REVIEW

Role of genetic factors in the pathogenesis of osteoporosis

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Abstract

Osteoporosis is a common disease with a strong genetic component characterised by low bone mass, microarchitectural deterioration of bone tissue and an increased risk of fracture. Twin and family studies have shown that genetic factors play an important role in regulating bone mineral density and other determinants of osteoporotic fracture risk, such as ultrasound properties of bone, skeletal geometry and bone turnover. Osteoporosis is a polygenic disorder, determined by the effects of several genes, each with relatively modest effects on bone mass and other determinants of fracture risk. It is only on rare occasions that osteoporosis occurs as the result of mutations in a single gene. Linkage studies in man and experimental animals have defined multiple loci which regulate bone mass but the genes responsible for these effects remain to be defined. Population-based studies and case-control studies have similarly identified polymorphisms in several candidate genes that have been associated with bone mass or osteoporotic fracture, including the vitamin D receptor, oestrogen receptor and collagen type IαI gene. The individual contribution of these genes to the pathogenesis of osteoporosis is small however, reflected by the fact that the relationship between individual candidate genes and osteoporosis has been inconsistent in different studies. An important aim of future work will be to define how the genes which regulate bone mass, bone turnover and other aspects of bone metabolism interact with each other and with environmental variables to cause osteoporosis in individual patients. If that aim can be achieved then there is every prospect that preventative therapy could be targeted to those at greatest risk of the osteoporosis, before fractures have occurred.

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Introduction

Osteoporosis is a common disease characterised by a generalised reduction in bone mineral density (BMD), microarchitectural deterioration of bone tissue and an increased risk of fracture. Osteoporosis is defined to exist when BMD values fall more than 2·5 standard deviations below the young adult mean. Since BMD values fall progressively with age, the prevalence of osteoporosis increases with age. It has been estimated that approximately 50% of all women will have osteoporosis by the age of 80 according to the above definition, although only a small proportion of these individuals will suffer an osteoporosis-related fracture. This reflects the fact that whilst low BMD values are strongly related to the risk of osteoporotic fracture, several other factors contribute, including: advanced age itself, low body mass index, previous fractures, muscle weakness, impaired vision, cognitive impairment, hyperthyroidism, corticosteroid therapy, use of sedatives, anticonvulsants and reduced muscle strength (Cummings *et al.* 1995, De Laet *et al.* 1997).

What is the evidence for a genetic contribution to osteoporosis?

Studies in twins and families indicate that genetic factors play an important role in the regulation of BMD and other determinants of osteoporotic fracture risk. The heritability of BMD has been estimated to lie between 50% and 85% in twin studies, with the strongest effects in the axial skeleton (Smith *et al.* 1973, Pocock *et al.* 1987, Christian *et al.* 1989, Slemenda *et al.* 1991, Flicker *et al.* 1995). Family-based studies have also yielded strong heritability estimates for BMD (Gueguen *et al.* 1995), with effects that are maximal in young adults and persist even after adjusting for lifestyle factors that are known to regulate BMD

(Krall & Dawson–Hughes 1993). Other determinants of osteoporotic fracture risk also have a heritable component, including: femoral neck geometry and hip axis length (Arden *et al.* 1996, Flicker *et al.* 1996, Slemenda *et al.* 1996), ultrasound properties of bone (Arden *et al.* 1996), biochemical markers of bone turnover (Morrison *et al.* 1992, Tokita *et al.* 1994, Garnero *et al.* 1996, Harris *et al.* 1998), body mass index (Carmichael & McGue 1995, Kaprio *et al.* 1995, Arden & Spector 1997), muscle strength (Arden & Spector 1997), age at menarche (Kaprio *et al.* 1995) and age at menopause (Snieder *et al.* 1998). The data are conflicting with regard to the influence of genetic factors on bone loss; Christian *et al.* (1989) found no evidence for a genetic effect on bone loss at the wrist in ageing male twins, whereas Kelly *et al.* (1993) concluded that there were strong genetic effects on axial bone loss in female twins. Only one twin study has tried to determine whether genetic factors contribute to fracture itself (Kannus *et al.* 1999). This showed higher rates of concordance for fracture in monozygotic twins as compared with dizygotic twins, but the differences were not significant. These data were erroneously interpreted as showing no evidence for a genetic effect on fracture, but the familial resemblance that was observed in fracture risk irrespective of twin status indicates that the study was inadequately powered to detect such an effect.

It should be noted that the heritability estimates cited in twin studies and family studies may not accurately reflect the actual percentage of BMD that is due to genetic factors but, rather, reflect the proportion of total variance in BMD attributable to genetic factors. Moreover, the assumptions made in the twin model do not take account of gene–gene or gene–environment interactions and can result in artificially inflated estimates of a genetic contribution due to greater sharing of environmental influences in monozygotic twins (Slemenda *et al.* 1991, Seeman & Hopper 1997).

Despite these caveats, there is little doubt that genetic factors are extremely important in the pathogenesis of osteoporosis and, in keeping with this, several population-based studies have shown that a family history of fracture is a significant risk factor for fracture by mechanisms that are partly independent of bone density (Cummings *et al.* 1995, Torgerson *et al.* 1996, Keen *et al.* 1999*a*).

How is osteoporosis inherited?

Osteoporosis is a complex disease, thought to be mediated by an interaction between environmental factors and several different genes that individually have modest effects on BMD and other aspects of fracture risk (Gueguen *et al.* 1995). In rare instances, however, osteoporosis is inherited in a simple Mendelian manner. Examples of this include osteogenesis imperfecta (Rowe 1991), and osteoporosis associated with inactivating

mutations in the aromatase gene (Morishima *et al.* 1995) and oestrogen receptor α gene (Smith *et al.* 1994). Families have also been described in which unusually high bone mass is inherited as an autosomal dominant trait, consistent with the effects of a single gene (Johnson *et al.* 1997). In these cases, the consequences of the gene mutation are so profound as to overwhelm the effects of other genes which contribute to regulation of bone mass. Even in such extreme cases, it is possible to identify polygenic effects on disease severity. The best examples of this are in osteogenesis imperfecta, where disease severity can vary markedly within and between families which have identical mutations in the collagen genes, presumably due to the influence of other genes on bone mass and bone fragility (Willing *et al.* 1990).

Strategies for identifying osteoporosis genes

The main strategies for identification and characterisation of genes that are involved in the pathogenesis of polygenic disorders like osteoporosis are illustrated in Fig. 1. In essence, all of these approaches involve looking for evidence of an association between a phenotypic characteristic and a series of polymorphic genetic markers. The phenotypic characteristic may be a continuous variable such as BMD or may be a categorical variable such as fracture. The genetic markers used in these studies are polymorphic regions of DNA which are analysed by PCR-based techniques on DNA extracted from peripheral blood. There are two main types of marker: repeat polymorphisms of variable length (VNTR) and single nucleotide polymorphisms (SNPs). Genetic studies involve typing a large number of markers spread at regular intervals throughout the genome (a genome search), or typing markers that are concentrated in specific areas of interest (candidate loci) or specific genes of interest (candidate genes). The probability that a marker locus is linked to a disease locus is expressed by the lodscore. The lodscore is the logarithm of the odds that loci are linked rather than unlinked; by convention, linkage is considered statistically significant with lodscores of $>+3 \cdot 0$ and can be rejected with lodscores of $< -2 \cdot 0$. Regions of chromosomes that contain alleles that influence continuous phenotypic traits such as BMD are termed quantitative tract loci (QTL). Whilst fracture is the outcome measure of most clinical importance in osteoporosis, this phenotype is difficult to study by genetic-based approaches. This is because confounding environmental factors such as trauma play an important role in the pathogenesis of fracture and this is difficult to control for in linkage analysis. Furthermore, since osteoporotic fractures occur late in life, the chances of collecting large families with several affected individuals who have fractures are also slim. Because of this, most studies in the field have focused on identifying QTL that regulate BMD. The genetic tools most commonly used for
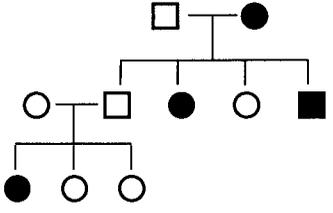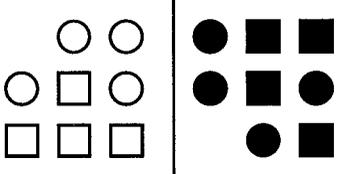
Figure 1 Different methods of investigating the genetic basis of complex disease.

QTL identification are whole genome scans in families, sib pairs and experimental animals. More recently, it has been suggested that genome-wide linkage disequilibrium mapping with SNPs in unrelated individuals may provide an alternative approach to QTL localization, although the feasibility of this remains unclear (Kruglyak 1999). Candidate gene approaches have also been widely used in the search for osteoporosis susceptibility genes. While candidate gene studies are in some respects more powerful than linkage-based approaches to the study of complex diseases, they are also prone to give false positive results due to population admixture, or biased selection of cases and controls. In view of this, associations should be regarded as provisional, pending replication in other populations or confirmation by techniques that use family-based controls such as the transmission disequilibrium test (TDT). TDT is based upon the assumption that if a given allele contributes to disease, then the probability that an affected person has inherited the allele from a heterozygous parent should vary from the expected Mendelian ratio of 50:50 (Spielman *et al.* 1994). Although the TDT test has been considered as a 'gold standard' for confirming the results of association studies, recent experience indicates that even this approach may yield results that are not reproducible.

Linkage studies in man

Genetic linkage studies have been successful in defining several loci responsible for regulation of bone mass. The syndrome of osteoporosis–pseudoglioma syndrome (OPS) is an autosomal recessive disorder of unknown cause characterised by juvenile onset blindness and osteoporosis. Gong *et al.* (1998) localised the gene responsible for OPS to a 3 cM region of chromosome 11q12–13, by a combination of linkage analysis and homozygosity mapping. Johnson *et al.* (1997) mapped a gene implicated in the regulation of high bone mass to the same chromosomal

region in an extended family with autosomal dominant inheritance of increased bone mass (BMD Z-score + 3·0 or greater). Independently, Heaney *et al.* (1998) mapped the gene responsible for autosomal recessive osteopetrosis – a condition characterised by osteosclerosis, deafness, blindness and severe anaemia – to the same locus. Taken together, these observations raise the possibility that all three traits may be caused by allelic variations of the same gene or family of genes. In order to assess the relevance of this region in determining BMD in normal individuals, Koller *et al.* (1999*b*) conducted a linkage study with several markers at the 11q12–13 locus in normal female sib pairs. They reported a peak lodscore of + 3·50 in the middle of the candidate region indicating that the gene(s) implicated in the monogenic disorders referred to above may also contribute to variation of BMD in the normal population.

Other linkage studies in sib pairs have defined several other loci which regulate BMD. Devoto *et al.* (1998) conducted a genome search in sib pairs derived from several extended families with osteoporosis and identified three regions with lodscores above + 2·5 on chromosomes 1p36, 2p23–p24 and 4q32–34 and several other loci with lodscores between + 1·5 and + 2·5. Koller *et al.* (1999*a*) used a similar approach to search for loci responsible for regulation of bone mass in 428 normal female sib pairs. In a first round genome search, lodscores of greater than + 1·8 were identified on chromosomes 1q21–23, 5q33–35 and 6p11–12. Further genotyping at these loci was carried out in an expanded sample of 464 Caucasian sib pairs and 131 African–American sib pairs. The highest lodscore attained was + 3·86 at chromosome 1q21–23. Lodscores of + 2·23 and + 2·13 were observed at the loci on chromosomes 5q33–35 and 6p11–12 respectively. Another genome-wide scan (Nui *et al.* 1999) in 96 Chinese nuclear families found tentative evidence of linkage between forearm BMD and loci on chromosome 2p21 (lodscore + 2·15) and 3q34 (lodscore + 1·67). Duncan *et al.* (2000) conducted a linkage study of 23 candidate genes implicated in the pathogenesis of osteoporosis in 115 probands and 499 of their relatives. The maximum lodscore was observed at the parathyroid hormone (PTH) receptor 1 locus (+ 2·7–3·5) but positive lodscores (above + 1·7–1·8) were also observed at the vitamin D receptor (VDR) locus, the collagen type Iα1 (COLIA1) locus and the epidermal growth factor locus.

Linkage studies in animals

Linkage studies in experimental animals provide an additional method of identifying the genes responsible for human disease, based on the assumption that key regulatory genes will be shared across species. Most studies in this area have been performed using mice, although some work has also been carried out in baboons. Studies in mice involve setting up experimental crosses of mouse strains

with low bone mass and high bone mass. Brother–sister mating is then carried out using the offspring from the F1 cross to generate F2 animals with markedly varying bone mass. Linkage studies are then performed in the progeny that result from the breeding programme. Linkage studies in inbred strains of mice have identified several loci that regulate bone mass. Klein *et al.* (1998) performed a genome search in 24 inbred strains of mice with varying BMD, generated by crossing the DBA/2 (high BMD) and the C57/BL6 (low BMD) strains. Ten loci were provisionally identified as being linked to bone mass in female mice and four loci were linked to body weight. One locus on chromosome 14 was linked to body weight and bone density. Similar studies performed on inter-crosses between the SAMP6 (low BMD) and SAMP2 (normal BMD) strains of mice identified two major loci which were responsible for regulation of bone mass; one on chromosome 11 (lodscore + 10·8) and another on chromosome 13 (lodscore + 5·8). A further locus was identified on the X-chromosome with possible evidence of linkage to BMD (Shimizu *et al.* 1999). Further studies are required to identify the genes responsible for these effects and to determine if these genes and loci are also important in regulating bone mass in humans.

Candidate gene studies

Candidate gene association studies in osteoporosis have logically tackled the main regulators of bone metabolism, such as calciotrophic hormones, bone matrix proteins, steroid hormones and local regulators of bone metabolism. Relevant information on candidate genes which have been studied so far in relation to the genetics of osteoporosis are summarised in Table 1, together with a more detailed discussion below of specific candidate genes which have been the most extensively investigated.

VDR

The active metabolites of vitamin D play an important role in regulating bone cell function and maintenance of serum calcium homeostasis by binding to the VDR and regulating the expression of a number of response genes. Morrison *et al.* (1994) reported a significant association between polymorphisms in the 3′ region of VDR and BMD in a twin study and a population-based study. On the basis of these results, they concluded that allelic variation of this gene may account for up to 75% of the genetic effect on BMD, although subsequent work showed that the effect of VDR on bone mass was much weaker than that originally reported due to genotyping errors. A large number of studies have since been carried out on the relationships between VDR genotype, bone density and other aspects of calcium metabolism. Studies

Table 1 Candidate gene studies in osteoporosis

Candidate	Function	Association	References
VDR	Calcium absorption; osteoblast-osteoclast activity	BMD, calcium absorption; serum osteocalcin levels	Morrison et al. 1992
Oestrogen receptor	Osteoblast-osteoclast activity	BMD	Sano et al. 1995, Kobayashi et al. 1996
Androgen receptor	Osteoblast function	BMD	Sowers et al. 1999
PTH	Calcium homeostasis; osteoblast-osteoclast activity	BMD	Hosoi et al. 1999
Calcitonin receptor	Osteoclast function	BMD, vertebral fracture	Masi et al. 1998, Taboulet et al. 1998
Peroxisome proliferator-activated receptor γ	Adipocyte differentiation	BMD	Ogawa et al. 1999
COLIA1	Matrix component	BMD, vertebral fracture; hip fracture	Grant et al. 1996
α2-HS-glycoprotein	Matrix component	Heel ultrasound	Zmuda et al. 1998
Osteocalcin	Matrix component	BMD	Dohi et al. 1998
TGFβ-1	Osteoblast-osteoclast activity	BMD, vertebral fracture; serum TGFβ levels	Langdahl et al. 1997, Yamada et al. 1998, Grainger et al. 1999
Interleukin-6	Osteoclast activity	BMD	Murray et al. 1997, Tsukamoto et al. 1998
IL-1RN	Osteoblast-osteoclast activity	Postmenopausal bone loss	Keen et al. 1998
ApoE	Vitamin K transport	BMD, hip fracture	Shiraki et al. 1997, Cauley et al. 1999a

TGFβ-1, transorming growth factor β-1; IL-1RN, interleukin-1 receptor antagonist; ApoE, apolipoprotein E.

by several investigators have essentially supported the original findings (Eisman 1995, Cooper & Umbach 1996, Ames *et al.* 1999), whereas others have found no significant association between VDR alleles and BMD (Garnero *et al.* 1995) and still others reported an inverse association to those originally reported (Houston *et al.* 1996, Uitterlinden *et al.* 1996). A recent meta-analysis (Gong *et al.* 1999) of studies published between 1994 and 1998 concluded that there was strong evidence for a positive effect of VDR on bone mass, although this did not include data from the largest study so far (Uitterlinden *et al.* 1996). There is evidence to suggest the relationship between VDR genotype and BMD may be modified by high calcium intake (Ferrari *et al.* 1995, Krall *et al.* 1995) and vitamin D intake (Graafmans *et al.* 1997). In keeping with this view, intestinal calcium absorption has been associated with the BsmI VDR polymorphism in some studies (Dawson-Hughes *et al.* 1995, Gennari *et al.* 1997). The mechanism by which this occurs is unclear however, since no association has been found between genotype and mucosal VDR density (Barger-Lux *et al.* 1995). Two studies have looked at possible associations between VDR genotype and fracture. In one, a positive association was found between VDR genotype and fractures, but this was only significant in a subgroup of older women aged 75 years and above (Feskanich *et al.* 1998). In another study, no effect was found (Ensrud *et al.* 1999). Studies that have sought to define functional associations of the 3′ VDR polymorphisms have yielded mixed results. Reporter gene constructs prepared from the 3′ region of the VDR gene in different individuals have shown evidence of haplotype-specific differences in gene transcription, suggesting that the BsmI, ApaI and TaqI polymorphisms in this region may possibly act as a marker for other sequence variations in the 3′ untranslated region of VDR that affect RNA stability (Morrison *et al.* 1994). This observation is supported, in part, by the results of other studies which showed differences in allele-specific transcription in myeloid and prostate-derived cell lines that were heterozygous (Tt) for the TaqI polymorphism (Verbeek *et al.* 1997). In these studies, however, transcripts from the 't' allele were 30% more abundant than the 'T' which is the opposite from the result expected on the basis of the studies of Morrison *et al.* (1994). Other *in vitro* studies have shown no differences in allele-specific transcription, or ligand binding in relation to the BsmI polymorphism (Mocharla *et al.* 1997, Gross *et al.* 1998*b*). Another polymorphism in exon 2 of the VDR gene is a T→C transition, recognised by the FokI restriction enzyme (Arai *et al.* 1997, Gross *et al.* 1998*a*). This transition introduces an alternative translational start codon that results in a shorter isoform of the VDR gene. The FokI polymorphism has been associated with BMD in some studies (Arai *et al.* 1997, Gross *et al.* 1997, Harris *et al.* 1997) but not in others (Eccleshall *et al.* 1998, Lucotte *et al.* 1999, Sowers *et al.* 1999). In one study, an association was found with calcium

between VDR FokI alleles and calcium absorption (Ames *et al.* 1999), whereas two other studies reported no significant effects (Cauley *et al.* 1999*b*, Zmuda *et al.* 1999). Functional studies of the FokI polymorphism have yielded mixed results; Arai *et al.* (1997) reported that different VDR FokI alleles linked to a luciferase reporter gene construct had different biological activities *in vitro*, whereas, in other studies, no functional differences between FokI alleles were observed (Gross *et al.* 1998*a*). In summary, the studies which have been performed to date indicate that allelic variation at the VDR gene locus has some role to play in the genetic regulation of bone mass. These effects appear to be modified by dietary calcium and vitamin D intake and in some studies have been associated with differences in intestinal calcium absorption. The molecular mechanisms responsible remain unclear however, since no consistent differences have been observed between allelic variants at the 5′ or 3′ region of the VDR and receptor function, indicating the need for further work in this area.

Type I collagen

Type I collagen is the major structural protein of bone, thus the genes encoding this protein (COLIA1 and COLIA2) are candidates for the genetic regulation of bone mass. Indeed, deletions or point mutations in these two genes have been identified as the molecular basis of up to 90% of cases of osteogenesis imperfecta, a hereditary disease characterised by osteoporotic bone and skeletal fracture in early life (Rowe 1991). This led Spotila *et al.* (1994) to look for an association between polymorphisms in coding regions of the collagen type I genes and BMD. A disease-associated coding mutation was found in one family which, on clinical evaluation, was thought to represent a mild form of osteogenesis imperfecta (Spotila *et al.* 1991). Polymorphisms were found in other families, but some of these were also observed in normal individuals and overall they were not thought to be causally related to the presence of osteoporosis. In a related study, Grant *et al.* (1996) found evidence of a common polymorphism affecting a binding site for the transcription factor Sp1 in the first intron of COLIA1 which was more prevalent in osteoporotic patients as compared with controls. Positive associations between the COLIA1 Sp1 polymorphism and bone mass or osteoporotic fractures were subsequently reported in other studies (Garnero *et al.* 1998, Langdahl *et al.* 1998, Roux *et al.* 1998, Uitterlinden *et al.* 1998, Alvarez *et al.* 1999). Several investigators have noted that the association between COLIA1 alleles and fracture persists after correction for BMD (Uitterlinden *et al.* 1998) and is stronger than would be expected on the basis of allele-specific differences in bone mass (Grant *et al.* 1996, Langdahl *et al.* 1998, Keen *et al.* 1999*b*). This has led to speculation that the polymorphism may principally act as a marker for increased bone fragility rather than reduced BMD.

However, a small study in a Finnish population (Liden *et al.* 1998) showed no significant association between the COLIA1 Sp1 polymorphism and bone mass or fracture, nor did another study in US twins, although the fracture patients in this study were much older than the controls (Hustmyer *et al.* 1999). Significant ethnic differences have been reported in the population prevalence of this Sp1 polymorphism. It is relatively common in Caucasian populations, but rare in Africans and very rare in Asians (Beavan *et al.* 1998). The mechanism by which the Sp1 polymorphism predisposes to osteoporosis remains to be fully defined, but preliminary data have shown evidence of allele-specific differences in binding of the Sp1 protein to the polymorphic recognition site; differences in allele-specific transcription; differences in collagen protein production; and differences in bone strength in samples derived from patients of different genotype (Dean *et al.* 1998).

Oestrogen α receptor (ESR)

Oestrogen, by interacting with its receptors in bone and other tissues plays an important role in regulating skeletal growth and maintenance of bone mass. Knockout mice with null alleles at the ESR locus have reduced BMD compared with wild-type controls (Korach 1994) and osteoporosis has also been observed in a man with an inactivating mutation of the ESR gene (Smith *et al.* 1994). These findings prompted research on the relationship of polymorphisms at the ESR locus and bone mass. Association studies have focused on two SNPs and a TA dinucleotide repeat in the ESR gene. Sano *et al.* (1995) reported a positive association between a TA repeat polymorphism in the ESR promoter and bone mass in a small study in Japanese women and similar results were observed in an American population (Sowers *et al.* 1999). Other investigators have reported positive associations between haplotypes defined by PvuII and/or XbaI polymorphisms in the first intron of the ESR gene and bone mass (Kobayashi *et al.* 1996, Mizunuma *et al.* 1997, Ongphiphadhanakul *et al.* 1998, Sowers *et al.* 1999) as well as age at menopause (Weel *et al.* 1999). Other studies in Korean (Han *et al.* 1997), Belgian (Vandevyer *et al.* 1999) and Italian (Gennari *et al.* 1998) populations found no association between PvuII polymorphisms and bone mass. Whilst ESR PvuII alleles interacted with VDR alleles to predict BMD in the Italian study (Gennari *et al.* 1998), no such effect was observed in the Belgian study (Vandevyer *et al.* 1999). The molecular mechanism by which these polymorphisms influence bone mass are as yet unclear. The PvuII and XbaI polymorphisms lie in an apparently non-functional area of the gene but are in linkage disequilibrium with the TA polymorphism in the ESR promoter. It is conceivable that the TA repeat could influence gene transcription, but this has not yet been studied.

Other genes

Transforming growth factor beta (TGFβ)

A rare polymorphism in intron 4 of TGFβ-1 has been associated with very low BMD and osteoporotic fracture in one study (Langdahl *et al.* 1997) but the effects on TGFβ function are unclear. Another polymorphism, a C→T transition in a TGFβ-1 coding region, has been described which causes a leucine-proline substitution at amino acid 10. The C allele was associated with high BMD and a reduced frequency of osteoporotic fractures in two Japanese populations (Yamada *et al.* 1998). This polymorphism is associated with raised circulating levels of TGFβ, suggesting that it may influence protein secretion or stability. Two promoter polymorphisms of TGFβ have also been described that are associated with circulating TGFβ levels (Grainger *et al.* 1999), but the relationship with BMD has not yet been reported.

Androgen receptor (AR)

A polymorphic (AGC)$_n$ repeat polymorphism has been described in exon 1 of the AR, which codes for a polyglutamine tract in the activation domain of the receptor. Length variations in the polymorphism have previously been associated with differences in receptor function and large expansions of the tract have been found to cause X-linked spino-cerebellar muscular atrophy (Tut *et al.* 1997). Sowers *et al.* (1999) reported a strong association between the AR repeat polymorphism and BMD in a cohort of 261 American women, but this remains to be confirmed in other populations.

Osteocalcin

Dohi *et al.* (1998) described a C→T transition in the gene promoter of the osteocalcin gene that was related to BMD in a Japanese population but Sowers *et al.* (1999) found no association between this polymorphism and BMD or circulating osteocalcin levels in an American population.

Apolipoprotein E (ApoE)

The human ApoE gene is polymorphic, with three common alleles (ε2, ε3, ε4) coding for three isoforms (E2, E3, E4) which differ from each other by a single amino acid and in their binding affinity for the four ApoE receptors. Shiraki *et al.* (1997) reported that BMD values were significantly reduced in Japanese women who carried the ApoE4 allele and a recent study in American Caucasian women (Cauley *et al.* 1999*a*) found an association between the presence of at least one ApoE4 allele and hip fracture. In this study, there was no significant difference in BMD at the femoral neck between those with or without the ApoE4 allele. These studies highlight ApoE as a promising candidate gene for further study, although the precise

mechanisms by which ApoE alleles influence bone remain unclear.

α2-HS-glycoprotein

α2-HS-glycoprotein (AHSG) is a serum-derived protein that binds to bone matrix due to its affinity to hydroxyapatite. Zmuda *et al.* (1998) studied the relationship between a coding polymorphism of AHSG and osteoporosis in Caucasian women from the USA. The polymorphism (AHSG★1 or AHSG★2) was not significantly related to hip, lumbar spine or calcaneal BMD but, compared with the homozygous AHSG★2 women, calcaneal broadband ultrasound attenuation (BUA) was 13% lower in heterozygous and 16% lower in homozygous AHSG★1 women. Height was also reduced in homozygous AHSG★1 women, intermediate in heterozygous women, and highest among homozygous AHSG★2 subjects. These results suggest that the AHSG polymorphism may contribute to the genetic influence on calcaneal BUA and height.

Parathyroid hormone

Two studies have looked for an association between an anonymous polymorphism recognised by the enzyme BstB1 in the parathyroid hormone gene and osteoporosis. Studies in a Japanese population reported a positive association with BMD (Hosoi *et al.* 1999), and in another study metacarpal dimensions were positively associated with this polymorphism. The mechanisms which underlie this association remain to be determined.

Interleukin-6 (IL-6)

Two studies have looked for evidence of an association between BMD and polymorphisms at the IL-6 locus. An AT-rich minisatellite repeat was associated with lumbar spine BMD in one study (Murray *et al.* 1997) and a polymorphic AC-rich minisatellite was reported to be associated with wrist BMD in another (Tsukamoto *et al.* 1998). The mechanisms by which these polymorphisms affect IL-6 gene function are unclear, but it is possible that they could be mediated by linkage disequilibrium with a functional polymorphism that is known to be present in the IL-6 promoter (Fishman *et al.* 1998).

Interleukin-1 receptor antagonist (IL-1RN)

An 86 base pair VNTR polymorphism has been identified in the second intron of the IL-1RN gene. In an association study of 108 postmenopausal women, Keen *et al.* (1998) found that IL-1RN genotype was significantly associated with early postmenopausal bone loss at the spine over a 5-year period but no significant relationship was observed with BMD overall at either the spine or hip. Further studies will be required to confirm these findings.

Calcitonin receptor

A coding polymorphism causing a proline-leucine substitution at codon 436 of the calcitonin receptor gene has been described. The relationship between this polymorphism and BMD has been studied in French and Italian populations. Masi *et al.* (1998) reported that individuals homozygous for the leucine substitution had reduced bone mass when compared with heterozygotes and proline homozygotes. Taboulet *et al.* (1998) reported an association between BMD and this polymorphism, and found that heterozygotes had higher BMD and a reduced risk of fracture when compared with homozygotes. The effects of this polymorphism on calcitonin receptor function has not yet been studied.

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