Genetics of Osteoporosis

2

Francesca Marini, Laura Masi, Gemma Marcucci, Luisella Cianferotti, and Maria Luisa Brandi

2.1 Introduction

Osteoporosis is a clinical condition of the skeleton, defined when value of bone mineral density (BMD) is lower than 2.5 standard deviations from the mean value of the young adult population (T-score values), usually measured at the lumbar spine (L1–L4) and femoral neck. Low bone mass is associated with deterioration in micro-architecture and geometry of the skeleton, and with a deregulated bone turnover, resulting in an excessive bone resorption and a reduced novel bone formation. The final clinical endpoints of osteoporosis are fragility fractures, mainly at the wrist, spine, and femoral neck that occur in about 30% of postmenopausal women and 12% of elderly men [1] and are responsible for the morbidity and mortality of the disease.

Bone strength is the parameter to measure the risk of fracture, and it is principally determined by the combination of BMD, bone size, and bone quality. For years BMD has been the only one measurable marker for assessing osteoporosis and fracture risk, and also today it is widely used to define the osteoporosis status. However, it is now well assessed that BMD value alone is not sufficient to determine the real risk of develop osteoporotic fracture, and other important parameters of bone quality (such as bone architecture and bone metabolism) have to be taken into account.

Osteoporosis risk depends by the failure to acquire the optimal bone mass peak during growth and by the capacity of maintain bone mass during the elderly and aspects that are both regulated by numerous dietary, lifestyle, hormonal, and genetic factors. Deficiency of calcium and/or vitamin D during childhood and adolescence may be responsible for the reduction of bone mass peak, while during the adulthood

F. Marini · L. Masi · G. Marcucci · L. Cianferotti · M. L. Brandi (✉)
Department of Surgery and Translational Medicine, University of Florence, Florence, Italy
e-mail: f.marini@dmi.unifi.it; laura.masi@aouc.unifi.it; luisella.cianferotti@unifi.it; marialuisa.brandi@unifi.it

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and elderly may have a fundamental negative role in increasing bone mass loss. The rapid decrease of estrogens at menopause strongly contributes to a rapid bone loss in postmenopausal women, and it is one of the main causes of the higher incidence of osteoporosis in women.

Today, it is well assessed that osteoporosis is a multifactorial complex disorder whose pathogenesis is due to the interaction and synergic effects of various predisposing genetic determinants regulating bone and mineral metabolism, of "non-skeletal" risk factors that could influence the risk of falling (i.e., muscle strength, balance, and visual acuity), of environmental influences, and of dietary and lifestyle habits.

2.2 Genetic Contribution to Osteoporosis

Principal skeletal determinants of osteoporosis predisposition and fragility fracture risk, such as BMD, bone geometry, and bone metabolism, are all under strong genetic influences. Major advances in the knowledge of genetic aspects of osteoporosis and fracture risk have been made in the last two decades, and they have been principally derived by study on monogenic bone diseases, linkage analyses in osteoporotic pedigrees, association case-control and population-based studies for candidate genes, and experimental crosses in animal models.

Twin and family studies allowed to assess that about 60–85% of human BMD variability is under control of genetic factors [2, 3], and the heritability of other bone characteristics, such as bone geometry and bone turnover markers, ranges between 50 and 80% [4, 5]. Moreover, genetic factors demonstrated to regulate up to 80% of individual variability of bone mass peak acquisition [6], acting principally before puberty. Conversely, the effect of genetic influences on fracture risk is less than 30% [7], maybe because fracture is a more complex phenotype that is determined not only by bone density and quality but also by other non-skeletal conditions.

Several genes have been associated with bone mass and other determinants of bone quality and fracture risk, but each of them has demonstrated to exert only a relatively modest single effect on bone tissue, suggesting that osteoporosis is the result of the synergic effect of various predisposing genetic variants, within different genes, in association with environmental and lifestyle risk factors. To date, more than 100 candidate gene polymorphic variants have been tested for their association with BMD, fractures, and other bone-related quantitative trait loci (QTLs).

Briefly, we reported data about studies on major genes involved in osteoporosis and related phenotypes, discussing the effect of their polymorphic variants on bone mass, bone quality, and metabolism.

2.2.1 Lipoprotein Receptor-Related Protein 5 (*LRP5*) and Lipoprotein Receptor-Related Protein 6 (*LRP6*) Genes

These two genes are discussed together since they form a receptor complex with frizzled (Fz) to activate the transcriptional activity of the beta-catenin within the

Wnt signaling pathway that is involved in the regulation of osteoblast commitment, differentiation, and apoptosis, in the synthesis of bone matrix protein and mineralization process, as well as in the coupling to osteoclasts and induction of bone resorption [8]. Inactivating mutations of the *LRP5* gene are responsible for the osteoporosis pseudoglioma (OPPG), an autosomal recessive monogenic Mendelian disorder, characterized by severe early juvenile osteoporosis, very low bone mass, and fragility fractures. Conversely, activating mutations of the *LRP5* gene result in sclerosing bone dysplasias, clinical conditions characterized by an excessive bone mass. Due to its role in the development of these two rare inherited bone disorders, *LRP5* has been suspected as a key regulator of bone mass, and common polymorphic variations of this gene have been investigated, by association studies, for their relationship with BMD and fragility fracture in the general population. The two most investigated variants were the missense single nucleotide polymorphism (SNP) c.2047G>A, the Val667Met in exon 9 (rs4988321), and the missense SNP c.4037C>T, Ala1330Val in exon 18 (rs3736228). Both c.2047A and c.4037T alleles were associated with reduced lumbar bone mineral content, vertebral bone area, and stature in Caucasian men, but not in women [9], accounting for up to 15% of variance for these traits. In the same year, a study on young Korean men failed to find any association between *LRP5* polymorphism and peak bone mass and BMD at any site [10]. In a case-control study on middle-aged men (mean age 50 years) with idiopathic osteoporosis, both the rare alleles of these two polymorphisms and their haplotype have been associated with a threefold high risk of low BMD [11]. In 2006 the Rotterdam Study confirmed the association between the 1330Val allele and a reduced lumbar spine area and a higher risk of fracture at the femur, humerus, and pelvis in elderly men, but not in women [12]. The same study evidenced an interaction between the 1330Val allele and a missense SNP Ile1062Val in the *LRP6* gene (rs2302685), showing that 1330Val and 1062Val alleles have a synergic effect on fracture risk [12]. In 2008 a Bayesian meta-analysis on 10 association studies, including a total of 16,705 individual (of whom the great majority were women (8444) aged 18–81 years) indicated that 1330Val variant has a modest association with BMD and authors concluded that this aspect may limit its clinical use [13]. More recently, a prospective, multicenter, and large-scale study on 37,534 individuals from 18 participating teams in Europe and North America by the GENOMOS study group confirmed that genetic variations of the *LRP5* gene are associated with both BMD and fracture risk, very consistently across analyzed populations but with a modest clinical effect [14]. Conversely, the Ile1062Val SNP of *LRP6* did not show a significant association with BMD [14].

2.2.2 Vitamin D Receptor (*VDR*) Gene

Bioactive form of vitamin D is fundamental for the acquisition of bone mass pick and for the maintenance of bone homeostasis. It acts through its binding to the vitamin D receptor (VDR). Mutations of the *VDR* gene cause the syndrome of vitamin-resistant rickets a recessive Mendelian condition, characterized by severe rickets,

hypocalcemia, and hypophosphatemia, which is resistant to vitamin D supplementation. Due to the importance of vitamin D in bone metabolism, *VDR* has been the first candidate gene whose polymorphic variants have been analyzed in association studies for osteoporosis in 1994, showing that common allelic variants of *VDR* can be used to predict differences in BMD, accounting for up to 75% of the total genetic effect on BMD in healthy individuals [15]. Association studies between *VDR* and osteoporosis have been principally focused on two polymorphisms in intron 8 (*BsmI* and *ApaI*), one silent polymorphism in exon 9 (*TaqI*), a polymorphism affecting exon 2 and creating an alternative start codon and responsible for two different isoforms of VDR protein which differ in length by three amino acids (*FokI*), and a functional polymorphism in the promoter region at the binding site for the transcription factor Cdx-2. *BsmI*, *ApaI*, and *TaqI* are in linkage disequilibrium, and maybe they are also in linkage disequilibrium with other sequence variations in the 3′ untranslated region (UTR) of the *VDR* gene that could affect mRNA stability and, thus, VDR protein expression. Numerous association studies have been published, presenting conflicting and/or inconclusive data, maybe due to inadequate population sampling, ethnicity, gender, age, confounding factors, gene-gene interactions, and gene-environment interactions; a linkage disequilibrium between *VDR* polymorphisms and other bone metabolism genes cannot be excluded. Today, results of association studies on large populations seem to strongly reduce the role of *VDR* polymorphisms in the risk of osteoporosis and fragility fractures. The GENOMOS study (26,242 participants; 18,405 women) evaluated association between *Cdx-2*, *FokI*, *BsmI*, *ApaI*, and *TaqI* polymorphisms, and DXA-measured femoral neck and lumbar spine BMD, and fractures concluding that *FokI*, *BsmI*, *ApaI*, and *TaqI* are not associated with BMD or with fractures, and only *Cdx-2* showed a very modest effect on the risk of vertebral fractures [16].

A haplotype meta-analysis by Thakkinstian et al. [17] evidenced that *VDR* single polymorphisms were not significantly associated to osteoporosis, while specific *BsmI/ApaI/TaqI* haplotypes were significantly associated to the clinical condition. Data from this study seem to indicate a gain in power when considering *VDR* haplotypes rather than polymorphisms separately, demonstrating the importance of haplotype studies rather than single polymorphism studies for the *VDR* gene.

In addition, some studies suggested a possible interaction between calcium and vitamin D intake and *VDR* polymorphisms in the regulation of BMD [6, 18], with the possibility that effect of *VDR* genotypes on BMD would be visible only in the presence of a low calcium intake [19] or a vitamin D deficiency. Conversely, the association between *VDR* genotypes and bone mass would be hidden by high calcium and/or vitamin D intake.

2.2.3 Estrogen Receptor Alpha (*ERα*) Gene

Estrogens are very important for the correct bone metabolism, for the skeletal growth, and for the maintenance of bone mass. Indeed, severe depletion of estrogens at menopause results in a rapid loss of bone mass, and it is one major cause of

higher incidence of osteoporosis and fragility fractures in women than in men. Estrogens exert their action on bone cells through their specific steroid receptors (ERs). An inactivating mutation of the estrogen receptor alpha (*ERα* or *ESR1*) gene was identified in men affected by severe juvenile osteoporosis. This fact prompted *ERα* as an important candidate gene for osteoporosis. *ERα* and, very less frequently, estrogen receptor beta (*ERβ* or *ESR2*) genes have been widely studied about the association of their polymorphisms with osteoporosis and fragility fractures at the wrist, hip, and spine. In the last two decades, a large number of studies investigated about an association between *ERα* polymorphisms and bone mass, mostly focusing on two SNPs in the intron 1 of the gene, recognized, respectively, by the *XbaI* and *PvuII* restriction enzymes, and on a variable TA repeat in the promoter region. *PvuII* maps within consensus recognition sites for AP4 and Myb transcription factors and influences Myb-associated transcription in vitro [20]. Both *XbaI* and *PvuII* have shown to influence report gene transcription in vitro [21]. These data suggest a direct functional effect of *XbaI* and *PvuII* on *ERα* expression, but it is also possible a linkage disequilibrium with other functional polymorphic variations within *ERα* gene and/or contiguous genes.

Association studies between *ERα* polymorphisms and BMD showed inconsistent and controversial results. A meta-analysis by Ioannidis et al. [22], including more than 5000 women from 22 different studies (of which 11 including Caucasian women and 11 including Asian women), evidenced an association between *XbaI* genotypes and both BMD and fractures, with the XX genotype (*XbaI*) resulting associated with higher femur and spine BMD values (+1 to 2%) and with a reduced risk of fractures.

In 2004, the GENOMOS study group performed a large-scale association study between *XbaI*, *PvuII*, and TA repeat polymorphisms of *ERα* (both as single polymorphism and as haplotypes) and both BMD and occurrence of fragility fractures in 18,917 unrelated individuals from eight European centers [23]. None of the three polymorphisms or haplotypes showed any statistically significant effect on BMD. Conversely women with the homozygote XX genotype of *XbaI* had a reduced incidence of 19% for all fractures and of 35% for vertebral fractures. No significant effects on fracture risk were seen for *PvuII* and TA repeats. The study seems to indicate *XbaI* as a risk marker for fracture, independently by BMD values [23].

Very few studies investigated the role of polymorphic variants of *ERβ* in determining BMD and fracture risk, principally focused on a CA repeat in the intron 5 of the gene. The Framingham study analyzed the association of this genetic variation and four other intronic polymorphisms with BMD in 723 men and 795 women [24]. The CA repeat genotypes resulted associated with femoral BMD but not with the spine BMD, both in women and in men. Two other SNPs, *rs1256031* and *rs1256059* (respectively, in the intron 11 and the intron 15 of *ERβ*), showed an association with femoral BMD in men, and *rs1256031*, in particular, accounted for up to 4.0% difference in mean femoral BMD. The haplotype C-23CA-T (*rs1256031*, CA repeat, *rs1256059*) was significantly associated with reduced femoral BMD in women, with BMD value differences ranging from 3.0 to 4.3%. In the same year, the CA repeat was investigated for its association with BMD in 226 healthy

postmenopausal women (60–98 years), evidencing that women with less than 25 CA repeats had significantly higher BMD at the total skeleton, lumbar spine, and femoral neck with respect to women bearing more than 25 CA repeats [25].

Two years later a large population-based cohort study analyzed the association of *ERβ* polymorphisms with risk of vertebral and incident fragility fracture in postmenopausal women, alone or in association with polymorphisms of *ERα* and insulin-like growth factor I (*IGF1*) genes, showing a synergic effect of genotypes interaction on fracture risk, and, thus, reinforcing the idea of the polygenic and complex nature of osteoporosis [26].

2.2.4 Aromatase Gene (*CYP19*)

The *CYP19* gene encodes for aromatase, the enzyme responsible for estrogen synthesis by catalyzing the aromatization of C19 androgens to C18 estrogens. Inactivating mutations of *CYP19* cause aromatase deficiency, and they have been associated to clinical conditions affecting also bone growth and mineralization. Common polymorphisms of *CYP19* have been, in vitro, associated with enzymatic activity. A study by Masi et al. first reported an association between a tetranucleotide (TTTA) repeat polymorphism in intron 4 of the *CYP19* gene and BMD in postmenopausal Italian women [27]. The association of these polymorphisms with BMD was also studied in Italian elderly men but without evidencing a statistical significance [28]. The association between TTTA repeat and BMD was not confirmed in Finnish early postmenopausal women [29]. Another study reported an association between a common SNP in the 5′ untranslated region (UTR) of *CYP19* (rs1062033) and BMD in Spanish late postmenopausal women [30]. More recently, six polymorphisms (rs4646, rs10046, rs3784307, rs1062033, rs936306, and rs190258), located throughout the entire *CYP19* gene (including also the 5′ and 3′ UTRs), were associated with bone mass in 286 Spanish postmenopausal women [31]. The rs10046 SNP in the 3′UTR resulted associated with BMD; the postmenopausal decrease in bone mass appeared to be slower in women with the AA genotype, than in those with AG or GG genotypes. This polymorphism is in strongly linkage disequilibrium with the TTTA repeat and the rs4646 SNP in the 3′UTR, and they are all three associated with BMD. Two SNPs, located in exon I.6 and promoter I.6 of *CYP19*, were analyzed in a cohort of 256 Spanish postmenopausal women [32], and rs4775936 was associated with lumbar spine BMD, with the homozygote AA genotype exhibiting a significantly higher lumbar spine BMD if compared with GG or GA women.

Association of *CYP19* functional polymorphisms with BMD and/or fracture was also confirmed by other studies on different populations [33–37].

2.2.5 Collagen Type I Alpha I (*COLIA1*) Gene

Collagen type 1 is the most represented protein of bone extracellular matrix (about 80% of total proteins in bone tissue). Alterations of collagen synthesis, properties,

and relative quantity of its two chains affect mechanical features of bone tissue and increase susceptibility to fragility fractures. Inactivating mutations of the gene encoding the alpha I chain of type I collagen (*COLIA1*) are responsible for *osteogenesis imperfecta*, a hereditary Mendelian disorder characterized by severe osteoporosis and skeletal fracture in early life. Therefore, *COLIA1* is one of the principal candidate genes for fragility fractures in osteoporosis. A common polymorphism in the intron 1 of the *COL1A1* gene, (Sp1 polymorphism, rs1800012) alters the binding site for the Sp1 transcription factor, affecting *COL1A1* transcription and resulting in an alteration of the normal equilibrium between α_1 and α_2 chains (2:1). In particular, the s allele has an increased affinity for Sp1, resulting in a higher amount of α_1 with respect to α_2 chain; the Ss genotype is responsible for a collagen chain ratio of 2.3 (respect to the normal 2, typical of the SS genotype) [38]. Association studies evaluated the effect of Sp1 polymorphism on BMD and fragility fractures, showing a mild association with BMD values but a stronger relationship to osteoporotic fractures, particularly at the spine [38–41]. In particular, a higher prevalence of fragility fracture was found among ss and Ss genotypes with respect to the SS genotype [38–41], with an increase in fracture risk of about 68% for each copy of the s allele and independently by a significant reduction of BMD value [38].

The GENOMOS study evaluated *COLIA1* Sp1 alleles as a predictor of BMD and fracture in 20,786 unrelated individuals from several European countries and found only a modest association between the ss genotype and reduced BMD; no reduction of BMD was observed in Ss individuals [42]. Moreover, the s allele could predispose to incident vertebral fractures in women, but not in men, and the association with vertebral fracture has a 40% increase of risk for each copy of the s allele carried [42], independently by BMD.

A study by Uitterlinden et al. [43] investigated the interaction of polymorphisms of *VDR* and *COLI1A* genes in susceptibility to fractures in 1004 postmenopausal women. The "baT" (*BsmI-ApaI-TaqI*) *VDR* risk haplotype was evaluated in association with ss and Ss *COLI1A* risk genotypes, showing a significant interaction ($p = 0.03$) between *VDR* and *COLIA1* genotype effects. In subjects bearing the SS genotype, the fracture risk was not *VDR* genotype-dependent. Conversely, in subjects carrying ss or Ss genotypes, the contemporaneous presence of the baT haplotype was associated with a higher risk of fracture of 4.4 and 2.1, respectively [43].

Moreover, an additive effect of the *COLIA1* Sp1 polymorphism with 10565insGGA polymorphism of the sclerostin (*SOST* gene) was evidenced in an elderly male and female Caucasian healthy population [44].

Data from these two studies further confirmed the polygenic nature of osteoporosis and fracture risk.

2.2.6 Transforming Growth Factor Beta (*TGF-β1*)

Transforming growth factor beta (TGF-β1) is largely expressed by osteoclasts, and it has shown to control bone resorption and formation by directly acting on

both osteoblasts and osteoclasts [45]. Therefore, polymorphic variants of *TGF-β1* gene have been extensively studied in relation to osteoporosis. A C/T transition in exon 1 which causes a proline-leucine substitution at position 10 has been associated with higher level of circulating TGF-β1 protein, and the C allele was associated with higher BMD values and lower occurrence of fragility fractures in two Japanese populations [46]. A rare polymorphism in intron 4 (713-8delC variant) was associated with very low BMD, severe osteoporosis, and fracture risk in women with osteoporosis and with low bone mass and increased bone turnover in both osteoporotic and normal women [47]. The same research group evaluated, in 2003, the association between 8 polymorphisms of the *TGF-β1* gene and osteoporosis in a case-control study of 96 osteoporotic patients with vertebral fractures vs 330 normal individuals, evidencing that the TT genotype of the 816-20 T>C variant in the intron 5 was less common in fractured osteoporotic patients than in healthy controls and that it was associated with higher lumbar spine and hip bone mass [48].

The GENOMOS study investigated associations between five *TGF-β1* polymorphisms [G–1639A (G–800A, rs1800468), C–1348T (C–509T, rs1800469), T29C (Leu10Pro, rs1982073), G74C (Arg25Pro, rs1800471), and C788T (Thr263Ile, rs1800472)] and BMD and fractures in 28,924 male and female individuals from 10 different European research studies [49]. Only weak associations between the C–1348T SNP and lumbar spine BMD in men and between the C788T SNP and risk of incident vertebral fractures were reported [49], presumably indicating that polymorphic variations of the *TGF-β1* gene do not play a major role in regulating BMD or susceptibility to fragility fractures.

Recently, a meta-analysis integrated all the eligible studies, including a total of 8 studies involving 1851 cases and 2247 controls, and it investigate whether T869C and T29C polymorphisms of the *TGF-β1* gene were correlated with postmenopausal osteoporosis [50]. A significant association between T29C or T869C polymorphisms and osteoporosis risk was observed only in Asian, but not in Caucasian, population [50].

2.2.7 Other Genes

Polymorphisms of other genes, involved in the regulation of bone metabolism and turnover, have been, although more rarely, investigated about their association with BMD and fractures. They include sclerostin (*SOST*), bone morphogenetic protein 2 (*BMP2*), bone morphogenetic protein 4 (*BMP4*), osteoprotegerin (*OPG*, *TNFRSF11B*), receptor activator of nuclear factor kappa-B (*RANK*; *TNFRSF11A*), RANK ligand (RANKL; TNFSF11), and runt-related transcription factor 2 (*RUNX2*; *CBFA1*).

Principal results from their association and/or linkage studies are depicted in Table 2.1.

Table 2.1 Other candidate genes in osteoporosis association and/or linkage studies

Gene	Role on bone tissue	Analyzed polymorphisms	Analyzed population	Association of each polymorphism with BMD, osteoporosis, and/or fracture	References
SOST (sclerostin)	SOST is a secreted osteoclast-derived BMP antagonist that represses BMP-induced osteoblast differentiation and/or function	rs1230399 (−9247 T/C)	1243 Chinese subjects with low BMD	1. Significant genotypic/allelic associations with the spine, femoral neck, trochanter, and total hip BMD 2. The T allele increases the risk of osteoporosis	[51]
SOST (sclerostin)		rs1513670 rs1107748	1012 Chinese healthy women	1. Significant association between rs1513670 and total hip BMD 2. Significant association between rs1107748 and osteoporotic fracture	[52]
SOST (sclerostin)		rs10534024 rs9902563	1383 Danish men	Significant association of rs9902563 with femoral neck BMD	[53]
SOST (sclerostin)		rs1234612 rs1513670 rs1634330 rs1708635 rs2023794 rs7220711 rs74252774 rs851057 rs851058 rs865429	Original study: 703 healthy postmenopausal Chinese women Update study: 1379 healthy postmenopausal Chinese women including 703 from the previous study	The original study failed to identify any significant association between SOST SNPs or haplotypes and BMD In the update study: the CC genotype of rs2023794 and the TT genotype of rs74252774 were associated with higher BMD values at lumbar spine (but not at hip), than other genotypes	[54] [55]
SOST (sclerostin)		C4102G C10356T 10565insGGA G11988A C17965G A18292G T42722C A58874G A75707G	1939 Dutch white elderly men and women	1. The 3-bp insertion of 10565insGGA was associated with decreased BMD at the femoral neck, in women 2. The G allele of A75707G was associated with increased BMD at the femoral neck, in men	[44]

(continued)

Table 2.1 (continued)

Gene	Role on bone tissue	Analyzed polymorphisms	Analyzed population	Association of each polymorphism with BMD, osteoporosis, and/or fracture	References
BMP2 (bone morphogenetic protein 2)	BMP2 acts in osteoblast differentiation	Ser37Ala Arg190Ser	Caucasian elderly women and men from the Rotterdam study	No association with BMD, bone loss, hip structural analysis, and incident fractures	[56]
BMP2 (bone morphogenetic protein 2)		Ser37Ala (rs2273073) rs235710 rs235767 rs235754	1059 young Swedish women (over 25 years) 1044 elderly Swedish women (over 75 years)	1. No association with BMD and fracture 2. rs235754 was associated with the ultrasound parameters speed of sound and stiffness	[57]
BMP2 (bone morphogenetic protein 2)		Ser37Ala Ala94Ser Arg189Ser	1. Osteoporosis families from Iceland 2. A cohort of Danish postmenopausal women with persistently low BMD 3. A group of Danish postmenopausal osteoporotic fracture patients	Ser37Ala showed a strong association with osteoporosis	[58]
BMP4 (bone morphogenetic protein 4)	BMP4 is a potent osteotropic factors, acting on osteoblasts and promoting bone formation in vivo and in vitro	rs1957860 rs2855528 rs2761885 rs2855532 rs2071047 rs17563	1232 postmenopausal women (mean age 75 years)	1. rs17563 was associated with total and intertrochanteric hip BMD; BMD was 32% lower in the CC genotype 2. No association with fractures 3. The G-C-T haplotype (rs1957860-rs2855532-rs17563) was associated with high bone mass	[59]

TNFRSF11B (OPG; osteoprotegerin)	Osteoprotegerin is a soluble receptor for RANKL and therefore a competitive inhibitor of osteoclast differentiation and activity	A163G T245G T950C G1181C A6890C	1. 217 osteoporotic women and 51 osteoporotic men 2. 255 healthy women and 72 healthy men	1. The G allele of A163G was more common in individuals with spine fractures 2. The G allele of T245G was more common in osteoporotic patients 3. The CC genotype of G1181C was less common among patients with spine fractures	[60]
TNFRSF11B (OPG; osteoprotegerin)		G209A T245G C889T T950C	103 osteoporotic postmenopausal women	1. Statistically significant association of genotypes with BMD at the lumbar spine was observed for G209A and T245G 2. The heterozygote GATG haplotype (G209A and T245G) was associated with lower BMD with respect to the homozygote wild-type GGTT haplotype	[61]
TNFRSF11B (OPG; osteoprotegerin)		Lys3Asn	205 Chinese postmenopausal women	1. The homozygote Asn genotype was associated with a significantly higher BMD at the lumbar spine with respect to the other two genotypes 2. The homozygote Lys genotype had a 2.7 times greater risk for osteopenia/osteoporosis than the homozygote Asn genotype	[62]
TNFRSF11B (OPG; osteoprotegerin)		4752_4753delCT G1181C C1217T G1284A C4501T A6893G A6950C T8738A	60 osteoporotic postmenopausal women	Individuals with the homozygote GG genotype (G1181C) had a significantly lower lumbar spine BMD than subjects with the heterozygote GC genotype	[63]

(continued)

Table 2.1 (continued)

Gene	Role on bone tissue	Analyzed polymorphisms	Analyzed population	Association of each polymorphism with BMD, osteoporosis, and/or fracture	References
TNFRSF11B (OPG; osteoprotegerin)		A163G T950C G1181C	126 postmenopausal Maltese women	1. The TT genotype (T950C) was more frequently associated with low BMD 2. The A-T-G haplotype, from the three polymorphisms, was found to be more frequent in individuals with low BMD	[64]
TNFRSF11B (OPG; osteoprotegerin)		T245G G1181C	478 postmenopausal women	1. Lumbar spine BMD was associated with polymorphisms T245G and G1181C, as well as with CT haplotype 2. Femoral neck BMD showed an association with T245G polymorphism	[65]
TNFRSF11B (OPG; osteoprotegerin)		g.21775C>T g. 23367T>C	336 osteoporotic postmenopausal Chinese women	The TT genotype (g.23367T>C) was associated with lower BMD at the spine, but not at the neck hip or total hip	[66]
TNFRSF11B (OPG; osteoprotegerin)		g.18861A>G g.25548C>T	1. 338 osteoporotic postmenopausal Chinese women 2. 367 healthy postmenopausal Chinese women	The AA genotype (g.18861A>G) was significantly higher in women with osteoporosis	[67]
TNFRSF11B (OPG; osteoprotegerin)		g.27450A>T	1. 441 osteoporotic postmenopausal Chinese women 2. 445 healthy postmenopausal Chinese women	The AA genotype was significantly higher in women with osteoporosis	[68]
TNFRSF11B (OPG; osteoprotegerin)		A163G T245G G1181C	327 postmenopausal Slovak women	The G allele (T245G) was more frequent in subjects with vertebral, non-vertebral, and total fractures	[69]

TNFRSF11A (RANK)	RANK is the receptor for the RANK ligand and is involved in the regulation of osteoclast differentiation and activity	25 polymorphisms (of which 7 were newly identified in the study)	560 postmenopausal Korean women	34863G>A and 35928insdelC polymorphisms were significantly associated with lumbar spine BMD	[70]
TNFRSF11A (RANK)		C421T C575T	1. 100 osteoporotic postmenopausal Turkish women 2. 78 healthy postmenopausal Turkish women	No association with osteoporosis, BMD value, or fracture, both as single polymorphisms and haplotypes	[71]
TNFRSF11A (RANK)		rs996215 rs4603673 rs7239261 rs4500848 rs6567270 rs1805034 rs4303637 rs4941131 rs964662	1026 postmenopausal women	rs7239261 was significantly associated with femoral neck BMD	[72]
TNFSF11 (RANKL; RANK ligand)	RANKL binds to RANK on the surface of osteoclasts and their precursors and leads to their differentiation and survival	rs9594738 rs9525641 rs2277439 rs232485 rs287545 rs2200287 rs953316	1026 postmenopausal women	1. rs2277439 and rs2324851 were significantly associated with femoral neck BMD 2. The haplotype TGACGT (rs9525641-rs2277439-rs2324851-rs2875459-rs2200287-rs953316) was a genetic risk factor for lower femoral neck BMD 3. The haplotype TAGCGT was a genetic protective factor for lumbar spine BMD	[72]
TNFSF11 (RANKL; RANK ligand)		−290C>T −643C>T −693G>C −1594G>A	115 postmenopausal Slovenian women	The CC genotype (−290C>T) was associated with lower BMD than the TT genotype	[73]

(continued)

Table 2.1 (continued)

Gene	Role on bone tissue	Analyzed polymorphisms	Analyzed population	Association of each polymorphism with BMD, osteoporosis, and/or fracture	References
RUNX2 (runt-related transcription factor 2)	RUNX2 is a transcription factor essential to osteoblast differentiation, bone remodeling, and fracture healing	11Ala/17Ala repeat 11GCA>GCG (Ala11Ala)	495 osteoporotic women	1. The A allele (11GCA>GCG) was associated with higher BMD at the lumbar spine, femoral neck, whole body, ultradistal (UD), and mid-forearm sites 2. The A allele (11GCA>GCG) was significantly protective against Colles' fracture in elderly women, but not spine and hip fracture	[74]
RUNX2 (runt-related transcription factor 2)		−330 G>T −1025 T>C	821 postmenopausal Spanish women	The TC genotype (−1025 T>C) was associated with higher mean of femoral neck BMD values with respect to the TT genotype	[75]
RUNX2 (runt-related transcription factor 2)		Complete sequence of *RUNX2* exons and intron-exon junctions	729 postmenopausal Korean women	The homozygote CC genotype of −1025 T>C was associated with reduced lumbar spine BMD and reduced BMDs at proximal femur sites (trochanter and total femur)	[76]
RUNX2 (runt-related transcription factor 2)		−1025 T>C +198 G>A	689 postmenopausal and 87 pre-perimenopausal Spanish women	TC and CC genotypes −1025 T>C were associated with higher femoral neck BMD with respect to the TT genotype	[77]
RUNX2 (runt-related transcription factor 2)		rs7771980	907 healthy postmenopausal Korean women	TC and CC genotypes were associated with a lower vertebral fracture risk with respect to the TT genotype	[78]

2.3 Novel Approaches to the Genetics of Osteoporosis: Genome-Wide Association Studies (GWAS)

Because of the polygenic nature of osteoporosis, in which few genes exert major effects on bone metabolism and homeostasis, while a large number of genes have only minor effects, classical single gene association and/or linkage studies present numerous limitations, such as inconclusive or controversial results, false-positive and/or false-negative associations, reduced sensibility in identifying genotype-phenotype associations, and inability to identify novel candidate genes and their genetic variants. The recent development of next generation sequencing (NGS) technique has allowed to design gene chips for the simultaneous analysis of hundreds genes and their polymorphic variants. Genome-wide association studies (GWAS) have opened new horizons for the discovery of genetic loci and variants associated with osteoporosis and fracture risk, and the application of this novel approach, in the last years, has obtained success in identifying replicated genetic loci associated with osteoporosis.

The first GWAS in osteoporosis was performed in 2007 and analyzed 100,000 SNPs in 1141 individuals from the Framingham Osteoporosis Study to examine genetic associations with bone quantitative traits: BMD (including the femoral neck, trochanter, and lumbar spine), calcaneal ultrasound, and geometric indices of the hip [79]. Of the 40 top SNPs with the highest number of significantly associations with BMD traits, a variable percentage of 30–50% of them maps within genetic loci or near genes that have not previously been studied for osteoporosis. The others were polymorphisms located within known osteoporosis candidate genes, such as rs1884052 and rs3778099 in *ERα*, rs4988300 in *LRP5*, rs2189480 in *VDR*, rs2075555 in *COLIA1* and rs10519297, and rs2008691 in *CYP19*.

One year later, two major GWAS analyzed the association of over 300,000 SNPs with BMD and fractures [80, 81]. The first study [80] evidenced an association between BMD and two SNPs, rs4355801 on chromosome 8 near to the *TNFRSF11B* gene, and rs3736228, on chromosome 11 in the *LRP5* gene. The second study [81] identified five genomic regions significantly associated with BMD, both in the discovery set population and in the replication set populations. Three of these regions map close to or within genes known to be important in bone homeostasis: *TNFSF11*, *TNFRSF11B*, and *ERα*.

In 2009, a large-scale meta-analysis of five GWAS of femoral neck and lumbar spine BMD, including 19,195 individuals of Northern European descent, allowed to identify 20 genetic loci reaching the genome-wide significance (GWS; $p < 5 \times 10^{-8}$). Seven of them confirmed to be known bone-related loci/genes, 1p36 (*ZBTB40*), 6q25 (*ERα*), 8q24 (*TNFRSF11B*), 11q13.4 (*LRP5*), 12q13 (*SP7*), 13q14 (*TNFSF11*), and 18q21 (*TNFRSF11A*), while 13 mapped to new regions, not yet investigated as candidate genes for osteoporosis: 1p31.3 (*GPR177*), 2p21 (*SPTBN1*), 3p22 (*CTNNB1*), 4q21.1 (*MEPE*), 5q14 (*MEF2C*), 7p14 (*STARD3NL*), 7q21.3 (*FLJ42280*), 11p11.2 (*LRP4*, *ARHGAP1*, *F2*), 11p14.1 (*DCDC5*), 11p15 (*SOX6*), 16q24 (*FOXL1*), 17q21 (*HDAC5*), and 17q12 (*CRHR1*) [82].

Two years later, a larger meta-analysis of 17 GWAS of the femoral neck and lumbar spine BMD was performed on 32,961 subjects of European and East Asian ancestry and validated for marker replication of BMD association on 50,933 independent subjects and for association with risk of low-trauma fracture in 31,016 fractured individuals (cases) and 102,444 non-fractured controls [83]. The study identified 56 loci (32 novels) associated with BMD with a positive GWS; 14 of them resulted also associated with fracture risk. Numerous of these loci mapped near or within *TNFRSF11B*, *TNFRSF11A*, and *TNFSF11* genes or near or within genes involved in the Wnt signaling pathways, in the mesenchymal stem cell differentiation and in the endochondral ossification.

GWAS highlighted the highly polygenic and complex nature of osteoporosis and fracture susceptibility and the difficulty to predict the risk of osteoporosis on genetic bases. Anyway, since the first GWAS on osteoporosis was performed in 1997, numerous and great advances have been made in the discovery and validation of genes and loci involved in the predisposition to osteoporosis. GWAS allowed, to date, the identification of more than 60 loci associated with BMD, osteoporosis, and fragility fractures, including novel loci, whose functional analysis has demonstrated that they have a clear effect on bone metabolism and, presumably, also on osteoporosis pathophysiology.

The association of GWAS results with functional studies revealed very useful to identify novel molecular targets for anti-fracture drugs and, thus, allowed the design of novel target therapies for osteoporosis.

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