Proceedings of the Nutrition Society (2007), **66**, 158–165 DOI:10.1017/S002966510700540X

The Summer Meeting of the Nutrition Society, hosted by the Rowett Research Institute and the University of Aberdeen, was held at the Aberdeen Exhibition and Conference Centre, Aberdeen on 3–6 July 2006

Symposium on 'Genetic polymorphisms and disease risk'

Genetics of osteoporosis

Stuart H. Ralston

Molecular Medicine Centre, Rheumatic Diseases Unit, Edinburgh University, Western General Hospital, Edinburgh EH4 2XU, UK

Osteoporosis is a common disease with a strong genetic component characterised by reduced bone mass and an increased risk of fragility fractures. Twin and family studies have shown that genetic factors contribute to osteoporosis by influencing bone mineral density (BMD), and other phenotypes that are associated with fracture risk, although the heritability of fracture itself is modest. Linkage studies have identified several quantitative trait loci that regulate BMD but most causal genes remain to be identified. In contrast, linkage studies in monogenic bone diseases have been successful in gene identification, and polymorphisms in many of these genes have been found to contribute to the regulation of bone mass in the normal population. Population-based studies have identified polymorphisms in several candidate genes that have been associated with bone mass or osteoporotic fracture, although individually these polymorphisms only account for a small amount of the genetic contribution to BMD regulation. Environmental factors such as diet and physical activity are also important determinants of BMD, and in some cases specific nutrients have been found to interact with genetic polymorphisms to regulate BMD. From a clinical standpoint, advances in knowledge about the genetic basis of osteoporosis are likely to be important in increasing the understanding of the pathophysiology of the disease; providing new genetic markers with which to assess fracture risk and in identifying genes and pathways that form molecular targets for the design of the next generation of drug treatments.

Osteoporosis: Fracture: Bone mineral density: Linkage, genetic and association studies

Genetic factors play an important role in the pathogenesis of osteoporosis. Twin- and family-based studies have indicated that 60–85% of the variance in bone mineral density (BMD) is genetically determined (Krall & Dawson-Hughes, 1993; Gueguen *et al*. 1995), and other risk factors for osteoporotic fractures, such as quantitative ultrasound properties of bone, femoral neck geometry and bone turnover markers range, have also been shown to have a strong heritable component (Arden *et al*. 1996; Garnero *et al*. 1996). Family history of fracture has been shown in several studies to be a risk factor for fractures independently of BMD (Cummings *et al*. 1995; Torgerson *et al*. 1996), and in keeping with this finding several investigators (Deng *et al*. 2000; Andrew *et al*. 2005) have reported that fracture may have a heritable component. In one study of post-menopausal women (Deng *et al*. 2000) heritability of wrist fracture was estimated as about 25%, whereas another study of twins (Andrew *et al*. 2005) has suggested that the heritability of wrist fracture may be as much as 54%. Interestingly, the heritability of wrist fracture in both these studies was shown to be largely independent of BMD, suggesting that predisposition may have been mediated through genetic influences on other factors such as bone turnover, bone geometry or even perhaps the risk of falling. However, another study (Kannus *et al*. 1999) has failed to detect any evidence for heritability of fractures in elderly twins. These divergent results are probably explained by the fact that the heritability of fracture decreases with age, as environmental factors become more important. This relationship has

Abbreviations: BMD, bone mineral density; LRP, lipoprotein receptor-related protein; QTL, quantitative trait loci; VDR, vitamin D receptor.

Corresponding author: Professor Stuart Ralston, fax +44 131 651 1085, email stuart.ralston@ed.ac.uk

Table 1. Quantitative trait loci for bone mineral density detected by genome-wide linkage scan*

Study	Chromosome	cM†	Nearest marker	LOD score	Site	Gender
Deng *et al.* (2002)	4q31–32	152	D4S424	3·08	Spine	Both
	13q33–34	103	D13S285	2·43	Spine	Both
	10q26	170	D10S1651	2·29	Fem neck	Both
Koller *et al.* (2000)	1q21–23	169	D1S484	3·11	Spine	Female
Wilson *et al.* (2003)	1p36	17	D1S214	2·38	Total hip	Female
	3p22	76	D3S1289	2·72	Spine	Female
Karasik *et al.* (2002)	21q22.2	40	D21S2055	2·39	Total hip	Both
	21qter	58	D21S1446	3·14	Total hip	Both
	9q22	120	D9S930	2·71	Fem neck	Both
Devoto *et al.* (1998)	1p36	36	D1S450	2·29	Fem neck	Both
	2p23–24	17	D2S149	2·25	Spine	Both
	4qter	265	D4S1535	2·28	Spine	Both
Styrkarsdottir *et al.* (2003)	20p12	20	D20S905	2·89	Spine	Both
	20p12	20	D20S905	3·18	Fem neck	Both
Karasik *et al.* (2003)	9q22	120	D9S930	2·71	Fem neck	Both
Kammerer *et al.* (2003)	6q27	190	D6S281	2·27	Trochanter	Male
	2pter	0	D2S1780	3·98	Fem neck	Male
	13q14	55	D13S788	3·46	Trochanter	Male
	13q14	60	D13S788	2·51	Fem neck	Male
Ralston *et al.* (2005)	3q25	177	D3S1279	2·43	Fem neck	Male
	4q25	117	D4S1572	2·22	Fem neck	Male
	7p14	57	D7S516	2·28	Fem neck	Male
	10q21	80	D10S196	4·20	Fem neck	Male
	16p13	31	D16S3075	2·52	Fem neck	Male
	4q25	117	D4S1572	2·55	Fem neck	Female
	16q23	31	D16S3091	2·28	Spine	Female
	18p11	48	D18S53	2·83	Spine	Female
	20q13	90	D20S196	3·20	Spine	Female

cM, centimorgans; Fem, femoral.
*The loci shown are those identified by genome-wide scan for which the LOD score exceeded +2·2. The LOD score is the logarithm of the odds that the disease gene and the marker being studied are linked. For complex diseases linkage is considered significant when the LOD score exceeds +3·6, whereas linkage is considered suggestive when the LOD score exceeds +2·2.
†A measure of the physical distance between the locus identified and the telomere (tip) of the chromosome.

been demonstrated in a large study of Swedish twins (Michaelsson *et al*. 2005), which has shown that the heritability of hip fracture is about 68% in those aged <65 years but drops off rapidly with age to reach a value of almost zero by the eighth decade. This finding illustrates that identifying genes that are related to risk factors for osteoporosis such as BMD does not necessarily mean that these genes will influence the risk of fracture.

Human linkage studies

Several genome-wide linkage scans have been performed to try to identify loci that regulate BMD. The most important quantitative trait loci (QTL) for BMD identified by these studies are summarised in Table 1. Few of the genome-wide scans so far performed have identified QTL that meet the criteria for genome-wide significance, and only one gene that regulates susceptibility to osteoporosis has been identified by this approach; the *BMP2* gene that encodes bone morphogenic protein 2, an important regulator of osteoblast differentiation (Styrkarsdottir *et al*. 2003). Several important findings have emerged from these studies. First, it appears that the genes that regulate BMD probably do so in a site-specific and gender-specific manner (Peacock *et al*. 2005; Ralston *et al*. 2005); second, it appears that the genes that regulate peak bone mass differ from those that regulate BMD in older individuals (Kammerer *et al*. 2003; Karasik *et al*. 2003; Ralston *et al*. 2005). The lack of replication of linkage peaks between studies mirrors experience in other complex diseases and might reflect the fact that genes that regulate BMD differ in different populations or that genes that predispose to osteoporosis have modest effects that are difficult to detect by conventional linkage analysis. Technical advances such as the development of densely-spaced panels of single-nucleotide polymorphisms for genome-wide scans are likely to improve the chances of detecting genes of modest effect size in the future (Sawcer *et al*. 2004). There is also a prospect that meta-analysis of genome-wide scans may reveal significant QTL that have not been detected by individual studies (Fisher *et al*. 2003).

Interest has also focused on identifying QTL for the regulation of other phenotypes relevant to the pathogenesis of osteoporosis. For example, genome-wide linkage scans have identified several QTL that strongly influence hip geometry (Koller *et al*. 2001), while others have been performed that have identified QTL that regulate the quantitative ultrasound properties of bone (Wilson *et al*. 2004).

Animal linkage studies

Linkage studies in mice (Klein *et al*. 1998; Beamer *et al*. 2001), rats (Koller *et al*. 2005) and primates (Mahaney

et al. 1997) have resulted in the identification of several QTL that regulate BMD. Linkage analysis has also been used to localise QTL for other osteoporosis-related phenotypes such as bone structure, bone shape and bone strength (Turner *et al*. 2003; Alam *et al*. 2005) and circulating levels of insulin-like growth factor-1 (Bouxsein *et al*. 2002). Loci for the regulation of BMD have now been identified on almost all mouse chromosomes, and several rat chromosomes with replication of some QTL across different strains, and replication of some human BMD QTL (Koller *et al*. 2005). These studies have also shown that the genes that regulate BMD in mice have effects that are site-specific and gender-specific (Beamer *et al*. 2001; Orwoll *et al*. 2001). To date, only one gene that regulates BMD, the *Alox15* gene, has been identified, by studies in mice (Klein *et al*. 2004). In this study a QTL for the regulation of BMD was identified on mouse chromosome 11 by linkage in a cross of DBA/2 and C57BL/6 mice, and subsequent microarray analysis has shown that the parental DBA2 strain of mice (low BMD) has a 20-fold increase in expression of the *Alox15* mRNA when compared with C57BL/6 (high BMD) mice. From this observation the authors had suspected that *Alox15* might act as a negative regulator of bone mass and they have confirmed this hypothesis by finding that *Alox15*-knock-out mice have increased BMD and that inhibition of *Alox15* protects against ovariectomy-induced bone loss. The mechanism by which *Alox15* reduces BMD is unclear, but the gene encodes a lipoxygenase enzyme that converts arachidonic and linoleic acids into ligands for the transcription factor PPARγ, which is thought to regulate differentiation of mesenchymal cells into adipocytes and osteoblasts. Recent studies have shown that genetic variation in a human homologue of *Alox15* accounts for some of the heritable component of spine BMD regulation in man (Ichikawa *et al*. 2006).

Candidate gene studies

Candidate gene association studies have identified several polymorphisms that are associated with BMD, bone loss or osteoporotic fractures. Some of the most important candidate genes that have been implicated in the pathogenesis of osteoporosis will be discussed.

Vitamin D receptor

The vitamin D receptor (VDR) was the first candidate gene to be studied in relation to BMD regulation and most attention has focused on polymorphisms situated on the 3′ flank of VDR recognised by the restriction enzymes *BsmI*, *ApaI* and *TaqI*. A meta-analysis of association studies that have genotyped the BsmI polymorphism have concluded that there is evidence of an association between spine BMD and the BsmI polymorphism, equivalent to approximately 0·15 Z-score units, between the BB genotype and the other genotypes (Thakkinstian *et al*. 2004). Another polymorphism affecting exon 2 of VDR has been identified that creates an alternative translational start site, resulting in the production of two isoforms of the VDR protein that differ in length by three amino acids (Gross *et al*. 1997). This polymorphism has been associated with BMD in some studies, but functional studies have yielded inconclusive results (Gross *et al*. 1998). A polymorphism has been identified in the promoter of VDR at a binding site for the transcription factor Cdx-2 that has been associated with BMD in Japanese subjects and appears to be functional (Arai *et al*. 2001). This polymorphism has been associated with fracture in other populations, but not with BMD (Fang *et al*. 2003). The most comprehensive study of VDR alleles in relation to osteoporosis has been in the Rotterdam study in which haplotype-tagging single-nucleotide polymorphisms of VDR were analysed in 6418 subjects (Fang *et al*. 2005). Alleles were identified in the promoter region and 3′ untranslated region that were shown to be associated with an increased risk of fracture, and a subgroup of individuals who carried risk alleles at both sites were found to have an increased risk of fracture when compared with control subjects. Functional studies have also shown that the promoter haplotype that increases fracture risk is associated with reduced VDR expression in reporter assays whereas the 3′ UTR risk haplotype is associated with increased degradation of VDR mRNA. The data would be consistent with a model whereby the combination of risk haplotypes results in a lower VDR mRNA level as a result of decreased transcription and increased degradation. Interestingly, the risk alleles for fracture identified in this study were not associated with differences in BMD. In view of this finding, the mechanism by which these polymorphisms predispose to fracture is unclear. Moreover, if correction had been applied for all the combinations of VDR haplotypes tested in this study in relation to fracture, the association would not have been significant.

Collagen type I αI

COLIA1, the gene encoding the αI chain of type I collagen is an important functional candidate for the pathogenesis of osteoporosis, as type I collagen is the major protein of bone. Extensive studies have been conducted on a polymorphism that lies within intron 1 of the *COLIA1* gene at a Sp1 binding site (Grant *et al*. 1996). The thymidine-containing allele of this polymorphism has been associated with reduced bone density (Grant *et al*. 1996; Uitterlinden *et al*. 1998) and other osteoporosis-related phenotypes such as post-menopausal bone loss (Harris *et al*. 2000; MacDonald *et al*. 2001), bone geometry (Qureshi *et al*. 2001), bone quality (Mann *et al*. 2001) and bone mineralization (Stewart *et al*. 2005). Functional analysis has shown that the osteoporosis-associated T allele (‘s’) of the Sp1 polymorphism is associated with increased DNA–protein binding, increased transcription from the T allele and abnormally increased production of the collagen type I α1 mRNA and protein (Mann *et al*. 2001). It is thought that the resulting imbalance between the αI and α2 chains of collagen type I may contribute to impairment of bone strength and reduced bone mass in carriers of the T allele by subtly affecting bone mineralization (Stewart *et al*. 2005). Meta-analyses of published studies (Efstathiadou *et al*. 2001; Mann *et al*. 2001; Mann & Ralston, 2003) have

concluded that carriage of the T allele is associated with reduced BMD at the lumbar spine and femoral neck and with vertebral fractures. More recently, two polymorphisms (–1997G/T and –1663delT) have been identified in the *COLIA1* promoter region and have been associated with BMD (Garcia-Giralt *et al*. 2002). These polymorphisms are in linkage disequilibrium with the Sp1 polymorphism, and functional studies have shown that the polymorphisms influence *COLIA1* transcription in promoter–reporter assays (Garcia-Giralt *et al*. 2005). The –1997G/T promoter polymorphism has been studied in relation to BMD in other populations and in family-based studies (Liu *et al*. 2004; Yamada *et al*. 2005; Zhang *et al*. 2005) with mixed results, although most of these studies have been of limited sample size. Current evidence indicates that the promoter polymorphisms and the Sp1 polymorphism interact to regulate BMD in women (Stewart *et al*. 2006), indicating that the previously-reported associations between the Sp1 polymorphism and osteoporosis-related phenotypes may in fact be driven by an extended haplotype involving the Sp1 and promoter polymorphisms.

Oestrogen receptor α

The oestrogen receptor α, encoded by the *ESR1* gene, is another important functional candidate for the regulation of bone mass. A large number of investigators have looked for evidence of an association between *ESR1* alleles and BMD, mostly focusing on two polymorphisms within intron 1, recognised by the *XbaI* and *PvuII* restriction enzymes. There is some evidence to suggest that these polymorphisms regulate *ESR1* transcription and that they may therefore be functionally important (Herrington *et al*. 2002). A meta-analysis of published studies performed up until 2001 (Ioannidis *et al*. 2002) has shown an association between the *XbaI* polymorphism, BMD and fractures, with higher BMD values and reduced fracture risk in 'XX' homozygotes. Recently, a large prospective analysis in the Genetic Markers for Osteoporosis Project has confirmed that XX homozygotes have a reduced risk of fracture (Ioannidis *et al*. 2004), but no association with BMD was observed, indicating that *ESR1* might influence fracture risk by mechanisms that are independent of BMD. One possible mechanism might be through effects on bone quality or bone turnover, since *ESR1* alleles have recently been associated with ultrasound properties of bone and rates of post-menopausal bone loss (Albagha *et al*. 2005)

Transforming growth factor β1

Several polymorphisms of the *TGFB1* gene that encodes the growth factor transforming growth factor β-1 have been identified and some of them have been associated with BMD and/or osteoporotic fracture in various studies (Langdahl *et al*. 1997; Yamada *et al*. 2001). The best functional candidate is a C/T polymorphism that causes a proline to leucine amino acid substitution at position 10 in the transforming growth factor β-1 signal peptide that has been associated with circulating transforming growth factor β-1 levels. However, this polymorphism is in strong linkage disequilibrium within other polymorphisms in the *TGFB1* promoter and effects on transcription are also possible (Shah *et al*. 2005). Although many association studies have been performed, most are of limited sample size and further large-scale studies will be required to confirm or refute the status of *TGFB1* as a true susceptibility gene for osteoporosis.

Lipoprotein receptor-related protein 5

Inactivating mutations of the lipoprotein receptor-related protein (LRP) 5 gene are the cause of the rare recessive disorder osteoporosis pseudoglioma syndrome (Gong *et al*. 2001), whereas activating mutations in the same gene cause autosomal dominant inheritance of high bone mass (Little *et al*. 2002). The involvement of *LRP5* in these rare monogenic bone disorders led several investigators to evaluate the role of *LRP5* as a candidate gene for BMD regulation in the normal population. Six studies have now been published showing evidence of an allelic association between polymorphisms in *LRP5* and BMD (Ferrari *et al*. 2004; Koay *et al*. 2004; Koh *et al*. 2004; Mizuguchi *et al*. 2004; Urano *et al*. 2004; van Meurs *et al*. 2006). Many variants have been studied, but the most likely functional candidate is an alanine to valine amino acid substitution at position 1330 (A1330V). The mechanism by which this variant affects *LRP5* signalling has not been investigated, but evidence of an interaction between the *LRP5* A1330V variant and a coding polymorphism of *LRP6* (1062V) has been gained in the Rotterdam study (van Meurs *et al*. 2006), in which polymorphisms of both genes were found to interact to affect fracture susceptibility. One consistent feature to emerge from these studies is that the association between *LRP5* alleles and BMD is stronger in males (Ferrari *et al*. 2004; Koay *et al*. 2004), which suggests that LRP5 may regulate bone mass in a gender-specific manner.

Sclerostin

Mutations affecting the *SOST* gene, which encodes sclerostin, are the cause of the sclerosing bone dysplasias Van Buchem disease and sclerosteosis (Balemans *et al*. 2001, 2002*b*; Brunkow *et al*. 2001). Polymorphisms of *SOST* have been evaluated in relation to BMD in two studies. In one study (Balemans *et al*. 2002*a*) no association between *SOST* polymorphism and BMD was found in peri-menopausal women using a case–control design, whereas in another study of older women (Uitterlinden *et al*. 2004) evidence of an association with BMD was observed in men and women, with effects that increase with age. These data suggest that *SOST* polymorphisms may regulate BMD, especially in older individuals.

TCIRG1

The *TCIRG1* gene encodes the APT6i subunit of the osteoclast-specific proton pump (Frattini *et al*. 2000). Inactivating mutations in *TCIRGI* are responsible for a subset of patients with recessive osteopetrosis. Recent work indicates that polymorphisms of *TCIRG1* might contribute to regulation of BMD in the normal population;

a study by Frattini (Sobacchi *et al*. 2004) has shown evidence of an association between a polymorphism affecting an activator protein 1-binding site in the *TCIRG1* promoter and BMD in peri-menopausal women. However, functional studies need to be performed to identify the mechanisms that underlie this association and to replicate the finding in other populations.

CLCN7

The *CLCN7* gene encodes a chloride channel that is highly expressed in osteoclasts and essential for acidification of the resorption lacuna. Homozygous inactivation mutations in *CLCN7* cause a severe form of recessive osteopetrosis whereas heterozygous missense mutations of *CLCN7* cause autosomal dominant osteopetrosis (Balemans *et al*. 2005). Prompted by this observation Pettersson *et al*. (2005) have looked for evidence of an association between polymorphisms of *CLCN7* and BMD in normal individuals and have found that a common polymorphism in exon 15 of *CLCN7* that results in a methionine to valine amino acid change is associated with BMD in normal women. Further studies will be required to determine whether this polymorphism is functionally important and to replicate the observation in other populations.

Gene–environment interactions

Several studies have been performed in which interactions have been sought between environmental factors and polymorphic variants in candidate genes. For the most part, these studies have been underpowered and conflicting results have been obtained. The most widely studied gene–environment interaction is between VDR alleles and Ca or vitamin D intake. An early study (Krall *et al*. 1995) has shown faster rates of post-menopausal bone loss in women with the BB genotype of VDR than in women with other genotype groups, but have found that Ca supplements (500 mg daily) abolish this difference. Another study (Ferrari *et al*. 1995) has shown that change in lumbar spine bone density is associated with Ca intake in elderly subjects who have the VDR 'Bb' genotype group, but not in other genotypes. The largest study of VDR alleles in relation to bone mass, bone loss and Ca intake is that of MacDonald *et al*. (2006) who found little evidence of an interaction between Ca intake, VDR alleles and bone mass or bone loss. A functional polymorphism at position 677 (C677T) in the methylene tetrahydrofolate reductase gene has been associated with BMD and fracture in various studies (Miyao *et al*. 2000; Jorgensen *et al*. 2002; Abrahamsen *et al*. 2003; Bathum *et al*. 2004), but the results have been inconsistent, since in some studies (Abrahamsen *et al*. 2003; Bathum *et al*. 2004) the T allele has been associated with osteoporosis and in others (Jorgensen *et al*. 2002) the C allele has been associated with osteoporosis. Recent work has suggested that folate status (McLean *et al*. 2004) or riboflavin status (MacDonald *et al*. 2004) might influence the association between methylene tetrahydrofolate reductase alleles and BMD.

Conclusions

Many advances have been made in understanding the role of genetic factors in osteoporosis over the past 10 years, but a great deal of additional research is required to identify the genes that regulate BMD and other phenotypes relevant to the pathogenesis of osteoporotic fractures. Until recently, most of the studies in the area of osteoporosis genetics have been underpowered, leading to results that have seldom been replicated (Ioannidis, 2003). It has now become clear that large-scale studies need to be assembled to evaluate the true role of genetic polymorphisms in osteoporosis and other complex diseases (Ioannidis *et al*. 2006). It is likely that large-scale studies, when combined with technological advances such as genome-wide association, will assist in identifying and validating the role of candidate gene polymorphisms in the regulation of BMD and other markers of osteoporosis susceptibility.

References

Abrahamsen B, Madsen JS, Tofteng CL, Stilgren L, Bladbjerg EM, Kristensen SR, Brixen K & Mosekilde L (2003) A common methylenetetrahydrofolate reductase (C677T) polymorphism is associated with low bone mineral density and increased fracture incidence after menopause: longitudinal data from the Danish osteoporosis prevention study. *Journal of Bone and Mineral Research* **18**, 723–729.

Alam I, Sun Q, Liu L, Koller DL, Fishburn T, Carr LG, Econs MJ, Foroud T & Turner CH (2005) Whole-genome scan for linkage to bone strength and structure in inbred Fischer 344 and Lewis rats. *Journal of Bone and Mineral Research* **20**, 1589–1596.

Albagha OM, Pettersson U, Stewart A, McGuigan FE, MacDonald HM, Reid DM & Ralston SH (2005) Association of oestrogen receptor alpha gene polymorphisms with postmenopausal bone loss, bone mass, and quantitative ultrasound properties of bone. *Journal of Medical Genetics* **42**, 240–246.

Andrew T, Antoniades L, Scurrah KJ, MacGregor AJ & Spector TD (2005) Risk of wrist fracture in women is heritable and is influenced by genes that are largely independent of those influencing BMD. *Journal of Bone and Mineral Research* **20**, 67–74.

Arai H, Miyamoto KI, Yoshida M, Yamamoto H, Taketani Y, Morita K *et al.* (2001) The polymorphism in the caudal-related homeodomain protein Cdx-2 binding element in the human vitamin D receptor gene. *Journal of Bone and Mineral Research* **16**, 1256–1264.

Arden NK, Baker J, Hogg C, Baan K & Spector TD (1996) The heritability of bone mineral density, ultrasound of the calcaneus and hip axis length: a study of postmenopausal twins. *Journal of Bone and Mineral Research* **11**, 530–534.

Balemans W, Ebeling M, Patel N, Van Hul E, Olson P, Dioszegi M *et al.* (2001) Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). *Human Molecular Genetics* **10**, 537–543.

Balemans W, Foernzler D, Parsons C, Ebeling M, Thompson A, Reid DM, Lindpaintner K, Ralston SH & Van Hul W (2002*a*) Lack of association between the SOST gene and bone mineral density in perimenopausal women: analysis of five polymorphisms. *Bone* **31**, 515–519.

Balemans W, Patel N, Ebeling M, Van Hul E, Wuyts W, Lacza C *et al.* (2002*b*) Identification of a 52 kb deletion downstream of the SOST gene in patients with van Buchem disease. *Journal of Medical Genetics* **39**, 91–97.

Balemans W, Van Wesenbeeck L & Van Hul W (2005) A clinical and molecular overview of the human osteopetroses. *Calcified Tissue International* **77**, 263–274.

Bathum L, von Bornemann HJ, Christiansen L, Madsen JS, Skytthe A & Christensen K (2004) Evidence for an association of methylene tetrahydrofolate reductase polymorphism C677T and an increased risk of fractures: results from a population-based Danish twin study. *Osteoporosis International* **15**, 659–664.

Beamer WG, Shultz KL, Donahue LR, Churchill GA, Sen S, Wergedal JR, Baylink DJ & Rosen CJ (2001) Quantitative trait loci for femoral and lumbar vertebral bone mineral density in C57BL/6J and C3H/HeJ inbred strains of mice. *Journal of Bone and Mineral Research* **16**, 1195–1206.

Bouxsein ML, Rosen CJ, Turner CH, Ackert CL, Shultz KL, Donahue LR *et al.* (2002) Generation of a new congenic mouse strain to test the relationships among serum insulin-like growth factor I, bone mineral density, and skeletal morphology in vivo. *Journal of Bone and Mineral Research* **17**, 570–579.

Brunkow M, Gardner J, Van Ness J, Paeper B, Kovacevich B, Proll S *et al.* (2001) Bone dysplasia sclerosteosis results from loss of the sost gene product, a novel cystine knot-containing protein. *American Journal of Human Genetics* **68**, 577–589.

Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, Cauley J, Black D & Vogt TM (1995) Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. *New England Journal of Medicine* **332**, 767–773.

Deng HW, Chen WM, Recker S, Stegman MR, Li JL, Davies KM, Zhou Y, Deng H, Heaney R & Recker RR (2000) Genetic determination of Colles' fracture and differential bone mass in women with and without Colles' fracture. *Journal of Bone and Mineral Research* **15**, 1243–1252.

Deng HW, Xu FH, Huang QY, Shen H, Deng H, Conway T *et al.* (2002) A whole-genome linkage scan suggests several genomic regions potentially containing quantitative trait loci for osteoporosis. *Journal of Clinical Endocrinology and Metabolism* **87**, 5151–5159.

Devoto M, Shimoya K, Caminis J, Ott J, Tenenhouse A, Whyte MP *et al.* (1998) First-stage autosomal genome screen in extended pedigrees suggests genes predisposing to low bone mineral density on chromosomes 1p, 2p and 4q. *European Journal of Human Genetics* **6**, 151–157.

Efstathiadou Z, Tsatsoulis A & Ioannidis JP (2001) Association of collagen Ialpha 1 Sp1 polymorphism with the risk of prevalent fractures: a meta-analysis. *Journal of Bone and Mineral Research* **16**, 1586–1592.

Fang Y, van Meurs JB, Bergink AP, Hofman A, van Duijn CM, van Leeuwen JP, Pols HA & Uitterlinden AG (2003) Cdx-2 polymorphism in the promoter region of the human vitamin D receptor gene determines susceptibility to fracture in the elderly. *Journal of Bone and Mineral Research* **18**, 1632–1641.

Fang Y, van Meurs JB, D'Alesio A, Jhamai M, Zhao H, Rivadeneira F, Hofman A, van Leeuwen JP, Jehan F, Pols HA & Uitterlinden AG (2005) Promoter and 3′-untranslated-region haplotypes in the vitamin D receptor gene predispose to osteoporotic fracture: the Rotterdam Study. *American Journal of Human Genetics* **77**, 807–823.

Ferrari S, Rizzoli R, Chevally T, Slosman D, Eisman JA & Bonjour J-P (1995) Vitamin D receptor gene polymorphisms and change in lumbar spine bone mineral density. *Lancet* **345**, 423–424.

Ferrari SL, Deutsch S, Choudhury U, Chevalley T, Bonjour JP, Dermitzakis ET, Rizzoli R & Antonarakis SE (2004) Polymorphisms in the low-density lipoprotein receptor-related protein 5 (LRP5) gene are associated with variation in vertebral bone mass, vertebral bone size, and stature in whites. *American Journal of Human Genetics* **74**, 866–875.

Fisher SA, Lanchbury JS & Lewis CM (2003) Meta-analysis of four rheumatoid arthritis genome-wide linkage studies: confirmation of a susceptibility locus on chromosome 16. *Arthritis and Rheumatism* **48**, 1200–1206.

Frattini PJ, Orchard C, Sobacchi S, Giliani M, Abinun JP, Mattsson DJ *et al.* (2000) Defects in TCIRG1 subunit of the vacuolar proton pump are responsible for a subset of human autosomal recessive osteopetrosis. *Nature Genetics* **25**, 343–346.

Garcia-Giralt N, Enjuanes A, Bustamante M, Mellibovsky L, Nogues X, Carreras R, Diez-Perez A, Grinberg D & Balcells S (2005) In vitro functional assay of alleles and haplotypes of two COL1A1-promoter SNPs. *Bone* **36**, 902–908.

Garcia-Giralt N, Nogues X, Enjuanes A, Puig J, Mellibovsky L, Bay-Jensen A, Carreras R, Balcells S, Diez-Perez A & Grinberg D (2002) Two new single nucleotide polymorphisms in the COLIA1 upstream regulatory region and their relationship with bone mineral density. *Journal of Bone and Mineral Research* **17**, 384–393.

Garnero P, Arden NK, Griffiths G, Delmas PD & Spector TD (1996) Genetic influence on bone turnover in postmenopausal twins. *Journal of Clinical Endocrinology and Metabolism* **81**, 140–146.

Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM *et al.* (2001) LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell* **107**, 513–523.

Grant SFA, Reid DM, Blake G, Herd R, Fogelman I & Ralston SH (1996) Reduced bone density and osteoporosis associated with a polymorphic Sp1 site in the collagen type I alpha 1 gene. *Nature Genetics* **14**, 203–205.

Gross C, Eccleshall TR, Malloy PJ, Villa ML, Marcus R & Feldman D (1997) The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in postmenopausal Mexican-American women. *Journal of Bone and Mineral Research* **12**, 1850–1856.

Gross C, Krishnan AV, Malloy PJ, Eccleshall TR, Zhao XY & Feldman D (1998) The vitamin D receptor gene start codon polymorphism: a functional analysis of FokI variants. *Journal of Bone and Mineral Research* **13**, 1691–1699.

Gueguen R, Jouanny P, Guillemin F, Kuntz C, Pourel J & Siest G (1995) Segregation analysis and variance components analysis of bone mineral density in healthy families. *Journal of Bone and Mineral Research* **12**, 2017–2022.

Harris SS, Patel MS, Cole DE & Dawson-Hughes B (2000) Associations of the collagen type I alpha1 Sp1 polymorphism with five-year rates of bone loss in older adults. *Calcified Tissue International* **66**, 268–271.

Herrington DM, Howard TD, Brosnihan KB, McDonnell DP, Li X, Hawkins GA, Reboussin DM, Xu J, Zheng SL, Meyers DA & Bleecker ER (2002) Common estrogen receptor polymorphism augments effects of hormone replacement therapy on E-selectin but not C-reactive protein. *Circulation* **105**, 1879–1882.

Ichikawa S, Koller DL, Johnson ML, Lai D, Xuei X, Edenberg HJ *et al.* (2006) Human ALOX12, but not ALOX15, is associated with BMD in white men and women. *Journal of Bone and Mineral Research* **21**, 556–564.

Ioannidis JP (2003) Genetic associations: false or true? *Trends in Molecular Medicine* **9**, 135–138.

Ioannidis JP, Gwinn M, Little J, Higgins JP, Bernstein JL, Boffetta P *et al.* (2006) A road map for efficient and reliable human genome epidemiology. *Nature Genetics* **38**, 3–5.

Ioannidis JP, Ralston SH, Bennett ST, Brandi ML, Grinberg D, Karassa FB *et al.* (2004) Differential genetic effects of ESR1 gene polymorphisms on osteoporosis outcomes. *Journal of the American Medical Association* **292**, 2105–2114.

Ioannidis JP, Stavrou I, Trikalinos TA, Zois C, Brandi ML, Gennari L, Albagha O, Ralston SH & Tsatsoulis A (2002) Association of polymorphisms of the estrogen receptor alpha gene with bone mineral density and fracture risk in women: a meta-analysis. *Journal of Bone and Mineral Research* **17**, 2048–2060.

Jorgensen HL, Madsen JS, Madsen B, Saleh MM, Abrahamsen B, Fenger M & Lauritzen JB (2002) Association of a common allelic polymorphism (C677T) in the methylene tetrahydrofolate reductase gene with a reduced risk of osteoporotic fractures. A case control study in Danish postmenopausal women. *Calcified Tissue International* **71**, 386–392.

Kammerer CM, Schneider JL, Cole SA, Hixson JE, Samollow PB, O'Connell JR *et al.* (2003) Quantitative trait loci on chromosomes 2p, 4p, and 13q influence bone mineral density of the forearm and hip in Mexican Americans. *Journal of Bone and Mineral Research* **18**, 2245–2252.

Kannus P, Palvanen M, Kaprio J, Parkkari J & Koskenvuo M (1999) Genetic factors and osteoporotic fractures in elderly people: prospective 25 year follow up of a nationwide cohort of elderly Finnish twins. *British Medical Journal* **319**, 1334–1337.

Karasik D, Cupples LA, Hannan MT & Kiel DP (2003) Age, gender, and body mass effects on quantitative trait loci for bone mineral density: the Framingham Study. *Bone* **33**, 308–316.

Karasik D, Myers RH, Cupples LA, Hannan MT, Gagnon DR, Herbert A & Kiel DP (2002) Genome screen for quantitative trait loci contributing to normal variation in bone mineral density: the Framingham Study. *Journal of Bone and Mineral Research* **17**, 1718–1727.

Klein RF, Allard J, Avnur Z, Nikolcheva T, Rotstein D, Carlos AS, Shea M, Waters RV, Belknap JK, Peltz G & Orwoll ES (2004) Regulation of bone mass in mice by the lipoxygenase gene Alox15. *Science* **303**, 229–232.

Klein RF, Mitchell SR, Phillips TJ, Belknap JK & Orwoll ES (1998) Genetic analysis of bone mass in mice. *Journal of Bone and Mineral Research* **13**, 1648–1656.

Koay MA, Woon PY, Zhang Y, Miles LJ, Duncan EL, Ralston SH *et al.* (2004) Influence of LRP5 polymorphisms on normal variation in BMD. *Journal of Bone and Mineral Research* **19**, 1619–1627.

Koh JM, Jung MH, Hong JS, Park HJ, Chang JS, Shin HD, Kim SY & Kim GS (2004) Association between bone mineral density and LDL receptor-related protein 5 gene polymorphisms in young Korean men. *Journal of Korean Medical Science* **19**, 407–412.

Koller DL, Alam I, Sun Q, Liu L, Fishburn T, Carr LG, Econs MJ, Foroud T & Turner CH (2005) Genome screen for bone mineral density phenotypes in Fisher 344 and Lewis rat strains. *Mammalian Genome* **16**, 578–586.

Koller DL, Econs MJ, Morin PA, Christian JC, Hui SL, Parry P *et al.* (2000) Genome Screen for QTLs Contributing to Normal Variation in Bone Mineral Density and Osteoporosis. *Journal of Clinical Endocrinology and Metabolism* **85**, 3116–3120.

Koller DL, Liu G, Econs MJ, Hui SL, Morin PA, Joslyn G *et al.* (2001) Genome screen for quantitative trait loci underlying normal variation in femoral structure. *Journal of Bone and Mineral Research* **16**, 985–991.

Krall EA & Dawson-Hughes B (1993) Heritable and life-style determinants of bone mineral density. *Journal of Bone and Mineral Research* **8**, 1–9.

Krall EA, Parry P, Lichter JB & Dawson-Hughes B (1995) Vitamin D receptor alleles and rates of bone loss: influence of years since menopause and calcium intake. *Journal of Bone and Mineral Research* **10**, 978–984.

Langdahl BL, Knudsen JY, Jensen HK, Gregersen N & Eriksen EF (1997) A sequence variation: 713–8 elC in the transforming growth factor-beta 1 gene has higher prevalence in osteoporotic women than in normal women and is associated with very low bone mass in osteoporotic women and increased bone turnover in both osteoporotic and normal women. *Bone* **20**, 289–294.

Little RD, Carulli JP, Del Mastro RG, Dupuis J, Osborne M, Folz C *et al.* (2002) A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. *American Journal of Human Genetics* **70**, 11–19.

Liu PY, Lu Y, Long JR, Xu FH, Shen H, Recker RR & Deng HW (2004) Common variants at the PCOL2 and Sp1 binding sites of the COL1A1 gene and their interactive effect influence bone mineral density in Caucasians. *Journal of Medical Genetics* **41**, 752–757.

MacDonald HM, McGuigan FE, Fraser WD, New SA, Ralston SH & Reid DM (2004) Methylenetetrahydrofolate reductase polymorphism interacts with riboflavin intake to influence bone mineral density. *Bone* **35**, 957–964.

MacDonald HM, McGuigan FE, Stewart A, Black AJ, Fraser WD, Ralston S & Reid DM (2006) Large-scale population-based study shows no evidence of association between common polymorphism of the VDR gene and BMD in British women. *Journal of Bone and Mineral Research* **21**, 151–162.

MacDonald HM, McGuigan FEA, New SA, Campbell MK, Golden MH, Ralston SH & Reid DM (2001) COL1A1 Sp1 polymorphism predicts perimenopausal and early postmenopausal spinal bone loss. *Journal of Bone and Mineral Research* **16**, 1634–1641.

McLean RR, Karasik D, Selhub J, Tucker KL, Ordovas JM, Russo GT, Cupples LA, Jacques PF & Kiel DP (2004) Association of a common polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene with bone phenotypes depends on plasma folate status. *Journal of Bone and Mineral Research* **19**, 410–418.

Mahaney MC, Morin P, Rodriguez LA, Newman DE & Rogers J (1997) A quantitative trait locus on chromosome 11 may influence bone mineral density at several sites: quantitative analysis in pedigreed baboons. *Journal of Bone and Mineral Research* **12**, Suppl. 1, s118Abstr.

Mann V, Hobson EE, Li B, Stewart TL, Grant SF, Robins SP, Aspden RM & Ralston SH (2001) A COL1A1 Sp1 binding site polymorphism predisposes to osteoporotic fracture by affecting bone density and quality. *Journal of Clinical Investigation* **107**, 899–907.

Mann V & Ralston SH (2003) Meta-analysis of COL1A1 Sp1 polymorphism in relation to bone mineral density and osteoporotic fracture. *Bone* **32**, 711–717.

Michaelsson K, Melhus H, Ferm H, Ahlbom A & Pedersen NL (2005) Genetic liability to fractures in the elderly. *Archives of Internal Medicine* **165**, 1825–1830.

Miyao M, Morita H, Hosoi T, Kurihara H, Inoue S, Hoshino S, Shiraki M, Yazaki Y & Ouchi Y (2000) Association of methylenetetrahydrofolate reductase (MTHFR) polymorphism with bone mineral density in postmenopausal Japanese women. *Calcified Tissue International* **66**, 190–194.

Mizuguchi T, Furuta I, Watanabe Y, Tsukamoto K, Tomita H, Tsujihata M *et al.* (2004) LRP5, low-density-lipoprotein-receptor-related protein 5, is a determinant for bone mineral density. *Journal of Human Genetics* **49**, 80–86.

Orwoll ES, Belknap JK & Klein RF (2001) Gender specificity in the genetic determinants of peak bone mass. *Journal of Bone and Mineral Research* **16**, 1962–1971.

Peacock M, Koller DL, Fishburn T, Krishnan S, Lai D, Hui S, Johnston CC, Foroud T & Econs MJ (2005) Sex-specific and non-sex-specific quantitative trait loci contribute to normal variation in bone mineral density in men. *Journal of Clinical Endocrinology and Metabolism* **90**, 3060–3066.

Pettersson U, Albagha OM, Mirolo M, Taranta A, Frattini A, McGuigan FE *et al.* (2005) Polymorphisms of the CLCN7 gene are associated with BMD in women. *Journal of Bone and Mineral Research* **20**, 1960–1967.

Qureshi AM, McGuigan FEA, Seymour DG, Hutchison JD, Reid DM & Ralston SH (2001) Association between COLIA1 Sp1 alleles and femoral neck geometry. *Calcified Tissue International* **69**, 67–72.

Ralston SH, Galwey N, Mackay I, Albagha OM, Cardon L, Compston JE *et al.* (2005) Loci for regulation of bone mineral density in men and women identified by genome wide linkage scan: the FAMOS study. *Human Molecular Genetics* **14**, 943–951.

Sawcer SJ, Maranian M, Singlehurst S, Yeo T, Compston A, Daly MJ *et al.* (2004) Enhancing linkage analysis of complex disorders: an evaluation of high-density genotyping. *Human Molecular Genetics* **13**, 1943–1949.

Shah R, Rahaman B, Hurley CK & Posch PE (2005) Allelic diversity in the TGFB1 regulatory region: characterization of novel functional single nucleotide polymorphisms. *Human Genetics* **119**, 1–14.

Sobacchi C, Vezzoni P, Reid DM, McGuigan FE, Frattini A, Mirolo M, Albhaga OM, Musio A, Villa A & Ralston SH (2004) Association between a polymorphism affecting an AP1 binding site in the promoter of the TCIRG1 gene and bone mass in women. *Calcified Tissue International* **74**, 35–41.

Stewart TL, Jin H, McGuigan FE, Albagha OM, Garcia-Giralt N, Bassiti A, Grinberg D, Balcells S, Reid DM & Ralston SH (2006) Haplotypes defined by promoter and intron 1 polymorphisms of the COLIA1 gene regulate bone mineral density in women. *Journal of Clinical Endocrinology and Metabolism* **91**, 4112–4117.

Stewart TL, Roschger P, Misof BM, Mann V, Fratzl P, Klaushofer K, Aspden RM & Ralston SH (2005) Association of *COLIA1* Sp1 alleles with defective bone nodule formation *in vitro* and abnormal bone mineralisation *in vivo*. *Calcified Tissue International* **77**, 113–118.

Styrkarsdottir U, Cazier J-B, Kong A, Rolfsson O, Larsen H, Bjarnadottir E *et al.* (2003) Linkage of osteoporosis to chromosome 20p12 and association to BMP2. *PLoS Biology* **1**, E69.

Thakkinstian A, D'Este C, Eisman J, Nguyen T & Attia J (2004) Meta-analysis of molecular association studies: vitamin D receptor gene polymorphisms and BMD as a case study. *Journal of Bone and Mineral Research* **19**, 419–428.

Torgerson DJ, Campbell MK, Thomas RE & Reid DM (1996) Prediction of perimenopausal fractures by bone mineral density and other risk factors. *Journal of Bone and Mineral Research* **11**, 293–297.

Turner CH, Sun Q, Schriefer J, Pitner N, Price R, Bouxsein ML, Rosen CJ, Donahue LR, Shultz KL & Beamer WG (2003) Congenic mice reveal sex-specific genetic regulation of femoral structure and strength. *Calcified Tissue International* **73**, 297–303.

Uitterlinden AG, Arp PP, Paeper BW, Charmley P, Proll S, Rivadeneira F *et al.* (2004) Polymorphisms in the sclerosteosis/van Buchem disease gene (SOST) region are associated with bone-mineral density in elderly whites. *American Journal of Human Genetics* **75**, 1032–1045.

Uitterlinden AG, Burger H, Huang Q, Yue F, McGuigan FEA, Grant SFA, Hofman A, van Leeuwen JPTM, Pols HAP & Ralston SH (1998) Relation of alleles of the collagen type I a 1 gene to bone density and risk of osteoporotic fractures in postmenopausal women. *New England Journal of Medicine* **338**, 1016–1022.

Urano T, Shiraki M, Ezura Y, Fujita M, Sekine E, Hoshino S, Hosoi T, Orimo H, Emi M, Ouchi Y & Inoue S (2004) Association of a single-nucleotide polymorphism in low-density lipoprotein receptor-related protein 5 gene with bone mineral density. *Journal of Bone and Mineral Metabolism* **22**, 341–345.

van Meurs JB, Rivadeneira F, Jhamai M, Hugens W, Hofman A, van Leeuwen JP, Pols HA & Uitterlinden AG (2006) Common genetic variation of the low-density lipoprotein receptor-related protein 5 and 6 genes determines fracture risk in elderly white men. *Journal of Bone and Mineral Research* **21**, 141–150.

Wilson SG, Reed PW, Andrew T, Barber MJ, Lindersson M, Langdown M *et al.* (2004) A genome-screen of a large twin cohort reveals linkage for quantitative ultrasound of the calcaneus to 2q33–37 and 4q12–21. *Journal of Bone and Mineral Research* **19**, 270–277.

Wilson SG, Reed PW, Bansal A, Chiano M, Lindersson M, Langdown M *et al.* (2003) Comparison of genome screens for two independent cohorts provides replication of suggestive linkage of bone mineral density to 3p21 and 1p36. *American Journal of Human Genetics* **72**, 144–155.

Yamada Y, Ando F, Niino N & Shimokata H (2005) Association of a -1997G→T polymorphism of the collagen Ialpha1 gene with bone mineral density in postmenopausal Japanese women. *Human Biology* **77**, 27–36.

Yamada Y, Miyauchi A, Takagi Y, Tanaka M, Mizuno M & Harada A (2001) Association of the C-509T polymorphism, alone of in combination with the T869→C polymorphism, of the transforming growth factor-beta1 gene with bone mineral density and genetic susceptibility to osteoporosis in Japanese women. *Journal of Molecular Medicine* **79**, 149–156.

Zhang YY, Lei SF, Mo XY, Wang YB, Li MX & Deng HW (2005) The -1997 G/T polymorphism in the COLIA1 upstream regulatory region is associated with hip bone mineral density (BMD) in Chinese nuclear families. *Calcified Tissue International* **76**, 107–112.