PHARMACOGENETICS OF THE VITAMIN D RECEPTOR AND OSTEOPOROSIS

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ABSTRACT:

Osteoporosis is a major health care problem internationally with important implications for health care costs, morbidity, and mortality. Bone density, an important predictor of osteoporotic fracture risk, is affected by hormonal and environmental factors. However, in twin and family studies most of its age-specific variance is genetically determined. Common allelic variations in the vitamin D receptor (VDR) gene were the first to be linked to bone density. Recently, other candidate genes, notably oestrogen receptor, collagen 1αI, and PTH receptor genes and a chromosome 11 locus, have been associated with bone density and fracture. Polymorphisms in adjacent regulatory regions may be important mechanisms since functional coding region mutations have not been defined. For example, the polymorphic region in the collagen 1αI gene alters a Sp1 binding site and may alter collagen gene expression. At the pharmacogenetic level, VDR alleles predict differences in gut calcium absorption and long-term bone density response to calcium intake and active vitamin D analog treatment. Understanding the mechanisms underlying these allelic differences in relation to diet and lifestyle factors as well as response to therapy could aid selection of optimal therapy for osteoporosis prevention and treatment.

Osteoporosis is a common problem affecting older women and a large number of older men. In a recent community-based Australian study, the remaining lifetime risk for a 60-year-old woman was 56% and 29% for a man of the same age (Jones et al., 1994b). The prevalence of vertebral deformities and fractures in older men has now been shown to be similar to that in women (Cooper et al., 1992a; Cooper and Melton, 1992; Jacobsen et al., 1992; Kanis and McCloskey, 1992; Melton et al., 1993; Spector et al., 1993; Jones et al., 1996). The health care costs are estimated at 30 to 50 million dollars per year per million of population across many developed countries (Randell et al., 1995; Ray et al., 1997). There is also increased risk of death in both men and women with all types of osteoporotic fractures (Cooper et al., 1993; Browner et al., 1996; Center and Eisman, 1997; Center et al., 1999). Epidemiological data suggest important ethnic/racial differences, e.g., wide differences in age-adjusted incidence of hip fractures across various Caucasian populations and between Asian and Caucasian populations (Kanis, 1991, 1993, 1997; Kanis and McCloskey, 1992; Effros et al., 1994; Gullberg et al., 1997).

Osteoporosis occurs when bones become fragile such that they can break when relatively minor force is applied to them. Although these osteoporotic bone fractures heal normally, significant morbidity and even mortality is associated with these fractures. Some, such as of the spine, leave deformity and contribute to long-term disability and even pain. The weaker bones, susceptible to fracture, are the result of the peak bone mass and size achieved in late childhood or early adulthood and the inevitable loss that occurs after menopause and with advancing age (Hui et al., 1990; Kelly et al., 1990b; Kroger et al., 1992, 1994; Johnston and Slemenda, 1993, 1994; Eisman et al., 1993; Teegarden et al., 1995; Lonzer et al., 1996; Lysen and Walker, 1997; Willing et al., 1997). A range of hormonal and environmental factors affects the risk of osteoporosis, and genetic factors may influence sensitivity to hormonal and environmental factors. The ethnic and racial differences in incidence may relate to genetic or environmental factors, but it has also been suggested to reflect inherited differences in susceptibility to environmental factors. Understanding how inherited factors interact with environmental factors may hold the key to better prevention and treatment.

Genetics of Osteoporosis

Noninvasive techniques for assessment of bone structure, including radiological measures of quantitative computerized tomography and more recently dual photon absorptiometry or dual energy X-ray absorptiometry and quantitative ultrasound, have facilitated studies of genetics in osteoporosis. Bone phenotype measured by any of these techniques is the most powerful predictor of subsequent fracture risk. Peak bone mass measured by these tools seems to be under genetic control. This has been most clearly shown in studies of monozygotic and dizygotic twins (Slemenda et al., 1991a, 1996; Kelly et al., 1993, 1995; Johnston and Slemenda, 1994; Flicker et al., 1996; Seeman et al., 1996; Arden et al., 1996a; Arden and Spector, 1997; Harris et al., 1998; Hopper et al., 1998; Nguyen et al., 1998). Overall 45 to 80% of the age-corrected variability of bone phenotypic parameters can be explained by genetic factors with site-to-site differences in heritability (Slemenda et al., 1990b, 1991a, 1996; Flicker et al., 1996; Arden et al., 1996a; Arden and Spector, 1997; Harris et al., 1998; Nguyen et al., 1998; Howard et al., 1997, 1998a).

In addition, bone mass is also influenced by hormonal changes, such as puberty and menopause, and by lifestyle factors such as physical loading and calcium intake. Total bone mass increases about 3-fold over just a few years just before puberty (Johnston et al., 1992; Slemenda et al., 1994; Lonzer et al., 1996; Weaver et al., 1996). Bone mass remains stable until the onset of menopausal bone loss and...
age-related bone loss. The latter type of bone loss may start in the early 40s in both men and women and accelerates with aging (Hui et al., 1990; Johnston and Slemenda, 1992; Tuppurainen et al., 1993; Kroger et al., 1994; Jones et al., 1994a; Nguyen et al., 1995; Harris and Dawson-Hughes, 1996; Willing et al., 1997). Younger men also lose bone with androgen deficiency (Seeman, 1995; Soule et al., 1995; Slemenda et al., 1997). There is considerable and as yet unexplained variation in the rate of postmenopausal bone loss. Similarly, corticosteroid excess can have a major adverse effect on bone, but there is a wide range of sensitivity to these effects. These variations in hormonal sensitivity, which may reflect gene-environment interactions, are the focus of this review.

Family history of osteoporotic fracture has been found to be a risk factor for the development of osteoporotic fracture in numerous epidemiological studies (Sowers et al., 1992; Krall and Dawson-Hughes, 1993, 1995; Seeman et al., 1994; Seeman, 1994; Soroko et al., 1994; McKay et al., 1994; Gueguen et al., 1995; Lonzer et al., 1996; Torgerson et al., 1996; Lysen and Walker, 1997; Willing et al., 1997; Diaz et al., 1997; Lips, 1997; Evans et al., 1998). Even specific types of fractures seem to be related to inherited differences in bone density, e.g., mothers with osteoporotic fractures have daughters with lower site-specific bone density (Evans et al., 1988; McKay et al., 1994; Seeman, 1994; Seeman et al., 1994; Soroko et al., 1994). These findings lead to questions regarding the mechanisms of such an inherited predisposition. One obvious mechanism would be gene-gene interaction for a relatively large number of genes, i.e., complex multifactorial genetic factors. An alternative and complementary mechanism could involve gene-environment interactions.

Human and animal studies have shown high levels of heritability (45–80% of variance) of bone density and structure for any age or group (Sowers et al., 1992; Krall and Dawson-Hughes, 1993, 1995; Seeman et al., 1994, 1996; Seeman, 1994; Soroko et al., 1994; McKay et al., 1994; Garabedian, 1994; Jouanny et al., 1995; Lonzer et al., 1996; Slemenda et al., 1996; Arden et al., 1996a; Torgerson et al., 1996; Salamone et al., 1996b; Ulrich et al., 1996; Beam et al., 1996; Arden and Spector, 1997; Diaz et al., 1997; Harris et al., 1998; Hopper et al., 1998; Nguyen et al., 1998; Howard et al., 1998a; Ferrari et al., 1998b). This heritability being apparent before puberty suggests that genetic factors program inherent bone structural characteristics. However, these family-based studies compare individuals of widely different ages and do not assess the effects of age cohorts and familial environment and lifestyle similarities (Slemenda et al., 1991a; Sowers et al., 1992; Krall and Dawson-Hughes, 1993; Jouanny et al., 1995; Lonzer et al., 1996; Ulrich et al., 1996; Lysen and Walker, 1997; Willing et al., 1997). These data are yet to be extended to examine differences between ethnic and racial groups.

Although the twin model has been used to assess heritability, twin studies, particularly in monozygotic twins, can also be used to investigate the impact of environments and other lifestyle factors (Pocock et al., 1989b; Slemenda et al., 1991a; Young et al., 1995). Several environmental aspects have been shown to affect bone density. These include skeletal loading, calcium intake, smoking, and alcohol use (Slemenda et al., 1990a, 1991a; Cooper et al., 1992b; Nieves et al., 1992; Blauw et al., 1994; Cummings et al., 1995; Massey and Whiting, 1996). It is unclear to what extent genetic factors may affect these relationships. Physical loading on the skeleton, ranging from immobilization and microgravity to loading in elite athletic sports, has been shown to affect bone mass (Pocock et al., 1989a; Sessions et al., 1989; Kroger et al., 1994; Lonzer et al., 1996; Slemenda et al., 1991b, 1992, 1994; Recker et al., 1992; Slemenda and Johnston, 1993; Khan et al., 1996; Salamone et al., 1996b; Ulrich et al., 1996; Ensrud et al., 1997a,b).

There is considerable debate about what is appropriate calcium intake ranging from 800 to over 1000 mg/day (Kelly et al., 1990a; Kanis and Passmore, 1990; Kanis, 1991, 1994a,b; Johnston et al., 1992; Tuppurainen et al., 1993; Blauw et al., 1994; Teegarden et al., 1995; Weaver et al., 1995, 1996; Lonzer et al., 1996; Massey and Whiting, 1996; Dawson-Hughes et al., 1996, 1997; Wastney et al., 1996; Nordin, 1997; Jackman et al., 1997). In Asian cultures with little dairy intake and thus dietary calcium intakes close to 400 mg/day, osteoporotic fracture incidence is unexpectedly lower than in comparable Caucasian populations (Nakamura et al., 1994; Xu et al., 1996). Although other lifestyle factors may be important, this may also relate to ethnic or racial differences in calcium absorption, an example of gene-environment interactions.

The accelerated bone loss for 10 to 15 years post menopause varies widely among individuals, leading to categorization of slow and fast losers (Riis et al., 1996). Although there is some debate on this point, some “fast losers” remain so over 15 years of follow-up (Riis et al., 1996). Similar differences have been shown in individuals exposed to excessive levels of glucocorticoid or thyroid hormone (Sambrook et al., 1989, 1990; Cummings et al., 1995; Orwoll et al., 1996). The reasons for these interindividual differences have not been adequately defined. However, inherited and other environmental factors and gene-environment interactions would seem to be a logical mechanism.

An important concept in studying a disease such as osteoporosis is the high prevalence of the disease in the overall population. Gene mutations can be important factors in rare diseases such as osteogenesis imperfecta, an extreme form of osteoporosis that can result in lethality in utero, or osteopetrosis, wherein the bones are so dense that normal marrow space can not develop, leading to aplastic anemia. From the current World Health Organization definition of osteoporosis, relating to difference from young normal, the majority of the elderly population would be “osteoporotic”. It seems inherently unlikely that this common problem with such high heritability could be caused by “extreme” mutations; rather, it seems that it would be caused by a number of small effect-size changes (allelic polymorphisms) shifting bone density within the normal range.

**Genes and Bone Mass**

Polymorphic alleles of the vitamin D receptor gene were the first to be associated with bone turnover and bone density in a nonstructural gene (Morrison et al., 1992, 1994, 1997; Barger-Lux et al., 1995; Riggs et al., 1995; Fleet et al., 1995; Eisman, 1996). A number of subsequent studies have identified weaker (Morrison et al., 1992, 1994, 1997; Arden et al., 1996b; Grossett et al., 1996; Uitterlinden et al., 1996; Tokita et al., 1996; Viitanen et al., 1996; Salamone et al., 1996a; Houston et al., 1996; Zmuda et al., 1997; Vandevyver et al., 1997; McClure et al., 1997; Harris et al., 1997; Kiel et al., 1997; Geusens et al., 1997; Lazaretti-Castro et al., 1997; Tamai et al., 1997; Gennari et al., 1998; Willing et al., 1998; Ferrari et al., 1998a; Hauache et al., 1998) or no effects (Hustmyer et al., 1994; Garnero et al., 1995, 1996; Tuppurainen et al., 1993; Blaauw et al., 1994; Cummings et al., 1995; Massey and Whiting, 1996). Similar differences have been shown in individuals exposed to excessive levels of glucocorticoid or thyroid hormone (Sambrook et al., 1989, 1990; Cummings et al., 1995; Orwoll et al., 1996). The reasons for these interindividual differences have not been adequately defined. However, inherited and other environmental factors and gene-environment interactions would seem to be a logical mechanism.
turnover, e.g., the procollagen type I propeptide cleaved and released when collagen is produced, have also been shown to be genetically linked in some (Tokita et al., 1996) but not all (Hustmyer et al., 1994; Garnero et al., 1995, 1996) studies.

Some studies suggest relationships between the vitamin D receptor alleles and risk of fracture (Feskanich et al., 1998), but others found no such relationship (Looney et al., 1995; Houston et al., 1996; Ensrud et al., 1999). Some of these differences may relate to statistical power (Nguyen et al., 1994, 1996) and to differing ethnic and environmental backgrounds.

Several other “candidate” gene loci have been associated with bone density or fractures. These include hormone receptor genes, such as the oestrogen receptor gene (Qi et al., 1995; Kobayashi et al., 1996; Mizunuma et al., 1997; Han et al., 1997; Carling et al., 1997b; Shiraki et al., 1997; Gennari et al., 1998) and the calcitonin and parathyroid hormone receptors (Duncan et al., 1998, 1999; Mai et al., 1998). Genes for various cytokines have also been implicated, e.g., interleukins 6 and 4 (Kajkenova et al., 1997; Murray et al., 1997; Duncan et al., 1998; Tsukamoto et al., 1999), the interleukin-1 receptor antagonist gene (Keen et al., 1998b), TGF receptor and TGF-β1 gene (Langdahl et al., 1997; Keen et al., 1998a), as well as the insulin-like growth factor I gene (Kurland et al., 1997; Rosen et al., 1997, 1998a,b; Rosen and Donahue, 1998) and the apolipoprotein E gene (Shiraki et al., 1997; Kiel et al., 1998), and even an HLA type (Tsui et al., 1998). In each of these cases initial positive (association) results have been counter-balanced by some if not several negative (no association) studies. This wide variation across studies and populations suggests ethnic (presumably genetic) and/or environmental determinants of the expression of these gene effects. As mentioned above these may also relate in part to statistical power issues (Nguyen et al., 1994, 1996).

Less severe mutations in structural genes, e.g., collagen 1 α1 gene, identified in these severe diseases have already been related to bone density differences in familial forms of osteoporosis (Spotila et al., 1991, 1994, 1996a; Shapiro et al., 1992; Paterson and Mole, 1994). Moreover, a polymorphism affecting an Sp1 binding site in the first intron of the collagen 1 α1 gene has now been associated with differences in bone density and fracture risk in a large number of osteoporosis studies (Houston et al., 1996; Grant et al., 1996; Garnero et al., 1998; Langdahl et al., 1998; Liden et al., 1998; Uitterlinden et al., 1998). As for other studies of particular genes or genetic loci, this effect has varied in size in different studies (Willing et al., 1997).

The genome screening approach has recently defined a chromosomal locus (11q12–13) associated with very high bone density (Johnson et al., 1997, 1998). The original affected family members had bone densities 4 to 5 standard deviations away from their age-corrected means and some suggestion of abnormal long bone marrow space development. Other family studies have also identified a chromosome 11q locus as well as other loci at 1p36, 2p23–24, 4qter, and chromosome 13 (Spotila et al., 1996a; Devoto et al., 1998; Niu et al., 1998). The genome-screening approach, applied in mouse and baboon studies, is helping to identify other loci (Beamer et al., 1996; Jilka et al., 1996; Kajkenova et al., 1997; Beamer et al., 1998; Orwell et al., 1998; Young et al., 1998; Benes et al., 1998; Zmuda et al., 1999), which should lead to human loci from synteny with the human genome.

The concept of inheritance and disease could lead to the misunderstanding that the disease process could not be changed except perhaps via gene therapy. While this may be true for structural gene mutations, it is less relevant to changes in genes that may result in differences in the normal physiological regulatory systems. Indeed these sorts of changes are likely to interact with physiological stresses being expressed as the phenotypic outcome. This concept underlies the idea of normal genetic variability and the new field of pharmacogenetics. In relation to osteoporosis, it is important to remember the bone turnover itself is related to both environmental and genetic factors. Genetic variation could be expected to explain some of the widely observed differences in bone responses to various hormonal and environmental insults (Kelly et al., 1991, 1995; Slemenda et al., 1991a; Harris et al., 1998; Nguyen et al., 1998).

**Dietary Calcium Intake and Vitamin D Receptor Gene Alleles**

Dietary calcium intake is an obvious factor that may affect responsiveness of vitamin D receptor genes. This commonly ranges from less than 400 to over 1000 mg/day in different population groups. In some studies, genotype related differences in calcium handling have been observed. In one study the “BB” subjects did not increase their gut calcium absorption on lower dietary calcium intakes, as did the “bb” subjects and, in another, urinary calcium excretion was higher in the “bb” subjects (Dawson-Hughes et al., 1995; Ongphiphadhanakul et al., 1997). Another recent study found a 42% difference in gut calcium absorption between alternate homozygotes for the vitamin D receptor start codon polymorphism (Ames et al., 1999). As with other studies of bone and genetics, a number of studies have found positive relationships between the vitamin D receptor gene alleles and calcium homeostasis (Howard et al., 1995, 1998a,b; Dawson-Hughes et al., 1995; Kiel et al., 1997; Wishart et al., 1997; Ames et al., 1999), while other studies have been negative (Francis et al., 1997; Gunnnes et al., 1997; Kinyamu et al., 1997a; Rauch et al., 1997).

Similarly longitudinal studies have shown differences of the bone density response to calcium intake according to vitamin D receptor genotype. In one study, the vitamin D receptor heterozygotes responded to calcium intake while the alternate homozygotes either gained or lost bone irrespective of calcium intake (Ferrari et al., 1995). By contrast in a second study the “BB” homozygotes gained some bone when supplemented from a very low basic calcium intake (Krall et al., 1995).

Interestingly, despite apparent differences in gut calcium absorption, several studies have not found any difference in intestinal vitamin D receptor level (Barger-Lux et al., 1995; Kinyamu et al., 1997a,b). By contrast, differences in parathyroid gland regulation have been related to vitamin D receptor polymorphisms (Carling et al., 1995, 1997a,c; Yokoyama et al., 1998).

Another potential gene-environment interaction for the vitamin D receptor gene would be in relation to simple vitamin D itself or the active hormonal forms of vitamin D. Differences in response of bone density to the vitamin D metabolites and analogs have been reported according to the vitamin D receptor genotypes, particularly in Japanese studies (Tokita et al., 1994; Yamagata et al., 1994; Shiraki et al., 1997). The more common “bb” genotype in Japanese cohorts (about 75% of the subjects) was more responsive compared with the heterozygotes, who did not respond well or actually worsened. Given that the heterozygote is the most common genotype in most Caucasian groups, these differences have an intriguing parallel to the differences that have been observed in response to the active vitamin D compounds in clinical studies of osteoporosis between Japanese and Caucasian groups. In another study, the response to simple vitamin D varied according to vitamin D receptor genotype (Graafmans et al., 1997).

Relationships between vitamin D receptor and bone mass may also not be simple. In fact, there is now evidence that the vitamin D receptor gene alleles are associated with body weight (Barger-Lux et al., 1995; Suarez et al., 1997), which is one of the strongest predictors of bone density in all studies. There is considerable debate whether
this strong effect is via differences in lean mass or fat mass (Reid et al., 1992a,b, 1994; Young et al., 1995). In this regard several studies suggest a relationship between components of body size and vitamin D receptor genotypes. In some of these, body weight in infancy or early childhood was greater in “BB” subjects (Keen et al., 1997a; Suarez et al., 1997). In another study in older men, differences in forearm density were apparently due to differences in bone size, with greater bone size in the “BB” homozygotes (Need et al., 1996). In two other studies, a vitamin D receptor effect was not apparent in obese individuals (Vandevyver et al., 1997) and was associated with muscle strength (Geuens et al., 1997). It is possible that some of these relationships between vitamin D receptor genotypes, bone size, and bone mass may be mediated through insulin or insulin-like growth factor pathways (McDermott et al., 1997; Hitman et al., 1998), which have also been linked to bone density (Reid et al., 1993).

The mechanism by which any changes in the vitamin D receptor alleles may account for changes in calcium and bone homeostasis is not clear. At a simple level it is possible that subtle differences may exist in the regulation of the gene or in the stability of the mRNA product. Some initial in vitro studies suggested that change in stability of mRNA product (Morrison et al., 1994; Ariai et al., 1997); however, other studies did not confirm this effect (Mocharya et al., 1997; Gross et al.; Durrin et al., 1998). Another mechanism may relate to changes in alternative transcripts from the recently reported multiple promoters of single human vitamin D receptor gene (Crofts et al., 1998).

The differences in the vitamin D allelic effects may relate to genetic backgrounds and/or environmental factors such as calcium and vitamin D intakes. Genetic backgrounds may relate to other allelic gene effects, e.g., the oestrogen receptor genotype (Willing et al., 1998; Gennari et al., 1998). By this mechanism, allelic effects could differ between environments. Some effects could be quite unexpected; for example, the apparent protection against some chronic infections reported in a recent African study (Bellamy et al., 1999) and relationship to risk of osteoarthritis of spine and hip (Keen et al., 1997b; Uitterlinden et al., 1997; Jones et al., 1998).

Despite some continuing controversy over the strength of the vitamin D receptor gene associations, these initial findings stimulated the field of the genetics of osteoporosis toward targeted genetic studies and now genome scan approaches. The various examples of possible gene-environment interaction for the vitamin D receptor gene alleles include dietary calcium intake, simple vitamin D intake, and responses to therapy with active vitamin D compounds as well as more complex interactions with body growth patterns. The allelic variation in the vitamin D receptor is one of what seem likely to be a wide range of genes and chromosomal loci to be implicated in the genetic determination of response to therapy. This is perhaps most important in situations where long periods of time, often years, must elapse before the response to a therapy can be adequately assessed. In the sense that this information could guide selection of therapy in individuals, this could be seen as an example of pharmacogenetics, and similar information could soon be applicable for many different genes and therapies. Variability of genetic findings, which seems to be the rule rather than the exception, across studies, may reflect false positives. However, others appear to relate to interaction of loci with specific environmental and hormonal factors and other genetic loci, which includes genetic heterogeneity and differing ethnicity. Understanding how these different genetic (and ethnic) backgrounds combine with different environments to explain responses to therapy could underpin targeted selection of optimal therapy on an individual basis.
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