0.01 | 3.2E-08 |
| rs11684985 | chr2:72895293 | EXOC6B | 0 | A | G | 0.13 | 0.06 | 0.01 | 1.1E-08 |
| rs75495843 | chr3:38051211 | PLCD1 | 0 | A | G | 0.03 | 0.12 | 0.02 | 7.2E-10 |
| rs17235557 | chr3:56419866 | ERC2 | 0 | T | C | 0.67 | 0.04 | 0.01 | 1.5E-08 |
| rs7636776 | chr3:99840446 | CMSS1 | 0 | C | T | 0.41 | 0.06 | 0.01 | 2.4E-15 |
| rs6763927 | chr3:141140366 | ZBTB38 | 0 | T | A | 0.44 | 0.04 | 0.01 | 1.2E-08 |
| rs2707450 | chr4:17942560 | LCORL | 0 | C | T | 0.26 | 0.05 | 0.01 | 6.0E-10 |
| rs112728585 | chr4:81743389 | C4orf22 | 0 | C | A | 0.02 | 0.14 | 0.03 | 1.2E-08 |
| rs6824784 | chr4:82275412 | RASGEF1B | 72136 | G | A | 0.32 | 0.05 | 0.01 | 8.7E-10 |
| rs2131354 | chr4:145599908 | HHIP | 0 | A | G | 0.53 | 0.07 | 0.01 | 1.8E-21 |
| rs1507191 | chr4:151247236 | LRBA | 0 | C | T | 0.75 | 0.05 | 0.01 | 6.2E-10 |
| rs77844679 | chr5:170812216 | NPM1 | 1905 | G | C | 0.82 | 0.06 | 0.01 | 9.1E-10 |
| rs702101 | chr5:171276393 | FBXW11 | 12161 | A | G | 0.60 | 0.04 | 0.01 | 9.0E-09 |
| rs31778 | chr5:176522036 | FGFR4 | 0 | T | C | 0.29 | 0.05 | 0.01 | 1.2E-11 |
| rs806790 | chr6:26218920 | HIST1H2AE | 1209 | G | A | 0.58 | 0.04 | 0.01 | 1.3E-08 |
| rs112540634 | chr6:34623905 | C6orf106 | 0 | T | C | 0.14 | 0.06 | 0.01 | 1.5E-08 |
| rs2258604 | chr6:34997606 | ANKS1A | 0 | A | T | 0.66 | 0.05 | 0.01 | 1.8E-12 |
| 6:35038589\_CCT\_C | chr6:35038589 | ANKS1A | 0 | CCT | C | 0.98 | 0.18 | 0.03 | 9.0E-10 |
| 6:158783382\_AT\_A | chr6:158783382 | TULP4 | 0 | AT | A | 0.33 | 0.05 | 0.01 | 1.7E-10 |
| rs798565 | chr7:2752152 | AMZ1 | 0 | G | A | 0.70 | 0.05 | 0.01 | 2.9E-11 |
| rs148066163 | chr7:47169380 | TNS3 | 145373 | CAA | C | 0.28 | 0.04 | 0.01 | 1.6E-08 |
| rs42039 | chr7:92244422 | CDK6 | 0 | T | C | 0.24 | 0.06 | 0.01 | 2.1E-15 |
| rs34275932 | chr7:120816329 | CPED1 | 0 | C | G | 0.59 | 0.04 | 0.01 | 1.6E-09 |
| rs149882987 | chr7:148584494 | EZH2 | 3081 | G | A | 0.03 | 0.14 | 0.02 | 6.3E-11 |
| rs72656010 | chr8:57122215 | PLAG1 | 0 | T | C | 0.87 | 0.06 | 0.01 | 4.1E-10 |
| rs75810927 | chr8:69587226 | C8orf34 | 0 | C | A | 0.77 | 0.05 | 0.01 | 1.3E-08 |
| rs9298310 | chr8:79164782 | PKIA | 263593 | G | C | 0.29 | 0.07 | 0.01 | 1.5E-22 |
| rs28705285 | chr9:98279801 | PTCH1 | 462 | G | T | 0.24 | 0.05 | 0.01 | 4.0E-09 |
| rs762624732 | chr9:99106848 | SLC35D2 | 0 | T | TA | 0.20 | 0.05 | 0.01 | 3.4E-08 |
| rs10123619 | chr9:119353611 | ASTN2 | 0 | A | G | 0.15 | 0.06 | 0.01 | 5.9E-10 |

Conditionally independent significant signals within chromosomes 1-9 associated with femur neck width (FNW) in 38150 UKB participants. Results are presented as estimated association (beta) and standard error (SE) expressed per effect allele (EA). Beta, SE and P are from the conditional (COJO) analysis.

OA=other allele, EAF=effect allele frequency.

**Table 2B Conditionally independent genome wide significant variants for femur neck width (chromosomes 10-21)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|   |   |   |   |   |  | **FNW GWAS** |
| **SNP** | **Position** | **Closest gene** | **Distance to gene** | **EA** | **OA** | **EAF** | **Beta** | **SE** | **P** |
| rs10828316 | chr10:22838389 | PIP4K2A | 0 | C | A | 0.33 | 0.05 | 0.01 | 3.2E-10 |
| rs3740237 | chr10:32557592 | EPC1 | 0 | G | C | 0.86 | 0.06 | 0.01 | 8.1E-09 |
| rs12776235 | chr10:95019809 | MYOF | 46378 | T | C | 0.53 | 0.04 | 0.01 | 6.7E-09 |
| rs7952436 | chr11:67024534 | KDM2A | 0 | C | T | 0.92 | 0.12 | 0.01 | 2.4E-20 |
| rs923346 | chr11:68182375 | LRP5 | 0 | T | C | 0.83 | 0.06 | 0.01 | 1.5E-09 |
| rs11609223 | chr12:1419127 | ERC1 | 0 | G | T | 0.68 | 0.04 | 0.01 | 8.5E-09 |
| rs76895963 | chr12:4384844 | CCND2 | 0 | G | T | 0.02 | 0.19 | 0.03 | 2.4E-12 |
| rs11046703 | chr12:23114157 | ETNK1 | 270558 | C | T | 0.05 | 0.10 | 0.02 | 8.4E-11 |
| rs59932020 | chr12:24179601 | SOX5 | 75635 | T | A | 0.22 | 0.06 | 0.01 | 4.3E-12 |
| rs5029277 | chr12:28022055 | KLHL42 | 66082 | G | A | 0.78 | 0.07 | 0.01 | 5.2E-15 |
| rs11049385 | chr12:28320492 | CCDC91 | 0 | G | A | 0.31 | 0.05 | 0.01 | 2.2E-12 |
| rs10878984 | chr12:69828534 | FRS2 | 35596 | T | C | 0.34 | 0.05 | 0.01 | 3.6E-11 |
| rs7954185 | chr12:94096173 | CRADD | 0 | A | T | 0.49 | 0.05 | 0.01 | 5.2E-14 |
| rs71190381 | chr13:51120097 | DLEU1 | 0 | G | GAGTGA | 0.79 | 0.08 | 0.01 | 6.5E-23 |
| rs12889267 | chr14:21542766 | ARHGEF40 | 0 | A | G | 0.83 | 0.05 | 0.01 | 3.4E-09 |
| rs10083313 | chr14:53917808 | DDHD1 | 297808 | A | C | 0.27 | 0.05 | 0.01 | 9.1E-10 |
| rs28929474 | chr14:94844947 | SERPINA1 | 0 | T | C | 0.02 | 0.14 | 0.03 | 3.0E-08 |
| rs569147467 | chr14:103855865 | MARK3 | 0 | C | CA | 0.33 | 0.04 | 0.01 | 1.4E-08 |
| rs200675402 | chr15:86894913 | AGBL1 | 0 | A | AT | 0.97 | 0.13 | 0.02 | 2.5E-08 |
| rs8034564 | chr15:99190601 | IGF1R | 1600 | G | A | 0.42 | 0.04 | 0.01 | 1.0E-08 |
| rs30224 | chr16:14406119 | MKL2 | 45489 | T | C | 0.35 | 0.04 | 0.01 | 1.6E-09 |
| rs7223535 | chr17:29211667 | ATAD5 | 0 | G | A | 0.73 | 0.05 | 0.01 | 2.8E-10 |
| rs1043515 | chr17:36922196 | PIP4K2B | 0 | G | A | 0.57 | 0.05 | 0.01 | 2.2E-14 |
| rs9905385 | chr17:59498250 | C17orf82 | 7609 | A | G | 0.33 | 0.04 | 0.01 | 7.9E-09 |
| rs4141079 | chr17:59531402 | TBX4 | 0 | A | C | 0.74 | 0.05 | 0.01 | 4.3E-10 |
| rs4968440 | chr17:59613258 | TBX4 | 50787 | C | G | 0.36 | 0.04 | 0.01 | 3.6E-08 |
| rs17779649 | chr17:70372779 | SOX9 | 250218 | A | C | 0.87 | 0.08 | 0.01 | 1.2E-15 |
| rs9912553 | chr17:79959703 | ASPSCR1 | 0 | G | C | 0.72 | 0.05 | 0.01 | 5.5E-10 |
| rs4369779 | chr18:20735408 | CABLES1 | 0 | C | T | 0.79 | 0.05 | 0.01 | 1.1E-08 |
| rs1074047 | chr19:2158748 | AP3D1 | 0 | G | A | 0.48 | 0.05 | 0.01 | 2.9E-11 |
| rs742630 | chr20:31350664 | DNMT3B | 0 | C | G | 0.60 | 0.04 | 0.01 | 1.2E-08 |
| rs149142833 | chr20:32188142 | CBFA2T2 | 0 | C | T | 0.84 | 0.06 | 0.01 | 2.3E-09 |
| rs143384 | chr20:34025756 | GDF5 | 0 | G | A | 0.41 | 0.10 | 0.01 | 4.0E-48 |
| rs6063031 | chr20:45522102 | EYA2 | 1162 | A | G | 0.45 | 0.05 | 0.01 | 9.0E-13 |
| rs2298333 | chr21:39673981 | KCNJ15 | 0 | C | T | 0.43 | 0.04 | 0.01 | 1.5E-09 |

Conditionally independent significant signals within chromosomes 10-21 associated with femur neck width (FNW) in 38150 UKB participants. Results are presented as estimated association (beta) and standard error (SE) expressed per effect allele (EA). Beta, SE and P are from the conditional (COJO) analysis.

OA=other allele, EAF=effect allele frequency.

**Table 3 MAGMA gene set analysis**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **SET** |  | **VARIABLE** | **NGENES** | **P-VALUE** |
|  |  |  |  |  |
| **FNW** |  |   |   |   |
| \_SET1\_ | Curated\_gene\_sets:pid\_hedgehog\_2pathway | 22 | 9.5E-10 |
| \_SET2\_ | Curated\_gene\_sets:nikolsky\_breast\_cancer\_20q11\_amplicon | 31 | 2.9E-15 |
| \_SET3\_ | Curated\_gene\_sets:reactome\_ligand\_receptor\_interactions | 8 | 1.7E-06 |
| \_SET4\_ | Curated\_gene\_sets:reactome\_gli\_proteins\_bind\_promoters\_of\_hh\_responsive\_genes\_to\_promote\_transcription | 7 | 5.5E-09 |
| \_SET5\_ | GO\_bp:go\_cartilage\_development | 205 | 2.7E-11 |
| \_SET6\_ | GO\_bp:go\_regulation\_of\_cartilage\_development | 65 | 4.6E-09 |
| \_SET7\_ | GO\_bp:go\_osteoblast\_differentiation | 203 | 6.4E-08 |
| \_SET8\_ | GO\_bp:go\_skeletal\_system\_development | 513 | 2.4E-10 |
| \_SET9\_ | GO\_bp:go\_chondrocyte\_development | 47 | 1.0E-07 |
| \_SET10\_ | GO\_bp:go\_chondrocyte\_differentiation | 117 | 2.7E-16 |
| \_SET11\_ | GO\_bp:go\_regulation\_of\_chondrocyte\_differentiation | 47 | 2.9E-10 |
| \_SET12\_ | GO\_bp:go\_positive\_regulation\_of\_epidermis\_development | 37 | 8.1E-07 |
| \_SET13\_ | GO\_bp:go\_positive\_regulation\_of\_cartilage\_development | 30 | 4.9E-10 |
| \_SET14\_ | GO\_bp:go\_ossification | 373 | 6.3E-08 |
| \_SET15\_ | GO\_bp:go\_regulation\_of\_osteoblast\_differentiation | 111 | 2.3E-06 |
| \_SET16\_ | GO\_bp:go\_connective\_tissue\_development | 267 | 5.0E-08 |
| \_SET17\_ | GO\_bp:go\_positive\_regulation\_of\_chondrocyte\_differentiation | 20 | 8.5E-09 |
| \_SET18\_ | GO\_bp:go\_animal\_organ\_morphogenesis | 1027 | 2.4E-07 |
| \_SET19\_ | GO\_mf:go\_proximal\_promoter\_sequence\_specific\_dna\_binding | 529 | 2.2E-07 |
|   |   |   |   |   |
| **FN-BMD** |  |   |   |   |
| \_SET1\_ | Curated\_gene\_sets:reactome\_runx1\_regulates\_transcription\_of\_genes\_involved\_in\_wnt\_signaling | 5 | 4.3E-07 |
|   |   |   |   |   |
|  |   |   |   |   |

Significant gene sets from the MAGMA gene-set analysis of the femur neck width (FNW) GWAS and the femur neck BMD (FN-BMD) GWAS (PMID: 26367794): SET and VARIABLE: name of the gene set, NGENES: number of genes in the gene set. In total 15496 gene sets were available for testing. 15488 of them were represented in the FNW GWAS and 15485 were represented in the FN-BMD GWAS. A gene set was considered to be significant if p< 0.05/15496=3.2 x 10-6. mf = molecular function, bp = biological processes.

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