Bone health, genetics, and personalised nutrition

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Introduction

Osteoporosis is a metabolic bone disease characterised 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 [1], 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 on 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. Such collaborative research is also of critical importance in relation to providing the scientific basis for application of the concept of personalised nutrition (i.e., tailoring dietary advice to individuals on the basis of genotype) for prevention of osteoporosis. Osteoporosis is a complex disease, which is mediated by an interaction between environmental factors (including nutrition, smoking, and physical activity) and several different genes that individually have modest effects on bone mineral density

(BMD) and other aspects of fracture risk [2]. However, the notion of genetic determinants is of little value unless the specific genes that are involved can be identified, and moreover, interactions between these genes and certain environmental factors, especially nutrition that may mediate expression of bone-related phenotypes are elucidated.

Candidate genes for osteoporosis

There have been a staggering number of studies published over the last two decades, which 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 calciotropic 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 where a gene-nutrient interaction is likely or possible) are discussed in more detail below.

Vitamin D receptor gene

The majority of association studies of BMD and candidate gene markers have investigated markers in the vitamin D receptor (VDR) gene [3]. In 1994, a cardinal study by Morrison and colleagues reported a significant association between polymorphic sites situated between exon 8 and 9 at the 3' end of the VDR gene (detected using the *Bsm1*, *Taq1*, and *Apa1* restriction enzymes) and BMD in 250 Caucasian twins, aged 17–70 years, from Australia [4].

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Table 1 Candidate genes for osteoporosis

Candidate gene	Physiological function
Vitamin D receptor	Calcium absorption; osteoblast/osteoclast activity
Estrogen receptor α	Osteoblast/osteoclast activity
Estrogen receptor β	Osteoblast/osteoclast activity
Collagen I A 1	Matrix component
Transforming growth factor β -1	Osteoblast/osteoclast activity
Androgen receptor	Osteoblast function
Interleukin 6	Osteoclast activity
Apolipoprotein E	Vitamin K transport
Parathyroid hormone receptor	Calcium homeostasis; osteoblast/osteoclast activity
Calcitonin receptor	Osteoclast function
Peroxisome proliferator-activated receptor γ	Adipocyte differentiation
Osteocalcin	Matrix component
Calcium sensing receptor	Regulation of calcium homeostasis
Methylenetetrahydrofolate reductase	Homocysteine metabolism
Metalloproteinase-1 gene	Matrix component

Modified from Cusack and Cashman [9]

The study consisted of 70 monozygotic and 55 dizygotic (DZ) 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. [4] concluded that much of the genetic variation in BMD (up to 75%) could be explained on the basis of the *Bsm1* VDR genotype alone. They also reported that postmenopausal women with the BB VDR genotype would reach the BMD “fracture threshold” (defined as two standard deviations 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 significantly increase their risk of bone fracture. However, the same group subsequently reported that there were problems with their original genotyping of the DZ twin part of their study, such that the heritability component attributable to the VDR is less [5]. Since the initial report by Morrison et al. [4] 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. In addition, another common polymorphism in the VDR gene has been described in the coding region (exon 2) [6, 7]. This polymorphism results in a T-to-C transition, recognised by the *Fok1* restriction enzyme. It creates an alternative translation start codon (9 base pairs downstream) that results in a shorter isoform of the VDR gene. Although many studies in Caucasian and Asian populations have confirmed a positive effect between extreme homozygotes, other studies have reported little or no effect in various populations [3, 8, 9]. Cooper and Umbach [10],

after reviewing 16 studies published up to July 1996 in a meta-analysis, concluded that although overall there was an effect of the *Bsm1* VDR polymorphism (of the order of about 0.3 standard deviation (SD) between alternate homozygotes), it was weaker than that reported in the original study of Morrison et al. [4] (a difference of up to 1 SD unit or 10%). A second, more recent meta-analysis of 75 studies published (in full or as abstracts) between 1994 and 1998 also concluded that there was strong evidence for a positive effect of VDR on bone mass [11]. Some of the inconsistencies in the various studies performed to date may arise from the VDR gene effects on bone being modified by dietary calcium, vitamin D, caffeine, and possibly the intake of other nutrients (see below) or, indeed, by its interaction with other genes, such as the estrogen receptor α (*ER α*) gene [12–15].

Apolipoprotein E and methylenetetrahydrofolate reductase gene polymorphisms

Similar to the case with VDR genotype, there has been mixed findings from associational studies of the impact of other candidate genes for osteoporosis, including estrogen receptor (*ER*) α and β , apolipoprotein E (*Apo E*), and methylenetetrahydrofolate reductase (*MTHFR*) genes amongst others, on bone indices. These have been reviewed elsewhere [3, 9]. The reasons for the discordant and inconsistent findings of studies investigating the relationship between the above mentioned genotypes and bone turnover/BMD/fracture are unclear, but it may be related to nutritional intake/status (see below).

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. In particular, an understanding of diet-genotype interactions is at the core of the concept of tailoring dietary advice towards osteoporosis prevention on the basis of genotype, i.e. personalised nutrition for bone health. However, to date the number of studies, which have investigated possible interactions between genotypes and nutrients/food components are limited. These will be reviewed in the following section.

Vitamin D receptor genotype–calcium interactions

In recent years, convincing evidence has emerged with respect to the beneficial effect of dietary calcium on bone health in all age groups [16]. Considering the important regulatory role of 1,25(OH)₂ D₃ on calcium homeostasis, which is mediated by the VDR, studies investigating the interaction between VDR genotype, calcium intake, and bone integrity were among the first to test gene-nutrient interactions in determining bone health. While evidence from two longitudinal studies suggests a relationship between VDR genotype, calcium intake, and change in BMD [17, 18], a limited number of associational studies have examined whether a relationship between VDR genotype and bone was influenced by dietary calcium, and the results have been inconsistent [9, 19]. For example, Kiel et al. [20] showed that the association between calcium intake and BMD was dependent upon VDR genotype in 69–90 year old women. They reported that there was a significant association between usual calcium intake and BMD in women with the bb VDR genotype, such that BMD was significantly higher in those with dietary calcium intakes >800 mg/d compared with those with intakes <500 mg/d. This association was not evident in women with the Bb or BB VDR genotypes.

Vitamin D receptor genotype–cholecalciferol interactions

There is compelling evidence for a protective role for vitamin D on bone health [16, 21]. The response of bone to dietary vitamin D (i.e., cholecalciferol) may be modified by VDR genotype. For example, Graafmans et al. [22] studied the effects of a 2-year regimen of vitamin D supplementation (400 IU/d) on BMD in Caucasian (Dutch) women over 70 years old. They observed that the mean increase in BMD in the vitamin D group relative to

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 and VDR genotype–calciuric diet interactions

Besides an effect of VDR genotype on the response of bone to calcium and vitamin D, there is also some evidence that there is an interaction between VDR genotype and caffeine, and high sodium-high protein intake in determining bone loss. Rapuri et al. [23] showed that postmenopausal 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 to women with the TT VDR genotype. Harrington et al. [24] recently showed an interaction between VDR genotype and the effect of a high sodium-high protein intake on the rate of bone resorption in postmenopausal women.

Methylenetetrahydrofolate reductase genotype–B vitamin complex

As mentioned previously the common allelic MTHFR (C677T) polymorphism has been variably associated with BMD in postmenopausal 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-complex vitamins and the MTHFR genotype. The MTHFR enzyme together with several of the B-complex vitamins is required for clearing homocysteine from the circulation. A recent investigation of possible interactions between BMD, MTHFR genotype and B vitamin complex in pre- and early postmenopausal women in the Aberdeen prospective osteoporosis screening study suggest that dietary folate, B12 and B6 had no effect on BMD in the three MTHFR genotype groups [25]. However, for women homozygous for the TT genotype only (the group with elevated plasma homocysteine levels), there was a positive relationship between energy-adjusted vitamin B2 intake and BMD. The effect of B vitamin status, MTHFR genotype and bone integrity in older postmenopausal 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 through its involvement in the metabolism and

transport of vitamin K, an important cofactor for the carboxylation of osteocalcin. 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. [26], for example, reported that the serum level of vitamin K1 depended on the Apo E phenotype, namely $E2 > E3 > E4$. This distribution is in accordance with the relation between Apo E genotype and the rate of hepatic clearance of chylomicron remnants from circulation, with the Apo E4 allele having most rapid catabolism. This may have implications for supply of vitamin K to bone cells for metabolic activity. In the only study to date which has investigated the relationship between vitamin K, Apo E genotype and bone, Booth et al. [27] failed to find evidence of an interaction of vitamin K intake and Apo E4 allele on BMD or fracture incidence in elderly men and women. In that study, neither vitamin K intake nor Apo E genotype was associated with BMD or fracture, even though several studies have reported significant associations between the intake and/or status of vitamin K and bone outcomes [28] and Apo E genotype and bone outcomes [9]. Vitamin K intake was estimated by a food frequency questionnaire and, unfortunately, data on vitamin K status (such as undercarboxylated osteocalcin) was unavailable. Future studies will need to include measures of Apo E genotype, vitamin K1 intakes and status, and bone parameters to test the hypothesis that vitamin K1 may mediate the observed relationship between Apo E genotype and hip fracture.

Conclusion and the way forward

While numerous candidate genes for osteoporosis susceptibility have been identified over the last two decades, in general, it appears that several of these individually 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 calcium 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 parameters are modified by certain nutrients and other dietary components. However, in some cases these diet-genotype relationships have only been investigated in a single study and have not been repeated. Even when several studies exist for a specific diet-genotype interaction, the findings have been less than consistent. Therefore, more studies are needed so as to provide the scientific justification for advising people to alter their diet on the

basis of their genotype (i.e., personalised nutrition for osteoporosis prevention). In particular, there is an urgent need for randomised controlled intervention trials (RCT) in subjects stratified by genotyped in advance. A move more towards RCT and away from crosssectional/associational type studies may help confirm or disprove whether genotype-nutrient interactions exist. Many of the associational type studies to date have been underpowered and may not have controlled for possible confounding factors. Finally, considering the number of metabolic pathways by which 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.

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