PP31

A GWAS in an extreme high bone mass population shows excess signal from 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 *LRP5*), 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[1].

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[2].

- 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 to exclude known causes of raised BMD, including osteoarthritis
- Individuals with established *SOST* and *LRP5* mutations were excluded by Sanger sequencing (n=3)

(2) Ethnically-matched high BMD (n=1055) Anglo-Australasian Osteoporosis Genetics Consortium (AOGC)[3] 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[4]

(2) Ethnically-matched low BMD (n=900) Anglo-Australasian Osteoporosis Genetics Consortium (AOGC)[3] 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 r^2 threshold≥0.8 were retained.
- SNPs were tested for association with BMD using PLINK, assessed separately for each control group.

Fig 1. Manhattan plot showing SNP associations comparing (HBM cases + AOGC high BMD cases) against AOGC low BMD controls

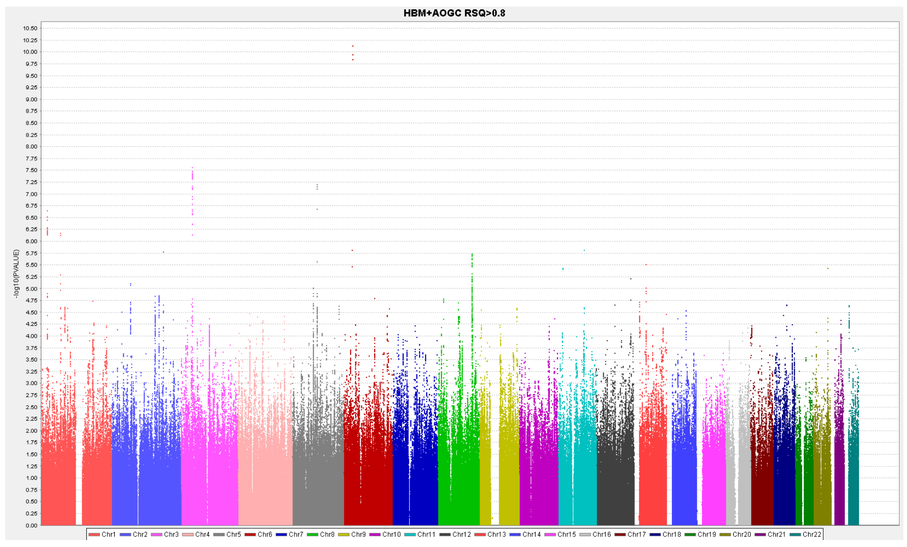
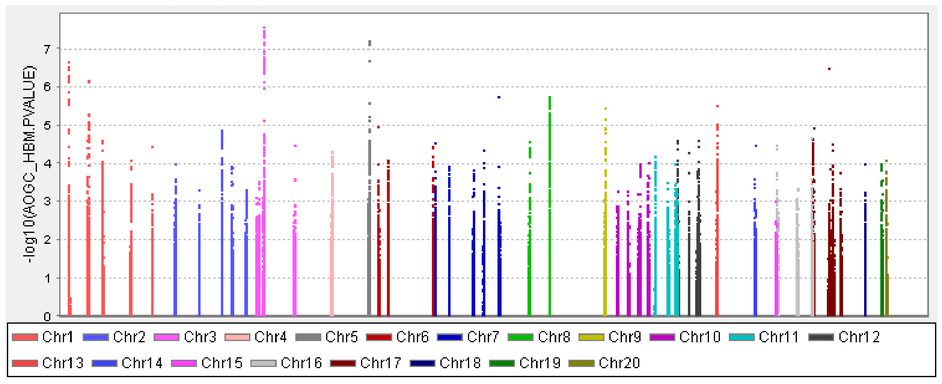


Fig 2. Manhattan plot restricted to established BMD-associated SNPs, comparing (HBM cases + AOGC high BMD cases) against AOGC low BMD controls



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.

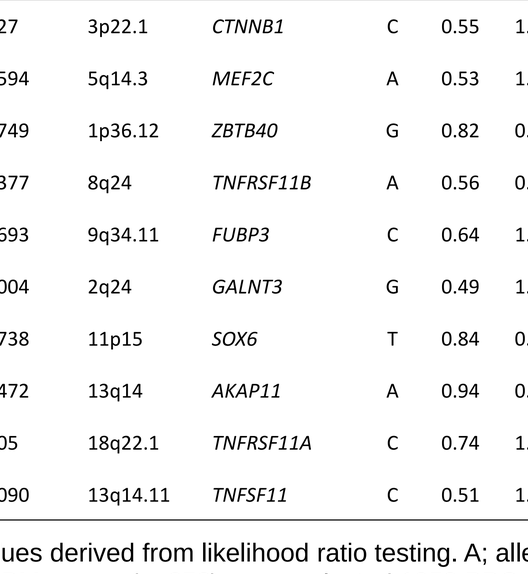
Results show over-representation of associations with BMD loci[3] in HBM cases (Figure 4A [HBM vs. 1958 BBC], Figure 4B [HBM vs. low AOGC]). This over-representation was greater when HBM was compared against the extreme low BMD population than when analysed against the unselected population, despite the larger population used in the latter analysis.

Fig 3. Estimated effects of top 10 established BMD SNPs in HBM cases in 3 analyses

SNP	Locus	Closest gene/ candidate	A	Freq.	HBM vs. 1958 British Birth Cohort		HBM vs. AOGC low BMD		HBM+AOGC high vs. AOGC low BMD	
					OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
rs430727	3p22.1	*CTNNB1*	C	0.55	1.45 (1.25, 1.64)	0.0001451	1.68 (1.47, 1.89)	7.8×10^{-7}	1.42 (1.30, 1.54)	2.3×10^{-8}
rs1366594	5q14.3	*MEF2C*	A	0.53	1.17 (0.99, 1.36)	0.09313	1.38 (1.17, 1.59)	0.002026	1.40 (1.28, 1.53)	5.2×10^{-8}
rs6426749	1p36.12	*ZBTB40*	G	0.82	0.87 (0.64, 1.10)	0.2259	0.65 (0.39, 0.91)	0.00129	0.65 (0.48, 0.81)	1.8×10^{-7}
rs2062377	8q24	*TNFRSF11B*	A	0.56	0.94 (0.76, 1.13)	0.5257	0.78 (0.58, 0.98)	0.01556	0.76 (0.63, 0.88)	9.5×10^{-6}
rs7851693	9q34.11	*FUBP3*	C	0.64	1.14 (0.94, 1.34)	0.1872	1.36 (1.14, 1.58)	0.005468	1.31 (1.19, 1.44)	2.5×10^{-5}
rs1346004	2q24	*GALNT3*	G	0.49	1.16 (0.97, 1.35)	0.13	1.30 (1.09, 1.51)	0.01364	1.30 (1.18, 1.43)	3.5×10^{-5}
rs7108738	11p15	*SOX6*	T	0.84	0.82 (0.59, 1.05)	0.09614	0.77 (0.50, 1.04)	0.05754	0.72 (0.56, 0.89)	7.5×10^{-5}
rs7326472	13q14	*AKAP11*	A	0.94	0.79 (0.43, 1.15)	0.2112	0.52 (0.09, 0.94)	0.003274	0.57 (0.28, 0.85)	5.6×10^{-5}
rs884205	18q22.1	*TNFRSF11A*	C	0.74	1.37 (1.13, 1.62)	0.007924	1.66 (1.40, 1.92)	0.0001063	1.34 (1.19, 1.49)	0.0001001
rs9533090	13q14.11	*TNFSF11*	C	0.51	1.32 (1.14, 1.51)	0.003315	1.56 (1.35, 1.76)	3.2×10^{-5}	1.28 (1.15, 1.40)	0.0001077

P values derived from likelihood ratio testing. A; allele. Freq; allele frequency of A. Results from 3 analyses are shown :
(1) HBM cases (n=258) vs. unselected 1958 BBC controls (n=5667), (2) HBM cases (n=258) vs. AOGC low BMD women (n=900), and
(3) HBM cases (n=258) combined with AOGC high BMD women (n=1055) vs. AOGC low BMD women (n=900)

Fig 4. Chi-Square Q-Q plot for 56 BMD loci, in 258 HBM cases vs.
(A) Low BMD controls (AOGC) and
(B) Unselected controls (1958 British Birth Cohort)



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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