REVIEW

Osteoporosis, genetics and hormones

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INTRODUCTION

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue. At a given age, bone mass results from the amount of bone acquired during growth, i.e. the peak bone mass (Bonjour et al. 1991, Theintz et al. 1992) minus the age-related bone loss which particularly accelerates after menopause. The rate and magnitude of bone mass gain during the pubertal years and of bone loss in later life may markedly differ from one skeletal site to another, as well as from one individual to another. Bone mass gain is mainly related to increases in bone size, that is in bone external dimensions, with minimal changes in bone microarchitecture. In contrast, postmenopausal and age-related decreases in bone mass result from thinning of both cortices and trabeculae, from perforation and eventually disappearance of the latter, leading to significant alterations of the bone microarchitecture (Fig. 1).

Peak bone mass acquisition

Before puberty, there is no consistent gender-related difference in bone mass at any skeletal site (Glastre et al. 1990, Bonjour et al. 1991, Geusens et al. 1991). Indeed, there is no evidence for a gender-related difference in bone mineral density (BMD; g/cm²) at birth (Trotter & Dixon 1974), and this similarity in bone mass between males and females is maintained until the onset of pubertal maturation (Gilsanz et al. 1988, 1991, 1998). During puberty, bone mineral mass of skeletal sites such as the lumbar spine more than doubles (Bonjour et al. 1991, Theintz et al. 1992). This increase occurs approximately 2 years earlier in females than in males (Fig. 2). Meanwhile, a gender-related difference in peak bone mass becomes detectable. This difference appears to result essentially from a longer period of bone mass gain in males than in females, resulting in a larger increase in bone size and cortical thickness in the former (Seeman 1997). Thus, the peak bone mineral content (BMC; g) at the lumbar spine and the proximal femur is higher in males than in females, whereas volumetric bone density (g/cm³) does not differ between genders at the end of pubertal maturation (Gilsanz et al. 1988, 1991, 1997, 1998). On the other hand, black people have greater volumetric bone density than white individuals (Gilsanz 1998, Gilsanz et al. 1998); trabecular number is similar, but the trabeculae appear to be thicker in black people (Han et al. 1996). Moreover, the cross-sectional area of the mid-femoral shaft is greater in black than in white individuals for an identical cortical thickness (Gilsanz et al. 1998).

There is an asynchrony between the gain in statural height and the gain in bone mass (Bonjour et al. 1991, Theintz et al. 1992, Fournier et al. 1997). The peak of statural growth velocity precedes the peak of maximal bone mass gain. In males, the greatest difference occurs in the 13- to 14-year age group and is more pronounced for the lumbar spine and femoral neck than for the mid-femoral shaft, whereas in females it occurs in the 11- to 12-year age group, corresponding in both genders to pubertal stages P2–P3 (Theintz et al. 1992). In healthy Caucasian females with apparently adequate intakes of energy, protein and calcium, bone mass accumulation can virtually be completed before the end of the second decade at both the lumbar spine and the femoral neck (Bonjour et al. 1991, Katzman
et al. 1991, Theintz et al. 1992, Matkovic et al. 1994). In males, cortical bone mass may still increase by a few percent beyond the age of epiphyseal plate closure (Parsons et al. 1996).

Bone loss

Some bone loss seems to occur at distinct skeletal sites, such as the proximal femur, well before menopause (Riggs et al. 1986, Mazess et al. 1987, Rodin et al. 1990, Luckey et al. 1996, Slemenda et al. 1996a). After peak bone mass is achieved, bone size varies little throughout life; a slight expansion of adult bone cortices can be found mainly in men (Garn et al. 1967, 1968, Mosekilde & Mosekilde 1990, Seeman 1997). However, this periosteal expansion is less than the increase in bone marrow space resulting from the endosteal resorption which increases with age in both genders. Under these conditions, bone cortex becomes

**FIGURE 1.** Schematic representation of cortical and cancellous bone changes throughout life. Stippling represents cortical porosity and hatching represents cancellous bone network.

**FIGURE 2.** Mean ± s.e.m. bone mass gain of the lumbar spine during adolescence (A: lumbar vertebrae L2–L4 BMD; B: lumbar vertebrae L2–L4 BMC). Reproduced with permission from Theintz et al. (1992).
Upon binding of 1,25(OH)2 D3, VDR forms a peripheral skeletal sites (Ensrud clearly established that bone loss does not attenuate account for the age-dependent bone loss. It is now trabeculae, through thinning and perforation, account for the age-dependent bone loss. It is now thinner (Fig. 1). This phenomenon, together with an increment in cortical porosity (Laval-Jeantet et al. 1983, Han et al. 1996) and a destruction of trabeculae, through thinning and perforation, account for the age-dependent bone loss. It is now clearly established that bone loss does not attenuate with age, but continues throughout life, at least in peripheral skeletal sites (Ensrud et al. 1995).

ENDOCRINE REGULATION OF BONE MASS

Many factors, more or less dependent on each other, are known to influence bone mass accumulation during growth. These determinants classically include genetic factors, which quantitatively appear the most prominent factors (Ferrari et al. 1998a, 1999), race, gender, nutrients (calcium, protein, phosphate), endocrine factors (sex steroids, calcitriol, insulin-like growth factor-I (IGF-I)), mechanical forces (physical activity, body weight), and exposure to risk factors (Bonjour & Rizzoli 1996, Gilsanz 1998). Most of these factors are also involved in the maintenance of bone mass during adulthood as well as in bone loss later in life, although in variable proportions compared with their role in peak bone mass acquisition.

The vitamin D system

Vitamin D3 is for the most part synthesized from its 7-dehydrocholesterol precursor in the dermis under ultra violet B radiations. It is sequentially hydroxylated by liver and kidneys in its hormonal metabolite, calcitriol, i.e. 1,25-dihydroxyvitamin D (1,25(OH)2 D3). The effects of 1,25(OH)2 D3 are mediated by its nuclear vitamin D receptor (VDR). Upon binding of 1,25(OH)2 D3, VDR forms a heterodimeric complex with the retinoic acid receptor and additional transcription factors, and ultimately regulates the expression of a number of genes bearing vitamin D responsive elements in their promoter region (for review see Christakos et al. 1996, Haussler et al. 1997). The role of vitamin D metabolites is primarily to maintain serum calcium and phosphate levels by directly promoting intestinal absorption of these ions as well as by activating bone resorption (for review see Reichel et al. 1989).

Failure of the vitamin D endocrine system during growth causes rickets, which is a prominent bone-deforming and sometimes life-threatening disorder. Vitamin D is also important in the maintenance of skeleton integrity in adults. Elderly people tend to have poor dietary calcium and vitamin D intakes, decreased sunlight exposure and dermal production of vitamin D, and diminished production of 1,25(OH)2 D3 with secondary hyperparathyroidism. In turn, vitamin D and calcium supplementation has been demonstrated to significantly increase BMD and decrease the incidence of osteoporotic fractures in the elderly (Chapuy et al. 1992, Chevalley et al. 1994, Dawson-Hughes et al. 1997).

Estrogens

Female sex hormones appear to be mandatory, not only for the acquisition of peak bone mass in both females and males (Smith et al. 1994, Carani et al. 1997, Vanderschueren et al. 1997), but also for the maintenance of bone mass in adults. They control bone remodeling during reproductive life in females (Rizzoli & Bonjour 1997, Riggs et al. 1998) and later on in aging men (Slemenda et al. 1996b, 1997, Greendale et al. 1997). Pathologic conditions associated with premature estrogen deficiency, such as anorexia nervosa, secondary amenorrhea due to strenuous exercise, or the use of inhibitors of gonadotropin secretion, further support the concept of a causal link between estrogen deficiency and accelerated bone loss (Drinkwater et al. 1984, Seeman et al. 1992). By indirectly accelerating bone turnover and by uncoupling bone formation from resorption (Manolagas 1998), estrogen deficiency is the main cause of postmenopausal osteoporosis, and possibly plays an important role in male osteoporosis as well (Bilezikian et al. 1999). Thus, estrogen deficiency is directly implicated in the age-related increase in the incidence of fragility fractures (Riggs et al. 1998). In addition, estrogen deficiency also seems to be correlated with the progressive increase in serum parathyroid hormone (PTH) levels observed in aging individuals, which by itself contributes to accelerate bone turnover (Khosla et al. 1997).

IGF-I

IGF-I is an essential factor for longitudinal bone growth (Froesch et al. 1985), as it stimulates proliferation and differentiation of chondrocytes in the epiphyseal plate (Canalis et al. 1991). IGF-I also plays a role in trabecular and cortical bone formation. This factor can stimulate both proliferation and differentiation of osteoblasts; it increases type I collagen synthesis, alkaline phosphatase activity and osteocalcin production (Schmid & Ernst 1992). Thus, IGF-I can exert anabolic effects on bone mass not only during growth, but also during adulthood (Rosen & Donahue 1995, Ammann et al. 1996, Bagi et al. 1996, 1997).

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Furthermore, by its renal action on tubular reabsorption of phosphate and on the synthesis of calcitriol, through a direct action on renal cells (Caverzasio & Bonjour 1989, Caverzasio et al. 1990), IGF-I can be considered as an important controller of the intestinal absorption and of the extracellular concentration of both calcium and phosphate, the main elements of bone mineral. On the other hand, IGF-I can selectively stimulate the transport of inorganic phosphate across the plasma membrane in some osteoblastic cell lines (Palmer et al. 1996, 1997). Osteogenic cells not only express specific IGF-I receptors, but they can also be endowed with IGF-I producing machinery (Canalis et al. 1991, Chevalley et al. 1998). Taking into account these experimental and clinical observations, IGF-I could play a prominent role in the pathophysiology of osteoporosis, of osteoporotic fracture and of its complications. In association with age, several reports have documented a decrement in IGF-I plasma levels (Hammerman 1987, Quesada et al. 1992, Goodman-Gruen & Barrett-Connor 1997, Langlois et al. 1998). Under these conditions, a restoration of this altered system in the elderly, for instance by protein replenishment (Schüler et al. 1998), is likely to favorably influence not only BMD, but also muscle mass and strength, since these two variables are important determinants of the risk of falling (Aniansson et al. 1984, Castaneda et al. 1995).

Thus, bone mass gain during childhood, bone loss after menopause and further loss in the elderly are determined by different sets of endogenous and exogenous factors (Ralston 1997, Seeman & Hopper 1997), and the relative influence of specific genes on the risk of osteoporosis may vary greatly with age. We will now examine the genetic aspects of hormones and their receptors involved in the regulation of bone accumulation and loss.

GENETIC DETERMINANTS OF OSTEOPOROSIS

Definition of the variable

Although areal BMD is a continuous variable, an operational definition of osteoporosis considers the disease as a BMD below −2.5 s.d. from the mean BMD of young adults for skeletal sites such as lumbar spine or proximal femur as evaluated by dual energy X-ray absorptiometry (DXA) (Kanis et al. 1996). This is the phenotypic trait (Rizzoli et al. 1995) that is most commonly used in studies evaluating heritability or polymorphic gene markers of osteoporosis. However, in order to understand the genetic basis for decreased bone strength, and ultimately osteoporotic fractures, one might eventually need to assess the inheritance of, and identify the specific genes associated with, a multitude of skeletal and extraskeletal traits, such as bone size, shape and microarchitecture (Turner et al. 2000), body weight (the single most influential variable correlated with BMD), muscle strength, biochemical variables of calcium and phosphate homeostasis, ovarian function, etc. Moreover, the clinical expression of osteoporosis is represented by the skeletal fracture. A fracture is a stochastic event which is determined by both bone-related factors (mass, size, architecture, microarchitecture, intrinsic properties of bone material) and bone-independent factors (falls, protecting responses, soft tissue padding, etc.) (Pinilla et al. 1996). The latter may have their own heritable and non-heritable components, which increases even further the complexity of the genetic determination of osteoporotic fractures (Kelly et al. 1990, Kannus et al. 1999, Zmuda et al. 1999).

Hereditary bone mass

It has been shown that daughters of osteoporotic women have a low BMD (Seeman et al. 1989). A study comparing peak bone mass at both the lumbar spine and femoral neck in young adult daughters from peri- and early postmenopausal women with decreased BMD and in daughters of women with normal BMD showed a decreased BMD among the former (Barthe et al. 1998). BMD is decreased among the relatives of 38 middle-aged men with severe idiopathic osteoporosis (Cohen-Solal et al. 1998). These studies confirm the importance of a familial history of osteoporosis in the screening of subjects at risk and suggest the expression of inherited determinants of the risk of osteoporosis from an early age on. A history of fracture is associated with a more than twofold increase in the risk of subsequent fracture (Klotzbuecher et al. 2000). In order to investigate the proportion of the BMD variance across the population explained by genetic factors, which is known as the heritability (Kelly et al. 1995), mainly two human models have been used. In the twin model, within-pairs correlations for BMD are compared between monozygotic (MZ) twins, who by essence share 100% of their genes, and dizygotic twins (DZ), who have 50% of their genes in common. Stronger correlation coefficients among adult MZ as compared with DZ twins are indicative of the genetic influence on peak bone mass, accounting for as much as 80% of lumbar spine and proximal femur...
BMD variance (Pocock et al. 1987). The influence of environmental and genetic factors on bone mass, but also on lean and fat mass has recently been reassessed in 102 female twin pairs (mean age ± s.d. 52.8 ± 13 years) from the Sydney Twin Study of Osteoporosis (Nguyen et al. 1998). Results indicate that 80% and 65% of variance of lean mass and fat mass, respectively, were attributable to genetic factors. However, genetic factors affecting lean and fat mass had only little influence on BMD at the lumbar spine or femoral neck. These results differ from the previous evidence of indirect genetic effects on bone mass occurring through the determination of lean body mass (Seeman et al. 1996). Hence it remains presently unclear whether the relation between lean mass and bone mass is most significantly determined by environmental or genetic influences.

Parent-offspring comparisons have also shown significant relationships for BMD, albeit heritability estimates have been somewhat lower (in the range of 60%) than in the twin model (Tylavsky et al. 1989). Actually, the magnitude of direct genetic effects on peak bone mass as evaluated in both human models may be overestimated by similarities in environmental covariates (Slemenda et al. 1991, Krall & Dawson-Hughes 1993). We investigated correlations for BMC, areal and volumetric BMD and bone area in the lumbar spine and femur (neck, trochanter and diaphysis) in 138 premenopausal women (mean age ± s.d., 40.0 ± 4.0 years) and their prepubertal daughters (8.1 ± 0.7 years) (Ferrari et al. 1998b). Regressions were adjusted for height, weight and calcium intake, to minimize the impact of indirect genetic effects as well as of dietary influences on bone mineral mass resemblance among relatives. The results indicate that despite great disparities in the maturity of the various constituents of bone mass before puberty with respect to peak adult values, heredity by maternal descent is detectable at all skeletal sites and affected virtually all bone mass constituents, including bone size and volumetric mineral density. Moreover, when the daughters’ bone values were re-evaluated 2 years later, while puberty had begun and bone mineral mass had considerably increased, measurements were highly correlated with prepubertal values (all r>0.80) and mother–daughter correlations had remained unchanged. By the age of 16–18 years in females and 18–20 years in males, bone mass gain virtually ceases, but a marked scattering of BMD values for both genders is then apparent across the population (Bonjour et al. 1991, Theintz et al. 1992). Thus, a major proportion of this variance is due to genetic factors which are already expressed before puberty with subsequent tracking of bone mass constituent through the phase of rapid pubertal growth until peak bone mass is achieved.

A twin study using quantitative ultrasound to evaluate bone properties in the phalanges and calcaneum has shown heritability values ranging from 55% to 82%, which is similar to BMD values estimated by DXA at the lumbar spine and femoral neck level (Danielson et al. 1999). However, cross-trait correlations suggested that specific genes unrelated to BMD explained at least half of the heritability of ultrasound measurements. Hence the risk for osteoporosis and osteoporotic fracture is determined by various sets of genes whose effects may not all be appreciated by one single measurement, such as BMD. Interestingly, it appears that male to male, and male to female inheritance of bone mass may differ substantially (Krall & Dawson-Hughes 1993). It might be hazardous therefore to extrapolate genetic influences on bone mineral mass as identified in women to the male population, in which this question has virtually not yet been investigated.

In contrast to the clear heritability of peak bone mass, the proportion of the variance in bone turnover as well as in postmenopausal and age-related bone loss which depends on genetic factors remains unclear. Twin studies have suggested a higher correlation for bone loss among MZ compared with DZ twins (Kelly et al. 1995), but the heritability of bone turnover, as assessed in this model by various markers of bone formation and resorption, appears to be small (Garnero et al. 1996a). Interestingly, however, the age at which cessation of the ovarian function occurs, which is a major determinant of the osteoporosis risk (see above), seems to display a strong heritability (63%) (Snieder et al. 1998).

Candidate genes versus genome screens

As pointed out before, osteoporosis is very likely to be a polygenic disease, involving a large variety of gene products implicated in both bone modeling (growth) and remodeling (loss). Genetic linkage between BMD and candidate genes has been searched in twin cohorts of unaffected individuals, in large numbers of both affected (osteoporotic) and unaffected unrelated individuals (case controls and association population studies) and, rarely, in osteoporotic probands and relatives. The latter case is best illustrated by the study of Duncan et al. (1999) who used a linkage approach to test a number of candidate genes known to be implicated in the control of BMD and/or bone metabolism in 115 probands with osteoporosis and 499 relatives. The candidate genes studied coded for structural
components, such as type I collagen A1 and A2, type II collagen A1, fibrillin type 1, or osteopontin, for growth factors or cytokines, such as colony-stimulating factor 1, epidermal growth factor, interleukin (IL)-1α, IL-4, IL-6, IL-11, transforming growth factor-β1 (TGF-β1), tumor necrosis factor-α and -β, or components of endocrine systems, such as androgen receptor, VDR, calcium-sensing receptor, estrogen receptor (ER)-1, IGF-I, PTH, PTH-related protein (PTHrP), and PTH receptor type 1. The strongest linkage with BMD was detected with the PTH receptor type 1 gene and the IL-6 gene were among the few c genes involved.

Polymorphic markers are designed as endonuclease restriction sites, variable number tandem repeats (VNTR, AT rich), dinucleotide repeats (CA) or sequence variations (*) when primarily identified as single-stranded conformation polymorphisms. Due to the elevated number of genotypes resulting from VNTR and CA repeats, frequencies cannot be shown but the number of most common genotypes is indicated in parentheses. Only published allelic polymorphisms associated with BMD (and/or osteoporosis) are listed (see text for references). Capital letter, absence of restriction site; small letter, presence of restriction site; ND, not determined.

**TABLE 1. Frequency of common allelic polymorphisms in candidate hormone or homone receptor gene loci associated with bone mass**

<table>
<thead>
<tr>
<th>Gene loci</th>
<th>Markers</th>
<th>Caucasian</th>
<th>Asian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D receptor 3’ end</td>
<td>BsmI</td>
<td>BB 18%, Bb 46%, bb 36%</td>
<td>BB 1–2%, Bb 21%, bb 77%</td>
</tr>
<tr>
<td></td>
<td>TaqI</td>
<td>TT 36%, Tt 48%, tt 16%</td>
<td>TT 78%, Tt 21%, tt 1%</td>
</tr>
<tr>
<td></td>
<td>ApaI</td>
<td>AA 24%, Aa 46%, aa 28%</td>
<td>AA 9%, Aa 48%, aa 43%</td>
</tr>
<tr>
<td>Vitamin D receptor start codon</td>
<td>PstI</td>
<td>FF 36%, Ff 49%, ff 15%</td>
<td>FF 33%, Ff 53%, ff 14%</td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td>XbaI</td>
<td>PP 18%, Pp 53%, pp 29%</td>
<td>PP 18%, Pp 52%, pp 30%</td>
</tr>
<tr>
<td>Parathyroid hormone</td>
<td>BstBI</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Glucocorticoid receptor exon 2</td>
<td>A1220,G*</td>
<td>Heterozygous 6%</td>
<td>ND</td>
</tr>
<tr>
<td>Calcitonin receptor</td>
<td>C447,T*</td>
<td>RR 49%, Rr 44%, rr 7%</td>
<td>ND</td>
</tr>
<tr>
<td>Insulin-like growth factor-I</td>
<td>TaqI</td>
<td>TT 11%, Tt 50%, tt 39%</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>CA repeat</td>
<td>ND</td>
<td>(6 genotypes)</td>
</tr>
</tbody>
</table>

Polymorphic markers are designed as endonuclease restriction sites, variable number tandem repeats (VNTR, AT rich), dinucleotide repeats (CA) or sequence variations (*) when primarily identified as single-stranded conformation polymorphisms. Due to the elevated number of genotypes resulting from VNTR and CA repeats, frequencies cannot be shown but the number of most common genotypes is indicated in parentheses. Only published allelic polymorphisms associated with BMD (and/or osteoporosis) are listed (see text for references). Capital letter, absence of restriction site; small letter, presence of restriction site; ND, not determined.

Among the multiple candidate genes harboring polymorphic loci so far investigated in relation to BMD and/or BMD changes (Table 1), the VDR-3’ end alleles (BsmI, ApaI and TaqI) were the first described (Morrison et al. 1994) and the most controversial (Hustmyer et al. 1994, Garnero et al. 1995, 1996b, Cooper & Umbach 1996, Jorgensen et al. 1996, Matsuyama et al. 1995, Tokita et al. 1996, Uitterlinden et al. 1996, Zmuda et al. 1997, Kikuchi et al. 1999). A meta-analysis combining 16 separate studies examined the relationship between VDR genotypes and BMD (Cooper & Umbach 1996). Subjects with the BB genotype had a 2·4%, 2·5% and 1·7% non-significantly lower BMD as compared with bb at the level of the femoral neck, lumbar spine, and distal radius respectively. Regarding vertebral fractures (368 cases and 1548 controls, collected in five studies), the relative risk in BB versus bb subjects varied between 0·7 and 2·5 among studies. All confidence intervals included 1 and none reached a level of statistical significance. For hip fracture, there were 235 cases and 800 controls in two cohorts. Although the relative risk of femoral neck fracture among BB in the Nurses’ Health Study was 2·4, reaching statistical significance when older age and lower calcium intake were taken into consideration (Feskanich et al. 1998), these results were not confirmed in the Study of
Osteoporotic Fractures, which found a relative risk of 0.8 for BB (Ensrud et al. 1999).

Based on the assumption that quantitative ultrasound measurements (QUS) of the calcaneum (broadband ultrasound measurement and speed of sound) might evaluate some properties of bone which differ from BMD at other sites as assessed by DXA, two studies have recently examined the relationship between VDR alleles and QUS in, respectively, 393 women aged 45–53 years (Gregg et al. 1999) and 425 postmenopausal women (Giguère et al. 2000). No significant association was found between VDR-3′ polymorphisms and QUS measurements in either study.

The more recently described association between VDR-5′ start codon polymorphism (FokI) and BMD, at first observed in small cohorts of postmenopausal Mexican–American women (Gross et al. 1996), white premenopausal American women (Harris et al. 1997) as well as Japanese women (Arai et al. 1997), has not been confirmed in two larger European studies in healthy premenopausal women or prepubertal girls (Eccleshall et al. 1998, Ferrari et al. 1998d).

Several independent investigators have shown the importance of age, gene–environment interactions and gene–gene interactions to explain the inconsistent relation between bone mineral mass and VDR-3′ and -5′ genotypes. Thus, significant BMD differences between VDR-3′ BsmI genotypes were detected in children (Sainz et al. 1997, Ferrari et al. 1998c), but were absent in premenopausal women from the same genetic background (Ferrari et al. 1998c). Moreover, the latter study found that BMD gain in prepubertal girls was increased at several skeletal sites in Bb and BB subjects in response to calcium supplements (800 mg/day), whereas it remained apparently unaffected in bb girls, who had a trend for spontaneously higher BMD accumulation on their usual calcium diet (Ferrari et al. 1998c) (Fig. 3). Accordingly, a model taking into account the early influence of VDR-3′ polymorphisms, calcium intake and puberty on BMD gain has been proposed to explain the relation between these genotypes and peak bone mass (Ferrari et al. 1998a). Interestingly also, several investigators have noted a significantly lower height among women and men with the VDR-3′ BB compared with Bb or bb genotypes (Barger-Lux et al. 1995, Ferrari et al. 1998c, Tao et al. 1998, Lorentzon et al. 2000). Considering the relationship between body size and bone size, as well as the influence of calcium intake on both body height and bone area during growth (Bonjour et al. 1997), it is tempting to speculate that VDR-3′ alleles together with environmental calcium might have an indirect and complex influence on peak bone mass through the regulation of skeletal growth. A number of investigators have shown a significant interaction between VDR-3′ genotypes and calcium intake on BMD in young adult and postmenopausal women (Salamone et al. 1996, Kiel et al. 1997, Rubin et al. 1999) as well as on BMD changes and/or intestinal calcium absorption in late postmenopausal women (Dawson-Hughes et al. 1995, Ferrari et al. 1995, Krall et al. 1995). The latter observation is particularly important, considering that postmenopausal women with low fractional calcium absorption and low calcium intake are at a significantly increased risk of hip fracture (Ensrud et al. 2000). Moreover, VDR-3′ alleles have been associated with BMD changes in response to vitamin D supplementation in a small group of elderly women (Graafmans et al. 1997).

A short-term dietary intervention trial in young healthy males segregated as alternate homozygous BB and bb according to VDR-3′ BsmI alleles further suggests that BB subjects may have a subtle resistance to calcitriol leading to significantly higher levels of circulating PTH, decreased tubular reabsorption of inorganic phosphate (Pi) and lower serum Pi levels when their calcium and phosphorus intakes are maintained at the lowest levels for several days (Ferrari et al. 1999). In addition, age and dietary calcium intake might also influence the association between VDR-5′ start codon polymorphisms and peak bone mass (Ferrari et al. 1998d). Moreover, similarly to VDR-3′ alleles (see above),
VDR-5' alleles have also been associated with intestinal calcium absorption in children, as evaluated by a radioisotopic method (Ames et al. 1999). Altogether, these observations provide a possible physiological mechanism to the relation between VDR gene polymorphisms and bone mass and emphasize the methodological limitations of early studies focusing on the association between VDR genotypes and BMD regardless of the influence of age and environmental factors. Moreover, other potential gene-environmental interactions, such as those involving physical exercise (Gregg et al. 1996) as well as gene-gene interactions might further modulate the relationship between VDR gene polymorphisms and bone mass.

Thus, BMD differences (equivalent to 1 s.d.) have been found in prepubertal girls, premenopausal women and young adult males segregated according to both VDR-3' BsmI and VDR-5' FokI genotypes whereas, taken independently, these polymorphisms were either not associated or only weakly associated with BMD (Ferrari et al. 1999). Gene-by-gene interactions have also been found between VDR and ER gene polymorphisms and are examined below.

In summary, VDR-3' and -5' alleles are possibly weak determinants of BMD, their effects being easily confounded by the influence of many other genes and environmental factors. Hence, VDR gene polymorphisms alone are not clinically useful genetic markers of the osteoporosis risk in the elderly. Nevertheless, when examining the effects of calcium on peak bone mass (and particularly bone size) acquisition (bone modeling), or on bone loss (bone remodeling), VDR alleles could be one significant factor to explain some of the variability observed in the population.

ER gene polymorphisms

Genotypes identified by PvuII and XbaI restriction fragment length polymorphisms in the first intron of the ERα gene (Table 1) were originally found to be significantly associated with BMD in postmenopausal Japanese women, but not with markers of bone turnover (Kobayashi et al. 1996). In contrast, a similar study from Korea reported no significant BMD differences among ER genotypes in postmenopausal women receiving hormone replacement therapy (HRT) (Han et al. 1997). Whereas the former study suggested that ER gene polymorphisms could be related to the acquisition of peak bone mass, the latter rather suggested an influence, if any, on the rate of bone loss. More recently, another study from Japan including 173 premenopausal to late postmenopausal women indicated a predominant association between ER genotypes and adult bone mass, which disappeared with advancing age (Mizunuma et al. 1997). Several investigators have examined ER gene polymorphisms and bone mass in Caucasian populations as well. In one study, a significant association was found between either the PvuII or the XbaI genotypes and lumbar spine BMD in 253 pre- and perimenopausal women, those with the PvuII pp genotype having a 6-4% lower BMD at this site compared with PP (Willing et al. 1998). However, there were no differences in BMD changes, nor in several biochemical markers of calcium and bone metabolism, including PTH and osteocalcin, over a 3-year period in this cohort.

One limitation of this study concerning the potential association of ER polymorphisms with postmenopausal bone loss was the very low overall bone loss in this cohort over 3 years (≤1%). In contrast, a very recent study which prospectively investigated the 5-year bone loss in early postmenopausal women receiving either HRT or placebo in addition to calcium and vitamin D found no significant differences in BMD among ER polymorphisms at baseline, but significant differences in lumbar spine BMD changes between ER genotypes PP (−6.4%) and pp (−2.9%) in the absence of HRT (Salmen et al. 2000). In women receiving HRT, these differences were no more apparent.

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Altogether, these data suggest that ERα gene alleles PvulI (alternatively XbaI) may be another possible determinant of bone mass, particularly through their interaction with VDR-3’ alleles and estrogen replacement therapy in postmenopausal women. The molecular mechanisms of these potential interactions as well as their value to identify responders versus non-responders to HRT remain completely unknown.

IGF-I gene polymorphisms

The IGF-I gene is a recent new candidate in relation to bone mass (Miyao et al. 1998, Rosen et al. 1998). Numerous alleles are identified by the number of CA repeats in the vicinity of promoting regions in the IGF-I gene, 1 kb upstream from the transcription start (Weber & May 1989). Men with idiopathic osteoporosis had low serum IGF-I, and this was associated with homozygosity for a specific allele of the IGF-I microsatellite characterized by 192 CA repeats (Rosen et al. 1998). This genotype has then been shown to be associated with lower peak serum IGF-I levels and lower femoral cross-sectional area in 85 pubertal boys and girls (Gilsanz et al. 1999). However, these findings were not confirmed in healthy postmenopausal Japanese women (Miyao et al. 1998), in 363 premenopausal women (Takacs et al. 1999), in 256 healthy Caucasian adolescents of both genders below the age of 20 (Berg et al. 2000), nor in 100 adult young males at the peak bone mass (Rizzoli et al. 2000). Hence, despite the clear role of the growth hormone–IGF-I axis on the development and maintenance of peak bone mass, the adequacy of IGF-I CA repeat polymorphisms as susceptibility markers for the risk of low bone mass/osteoporosis requires further evidence.

Other hormones/receptor gene polymorphisms

Several other polymorphic genes coding for hormones and/or the receptor have recently been investigated in relation to bone mass (Table 1) (Huizenga et al. 1998, Masi et al. 1998, Miyao et al. 1998, Rosen et al. 1998, Taboulet et al. 1998, Willing et al. 1998, Hosoi et al. 1999, Kanzawa et al. 1999, Sowers et al. 1999, Heishi et al. 2000) and some clearly deserve further attention. An allelic variant causing an amino acid change in the C-terminal domain of the calcitonin receptor gene has been identified (Masi et al. 1998, Taboulet et al. 1998, Tsukamoto & Emi 1998). In two of these studies, heterozygous Rr postmenopausal women had an apparently higher BMD as compared with either homozygote RR or rr. The lack of a clear allele dosage effect casts some doubts on the pertinence of these observations. Polymorphisms in the glucocorticoid receptor gene resulting in an amino acid change have been identified in 6% of a Dutch population and associated with an increased sensitivity to glucocorticoids resulting in a trend towards decreased BMD (Huizenga et al. 1998). This polymorphic locus carries a great potential to explain major interindividual variations in the rate of bone loss in glucocorticoid-treated patients.

The PTH gene has been shown to display polymorphisms (Muldersman et al. 1992, Hosoi et al. 1999, Kanzawa et al. 1999). In the study by Hosoi et al. (1999), heterozygosity in an intronic polymorphism in the PTH gene was found to be associated with higher lumbar spine BMD in 383 healthy postmenopausal Japanese women. However, serum PTH levels were absolutely identical among homozygotes and heterozygotes. Moreover, a small study in 91 healthy Caucasian women who were premenopausal at entry and were prospectively investigated for a median period of 20 years with repeated bone radiographs has recently suggested that PTH gene polymorphisms could explain 7–9% of the variance in bone dimensions and their changes with age, such as the cross-sectional cortical area of long bones (Gong et al. 1999). Regarding PTH/PTHrP receptor, although microsatellite markers in the vicinity of the PTH receptor 1 gene have clearly identified this locus as an important potential determinant of bone mass (Duncan et al. 1999), to date no polymorphisms have directly been identified in this gene sequence in relation to BMD.

Eventually, variable number tandem repeats (AGC)n in the androgen receptor gene have recently been shown to be associated with BMD at the femoral neck and lumbar spine as well as with femoral neck BMD changes over 3 years in 261 pre- and perimenopausal women (Sowers et al. 1999). Due to the increasing evidence of a contribution of androgens in bone mass determination in women as much as in men, these data are of great interest and clearly deserve confirmation in other cohorts.

Beyond hormones

A number of polymorphic genes coding for proteins acting beyond the hormone-receptor level seem to offer great promise in explaining some of the variance in the susceptibility to osteoporosis. Among them, functional allelic variants in the genes coding for the TGF-β1 and IL-6, two cytokines acting downstream of the ER to directly regulate bone resorption and/or formation (Manolagas
have recently been associated with osteoporosis and/or the rate of bone turnover in postmenopausal women. Thus, a common T>C transition polymorphism in the TGF-β1 gene exon 1 is significantly associated with serum TGF-β1 concentrations as well as the prevalence of osteoporosis in 239 unrelated postmenopausal Japanese women and susceptibility to fracture (Yamada et al. 1998, 2000). Moreover, in the same cohort, this polymorphism has been associated with the rate of bone loss as well as the response to vitamin D supplements (Yamada et al. 2000). A higher BMD was also observed in adolescents with the CC genotype, suggesting that this polymorphism could influence peak bone mass as well (Yamada et al. 1999). However, these findings require confirmation in populations of other ethnic origin. Another important, although rare, polymorphism in the TGF-β1 gene occurring as a one-base (C) deletion in intron 4 has also been associated with severe osteoporosis and increased bone turnover in Caucasians (Langdahl et al. 1997, Bertoldo et al. 2000).

A common G>C polymorphism at position −174 in the IL-6 gene promoter has recently been shown to affect the gene transcriptional activity (Fishman et al. 1998). These allelic variants influence the level of bone resorption, as evaluated by a marker of collagen I degradation – the serum C-telopeptide of type I collagen – in postmenopausal women (Ferrari et al. 2000).

**CONCLUSIONS**

Bone mass gain during growth as well as postmenopausal and age-related bone loss display skeletal-site specificity in terms of magnitude and kinetics. Bone strength, and thereby fracture risk, depends on bone size, volumetric bone density, microarchitecture, and intrinsic bony tissue properties. The risk of osteoporosis depends on the achievement of peak bone mass, which is mostly determined by changes in bone size with minimal modifications in volumetric density, and in the amount of bone loss, which is mainly the consequence of alteration in volumetric bone density and in microarchitecture. All these processes are controlled by complex and selective genetic, hormonal, nutritional and other environmental factors, which tightly interact (Fig. 4). Because of the multiple influences on bone homeostasis and the heterogeneity of the mechanisms governing skeletal growth and bone loss, the genetic control of bone mass implicates numerous genes of variable importance during an individual’s lifespan. The recent emergence of candidate genes, particularly controlling hormone levels and their receptors, associated with BMD and/or bone remodeling, has opened new concepts in the comprehension of the pathophysiology of osteoporosis. Among these genes, allelic polymorphisms for the receptors recognizing the vitamin D active metabolite (calcitriol), estradiol, and PTH could be associated with bone mass. Most importantly, these
polymorphic genes, as well as those coding for TGF-β and IL-6, have a potential interest to understand, and eventually predict, the individual response to anti-osteoporotic therapies. Thus, the possible implication of the PTH receptor 1 gene in the response to PTH raises great interest with respect to the recently reported effects of PTH in the prevention of fracture risk (Neer et al. 2000). However, any of these polymorphic loci is very unlikely to represent per se a definite marker of osteoporosis, particularly since their influence cannot be dissociated from environmental factors (Ferrari et al. 1998e). Therefore, to reliably appraise the role of genetics in osteoporosis as a highly complex model, one has to gather information from different sources. These include association studies in well-defined demographically and homogeneous populations, and randomized controlled trials looking at the bone response to hormonal and/or environmental changes, combined with experimental investigations assessing the molecular mechanisms implicated in the interaction between genotype and phenotype.

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