Human genetics of osteoporosis

Serge Ferrari* MD
Associate Professor of Medicine and Osteoporosis Genetics
Service of Bone Diseases, Department of Rehabilitation and Geriatrics, WHO Collaborating Center for Osteoporosis Prevention, Geneva University Hospital and Faculty of Medicine, 24, rue Micheli-du-Crest, CH – 1211 Genève 14, Switzerland

A family history of hip fracture carries a twofold increased risk of fracture among descendants. Genetic factors indeed play a major role in the determination of bone mineral density (BMD) and osteoporosis risk. Multiple chromosomal loci have been mapped by linkage approaches which potentially carry hundreds of genes involved in the determination of bone mass and quality. Association studies based on candidate gene polymorphisms and subsequent meta-analyses, and the more recent genome-wide association studies (GWAS), have clearly identified a handful of genes associated with BMD and/or fragility fractures. Among them are genes coding for the LDL-receptor related protein 5 (LRP5), estrogen receptor alpha (ESR1) and osteoprotegerin, OPG (TNFRSF11b). However, the percentage of osteoporosis risk explained by any of these polymorphisms is small, indicating that most genetic risk factors remain to be discovered and/or that interaction with environmental factors needs further consideration.

Key words: bone; osteoporosis; gene; polymorphism; fracture; LRP5; interleukin-6; estrogen receptor; vitamin D receptor; osteoprotegerin; QTL; GWAS.

The most recent WHO and European guidelines for the management of osteoporosis underscore the contribution of clinical risk factors (CRFs) in addition to bone mineral density (BMD) in order to evaluate the individual probability of a fragility fracture. Among those CRFs, a woman whose mother has had a hip fracture has twice the fracture probability of women without such family history. This pertains to the inheritance of phenotypical traits of bone mineral mass and bone quality (micro-architecture essentially) that define osteoporosis. So daughters of osteoporotic women have lower BMD compared to age-matched controls, and sons of men with idiopathic osteoporosis have a lower BMD compared to other young men. BMD is highly correlated between mothers and daughters, and this occurs many years before girls undergo puberty. In recent years, numerous gene polymorphisms (SNPs) have been associated with BMD and/or fractures.

* Tel.: +41 22 372 99 50; Fax: +41 22 382 99 73.
E-mail address: serge.ferrari@medecine.unige.ch

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This chapter reviews the evidence for BMD heritability, i.e., the proportion of the BMD variance explained by additive genetic factors; the quantitative trait loci (QTLs) in linkage to BMD and other skeletal traits; and the SNPs associated with osteoporosis that have been identified either by a candidate gene approach, or by genome-wide association studies (GWAS). We will also consider the importance of gene–environment interactions, since the latter may have a strong influence on the strength of the association between SNPs and the bone traits. By ‘environment’ we intend not only external factors, such as nutrition and physical activity, but also the internal milieu, in particular gonadal steroids.

HERITABILITY OF BONE MASS

By comparing BMD between monozygotic and dizygotic twins, and between parents and offspring, studies over the last four decades have documented the major contribution of genetic factors to osteoporosis. Most studies demonstrate heritability \( (h^2) \) of 50–80% for BMD at the most common fracture sites (lumbar spine, hip and radius), which expression is detectable from an early age (childhood) with a subsequent tracking for BMD, similar to height. However, fracture heritability has been more difficult to demonstrate and was usually estimated to be less than 50% (Table 1). The susceptibility to fractures indeed depends on many factors, including non-skeletal factors such as propensity to fall, diminished soft-tissue cushion, and more broadly on the physical environment. Moreover, some heritability of fracture is notably independent of BMD, and it is confirmed by the findings of SNPs that contribute to variation in BMD but do not always contribute to osteoporotic fractures per se. Heritability estimates for various osteoporosis-related traits are summarized in Table 1.

Some studies reported differences in BMD heritability between pairs of the same sex and the opposite sex, likely reflecting (in utero) imprinting effect on the expression of osteoporosis genes. Moreover, some studies found a heritability for BMD 20% greater in men than women, whereas others did not. Some of the apparent differences in the heritability of bone mass between genders, however, might be explained by our (in)ability to account for the contribution of ‘environmental’ covariates, particularly for the level of estrogen in men.

QUANTITATIVE TRAIT LOCI FOR OSTEOPOROSIS

Several studies in more or less extended families using genome microsatellitic markers of low density (typically 400–500 markers per genome), highlighted numerous QTLs on...
virtually any chromosome in linkage with BMD (Figure 1). In many of these studies identified gender-specific QTLs for BMD. In the FAMOS study including a large number of subjects (3691 from 715 families), a BMD QTL was identified on chromosome 10q21 specifically in men. Another on chromosome 4q25 was shared between men younger than 50 and older women. In another study, a very promising sex-specific QTL for FN BMD was identified at 15q in young women, with LOD score 4.3, but not in younger men from the same geographic region. This finding was also supported by a QTL meta-analysis in women. Similarly, QTLs for femoral bone geometry were different between genders. The meta-analysis of BMD linkage studies by Ioannidis et al on one hand identified new QTLs that had not been identified in the original cohorts, probably due to a lack of power, but on the other hand did not confirm several of the original QTLs, suggesting that they could be false-positive results. Linkage (QTL) approaches, first developed to map the gene mutations responsible for monogenic disorders, such as osteogenesis imperfecta, actually appear to lack sensitivity and specificity to delineate the multiple loci underlying susceptibility to complex disorders such as osteoporosis. Therefore linkage approaches so far did not allow identification of new osteoporosis genes in humans. Only very recently some authors, using high-density SNPs in the 1p36 region, previously identified in linkage with BMD, managed to identify a number of potential candidate genes for osteoporosis, including RERE and G1P2.

OSTEOPOROSIS GENE POLYMORPHISMS

The osteoporosis candidate genes may be roughly categorized into several broad categories according to the function of the coded molecules, mostly involved in metabolism of bone cells (osteoblasts and osteoclasts), structure and turnover of collagen and mineral (calcium and phosphorus), and regulatory/hormonal (obviously, sex-hormone) pathways. Multiple gene polymorphisms have been tested in association studies with BMD and/or fractures. Among them, a few have been subjected to meta-analysis: the vitamin D receptor gene (VDR), estrogen receptors α and β (ESR1 and ESR2), collagen 1 α1 chain (COL1A1), transforming growth factor beta (TGF-β), and more recently the LDL-receptor-related protein 5. Other genes coding for interleukin-6 (IL6), methylenetetrahydrofolate reductase (MTHFR), and aromatase (CYP19) have also been extensively investigated. Most studies reported evidence for an association with BMD and/or fracture, mostly in women, less commonly in men, and rarely in both genders. However, meta-analyses draw inconclusive results concerning the association of VDR, COL1A1 and TGF-β with fractures. These studies will be described in more detail below.

Collagen 1 α1 chain (COL1A1)

The gene coding for the α1 chain of collagen 1, the principal component of the bone extracellular matrix, carries a polymorphism (S/s) in its first intron which modifies the binding site for the transcription factor SP1, resulting in a subtle change in the equilibrium between α1 and α2 chains (normally 2:1) in the triple helix. The mechanical strength of human bone explants was diminished in s carriers compared to S. In keeping with those experimental results, a large cohort study and a first meta-analysis confirmed a higher prevalence of fractures among heterozygotes Ss and homozygotes ss compared to SS (RR: 1.5 and 1.9, respectively). Interestingly, in these studies the COL1A1 alleles were better associated with fracture risk itself
Figure 1. Quantitative trait loci (QTLs) for BMD in Caucasians. QTLs confirmed by meta-analysis are in bold and the loci identified by GWAS are boxed.
than BMD, consistent with an influence on bone quality. In contrast, a participant-level, pre-planned meta-analysis of several European cohorts (GENOMOS) only partially confirmed these observations, and mostly in women rather than men (Table 2). Heterogeneity in the definition and ascertainment of peripheral fractures among cohorts might explain this lack of consistency. Preliminary results in an Australian cohort actually suggest that ss homozygotes had twice the hip fracture probability at 5 years of non-ss, independently of BMD and age. If confirmed, this observation could be a first step towards the validation of genetic markers in fracture prediction models (see introduction).

**Vitamin D receptor (VDR)**

The gene coding for the vitamin D receptor (VDR) carries multiple polymorphisms, in particular in the 5'-initiation and 3'-non-coding regions. Some of these alleles, alone or in combination (haplotypes), could modify the stability and expression level of the mRNA. VDR association with BMD has been highly controversial, as there are probably as many positive as negative studies. Whereas an early meta-analysis, and later a large Dutch study, showed association of VDR genotypes/haplotypes with BMD and fractures, a more recent meta-analysis did not confirm those results.

Some studies suggested an interaction between VDR polymorphisms and calcium intake on BMD. These interactions could therefore modify the association of VDR alleles with BMD, so that their effect would be expressed (and thereby detectable) only in the presence of a low, respectively high, calcium intake.

**Table 2. Gene polymorphisms associated with osteoporosis: results from meta-analyses and genome-wide association studies (GWAS).**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Study</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>VDR</td>
<td>Vitamin D receptor</td>
<td>MAR +</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MAP −</td>
<td>38</td>
</tr>
<tr>
<td>ESR1</td>
<td>Estrogen receptor α</td>
<td>MAR +</td>
<td>40</td>
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<td></td>
<td>MAP +/−</td>
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<td>GWAS</td>
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<td></td>
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<td>46</td>
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<td>Osteoprotegerin (OPG)</td>
<td>GWAS (2x)</td>
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<td>Receptor activator of nuclear</td>
<td>GWAS</td>
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<tr>
<td></td>
<td>factor κB ligand (RANKL)</td>
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<td>MHC</td>
<td>Major histocompatibility complex</td>
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<tr>
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<td>Unknown</td>
<td>GWAS</td>
<td>96</td>
</tr>
</tbody>
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MAR, meta-analysis retrospective; MAP, meta-analysis at participant level; + and −, an association (+) or not (−).

* Association − with BMD, + with fractures.
* Association + with BMD, − with fractures, except incidental vertebral fractures in women (+).
* Association + with BMD, − with fractures, men only.
Estrogen receptors $\alpha$ (ESR1) and $\beta$ (ESR2)

Lack of estrogen plays a critical role in postmenopausal osteoporosis, but also in elderly men. In addition, estradiol is important for the acquisition and maintenance of peak bone mass in both genders.\textsuperscript{27,57–59} ESR1 gene polymorphisms have been analyzed extensively for association with BMD, bone loss, turnover markers and/or fractures in women, at first with conflicting results.\textsuperscript{39} Nevertheless, a meta-analysis of the association between ESR1 genotypes and BMD including more than 5000 women from 22 eligible studies ($n=11$ in Caucasians and $n=11$ in Asians), concluded that homozygotes for the $Xba$I (rs9340799) $XX$ genotype have a modestly but significantly higher BMD (+1–2%) at lumbar spine or hip compared to $xx$.$^{40}$ A recent meta-analysis of these polymorphisms in 18,917 individuals from eight European centers found evidence of association with fracture risk but not BMD.$^{41}$ These meta-analyses therefore suggest that ESR1 genetic variation may influence the age-related changes in bone structure that underlie bone strength/fragility, whereas ESR1 association with bone mass may be better discernible above a certain estradiol threshold, as mostly in females between menarche and menopause.

A few studies reported association of ESR1 genotypes with BMD in men\textsuperscript{60}, but most found no association.$^{21,61,62}$ Nevertheless, some authors found significant interactions between bioavailable estradiol levels and the $Xba$I and $Pvu$II genotypes on rates of bone loss in men aged 22–90 years.$^{63}$ An association between ESR1 polymorphisms was also reported in boys, either alone\textsuperscript{64} or with an interaction with VDR alleles.$^{65}$ However, those studies were small and need to be replicated. Interpretation of the above findings is limited by the lack of biological evidence that ESR1 intron-1 alleles may directly affect estrogen receptor $\alpha$ levels and/or activity.

A few studies have examined the polymorphisms in the estrogen receptor $\beta$ gene (ESR2) for association with BMD and fracture risk. The majority of the studies focused on the number of CA repeats and was done in postmenopausal women from various ethnic groups, including Asians\textsuperscript{66,67} and Caucasians.\textsuperscript{42,68} In the Offspring Cohort of the Framingham Study\textsuperscript{42} there was significant association of the CA repeat with measures of femoral BMD in both men and women, whereas two other polymorphisms (rs1256031 and rs1256059) were associated with femur neck BMD exclusively in men. Others reported an interaction between ESR2 and ESR1 genotypes with other genes (IGF-I, NR1P1) on BMD in postmenopausal women.$^{69,70}$ No meta-analysis of ESR2 association studies is available so far.

Interleukin-6 (IL6)

The decline in estradiol levels after menopause leads to increased production of pro-inflammatory cytokines, including IL-6, which circulating levels have been correlated to the extent of bone loss.$^{71}$ Several studies have identified the IL-6 gene locus (7p21) to be linked to BMD in postmenopausal women$^{72,73}$ and in families of osteoporotic probands$^{74,75}$, whereas no linkage was found in young healthy sister pairs.$^{76}$ These observations suggested that IL-6 gene polymorphisms could contribute to the variation in the rate of bone loss following estrogen deprivation. Then it was shown that functional polymorphisms in the IL-6 promoter region, –572 GC (rs1800796) and –174 GC (rs1800795), were associated with levels of the C-reactive protein and markers of bone resorption in postmenopausal women.$^{51,77}$ Another IL-6 gene polymorphism has been significantly associated with BMD of total body and lumbar spine in Japanese postmenopausal women but not in men.$^{78}$ In the Offspring Cohort of the Framingham Heart Study$^{52}$, BMD was
found to be significantly lower in women with IL-6 genotype \(-174\, GG\) compared to \(CC\), and intermediate with \(GC\), in women who were either more than 15 years past menopause, or estrogen-deficient, or who had insufficient calcium intake (<940 mg/day). No associations were observed in premenopausal women or in men (\(n = 755\)). These observations suggested that IL-6 gene promoter alleles modulate the response of the skeleton to estrogen deficiency and/or to other mechanisms of increased bone remodeling. This hypothesis is further supported by the evidence that the \(-174\, G/C\) allele is located in the binding site for NF-IL-6, a transcription factor which interacts with the estrogen receptor to repress IL-6 gene transcription. These polymorphisms have also been associated with other markers of bone fragility, including bone ultrasound measurements at the calcaneum and wrist (Colle’s) fractures, as well as with hip BMD loss.

**LDL-receptor-related protein 5 (LRP5)**

LRP5 is a member of the low-density lipoprotein (LDL) receptor-related family coding for a transmembrane co-receptor for Wnt signaling. Mutations of this gene are associated either with osteoporosis–pseudoglioma syndrome (loss-of-function mutations) or with high bone mass and osteosclerotic phenotypes (gain-of-function mutations). Two polymorphisms resulting in an amino-acid change (missense substitutions) in the extracellular domain of the protein (V667M in exon 9 and A1330V in exon 18) have been associated with BMD and/or fracture risk in numerous cohorts, and repeatedly in men. In a case–control study of men with idiopathic osteoporosis (mean age 50 years), that is in the absence of secondary causes, these two polymorphisms and their haplotypes were associated with a three-fold increased risk of low bone mass. Consistent with these findings, the Rotterdam study confirmed association of LRP5 1330-valine with significantly decreased lumbar spine area and a higher risk of fragility fractures (hip, proximal humerus, and pelvis fractures) in elderly men (OR 1.6, 95% CI 1.0–2.4) but not in women. Moreover, vertebral bone mass and size in adult males, as well as changes over 1 year in lumbar spine BMD and size in pre-pubertal boys, were also significantly associated with these LRP5 variants, whereas no association was found in females. Then two large cohort studies, one monocentric, the other multicentric, demonstrated significant associations of LRP5 genotypes with BMD and/or fractures in both genders. However, the Framingham study showed an interaction between the LRP5 A1330V genotypes and the level of physical activity specifically in men.

**Other osteoporosis candidate genes**

Among the other genes associated with BMD and/or fractures in at least one important study are: bone morphogenetic protein 2 (BMP2), the androgen receptor (AR), parathyroid hormone receptor (PTH1R), leptin receptor (LEPR), sclerostin (SOST), and osteoprotegerin (OPG). With the exception of OPG (see below), most require confirmation. Concerning TGF-\(\beta\), however, a recent pre-planned meta-analysis at the participant level including 20,000 subjects did not confirm any association with osteoporosis phenotypes in either women or men.

**GENOME-WIDE ASSOCIATION STUDIES (GWAS)**

Gene chips development now allows analysis of a large number of SNPs simultaneously, that is up to 5% of the nearly 12 million SNPs currently mapped through...
the human genome. This approach has opened new horizons for the discovery of genetic variations associated with osteoporosis and other common disorders such as diabetes. The first reported genome-wide association study (GWAS) in osteoporosis analyzed 100,000 SNPs with BMD, bone ultrasound properties, and hip geometry indices. Tens of polymorphisms probably associated \((P \leq 10^{-6})\) with some of these phenotypes were identified. Among them, some markers within or near known osteoporosis candidate genes were also found, including ESR1, LRP5, COL1A1 and CYP19; however, their statistical \(P\) values were not the most impressive. Most recently, two major GWAS using 300,000 validated SNPs were published. These two studies also included a large number of subjects from several independent cohorts, allowing confirmation of signals. Thus Richard et al definitely identified the OPG gene (TNFSRF11B) in association with BMD and the risk of osteoporosis \((+20\% \text{ in carriers of the 'risk' polymorphism})\). They also demonstrated that OPG expression in lymphocytes was dependent on the TNFSRF11B alleles. This study also confirmed strong association of LRP5 with BMD and fracture risk \((+30\%)\) (Table 2). Note that only few men were included; these results therefore pertain to osteoporosis in women. Styrkarsdottir et al first identified 77 SNPs associated with BMD in a cohort for Iceland. After replication in different cohorts from Denmark, Australia and Iceland again, association with BMD was confirmed for OPG (TNFSRF11B), its biological partner and osteoclastogenic factor RANKL (TNFSF11), estrogen receptor \(\alpha\) (ESR1) and a nearby chromosomal locus \((6q25)\), the major histocompatibility complex locus \((\text{MHC, chromosome 6p21})\), and a region on chromosome 1p36 without any known gene. TNFSRF11B, MHC et 1p36 were also associated with fracture risk. Moreover, these results could be expanded in the near future (that is, with further replication cohorts) since dozens of other SNPs were potentially associated with BMD/osteoporosis with statistical \(P\) values lower than those required to demonstrate genome-wide levels of significance \((\text{i.e. } P \leq 10^{-8})\).

CONCLUSIONS AND PERSPECTIVES

The contribution of genetic factors to osteoporosis is most important. Numerous large cohort studies as well as meta-analyses have started to clearly delineate the association of certain polymorphisms with BMD and/or fracture risk, for instance related to the LRP5 gene. GWAS now provide new perspectives to identify an ever-increasing number of genetic susceptibility factors for osteoporosis. It should be emphasized, however, that the contribution of each SNP so far identified to BMD or fracture risk is extremely modest, usually less than 1–3\%, in keeping with the notion that osteoporosis is a complex disorder with multiple genetic and environmental determinants. We propose that by including environmental factors and their interaction with genetic factors in the models to test for association, a few gene polymorphisms with larger effects could actually emerge, particularly among the ones already identified. Accordingly, we must foresee the development of genetic markers as new tools in the prediction models to evaluate the individual fracture probability.

REFERENCES


