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New sequence variants associated with bone mineral density

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In an extended genome-wide association study of bone mineral density among 6,865 Icelanders and a follow-up in 8,510 subjects of European descent, we identified four new genome-wide significant loci. These are near the *SOST* gene at 17q21, the *MARK3* gene at 14q32, the *SP7* gene at 12q13 and the *TNFRSF11A* (*RANK*) gene at 18q21. Furthermore, nonsynonymous SNPs in the *C17orf53, LRP4, ADAM19* and *IBSP* genes were suggestively associated with bone density.

Bone mineral density (BMD) is the single best predictor of fragility fractures[1,2] and is used as a reference standard for the description of osteoporosis[3]. It is a highly familial quantitative trait, with heritability estimates in the range of 0.6–0.8 (ref. 4), but is also influenced by environmental and medical factors.

We[5] and others[6] have recently published reports of genome-wide studies describing several loci for BMD. Both studies yielded a locus on 8q24 harboring the *TNFRSF11B* (also known as *OPG*, osteroprotegerin) gene. The study by Richards *et al.*[6] also confirmed the well-documented *LRP5* (low-density lipoprotein receptor–related protein 5) gene[7,8], whereas we reported loci at the *TNFSF11* (also known as *RANKL*, receptor activator of nuclear factor kB ligand) gene (13q14), the *ESR1* (estrogen receptor 1) gene (6q25), the MHC (major histocompatibility complex) region (6p21) and a locus on 1p36 that reached genome-wide significance. We also described additional loci that showed suggestive association but did not reach the genome-wide significance level, including the *LRP4* (low-density lipoprotein receptor-related protein 4) gene (11p11), the *TNFRSF11A* (also known as *RANK*, receptor activator of the nuclear factor kB) gene and the *SP7* (osterix) gene (12q13). We noticed a substantial excess of SNP associations compared to that expected, indicating that true additional association signals might still be found. To search for these additional variants, we expanded the genome-wide scan from 5,861 individuals to 6,865, followed up more of the associated signals and expanded the non-Icelandic replication samples from 3,750 individuals to 5,375 (**Supplementary Methods** online). For the follow-up study we selected the top 100 most significantly associated SNPs and the 20 most significantly associated nonsynonymous or potentially functional SNPs with BMD at either the hip or the spine (**Supplementary Tables 1** and **2** online). A total of 186 SNPs (accounting for overlaps between the hip and spine scans and failure of some SNP assays), representing 114 chromosomal loci, were genotyped in a two-stage follow-up strategy. We first genotyped a subset of a study population from Denmark ($n = 2{,}238$), selected SNPs with a P value (single-sided) less than 0.1 in this group—70 SNPs in total—and genotyped those in the remaining Danish samples ($n = 1{,}646$), the Australian Dubbo Osteoporosis Epidemiology Study (DOES) cohort ($n = 1{,}491$) and in an Icelandic replication set ($n = 3{,}135$).

For each phenotype, combining results from all sample sets, we set the threshold for genome-wide significant association at $P < 1.6 \times 10^{-7}$ (0.05/305,051). Additionally, a nominally significant association ($P < 0.05$) in the Danish and Australian sets combined was used as a further requirement to ensure that the overall significant association is robust across populations and not driven by the Icelandic samples only. In total, 21 SNPs satisfied both criteria for either skeletal site, 12 for BMD at the hip (**Supplementary Table 1**) and 14 for spine BMD (**Supplementary Table 2**). Sixteen of these markers are in regions we previously reported as genome-wide significant for hip or spine BMD, that is, 1p36, 6q25 (*ESR1*), 8q24 (*TNFRSF11B*) and 13q14 (*TNFSF11*)[5] (**Supplementary Table 3** online). Two of the regions that showed suggestive association in our previous study, 12q13 and 18q21, were genome-wide significant in the current dataset. The SNP rs10876432, located close to the *SP7* gene on 12q13, was significant for spine BMD ($P = 1.3 \times 10^{-7}$) and rs3018362 on 18q21, close to the *TNFRSF11A* gene, was significant for BMD at the hip ($P = 5.4 \times 10^{-8}$) (**Table 1**). In addition, the association of most of the previously reported markers was strengthened in this study as a result of the larger sample size (**Supplementary Table 3**); for example, rs3130340, located in the MHC region on 6p21, associated with spine BMD with a P value of 4.3×10^{-8} with the current data compared to 1.2×10^{-7} in our earlier report.

Two new genome-wide significant loci were identified, 14q32 and 17q21, represented by rs2010281 ($P = 1.8 \times 10^{-9}$), located on 14q32, and rs7220711 ($P = 2.3 \times 10^{-8}$), rs1107748 ($P = 1.0 \times 10^{-7}$) and rs1513670 ($P = 2.1 \times 10^{-8}$) on 17q21 (**Table 1**). The rs2010281 SNP is positioned in intron 1 of the *MARK3* (MAP/microtubule affinity-regulating kinase 3) gene (**Supplementary Fig. 1** online). This universally expressed gene encodes a protein kinase that phosphorylates

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Table 1 Association of previously unreported genome-wide significant SNPs with bone mineral density and low-trauma fractures

Region, allele and SNP frequency		*P* value in Icelandic discovery set ($N = 6,865$)	*P* value in DK and AUS ($N = 5,375$)	*P* value in Icelandic replication set ($N = 3,135$)	Effect in all sets combined ($N = 15,375$)	*P* value in all sets combined ($N = 15,375$)	OR low-trauma fracture ($N = 4,470$)	*P* value low-trauma fracture ($N = 4,470$)
Hip bone mineral density:								
14q32 (*MARK3*)								
rs2010281[A]	0.327	7.4×10^{-5}	3.3×10^{-6}	0.027	–0.08 (–0.11, –0.06)	1.8×10^{-9}	1.09 (1.03, 1.15)	0.0038
17q21 (*SOST*)								
rs1513670[A]	0.368	6.0×10^{-5}	0.0049	0.012	–0.08 (–0.10, –0.05)	2.1×10^{-8}	1.07 (1.01, 1.13)	0.023
rs7220711[A]	0.666	1.4×10^{-4}	0.0021	0.014	–0.08 (–0.10, –0.05)	2.2×10^{-8}	1.09 (1.03, 1.15)	0.0027
rs1107748[T]	0.644	7.2×10^{-5}	0.031	0.0042	–0.07 (–0.10, –0.05)	1.0×10^{-7}	1.10 (1.04, 1.17)	6.1×10^{-4}
18q21 (*TNFRSF11A*)								
rs3018362[A]	0.350	3.5×10^{-5}	6.1×10^{-4}	0.16	–0.08 (–0.10, –0.05)	5.4×10^{-8}	1.08 (1.02, 1.14)	0.005
Spine bone mineral density:								
12q13 (*SP7*)								
rs10876432[A]	0.729	9.9×10^{-6}	0.011	0.12	–0.08 (–0.11, –0.05)	1.3×10^{-7}	1.05 (0.99, 1.11)	0.13

The estimated effect on bone density is expressed as s.d. units below the population average, per copy of the allele, and the 95% confidence interval. All *P* values are two-sided. Here we list the allelic frequency for the Icelandic set, the corrected *P* value in the Icelandic discovery set, the *P* value in the combined set of the Danish (DK) samples and the Australian DOES cohort (AUS), and the effect on bone density in all the sets combined (Icelandic discovery and replication, Danish and Australian) with the corresponding *P* value for all the sets combined. The odds ratios (OR) with their 95% confidence intervals and *P* values are shown for all the sets combined.

microtubule-associated proteins and has a role in determining cell polarity. The three SNPs on 17q21 are all correlated with one another ($r^2 = 0.2$–0.8, **Supplementary Table 4** online); however, the full associated signal was not captured by any single SNP (**Supplementary Table 5** online). This suggests that a variant capturing the effect of these SNPs remains to be identified or that they represent different variants associated with BMD. These SNPs are located between 23 kb and 57 kb 3′ to the *SOST* (sclerosteosis) gene (**Fig. 1**). Polymorphisms in the *SOST* gene have previously been shown to associate with BMD[9,10]. The gene was initially isolated as the disease-causing gene in sclerosteosis[11,12], a disorder characterized by massive bone overgrowth. A 52-kb deletion downstream of the gene (32 kb) was identified in a similar skeletal disorder, van Buchem disease[13]. Notably, two of the associated SNPs, rs7220711 and rs1107748, are located in this deleted region. Sclerostin, encoded by the *SOST* gene, is not only a bone morphogenic protein antagonist but also interferes with Wnt signaling by disrupting Wnt-induced frizzled–Lrp complex formation.

Nonsynonymous SNPs, or SNPs that are located in potentially functional regions such as transcription factor–binding sites, promoters or splice sites, are often considered plausible causative variants. We followed up the 20 most strongly associated SNPs with hip or spine BMD. Only the previously reported rs6929137 (V640I) in *C6orf97* (6q25 *ESR1* locus) reached genome-wide significance (**Supplementary Tables 1b** and **2b**). Four other SNPs replicated nominally in the combined Danish and Australian sample sets and were considered suggestively associated with hip BMD with *P* values $< 5 \times 10^{-5}$ in the combined analysis of all sample sets (**Supplementary Table 1b**). These are rs227584 (T126P) in the *C17orf53* gene ($P = 8.9 \times 10^{-7}$), rs6485702 (I1086V) in the *LRP4* gene ($P = 2.1 \times 10^{-6}$), rs1422795 (G284S) in the *ADAM19* (ADAM metallopeptidase domain 19 (meltrin beta)) gene ($P = 2.0 \times 10^{-5}$) and rs1054627 (G195Q) in the *IBSP* (integrin-binding sialoprotein) gene ($P = 4.6 \times 10^{-5}$). The rs227584 SNP in *C17orf53* is located close to the three genome-wide associated SNPs on 17q21 (see above, **Fig. 1**) but represents an independent association signal (**Supplementary Tables 4** and **5**). The *IBSP* gene is especially notable, as it encodes a major structural protein of the bone matrix (bone sialoprotein) which constitutes 12% of the noncollagenous proteins in human bone.

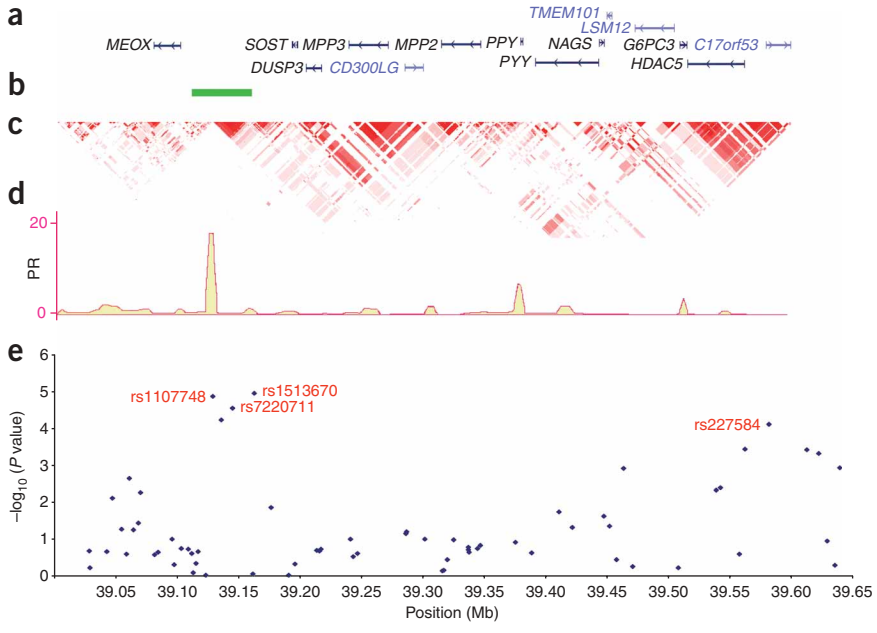

Figure 1 The associated region on 17q21. (**a**) Location of known genes from the NCBI mRNA reference sequence collection (RefSeq). (**b**) The 52-kb deletion found in individuals with Van Buchem disease. (**c**) Pair-wise r^2 values for SNPs (MAF > 5%) in the HapMap CEU population. (**d**) Estimated recombination rates (RR) in cM/Mb from the HapMap Phase II data[15]. (**e**) Association for all SNPs tested in the region in the Icelandic discovery set.

We considered associations between low-trauma fractures and the SNPs associated with BMD (**Supplementary Tables 1**, **2** and **6** online). The three *SOST* SNPs (17q21) and rs2010281 (14q32) all associated significantly with any low-trauma fractures with modest odds ratios (OR) of 1.07 to 1.10 (**Table 1**). This rather weak association with fractures indicates an effect that may largely be due to the influence of these variants on BMD.

This study adds more sequence variants to the growing collection of variants that influence BMD and the risk of fractures[5,6,8]. Each of these common variants exerts a small effect on BMD on its own; the SNPs listed in **Table 1**, together with those of previous publications[5,6,8], account for approximately 4% of the total variation in hip and spine BMD. In our study of heritability of BMD[14] the data was consistent with one or a few genes that have a considerable effect, in addition to multiple genes each with a small effect. We have identified several of these common variants of smaller effect, but the rarer and higher-risk variants remain to be uncovered.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

U.S., B.V.H., S.G., J.R.G., A.K., U.T. and K.S. designed the study; U.S., S.G., G.B.W., T.I., T.J., J.S., S.S., J.R.C., T.V.N., P.A., J.A.E., C.C. and G.S. collected the data; U.S., B.V.H., D.F.G. and A.K. analyzed the data; U.S., B.V.H., S.G., A.K., U.T. and K.S. wrote the first draft of the paper. All authors contributed to the final version of the paper.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/

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