Impact of genetic variation on metabolic response of bone to diet

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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 (Guéguen 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 calcitriol 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

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

BMD, bone mineral density; MTHFR, Methylene-tetrahydrofolate reductase; TGFβ1, 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 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 association 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 \((P < 0.0001−P < 0.05\) 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 I 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 postmenopausal) 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 I 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 indices of bone turnover) between alternate 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 I 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. 1998a; 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; Tofeng et al. 2002). This polymorphism does not seem to be in linkage disequilibrium 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, function-ality studies of the Fok 1 polymorphism have yielded mixed results (Arai et al. 1997; Gross et al. 1998b; 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 a 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 perimenopausal 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; Onghiphathanakul 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 vs. twenty-CA repeats in the studies of Ogawa et al. (2000) and Lau et al. (2002) respectively were associated with significantly (P < 0.01) 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 (ε 2, ε 3 and ε 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 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 ε 4 allele is more prevalent in Finns (23 v. 15 %) and the ε 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 < 0.05) 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; Phuijim 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<0·01) and lumbar spine (P<0·05) and increased fracture incidence (P<0·05) 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 α1 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 Sp1, 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 Sp1 genotype and BMD and osteoporotic fracture, concluded that Sp1 alleles are associated with a modest reduction in BMD and a significantly (P<0·01) 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 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 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 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 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 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 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 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 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 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 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 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 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 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 bone integrity were among the first to test gene–nutrient interactions in determining bone health.
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 \( (P < 0.05) \) 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 \( (< 600 \text{ mg/d}) \).

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. Ca absorption was similar in the two groups on the high Ca intake but differed significantly \( (P<0.05) \) 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 \( 25\text{Ca} \) 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 \( 3\text{VDR} \) gene polymorphisms. The \( bb, aa \), TT VDR haplotype was associated with significantly \( (P<0.05) \) higher Ca absorption. Zmuda et al. (1997) reported that African-American women (aged ≥65 years) with the BB genotype tended to have lower fractional \( 45\text{Ca} \) 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, Graffmans et al. (1997) studied the effects of a 2-year regimen of vitamin D supplementation \( (10 \mu g/d) \) 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 \( (>300 \text{ mg/d}) \) 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 B12 and B6 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 homo-
cysteine 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 trans-
port of vitamin K, an important cofactor for the carboxy-
lation 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 pheno-
type, 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
genotype 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-pho-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-
sterooidal 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 (Dalsal 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; Dalsal et al. 1998;
Poter et al. 1998; Salmen et al. 2000a; 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 supplemen-
tation (Dalsal 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
donucleotide 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. (2000a) suggested that women possessing a
P allele (as defined by the Pvu II donucleotide 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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