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REVIEW

Genetics of pediatric bone strength

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Osteoporosis is one of the most common chronic forms of disability in postmenopausal women and represents a major health burden around the world. Bone fragility is affected by bone mineral density (BMD), and, one of the most important factors in preventing osteoporosis is optimizing peak bone mass, which is achieved during growth in childhood and adolescence. BMD is a complex trait resulting from environmental and genetic factors. Genome-wide association studies have discovered robust genetic signals influencing BMD in adults, and similar studies have also been conducted to investigate the genetics of BMD in the pediatric setting. These latter studies have revealed that many adult osteoporosis-related loci also regulate BMD during growth. These investigations have the potential to profoundly impact public health and will allow for the eventual development of effective interventions for the prevention of osteoporosis.

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Introduction

Osteoporosis represents one of the most common chronic forms of disability affecting both men and women. It is most common in postmenopausal women, and, indeed, after the age of 50 years, ~40% of Caucasian women will suffer at least one fracture.[1] The subsequent losses in mobility and increased mortality have enormous direct and indirect financial impacts, exceeding tens of billions of dollars per year in the United States alone, with costs likely to rise during the next few decades due to an aging population.[2]

Bone fragility is affected by bone mass and density, structural strength and bone quality. One of the most important factors in preventing osteoporosis is optimizing peak bone mass (PBM). During childhood and adolescence, skeletal development is characterized by the rapid expansion of cortical dimensions and an increase in trabecular density.[3] Approximately 25% of PBM is accumulated in the 2 years surrounding the adolescent growth spurt.[3] Insufficient accumulation of skeletal mass enhances the likelihood of osteoporosis and fracture later in life. In children as in adults, fracture risk is higher in individuals with lower bone mineral density (BMD).[4] Although environmental factors such as dietary intake, physical activity and smoking influence the accumulation of bone mass during growth,[5] the pediatric bone mass is largely determined by genetics.

From a public health standpoint, identifying genetic factors that affect PBM is a critical step in developing effective interventions for the prevention of osteoporosis. To that end, in this review, we will cover findings from candidate gene studies and genome-wide association studies (GWASs) of bone fragility in adults and children and focus on key biological pathways revealed by these studies. **Table 1** summarizes the BMD-associated loci from GWASs, the overlap between adult and pediatric bone loci and the essential biological pathways involved.

Evidence for a Genetic Component in Bone Mass, Density, Structure and Osteoporosis

BMD is a classic complex trait determined by behavioral, environmental and genetic factors. There is strong evidence for a genetic component in PBM and the predisposition of osteoporosis, with an estimated 60–80% of the variability in the risk explained by heritable factors,[6] consistent with the findings that BMD is reduced in the daughters of osteoporotic women and in men and women with first degree relatives who have osteoporosis.

Height and other anthropometric variables related to skeletal size have been known for many years to be highly heritable.[7,8] Although bone size is an independent determinant of bone strength, there is strong evidence for a heritable component to bone strength independent of body size.[9]

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Table 1 BMD-associated loci from GWAS, overlap between adult and pediatric bone loci and essential biological pathways involved

Gene Name(s)	*Locus*	*Adult/Pediatric*	*Biological Pathway*
SP7 (Osterix)	12q13.13	Both	Mesenchymal stem cells
RANKL (AKAP11)	13q14.11	Both	RANKL/RANK/OPG
OPG (TNFRSF11B, COLEC10)	8q24	Both	RANKL/RANK/OPG, skeletal system development
RANK (TNFRSF11A)	18q22.1	Both	RANKL/RANK/OPG, skeletal system development
PTHLH (KLHDC5)	12p11.22	Both	Skeletal system development
LRP5 (PPP6R3)	11q13.4	Both	WNT
RSPO3	6q22.33	Both	WNT
WNT16 (C7orf58, CPED1, FAM3C)	7q31	Both	WNT
WNT4	1p36.23-p35.1	Both	WNT
FUBP3	9q34.11-q34.12	Both	
GALNT3	2q24-q31	Both	
LGR4 (LIN7C)	11p14-p13	Both	
ABCF2	7q36	Adult	
SUPT3H	6p21.1-p21.3	Adult	Mesenchymal stem cells
SOX9	17q24.3-q25.1	Adult	Mesenchymal stem cells, skeletal system development
HOXC6	12q13.3	Adult	Skeletal system development
IDUA	4p16.3	Adult	Skeletal system development
MEPE	4q21.1	Adult	Skeletal system development
SOX6	11p15.3	Adult	Skeletal system development
AXIN1	16p13.3	Adult	WNT
MAPT	17q21.1	Adult	WNT
SOX4 (CDKAL1)	6p22.3	Adult	WNT
WLS (GRP177)	1p31.3	Adult	WNT
WNT5B (ERC1)	12p13.3	Adult	WNT
CTNNB1	3p21	Adult	WNT, mesenchymal stem cells, skeletal system development
SOST	17q11.2	Adult	WNT, skeletal system development
ANAPC1	22q12.1	Adult	
ARHGAP1	11p12-q12	Adult	
C12orf23	12q23.3	Adult	
C16orf38 (CLCN7, PTX4)	16p13	Adult	
C17orf53	17q21.31	Adult	
C18orf19 (FAM210A)	18p11.21	Adult	
ESR1 (C6orf97)	6q25.1	Adult	
CPN1	10q24.2	Adult	
CYLD	16q12.1	Adult	
DCDC5	11p14.1-p13	Adult	
DHH	12q12-q13.1	Adult	
DNM3	1q24.3	Adult	
FOXL1	16q24	Adult	
GPATCH1	19q13.11	Adult	
INSIG2	2q14.1	Adult	
JAG1	20p12.1-p11.23	Adult	
KCNMA1	10q22.3	Adult	
KIAA2018	3q13.2	Adult	
LEKR1	3q25.31-q25.32	Adult	
MARK3	14q32.3	Adult	
MBL2	10q11.2-q21	Adult	
MEF2C	5q14	Adult	
MPP7	10p11.23	Adult	
NTAN1	16p13.11	Adult	
PKDCC	2p21	Adult	
RPS6KA5	14q31-q32.1	Adult	
SALL1	16q12.1	Adult	
SLC25A13 (C7orf76)	7q21.3	Adult	
SMG6	17p13.3	Adult	
SPTBN1	2p21	Adult	
STARD3NL	7p14-p13	Adult	
TXNDC3	7p14.1	Adult	
XKR9 (LACTB2)	8q13.3	Adult	
ZBTB40	1pter-q31.3	Adult	
EYA4	6q23	Pediatric	
MTAP (MIR31HG)	9p21	Pediatric	
RIN3	14q32.12	Pediatric	

Abbreviations: BMD, bone mineral density; GWAS, genome-wide association study.

Population ancestry differences in BMD in children also support a strong genetic component of bone accretion during childhood. BMD is greatest for African Americans, and Caucasians have greater values than Asians and Hispanics.[10] African Americans have greater maturation-specific trabecular density and cortical structural strength.[11] Indeed, we recently examined adult bone loci in a multi-ethnic pediatric setting.[12] Using polygenic risk scores, we demonstrated that adult variants are predictive of BMD early in childhood and adolescence; further, we showed that, within the study cohorts and across populations worldwide, the frequency of those alleles associated with increased BMD is systematically

elevated in individuals of sub-Saharan African ancestry. This study adds to the overwhelming evidence that genetic factors and ethnicity are strong determinants of bone accretion during growth.

Candidate Gene Studies (pre-GWASs)

The first gene discoveries related to BMD used a candidate gene approach. Most notably, a single-nucleotide polymorphism (SNP) in the regulatory region of the collagen 1 alpha 1 (*COL1A1*) gene was discovered to affect the binding site for the transcription factor Sp1.[13] The initial reports showed an association between this SNP and reduced BMD and osteoporotic fracture in pre- and postmenopausal women[13,14] plus postmenopausal bone loss. Subsequent meta-analyses have confirmed these initial observations.[15] Using vertebral cancellous BMD measured by quantitative computed tomography, Gilsanz and colleagues[16] showed that the same SNP was associated with decreased vertebral BMD in pre-pubertal girls. Suuriniemi *et al.*[17] found that the *COL1A1* SNP was associated with BMC and BMD of the total body, lumbar spine and proximal femur, as well as bone ultrasound attenuation at the calcaneus and markers of bone turnover in 10- to 13-year-old girls. To date, only one small longitudinal study of 148 children and young adults[18] for this variant has been carried out in a pediatric population to study influences on bone gain in contrast to postmenopausal bone loss, finding associations between *COL1A1* polymorphisms and stature, lumbar vertebral body size and ultrasound speed of sound, independent of BMD and BMC at baseline and follow-up 4 years later. However, changes in bone measures over time did not differ by genotype.

Several candidate gene studies have examined the influence of variants in the vitamin D receptor (*VDR*) gene on BMD in children. In adult women, variation in the *VDR* gene contributes relatively little to variation in BMD;[19] by contrast, in children, *VDR* SNPs account for a greater difference when femoral and vertebral BMD are compared between those with homozygous recessive (bb) and those with dominant genotypes (BB),[20] suggesting that these polymorphisms have a greater influence on BMD during childhood. In pre-pubertal girls, dietary calcium intake also correlated with a change in BMD in those with homozygous dominant and heterozygous *VDR* (BB and Bb) genotypes but not in those with the homozygous recessive (bb) genotype.[20]

Finally, candidate gene studies of polymorphisms in/near genes encoding the estrogen receptor, aromatase, interleukin-6, low-density lipoprotein receptor-related protein 5 (*LRP5*) and osteocalcin have also been shown to be independent predictors of bone size, BMC or BMD in adolescents.[21]

Linkage Studies

Genome-wide linkage scans in families with osteoporosis-related traits have yielded several loci, but the genes in the vast majority of these loci have yet to be elucidated. In 2003, Styrkarsdottir *et al.*[22] described the isolation of the bone morphogenetic protein 2 (*BMP2*) gene under a locus on chromosome 20; however, this genetic association with adult BMD has yet to be replicated independently by others and has not been implicated with pediatric BMD. In addition, a kindred with an autosomal dominant inherited high bone mass phenotype was used to map a mutation in the gene encoding *LRP5* on 11q12–13.[23] Furthermore, osteoporosis-pseudoglioma syndrome, characterized by low-bone mass with childhood fractures and abnormal eye development, is the result of an inherited loss of function of the *LRP5* gene,[24] leading to inhibition of Wnt signaling. It is possible that *LRP5* polymorphisms are significant contributors to the natural variation in bone density in normal children.[25]

In 2005, a linkage study in 3691 individuals from 715 families with reduced BMD at the lumbar spine or femoral neck in probands identified gender- and age-specific loci, illustrating the importance of conducting stratified analyses.[26]

Syndromes

Early bone health may be compromised by several genetic or acquired childhood disorders[27] including Ehlers–Danlos, homocystinuria, Marfan's syndrome and osteogenesis imperfecta (OI). Bone fragility in most heritable disorders results from defects in the bone matrix that affect the entire skeleton. OI is the best example of these disorders, with more than 1000 genetic variants identified (primarily in the *COL1A1* and *COL1A2* genes), causing a range of skeletal effects. The spectrum of problems in this disorder includes low-bone mass, chronic bone pain, recurrent fractures and skeletal deformity.[28]

GWASs in Adults

The aforementioned genetic findings explain a very small proportion of the modeled genetic component in BMD. However, in recent years, SNP genotyping technology has greatly improved, with high-throughput genotyping methods allowing large volumes of SNPs (10^5–10^6) to be genotyped in large population-based cohorts, enabling the large-scale agnostic GWAS approach to discover novel loci implicated in complex diseases. This technology has revealed compelling evidence for genetic variants involved in various common complex traits and diseases. The first GWAS published in the orthopedic field was for bone density and other indicators of bone strength.[29] This study, utilizing adult bone mass and geometry data in the Framingham Heart Study, was carried out using the relatively low-resolution Affymetrix 100 K SNP GeneChip marker set (Santa Clara, CA, USA) and did not conclusively implicate any new genetic loci. Subsequently, three additional GWAS efforts have been published on adult bone phenotypes.[30–32] The latest meta-analysis reported 56 adult BMD and 14 fracture risk-associated loci,[33] the strongest of which coincided with the genes that encode 'MADS box transcription enhancer factor 2, polypeptide C' (*MEF2C*), 'SRY-box 6' (*SOX6*), 'wingless-type MMTV integration site family, member 16' (*WNT16*), 'Drosophila homolog of wntless' (*WLS*), 'zinc finger- and BTB domain-containing protein 40' (*ZBTB40*), N-acetylgalactosaminyltransferase 3 (*GALNT3*), *C6orf97*, 'STARD3 N-terminal-like' (*STARD3NL*), 'solute carrier family 25, member 13' (*SLC25A13*), osteoprotegerin (*OPG*) and 'A-kinase anchor protein 11' (*AKAP11*). Many of the genes and pathways implicated in these studies are already known to be involved in bone biology and explain only a small portion ($\sim$6%) of the overall predicted genetic contribution to the trait.

A possible explanation for this 'missing heritability' is the contribution of rare variants of strong effect that are not easily detected by GWASs. Indeed, a recent seminal whole-genome sequencing study in a large sample of European adults identified rare variation near the engrailed 1 (*EN1*) gene that was associated with adult BMD.[34] Strikingly, the rare variant effect sizes were 4 times higher than the average effect size of common variants identified by GWAS.

In conclusion, as most previous studies have focused on bone phenotypes later in life, there has remained a need for a higher resolution genome-wide assessment in pediatric cohorts to understand the contribution of common genetic variation to bone development. In addition, a greater understanding of how loci uncovered in adults confer their effects early in life, with more detailed bone phenotypes, is needed. In fact, common locus discovery is likely to be easier in children than in adults because of their less prolonged exposure to environmental or lifestyle factors.

GWAS and Candidate Studies in Pediatric Cohorts

In addition to the points raised directly above, the genetic regulation of pediatric skeleton could also have some distinct properties from that of the adult skeleton. GWAS approaches that have been used to identify novel bone density loci in adults are equally applicable for discovering loci that associate with pediatric bone phenotypes. In 2009, Timpson *et al.*[35] were the first to conduct a GWAS for pediatric bone phenotypes. Using data from the Avon Longitudinal Study of Parents and Children (ALSPAC; Caucasian 10-year-olds), variants near *SP7* (*Osterix*) were found to associate with total body less head (TBLH)-BMD, TBLH-BMC and bone area. Interestingly, the *SP7* locus was one of the earliest adult BMD loci discovered by GWAS.[32] This initially indicated that *SP7* could be a key regulator of BMD across the lifespan, but this locus has not remained the leading pediatric bone density locus.

The 7q31.31 locus is currently the leading genomic region for pediatric bone density phenotypes, which includes three genes encoding 'cadherin-like and PC-esterase domain containing 1' (*CPED1*), *WNT16* and 'family with sequence similarity 3, member C' (*FAM3C*). In 2012, Medina-Gomez *et al.* analyzed data from the multi-ethnic Generation R cohort (6-year olds) and observed that rs917727 (*WNT16*/*FAM3C*) was associated with TBLH-BMD.[36] Importantly, this association was replicated in ALSPAC (and in multiple adult cohorts). In addition, rs4609139 (*CPED1*) was also associated with TBLH-BMD, independent of rs917727 (*WNT16*/*FAM3C*), and both variants were associated with skull BMD. In 2014, Kemp *et al.*[37] further explored the Generation R and ALSPAC data and found associations between 7q31.31 variants and upper and lower limb BMD, with the strength of the associations strongest for upper limb BMD. A third pediatric GWAS was conducted by us in 2015 using data from the multi-ethnic Bone Mineral Density in Childhood Study (BMDCS: 5–20-year olds).[38] In that study, we found that rs7797976 (*CPED1*) was associated with distal radius BMD in females. Collectively, these three studies strongly indicate that the 7q31.31 locus is a regulator of pediatric bone density.

The GWAS-implicated pediatric bone density loci reviewed so far overlap with the loci discovered in adults.[33] In addition, Kemp *et al.*[37] also reported GWAS associations with variants representing the following established adult BMD loci: wingless-type MMTV integration site family, member 4 (*WNT4*), *GALNT3*, 'far upstream element-binding protein 3' (*FUBP3*), kelch domain-containing protein 5 (*KLHDC5*)-parathyroid hormone-like hormone (*PTHLH*), RANK ligand (*RANKL)* and R-spondin family member 3 (*RSPO3*). However, novel pediatric bone density loci have been discovered. Specifically, Kemp *et al.* discovered that the variant rs754388 (*RIN3*—'RAS and RAB interactor 3') was associated with TBLH-BMD and lower limb BMD and rs3012465 (*EYA4*—'eyes absent 4') was associated with skull BMD,[37] whereas we discovered that rs7035284 (9p21.3) was associated with distal radius BMD in males.[38] In the future, larger pediatric GWASs (on the scale of the adult bone density studies) could discover additional novel pediatric BMD loci.

Larger scale pediatric GWASs could also confirm whether more of the established adult BMD loci associate with pediatric bone phenotypes. Until then, candidate gene studies offer an alternative approach to determine whether adult GWAS-implicated loci operate in the pediatric setting. To that end, genetic risk scores have been calculated based on the number of BMD-lowering alleles carried at all adult GWAS-implicated BMD loci; in addition, genetic risk scores have also been calculated using subsets of these loci involved in fracture risk, bone signaling pathways (WNT, RANK-RANKL-OPG and mesenchymal stem cell differentiation) and pediatric BMD. Using longitudinal data from the ALSPAC (ages 9 to 17), Warrington *et al.* observed associations between these genetic risk scores and TBLH-BMD and TBLH-BMC, and these associations were stronger among the older adolescents (that is, score $\times$ age interactions).[39] Using longitudinal data from the BMDCS (up to seven study visits, spanning ages 5–20 years), we observed associations between three of the genetic risk scores (overall adult score, fracture score and WNT score), with BMD at multiple skeletal sites (spine, total hip, femoral neck and distal radius) and TBLH-BMC.[40] In that study, the overall adult score associations were strong in females (that is, score $\times$ sex interaction), and the fracture score associations were stronger in older adolescents (that is, score $\times$ age interaction). We also tested for associations between the individual variants comprising the genetic risk scores with bone phenotypes in the BMDCS.[41] Many of the adult BMD variants associated with pediatric bone density in children and adolescents, but the associations tended to be sex and/or puberty stage specific.

Leveraging the same candidate gene approach, we also tested whether rare variants near *EN1* originally reported in adults[34] also associated with pediatric bone density. In agreement with the original adult discovery, we observed associations between these rare variants near *EN1* with total hip BMD and femoral neck BMD in BMDCS, with especially strong associations in females.[42]

Functional Role of Key BMD-Associated Loci in Bone Development

Animal models, rare mutations leading to syndromic bone diseases and genes near common loci associated in GWAS all point toward a key role for the canonical, or β-catenin, WNT signaling pathway in the regulation of bone formation and maintenance (extensively reviewed in reference [43]). The WNT pathway is involved in many cellular activities such as cell fate

determination, proliferation, differentiation and apoptosis. In bone, activation of the WNT signaling cascade frees β-catenin from cytoplasmic degradation, allowing it to enter the nucleus and regulate target gene expression,[44] leading to increased bone mass and strength, whereas inhibition leads to diminished mass and increased fragility. Wnt signaling, either through direct interactions with Wnt proteins or indirectly via Wnt co-receptors, induces the differentiation of bone-forming osteoblasts and suppresses bone-resorbing osteoclasts.[45]

GWASs of bone accrual in the pediatric setting have implicated many genes involved in this pathway, including those that encode *WNT16*, *LRP5*, 'receptor activator of nuclear factor κ' (*RANK*), *RANKL*, *OPG* and 'leucine-rich repeat containing G-protein-coupled receptor 4' (*LGR4)*. *WNT16* has been associated with cortical bone thickness, porosity and fracture in human GWASs.[46] In knockout mice missing *Wnt16*, the periosteal bone formation rate as well as the mineral apposition rate were reduced,[47] and mice conditionally overexpressing human *WNT16* in osteoblasts showed increased cortical and trabecular bone mass.[48] Similarly, loss of function mutations in *LRP5*, which encodes a transmembrane WNT co-receptor that synergistically binds WNT along with the frizzled receptor, disrupt normal signaling and lead to low-bone mass (osteoporosis-pseudoglioma syndrome), whereas gain of function mutations result in high bone mass. In addition, other members of the LRP family are involved in the maintenance of bone,[49] and a variant ~84 kb upstream of *LRP3* has been associated with pediatric bone mass during the mid-to-late stages of puberty.[41]

RANK, a member of the tumor necrosis factor receptor subfamily, is found on the surface of osteoclasts and together with its ligand RANKL regulates the formation, activation and survival of osteoclasts during normal bone modeling and remodeling.[50] WNT-β-catenin signaling is required for the expression of OPG, an anti-osteoclastic factor that acts as a decoy receptor for RANKL in osteoblasts and osteoclasts. Meanwhile, OPG sequesters RANKL, preventing excessive bone resorption.

LGR4, regulated by BMP during osteoblast differentiation,[51] is a G-protein-coupled receptor that is activated by extracellular WNT receptors and potentiates WNT signaling.

Finally, En1 is a homeobox gene essential for mouse limb development and involved in Wnt signaling;[52] conditional knockout mice have lower trabecular bone volume, number and thickness at the lumbar spine and femur and lower cortical thickness at the femur, probably as a consequence of high bone turnover.[34]

Several other pediatric BMD-associated loci lie near genes with strong functional evidence for involvement in bone development. For example, the gene encoding 'sex determining region Y- Box 9' (*SOX9*), associated with BMD during late puberty,[41] functions during chondrocyte differentiation, and loss of function leads to the skeletal malformation disorder campomelic dysplasia.[53] In addition, common variants have been implicated near the *GALNT3* gene, which lead to dwarfism when it is overexpressed in chondrocytes.[54]

Concluding Remarks and Future Perspectives

In less than a decade, the GWAS approach has proven successful at identifying >60 loci robustly associated with BMD in adults, and new discoveries are underway in the pediatric setting. However, these loci have small effect sizes and collectively explain only about 6% of the variance associated with this trait.[33] This 'missing heritability' problem suggests that there might be many more loci to be discovered, each contributing a small effect on risk, or, more likely, there might be a few rare variants (including copy number variations) of large effect. In both cases, larger meta-analysis studies are needed, combined with whole-genome sequencing techniques and deep imputation. Further, although most of the signals discovered by GWASs can be convincingly attributed to nearby genes in key pathways relevant to bone biology, there are still several loci that cannot easily be linked to known bone pathways. Novel approaches and functional studies investigating epigenetic regulation and the role of non-coding regions in the long-distance control of gene expression will be needed to link these signals to their culprit target genes. Further, as BMD is known to vary on the basis of ethnicity and gender, this needs to be recognized as we move forward toward personalized, preventative medicine.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Melton 3rd LJ , Chrischilles EA, Cooper C, Lane AW, Riggs BL. Perspective. How many women have osteoporosis? *J Bone Miner Res* 1992; **7**: 1005–1010.
2. Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005-2025. *J Bone Miner Res* 2007; **22**: 465–475.
3. Leonard MB, Zemel BS. Current concepts in pediatric bone disease. *Pediatr Clin North Am* 2002; **49**: 143–173.
4. Clark EM, Ness AR, Bishop NJ, Tobias JH. Association between bone mass and fractures in children: a prospective cohort study. *J Bone Miner Res* 2006; **21**: 1489–1495.
5. Office of the Surgeon General (US). Determinants of Bone Health. In: *Bone Health and Osteoporosis: A Report of the Surgeon General.* Office of the Surgeon General (US): Rockville, MD, USA, 2004, Available from http://www.ncbi.nlm.nih.gov/books/NBK45503/.
6. Heaney RP, Abrams S, Dawson-Hughes B, Looker A, Marcus R, Matkovic V *et al.* Peak bone mass. *Osteoporos Int* 2000; **11**: 985–1009.
7. Visscher PM, Medland SE, Ferreira MA, Morley KI, Zhu G, Cornes BK *et al.* Assumption-free estimation of heritability from genome-wide identity-by-descent sharing between full siblings. *PLoS Genet* 2006; **2**: e41.
8. Jian WX, Long JR, Deng HW. High heritability of bone size at the hip and spine in Chinese. *J Hum Genet* 2004; **49**: 87–91.
9. Havill LM, Mahaney MC, L Binkley T, Specker BL. Effects of genes, sex, age, and activity on BMC, bone size, and areal and volumetric BMD. *J Bone Miner Res* 2007; **22**: 737–746.
10. Zemel BS, Kalkwarf HJ, Gilsanz V, Lappe JM, Oberfield S, Shepherd JA *et al.* Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: results of the bone mineral density in childhood study. *J Clin Endocrinol Metab* 2011; **96**: 3160–3169.
11. Leonard MB, Elmi A, Mostoufi-Moab S, Shults J, Burnham JM, Thayu M *et al.* Effects of sex, race, and puberty on cortical bone and the functional muscle bone unit in children, adolescents, and young adults. *J Clin Endocrinol Metab* 2010; **95**: 1681–1689.
12. Medina-Gomez C, Chesi A, Heppe DH, Zemel BS, Yin JL, Kalkwarf HJ *et al.* BMD loci contribute to ethnic and developmental differences in skeletal fragility across populations: assessment of evolutionary selection pressures. *Mol Biol Evol* 2015; **32**: 2961–2972.
13. Grant SF, Reid DM, Blake G, Herd R, Fogelman I, Ralston SH. Reduced bone density and osteoporosis associated with a polymorphic Sp1 binding site in the collagen type I alpha 1 gene. *Nat Genet* 1996; **14**: 203–205.
14. Uitterlinden AG, Burger H, Huang Q, Yue F, McGuigan FE, Grant SF *et al.* Relation of alleles of the collagen type Ialpha1 gene to bone density and the risk of osteoporotic fractures in postmenopausal women. *N Engl J Med* 1998; **338**: 1016–1021.
15. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 2003; **33**: 177–182.
16. Sainz J, Van Tornout JM, Sayre J, Kaufman F, Gilsanz V. Association of collagen type 1 alpha1 gene polymorphism with bone density in early childhood. *J Clin Endocrinol Metab* 1999; **84**: 853–855.
17. Suuriniemi M, Kovanen V, Mahonen A, Alen M, Wang Q, Lyytikainen A *et al.* COL1A1 Sp1 polymorphism associates with bone density in early puberty. *Bone* 2006; **39**: 591–597.

18. van der Sluis IM, de Muinck Keizer-Schrama SM, Pols HA, Lequin MH, Krenning EP, Uitterlinden AG. Collagen Ialpha1 polymorphism is associated with bone characteristics in Caucasian children and young adults. *Calcif Tissue Int* 2002; **71**: 393–399.
19. Cooper GS, Umbach DM. Are vitamin D receptor polymorphisms associated with bone mineral density? A meta-analysis. *J Bone Miner Res* 1996; **11**: 1841–1849.
20. Ferrari SL, Rizzoli R, Slosman DO, Bonjour JP. Do dietary calcium and age explain the controversy surrounding the relationship between bone mineral density and vitamin D receptor gene polymorphisms? *J Bone Miner Res* 1998; **13**: 363–370.
21. Marini F, Brandi ML. Genetic determinants of osteoporosis: common bases to cardiovascular diseases? *Int J Hypertens* 2010; **2010**: 394579.
22. Styrkarsdottir U, Cazier JB, Kong A, Rolfsson O, Larsen H, Bjarnadottir E *et al.* Linkage of osteoporosis to chromosome 20p12 and association to BMP2. *PLoS Biol* 2003; **1**: E69.
23. Johnson ML, Gong G, Kimberling W, Recker SM, Kimmel DB, Recker RB. Linkage of a gene causing high bone mass to human chromosome 11 11q12-13. *Am J Hum Genet* 1997; **60**: 1326–1332.
24. Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM *et al.* LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell* 2001; **107**: 513–523.
25. Koay MA, Tobias JH, Leary SD, Steer CD, Vilarino-Guell C, Brown MA. The effect of LRP5 polymorphisms on bone mineral density is apparent in childhood. *Calcif Tissue Int* 2007; **81**: 1–9.
26. Ralston SH, Galwey N, MacKay I, Albagha OM, Cardon L, Compston JE *et al.* Loci for regulation of bone mineral density in men and women identified by genome wide linkage scan: the FAMOS study. *Hum Mol Genet* 2005; **14**: 943–951.
27. Soyka LA, Fairfield WP, Klibanski A. Clinical review 117: Hormonal determinants and disorders of peak bone mass in children. *J Clin Endocrinol Metab* 2000; **85**: 3951–3963.
28. Forlino A, Marini JC. Osteogenesis imperfecta. *Lancet* 2016; **387**: 1657–1671.
29. Kiel DP, Demissie S, Dupuis J, Lunetta KL, Murabito JM, Karasik D. Genome-wide association with bone mass and geometry in the Framingham Heart Study. *BMC Med Genet* 2007; **8**: S14.
30. Styrkarsdottir U, Halldorsson BV, Gretarsdottir S, Gudbjartsson DF, Walters GB, Ingvarsson T *et al.* Multiple genetic loci for bone mineral density and fractures. *N Engl J Med* 2008; **358**: 2355–2365.
31. Richards JB, Rivadeneira F, Inouye M, Pastinen TM, Soranzo N, Wilson SG *et al.* Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. *Lancet* 2008; **371**: 1505–1512.
32. Styrkarsdottir U, Halldorsson BV, Gretarsdottir S, Gudbjartsson DF, Walters GB, Ingvarsson T *et al.* New sequence variants associated with bone mineral density. *Nat Genet* 2009; **41**: 15–17.
33. Estrada K, Styrkarsdottir U, Evangelou E, Hsu YH, Duncan EL, Ntzani EE *et al.* Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. *Nat Genet* 2012; **44**: 491–501.
34. Zheng HF, Forgetta V, Hsu YH, Estrada K, Rosello-Diez A, Leo PJ *et al.* Whole-genome sequencing identifies EN1 as a determinant of bone density and fracture. *Nature* 2015; **526**: 112–117.
35. Timpson NJ, Tobias JH, Richards JB, Soranzo N, Duncan EL, Sims AM *et al.* Common variants in the region around Osterix are associated with bone mineral density and growth in childhood. *Hum Mol Genet* 2009; **18**: 1510–1517.
36. Medina-Gomez C, Kemp JP, Estrada K, Eriksson J, Liu J, Reppe S *et al.* Meta-analysis of genome-wide scans for total body BMD in children and adults reveals allelic heterogeneity and age-specific effects at the WNT16 locus. *PLoS Genet* 2012; **8**: e1002718.
37. Kemp JP, Medina-Gomez C, Estrada St K , Pourcain B, Heppe DH, Warrington NM *et al.* Phenotypic dissection of bone mineral density reveals skeletal site specificity and facilitates the identification of novel loci in the genetic regulation of bone mass attainment. *PLoS Genet* 2014; **10**: e1004423.
38. Chesi A, Mitchell JA, Kalkwarf HJ, Bradfield JP, Lappe JM, McCormack SE *et al.* A trans-ethnic genome-wide association study identifies gender-specific loci influencing pediatric aBMD and BMC at the distal radius. *Hum Mol Genet* 2015; **24**: 5053–5059.
39. Warrington NM, Kemp JP, Tilling K, Tobias JH, Evans DM. Genetic variants in adult bone mineral density and fracture risk genes are associated with the rate of bone mineral density acquisition in adolescence. *Hum Mol Genet* 2015; **24**: 4158–4166.
40. Mitchell JA, Chesi A, Elci O, McCormack SE, Roy SM, Kalkwarf HJ *et al.* Genetic risk scores implicated in adult bone fragility associate with pediatric bone density. *J Bone Miner Res* 2015; **31**: 789–795.
41. Mitchell JA, Chesi A, Elci O, McCormack SE, Kalkwarf HJ, Lappe JM *et al.* Genetics of bone mass in childhood and adolescence: effects of sex and maturation interactions. *J Bone Miner Res* 2015; **30**: 1676–1683.
42. Mitchell JA, Chesi A, McCormack SE, Roy SM, Cousminer DL, Kalkwarf HJ *et al.* Rare EN1 variants and pediatric bone mass. *J Bone Miner Res* (e-pub ahead of print 11 March 2016; doi:10.1002/jbmr.2833).
43. Baron R, Kneissel M. WNT signaling in bone homeostasis and disease: from human mutations to treatments. *Nat Med* 2013; **19**: 179–192.
44. Baron R, Rawadi G, Roman-Roman S. Wnt signaling: a key regulator of bone mass. *Curr Top Dev Biol* 2006; **76**: 103–127.
45. Canalis E. Wnt signalling in osteoporosis: mechanisms and novel therapeutic approaches. *Nat Rev Endocrinol* 2013; **9**: 575–583.
46. Zheng HF, Tobias JH, Duncan E, Evans DM, Eriksson J, Paternoster L *et al.* WNT16 influences bone mineral density, cortical bone thickness, bone strength, and osteoporotic fracture risk. *PLoS Genet* 2012; **8**: e1002745.
47. Wergedal JE, Kesavan C, Brommage R, Das S, Mohan S. Role of WNT16 in the regulation of periosteal bone formation in female mice. *Endocrinology* **156**: 1023–1032 2015.
48. Alam I, Alkhouli M, Gerard-O'Riley RL, Wright WB, Acton D, Gray AK *et al.* Osteoblast-specific overexpression of human WNT16 increases both cortical and trabecular bone mass and structure in mice. *Endocrinology* 2015; **157**: 722–736.
49. Lara-Castillo N, Johnson ML. LRP receptor family member associated bone disease. *Rev Endocr Metab Disord* 2015; **16**: 141–148.
50. Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. *Arch Biochem Biophys* 2008; **473**: 139–146.
51. Pawaputanon Na Mahasarakham C, Ezura Y, Kawasaki M, Smriti A, Moriya S, Yamada T *et al.* BMP-2 enhances Lgr4 gene expression in osteoblastic cells. *J Cell Physiol* (e-pub ahead of print 2 September 2015; doi:10.1002/jcp.25180).
52. Loomis CA, Harris E, Michaud J, Wurst W, Hanks M, Joyner AL. The mouse Engrailed-1 gene and ventral limb patterning. *Nature* 1996; **382**: 360–363.
53. Foster JW, Dominguez-Steglich MA, Guioli S, Kwok C, Weller PA, Stevanovic M *et al.* Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. *Nature* 1994; **372**: 525–530.
54. Yoshida CA, Kawane T, Moriishi T, Purushothaman A, Miyazaki T, Komori H *et al.* Overexpression of Galnt3 in chondrocytes resulted in dwarfism due to the increase of mucin-type O-glycans and reduction of glycosaminoglycans. *J Biol Chem* 2014; **289**: 26584–26596.