A GWAS in an extreme high bone mass population shows excess signal for genes associated with BMD in the normal population

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Introduction
Extreme high bone mass (HBM) may be monogenic (e.g. due to mutations in SOST or LRPS), oligogenic or polygenic, and may be due to variants in the same genes determining bone mineral density (BMD) as are found in the general population.

Aim
To determine the extent to which variation in 56 established BMD-associated loci cause raised BMD in an extreme UK-based HBM population.

Study populations
Cases:
(1) 258 unexplained HBM cases were recruited from 15 UK centres, by screening 335,115 DXA scans
• HBM was defined as either:
  a) L1 Z-score ≥+3.2 plus total hip Z-score ≥+1.2
  b) Total hip Z-score ≥+3.2 plus L1 Z-score ≥+1.2
• All patients and DXA images were reviewed
• SNPs with r²<0.8 were removed, leaving 181,323 SNPs.
• All patients and DXA images were reviewed to exclude known causes of raised BMD, including osteoarthritis
• Individuals with established SOST and LRPS mutations were excluded by Sanger sequencing (n=3)

(2) Ethnically-matched high BMD (n=1055) Anglo-Australasian Osteoporosis Genetics Consortium (AOGC) post-menopausal women, hip BMD Z-scores +4.0 to +1.5

Controls: 2 previously genotyped populations:
(1) Unselected (n=5667) The 1958 British Birth Cohort
(2) Ethnically-matched low BMD (n=900) Anglo-Australasian Osteoporosis Genetics Consortium (AOGC) post-menopausal women, hip BMD Z-scores -4.0 to -1.5

GWAS Methods
We performed a GWAS for HBM, genotyping 240 unrelated HBM cases using Infinium OmniExpress-12v1.0 DNA analysis beadchips and clustering using GenomeStudio software (illumina).

• Samples were assessed for cryptic relatedness, excess heterozygosity/missingness.
• SNPs with MAF<1%, and/or not in HWE were removed, leaving 181,323 SNPs.
• The dataset was imputed using the 1000 Genomes Project; SNPs with r² threshold>0.8 were retained.
• SNPs were tested for association with BMD using PLINK, assessed separately for each control group.

Results
HBM cases, 79% female had mean (SD) age 60.3 (11.2) years, BMI 31.1 (6.2), total hip Z-score 3.3 (1.0), L1 Z-score 4.2 (1.3). AOGC high BMD women had mean age 70.4 (8.3) with BMI 30.1 (5.5). AOGC low BMD women were aged 68.7 (8.8) with BMI 24.3 (4.8).

Figures 1 & 2 are Manhattan plots for the GWAS of firstly all SNPs and secondly just the established BMD SNPs. The association between HBM and the top 10 BMD-established SNPs are shown in Figure 3.

Results show over-representation of associations with BMD loci in HBM cases (Figure 4A [HBM vs. 1958 BBC], Figure 4B [HBM vs. low AOGC]). This over-representation was greater when HBM was compared against the extreme low BMD population than when analysed against the unselected population, despite the larger population used in the latter analysis.

Conclusions
Within our UK-based population association of unexplained extreme HBM showed over-representation in the 56 established BMD genes.

Results suggest HBM is, at least in part, of polygenic origin and is controlled by the same genes which determine BMD in the general population.

Studying extreme populations will enhance understanding of such genes determining BMD.

Whole-exome sequencing of this HBM population is currently underway to determine the exact variants contributing to HBM.

References
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