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Impact of genetic variation on metabolic response of bone to diet

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There is compelling evidence to suggest that both the development of bone to peak bone mass at maturity and subsequent loss depend on the interaction between genetic, hormonal, environmental and nutritional factors. The major part (≤ 80 %) of the age-specific variation in bone turnover and bone density is genetically determined. However, the notion of genetic determinant is of little value unless the specific genes that are involved can be identified. Most work in this area of osteoporosis research has focused on the candidate gene approach, which has identified several candidate genes for osteoporosis, including genes encoding the vitamin D receptor (VDR), oestrogen receptors (α and β), apolipoprotein E, collagen type I α 1 and methylenetetrahydrofolate reductase, amongst many others. However, in general, findings from numerous studies of the association between such genes and various bone variables have been inconsistent. In addition to possible gene—gene interactions it is likely that there are interactions between these genes and certain environmental factors, especially nutrition, that may mediate expression of bone-related phenotypes. While these potential interactions add a level of complexity to our understanding of these apparent genetic effects on bone, identification of a role for genetic factors without knowledge of their interaction with nutrients can do little to advance prevention and treatment of osteoporosis. This information is especially important because, unlike genotype, diet and nutrition can be modified. The aim of the present review is to critically evaluate current knowledge relating to candidate genes for osteoporosis, with particular emphasis on their interaction with nutrients and dietary factors in determining bone health.

Osteoporosis: Genotype: Nutrient: Interactions

Osteoporosis is a metabolic bone disease characterized by low bone mass and deterioration of bone tissue that leads to bone fragility and an increase in fracture risk in later life. Ageing demographics of Europe and other continents suggest that unless drastic measures are taken to prevent the development of osteoporosis, the incidence and the costs associated with treating osteoporosis will climb in the coming decades (Norris, 1992; European Commission, 1998), posing a major socio-economic burden. Consequently, the urgent need for suitable preventive strategies has intensified osteoporosis research carried out by physicians as well as scientists from a diverse range of backgrounds. While the molecular genetics of osteoporosis and the role of nutrition in bone health are currently very vibrant and important research areas in their own right, there is a huge opportunity for more collaborative research efforts between these two areas aimed at cohesive strategies for osteoporosis prevention.

The importance of genetics in the pathogenesis of osteoporosis is well established. Studies in twins and families have shown that genetic factors play an important role in the regulation of bone mineral density (BMD) and other determinants of osteoporotic fracture risk. It has been estimated from twin studies that 50–85 % of the variance in BMD is genetically determined (Pocock *et al*. 1987; Christian *et al*. 1989; Slemenda *et al*. 1991). Family-based studies have also yielded strong heritability estimates for BMD, especially in young adulthood (Gueguen *et al*. 1995). Whilst low BMD is a major risk factor for osteoporotic fracture, there are other determinants of osteoporotic fracture risk, including femoral neck geometry and hip axis length, ultrasound properties of bone, biochemical markers of bone turnover, BMI, age at menarche and age at menopause, and these factors also have a heritable component (for review, see Stewart & Ralston, 2000). For example, heritability estimates of risk factors such as quantitative ultrasound, femoral neck geometry and markers of bone turnover range between 50 and 80 % (Arden *et al*. 1996; Garnero *et al*. 1996).

Abbreviations: Apo, apolipoprotein; BMD, bone mineral density; HRT, hormone-replacement therapy; MTHFR, methylenetetrahydrofolate reductase; OR, oestrogen receptor; VDR, vitamin D receptor.

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Osteoporosis is a complex disease that is mediated by an interaction between environmental factors (including nutrition, smoking and physical activity) and several different genes that individually have modest effects on BMD and other aspects of fracture risk (Gueguen *et al*. 1995). However, the notion of genetic determinants is of little value unless the specific genes involved can be identified and the interactions between these genes and certain environmental factors (especially nutrition) that may mediate expression of bone-related phenotypes can be elucidated.

Strategies for identification of osteoporosis-susceptibility genes

There are several strategies for identification of genes that are involved in the pathogenesis of polygenic disorders, including osteoporosis. These strategies include linkage analysis studies, studies investigating allele sharing in sibling pairs and the candidate gene approach, amongst others; the advantages and disadvantages have been described in detail elsewhere (Stewart & Ralston, 2000; Thompson, 2001; Vink & Boomsma, 2002; Ralston, 2003). In essence, all these approaches involve looking for evidence of an association between a phenotypic characteristic (e.g. bone turnover, BMD, fractures) and a single or series of polymorphic genetic marker(s) (Stewart & Ralston, 2000). The genetic markers used in these studies are polymorphic regions of DNA, which are analysed by polymerase chain reaction-based techniques on DNA extracted usually from peripheral blood. There are two main types of marker, repeat polymorphisms of variable length (e.g. dinucleotide repeats) and single nucleotide polymorphisms. Genetic studies involve typing a large number of markers spread at regular intervals throughout the genome (a genome-wide search) or typing markers that are concentrated in specific areas of interest (candidate loci) or in specific genes of interest (candidate genes; Stewart & Ralston, 2000). Genetic linkage studies have been successful in defining several loci responsible for regulation of bone mass, and now these chromosomal regions are being mined, or fine mapped, for genes contained therein that may predispose to low BMD. Studies using the osteoporosis candidate gene approach, on the other hand, have logically tackled the main regulators of bone metabolism and mass. While the potential interaction between such genes and environmental factors, including nutrients and food components, add a level of complexity to our understanding of these apparent genetic effects (see p. 905), identification of a role for genetic factors without knowledge of such interactions can do little to advance prevention and treatment of osteoporosis (Wood & Fleet, 1998). The remainder of the present review will briefly describe the various candidate genes for susceptibility to osteoporosis that have been most extensively investigated, and then review available evidence for interactions between these genes and certain nutrients or food components in determining bone health, and thus osteoporosis risk.

Candidate genes for osteoporosis

There have been a staggering number of studies published over the last two decades that have reported associations, or lack thereof, between candidate genes and bone turnover, BMD and/or fracture incidence, as well as other bone-related phenotypic characteristics such as ultrasound properties of bone. These genes encode a wide range of proteins, including receptors for calciotrophic and steroid hormones, bone matrix proteins, and local regulators of bone metabolism, such as cytokines and growth factors, amongst others (see Table 1). Some of the more important candidate genes that have been studied (especially genes for which a gene–nutrient interaction is likely or possible) are discussed in more detail later.

Table 1. Major candidate genes implicated in the aetiology of osteoporosis

Candidate gene	Bone health-related function	Physiological correlate	Seminal reference(s)
Vitamin D receptor	Ca absorption; osteoblast and osteoclast activity	BMD, Ca absorption, bone turnover	Morrison *et al.* (1994), Gross *et al.* (1996)
Oestrogen receptor α	Osteoblast and osteoclast activity	BMD, fracture	Sano *et al.* (1995), Kobayashi *et al.* (1996)
Oestrogen receptor β	Osteoblast and osteoclast activity	BMD	Ogawa *et al.* (2000)
Collagen type I α1	Matrix compoenent	BMD, fracture, bone quality	Grant *et al.* (1996)
MTHFR enzyme	Homocysteine clearance	BMD, fracture	Miyao *et al.* (2000)
TGFβ1	Osteoblast and osteoclast activity	BMD, vertebral fracture	Langdahl *et al.* (1997)
Androgen receptor	Osteoblast function	BMD	Sowers *et al.* (1999)
Interleukin 6	Osteoclast activity	BMD	Murray *et al.* (1997)
Apolipoprotein E	Vitamin K transport	BMD, hip and wrist fracture bone quality	Shiraki *et al.* (1997)
PTH receptor	Ca homeostasis; osteoblast and osteoclast activity	BMD	Hosoi *et al.* (1999)
Calcitonin receptor	Osteoclast function	BMD, vertebral fracture	Masi *et al.* (2002)
Osteocalcin	Matrix component	BMD	Dohi *et al.* (1998)
Ca-sensing receptor	Regulation of Ca homeostasis	BMD	Takacs *et al.* (2002)
Metalloproteinase-1	Matrix component	BMD	Yamada *et al.* (2002)

BMD, bone mineral density; MTHFR, Methylenetetrahydrofolate reductase; TGFβ-1, transforming growth factor β1; PTH, parathyroid hormone.

Vitamin D receptor gene polymorphisms

The majority of association studies of BMD and candidate gene markers have investigated markers in the vitamin D receptor (VDR) gene (Wood & Fleet, 1998). The seco-steroid 1,25-dihydroxycholecalciferol has been shown to be an important hormonal regulator of bone and Ca metabolism (Norman *et al*. 1990) and the VDR mediates the biological actions of 1,25-dihydroxycholecalciferol. Thus, the prominent role of the VDR in Ca metabolism made the VDR gene a likely candidate gene in determining low BMD and, hence, risk of osteoporosis. While there are likely to be more than twenty-five polymorphisms present in the VDR gene, including areas that are functionally relevant such as the promoter region (Uitterlinden *et al*. 2002), most associational studies to date have focused on only a handful of these polymorphisms.

Taq *I*, Bsm *I and* Apa *I vitamin D receptor polymorphisms*. In 1994 a cardinal study by Morrison *et al*. (1994) reported a significant association ($P < 0{\cdot}0001$–$P < 0{\cdot}05$, depending on skeletal site) between polymorphic sites situated between exons 8 and 9 at the 3′ end of the VDR gene (detected using the *Bsm* 1 restriction enzyme) and BMD in 250 Caucasian twins aged 17–70 years from Australia. The study consisted of seventy monozygotic and fifty-five dizygotic adult twin pairs; with most subjects being female. In addition, a further 311 unrelated healthy adult females (207 of which were post-menopausal) were also studied. From their study of twins Morrison *et al*. (1994) concluded that much of the genetic variation in BMD (≤ 75 %) could be explained on the basis of the *Bsm* 1 VDR genotype alone. They also reported that post-menopausal women with the *BB* VDR genotype would reach the BMD 'fracture threshold' (defined as 2 SD below the mean of young adults) 10 years sooner than their *bb* VDR genotype counterparts. This greater decline in BMD in the *BB* VDR group could markedly increase their risk of bone fracture. However, the same group subsequently reported that there were problems with their original genotyping of the dizygotic twin part of their study, such that the heritability component attributable to the VDR is lower (Morrison *et al*. 1997).

Since the initial report by Morrison *et al*. (1994) many groups have investigated the relationship between VDR genotypes (defined at the 3′ end) and BMD and bone turnover (as measured by serum- and urinary-based biochemical markers) either in twins or in general populations. Although many studies in Caucasian and Asian populations have confirmed a positive effect between extreme homozygotes (i.e. *BB v. bb*), other studies have reported little or no effect in various populations (for reviews, see Eisman, 1995, 1999, 2001; Peacock, 1995; Wood & Fleet, 1998; Gennari *et al*. 2002; Uitterlinden *et al*. 2002). Moreover, some studies, including a large Dutch study, have reported a VDR gene allele effect, but in the opposite direction to that of the previous studies (Houston *et al*. 1996; Salamone *et al*. 1996; Uitterlinden *et al*. 1996). After reviewing sixteen studies published up to July 1996 in a meta-analysis, Cooper & Umbach (1996) concluded that although overall there was an effect of the *Bsm* 1 VDR polymorphism (of the order of about 0·3 SD) between alternate homozygotes), it was weaker than that reported in the original study of Morrison *et al*. (1994; a difference of ≤ 1 SD unit, or 10 %). A second more recent meta-analysis of seventy-five studies published (in full or as abstracts) between 1994 and 1998 confirmed the findings of the earlier meta-analysis, concluding that there was strong evidence for a positive effect of VDR on bone mass (Gong *et al*. 1999).

Some of the inconsistencies in the various studies performed to date may arise from the VDR gene effects on bone being modified by dietary Ca, vitamin D, caffeine and possibly the intake of other nutrients (see p. 905), or by an interaction of the VDR gene with other genes such as the oestrogen receptor (OR) α gene (Gennari *et al*. 1998; Willing *et al*. 1998; Deng *et al*. 1999; for review, see Gennari *et al*. 2002).

There is also some, albeit inconsistent, evidence to suggest a relationship between VDR genotype and other bone-related phenotypic characteristics such as Ca absorption (see p. 903) and fracture. For example, there are only a limited number of studies that have investigated the link between VDR genotype and fracture incidence, the clinically-relevant outcome of osteoporosis; three studies found a positive association (Feskanich *et al*. 1998; Langdahl *et al*. 2000; Uitterlinden *et al*. 2001), while other studies found no effect (Berg *et al*. 1996; Houston *et al*. 1996; Ensrud *et al*. 1999).

Studies that have sought to define a functional association for the 3′-VDR polymorphisms using reporter gene constructs and gene transcription assays, or *in vitro* binding assays, have yielded mixed results (Morrison *et al*. 1994; Verbeek *et al*. 1997; Gross *et al*. 1998*a*; for reviews, see Gennari *et al*. 2002; Uitterlinden *et al*. 2002).

Fok *1 VDR polymorphisms*. Another common polymorphism in the VDR gene has been described in the coding region (exon 2; Gross *et al*. 1996; Arai *et al*. 1997). This polymorphism results in a T→C transition, recognized by the *Fok* 1 restriction enzyme. It creates an alternative translation start codon (9 bp downstream) that results in a shorter isoform of the VDR gene. The *Fok* 1 polymorphism in the VDR gene has been associated with a 13 % lower lumbar spine BMD and a greater rate of bone loss at the hip (4·7 *v*. 0·5 % for ff *v*. FF genotypes) in post-menopausal Mexican-American women (Gross *et al*. 1996). However, no intergroup differences were detected in any of the biochemical indices of bone turnover (Gross *et al*. 1996). The *Fok 1* polymorphism has been associated with BMD in some studies reported subsequently (Miyamoto *et al*. 1996; Arai *et al*. 1997; Harris *et al*. 1997; Gennari *et al*. 1999; Lucotte *et al*. 1999; Choi *et al*. 2000), but not others (Eccleshall *et al*. 1998; Cheng & Tsai, 1999; Sowers *et al*. 1999; Tofteng *et al*. 2002). This polymorphism does not seem to be in linkage dysequilibrium with 3′-VDR polymorphisms, and thus acts independently.

In a study in children aged 7–12 years Ames *et al*. (1999) showed that the *Fok* 1 polymorphism at the VDR translation initiation site was associated with BMD and Ca absorption. Children with the *FF* genotype absorbed on average 115 mg Ca/d more than those with the *ff* genotype. BMD was 8·2 % greater in the *FF* genotype than in the *ff* genotype. These results suggest a substantial relationship between the VDR gene and bone health at one or more levels, including absorption of dietary Ca and BMD in growing children. It

should be noted, however, that the influence of the *Fok* 1 VDR genotype has not been found in all studies of children and young adults. Ferrari *et al*. (1998), for example, failed to find an association between the *Fok* 1 VDR polymorphism and BMD in European-Caucasian prepubertal girls and premenopausal women.

As with the studies of the 3′ polymorphisms, functionality studies of the *Fok* 1 polymorphism have yielded mixed results (Arai *et al*. 1997; Gross *et al*. 1998*b*; for reviews, see Gennari *et al*. 2002; Uitterlinden *et al*. 2002). Thus, there is a need for further work to define the molecular mechanisms by which the various VDR polymorphisms influence Ca metabolism and bone mass.

Oestrogen receptor (α and β) gene polymorphisms

Oestrogen deficiency in post-menopausal women is associated with increased bone turnover and acceleration of bone loss, leading to increased susceptibility to bone fractures (Nguyen *et al*. 1995). Furthermore, oestrogen-replacement therapy has been shown to prevent this accelerated bone loss, which is associated with the post-menopausal period (Riggs & Melton, 1986). The presence of the OR has been demonstrated in human bone cells, suggesting that oestrogen may exert a direct effect on bone. Moreover, an inactivating mutation of the ORα gene has been associated with decreased bone density in the case of a male patient (Smith *et al*. 1994), and there has also been a report of decreased BMD values in mice lacking functional ORα (Korach, 1994). Thus, it is not surprising that research has focused on the possible relationships between polymorphisms at the ORα locus and bone mass. Similarly, while the exact role of the ORβ is not clear, its involvement in mediating an oestrogenic action on bone growth and size in ORβ knock-out mice (Windahl *et al*. 1999) has made it another likely candidate gene.

Oestrogen receptor α gene polymorphisms. Sano *et al*. (1995) reported a positive association between a TA dinucleotide repeat polymorphism in the ORα promoter and bone mass in a study of 144 Japanese women. Sowers *et al*. (1999) reported similar findings from a study of an American population (including 261 pre- and peri-menopausal women). There have also been a number of studies that have investigated associations between haplotypes defined by *Pvu* II and/or *Xba* I restriction fragment length polymorphisms in the first intron of the ORα gene and bone mass. While some of these studies reported positive associations between the *Pvu* II and/or *Xba* I polymorphisms and bone mass (Kobayashi *et al*. 1996; Mizunuma *et al*. 1997; Ongphiphadhanakul *et al*. 1998), other studies did not (Han *et al*. 1997; Gennari *et al*. 1998; Vandevyver *et al*. 1999). An association between ORα genotype and low BMD has also been found in Caucasian populations (Mahonen *et al*. 1997; Willing *et al*. 1998). However, in these studies low BMD was shown to be associated with the *pp* ORα genotype, while in Asian populations low BMD is associated with the *PP* ORα genotype, suggesting that the ORα genotype effect on BMD may be population specific (Willing *et al*. 1998). A recent meta-analysis of data from studies published up to November 2001, encompassing data for 5834 female subjects from thirty distinct study groups, found no association between the *Pvu* II polymorphism and BMD, while *XX* ORα genotype, detected by use of the *Xba* I restriction enzyme, was found to confer a higher BMD, in addition to a protective effect that decreased the risk of fracture (Ioannidis *et al*. 2002).

The molecular mechanism by which these polymorphisms influence bone mass is unclear. Both *Pvu* II and *Xba* I polymorphisms lie in an apparently non-functional area of the gene (Stewart & Ralston, 2000).

Oestrogen receptor β gene polymorphisms. Ogawa *et al*. (2000) reported an association between BMD and a dinucleotide (CA) repeat polymorphism located in the flanking region of the ORβ gene in healthy Japanese post-menopausal women. Lau *et al*. (2002) also found an association between the ORβ and BMD in premenopausal Chinese women, but with a different allelic distribution pattern, i.e. twenty-six-CA repeats *v*. twenty-CA repeats in the studies of Ogawa *et al*. (2000) and Lau *et al*. (2002) respectively were associated with significantly ($P < 0{\cdot}01$) increased lumbar spine adjusted BMD values. Ban *et al*. (2001) failed to find an association between either the twenty-CA repeat allele or twenty-six-CA repeat allele and BMD in a population of older Japanese women. Functional studies will also be required to unravel the underlying molecular mechanisms of these polymorphisms.

Apolipoprotein E gene polymorphisms

Apolipoprotein E (Apo E) is a major constituent of HDL and LDL. The Apo E protein is polymorphic, and structural variants have been detected by isoelectric focusing. There are three common alleles in the population ($\in 2$, $\in 3$ and $\in 4$) at a single gene locus (human chromosome 19), which produce the gene products Apo E2, E3 and E4 respectively (Mahley *et al*. 1991). This polymorphism results in six common Apo E phenotypes, three homozygous (E2/2, E3/3 and E4/4) and three heterozygous (E4/2, E4/3 and E3/2). The Apo E3/3 phenotype is the most common, occurring in > 60 % of individuals (Simopoulos, 1995). Thus, Apo E3 is considered the parent form of this protein, while Apo E4 and Apo E2 are its variants and are themselves distinguished by single amino acid substitutions at residues 112 (cysteine → arginine) and 158 (arginine → cysteine) respectively of the 299-amino acid chain that constitutes mature Apo E (de Knijff *et al*. 1994).

Apo E allelic frequencies vary among populations. For example, when compared with most Caucasian populations the $\in 4$ allele is more prevalent in Finns (23 *v*. 15 %) and the $\in 3$ allele is more prevalent in the Japanese (85 *v*. 75 %; Hegle & Breslow, 1987).

Shiraki *et al*. (1997) investigated the relationship between phenotypes of Apo E and BMD in 284 post-menopausal Japanese women. The Apo E phenotype groupings were defined as Apo E4–/– (i.e. E3/2 and E3/3; 76 % of the population), Apo E4+/– (i.e. E4/2 and E4/3; 22 % of the population) and Apo E4+/+ (i.e. E4/4; 2 % of the population). A significant ($P < 0{\cdot}05$) gene–dose effect from the Apo E4 allele on BMD of the lumbar spine and total body was reported. Subjects in the Apo E4/4 phenotype group had the lowest BMD and a higher bone turnover, as indicated by

higher serum levels of intact osteocalcin (Shiraki *et al*. 1997). Several other studies have reported an association between BMD and the Apo E4 genotype (Sanada *et al*. 1998; Dick *et al*. 2002; Pluijm *et al*. 2002). Salamone *et al*. (2000) found that peri- and post-menopausal women (not taking hormone-replacement therapy (HRT)) with an Apo E4 allele had a two-fold higher rate of spinal bone loss compared with those without an Apo E4 allele, an allelic effect not observed in women on HRT. Interestingly, Cauley *et al*. (1999) found that American-Caucasian women with at least one of the Apo E4 alleles were at a substantially increased risk of hip and wrist fractures that was not explained by bone density, impaired cognitive function or falling. However, it should be noted that some studies have found no association between the Apo E genotype and BMD, bone loss and/or osteoporotic fracture (Booth *et al*. 2000; Heikkinen *et al*. 2000; Stulc *et al*. 2000; von Muhlen *et al*. 2001).

The reason for the observed relationship between the Apo E genotype and BMD, bone loss and/or fracture incidence in some studies but not in others is unclear, but it may be related to a gene–nutrient interaction between the Apo E genotype and vitamin K status (see p. 907).

Methylenetetrahydrofolate reductase gene polymorphism

Miyao *et al*. (2000) recently demonstrated that an allelic polymorphism in the gene encoding the methylenetetrahydrofolate reductase (MTHFR) enzyme, which is important in clearing homocysteine from the circulation, was associated with reduced BMD in post-menopausal Japanese women. The polymorphism is located at nucleotide 677 in the MTHFR gene and is caused by a single base change (C→T), leading to an amino acid replacement of alanine with valine at position 222. This point mutation gives rise to a thermo-labile variant of the MTHFR enzyme that is less effective. Abrahamsen *et al*. (2003) also reported that early post-menopausal Danish women with the *TT* MTHFR genotype had significantly lower BMD at the hip ($P<0{\cdot}01$) and lumbar spine ($P<0{\cdot}05$) and increased fracture incidence ($P<0{\cdot}05$) than those with the *CC* MTHFR genotype. However, the MTHFR genotype did not influence bone turnover, as assessed by biochemical markers in this population (Abrahamsen *et al*. 2003). In contrast, Jorgensen *et al*. (2002) reported an association between the C677T polymorphism (*TT*) in the MTHFR gene and a reduced risk of osteoporotic fracture of the forearm and hip in a case–control study of Danish post-menopausal women relative to those with the wild-type *CC* genotype, even though BMD at the forearm and ultrasound variables measured at the calcaneus were similar for both genotype groups. The MTHFR genotype is associated with higher plasma homocysteine levels (Abrahamsen *et al*. 2003), which could affect collagen maturation and possibly bone strength. In a preliminary investigation of the data from the Aberdeen-based Prospective Osteoporosis Screening Study there was no effect of the MTHFR genotype on BMD in peri-menopausal and early post-menopausal Scottish women (S New, personal communication). The reason for the discordant findings of studies investigating the relationship between MTHFR genotype and BMD is unclear, but it may be related to B-vitamin status (see p. 906).

Other osteoporosis-susceptibility genes

In addition to the genes described earlier, a polymorphism in the gene encoding collagen type I α 1 has been shown to be important for the genetic regulation of bone mass. For example, Grant *et al*. (1996) reported that a G→T polymorphism in the first intron of the promoter region of the collagen type I α1 gene, at a recognition site for the transcription factor *Sp* 1, is related to bone mass and osteoporotic fracture. Furthermore, a recent meta-analysis of the numerous subsequent studies (Mann & Ralston, 2003), which examined the relationship between the collagen type I α1 *Sp* 1 genotype and BMD and osteoporotic fracture, concluded that *Sp* 1 alleles are associated with a modest reduction in BMD and a significantly ($P<0{\cdot}01$) increased risk of osteoporotic fracture, particularly vertebral fractures. Polymorphisms in genes encoding transforming growth factor β, androgen receptor, calcitonin receptor, osteocalcin, parathyroid hormone receptor, Ca-sensing receptor and interleukin-6, amongst others, have also been associated with bone turnover and/or BMD (see Table 1) in a limited number of studies. These studies have been reviewed elsewhere (Stewart & Ralston, 2000; Garnero *et al*. 2002; Gennari *et al*. 2002; Uitterlinden *et al*. 2002; Langdahl *et al*. 2003) and are beyond the scope of the present review.

Interaction of genotype and diet

Understanding how inherited factors interact with environmental factors (especially nutrition) may hold the key to better prevention and treatment of osteoporosis. However, to date the number of studies that have investigated possible interactions between genotypes and nutrients or food components are limited. These studies will be reviewed in the following section.

Vitamin D receptor genotype–calcium interactions

In recent years convincing evidence has emerged concerning the association between dietary Ca and bone health in all age-groups (Institute of Medicine, 1997; European Commission, 1998; Cashman, 2002). Considering the important regulatory role of 1,25-dihydroxycholecalciferol in Ca homeostasis, which is mediated by the VDR, studies investigating the interaction between VDR genotype, Ca intake and bone integrity were among the first to test gene–nutrient interactions in determining bone health. Two longitudinal studies have investigated a relationship between VDR genotype, Ca intake and change in BMD. One study in older subjects found that while those with the higher bone density (i.e. *bb* VDR genotype) maintained bone density, those with the lower bone density (i.e. *BB* VDR genotype) lost bone density, irrespective of Ca intake (Ferrari *et al*. 1995). Interestingly, the bone density of the heterozygotes (*Bb*) responded to Ca intake, crossing from net loss to net gain at an intake of about 1000 mg/d (Ferrari *et al*. 1995). In contrast, Krall *et al*. (1995) reported

that Ca supplementation of a diet habitually low in Ca reduced bone loss from the femoral neck in women with the *BB* VDR genotype. Greater rates of bone loss under conditions of low dietary Ca intakes would be consistent with a possible effect of the VDR genotype on vitamin D-dependent Ca absorption (see p. 906). Moreover, this absorption defect might be masked in subjects with high Ca loads via a vitamin D-independent pathway (Sheikh *et al*. 1988).

A limited number of associational studies have examined whether dietary Ca influences the relationship between VDR genotype and bone, and the results have been inconsistent. For example, Kiel *et al*. (1997) showed that the association between Ca intake and BMD was dependent on VDR genotype in 69–90-year-old women. They reported that there was an association between usual Ca intake and BMD in women with the *bb* VDR genotype, such that BMD was significantly ($P < 0{\cdot}05$) higher in those with dietary Ca intakes of > 800 mg/d compared with those with intakes of < 500 mg/d. This association was not evident in women with the *Bb* or *BB* VDR genotypes. Ferrari *et al*. (1998) reported that a trend for an association between *Fok* 1 VDR genotype and BMD was more evident at high Ca intake than at low Ca intake in a study of European-Caucasian females. Similarly, the association between VDR genotype and BMD at the femoral neck appeared to be modified by Ca intake in premenopausal women (Salamone *et al*. 1996). However, Garnero *et al*. (1996) failed to find an association between VDR genotype, BMD and Ca intake in a group (*n* 268) of elderly post-menopausal women. However, only sixty-four of the women had a low habitual Ca intake (< 600 mg/d).

There have been a number of studies that have investigated the impact of VDR genotype on Ca absorption. Dawson-Hughes *et al*. (1995), for example, compared fractional Ca absorption in healthy late post-menopausal women with the *bb* and *BB* VDR genotypes. Ca absorption and plasma 1,25-dihydroxyvitamin D levels were measured in sixty women after 2 weeks on a high Ca (1500 mg/d) intake and 2 weeks on a low Ca (< 300 mg/d) intake. ^{45}Ca absorption was similar in the two groups on the high Ca intake but differed significantly ($P < 0{\cdot}05$) in the groups on the low Ca intake (21 and 24 % increases in the *BB* and *bb* groups respectively). Ca restriction induced similar percentage increases in plasma 1,25-dihydroxycholecalciferol levels, but the *BB* group had a smaller increase in the fractional ^{45}Ca absorption index, which would be consistent with a possible intestinal resistance to the action of 1,25-dihydroxycholecalciferol. Similarly, Wishart *et al*. (1997) investigated the relationship between intestinal Ca absorption, serum 1,25-dihydroxyvitamin D levels and all three 3′-VDR gene polymorphisms. The *bb, aa, TT* VDR haplotype was associated with significantly ($P < 0{\cdot}05$) higher Ca absorption. Zmuda *et al*. (1997) reported that African-American women (aged ≥ 65 years) with the *BB* genotype tended to have lower fractional ^{45}Ca absorption (by 14 %) compared with women with the *bb* genotype. Ames *et al*. (1999) showed that the *Fok* 1 VDR genotype was associated with major differences in Ca absorption (42 % between the extreme homozygotes) as well as bone density in young children. In contrast, Kinyamu *et al*. (1997) found no relationship between VDR polymorphisms and intestinal Ca absorption in either young or elderly women. Likewise, Francis *et al*. (1997) investigated the association between the VDR genotype and Ca absorption in men. The results showed no significant difference in Ca absorption among the VDR genotypes. Interestingly, despite apparent differences in intestinal Ca absorption, at least in some studies, two separate but small studies did not identify any genotype-related differences in intestinal VDR level (Barger-Lux *et al*. 1995; Kinyamu *et al*. 1997), suggesting that the intestine is not the primary mediator of any genotype-related differences. VDR polymorphisms have been reported to have effects on parathyroid gland regulation (Carling *et al*. 1995, 1997; Yokoyama *et al*. 1998), suggesting differences in parathyroid hormone regulation as a possible pathway for subtle differences in vitamin D regulation of bone and Ca homeostasis.

Vitamin D receptor genotype–cholecalciferol interactions

There is compelling evidence for a protective role for vitamin D on bone health (for reviews, see Institute of Medicine, 1997; Zitterman, 2003). The response of bone to dietary vitamin D (i.e. cholecalciferol) may be modified by VDR genotype. For example, Graafmans *et al*. (1997) studied the effects of a 2-year regimen of vitamin D supplementation (10 µg/d) on BMD in Caucasian (Dutch) women > 70 years old. They observed that the mean increase in BMD in the vitamin D group relative to the placebo group was higher in subjects with the *BB* and *Bb* VDR genotype compared with those with the *bb* VDR genotype.

Vitamin D receptor genotype–caffeine interactions

In addition to an effect of VDR genotype on the response of bone to Ca and vitamin D, there is also some evidence for an interaction between VDR genotype and caffeine intake in determining bone loss. Rapuri *et al*. (2001) showed that post-menopausal women with the *tt* genetic variant of VDR appeared to be at a greater risk for the deleterious effect of a high caffeine intake (> 300 mg/d) on vertebral bone loss over 3 years compared with women with the *TT* VDR genotype.

Methylenetetrahydrofolate reductase genotype–B-vitamin complex

As mentioned previously the association between the common allelic MTHFR (C677T) polymorphism and BMD has been found to be variable in post-menopausal women. Some of the discordant findings on its effect on bone may arise from a possible gene–nutrient interaction between one or more of the B-vitamin complex and the MTHFR genotype. The MTHFR enzyme and several of the B-vitamin complex are required for clearing homocysteine from the circulation. A preliminary investigation of possible interactions between BMD, the MTHFR genotype and the B-vitamin complex in peri-menopausal and early post-menopausal women in the Aberdeen Prospective Osteoporosis Screening Study suggested that folate and vitamins B_{12} and B_6 had no effect on BMD in the three MTHFR-genotype groups (S New, personal communication). However, for women homozygous for the *TT*

genotype only (the group with elevated plasma homocysteine levels), there was a positive relationship between energy-adjusted riboflavin intake and BMD. The effect of B-vitamin status, MTHFR genotype and bone integrity in older post-menopausal women, in whom homocysteine levels would be greater, and in other age-groups in both men and women needs to be investigated.

Apo E genotype–vitamin K interactions

Apo E phenotype may be linked to osteoporosis and fracture risk (Shiraki *et al*. 1997; Sanada *et al*. 1998; Cauley *et al*. 1999; Salamone *et al*. 2000; Dick *et al*. 2002; Pluijm *et al*. 2002) through its involvement in the metabolism and transport of vitamin K, an important cofactor for the carboxylation of osteocalcin (Vermeer *et al*. 1995). Several studies have reported an association between undercarboxylated osteocalcin, a status indicator for vitamin K, and loss of BMD and/or hip fracture (for review, see Institute of Medicine, 2001). Genetically-determined subtypes of Apo E play a crucial role in the transport of chylomicrons, and thus of vitamin K, to the liver and other target tissues, including bone. Saupe *et al*. (1993), for example, reported that the serum level of vitamin K depended on the Apo E phenotype, i.e. E2 > E3 > E4. This distribution is in accordance with the relationship between the Apo E genotype and the rate of hepatic clearance of chylomicron remnants from circulation, with the Apo E4 allele having the most rapid catabolism (Booth *et al*. 2000). This finding may have implications for the supply of vitamin K to bone cells for metabolic activity. In the only study to date that has investigated the relationship between vitamin K, the Apo E geno-type and bone Booth *et al*. (2000) failed to find evidence of an interaction between vitamin K intake and the Apo E4 allele in relation to BMD or fracture incidence in elderly men and women. In that study there was no association between either vitamin K intake or Apo E genotype and BMD or fracture, even though several studies have reported relationships between the intake and/or status of vitamin K and bone outcomes (Institute of Medicine, 2001) and the Apo E genotype and bone outcomes (Shiraki *et al*. 1997; Sanada *et al*. 1998; Cauley *et al*. 1999; Salamone *et al*. 2000; Dick *et al*. 2002; Pluijm *et al*. 2002). Vitamin K intake was estimated by a food-frequency questionnaire and, unfortunately, data on vitamin K status (such as undercarboxylated osteocalcin) were unavailable. Future studies will need to include measures of the Apo E genotype, vitamin K intake and status, and bone variables in order to test the hypothesis that vitamin K may mediate the observed relationship between Apo E genotype and hip fracture.

Possible oestrogen receptor genotype-phyto-oestrogen interactions

While the mechanism by which polymorphisms in the ORα gene affect BMD is unclear, it may be that they confer some extent of oestrogen resistance. For example, Han *et al*. (1997) suggest that variants in the ORα gene might account for the lack of response to HRT in some women despite good drug compliance and good health. If the ORα genotype can lead to oestrogen resistance, then there are also implications for women using dietary phyto-oestrogens as a natural alternative to HRT. Phyto-oestrogens are non-steroidal compounds that occur naturally in foods of plant origin (especially soyabean foods), and they are able to compete with the principal oestrogens of most mammals (17β-oestradiol and oestrone) to bind with OR (Cassidy, 1996). Such compounds have been shown to have a favourable effect on bone mass in post-menopausal women in several, but not all, studies (Dalais *et al*. 1998; Potter *et al*. 1998; Morabito *et al*. 2002; for review, see Cotter & Cashman, 2003). Although post-menopausal HRT is, and dietary phyto-oestrogen supplementation appears to be, effective in preventing bone loss, individual variation exists in relation to the response to HRT and phyto-oestrogen supplementation (Hassager *et al*. 1994; Dalais *et al*. 1998; Potter *et al*. 1998; Salmen *et al*. 2000*b*; Morabito *et al*. 2002). For example, some studies have reported that spinal BMD is diminished in 3–30 % of the women who take accepted bone-sparing doses of oestrogen (Genant *et al*. 1982; Riis *et al*. 1987; Stevenson *et al*. 1990). It has also been reported that ≤ 11 % of the healthy early post-menopausal women who receive HRT over ≥ 1 year lose > 1 % bone/year (Hassager *et al*. 1994; Han *et al*. 1997). Post-menopausal women who are not receiving HRT lose on average 2 % of BMD annually (European Commission, 1998). There is also variation between individuals in their skeletal response to dietary phyto-oestrogen supplementation (Dalais *et al*. 1998; Potter *et al*. 1998; Morabito *et al*. 2002). This variation could be explained by a genetically-determined response to HRT and phyto-oestrogen therapy.

Several studies have investigated the influence of the ORα genotype, singly and in relation to the VDR genotype, on the responsiveness of bone to HRT in post-menopausal women. Recently, Ongphiphadhanakul *et al*. (2000) reported that the ORα gene polymorphism (as defined by the *Pvu* II endonuclease system) affects the vertebral BMD response to oestrogen in post-menopausal women, suggesting that ORα genotype may help identify those women who will have more skeletal benefit from oestrogen therapy. Similarly, Salmen *et al*. (2000*a*) suggested that women possessing a *P* allelle (as defined by the *Pvu* II endonuclease system for detecting the ORα genotype) would benefit more from long-term HRT than those without this allele. Han *et al*. (1997), on the other hand, reported that after 1 year of HRT the changes in bone density in post-menopausal women were not associated with the ORα genotype.

To date, no studies have investigated the influence of the ORα genotype on the responsiveness of bone to dietary phyto-oestrogen supplementation. Furthermore, phyto-oestrogens, which have been shown to have a relative molar binding affinity for ORα between 100 and 1000 times lower than that for 17β-oestradiol *in vitro* (Kuiper *et al*. 1998), have an even higher specificity for ORβ (Mosselman *et al*. 1996). ORβ is preferentially expressed in tissues such as bone, brain, vascular endothelia and bladder. However, to date, no studies have investigated the influence of the ORβ genotype on the responsiveness of bone to phyto-oestrogen supplementation. Since dietary phyto-oestrogens bind to both ORα and ORβ, polymorphisms in both receptor subtypes may influence the response of bone to phyto-

oestrogen therapy. However, future research is needed to investigate the potential impact of genetic variation at the OR genes loci on the responsiveness of bone to phyto-oestrogen therapy.

Conclusion

While numerous candidate genes for osteoporosis susceptibility have been identified over the last two decades, in general it appears that individually several of these genes have modest effects on BMD and other aspects of fracture risk. It is not surprising that numerous genes have been implicated in osteoporosis, considering the number of regulatory proteins involved in Ca and bone metabolism, as well as other aspects of bone strength and quality. Furthermore, the complexity of osteoporosis is mediated, at least in part, by an interaction between environmental factors and many of these candidate genes. There is increasing evidence that the effects of some of these genes on bone health-related variables are modified by certain nutrients and other dietary components. While there has been some interest in this specific research area in recent years, it is likely that interactions between genetic and nutritional factors are an important target for future research. Considering the number of metabolic pathways by which the nutrient environment can influence bone health, it is highly likely that allelic variation in other known, and yet to be discovered, osteoporosis-susceptibility genes will be shown to interact with nutritional factors in terms of determining an effect on bone. Without doubt, diet–bone health studies that adopt this 'nutrigenetic' approach will be complicated by the potential effects of gene–gene interactions and undefined environmental factors. On the other hand, consideration of nutritional factors, as well as other environmental factors such as alcohol and exercise, will be critical in interpreting these genetic pathways and in the development of genotype-specific, or 'individually-tailored', nutritional recommendations for bone health. To this end, nutritional scientists researching in the area of diet–gene interactions in bone health might be well advised to keep a close eye on developments in the pharmaceutical sector, in particular the 'pharmacogenetic' *v*. 'pharmacogenomic' approach to drug therapy. While pharmacogenetics is aimed at optimization of drug therapy based on an individual patient's genetic profile, i.e. individual single nucleotide polymorphisms, pharmacogenomics utilizes information from multiple single nucleotide polymorphisms within a patient's genome to maximize efficacy and minimize toxicity of drug therapy. This sector is also carefully considering the important bioethical issues that surround this type of research. Thus, with time it may be possible that 'nutrigenomics' will replace, or at least complement, research currently being carried out in the area of nutrigenetics and bone health.

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