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THE GENETICS OF OSTEOPOROSIS: Vitamin D Receptor Polymorphisms

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ABSTRACT

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. It is a disease with a complex etiology that includes genetic and environmental contributors. Environmental factors that influence bone density include dietary factors—such as intakes of calcium, alcohol, and caffeine—and lifestyle factors—such as exercise and smoking. Ethnic differences in the propensity to nontraumatic bone fracture suggest that genetic factors are important. Recently, common allelic variations in the vitamin D receptor gene have been found to be associated with bone mineral density in racially diverse population groups, as well as in prepubertal girls, young adult and postmenopausal women, and men. However, many studies have not been able to find this association. Additional approaches, such as sib-pair analysis, will probably be necessary in the future to identify the important genetic determinants of osteoporosis.

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INTRODUCTION

In the last 30 years, attitudes about the study of diseases have undergone a revolutionary change. As the techniques of molecular biology have become more routine in research labs, scientists have been able to identify more genes that may influence or cause diseases in humans. This has led to a belief in "genetic predeterminism," or a sense that the occurrence of all diseases can be explained by the presence of a defective gene or genes.

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. It has a complex etiology that includes genetic and environmental contributors (55, 56, 94). Although these factors may exert their influence over the course of a lifetime, the fractures associated with osteoporosis generally occur later in life. As a result, we consider osteoporosis to be a disease of the elderly. Thus, the aging demographics of the United States 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 (90).

Nutritionists have long known that dietary factors impact bone health (43). However, several questions remain to be answered. For example, how does our emerging understanding of the genetics of osteoporosis influence the role of dietary and environmental factors on calcium metabolism and bone biology? Moreover, will the genetic profile of an individual allow nutrition scientists and other public health practitioners to design dietary and lifestyle recommendations that lead to optimum bone health and lower the risk of developing osteoporosis?

The answers to these questions will surely preoccupy the medical community in the next century. This review presents current knowledge on the environmental and genetic factors that contribute to a healthy skeleton. In particular, we review recent studies on the association of vitamin D receptor gene polymorphisms and bone mineral density and the risk of osteoporosis.

RISK FACTORS FOR DEVELOPING OSTEOPOROSIS

Aside from studying genetic contributors to osteoporotic risk, researchers have examined skeletal and environmental factors that may contribute to it. What follows is a brief summary of that work. Interested readers are referred to recent reviews for more detailed discussions of these topics (43, 55, 94).

Traditionally, researchers have focused on the features of bone itself that make it susceptible to fracture, i.e. bone mineral density (grams per squared centimeter) or content (grams per centimeter), and to some extent bone architecture. Osteoporosis is associated with low bone density and researchers have defined a fracture threshold, or a level of bone density below which bone is more susceptible to either spontaneous or trauma-induced fracture (1, 7). At what age a person reaches the fracture threshold is determined by two factors: the peak bone mass attained by early adulthood, and the rate of adult bone loss. Women experience a rapid drop in bone mass during menopause, and this additional loss accounts for a large part of the gender differences in the occurrence of osteoporosis (12). The bone found at a given skeletal site can be composed of varying amounts of trabecular and cortical bone, which influences the density and breaking strength of the bone and the rate of bone loss. Postmenopausal osteoporosis is characterized by a loss of trabecular bone and fractures of trabecular-rich bones, such as the spine and ends of the long bones. Senile osteoporosis is characterized by loss of both cortical and trabecular bone, such as the vertebrae and femur neck.

The primary focus of much of the research on genetic factors that influence bone density and osteoporosis has been on factors that influence the attainment of peak bone mass and the rate of adult bone loss. Although this review also focuses on bone-mineral density (BMD), other important features have been identified. For example, heavier individuals have a higher BMD, although recent research points to lean body mass, rather than fat mass, as the important feature leading to this higher BMD (10). Thus, a genetic effect that exerts an influence on the skeleton could work indirectly by influencing body composition. Recently, researchers have begun to study the role of bone architecture on the resistance of bone to physical stress (92). Hip axis length (HAL) may be one feature that explains some of the variability in fracture risk that is not explained by bone density (15). Finally, since many osteoporotic fractures do

not occur until a person with low BMD has fallen, neurologic deficits may contribute to osteoporosis by affecting balance, causing a propensity to fall (83). Genetic influences on BMD and the susceptibility to osteoporotic fracture could operate through genes that influence muscle mass, skeletal architecture, and balance-related neurological attributes.

Environmental Factors

DIET Although overall good nutrition is probably important to optimize bone health, the most important and well-studied nutritional variable influencing bone is dietary calcium intake (7). Along with phosphate, calcium is needed for proper mineralization of bone. The importance of this nutrient is reflected in recent recommendations to increase the dietary calcium across all age groups to optimize bone health and reduce the risk of osteoporosis (84). Other dietary practices may disrupt calcium metabolism. For example, high protein and salt intakes increase urinary calcium loss and may lead to a negative calcium balance and bone loss (59, 88). High dietary sodium intake is associated with low bone density in premenopausal women (21).

Another important nutrient for bone is a sufficient supply of vitamin D, from either dietary intake or sunlight exposure, to maximize intestinal calcium absorption and maintain calcium balance (18, 84). Animal studies show that adequate ascorbate and copper intake is needed to ensure proper collagen matrix formation (50, 91). More recently low vitamin K nutriture has been correlated to low BMD in women (114). Conceivably, genetic factors influencing the absorption, metabolism, and retention of these essential nutrients could affect osteoporosis risk.

LIFESTYLE The most important lifestyle factor influencing bone health is exercise. Early studies clearly showed that disuse of a weight-bearing bone leads to excessive bone loss (33). Conversely, exercise leads to greater density in the bones undergoing the physical stress induced by the exercise (108). Moreover, this response to exercise may be dependent on the level of calcium intake (111). Three other lifestyle habits have been shown to have an important influence on bone density: alcohol intake, caffeine consumption, and smoking (41, 61, 76).

Genetic Factors

RACIAL DIFFERENCES Asian women have a 40–50% and African-American women have a 50–60% lower risk of hip fracture than do Caucasian women (94). Paradoxically, Asian women have lower bone density (97) and African-American women have higher bone density (94) than Caucasian women have. Differences in body size may account in large part for the difference in bone density between Asian and Caucasian women (97), but it does not account for the difference between African-American and Caucasian women (26). Cummings

et al (15) showed that both Asian and African-American women have a shorter hip axis length and that this accounts for a large portion of the osteoporotic risk in those groups. Collectively, studies like these suggest that there is a significant genetic component to bone characteristics and osteoporosis.

HERITABILITY STUDIES When we think of genetically predetermined diseases, we usually mean diseases that are due to one or many mutations in a single gene (e.g. the hemoglobin defect associated with sickle cell anemia) that follow the rules of Mendelian inheritance. The single-gene disease approach has not explained osteoporosis or other common, multifactorial, complex diseases of aging (e.g. cancer and cardiovascular disease). Although some bone diseases can be caused by mutations in single genes—e.g. vitamin D–resistant rickets and the vitamin D receptor gene (13), osteogenesis imperfecta, and genes for type I pro-collagen (66)—these mutations do not occur in the majority of people with osteoporotic fractures. To determine whether low bone density or osteoporosis risk has a genetic component, investigators have turned to family and twin studies.

Family studies In family studies, researchers look to see whether a trait (e.g. low bone density) runs in a family. However, because of shared family environment, one has to consider that this factor, rather than genetics, is the explanation for a familial character. With this caveat in mind, researchers have consistently found a familial resemblance in bone density at a number of appendicular and axial sites (27, 52, 64, 71, 110, 119). This is true in both Caucasians and African-Americans (63). The familial relation to bone density manifests itself prior to achieving peak bone density (29, 78). Lutz & Tesar (72) found the BMD relationship was stronger between premenopausal mothers and daughters than between postmenopausal mothers and daughters. This suggests that other factors can modify the genetic influence on the skeleton. Several groups have shown that including both parents in the assessment of genetic factors improves the predictive value (51, 64, 69, 86, 109). Some (4, 71, 101), but not all (22, 40), studies show that daughters whose mothers have a history of low bone density or osteoporosis also have lower bone density. Recently, intestinal calcium absorption was also found to be correlated in mothers and daughters, with the relationship being strongest in pairs where the mother had high bone density (16).

Several groups have applied segregation analysis to estimate the degree of familial resemblance under varying genetic or environmental hypotheses. An analysis of total body bone density in 129 nuclear families from France found no evidence of a major gene influencing BMD (39) but supported a polygenic effect exerting its greatest influence on peak bone mass. This is consistent with a variance component analysis in 535 American women in 137 family sets that

suggested that polygenic loci account for about half of the variability in maximal femoral bone density. In contrast, segregation analysis on X-rays of hands in 213 Turkmenian pedigrees showed 50–60% of total variation in BMD could be attributed to a single Medelian locus or to two codominant alleles (68). These conflicting data could be due to the difference in the bone sites measured, the relatively small sample sizes, the distinct ethnic character of the groups, or the failure of the studies to include in their models the correct parameters affecting bone density.

Twin studies Twin studies take advantage of the fact that monozygotic (MZ) twins are genetically identical clones and will be the same for genetically determined traits. Like any other set of siblings, dizygotic (DZ) twins share only half of their genes, but they are age matched. Heritability of a trait can be calculated from the interclass correlation for each zygosity using a variety of equations. However, these calculations assume that any genetic variance is additive and that the environmental covariance of the DZ twins is equal to that of the MZ twins. Studies on MZ twins separated at birth can separate out the environmental from the genetic effects on a trait. However, there are few of these subjects to study, and no studies have been conducted on bone density or osteoporosis in separated twins. The data from twin studies consistently demonstrate that bone density at a number of sites has a strong genetic component (50–90% of variability is determined by a genetic component, depending on the site measured) (3, 20, 73, 80, 93, 102, 106). However, Slemenda et al (106) concluded that the high heritability estimates they and others have calculated were due to errors in the assumptions made about the twin model.

Bone from appendicular skeletal sites (femur neck, trochanter, and forearm) is comprised primarily of cortical bone and may be less influenced by genetic factors than trabecular bone–rich axial skeletal sites (Wards triangle, lumbar spine) (3, 20, 93). Similarly, the rate of bone loss over time appears to be genetically controlled only at axial sites (58), whereas at skeletal sites rich in cortical bone, genetic factors have a nonsignificant effect (11, 58, 105). The effect of genetic influences on bone loss is reflected in the strong genetic effect on serum markers for bone formation (osteocalcin, bone specific alkaline phosphatase, propeptide of collagen type I) and to a lesser extent on urinary bone resorption markers (34, 57). Bivariate analysis showed that the same genetic factors influence all bone sites (93), whereas more recent computer modeling of bone density data suggests there are additional factors unique to cortical bone and trabecular bone within each site (73).

As with bone density, there is a strong genetic contribution to bone geometry, e.g. the center of femur neck mass (70% genetic), resistance of the femur neck to fall forces (92%) (107), and hip axis length (51% after height and environmental

adjustments) (31). The association between bone density and lean or fat mass is likely determined either by the genes regulating body size (102) or via shared environmental influences (46). Finally, a recent study using twins discordant for exercise suggests that genetic influences may also determine the capacity of bone to respond to exercise (25).

Linkage analysis studies Family and twin studies can identify the portion of a disease or trait that is accounted for by genetic factors, but they do not identify which genes are responsible for the effect. This is traditionally done by linkage analysis and positional cloning (24). The disadvantage of this approach is that it requires a large number of families in order to identify genes that have a modest effect on a trait or disease (96). Linkage analysis has not been commonly used to identify genes associated with bone density. However, for the interested reader, some examples of linkage analysis approaches to BMD include the following studies. Using 22 members of a kindred that have a phenotype of high spinal bone density, Johnson et al (49) found evidence for linkage of this trait to chromosome 11, near chromosomal marker D11S987. As discussed below, polymorphisms in the vitamin D receptor (VDR) and collagen type I alpha I genes have been associated with low bone density; however, neither of these genes resides on this chromosome. Spotila et al (113) examined 37 members of 22 families with familial osteopenia. Their data suggest a monogenic mode of inheritance of the low bone density trait. However, they have not been able to attribute this trait to a chromosomal location. Further work is clearly needed on this front.

ASSOCIATION STUDIES OF GENETIC MARKERS WITH BONE MINERAL DENSITY AND OSTEOPOROSIS

An alternative to genetic linkage studies aimed at identifying genes that cause osteoporosis are studies that look for polymorphisms or mutations in genes whose protein products are already known to influence bone metabolism. These candidate genes are then investigated in association studies that attempt to statistically link these polymorphisms to the occurrence of disease or a physiological trait.

Genetic markers are often based on restriction fragment length polymorphisms (RFLPs) caused by random mutations in DNA that lead to variations in specific endonuclease cut sites. However, these mutations occur throughout the genome and do not necessarily occur within the coding region of a gene. Thus, RFLPs do not necessarily have a functional consequence to the gene in which they are detected. Some examples of genetic markers that have been investigated in association studies of BMD or osteoporosis are the RFLPs in

the VDR gene (using *Bsm*I, *Taq*I, *Apa*I, and *Fok*I restriction enzymes) and the estrogen receptor gene (using *Xba*I and *Pvu*I restriction enzymes), and nucleotide repeat polymorphisms in the Sp1 binding site in the collagen type I alpha I gene promotor. In addition, associations between BMD and various phenotypes based on circulating isoforms of apolipoprotein E4 have also been reported. In the case of the *Bsm*, *Taq*, and *Apa* RFLPs, there are no associated changes in the amino acid composition of the VDR protein. However, the *Fok*I RFLP is associated with the expression of a shortened form of the VDR lacking the three N-terminal amino acids. Mutations in the Sp1 binding site of the collagen gene promotor could influence the regulation of collagen expression. Use of several genetic typing approaches, e.g. direct haplotyping for several different VDR RFLPs (118), may result in greater genetic resolution.

Classification of individuals based on a given RFLP is done by Southern analysis of the DNA. In polymerase chain reaction (PCR)-based methods, the gene of interest is first amplified, then the PCR product is digested with a specific endonuclease to yield a distinctive genotypic pattern. For example, digestion of a VDR gene PCR product with *Bsm*I results in three possible band patterns based on the two possible variations (absence or presence of the cut site) that occur in the inherited alleles, i.e. *BB*, *Bb*, and *bb*, where accepted nomenclature uses *B* to represent the absence of the *Bsm*I restriction site and *b* represents the presence of the cut site. *Bsm*I treatment of PCR products results in a characteristic pattern consisting of one large band in the case of a *BB* homozygote, two bands for the *bb* homozygote, and three bands for a *Bb* heterozygote.

Although an association study with candidate genetic markers has the outward characteristics of a shotgun approach, it has certain statistical advantages over linkage analysis (96). A critical discussion of the pitfalls of these different methods used to study the genetic contributions to complex diseases is given by Econs & Speer (24). The following is a brief summary of some of the issues that arise in interpreting association studies (24).

Factors in Genetic Association Studies That May Lead to False Associations

Although a given allele (e.g. a RFLP) may be associated with a given trait, it does not mean the allele is necessary to the manifestation of the given phenotype. On the other hand, finding a significant statistical association between a genetic marker and a given trait implies that individuals who carry the associated allele are at an increased risk for the disease or condition. However, complications that arise from association studies may result in false associations and may lead to discrepancies in the findings of various studies. Some of the factors that may influence these false associations are discussed below.

(*a*) The control group (nonaffected population) needs to be appropriate to the study population and must be matched for age, sex, ethnic background, and relevant environmental variables. (*b*) The generalizability of a study's findings may be limited. If investigators cannot replicate the original study observations using populations of individuals of different races or ethnicity, different environmental exposure, etc, the generalizability of the study is questionable. Such failures in replication may be due to errors in study design, implementation, or analysis. Or it is possible the findings of the original study are true but cannot be applied widely outside of the original population. The lack of replicability could prove useful, however, in that it could unearth other variables that are modifying the genotype effect. (*c*) Population stratification can lead to spurious results in association studies. In such cases, population admixture results in a combination of different populations with varying allelic or genotypic frequencies. For example, if population A has a high frequency of genotype "A" and for unrelated reasons a high BMD and population *B* has a high population frequency of allele "a" and again for unrelated reasons a high prevalence of low BMD, the unwitting combination of these two populations in one study group will indicate a strong association between allele A and high BMD, even though within each individual population there is no association. Population stratification can also lead to deviations from the Hardy-Weinberg theory, which predicts the behavior of alleles and genotypic frequencies in populations. (*d*) Competing risks are another concern. An association between a genotype and other disorders or life situations may affect whether an affected individual is recruited into a study. For example, if the genotype is a risk factor for a common ailment that is an exclusion criteria in the study, e.g. hypertension, then the preferential exclusion of these subjects may bias the results of the study. (*e*) Another concern is population characteristics. Different nutrient intake levels and/or levels of physical activity could cause different outcomes between studies. Finally, (*f*) the putative marker allele may not influence the phenotype directly but may be in linkage disequilibrium with another gene that does directly influence it. In this situation, depending on the degree of recombination that has occurred in a given population, some studies may find an association of the trait with one allele, while other studies find associations with a different allele. Keeping these issues in mind, we consider some of the published association studies on BMD or osteoporosis with candidate marker genes.

Estrogen Receptor Gene Polymorphisms

Estrogen deficiency in postmenopausal women is associated with increased bone turnover and acceleration of bone loss, leading to an increased susceptibility to bone fractures (87). Estrogen receptors have been found in human osteoblasts and osteoclasts, which suggests a possible direct effect from estrogen

on bone. Estrogen receptor mutations are associated with bone loss in humans and mice (122). Thus, polymorphisms in the estrogen receptor may prove to be markers of increased risk for low BMD and osteoporosis (99). Yanagi et al (122) investigated the association between *Pvu*I and XbaI restriction fragment length polymorphisms of the estrogen receptor gene and BMD in 238 postmenopausal Japanese women. The *PPxx* genotype, observed in 8% of the subjects, was found to be associated with a significantly lower BMD in the lumbar spine and whole body. Additional investigations of the relationship between the *Px* haplotype and BMD in other populations are needed before the usefulness of this genetic marker can be properly evaluated.

Collagen Gene Polymorphisms

Type I collagen is the major protein of bone encoded by the COLIA1 and COLIA2 genes. Grant et al (37) reported that a G-to-T polymorphism in the promotor region of COLIA1 at a recognition site for the transcription factor Spl is related to bone mass and osteoporotic fracture. G/T heterozygotes at the polymorphic Spl site (*Ss*) had significantly lower BMD than did G/G homozygotes (*SS*) in two populations of British women, and BMD was lower still in T/T homozygotes (*ss*). Importantly, the unfavorable *Ss* and *ss* genotypes were over-represented in patients with severe osteoporosis and vertebral fractures (54%), as compared with controls (27%), which suggests that this marker may be useful in predicting the risk of developing osteoporosis. Unfortunately, several discordant studies investigating associations between the collagen gene polymorphism and BMD or osteoporosis have recently been reported in abstract form at scientific meetings. This suggests that this marker of osteoporosis risk may have limited generalizability.

Apolipoprotein E Phenotype

Apolipoprotein E (apo E) is a major constituent of high-density and low-density lipoproteins. The principal isoforms of apo E are apo E3 and apo E4. A less common isoform, apo E2, is also found. Previous studies have shown that the apo E phenotypes are associated with the risk of developing diseases such as Alzheimer's dementia and cardiovascular disease (53, 81). Shiraki et al (104) investigated the relationship between phenotypes of apolipoprotein E and BMD in 284 postmenopausal Japanese women. Subjects were categorized by three phenotypic groups based on the isoforms (E2, E3, and E4) of apo E4 found in the blood: apo E4 $-/-$ (E3/2 and E3/3; 76% of the population), apo E4 $+/-$ (E4/3 and E4/2; 22% of the population), and apo E4 $+/+$ (E4/4; 2% of the population). This study found a significant gene-dose effect from the apo E4 allele on BMD of the lumbar spine and total body. The group representing the negative homozygotes for apo E allele (apo E4 $-/-$) had the highest BMD. The

reason for the observed relationship between the apo E genotype and BMD is obscure, but it may be related to vitamin K status because apo E phenotype has been associated with circulating levels of phylloquinone (100) and vitamin K status has been linked to BMD (114). Additional investigation of the association between apo E phenotypes and BMD or osteoporosis appears warranted.

Vitamin D Receptor Gene Polymorphisms

The majority of association studies of BMD and candidate gene markers have investigated markers for the VDR gene. The seco-steroid $1,25(OH)_2D$ is an important hormonal regulator of bone and mineral metabolism (89). The VDR mediates the biological actions of $1,25(OH)_2D$. The prominent role of the VDR in calcium metabolism suggests that this gene is a likely candidate gene for causing low BMD and osteoporosis. It is clear that mutations in functionally critical areas of this gene can have profound effects on mineral metabolism and BMD. For example, various point mutations of the VDR gene have been identified and shown to be responsible for functional defects in the ligand-binding or DNA-binding domains of the VDR receptor (67, 74). However, because these VDR mutations that cause hereditary rickets are rare in the general population, they would not serve to explain the majority of cases of osteoporosis. On the other hand, restriction fragment length polymorphisms of the VDR are common (14). If these polymorphisms influence the level or function of the VDR, then they could have an important impact on mineral metabolism and BMD.

FOKI VDR POLYMORPHISMS A common polymorphism has been described in the coding region of the VDR gene. The polymorphism results from a C-to-T transition and creates an initiation codon (ATG) three codons proximal to a downstream start site. The polymorphism can be defined by a restriction fragment length polymorphism (RFLP) using the restriction endonuclease *Fok*I. The presence of a *Fok*I site, designated *f*, allows protein translation to initiate from the first ATG. The allele lacking the site (designated *F*) initiates from a second ATG site downstream from the first site. Thus, translation products from these alleles differ by three amino acids, with the *f* variant elongated (38). The *Fok*I polymorphism in the VDR gene has been associated with a 13% lower lumbar spine BMD and a greater rate of bone loss in the femoral neck (4.7% vs 0.5%; *ff* vs *FF*) in postmenopausal Mexican-American women (38). Likewise, in Caucasian premenopausal women (42), the *ff* genotype is associated with a 4% lower total body and 12% lower femoral neck BMD. However, an influence of the *Fok*I genotype has not been found in all studies (23). Moreover, the functional significance of a shortened VDR is uncertain, although a recent study in Japanese women suggests that the different start site allele may

be produced with different frequencies (2). Additional investigations of the *Fok*I VDR polymorphism in various populations appears warranted, however, because the number of reported association studies is small.

BSMI-, TAQI-, AND APAI-VDR POLYMORPHISMS The vast majority of studies of VDR genotype have studied the influence of the *Bsm*I VDR polymorphism on BMD (14). In 1994, a cardinal study reported a significant association between the *Bsm*I VDR genotype and BMD in 250 Caucasian twins, aged 17–70 years, from Australia (82). The study consisted of 70 monozygotic (MZ) and 55 dizygotic (DZ) adult twin pairs; most subjects were female. In addition, a further 311 elderly women (207 postmenopausal) were also studied. From their study of twins, these investigators concluded that much of the genetic variation in BMD could be explained on the basis of the *Bsm*I VDR genotype alone. They also reported that postmenopausal women with the *BB* genotype would reach the BMD "fracture threshold" (defined as two standard deviations below the mean of young adults) 10 years sooner than their *bb* genotype counterparts. This greater decline in BMD in the *BB* group could significantly increase their risk of bone fracture. The potential usefulness of the *Bsm*I VDR in predicting BMD was given additional support by Spector et al (112), who found an association between vitamin D receptor genotype and BMD in a study of postmenopausal twins (95 DZ pairs of twins and 87 MZ pairs of twins, aged 50–69 years) in Britain. Adjusted BMD was significantly lower in the *BB* group at the hip, at the lumbar spine, and for the whole body. In contrast, however, a twin study conducted in the United States found no relationship between VDR genotype and BMD (47). In the latter study, polymorphisms at the vitamin D receptor gene were examined in relation to BMD at the spine, femur, and forearm in 86 MZ and 39 DZ adult female twins. Subsequently, many studies in various populations have investigated the association between the *Bsm*I VDR genotype and BMD and found discordant results. The findings of some of these studies were the subject of a meta-analysis by Cooper & Umbach (14), who concluded that overall the *Bsm*I VDR polymorphism had a significant, but small, effect on BMD.

POSSIBLE MODIFYING FACTORS IN ASSOCIATION STUDIES

Given the variety of genes and environmental factors that could influence BMD over a lifetime, it is not surprising that not a single gene marker has been identified that can explain differences in BMD in all populations. The lack of agreement between studies investigating the association of VDR polymorphism with BMD may be due to several reasons (see above). Specific environmental factors that need to be considered as potentially important modifiers of genetic

effects on BMD include racial differences, age, hormonal status, body composition, nutritional modulation of the phenotype, and important lifestyle variables such as smoking and caffeine and alcohol use and the level of physical activity. Unfortunately, several of these important variables affecting BMD have not been reported in many studies. Some possible influences of these modifying factors on the associations between the *Bsm*I VDR genotypes and BMD are discussed below.

Race

There is a wide variation in the prevalence of the *Bsm*I VDR polymorphism in different racial groups. For example, persons of Asian or African-American background have a low frequency of the *B* allele compared with Caucasians. In a meta-analysis of studies investigating the association of VDR genotype and BMD published prior to July 1996, Cooper & Umbach (14) found that the racial distributions of the *BB* (low bone density) genotype was 17% in Caucasians (21 studies), 5% in African-Americans (3 studies), and 2% in Asians (5 studies). Nevertheless, an association between the *B* allele and low BMD has been reported in Caucasians (30, 65, 95), Asians (116, 121), and African-Americans (30). Thus, although the frequency of a given VDR genotype may vary considerably depending on race, there is no obvious racial bias in observing an association between VDR genotype and low BMD.

Age

BMD in adults represents the net effect of the peak bone mass achieved and the rate of adult bone loss. If the VDR polymorphism has differing degrees of influence on peak bone mass compared with rates of bone loss, then the association between VDR genotype and BMD may be more readily apparent in either younger or older adults. For example, if VDR polymorphisms have a strong influence on peak bone mass but less of an influence on the rate of postmenopausal bone loss, then the association between BMD and VDR polymorphisms could be attenuated in elderly populations (95, 118). A possible age (or estrogen) effect on the VDR genotype association with BMD may be appreciated by comparing the effects of VDR genotype on BMD in pre- and postmenopausal women (Figure 1). The *BB* VDR genotype appears to have a more pronounced effect on BMD in young, premenopausal women compared with older, postmenopausal women.

Body Composition

Measurements of bone density by absorptiometry based on a two-dimensional projection of a three-dimensional structure cannot accurately account for variations in cross-sectional areas and are influenced not only by bone mass but also by the size of the bone (98). It has been suggested that the VDR polymorphisms

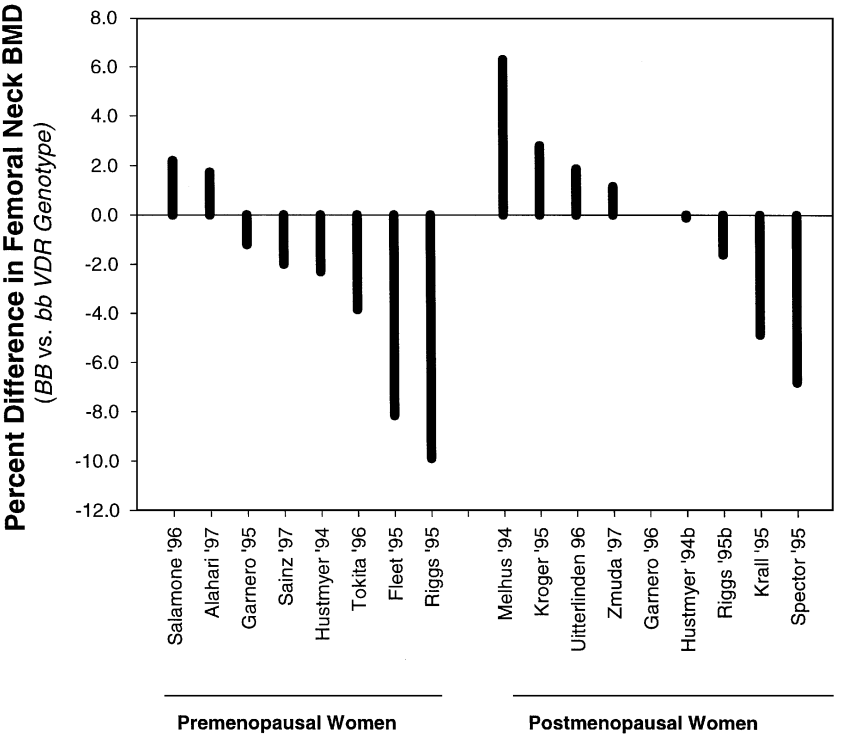


Figure 1 Comparison of the effects of vitamin D receptor (VDR) genotype (*BB* vs *bb*) on bone-mineral density (BMD) in premenopausal and postmenopausal women reported in various studies. Average difference in femoral neck BMD between *BB* and *bb* genotype groups was −2.9% from studies conducted in premenopausal women ($n = 9$ studies; total 819 subjects) and −0.14% in postmenopausal women ($n = 8$ studies; total 1051 subjects).

could affect BMD measurements via an effect on body size (5). In this case, the vitamin D receptor polymorphisms would not be related to bone mass, but rather to variations in diaphyseal cross-sectional growth resulting from periosteal apposition of new bone (5, 85, 98). Barger-Lux and colleagues (5) determined vitamin D receptor genotype, bone mass at spine and total body, and body size in 32 healthy premenopausal females. They found that bone-mineral content (BMC) at both spine and total body was significantly associated with VDR gene alleles. BMC was highest for the *bb* genotype, lowest for *BB*, and intermediate for *Bb*. However, they observed a similar association between VDR genotypes and body weight, and when BMC was adjusted for body weight, the association with VDR polymorphism disappeared. Need et al (85) reported that bone area

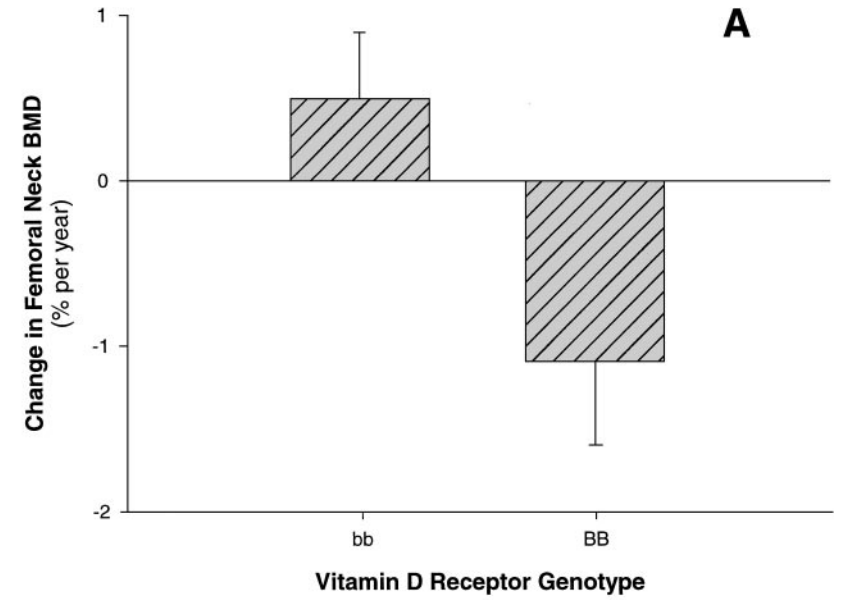
was greater in the *BB* genotype and concluded that although the *BB* genotype appeared to be associated with lower bone density in men, this observation could be due to larger bone size rather than reduced bone mass. Recently, this matter was significantly illuminated by the study of Sainz et al (98), who used quantitative computed tomography to measure bone. This technique allows accurate assessment of both the size of the bones and the various components that influence bone mass. In their study of the association between VDR genotype and skeletal development in prepubertal girls, aged 7–12 years, computed tomography measurements of bone revealed a VDR genotype effect on femoral and vertebral bone density. Girls with the *bb* genotype had a 3% higher femoral bone density and a 10% higher vertebral bone density than did girls with a *BB* genotype. Importantly, however, no association of VDR genotype was found for cross-sectional areas of the vertebrae or the cross-sectional or cortical areas of the femur, which suggests that variations in bone size apparently do not bias the VDR genotype association with BMD.

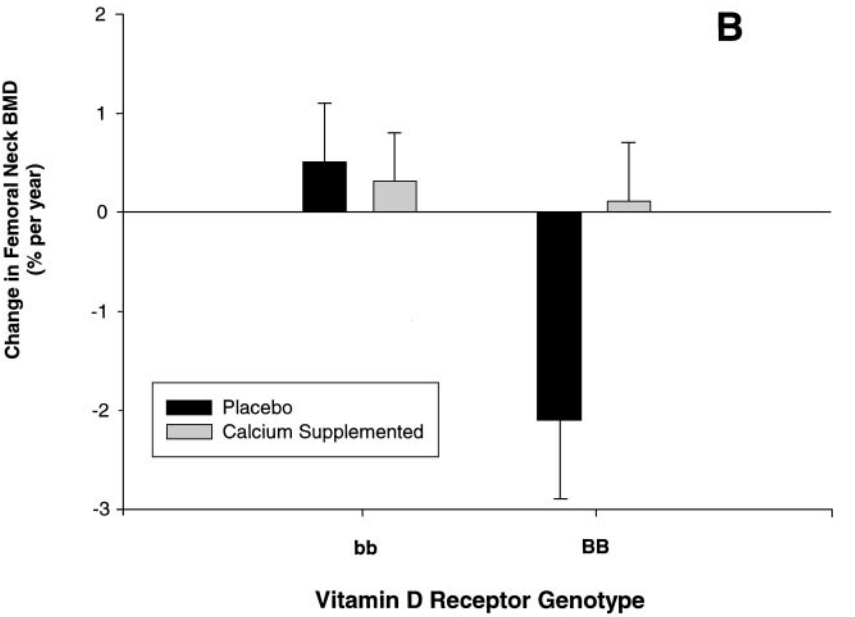
Calcium Intake

Dietary calcium intake influences both the gain and loss of bone throughout the life cycle. Some studies have suggested that this nutritional factor may modulate the effect of VDR genotype on the skeleton.

Krall et al (65) investigated the influence of VDR genotype on rates of bone loss in 229 elderly postmenopausal women who had previously taken part in a placebo-controlled calcium supplementation trial (17). BMD was measured over the course of 2 years at the femoral neck, spine, and radius. Rates of bone loss were greater in the *BB* group at all sites. However, when the study population was analyzed according to calcium intake level, it was found that the VDR genotype influenced bone loss from the femoral neck only in those with a low dietary calcium intake (Figure 2).

It should be noted that participants in this clinical trial were chosen on the basis of low dietary calcium intakes and were randomized to a 500-mg/day calcium supplement. Ferrari et al (28) investigated the relation between VDR genotype and change in the lumbar spine BMD over the course of 18 months in a group of elderly subjects supplemented with 800 mg of calcium/day. They observed a significant effect from VDR genotype on rates of bone loss. However, calcium intake was related only to the change in BMD in the *Bb* heterozygote group, which suggests a possible interaction between VDR genotype and calcium utilization. Kiel et al (60) investigated the relationship between VDR polymorphisms and BMD in 328 elderly women, aged 69–90 years, who were participants in the Framingham Heart Study, a longitudinal study of risk factors for heart disease. In subjects with the *bb* but not the *Bb* or *BB* genotypes, significant associations existed between usual calcium intake and BMD at five of





six skeletal sites, such that BMD was 7–12% higher in those with dietary calcium intakes greater than 800 mg/day compared with those with intakes <500 mg/day. The data also suggested that BMD was higher in persons with the *bb* genotype only in the group with calcium intakes above 800 mg/day. These findings suggest that optimal utilization of an adequate calcium intake may be influenced by VDR genotype. In a study of 268 postmenopausal women, Garnero et al (35) observed no relationship between VDR genotype, BMD, and calcium intake. However, only 64 of these elderly subjects consumed low-calcium diets (<600 mg/day). There currently is a paucity of data available on the influence of VDR genotype on the rate of bone accrual in children consuming varying levels of dietary calcium, as well as on the genotype-dependent response to calcium supplementation in various other populations. Additional studies of the potential interaction between VDR genotype and bone status in young and old subjects in the context of well-controlled calcium supplement trials could prove instructive.

Vitamin D Supplementation

Vitamin D deficiency is common in some elderly populations. Moreover, vitamin D intake can affect the rate of bone loss in elderly subjects (18). Thus, knowledge of VDR genotype may prove useful in predicting the response of a group of subjects to vitamin D supplementation. However, little information is available on this important question. Graafmans et al (36) studied the effects from a 2-year regimen of vitamin D supplementation (400 IU/day) on BMD in Caucasian women over 70 years old. Although they did not find an association between VDR genotypes and baseline femoral neck BMD, they observed that the mean increase in BMD in the vitamin D group relative to a placebo group was higher in subjects with the *BB* and *Bb* genotype compared with the *bb* group. They interpreted their findings as representing a functional involvement of VDR gene variants in determining BMD. In contrast, Matsuyama et al (75) reported that Japanese subjects given an active form of vitamin D had a more positive skeletal response to supplementation in the *bb* VDR genotype group. Likewise, Howard et al (45) observed in a group of Australian subjects that the *bb* genotype group demonstrated a greater relative suppression of PTH in

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Figure 2 Effect of vitamin D receptor (VDR) genotype (*BB* vs *bb*) on rates of femoral neck bone loss in postmenopausal women with and without calcium supplementation. (*A*) Genotype effect on bone loss in total group. *BB* genotype group had greater rates of bone loss compared with *bb* group. (*B*) Genotype effect on bone loss in placebo group that consumed a low-calcium diet and in calcium-supplemented group. Genotype effect on rates of bone loss were only apparent in the women who consumed low-calcium diets. [Adapted from Krall et al (65).]

response to short-term administration of $1,25(OH)_2D$. These studies suggest that VDR genotype can influence the biological response to vitamin D therapy. The mechanism of this effect is unknown, but it is reasonable to assume that it may reflect differences in VDR level or activity. However, as discussed below, there is no evidence of altered VDR level in persons with different VDR genotypes.

BIOCHEMICAL AND PHYSIOLOGICAL CORRELATES OF VDR GENOTYPE

The association between VDR genotype and BMD found in some studies has spurred the investigation of possible genotype effects on some underlying biochemical or physiological processes that could influence BMD. These factors include the vitamin D receptor level, intestinal calcium absorption, and bone turnover.

Vitamin D Receptor Expression

Although the anonymous VDR polymorphisms, such as those determined by the *Bsm*I restriction site, do not occur within the coding region of the protein whereas other polymorphic sites in the VDR gene, such as the *Taq*I endonuclease site, are not predicted to cause an amino acid substitution in the VDR protein, these allelic variations could still influence the degree of expression of the VDR (82). To investigate this question, Barger-Lux et al (5) measured duodenal VDR levels in 32 premenopausal women: They found no effect of the *Bsm*I polymorphism. Likewise, Kinyamu et al (62) measured intestinal VDR levels in 92 women genotyped for *Bsm*I and *Taq*I polymorphism at the VDR gene locus and found that there were no significant differences in intestinal VDR among VDR genotype groups. Thus, both studies that have attempted to correlate VDR polymorphisms with intestinal VDR level have not shown a genotype effect on VDR. It could be argued, however, that VDR expression could vary in a tissue-specific manner and that the VDR genotype could have differing effects in different tissues. Mocharla et al (79), however, recently reported that VDR mRNA was not different in lymphocytes from subjects with different VDR genotypes. Nevertheless, no information is available on the relationship between VDR genotype and the level of VDR expression in bone. Future studies of the functional effects of various VDR polymorphisms on VDR expression and function in bone cells in culture could prove enlightening.

Intestinal Calcium Absorption

As mentioned above, Krall et al (65) reported that *Bsm*I VDR polymorphisms were associated with the rate of femoral bone loss only in postmenopausal women who consume a low-calcium diet (Figure 2). Greater rates of bone loss

under conditions of low dietary calcium intakes would be consistent with a possible effect of the VDR genotype on vitamin D–dependent calcium absorption. Moreover, this absorption defect would be masked in subjects with high calcium intakes because most of the calcium absorbed at high calcium loads is via a vitamin D–independent pathway (103).

Dawson-Hughes et al (19) compared fractional calcium absorption in healthy, late-postmenopausal women with (*bb*) and without (*BB*) the *Bsm*I restriction site. Calcium absorption and plasma 1,25-$(OH)_2$D were measured in 60 women (26 *BB* and 34 *bb*) after 2 weeks of high-calcium (1500 mg/day) and 2 weeks of low-calcium (<300 mg/day) intake. ^{45}Ca absorption was similar in the two groups on the high-calcium intake (19%/liter in *BB* and 20%/liter in *bb*) but differed significantly in the groups on the low-calcium intake (21 vs 24%/liter). Calcium restriction induced similar percentage increases in plasma 1,25-$(OH)_2$D, 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$(OH)_2$D. In a complementary study, Zmuda et al (123) examined the relation between VDR genotype and fractional ^{45}Ca absorption in 101 postmenopausal African-American women. VDR gene polymorphisms were defined by the endonucleases *Bsm*I, *Apa*I, and *Taq*I. Women homozygous for the *B* allele had 14% lower fractional ^{45}Ca absorption (not significant) compared with women homozygous for the *b* allele. Wishart et al (120) investigated the relationship between calcium absorption and vitamin D receptor genotype in 99 healthy women approaching menopause. VDR alleles were also classified according to *Bsm*I, *Taq*I, and *Apa*I restriction enzymes. Radiocalcium absorption was significantly greater in the bbaaTT haplotype and the aa genotype. In contrast, Kinyamu et al (62) found no relationship between VDR polymorphisms and intestinal calcium absorption in either young or elderly women. Some 92 Caucasian women (49 young women, aged 25–35 years, and 43 elderly women, aged 65–83 years) were genotyped for *Bsm*I and *Taq*I polymorphisms at the VDR gene locus. No significant differences were found in intestinal calcium absorption among VDR genotype groups. Likewise, Francis et al (32) investigated the association between vitamin D receptor genotype and calcium absorption in 48 men (median age 64, range 27–77), half of whom had crush fractures, and showed no significant difference in calcium absorption among the genotypes. Thus, based on the reported relationship between VDR genotype and intestinal calcium absorption, genotype has no or a very minor influence on calcium absorption.

Bone Markers

Morrison et al (82) initially reported that serum osteocalcin, a biochemical marker of bone formation, was associated with polymorphisms in the VDR

gene. Serum osteocalcin levels were significantly higher in subjects with the *bb* genotype versus those with the *BB*, whereas the *Bb* heterozygotes were intermediate. However, these genotype effects on serum osteocalcin have not been consistently observed by others, despite some studies finding positive associations between VDR genotype and BMD (30). Similarly, biochemical markers of bone resorption have not been consistently associated with VDR genotype.

GENETIC POLYMORPHISMS AS RISK FACTORS FOR OSTEOPOROSIS AND OTHER CHRONIC DISEASES

Some people are clearly at increased risk for osteoporosis due to genetic factors. The identification of genetic markers highly predictive of osteoporosis risk would be an important scientific advance. Given that some studies indicate an association between VDR polymorphisms and BMD, an important question is whether VDR genotype predicts the risk of developing osteoporosis. Several reports have investigated whether patient populations with osteoporosis have a greater prevalence of the *BB* genotype (6, 44, 70). However, none of these studies has found an excess prevalence of VDR polymorphisms associated with osteoporosis. Interestingly, although VDR genotype does not predict osteoporosis risk, several recent reports have suggested that *Bsm*I VDR genotype may be a useful predictor of risk for other diseases, such as primary hyperparathyroidism (8, 9), osteoarthritis (54, 117), prostate cancer (48, 115), and perhaps diabetes (77). The underlying mechanisms responsible for the association between VDR genetic markers and various disease processes are unknown. Moreover, given the potential pitfalls of association studies, due caution should be exercised.

SUMMARY

A significant number of studies have found an association between the *Bsm*I VDR genotype and BMD in various racially diverse populations, as well as in prepubertal girls, young adult and postmenopausal women, and men. However, there are also many studies that have not found an association between this VDR genotype and BMD. The reasons for the discordant findings are uncertain but not surprising given the multifactorial influences on BMD and the possible pitfalls of association studies with candidate genes. In addition, negative or discordant findings have been reported concerning the effects of VDR genotype on intestinal vitamin D receptor levels, intestinal calcium absorption, and measures of bone turnover. Novel candidate genetic markers will no doubt continue to be investigated in the quest to understand the genetic underpinnings of osteoporosis. Despite the unsettled state of affairs concerning the usefulness of particular

genetic markers as demonstrated in association studies, the search to identify individual genetic markers of low bone density and osteoporosis risk is important and will surely continue. However, it is clear that additional approaches beyond association studies need to be pursued with renewed vigor. Additional approaches to detect linkage of genes to low bone density and osteoporosis risk can be employed, such as sib-pair analysis, which assesses whether pairs of affected relatives share an allele more frequently than could be expected by chance alone. Sib-pair analysis in large cohorts has the potential to identify those areas of the genome that harbor genes that predispose to complex traits, such as osteopenia and osteoporosis (24). In the final analysis, a combination of several approaches will probably be needed to identify the important genetic influences, and their environmental modifiers, that increase an individual's risk of developing osteoporosis. The ongoing research efforts to map the human genome herald exciting opportunities for research on diet-gene interactions in the future. Moreover, the likely possibility that dietary or pharmacological manipulation can influence the phenotypic expression of some genetic propensities offers continued hope for improving the health of future populations and can serve as an antidote to fatalistic attitudes of genetic predeterminism.

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