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Plenary Lecture

Genetics, calcium intake and osteoporosis

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Osteoporosis is a major health problem worldwide, particularly with advancing age in both men and women. The strength of the skeleton in older age results from bone strength achieved in early adulthood and age-related and, in women, post-menopause-related bone loss. While trauma and the manner in which older people fall are important contributors to fracture risk, low bone mass is a major factor. Determinants of bone mass include external factors such as lifestyle, especially physical activity, and calcium intake. The wide variation in dietary calcium intake across countries does not correlate with osteoporotic fracture risk, presumably due to ethnic differences between and within populations. The twin approach has been useful in the identification of the major part of age-specific variation in bone density (and turnover), which is genetically determined. Exploring possible genetic factors, we reported that common allelic variations in the vitamin D receptor (VDR) gene were associated with indices of bone turnover and density. Subsequent studies, including our own, have found weaker effects. However, allelic effects of the VDR gene polymorphisms have now been reported in a wide range of, but not all, Caucasian and Asian populations in which they have been studied. In relation to possible physiological mechanisms, the VDR alleles correlate with differences in gut calcium absorption and response of bone density to long-term dietary calcium intake. Moreover, differences in response to active vitamin D compounds have been found in relation to VDR gene alleles. Understanding how these allelic variants, which are not associated with differences in the coding region of the gene and thus the translated product, alter bone homoeostasis in relation to dietary manipulations has great potential to improve osteoporosis prevention and treatment. Also, it can serve as a model of the interaction between genetic diversity and differing nutritional requirements within and between ethnic and racial groups.

Osteoporosis is a major health-care problem with obvious relevance to nutritional intake. As a problem it is increasing gradually with the general aging of societies, secondary to improvements in public health and associated delay in mortality, even in developing countries. It is somewhat ironic that the improvements in general health, in part related to improved nutrition, have revealed another health problem, which may itself be related to nutritional requirements. This change may be most apparent in Asian countries, where more hip fractures will occur than in the rest of the world by the middle of the next century (Cooper *et al.* 1992). Most of this difference is projected on the basis of the population and its aging, but the Asian countries are also distinguished by a generally lower intake of dairy foods, and thus of Ca. The role of Ca intake has been a focus of attention in clinical research into prevention and treatment for osteoporosis, for the obvious reason that the bone is the major store for Ca in the body. It was considered early that Ca-deficient intakes would inevitably contribute to loss of Ca from bone, as physiological demands for Ca ensured that the bone storehouse of Ca would be sacrificed. However, it has become obvious that the body has considerable capacity to cope with very low Ca intakes, by increasing the proportion of Ca absorbed from dietary sources and resorption of Ca secreted into the gut as a part of normal digestive processes, and by improving the efficiency of renal Ca conservation. The rising estimates of the incidence and prevalence of osteoporosis (Jones *et al.* 1994*a*) have required a more careful assessment of endogenous and exogenous factors, which contribute to, or determine, the risk.

A primary question has been what is the interplay between endogenous (or inherited) factors and environmental factors, including lifestyle and diet. The actual event of an osteoporotic fracture results from trauma, which would not have been expected to break a bone unless it was relatively weakened (Nguyen *et al.* 1993; Kroger *et al.* 1994; Cummings *et al.* 1995). Thus, in osteoporosis even relatively minor trauma, as might occur in normal daily activities, can result in a fracture. Although trauma, particularly from falls, can be targeted for intervention, the strength of the bone remains a key determinant of

Abbreviations: BB, lower bone density genotype; bb, higher bone density genotype; VDR, vitamin D receptor.

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osteoporotic fracture risk (Grisso *et al.* 1991, 1994). Bone structure and presumed strength has been assessed in the past 20 years by bone densitometry, which provides accurate and reproducible information on bone mass and density with a non-invasive X-ray-based test. Although these data are informative, bone strength relates not only to the total amount of bone but also to how it is distributed. Structural and micro-structural organization may contribute to bone strength, but bone 'density' is still a valuable surrogate.

Given that bone mass is a critical determinant of fracture risk, it is important to recognize that bone density at any time in life depends on the total amount of bone formed by the early twenties and the subsequent loss with aging and after the menopause (Jones *et al.* 1994*a*, *b*; Teegarden *et al.* 1995; Young *et al.* 1995). Put simply, fracture risk is highest in those who achieve low bone mass in early life and/or lose bone more rapidly with age and menopause. As the bone loss continues and may even accelerate throughout older years (Jones *et al.* 1994*b*), at ages when falls may be more common, it is not surprising that incidence of fractures increases exponentially with advancing age.

A critical time for the development of bone mass and density is at and just before puberty. About that time the skeleton increases in size and length and total bone mass increases about three-fold during just a few years (Teegarden *et al.* 1995; Young *et al.* 1995). A key issue is the role of nutrition and physical activity in this development. There are wide differences in Ca availability and, indeed, in recommended intakes in different countries, yet differences in bone mass in adult life do not seem to correlate with these differences (Angus & Eisman, 1988; Angus *et al.* 1988*a*, *b*). Two possible explanations are that bone and Ca physiology are capable of adjusting to minimal Ca intakes without compromising the skeleton, or that there are ethnic or other environmental differences which contribute to the level of Ca requirement.

Physical loading of the skeleton is likely to be important for the development and maintenance of bone structural strength (Pocock *et al.* 1986, 1989*a*; Carbon *et al.* 1990; Kelly *et al.* 1990*b*). While this is clearly the case with extremes of loading, from heavy long-term physical activity to immobilization or microgravity, the 'dose–response' is rather flat across common levels of physical activity (Eisman *et al.* 1991). Although of perhaps lesser relevance in the attainment than in the maintenance or loss of bone, excessive alcohol use and tobacco smoking are associated with osteoporosis (Angus *et al.* 1988*a*, *b*; Pocock *et al.* 1989*b*; Hopper & Seeman, 1994). Interestingly, moderate alcohol intake in adulthood is associated with higher bone mass than is either zero or excessive intake (Angus *et al.* 1988*a*, *b*). Even taken together, these other environmental factors do not seem to explain the wide within-country and between-country differences in peak bone mass achieved in early adulthood, or loss in later life.

Differences in bone density between countries could reasonably be considered to be related to ethnic differences in bone structure and size. Within countries with mixed ethnic groups, similar differences could contribute. On the other hand, within homogeneous ethnic groups such differences seem unlikely to be major contributors. However, in family and epidemiological studies, inherited factors appear to play a key role in the predisposition to development of osteoporosis and osteoporotic fractures (Evans *et al.* 1988; McKay *et al.* 1994; Seeman *et al.* 1994; Soroko *et al.* 1994). Moreover, studies of osteoporotic fractures in mother–daughter pairs are consistent with inherited factors in bone structure.

If inherited predisposition to low bone density exists, the question arises as to how such an effect could be mediated. There could be direct effects of mutated genes which alter bone structure in some irreversible fashion. This may be the case, as in osteogenesis imperfecta, where severe but rare mutations in the collagen Iα1 gene are associated with severe bone disease. More common but less severe mutations have also been shown to be associated with premature osteoporosis (Spotila *et al.* 1991, 1994). Also, an intronic polymorphism in the collagen Iα1 gene, possibly altering transcription of an otherwise normal gene, has been associated with low bone density and increased osteoporosis risk (Grant *et al.* 1996). However, mutated genes, with altered function of the gene product, seem to be relatively uncommon and it is more likely that the wide normal range of bone density is contributed to by a number of genes, with subtle differences in function acting alone or interacting with each other (multi-factorial genetic interactions), and with the environment (gene–environment interactions), resulting in higher or lower bone density and strength.

The possibility of genetic components contributing to bone density has been investigated through the twin model. In this model the differences between twins (siblings) are related to the differences in individual *v.* shared environments, and the differences in individual *v.* shared genes. The critical difference between identical and non-identical twins of the same sex is that the identical twin pairs have all their genes in common, while the non-identical twins share half their genes. This approach in the study of twins has suggested that about 75% of the age-specific variance of bone density is attributable to genetic factors (Smith *et al.* 1973; Moller *et al.* 1978; Dequeker *et al.* 1987; Pocock *et al.* 1987; Slemenda *et al.* 1991). Given that osteoporosis is so prevalent in the community at large it follows that any contributory genes must themselves be common. Alternatively, it is possible that a very large number of uncommon variants each contribute to a relatively small extent, such that the net effect of genetic factors is common. Identification of genetic effects on bone mass and structure has the potential to increase understanding of the underlying pathophysiology related to the 'normal' variation in bone density. Bone density at any age is a physiological variable with a normal 'mean' value and a distribution around that value. However, fracture risk at any age relates to deviation from young normal values rather than age-matched values. Indeed, many older people with bone density values within 2 SD of their age-matched normal values will suffer osteoporotic fractures. Thus, genetic factors, which contribute to the determination of an individual's position within the normal range, are of considerable importance.

A key concept is how relatively minor changes in genes or their expression could be expected to be corrected for through physiological counter-regulatory systems, unless

both sensor and effector limbs of the homeostatic systems are modified by one or more, even subtle, genetic variations. This concept leads to a framework for understanding how gene–gene or gene–environmental interactions could result in functionally significant differences in the physiological variable of bone density.

Initially, we examined non-identical twins to try to identify surrogate or intermediate effector differences which might be linked to the differences in bone density between members of a dizygotic twin pair. We showed that indices of bone turnover, initially osteocalcin, were more similar in identical twins than non-identical twins, consistent with a strong genetic effect on bone turnover (Kelley *et al.* 1991). We also showed that the difference in osteocalcin, and subsequently other indices of bone turnover, predicted the difference in bone density between twin pairs; the higher the indices of bone turnover, the lower the bone density (Kelly *et al.* 1991, 1993; Tokita *et al.* 1994). The potential interaction between genes, and between genes and the environment, could complicate further analyses of these apparent genetic effects (Kelly *et al.* 1990*a*; Slemenda *et al.* 1991). However, without investigating such effects further, identifying a role for genetic factors can do little to advance prevention and treatment of osteoporosis.

The concept of involvement of both sensor and effector limbs of a physiological pathway in causing an homeostatic shift led us to examine the vitamin D–endocrine system. This system includes the renal production of 1,25-dihydroxyvitamin D from 25-hydroxyvitamin D, which is tightly regulated by parathyroid hormone and circulating Ca and phosphate levels. The effector limbs relate to the multiple roles of the active metabolite, 1,25-dihydroxyvitamin D, in the regulation of intestinal Ca absorption, bone formation and resorption, and even feedback on the parathyroid gland to decrease parathyroid hormone production. Thus, allelic differences in the vitamin D receptor (VDR) gene, along with similar differences in other steroid hormone-receptor genes, were investigated using the twin model. Initially, we found that VDR allelic differences were linked with differences in indices of bone turnover in a group of twins and in a Japanese population sample (Morrison *et al.* 1992; Tokita *et al.* 1996). Other subsequent studies have either confirmed or been unable to confirm such a relationship in a variety of twin linkage (Hustmyer *et al.* 1994) or population association studies across ethnic groups (Garnero *et al.* 1995, 1996; Keen *et al.* 1995; Spector *et al.* 1995).

Given the relationship between indices of bone turnover and bone density, we examined the relationship between VDR alleles and bone density in twins. We found linkage between these allelic markers and differences in bone density (Morrison *et al.* 1994). In our expanded twin studies (including re-genotyping of some of the initial twins) and other twin studies, this linkage has been found to be weaker or not discernible (Hustmyer *et al.* 1994; Spector *et al.* 1995; Eisman, 1996; Morrison *et al.* 1997). In the original study (Morrison *et al.* 1994) and a wide range of further population studies across ethnic and racial groups, associations have been reported between VDR alleles and bone density. Several studies in Caucasian and Asian populations (Yamagata *et al.* 1994; Fleet *et al.* 1995; Koshiyama *et al.* 1995; Riggs *et al.* 1995; Gross *et al.* 1996; Morrison *et al.* 1997; Tamai *et al.* 1997) have shown an allelic effect between extreme homozygotes of perhaps 0·5 SD units, with a difference in bone density ranging from 4 to 13 %. Other studies have reported little or no effect in various Caucasian populations (Hustmyer *et al.* 1994; Barger-Lux *et al.* 1995*a*, *b*; Garnero *et al.* 1995; Keen *et al.* 1995; Kroger *et al.* 1995; Jorgensen *et al.* 1996; Alahari *et al.* 1997; Francis *et al.* 1997; Gunnes *et al.* 1997; McClure *et al.* 1997; Vandevyver *et al.* 1997). Moreover, three 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; Uitterlinden *et al.* 1996; Salamone *et al.* 1996). Those studies available were reviewed recently from opposing viewpoints (Eisman, 1995; Peacock, 1995), while a meta-analysis concluded that an effect existed but was of the order of 0·3 SD (Cooper & Umbach, 1996).

In the meta-analysis of the VDR gene associations with bone density (Cooper & Umbach, 1996), it was acknowledged that it had not been possible to control for possible environmental or gene–gene interactions, which could explain some of the differences between the studies. The possibility of linkage of the VDR gene effect to a nearby effector gene has also been proposed. This concept gained some credence following the reports of linkage of bone density with a start codon polymorphism in the VDR gene in Mexican-Americans, amongst whom no association could be found for the original Bsm-Apa-Taq polymorphisms, which are in exon 8/intron 9 of the gene (Gross *et al.* 1996; McClure *et al.* 1997). However, in other as yet unpublished studies (CP White, TV Nguyen, JR Center and JA Eisman, unpublished results) in an epidemiological group of 2000 men and women, we do not find any relationship with the start codon polymorphism. In the initial report, an association of differences in the 3′-untranslated region of the allelic forms of the VDR was noted to be associated with differences in mRNA stability, and thus potentially VDR levels (Morrison *et al.* 1994). However, despite this potential mechanism, subsequent studies have not found an association with VDR levels in intestinal biopsies or isolated blood monocytes (Kinyamu *et al.* 1997; Mocharla *et al.* 1997). Thus, the mechanisms of any effect remain unexplained.

Returning to the impact of nutritional factors, the initial studies in which VDR alleles were associated or linked with bone density were in populations with a moderate to low Ca intake (mean about 700 mg/d; Morrison *et al.* 1994, 1997; Yamagata *et al.* 1994; Tokita *et al.* 1996; Tamai *et al.* 1997). The studies showing the reverse VDR gene effect were associated with higher Ca intakes (means >1000 mg/d; Houston *et al.* 1996; Salamone *et al.* 1996; Uitterlinden *et al.* 1996) and in the 'no effect' studies, Ca intake lay between these extremes of Ca intake Hustmyer *et al.* 1994; Garnero *et al.* 1995, 1996; Jorgensen *et al.* 1996; Alahari *et al.* 1997; Francis *et al.* 1997; Gunnes *et al.* 1997). Moreover, no attempt has been made as yet to control for any impact of vitamin D status. Thus, some of these differences may be related to nutrition, particularly Ca intake and vitamin D status. This is of particular interest in view of the wide differences in average Ca intake

between Asian and Caucasian populations (< 400–> 1000 mg Ca/d) and the different sunlight exposure between populations living close to or far away from the equator, and hence expected dermal vitamin D generation.

Potential effects of vitamin status have not been systematically examined. However, the interaction of VDR alleles with Ca intake has been studied in a number of ways. Typically these studies have evaluated the relationship between changes in Ca absorption and/or bone density over time in relation to Ca intake and VDR alleles. One study in older subjects found that those with the higher bone density genotype (bb) maintained bone density, while those with the lower bone density genotype (BB) lost bone density, irrespective of Ca intake (Ferrari *et al.* 1995). Interestingly, in that study the bone density of the heterozygotes (Bb) responded to Ca intake, crossing from net loss to net gain at about 1000 mg Ca intake daily. However, in another study in younger people on low dietary Ca intakes, BB genotype subjects responded best to Ca supplementation (Krall *et al.* 1995). These differences may relate to the mean Ca intake and the age of the individuals, since in another study the VDR allelic effect was present in younger individuals but not in older individuals above 70 years of age (Riggs *et al.* 1995). Importantly, in a study of intestinal Ca absorption, bb genotype subjects were better able to increase Ca absorption than BB individuals on varying Ca intakes between 300 (low) and 1500 (high) mg of Ca daily (Dawson-Hughes *et al.* 1995). Similarly, in a cross-sectional study of intestinal Ca absorption in relation to dietary Ca intake and serum 1,25-dihydroxyvitamin D levels the VDR genotype bbaaTT was associated with higher Ca absorption (Wishart *et al.* 1997). However, another study found no comparable relationship between Ca absorption and VDR genotype (Francis *et al.* 1997).

The response of bone homeostasis to vitamin D metabolites and analogues has been of particular interest in two separate studies carried out in Japanese subjects. These subjects, as in other studies of Japanese subjects, had a low frequency of the B allele, such that the bb genotype comprised about 75 % of the subjects compared with about one-third of Caucasian subjects. Those bb subjects responded to the active vitamin D analogue or metabolite with an increase in bone density, while the Bb heterozygotes (about 50 % of Caucasian groups) either did not respond or actually lost bone (Matsuyama *et al.* 1995; Nakamura, 1997). These differences in response to active vitamin D compounds may explain the widely different data from studies in osteoporosis between Asian and Caucasian groups. Thus, the commonest genotype in Japanese subjects, who do respond, is relatively less common in Caucasian subjects, while the genotype that responds relatively poorly is uncommon amongst Japanese subjects but is present in the majority of Caucasian studies.

In support of a role for VDR gene alleles in bone and Ca physiology are two studies showing relationships between VDR alleles and incidence and/or severity of primary and secondary hyperparathyroidism (Carling *et al.* 1995, 1997). Possibly related to this concept and the role of vitamin D compounds in the regulation of cancer cell replication (for review, see Eisman, 1993) is the finding of an association between VDR alleles and the incidence of prostatic cancer (Taylor *et al.* 1996; Ingles *et al.* 1997).

Notwithstanding the apparent association of VDR alleles with bone density and turnover, and Ca homeostasis, studies have not shown clear differences in incident or prevalent osteoporotic fractures in relation to VDR alleles (Gallagher *et al.* 1994; Melhus *et al.* 1994; Looney *et al.* 1995). One interesting Japanese study did find lower bone density in BB genotype individuals, as well as fractures occurring in BB genotype subjects with relatively less severe osteoporosis (Tamai *et al.* 1997). In general, however, these studies have lacked power to detect the effects discussed (Nguyen *et al.* 1994). A Mayo Clinic study (Riggs *et al.* 1995), as noted previously, observed an effect in younger individuals but not in older individuals, suggesting that the genetic effect was lost gradually with age, such that there would be no association with osteoporotic fracture incidence. However, this loss of effect with age was also associated with the absence of any decline in femoral neck bone density in the older subjects. Thus, their findings may reflect survival or cohort bias related to external factors, such as physical activity and nutrition, at critical developmental stages.

It is not clear whether genetic factors contribute to the variability in rates of change of bone density in adulthood. One long-term (14 year) study in older male twins found no such effect (Christian *et al.* 1989), while another of shorter term (2·5 years) in younger twins did find a genetic effect on change in bone density (Kelly *et al.* 1993). Bone loss has been studied in relation to VDR alleles in a number of studies. One study in Japanese subjects found an effect (Koshiyama *et al.* 1995), while two other studies in Caucasian subjects did not (Barger-Lux *et al.* 1995*a*, *b*; Garnero *et al.* 1996). However, rapid bone loss in early postmenopausal women could be expected to be unrelated to VDR alleles. Another potentially important confounder in the studies of bone density and VDR alleles may relate to effects on bone size and/or body size. For example, long-term change in femoral shaft cortical area has been reported to relate to both Ca intake and VDR alleles (Barger-Lux *et al.* 1995*a*, *b*). Also, VDR genotype has been related to body size at various stages of life from infancy onwards (Barger-Lux *et al.* 1995*a*, *b*; Keen *et al.* 1997; Tamai *et al.* 1997). Furthermore, in one study of femoral-neck bone density a VDR allele effect was noted in average-weight subjects but not in obese subjects (BMI > 30 kg/m^2; Vandevyver *et al.* 1997). These interactions with body and bone size will need to be carefully elucidated before the potential for interaction between genetic factors and nutritional intake can be fully understood.

Summary

Genetic factors explain a high proportion of the age-specific differences in bone density, size and turnover. The potential for interaction between hormonal, diet and lifestyle factors is likely to be important. Common allelic variation in the VDR is an example of normal gene variants altering Ca homoeostasis, with effects on body and bone size as well as bone density. The VDR findings suggesting interactions

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between genetic and nutritional factors are an important target for future research. These studies are complicated by the potential for effects of gene–gene interactions and of undefined environmental factors. These problems notwithstanding, considerations of environmental and nutritional contributions, such as Ca intake and vitamin D status, will be critical in interpreting these genetic pathways and in 'personalizing' nutritional recommendations.

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