# 2 Genetics, Nutrition, and Bone Health

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*Positive health requires a knowledge of man's primary constitution and of the powers of various foods, both those natural to them and those resulting from human skill. But eating alone is not enough for health. There must also be exercise, of which the effects must likewise be known.*

*— Hippocrates, 480 BC*

## 1. INTRODUCTION

Nutritional, lifestyle, and genetic factors all influence bone mass development during growth *(1)*. Heritability explains up to 80% of the population variance for peak bone mass, and the influence of these genetic factors is expressed well before puberty *(2)*. Genome-wide linkage studies in humans and mice have started to reveal the multitude of quantitative trait loci (QTLs) potentially contributing to bone mass, although most of the specific gene variants that influence discrete traits for bone strength, such as bone size, cortical thickness, and trabecular architecture, remain to be identified *(3)*. Despite this strong genetic determination, genotypes associated with bone mineral density (BMD) in a given cohort have not necessarily been found to be associated with BMD in other cohorts with a similar genetic background but with different nutrient intakes and/or lifestyle factors. Moreover, numerous intervention trials using calcium supplements and/or dairy food products have proven beneficial to improve bone mass gain in children, particularly in those with a spontaneously inadequate calcium intake *(4)*. Thus, the major genetic determination of peak bone mass does not preclude nutrients to modify the "tracking" of bone mass during growth. In fact, there are clear suggestions that nutritional and genetic factors may interact to influence bone modeling, that is, changes in BMD, bone size, and architecture, and mineral homeostasis during the years of peak bone mass acquisition *(5)*. Likewise, gene–environment interactions have been found to influence bone remodeling and the maintenance of bone mass in postmenopausal women *(6)*. These observations open the way for a novel approach in osteoporosis prevention, based on

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genetic profiling associated with the response to nutritional factors and drugs (i.e., nutrigenetics and pharmacogenetics).

Over the past few years, hundreds of studies have been published in the field of osteoporosis genetics. Many of these studies have led to inconsistent results, in part because areal bone mineral density (aBMD), the most commonly used trait in both linkage and association studies, and to an even greater extent fracture, are complex phenotypes. Fracture in particular is a rare and stochastic event that depends on one side on bone strength *(7)*, and on another side on a number of extrinsic factors related to failure load, such as the propensity to falls, protecting responses, soft tissue padding, etc. *(8)*. The latter factors may have their own heritable and nonheritable components *(9)*. Not surprisingly, then, the apparent contribution of genetic factors to the liability for fracture is less than 30%, as compared to 60–80% for aBMD *(3)*. In order to understand the genetic basis of bone health, and ultimately of osteoporotic fractures, a more precise definition and evaluation of the discrete phenotypes contributing to bone strength is required. Since osteoporosis is a disorder characterized by low bone mass and microarchitectural deterioration of bone tissue, one eventually needs to identify the specific genes associated with both quantitative and "qualitative" bone traits, including volumetric bone density, bone size, geometry, microarchitecture (such as trabecular connectivity), bone turnover, and material properties (including microdamage and collagen cross-linking) *(10)*. In addition, genetic influences on endocrine and paracrine pathways for calcium and phosphate homeostasis, bone formation (osteoblastic function) and resorption (osteoclastic function), as well as on indirect determinants of bone strength, such as body weight, height, lean mass, and muscle strength *(11)*, should all be explored. By using such "proximal phenotypes," that is, by dissecting the multiple causes of a complex disease such as osteoporosis into observably distinct traits that can be mapped individually, stronger signals will be obtained in future genetic studies *(12)*.

## 2. INHERITANCE AND HERITABILITY OF BONE MASS AND STRENGTH

It has been shown that daughters of osteoporotic women have low BMD *(13)* and that both women and men with a family history of osteoporosis have significantly decreased BMD compared to subjects without such history *(14,15)*. BMD was also found to be decreased among relatives of middle-aged men with severe idiopathic osteoporosis *(16)*. These studies clearly suggest that the risk of osteoporosis is at least partly inherited in both genders. However, daughters of women with vertebral fractures have a BMD deficit at the spine that is already half the deficit of their mothers, whereas daughters of women with hip fractures have only a small BMD deficit at the femur neck *(17)*. These observations suggest that the influence of genetic factors on peak bone mass might affect the risk of osteoporotic fractures differentially at spine and hip, i.e., would be more pronounced for

vertebral fractures, whereas hip fracture risk would be more affected by age-related changes in BMD *(17)*.

Heritability ($h^2$, %) is defined as the proportion of the total variance for a trait across the population that is attributable to the average effects of genes *(18)*. By comparing within-pairs correlations for BMD between monozygotic (MZ) twins, who by essence share 100% of their genes, and dizygotic (DZ) twins, who have 50% of their genes in common, genetic factors have been estimated to account for as much as 80% of the population variance for lumbar spine and proximal femur BMD *(19)*. However, a bias in the estimate of heritability using twins as well as other familial models can be introduced by underestimating environmental sources of covariance *(20,21)*, which may then inflate the apparent additive genetic effects on bone mass. Some studies also suggest that additive genetic covariance exists between bone mass and lean body mass, i.e., that bone and muscle share some common (genetic) determinants *(10)*. This finding is consistent with the influence of mechanical loading on bone structure, particularly cortical thickness, and also with the fact that some gene products may be implicated in the regulation of both bone and muscle metabolism, such as the vitamin D receptor (VDR) (see below). In contrast, the Sydney Twin Study of Osteoporosis has found that the 80% and 65% of variance for lean mass and fat mass, respectively, that was attributable to genetic factors had only little influence on BMD at the lumbar spine or femoral neck *(22)*. Hence, it remains presently unclear whether the relation between lean mass and bone mass is most significantly determined by common environmental or genetic influences. Nevertheless, these observations suggest that the influence of some genes on bone mass might further depend on lifestyle factors, particularly the level of physical exercise, which will at first allow particular gene variants to expressed their differences at the muscle level.

In addition to BMD, twins studies have also reported heritability estimates ranging from 55% to 82% using quantitative ultrasound to evaluate bone properties in the phalanges and/or calcaneum, which were similar to or just slightly lower than BMD values estimated by dual-energy X-ray absorptiometry (DXA) at the lumbar spine and femoral neck *(23,24)*. Moreover, cross-trait correlations suggest that specific genes unrelated to BMD explain at least half of the heritability of the skeletal phenotype(s) measured by ultrasound *(24)*. The major difficulty in interpreting these results comes from our poor current understanding of the actual bone properties measured by ultrasounds, besides BMD. Several parameters of bone geometry, such as femoral cross-sectional area, femoral axis length, and the height and width of vertebral bodies, have also been shown to have a major genetic determination using twins and/or sib-pairs analysis *(3)*.

Parents–offspring correlations for BMD have also been significant, albeit heritability estimates have been somewhat lower in this case (in the range of 50–60%) compared to the twins model *(25,26)*. The heritability of aBMD, bone mineral content (BMC), volumetric bone mineral density (vBMD), and bone area in the lumbar spine and femur (neck, trochanter, and diaphysis) has also been evaluated

in 8-yr-old prepubertal girls and their premenopausal mothers *(27)*. In this study, regressions were adjusted for height, weight, and calcium intake in order to minimize the contribution of genetic covariance and shared nutritional factors. Despite great disparities in the maturity of the various bone traits before puberty, ranging from only 30% for BMC to nearly 100% for vBMD in children compared to their mothers' (peak) values, heredity by maternal descent was detectable at all skeletal sites and affected all traits, including bone size and vBMD ($h^2$ range: 52–76%). Moreover, all bone parameters were reevaluated 2 yr later in the female children, showing high correlation with prepubertal values (all $r > 0.80$) and similar heritability estimates in mothers and daughters as evaluated earlier, despite considerable increase in bone mass in children during this period. These results indicate that a major proportion of the population variance for peak bone mass is explained by genetic influences that are already expressed before puberty, and suggest "tracking" of the genotypic values (i.e., the mean phenotype observed among individuals with a given genotype) during growth. Concerning vBMD in particular, taking together the evidence of a significant heritability in prepubertal children and the constancy of vBMD measurements during growth, it appears that genetic determination might be already established before birth *(17)*. The prepubertal and early pubertal expression of genes accounting for a vast proportion of peak bone mass variance has been independently confirmed using both parents–offspring *(28)* and the twins model *(21)*. The latter study as well as another analysis of variance components for BMD in nuclear families *(29)*, however, suggested that heritability estimates could further increase (up to 84%) after peak bone mass is achieved. This might be explained by a few specific genes being expressed after puberty, perhaps through some interactions with nutritional/lifestyle factors that are specific to young adults.

Information concerning bone mass heritability by paternal descent is scarce. Overall, father–son correlations for BMD at various skeletal sites appear to be slightly lower than mother–daughter correlations *(26,30)*. In addition, mother–son correlations for bone density are similar or lower compared to mother–daughter estimates, whereas father–daughter correlations appear to be the lowest. Altogether, these data suggest a predominant effect on bone mass of genes inherited by maternal descent, which might at least partly be explained by gene imprinting effects and/or by interactions with maternal environmental factors *in utero*.

In contrast to the clear heritability of peak bone mass, the contribution of genetic factors to the population variance for bone turnover and age-related bone loss remains unclear. A small twins study including both females and males suggested better correlations for bone loss among MZ compared to DZ twins *(18)*, whereas another small study in male twins did not find a significant heritability for bone loss *(31)*. The heritability of bone turnover markers seems to be lower in postmenopausal compared to younger women, i.e., in the range of 30% *(32)*. Moreover, BMD heritability estimates between mothers and daughters are lower in post- compared to premenopausal daughters *(33)*. On another side, the age at which cessation

of the ovarian function occurs, obviously a major determinant of osteoporosis risk in later years, seems to be genetically determined ($h^2$, 63%) *(34)*. Even if the overall genetic determination of bone remodeling in later years is less prominent than it is on bone modeling and peak bone mass acquisition, it does not preclude some specific gene variants to play an important role in modulating bone turnover and bone loss, particularly as a result of interactions with hormonal, nutritional, and other lifestyle factors (see below). These gene variants may either be the same that are associated with peak BMD or different genes, mostly associated with bone mass and strength in aging subjects.

## 3. QUANTITATIVE TRAIT LOCI FOR BONE MASS

The actual number of genes contributing to bone health and, conversely, osteoporosis risk, is currently unknown. It has been hypothesized that the determination of bone mass involves dozens of genes with relatively small additive effects, so-called modulator genes, and a few genes with rather large effects *(35,36)*. Recent segregation studies in populations defined by a very homogeneous genetic background and environment indeed suggest that analytical models accounting for a major gene effect could be the most appropriate to describe BMD heritability *(37–39)*. Genome-wide screening approaches search for loci flanked by DNA microsatellite markers that co-segregate with the phenotype of interest in a population of related individuals. These pedigrees can be constituted by large kindreds and sibships with extreme phenotypes (such as high or low bone mass) or from the population at large *(36.)* Although linkage studies for bone mass and other determinants of bone strength in both humans and mice have identified a large number of QTLs linked to these traits *(3)*, located on virtually every chromosome, some of these QTLs have shown better consistency across various populations, suggesting that gene(s) in those loci could be major contributors to the population variance for bone mineral density (Table 1). In addition, many other QTLs for spine and femur bone geometry have been identified *(3,40)*, as well as for bone ultrasound properties *(41)*. A major advantage of genome-wide scanning is that it makes no assumptions about the genes potentially governing the trait, which can potentially lead to the identification of novel, previously unsuspected, genes contributing to bone mass and strength. Mapping novel gene(s) in the QTLs thus identified may in turn have a major impact on our understanding of the pathophysiology of osteoporosis and other skeletal disorders, such as bone dysplasias. A recent example is the linkage of three Mendelian skeletal disorders, i.e., the autosomal recessive syndrome of juvenile-onset osteoporosis-pseudoglioma (OPPG), familial high bone mass (HBM), and autosomal dominant osteopetrosis Type I (ADOI) to chromosome 11q12-13 *(42–44)*. Later on, genes mapping in this region and coding for low density lipoprotein-receptor related protein 5 *(LRP5)* and the osteoclast-specific subunit of the vacuolar proton pump, *ATP6i* (*TC1RG1* gene), respectively, have been identified to be responsible for the OPPG and HBM syndromes *(LRP5)*

Table 1
Quantitative Trait Loci (QTLs) for Bone Mineral Density (BMD) in Humans

| *QTL* | *Koller (115)* | *Devoto (116)* | *Karasik (117)* | *Deng (118)* | *Wilson (119)* | *Candidate Genes* |
|---|---|---|---|---|---|---|
| **1p36** | | | | | | *MTHFR, TNFRSF1B** |
| 1q21–23 | LS | | | | | *IL6R, BGLAP* |
| 2p23–24 | | LS | | | | |
| **3p22–14** | | | | DR | LS | *PTHR1* |
| **4q32** | | FN | | LS, DR | | *PDGF(C), NPY1R, IL-15* |
| 5q33–35 | FN | | | | | *IL-4, GR* |
| 6p11–12 | LS | | | | | |
| 6p21.2 | | | FN, LS | | | *HLA DRB1, BMP6, TNFA* |
| 7p22 | | | | LS | | *IL6, TWIST* |
| 8q24 | | | Ward | | | *TNFRSF11b*[§] |
| 9p24 | | | | DR | | |
| 10q26 | | | | FN | | *FGFR2* |
| 11q12–13 | FN, LS | | | | | *LRP5, TCIRG*[¶] |
| **12q24** | | | LS | LS | | *IGF-1* |
| 13q33–34 | | | | (LS) | | *COL4A1, A2* |
| **14q31–34** | FT | | LS | | | *TSHR, TRAF3*[#] |
| **16p12–q23** | | | (Ward) | | LS | *IL4R, MMP2, CDH11*[†] |
| 17p13 | | | | DR (FN) | | |
| 21q22.2-qter | | | FT | | | *COL6A1* |
| 22q12–13 | LS | | | | | |

QTLs with LOD scores for linkage >1.8 from 5 independent genome-wide screening studies in Caucasians are summarized. In bold, QTLs that have been identified in more than one study. In parentheses, LODs < 1.75. Candidate osteoporosis genes mapped near the identified QTLs are also mentioned. LS, lumbar spine; FN, femoral neck; FT, trochanter; WB, whole body; DR, distal radius; Ward, Ward's area.

* TNF receptor superfamily/1β (TNF receptor 2); [§]osteoprotegerin (OPG); [¶]osteoclast-specific subunit of the vacuolar proton pump, ATP6i; [#]TNF receptor-associated factor 3; [†]osteoblast-cadherin (cadherin-11).

*(45–47)* and for malignant autosomal recessive osteopetrosis *(TCIRG1) (48).* Since the 11q12-13 locus has also been linked to femur and spine BMD in pairs of healthy Caucasian-American sisters *(49)* (Table 1) as well as with hand BMD in Russians *(50),* the *LRP5* and *TCIRG1* genes have become obvious candidates for bone mass determination in the population. A first study suggests that genetic variation in *TCIRG1* may not be associated with peak bone mass in healthy premenopausal women *(51).* In contrast, preliminary studies suggest that *LRP5* polymorphisms might actually be associated with lumbar spine BMD, size, and the risk of osteoporosis in men.

The discrepancy of QTLs mapping across several studies (Table 1) illustrates some of the limitations of genome scanning for bone mass in humans. On one side, there is the limited power of this analysis, which may require thousands of individuals to achieve the required statistical power, unless sib pairs extremely discordant for the trait or large pedigrees are gathered *(36).* On another side, it is limited by the density of microsatellites markers used, typically distant 10 or more centi-Morgans (cM) (≥10 mio base pairs). The QTLs thus identified may contain hundreds of genes, whereas chromosomal regions that have not been formally identified by linkage (logorithm of the odds [LODs] below 1.8) may still harbor clear candidate genes for osteoporosis (Table 2). One study in 115 probands with osteoporosis and 499 of their relatives somewhat circumvented the problem of leaving obvious candidate genes unidentified by using a limited number of microsatellites in the vicinity of specific genes implicated in the control of BMD and/or bone metabolism *(52).* The candidate genes studied coded for structural components, such as type I collagen A1 and A2, type II collagen A1, fibrillin type 1, and osteopontin; for growth factors and cytokines, such as colony-stimulating factor 1, epidermal growth factor, interleukin (IL)-1α, IL-4, IL-6, IL-11, transforming growth factor-$\beta_1$, tumor necrosis factors-α and -β; and for components of endocrine systems, such as androgen receptor, VDR, calcium-sensing receptor, estrogen receptor-α (ESR1), insulin-like growth factor (IGF)-1, parathyroid hormone (PTH), PTH-related protein, and PTH receptor type 1. The strongest linkage with BMD was detected with the PTH receptor type 1 gene, whereas ESR1 gene and the IL-6 gene were among the few other loci to be significantly associated with BMD, although with lower LOD scores.

## 4. POPULATION-BASED ASSOCIATION STUDIES

Population-based association studies have mostly tested the relationship between BMD and/or bone turnover markers in unrelated individuals and polymorphic candidate genes coding for bone structural molecules, hormones, and/or their receptors implicated in calcium/phosphate and bone metabolism, cytokines involved in bone remodeling and, more recently, transcription factors (Table 2) *(53).* Association studies based on one or a few single nucleotide polymorphisms (SNPs) have their limitations, because they are by definition unable to identify new susceptibility genes for osteoporosis; true associations may be missed because of

Table 2
Candidate Genes in Osteoporosis Association Studies

| *Protein* | *Candidate Gene* | *Chromosome* |
|---|---|---|
| ***Receptors*** | | |
| Vitamin D | *VDR* | 12q13 |
| Estrogen | *ESR1* (α) | 6q25.1 |
| | *ESR2* (β) | 14q23 |
| Parathyroid hormone | *PTH1R* | 3p22-21.1 |
| Calcitonin | *CALCR* | 7q21.3 |
| Calcium-sensing | *CASR* | 3q21-24 |
| Androgen | *AR* | Xq11.2-q12 |
| Glucocorticoid | *GR* | 5q31 |
| Tumor necrosis factor (receptor 2) | *TNFRSF 1b* | 1p36 |
| Osteoprotegerin | *TNFRSF 11b* | 8q24 |
| ***Growth factors and cytokines*** | | |
| Transforming growth factor-β | *TGFB1* | 19q13.2 |
| Interleukin-6 | *IL6* | 7p21 |
| Insulin-like growth factor 1 | *IGF1* | 12q22-23 |
| Interleukin-1 receptor antagonist | *IL1RN* | 2q14.2 |
| Tumor necrosis factor-α | *TNFA* | 6p21 |
| ***Enzymes*** | | |
| Aromatase | *CYP19* | 15q21.1 |
| Methylenetetrahydrofolate reductase | *MTHFR* | 1p36 |
| ***Bone-associated proteins*** | | |
| Collagen type I, α1 | *COL1A1* | 17q21-22 |
| Collagen type I, α2 | *COL1A2* | 7q22 |
| Osteocalcin | *BGLAP* | 1q25-31 |
| ***Miscellaneous*** | | |
| Apolipoprotein E | *APOE* | 19q13 |
| α2-HS-glycoprotein | *AHSG* | 3q27 |

the incomplete information provided by individual SNPs; negative results do not rule out association involving nearby SNPs; and positive results may not indicate the discovery of the causal SNP but simply a marker in *linkage disequilibrium* with a true causal SNP located some distance (perhaps several genes) away. Moreover, many association studies with BMD were poorly designed in terms of power to detect true differences between genotypes, and in terms of cohort homogeneity for age, gender, and genetic background *(54)*. By investigating functional gene variants located in gene regulatory (promoter) and coding regions (exons), rather than synonymous or intronic SNPs, and by taking into account potential interactions with other genes and environmental factors, the consistency of

population-based association studies can, however, be markedly improved. The following sections discuss genetic variation in three genes, namely, VDR, estrogen receptor-α (ESR1), and IL-6 genes, in order to illustrate the importance of interactions between genetic and nutritional/lifestyle factors on both peak bone mass acquisition and maintenance.

### *4.1. Interaction of Calcium Intake With VDR Gene Polymorphisms*

The VDR mediates the effects of calcitriol *(55)* on the intestinal absorption of calcium and phosphate and on bone mineralization. The VDR gene, whose nine exons and multiple promoters expand over more than 80 kb, is highly polymorphic *(56)*. The best known and actually first variants ever described in association with bone mass are the VDR 3′-UTR alleles (intron 8/exon9 *Bsm 1, Taq,* and *Apa 1,* the first two being in nearly complete *linkage disequilibrium*) *(57,58)*. The original study found that *Bsm 1* polymorphisms (B-allele frequency equal to 0.4 and 0.1 among Caucasians and Asians, respectively) were associated with BMD and postmenopausal bone loss. However, it later became clear that this study had methodological problems, as a number of investigators failed to confirm differences in BMD and markers of bone turnover between *Bsm1* genotypes *(59–61)*. Nevertheless, a large-scale study in 55+-yr-old men and women using VDR haplotypes *(62)* and a meta-analysis combining 16 separate studies *(63)* provided some support for modest differences (2–3%) in BMD between VDR-3′ alleles. A common variant in the VDR first start codon (ATG, *Fok 1*) has also been reported (*f*-allele frequency close to 0.4 among both Caucasians and Asians), wherein the *ff* genotype was associated with a moderately lower BMD compared to *FF* in postmenopausal women *(64,65)*. However, the original cohort in which this association was described was small and non-white, and these results were not confirmed in Caucasians *(66–68)*, an unexpected result considering the fact that *Fok1* variants are predicted to code for VDR molecules differing by three amino acids in length. Nevertheless, it was later shown that VDR-3′ and -5′ alleles might interact on their association with BMD *(67)*. Other studies have shown that significant BMD differences between VDR-3′ *Bsm*I genotypes could be detected in children *(69,70)*, but not in premenopausal women from the same genetic background. These observations led to suggest that age-related factors, such as levels of gonadal steroids and nutritional/lifestyle variables, may have a profound influence on the association of VDR alleles with bone mass.

Consistent with its prominent effects on bone mass growth during childhood and the maintenance of bone mass in aging women, dietary calcium intake seems to play an important role in modulating the association of VDR polymorphisms with BMD in both age groups. Conversely, genetic variation at the VDR may contribute to the highly variable skeletal response to calcium supplementation. In a cohort of 144 prepubertal girls, 1-yr BMD gain at the distal radius and proximal femur was 50–70% higher in those receiving calcium supplements (850 mg/d) provided as milk extracts (containing phosphorus) compared to placebo, whereas

lumbar spine BMD was barely affected *(4)*. Not surprisingly, these effects were most prominent in children with inadequate dietary calcium intake for age (below 800 mg/d). In this case, calcium supplements likely influenced bone modeling, as demonstrated by an increase in both vertebral height and stature, and by the maintenance of positive calcium supplements effects on bone mass years after the end of the intervention *(71)*. In this cohort, baseline BMD at lumbar spine and femur neck was significantly lower in subjects with VDR *Bsm1* BB genotype compared to heterozygotes and *bb*. BMD gain in response to calcium supplements was increased at several skeletal sites among *BB* and *Bb*, whereas it remained apparently unaffected in *bb* girls, who had a trend for spontaneously higher BMD accumulation on their usual calcium diet *(69)*. Another calcium-intervention trial with a similar design was recently carried out in 235 prepubertal boys with an inadequate calcium intake. In contrast to the above results, calcium supplementation did not have prominent effects on BMD gain, nor was a significant association or interaction with VDR genotypes observed. On one side, these findings may reveal gender-related differences in the influence of VDR polymorphisms on bone mass gain and its response to calcium. More likely, they may indicate further levels of interaction between nutrients themselves, such as proteins and calcium *(72)*, and between these nutrients and other genetic factors. Indeed, BMD in these boys was better correlated with protein than with calcium intake, the former being on average quite high in this cohort (mean, 1.7 g/kg body weight) *(73)*. By its proper effects on IGF-1 expression and bone formation *(74)*, this high protein intake may have offset part of the deficit in spontaneous calcium intake, thereby preventing calcium supplements from being efficient. Accordingly, calcium supplements significantly increased BMD gain in boys with the lowest protein intake. From a genetic standpoint, the high protein intake may have allowed these boys to achieve a BMD gain close to their best genetic potential, thereby obscuring differences among VDR genotypes. In this particular case, it would be interesting to test whether genetic variation in the protein metabolic pathway, such as IGF-1 polymorphisms *(75)*, might be associated with BMD gain during growth.

Another interesting study comparing the distribution of VDR genotypes in 105 rachitic children from Nigeria (calcium intake, 200 mg/d) and 94 healthy controls found that VDR-3′ genotypes were similarly distributed among cases and controls, whereas the *FOK1 ff* genotype was significantly underepresented among cases *(76)*, suggesting that the latter genotype might be protective against osteomalacia induced by dietary calcium deficiency. In contrast, in 72 Caucasian, African-American, and Mexican children with adequate calcium intake (above 1000 mg/d), Ames et al. found that carriers of the VDR genotype *ff* had significantly *decreased* intestinal calcium absorption *(77)*. How the VDR genotype *BB* could be associated with both lower BMD and increased BMD gain after calcium supplementation [in prepubertal girls, *(69)*] and the *ff* genotype be associated with protection against rachitism while being associated with decreased intestinal calcium absorption in healthy kids will be explained at the end of this section.

A significant interaction between VDR-3′ genotypes and calcium intake on BMD and BMD changes has also been shown in postmenopausal women by a number of investigators *(78–81)*. In a pioneering study including elderly subjects (90% women, mean age 73 yr) with a high prevalence of osteoporosis and a low calcium intake (590 mg/d), *bb* subjects apparently did not lose bone at the lumbar spine over 18 mo, whereas spine BMD decreased 2% in heterozygotes and *BB* during this time *(78)*. Calcium supplements (800 mg/d) reversed bone loss in *Bb* subjects after 18 mo, but did not significantly alter BMD changes among the other genotypes. Another prospective study in younger postmenopausal women (mean age 59 yr) whose mean calcium intake was very low (400 mg/d) also found that lumbar spine and hip bone loss was significantly higher in *BB* subjects. In subjects receiving calcium supplements (500 mg/d), however, bone mass changes were similar in all genotypic groups, indicating that the response to calcium was actually greater among *BB (79)*. Similar to the previous two studies, a long-term follow-up (6.3 yr) study in postmenopausal women (mean age 69 yr) reported that among individuals with low calcium intake (below 456 mg/d), *TT* homozygotes for VDR *Taq 1* polymorphism (same as *bb*) had a significantly lower rate of bone loss at both the femoral neck and lumbar spine compared to *tt* (same as *BB*). In contrast, among those with a higher dietary calcium intake (above 705 mg/d), there were no more significant differences in BMD changes between genotypes *(82)*. Cross-sectional observations in the Framingham Osteoporosis cohort also reported a significant interaction between VDR *Bsm 1* alleles and calcium intake on bone mass in the elderly. Thus, in men and women aged 69–90 yr, BMD at the femur trochanter and ultra-distal radius (two regions rich in cancellous bone) was significantly higher in *bb* compared to *BB* in subjects whose calcium intake was greater than 800 mg/d *(81)*. In addition, a case-control study reported that the relative risk of hip and wrist fractures among participants to the Nurses Health Study (mean age 60 yr) was significantly higher in *BB* compared to *bb* in a subgroup with calcium intake below 1078 mg/d (odds ratio, 4.3), but not in the subgroup with higher calcium intake (odds = 1) *(83)*. In summary, the VDR allele *B* seems to be associated with increased bone loss after the menopause but also with a better response to calcium supplementation.

Evidence for functional differences among VDR genotypes comes from a few studies on fractional calcium absorption and on calcium/phosphate homeostasis in response to dietary changes, as well as on patients with primary hyperparathyroidism *(55)*. Thus, in postmenopausal women following a calcium-restriction period, fractional absorption of calcium increased significantly less in *BB* compared to *bb* women, despite a trend for higher calcitriol levels among the former *(84)*. Similarly, a short-term (2-wk) dietary modification trial in young healthy males found that *BB* had a subtle resistance to calcitriol while on a low dietary calcium and phosphate diet for several days, leading to significantly higher levels of circulating PTH, decreased tubular reabsorption of phosphate and lower serum phosphate levels *(85)*. Although these two studies are internally

consistent and may explain why many investigators found lower BMD in *BB* subjects on low-calcium diets, they remain to be reconciled with the many observations of a better skeletal response to calcium supplementation in carriers of the *B* allele (see above).

To answer this question, we propose a model in which the VDR *BB* (and *FF*) genotype is characterized by *low efficiency–high capacity,* whereas the VDR *bb* (and *ff*) ff genotype is characterized by *high efficiency–poor capacity,* in promoting calcium absorption and/or bone mineralization. This means that carriers of the VDR genotype *ff* may be capable to better extract calcium from a calcium-poor environment (as suggested by the rachitic kids from Nigeria), whereas carriers of the genotype *BB* would be at a disadvantage in similar conditions (the metabolic studies above); on the opposite, in a calcium-rich environment, the capacity of *ff* carriers to utilize large amounts of calcium would be limited (the calcium absorption study in healthy kids), whereas *BB* could do so (based on their better response in calcium supplementation trials). This might also explain why the *BB* genotype is virtually absent among Asians, whose diet is traditionally poor in dairy products. Accordingly, the relationship between BMD and dietary calcium might be better described by sigmoidal curves for which the point of inflection occurs at different calcium intake thresholds depending on VDR genotypes *(86).* In keeping with our interpretation (above), further developments of this model have proposed that the calcium intake–BMD curves might not run parallel to each other but actually cross over *(2).* In this case, the VDR genotypes associated with decreased bone mass at low calcium intake *(BB, FF)* might actually be the ones associated with increased bone mass at higher calcium intakes (hence with a better response to calcium supplements), whereas the opposite would be true for carriers of the alternate homozygous genotypes *bb* and *ff* (Fig. 1).

### *4.2. Estrogen Receptor Gene Polymorphisms*

Female sex hormones appear to be mandatory not only for the acquisition of peak bone mass in both females and males *(87,88),* but also for the maintenance of bone mass in both genders *(89).* They control bone remodeling during reproductive life in females and later on in aging men *(90).* Genotypes identified by *Pvu*II and *Xba*I restriction fragment length polymorphisms in the first intron of the estrogen receptor α (ERα) gene (*ESR1*) (Table 1) were originally found to be significantly associated with BMD in postmenopausal Japanese women, but not with markers of bone turnover *(91).* In contrast, a similar study from Korea reported no significant BMD differences among ERα genotypes in postmenopausal women receiving hormone replacement therapy (HRT) *(92).* Another study from Japan including 173 premenopausal to late postmenopausal women indicated a predominant association between ERα genotypes and adult bone mass, which disappeared with advancing age *(93).* Several investigators have examined ERα gene polymorphisms and bone mass in Caucasian populations as well. In one study, a significant association was found between either the *Pvu*II or the *Xba*I genotypes and lumbar spine BMD in 253

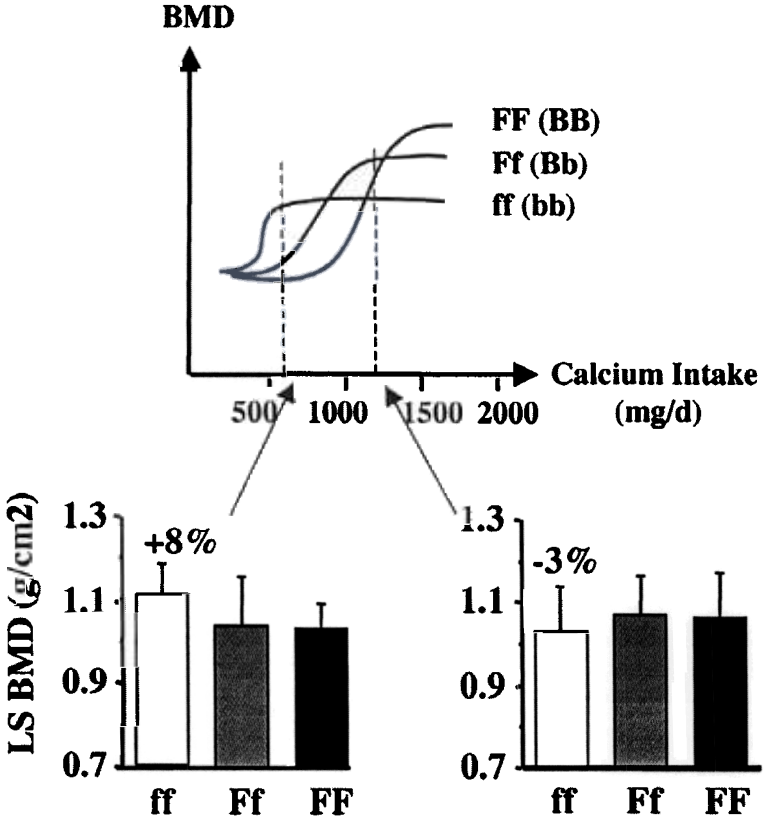


**Fig. 1.** Scheme of the interaction between calcium intake and vitamin D receptor (VDR) polymorphisms on bone mineral density (BMD). The scheme on top illustrates the relationship between BMD and dietary calcium intake according to VDR polymorphisms (adapted from ref. 2), with *ff (bb)* being the "high efficiency–low capacity" genotype and *FF (BB)* the "low efficiency–high capacity" genotype. The lumbar spine BMD data shown in the two diagrams below were obtained in 177 healthy premenopausal women genotyped for VDR *Fok I* polymorphisms (*FF, Ff,* and *ff*), who were further subdivided into two groups for dietary calcium intake (respectively below and above the median for the whole cohort) (adapted from ref. *67*). The arrows and dotted lines indicate the expected BMD differences among VDR genotypes in these two groups of women (mean calcium intake, 550 and 1200 mg/d, respectively).

pre- and perimenopausal women, those with the *PvuII pp* genotype having a 6.4% lower BMD at this site compared to *PP (94).* 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-yr period in this cohort. One limitation of this study was a low rate of bone loss in this cohort over 3 yr (≤1%). In contrast, a very recent study that prospectively investigated the 5-yr bone loss in early post-

menopausal women receiving either HRT or placebo in addition to calcium and vitamin D found no significant differences in BMD among ERα polymorphisms at baseline, but significant differences in lumbar spine BMD changes between genotypes *PP* (–6.4%) and *pp* (–2.9%) in the absence of HRT *(95)*. In women receiving HRT, these differences were no more apparent.

A significant gene-by-gene interaction between VDR and ER gene polymorphisms has been suggested by several authors. In the study by Willing et al. *(94)*, BMD at all skeletal sites was lower in subjects with the VDR *Bsm*I genotype *BB*, as compared to *Bb* and *bb*, in the subgroup of women carrying the ERα *Pvu*II genotype *PP*. Of note, however, there were only five *BB/PP* subjects in this cohort. An interaction between VDR-3′ and ERα polymorphisms has also been found in relation to BMD in a cohort of 426 normal and osteoporotic women *(96)*. Subjects carrying the *BB/PP* genotypes had a significantly lower BMD at the lumbar spine compared to alternate homozygotes *bb/pp*. VDR/ ERα polymorphisms have also been related to the rate of postmenopausal bone loss in a small cohort of women ($n$ = 108) with or without HRT *(97)*. These results, however, remain controversial, as a recent study in 313 late postmenopausal women with a low average calcium intake (approx 600 mg/day), including 142 women with a history of osteoporotic fractures, found no significant association between ERα polymorphisms alone or in combination with VDR polymorphisms on BMD, nor on biochemical markers of bone and mineral metabolism *(98)*.

A meta-analysis on the association of ERα genetic variation with BMD and fracture risk in more than 5000 women from 30 studies concludes to the absence of significant differences in lumbar spine or hip BMD between *Pvu*II alleles, whereas homozygotes for the *Xba*I *XX* genotype have a significantly higher BMD at these two sites *(99)*. Since the *Xba*I and *Pvu*II sites are very nearly located and in strong *linkage disequilibrium*, this may explain why some authors found an association with the latter. Moreover, the meta-analysis found a trend for more prominent differences in pre- compared to postmenopausal women, suggesting that ERα genotypes might exert their influence prominently on peak bone mass acquisition. Despite standardized BMD differences of only approximately $Z = 0.1$ between *XX* and *xx* genotypes, differences in the risk of fracture were disproportionately high (odds ratio, 0.66 in *XX* vs *xx (99)*. The latter observation suggests that ERα genotypes might be implicated in the determination of bone microarchitecture, which is poorly accounted for by DXA measurements.

The molecular mechanisms by which ERα polymorphisms may modulate the actions of estrogens on bone modeling and remodeling remain to be elucidated. A consistent finding, however, is that HRT appears to alleviate BMD differences among these genotypes (see above), indicating that carriers of the *x* allele might particularly benefit from HRT after the menopause. HRT has also been found to alter the association with BMD of other genes belonging to the biological pathway by which estrogens exert their activity on the skeleton, such as IL-6 (see below).

Thus, future studies should consider the possible interaction of ERα allelic variants not only with estrogens, but also with IL-6 and VDR alleles.

### *4.3. IL-6 Gene Promoter Polymorphisms*

IL-6 is a pleiotropic cytokine playing a central role in the activation of osteoclasts (the bone-resorbing cells) and bone turnover *(100)*. Since IL-6 gene expression is normally repressed by endogenous estrogens, increased IL-6 production is an important factor for postmenopausal bone loss *(101)*. Moreover, IL-6 expression in bone is triggered by parathyroid hormone and it is therefore implicated in the age-related bone loss associated with poor calcium and vitamin D intake *(101,102)*. Several studies have identified the IL-6 gene locus to be linked to BMD in postmenopausal women *(103,104)* and in families of osteoporotic probands *(52,105)*, whereas no linkage between the IL-6 gene locus and bone mass was found in young sib pairs *(106)*. These observations therefore suggested that IL-6 genetic variation might contribute to the population variance in bone loss rather than peak bone mass. More recently, several functional allelic variants have been identified in the IL-6 gene promoter region *(107,108)*. Among them, a common –174G>C polymorphism (frequency of the C allele, 0.4 among Caucasians) is located close to a binding site for a transcription factor (NF-IL-6) that is under the dependency of estrogens. There is some evidence that this variant may produce a functional mutation in that the C allele is associated with lower IL-6 gene transcriptional activity in vitro *(107)*. In addition, a rare G>C allelic polymorphism at position –573 (frequency of the C allele, 0.06 among Caucasians), which is closely related to two glucocorticoid responsive elements, has very recently been identified in this region. The –573 alleles also seem to influence the level of IL-6 transcriptional activity in vitro *(109)*. Further proof of functionality of IL-6 genetic variation comes from the fact that –174CC homozygotes have circulating IL-6 concentrations approximately half those of –174GG homozygotes *(107)*, whereas both the –174C and –573G alleles are associated with significantly lower levels of the IL-6-dependent C-reactive protein (CRP) in serum *(109)*.

In 434 healthy, community-dwelling, white U.S. postmenopausal women (mean age ± SD, 71.7 ± 5.7 yr), C-terminal crosslinks of Type 1 collagen (CTx), a marker of bone resorption, were significantly lower among –174CC and –573GG compared to the other genotypes *(110)*. Postmenopausal women with genotype –174CC (15% of the population) had levels of bone resorption similar to those of premenopausal women. Interestingly, the Il-6 –573G>C and –174 G>C allelic variants appear to cooperate in the regulation of IL-6 gene transcriptional activity *(108)*. Accordingly, homozygous carriers of the two IL-6 variants associated with low IL-6 activity (namely, –174C and –573G) had CTx levels that were 30% lower compared to those not carrying this allelic combination *(109)*. Among these women, women with the –174CC genotype had BMD at the hip and forearm (distal radius) that was 1.5% to 4.7% (nonsignificantly) higher as compared to GG homozygotes *(110)*. However, differences were larger at the trochanter and ultradistal radius, consistent with the

predominant effects of estrogen deficiency (and increased IL-6 activity) on cancellous bone. When the cohort was further divided in two groups of early and late postmenopausal women, BMD was found to be significantly lower in the older compared to younger women, but more prominently so in subjects carrying the IL-6 –174 GG and GC genotypes (–9% to –10% at the various skeletal sites) compared to CC women (–5% to 6.1%). Taken together with the lower level of bone resorption associated with the –174C allele, these observations indicate that postmenopausal women with the IL-6 –174CC genotype may be "slow bone losers." Two studies also reported an association of IL-6 polymorphisms with peak bone mass, one in young males *(111)* and the other in premenopausal females *(112)*. However, the latter study failed to detect a lower rate of bone resorption or bone loss associated with the –174CC genotype in 234 postmenopausal women (mean age 64 yr). These data suggest that IL-6 alleles may contribute to peak bone mass, but this association may be blunted in early postmenopausal women by the dramatic hormonal changes occurring at the menopause. Eventually, IL-6 genetic variation might again exert detectable effects on bone turnover and bone mass 20 yr after the menopause, i.e., at a time when nutritional and lifestyle factors, such as poor calcium and vitamin D intake, further modulate IL-6 gene expression.

To test this hypothesis, the interaction between IL-6 promoter polymorphisms and factors known to affect bone turnover, namely, years since menopause, estrogen status, physical activity, smoking, dietary calcium, vitamin D, and alcohol intake, was examined in the Offspring Cohort of the Framingham Heart Study *(113)*. This cohort comprises 1574 unrelated men and women (mean age 60 yr) with bone mineral density measurements at the hip. Consistent with the study of Garnero et al. *(112)*, in models that considered only the main effects of IL-6 polymorphisms, no significant association with bone mineral density was observed in either women or men. In contrast, interactions were found between IL-6 –174 genotypes and years since menopause, estrogen status, dietary calcium, and vitamin D intake in women. Thus, bone mineral density was significantly lower with genotype –174 GG compared to CC, and intermediate with GC, in women above 15 yr past menopause, in those without estrogens or with calcium intake below 940 mg/d. In estrogen-deficient women with poor calcium intake, hip bone mineral density differences between IL-6 –174 genotypes CC and GG were as high as 16%. In contrast, no such interactions were observed in men. These data therefore suggest that age, HRT, and dietary calcium all influence the association between IL-6 alleles and bone mass. Accordingly, HRT and adequate calcium intake could be better targeted to population subgroups genetically identified to be at otherwise increased risk of accelerated bone resorption and low bone mass with aging, such as IL-6 –174GG homozygotes.

## 5. CONCLUSION

Genetically speaking, humans today live in a nutritional environment that differs from that for which our genetic constitution was selected *(114)*. Thus, gene polymorphisms that appeared a few ten thousand years ago in the human

genome as an adaptation to a changing environment (hunter-gatherer, then agricultural), but have become *mal*-adapted to our current nutritional and lifestyle habits, may in turn contribute highly to a number of common disorders including osteoporosis, but also diabetes, hypertension, etc. The identification of such genetic variations and interactions through various approaches combining association studies with candidate genes involved in bone metabolic pathways and genome-wide mapping of QTLs linked to discrete traits for bone strength is ongoing. In the future, advances in the osteoporosis genetics field may allow for individualized Recommended Dietary Allowances for various nutrients, primarily calcium, and for targeted interventions aiming at improving lifestyle factors for better bone health.

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