

Significance and characteristics of selected genes in the pathogenesis of osteoporosis

Význam a charakteristika vybraných génov v patogenéze osteoporózy

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Abstract

Osteoporosis is a complex disease affected not only by environmental factors but also by a strong genetic component. Genetic factors contribute to osteoporosis by affecting not only bone mineral density but also bone size, quality and bone turnover. However, determining the genetic architecture, and in particular the basic genomic and molecular mechanisms of osteoporosis in vivo in humans, is still challenging. In recent years, we have seen progress in research into the genetic background of osteoporosis in connection with the development of modern methods of molecular biology. Scientific research focuses primarily on the identification and characterization of selected polymorphisms of candidate genes, determining bone quality, bone density (BMD) and, last but not least, the risk of fractures. The results of molecular genetic research on osteoporosis significantly contribute / could contribute to the improvement not only of therapeutic and therapeutic procedures, but especially to the introduction of early prevention in personalized medicine.

Keywords: BMD – fracture risk – gene – osteoporosis – polymorphism

Abstrakt

Osteoporóza je komplexné ochorenie ovplyvnené okrem faktorov prostredia aj silnou genetickou zložkou. Genetické faktory prispievajú ku vzniku osteoporózy, tým, že ovplyvňujú nielen kostnú minerálnu hustotu, ale tiež aj veľkosť kosti, jej kvalitu a kostný obrat. Stanovenie genetickej architektúry a najmä základných genómových a molekulárnych mechanizmov osteoporózy in vivo u ľudí, je však stále náročné. V posledných rokoch zaznamenávame pokrok vo výskume genetického pozadia osteoporózy v súvislosti s rozvojom moderných metód molekulárnej biológie. Vedecké výskumy sa zameriavajú predovšetkým na identifikáciu a charakterizáciu vybraných polymorfizmov kandidátnych génov determinujúcich kvalitu kostného tkaniva, úroveň kostnej denzity (BMD) a v neposlednom rade aj riziko fraktúr. Výsledky molekulárno-genetických výskumov osteoporózy významne prispievajú/mohli by prispievať ku skvalitneniu nielen liečebno-terapeutických postupov, ale najmä k zavedeniu včasnej prevencie v personalizovanej medicíne

Kľúčové slová: BMD – gén – osteoporóza – polymorfizmus – riziko zlomenín

Introduction

Osteoporosis is a worldwide disease with a multifactorial etiology. Unlike monogenic diseases, which are usu-

ally caused by mutations in a single gene, osteoporosis is classified as a polygenic disease that is influenced by genetic and environmental factors. Multifactorial dis-

eases are the most common cause of morbidity and mortality in the human population and their incidence is relatively common in the population in connection with many difficult-to-predict pathological conditions [1]. Osteoporosis as a multifactorial and polygenic disease is affected by the interaction of the environment and the so-called genes. Strong and weak effect, as well as interactions between the genes themselves. Studies of monozygotic and dizygotic twins and at-risk families suggest that the influence of genetic factors, the heritability (h^2) of bone mineral density (BMD) is approximately 60–80 %. However, the influence of genetic factors at risk of fractures is reported to be less than 30 %, as the fracture represents a more complex phenotype that is determined not only by bone quality but also by other non-skeletal factors [2]. The remaining 20–40 % of the variability in bone mineral density depends on external factors [3]. Uncontrollable factors leading to osteoporosis include genes and their polymorphisms that affect the phenotype; bone mineral density, bone size, bone macroarchitecture and microarchitecture, bone mineral density (BMD), bone quality, bone metabolic turnover, achievement of PBM, femoral neck geometry, quantitative ultrasound properties of bone [4].

The advent of genomic association studies (GWAS) has made a huge contribution to the study of osteoporosis genetics. Today, the most intensively studied are the *VDR* gene for the vitamin D receptor, the *COL1A1* gene of type 1 collagen and the gene for the estrogen receptor- α (ER α) [5].

Bone fractures are considered to be the most relevant clinical consequences of osteoporosis. The most important determinants of fracture risk include genetic predisposition, aging population, low BMD, female gender, and falls. A positive family history is a risk factor for osteoporosis and fractures. A hip fracture in parents has been included in the FRAX clinical trial algorithm as a risk factor in the last decade. Further findings are based on further genetic studies [6].

To date, association studies of candidate genes for osteoporosis have provided the most information on the relationship of genes to bone variability (bone mineral density, bone quality, and bone metabolism).

Selected candidate genes associated with osteoporosis

Studies of candidate genes focus on the search for genes and their polymorphisms involved in various biological processes of bone physiology of metabolism. In the context of osteoporosis, genes and their polymorphisms encoding many regulators of bone metabolism are being investigated. These regulators include, for example, calcitropic hormones; genes that encode the synthesis, metabolism and receptors of sex hormones, the syn-

thesis of bone matrix proteins; steroid hormones, cytokines, growth factors and their receptors and various local regulators of bone metabolism [7]. These are in particular the vitamin D receptor (*VDR*) gene, the estrogen receptor (*ER*) gene, the type 1 and 2 collagen genes (*COL1A1* and *COL1A2*), the interleukin 1, 6 (*IL1*, *IL6*) gene, *LRP5* (low-density lipoprotein receptor-related protein 5), a gene for transforming growth factor β (*TGF β*). *VDR* was the first gene in 1994 to be analyzed in association studies in relation to osteoporosis. Different gene variants have up to 75 % genetic effect on bone BMD changes. Furthermore, in men with severe juvenile osteoporosis, a mutation in the inactivation of the estrogen receptor- α gene was identified, which determined the significance of this gene for osteoporosis. There are many polymorphisms of candidate genes in relation to osteoporosis and an increased risk of fractures [5]. Table (p. 74ff) shows candidate osteoporosis genes.

Osteoporosis is one of the diseases of civilization, so constant progress in molecular genetic research is important from a therapeutic, diagnostic and economic point of view [8].

RANK/RANKL/OPG system

In the mid-1990s, three signal proteins were discovered in bone metabolism research that play a key role in maintaining bone integrity. These proteins belong to the "superfamily" of tumor necrosis factor (TNF) and together form the RANK/RANKL system/signaling pathway and osteoprotegerin (OPG). Bone mass and bone remodeling are jointly determined by the interaction of both osteoblasts and osteoclasts under the control of these major signaling pathways [9]. The OPG-RANK-RANKL signaling pathway predominantly controls the link between osteoblasts and osteoclast activity [10]. RANK – receptor activator of NF κ B, RANKL – receptors activating NF κ B ligands and OPG – osteoprotegerin after their activation or inhibition could be triggers of many diseases, such as e.g. postmenopausal and glucocorticoid-induced osteoporosis, endocrine diseases, hyperparathyroidism, hypogonadism, chronic inflammatory arthritis, bone marrow transplantation, as well as bone metastases and malignancies [10,11]. This signaling pathway is involved in several physiological and pathological processes, including bone metabolism, mammary gland development, regulation of immune function, tumorigenesis and metastasis of cancer stem cells, thermoregulation, and vascular calcification [12,13].

RANKL (Receptor Activator Nuclear factor KappaB Ligand) is a polypeptide of 317 amino acids. It is a ligand for the RANK signaling receptor (Receptor Activator of Nuclear factor Kappa B) and osteoprotegerin, and is found in the body either as a membrane-bound protein or

as a soluble protein that results from enzymatic cleavage [11,14]. Among other things, it is produced mainly by osteoblasts, it plays an important role in the process of differentiation and activation of osteoclasts. RANKL binds to its signaling receptor RANK (an initiation step in the process of osteoclastogenesis), on the surface of osteoclast precursor cells, leading to the fusion of these cells into multinucleated cells, which then differentiate into mature osteoclasts. RANK is a transmembrane protein that is composed of 616 amino acids. RANK is a receptor for RANKL and is found on mature osteoclast cells and their precursors [11,15]. OPG is an important protein in bone metabolism. OPG is composed of 401 amino acids, was discovered in 1997 as a transporter between the bone, heart and vascular system and provides for the induction of osteoclasts by "competing" with the RANK signaling receptor for RANKL binding. OPG binds RANKL with approximately 500-fold higher affinity than RANK. Thus, OPG prevents RANKL from binding to its RANK receptor, inhibits osteoclastogenesis, and protects bone from osteoclast-mediated excessive resorption [11]. OPG is primarily expressed by stromal cells and bone marrow osteoblasts [9,11]. *OPG* gene polymorphisms are studied in relation to bone mineral density and the development of osteoporosis [16].

The first association study was performed by Langdahl et al, who found a correlation between 163A/G, 245T/G and 1181C/G *OPG* gene polymorphisms and the risk of spinal fractures [17,18]. Mydlářová Blaščáková et al [19] based on molecular-genetic analysis of the A163G *OPG* gene polymorphism found significant differences in genotype frequencies when comparing the control and osteoporotic groups of Slovak postmenopausal women. The authors state that the presence of the G-allele of the investigated polymorphism has a different effect on bone metabolism in two ethnically different groups – Roma and non-Roma [19].

Lipoprotein receptor-related protein 5 (*LRP5*)

The low-density lipoprotein receptor-related protein 5 (*LRP5* gene) is a gene encoding a low-density transmembrane lipoprotein receptor. It is located on chromosome 11q12–13. It is a member of the LDL receptor family. This gene encodes a 1615 amino acid transmembrane protein and contains 23 exons. In bone, the *LRP5* gene is expressed mainly in osteoblasts on endosteal and trabecular bone surfaces, but not in osteoclasts that do not lead to bone repair. It has been discovered as a key regulator of bone formation and its association with fractures has also been observed [20,21,23].

LRP5 gene is important in regulating the amount of minerals in bone, regulating the proliferation and differ-

entiation of osteoblasts (the cells that make up bone tissue) during bone development, thereby affecting bone density, which gives bones overall strength and reduces the risk of fractures. Therefore, it plays an important role in diseases related to changes in bone density caused by mutations in this gene [22].

Mydlářová Blaščáková et al [24] conducted a study to evaluate selected anthropometric parameters (body weight, body height, waist circumference and hip circumference), densitometric parameters (BMD and T-score in the left hip, vertebrae L1-L4) and molecular genetic analysis of polymorphism rs599083 *LRP5* gene. The research group consisted of 96 postmenopausal women (osteoporotic women 31, osteopenic women 50, control groups 15) aged 38–86 years. Based on molecular genetic analysis of the investigated polymorphism of the rs599083 *LRP5* gene, the authors found that the lowest percentage in the whole group had the risk genotype GG, while calculating the relative risk did not confirm a possible association between the G-allele and increasing risk of osteoporosis [24].

Park et al [25] in their study focused on monitoring the association of ten single nucleotide polymorphisms/SNPs (*ZBTB40* rs6426749, *MEF2C* rs1366594, *ESR1* rs2941740, *TNFRSF11B* rs3134070, *TNFRSF11B* rs2073617, *SOX6* rs711785, *TNFSF11* rs227438, *TNFSF11* rs9594782, *FOXL1* rs10048146 and *LRP5* rs599083) and bone mineral density (BMD). The study involved 494 men and 493 postmenopausal women from Korea. A significant association of the rs599083 *LRP5* gene polymorphism with BMD values in the femoral neck region was demonstrated, but the association of the rs599083 *LRP5* gene polymorphism with BMD in the lumbar spine was not statistically significant [25].

Horváth et al [26] examined 932 Hungarian postmenopausal women in their scientific study (osteoporotic n = 90, osteopenic n = 455, control group n = 344). The authors of this study found a significant association between the SNR rs4988300 of the *LRP5* gene and total hip BMD values in the largest cohort in the largest homogeneous postmenopausal study group to date [26].

Dominant mutations causing loss of function in the *LRP5* protein are among the most common causes of familial exudative vitreoretinopathy (FEVR), with low BMD. Norwitz et al [20] emphasize the possible finding that *LRP5* haploinsufficiency affects BMD in men to a greater extent than in women.

Collagen gene (*COL1A1* and *COL1A2*)

The basic protein in connective tissue – bones and skin – is collagen type 1. Collagen 1 forms a heterotrimer consisting of protein chains $\alpha 1$ and $\alpha 2$, which are respectively encoded by the genes *COL1A1* and *COL1A2*. In type 1 collagen, the

$\alpha 1$ chain synthesizes the *COL1A1* gene and the $\alpha 2$ chain synthesizes the *COL1A2* gene. C-propeptides are important during heterotrimer formation because they are responsible for the correct folding of the chains. Levels of its serum propeptides N and C are thought to be involved in bone matrix synthesis. The *COL1A1* and *COL1A2* genes are one of the major candidate genes for osteoporosis and their polymorphisms have been studied in relation to BMD and osteoporotic fractures [27]. The gene for type 1 collagen (*COL1A1*) is located on chromosome 17 of the long arm (q) – 17q21.33 [28].

The most studied polymorphism of the *COL1A1* gene is the Sp1 polymorphism. This polymorphism binds to the binding site of specific protein 1 and is involved in the regulation of *COL1A1* transcription and bone biochemical processes. It leads to a reduction in bone mass by changing the normal 2 : 1 ratio between the collagen chains $\alpha 1$ and $\alpha 2$, which in turn disrupts bone mineralization and increases the risk of fractures. The polymorphism of the Sp1 *COL1A1* gene is based on the genetic substitution of nitrogenous bases – guanine with thymine. The S-allele corresponds to guanine and the thymine allele, which has a higher binding affinity for the SP1 transcription factor than S. Carriers of the ss genotype have a 2.7 times higher risk of fractures than carriers of the S-allele [29].

Chen et al [30] analyzed risk factors determining the incidence of fractures in postmenopausal Taiwanese women. The research focused on association studies comparing mean values (age, age of onset of menopause, body height, body weight, and BMI) between a group of osteoporotic postmenopausal women (n = 50) and a control group of postmenopausal women (n = 50). They recorded statistical significance in the study results in association with the onset of menopause and height [30].

The significant association of GG, GT genotypes of the rs1107946 polymorphism of the *COL1A1* gene with BMD in the lumbar vertebrae and hip region in the population of postmenopausal Caucasian women was also confirmed by Xie et al [31]. However, in their study, they found no significant correlation between SNS rs1107946 of the *COL1A1* gene and the risk of osteoporotic fractures in the hip, femoral neck and lumbar vertebrae [31], in contrast to Ramirez et al [32], who in their study in Mexican postmenopausal women found an association of this polymorphism rs1107946 *COL1A1* gene (specifically the G-allele) with an increased risk of hip fracture [32]. Yu et al [33] also analyzed the distribution of individual SNP genotypes rs1107946 of the *COL1A1* gene, with no statistically significant difference [33]. Majchrzycki et al [34] conducted a study in Polish postmenopausal women diagnosed with osteoporosis (n = 90) and osteopenia (n = 90), in which they analyzed two polymorphisms of

the *COL1A1* gene: rs1800012 and rs1107946, but also did not record statistical significance [34].

In the Caucasian population, the Sp1 *COL1A1* gene polymorphism may be important in identifying individuals at risk for osteoporosis and an increased risk of osteoporotic fractures (particularly of the spine) in postmenopausal women. In this group of women, the presence of the SS genotype in the Sp1 polymorphism was found to be associated with decreased BMD in puberty – juvenile idiopathic arthritis [7].

In a study of the interaction of *COL1A1* and *VDR* gene polymorphisms with osteoporotic fractures, the risk haplotype of the baT (BsmI-ApaI-TaqI) *VDR* gene was evaluated in association with the genotype of the ss and Ss *COL1A1* genes. The authors found a significant interaction between the genotypes of the *VDR* and the *COL1A1* gene. In individuals with the SS genotype, the risk of fracture was independent of the *VDR* gene genotype, but in individuals with the ss and Ss genotypes, the co-presence of the baT haplotype was associated with a higher risk of fracture [7].

CER1 gene

Another candidate gene for osteoporosis is the *cerberus 1* gene, referred to as the *CER1* gene. The *CER1* gene, otherwise called *DAND4*, is a protein encoding a cytokine. This cytokine encoded by the *CER1* gene belongs to the group of cysteine genes. The *CER1* gene, together with DAN and DRM/Gremlin, belongs to a group of bone morphogenetic protein (BMP) antagonists that are associated with bone mineral density (BMD), early onset menopause and are important for proper skeletal development and bone turnover in patients with osteoporosis. The *CER1* gene is located on the short arm of chromosome 9 at position 9p22.3 and has described five polymorphisms (rs1494360, rs17289263, rs3747532, rs7022304 and rs74434454) [35,36].

These polymorphisms were investigated by Koromila et al [37], who in their study examined the association of the *CER1* gene in connection with osteoporosis in 300 postmenopausal Greek women (200 osteoporotic and 100 control groups). In the investigated polymorphism of the rs74434454 *CER1* gene, the distribution of genotypes in women diagnosed with osteoporosis was as follows – TT (65.60%), TC (34.40%), CC (0%) and in the control group of postmenopausal women: TT (90.10%), TC (9.90%), CC (0.0). In the group of osteoporotic women with the TT genotype, women had a confirmed risk of spinal fractures and women with the TC genotype are expected to have an increased risk of femoral neck fracture [37].

Koromila et al. 2013 examined the effect of all 5 polymorphisms of the *CER1* gene and examined the correla-

tions between these polymorphisms and BMD, early onset of menopause, and bone turnover markers in 607 Greek women (457 postmenopausal and 150 healthy women). They state that osteoporotic women start menopause 2 years earlier than in the control group of healthy women. In addition, postmenopausal women in the research group who overcame a femoral neck fracture had menopause significantly earlier than healthy control women who did not overcome the fracture. All 5 polymorphisms of the *CER1* gene were significantly associated with BMD, which means that not only homozygotes but also heterozygotes have a significantly higher potential risk of fractures. The increased risk of lumbar fracture is mainly associated with the rs1494360 *CER1* gene polymorphism. For polymorphisms rs7022304 (AA, AG, GG), rs3747535 (CC, CG, GG) of the *CER1* gene, the authors report higher serum values of calcitonin (CT) and parathyroid hormone (PTH) in the group of postmenopausal women with osteoporosis. On the other hand, decreased levels of osteocalcin (OC) and insulin-like growth factor 1 (IGF1). Koromila et al [38] confirmed a statistically significant association between decreased osteocalcin (OC) levels and femoral neck fractures in this study in women diagnosed with osteoporosis. *CER1* gene polymorphisms play an important role in the determination of osteoporosis, and the authors also report a potential predictive role along with bone markers in postmenopausal osteoporotic women [38].

Huang, Ng and You-Qiang [3] examined 1,083 individuals associating the rs3747532 *CER1* gene polymorphism with an increased risk of low BMD and vertebral fractures [3].

Mydlárová Blaščáková et al [39] focused on the determination of the rs74434454 *CER1* gene polymorphism in 101 Slovak postmenopausal women, while they did not record statistical significance in the genotypes of this polymorphism between the osteoporotic and control groups [39].

Gene encoding vitamin D receptor (*VDR*)

The vitamin D receptor (*VDR*) is one of the transcriptional regulatory factors. These factors regulate the proteins that participate in the acquisition of bone mass and the maintenance of bone homeostasis. The vitamin D receptor gene was the first candidate gene to investigate the molecular genetic basis of osteoporosis. *VDR* gene polymorphism may be affected by individual predisposition to OP and response to vitamin D supplementation [7,40].

The human *VDR* gene is located on chromosome 12 (12q12–14) and consists of 14 exons approximately 75 kb in size. Even a small modification in the gene can affect the structure and functional activity of the receptor. Common

single nucleotide variations (SNVs) in the *VDR* gene may be associated with various biological responses to vitamin D – *VDR* Apal (rs7975232, c.1025–49C>A), BsmI (rs1544410, 1024 + 443C>T) and TaqI (rs731236, c.1056A>G). Scientific studies indicate a significant association (up to 75 %) of the third region polymorphism in the *VDR* gene with BMD. Mutations in the *VDR* gene cause recessive inherited disease with severe hypophosphatemia and bone ossification disorder with rickets, hypocalcemia, vitamin D deficiency. *VDR* gene Apal, BsmI and TaqI polymorphisms may be risk factors for rheumatoid arthritis [3,7].

Marozik [40] conducted a study to reveal the effect of Apa gene *VDR* polymorphisms rs7975232, BsmI rs1544410, TaqI rs731236, FokI rs2228570 and Cdx2 rs11568820 with BMD, 25-hydroxyvitamin D levels and the risk of osteoporosis in 355 osteoporotic and 247 healthy Belarusian women. *VDR* gene polymorphisms rs7975232 A/A, rs1544410 T/T and individual variants rs731236 G/G and their ATG haplotype showed a significant association with an increased risk of osteoporosis (for ATG, OR = 1.8, $p = 0.0001$) and a decrease in BMD (ATG, -0.09 g/cm^2 , $p = 0.0001$). Allele A of the rs11568820 polymorphism showed a protective effect on BMD ($+0.22 \text{ g/cm}^2$, $p = 0.027$). The AA genotype of the rs731236 polymorphism was associated with a reduced level of 25 (OH) D. These results emphasize the importance of investigating genetic markers for a personalized medicine strategy [40].

Meta-analyses of 16 studies looking at the effect of the Bsm I *VDR* gene polymorphism with BMD concluded that BMD was 2.5 % lower in the spine and 2.4 % lower in the femoral neck in subjects with BB genotype compared to subjects with genotype bb. These findings suggest that homozygotes with the bb genotype had a statistically higher BMD value than individuals with the Bb/BB genotype [16].

Boroňová et al [41] found in Slovak postmenopausal women that the Fok I and Cdx-2 *VDR* gene polymorphisms are related to osteoporotic fractures [41].

PRDM16 gene

The *PRDM16* gene is located on chromosome 1, position 1p36.3 and contains 17 exons. The full name is PR/SET domain 16, other designations MEL1; KMT8F; LVNC8; PFM13; CMD1LL. The molecular weight of the *PRDM16* protein is 140 kDa. Reciprocal translocation of t (1; 3) (p36; q21) occurs in a subset of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). This gene is located near the 1p36.3 breakpoint and has been shown to be specifically expressed in t (1:3) (p36, q21)-positive MDS/AML. The protein encoded by this gene is a zinc finger transcription factor and contains an N-terminal PR domain. Translocation leads to overexpression

of a truncated version of this protein, which lacks a PR domain, which may play an important role in the pathogenesis of MDS and AML. Alternatively, spliced transcription variants encoding different isoforms have been described [42]. The *PRDM16* gene is a histone H3K4 methyltransferase on chromatin [43]. It encodes a protein of 1,247 amino acids (AMA). Translocation leads to overexpression of a truncated version of this protein, which lacks a PR domain and may play an important role in the pathogenesis of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) [44]. *PRDM16* gene is mainly known to regulate the differentiation of brown adipocytes (brown adipose tissue), platelet production, the preservation of nerve and hematopoietic stem cells, and its role in osteogenesis is little known [43,45]. The *PRDM16* gene is required for cardiac development, so loss of gene expression leads to the development of cardiomyopathy of the deletion syndrome 1p36, and changes in gene expression are also related to the development of migraine [44].

Zhou [43] states that the *PRDM16* gene is a transcriptional cofactor that plays a crucial role in the development of brown adipose tissue as well as in the maintenance of adult hematopoietic and neural stem cells in adults [43].

Zeng et al [45] discussed the expression of the *PRDM16* gene in the differentiation of osteoblasts into osteocytes in their study. The authors found that the *PRDM16* gene is a negative regulator of TGF β signaling. This inhibition of the signal, together with the partial suppression of the expression of the *SOST* gene, which is specific for osteocytes, prevents the terminal differentiation of osteoblasts into osteocytes [45].

Estrogen receptor 1 – ESR1 gene

The hormone estrogen is a female sex hormone that plays an important role in maintaining bone mass and regulating skeletal growth. In addition to its direct effect on bone cells, estrogen also has an indirect effect on parathyroid hormone and the active form of vitamin D. These functions are performed by estrogen through a major receptor called the estrogen receptor 1/alpha (ESR1 α), which is secreted in osteoblasts, osteoclasts and chondrocytes. The *ESR1* gene is located on the long arm of chromosome 6 (6q25.1-q25.2) and consists of 8 exons separated by 7 introns (longer than 140 kb). The *ESR1* gene is an important candidate gene for genetic regulation of bone mass, due to its association with bone mineral density values [46,47].

In postmenopausal women, serum estrogen levels are reduced, producing pro-inflammatory cytokines (IL1 and TNF α) in the body, which contribute to increased osteoclast function and consequent bone resorption [46].

Among the most important and most studied polymorphisms of the *ESR1* gene are rs9340799 (Xba I) and rs2234693 (Pvu II) located in intron 1 [47]. Zhu et al [48] report that the Xba I polymorphism in postmenopausal women is associated with lumbar spine BMD. Research shows that the X-allele, unlike the x allele, has a protective effect. Genotype Xx is associated with elevated lumbar spine BMD in the Caucasus population. In women diagnosed with postmenopausal osteoporosis in the femoral neck, they reported higher BMD values associated with genotype XX, in contrast to women with genotype xx. In a study of the Pvu II polymorphism of the *ESR1* gene, they found that women with the Pp genotype had lower lumbar spine BMD values than women with the pp genotype. Zhu et al [48] report that PP and Pp genotypes are associated with decreased BMD in the femoral neck region in both caucasoid and mongoloid populations [48].

A meta-analysis of the association between *ESR1* and BMD genotypes in 5,000 Asian and Caucasian women concluded that homozygotes of genotype XX, Xba I polymorphism (rs9340799) had higher lumbar spine BMD values compared to genotype xx [49].

Mondockova et al [50] conducted a study in Slovak postmenopausal women in which they studied the association of the rs9340799 *ESR1* gene polymorphism with BMD. The results of the study show that individuals with the AA genotype had higher BMD values (especially in the femoral neck and lumbar spine) than in individuals with the AG and GG genotypes [50].

Conclusion

Osteoporosis is currently the most common disease of the musculoskeletal system and represents a serious health and socio-economic problem. Positive family history, increasing average age of the population, female gender, low bone mineral density are important determinants of fracture risk. Scientific studies conducted on twins, family studies, suggest that genetic factors play an important role in regulating bone mineral density and other risk determinants of osteoporotic fractures, such as ultrasound properties of bone, skeletal geometry and bone turnover. Osteoporosis is a polygenic disorder determined by the effects of several genes, each of which has relatively weak effects on bone mass, the risk of fractures. The association studies performed so far with candidate genes associated with osteoporosis have yielded conflicting results. Therefore, the identification of genes and their polymorphisms that contribute to the study of pathogenesis is the subject of further molecular genetic research and is of great importance for public health, clinics and therapeutics. Molecular genetic analysis of osteoporosis is not commonly per-

formed in practice, but these analyzes could contribute to the elucidation of the metabolic processes of this disease, to the improvement of therapeutic therapies and to the early prevention of osteoporosis.

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Table | Major candidate osteoporosis genes. Adapted from [51]

candidate genes	protein	chromosome location
bone matrix proteins		
<i>COL1A1</i>	collagen type I α -1	17q21.33
<i>COL1A2</i>	collagen type I α -2	7q22.1
<i>ITGA1</i>	integrin, α 1	5q11.2
<i>MMP2</i>	matrix metalloproteinase 2	16q13-q21
<i>OCIL</i>	osteoclast inhibitory lectin	12p13
<i>SPARC</i>	osteonectin	5q31.3-q32
miscellaneous		
<i>ADCY10</i>	adenylate cyclase 10	1q24
<i>ALOX15</i>	arachidonate 15-lipoxygenase	17p13.3
<i>ALOX5</i>	arachidonate 5-lipoxygenase	10q11.2
<i>ALPL</i>	alkaline phosphatase	1p36.12
<i>ANXA6</i>	annexin A6	5q32-q34
<i>APC</i>	adenomatous polyposis coli	5q21-q22
<i>ARHGEF3</i>	rho guanine nucleotide exchange factor 3	3p21-p13
<i>BMPR1B</i>	bone morphogenetic protein receptor, type IB	4q22-q24
<i>CA10</i>	carbonic anhydrase X	17q21.33
<i>CA8</i>	carbonic anhydrase VIII	8q11-q12
<i>CALM1</i>	calmodulin 1	14q24-q31
<i>CLCN7</i>	chloride channel 7	16p13
<i>COMT</i>	catechol-O-methyltransferase	22q11.21
<i>CRTAP</i>	cartilage-associated protein	3p22.3
<i>DMP1</i>	dentin matrix acidic phosphoprotein 1	4q21
<i>ENPP1</i>	ectonucleotide pyrophosphatase/phosphodiesterase 1	6q22-q23
<i>FABP3</i>	fatty acid-binding protein 3	1p33-p32
<i>FLNB</i>	filamin β	3p14.3
<i>FLT1</i>	fms-related tyrosine kinase 1	13q12
<i>FOXC2</i>	forkhead box C2	16q22-q24
<i>FZD1</i>	frizzled homolog 1	7q21
<i>FZD6</i>	frizzled homolog 6	8q22.3-q23.1
<i>HMGA2</i>	high mobility group AT-hook 2	12q15
<i>HOXA</i>	homeobox A cluster	7p15-p14
<i>HSD11B1</i>	hydroxysteroid (11- β) dehydrogenase 1	1q32-q41
<i>MDR1</i>	multidrug resistance 1	7q21.1

Table | Major candidate osteoporosis genes. Adapted from [51]

<i>MTHFR</i>	methylenetetrahydrofolate reductase	1p36.3
<i>NFATC1</i>	nuclear factor of activated T-cells, cytoplasmic, calcineurin	18q23
<i>NOG</i>	noggin	17q21-q22
<i>NOS3</i>	endothelial nitric oxidase synthase	7q36
<i>NR1I3</i>	constitutive androstane receptor	1q23.3
<i>P2X 7</i>	purinergic receptor P2X, ligand-gated ion channel, 7	12q24
<i>PBX1</i>	pre-B-cell leukemia homeobox 1	1q23
<i>PIR</i>	pirin	Xp22.2
<i>PLOD</i>	procollagen-lysine 1, 2-oxoglutarate 5-dioxygenase 1	1p36.22
<i>PPAR-g</i>	peroxisome proliferator-activated receptor γ	3p25
<i>PTN</i>	pleiotrophin	7q33-q34
<i>RIZ1</i>	retinoblastoma protein-interacting zinc finger protein	1p36.21
<i>ROR2</i>	receptor tyrosine kinase-like orphan receptor 2	9q22
<i>RUNX2</i>	runt-related transcription factor 2	6p21
<i>SFRP1</i>	secreted frizzled-related protein 1	8p12-p11.1
<i>SFRP2</i>	secreted frizzled-related protein 2	4q31.3
<i>SOST</i>	sclerostin	17q11.2
<i>SREBF1</i>	sterol regulatory element binding transcription factor 1	17p11.2
<i>THSD4</i>	thrombospondin, type I, domain containing 4	15q23
<i>THSD7</i>	thrombospondin, type I, domain containing 7A	7p21.3
<i>TIMP2</i>	tissue inhibitor of metalloproteinase 2	17q25
<i>TWIST1</i>	twist homolog 1	7p21.2
<i>WISP3</i>	WNT1 inducible signaling pathway protein 3	6q21
<i>WNT10B</i>	wingless-type MMTV integration site family, member 10B	12q13
<i>WNT3A</i>	wingless-type MMTV integration site family, member 3A	1q42
<i>WNT7B</i>	wingless-type MMTV integration site family, member 7B	22q13
calcitropic hormones and receptors		
<i>AR</i>	androgen receptor	Xq11.2-q12
<i>CASR</i>	calcium-sensing receptor	3q13
<i>CRHR1</i>	corticotropin-releasing hormone receptor 1	17q12-q22
<i>CTR</i>	calcitonin receptor	7q21.3
<i>CYP17A1</i>	steroid 17- α -hydroxylase	10q24.3
<i>CYP19</i>	cytochrome P450, family XIX	10q26
<i>CYP19A1</i>	aromatase	15q21.1
<i>CYP17B1</i>	aryl hydrocarbon hydroxylase	2p21
<i>DBP</i>	vitamin D-binding protein	4q12-q13
<i>ER-a</i>	estrogen receptor-a	6q25.1
<i>ER-b</i>	estrogen receptor-b	14q23.2
<i>ESRRA</i>	estrogen-related receptor α	11q13
<i>GR</i>	glucocorticoid receptor	5q31.3
<i>LHB</i>	luteinizing hormone β polypeptide	19q13.32

Table | Major candidate osteoporosis genes. Adapted from [51]

<i>LHCGR</i>	luteinizing hormone-choriogonadotropin receptor	2p21
<i>PRL</i>	prolactin	6p22.2-p21.3
<i>PTH</i>	parathyroid hormone	11p15.3-p15.1
<i>PTH LH</i>	parathyroid hormone-like hormone	12p12.1-p11.2
<i>PTH R1</i>	parathyroid hormone receptor 1	3p22-p21.1
<i>PTH R2</i>	parathyroid hormone receptor 2	2q33
<i>SHBG</i>	sex hormone-binding globulin	17p13-p12
<i>SRD5A2</i>	steroid-5- α -reductase, α polypeptide 2	2p23
<i>TSHR</i>	thyroid-stimulating hormone receptor	14q31
<i>VDR</i>	vitamin-D receptor	12q13.11
cytokines, growth factors and receptors		
<i>BMP2</i>	bone morphogenetic protein 2	20p12
<i>BMP7</i>	bone morphogenetic protein 7	20q13
<i>CD40</i>	tumor necrosis factor receptor superfamily member 5	20q12-q13.2
<i>CNR2</i>	cannabinoid receptor 2	1p36.11
<i>DKK2</i>	dickkopf homolog 2	4q25
<i>FGFR1</i>	fibroblast growth factor receptor 1	8p11.2-p11.1
<i>FGFR2</i>	fibroblast growth factor receptor 2	10q26
<i>GDF5</i>	growth differentiation factor 5	20q11.2
<i>GHRH</i>	growth hormone-releasing hormone	20q11.2
<i>IGFBP2</i>	insulin-like growth factor binding protein 2	2q33-q34
<i>IL-23</i>	interleukin 23	12q13.2
<i>IL-23R</i>	interleukin 23 receptor	1p31.3
<i>IL-6</i>	interleukin 6	7p21
<i>IL-6R</i>	interleukin 6 receptor	1q21
<i>IL-15</i>	interleukin 15	4q31
<i>LEPR</i>	leptin receptor	1p31
<i>LRP1</i>	low-density lipoprotein receptor-related protein 1	12q13-q14
<i>LRP5</i>	low-density lipoprotein receptor-related protein 5	11q13.4
<i>LRP6</i>	low-density lipoprotein receptor-related protein 6	12p11-p13
<i>LTBP2</i>	latent transforming growth factor β binding protein 2	14q24
<i>MSTN</i>	myostatin	2q32.2
<i>NPY</i>	neuropeptide Y	7p15.1
<i>OPG</i>	osteoprotegerin	8q24
<i>RANK</i>	receptor activator of nuclear factor κ - β	18q22.1
<i>RANKL</i>	receptor activator of nuclear factor κ - β ligand	13q14
<i>TGF-b1</i>	transforming growth factor b-1	19q13.1
<i>TNF-a</i>	tumor necrosis factor a	6p21.3
<i>TNFRSF1B</i>	tumor necrosis factor receptor superfamily, member 1B	1p36.3-p36.2
<i>TNF-β</i>	lymphotoxin α	6p21.3
<i>VEGF</i>	vascular endothelial growth factor A	6p12

References

- Hsu YH, Xu X, Jeong S. Genetic Determinants and Pharmacogenetics of Osteoporosis and Osteoporotic Fracture. (2020). In: Leder B, Wein M (eds). *Osteoporosis. Contemporary Endocrinology*. Humana, Cham. Available from DOI: <https://doi.org/10.1007/978-3-319-69287-6_25>.
- Yang TL, Shen H, Liu AA et al. A road map for understanding molecular and genetic determinants of osteoporosis. *Nat Rev Endocrinol* 2020; 16: 91–103. Available from DOI: <[10.1038/s41574-019-0282-7](https://doi.org/10.1038/s41574-019-0282-7)>.
- Huang S, Ng GCH, You-Qiang S. *Genetic disorders associated with osteoporosis*. IntechOpen: London 2015. ISBN 978-953-51-7231-4.
- Spáčilová Z, Zrubcová D, Tináková S. Rizikové faktory osteoporózy v slovenskej populácii 40 a viacročných. Katedra ošetrovateľstva, Fakulta sociálnych vied a zdravotníctva, Univerzita Konštantína Filozofa v Nitre. Available from WWW: <<https://dspace5.zcu.cz/bitstream/11025/35053/1/Sp%C3%A1%C4%8Dilov%C3%A1%20ad..pdf>>.
- Marini F, Masi L, Marucci G et al. Genetics of Osteoporosis. In: Lenzi A, Migliaccio S. *Multidisciplinary Approach to Osteoporosis*. Springer 2018. ISBN 978-3-319-75110-8. Available from WWW: <https://link.springer.com/chapter/10.1007/978-3-319-75110-8_2>.
- Koromani F, Trajanoska K, Rivadeneira F et al. Recent Advances in the Genetics of Fractures in Osteoporosis. *Front Endocrinol (Lausanne)*. 2019; 10: 337. Available from DOI: <<https://doi.org/10.3389/fendo.2019.00337>>.
- Lenzi A, Migliaccio S. *Multidisciplinary Approach to Osteoporosis*. Springer: Rome (Italy) 2018. ISBN 978-3-319-75108-5.
- Clark GR, Duncan EL. The genetics of osteoporosis. *Brit Med Bull* 2015; 113(1): 73–81. Available from DOI: <<http://dx.doi.org/10.1093/bmb/ldu042>>.
- Rivadeneira F, Mäkitie O. Osteoporosis and Bone Mass Disorders: From Gene Pathways to Treatments. *Trends Endocrinol Metab* 2016; 27(5): 262–281. Available from DOI: <<http://doi.org/10.1016/j.tem.2016.03.006>>.
- Gažová A et al. Klinický význam komponentov systému RANK/RANKL/OPG u reumatoidnej artritídy. *Clin Osteol* 2018; 23(4): 176–180.
- Infante M, Fabi A, Francesco Cognetti F et al. RANKL/RANK/OPG system beyond bone remodeling: involvement in breast cancer and clinical perspectives. *J Exp Clin Cancer Res* 2019; 38(1). Available from DOI: <[10.1186/s13046-018-1001-2](https://doi.org/10.1186/s13046-018-1001-2)>.
- Sisay M, Mengistu G, Edessa D. The RANK/RANKL/OPG system in tumorigenesis and metastasis of cancer stem cell: potential targets for anticancer therapy. *Oncotargets Ther* 2017; 2017(10): 3801–3810. Available from DOI: <<https://doi.org/10.2147/OTT.S135867>>.
- Coudert AE, de Vernejoul MC, Muraza M et al. Osteopetrosis and its relevance for the discovery of new functions associated with the skeleton. *J Int Endocrinol* 2015; 2015:372156. Available from DOI: <<http://10.1155/2015/372156>>.
- Liu W, Zhang X. Receptor activator of nuclear factor- κ B ligand (RANKL)/RANK/osteoprotegerin system in bone and other tissues (review). *Mol Med Rep* 2015; 11(5): 3212–3218. Available from DOI: <<http://dx.doi.org/10.3892/mmr.2015.3152>>.
- Walsh MC, Choi Y. Biology of the RANKL – RANK – OPG System in Immunity, Bone and Beyond. *Front Immunol* 2014; 5:511. Available from DOI: <<http://dx.doi.org/10.3389/fimmu.2014.00511>>.
- Kumar D, Eng CH. *Genomic medicine: principles and practise*. University Press: Oxford 2015. ISBN 978-0-1998-9602-8.
- Boroňová I, Bernasovská J, Mačeková S et al. TNFRSF11B gene polymorphism, bone mineral density, and fractures in Slovak postmenopausal women. *J Appl Genet* 2015; 56(1): 57–63. Available from DOI: <<https://doi.org/10.1007/s13353-014-0247-4>>.
- Sheng X, Cai G, Gong X et al. Common variants in OPG confer risk to bone mineral density variation and osteoporosis fractures. *Sci Rep* 2017; 7(1): 1739. Available from DOI: <<http://dx.doi.org/10.1038/s41598-017-01579-6>>.
- Mydlárová Blaščáková M, Blaščáková L, Poráčová J et al. Relationship between A163G osteoprotegerin gene polymorphism and other osteoporosis parameters in Roma and non-Roma postmenopausal women in eastern Slovakia. *J Clin Lab Anal* 2017; 31(5): e22093. Available from DOI: <<http://dx.doi.org/10.1002/jcla.22093>>.
- Norwitz NG, Mota AS, Misra M et al. LRP5, Bone Density, and Mechanical Stress: A Case Report and Literature Review. *Front Endocrinol (Lausanne)* 2019; 10: 184. Available from DOI: <<https://doi.org/10.3389/fendo.2019.00184>>.
- Xi Y, Jiang T, Yu J et al. The Investigation of LRP5-Loaded Composite with Sustained Release Behavior and Its Application in Bone Repair. *Int J Polym Sci* 2019; 1–8. Available from DOI: <<https://doi.org/10.1155/2019/1058410>>.
- UniProtKB – O75197 (LRP5_HUMAN). UniProt 2019. Available from WWW: <<https://www.uniprot.org/uniprot/O75197>>.
- LRP5 gene. GeneCards 2021. Available from WWW: <<https://www.genecards.org/cgi-bin/carddisp.pl?gene=LRP5>>.
- Mydlárová Blaščáková M, Petrejčíková E, Zigová M et al. Asociácia polymorfizmu rs599083 LRP5 génu s antropometrickými a denzitometrickými parametrami u postmenopauzálnych žien so zníženou kostnou denzitou – pilotná štúdia. *Clin Osteol* 2020; 25(3): 163–164.
- Park SE, Oh KW, Lee WY et al. Association of osteoporosis susceptibility genes with bone mineral density and bone metabolism related markers in Koreans. *Endocr J* 2014; 61(11): 1069–1078. Available from DOI: <[10.1507/endocrj.ej14-0119](https://doi.org/10.1507/endocrj.ej14-0119)>.
- Horváth P. Strong effect of SNP rs4988300 of the LRP5 gene on bone phenotype of Caucasian postmenopausal women. *J Bone Miner Metab* 2016; 34: 79–85 Available from DOI: <<http://10.1007/s00774-014-0645-z>>.
- Sharma U, Carrique L, Vadon-Le Goff S et al. Structural basis of homo- and heterotrimerization of collagen I. *Nat Commun* 2017; 8: 14671. Available from DOI: <<http://dx.doi.org/10.1038/ncomms14671>>.
- U.S. National Library of Medicine. COL1A1 gene – collagen type I alpha 1 chain 2021. Available from WWW: <<https://medlineplus.gov/genetics/gene/col1a1/>>.
- Soibam D, Singh TA, Nandy P et al. Sp1 Binding Site Polymorphism at COL1A1 Gene and Its Relation to Bone Mineral Density for Osteoporosis Risk Factor Among the Sikkimese Men and Women of Northeast India. *Ind J Clin Biochem* 2019; 34: 230–233. Available from DOI: <<https://doi.org/10.1007/s12291-017-0728-4>>.
- Chen FP, Hsu KH, Fu TS et al. Risk factor for first-incident hip fracture in Taiwanese postmenopausal women. *Taiwan J Obstet Gynecol* 2016; 55(2): 258–262. Available from DOI: <[10.1016/j.tjog.2015.12.017](https://doi.org/10.1016/j.tjog.2015.12.017)>.
- Xie P, Liu B, Zhang L et al. Association of COL1A1 polymorphisms with osteoporosis: A meta analysis of clinical studies. *Int J Clin Exp Med* 2015; 8(9): 14764–14781. Available from WWW: <<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4658848/>>.
- *32. Falcón-Ramírez E, Hidalgo-Bravo A, Barredo-Prieto BA et al. Association of the COL1A1 gene polymorphisms in Mexican postmenopausal women with fracture or with low bone mineral density at the hip. *Aging Clin Exp Res* 2015; 28(3). Available from DOI: <[10.1007/s40520-015-0449-6](https://doi.org/10.1007/s40520-015-0449-6)>.
- Yu K, Tang J, Dai CQ et al. COL1A1 gene -1997G/T polymorphism and risk of osteoporosis in postmenopausal women: a meta-analysis. *Genet Mol Res* 2015; 14(3): 10991–10998. Available from DOI: <<https://doi.org/10.4238/2015>>.
- Majchrzycki M, Bartkowiak-Wieczorek J, Wolski H et al. Polymorphisms of collagen 1A1 (COL1A1) gene and their relation to bone mineral density in postmenopausal women. *Ginekologia Polska* 2015; 12(86): 907–914. Available from DOI: <<https://doi.org/10.17772/gp/60550>>.
- CER1 gene. Gene Cards 2021. Available from WWW: <<http://www.genecards.org/cgi-bin/carddisp.pl?gene=CER1>>.
- National center for biotechnology information. CER1 cerebri 1, DAN family BMP antagonist [Homo sapiens (human)]. NCBI 2021. Available from WWW: <<https://www.ncbi.nlm.nih.gov/gene/9350>>.
- Koromila T, Dailiana Z, Samara S et al. Novel Sequence Variations in the CER1 Gene Are Strongly Associated with Low Bone Mineral Den-

- sity and Risk of Osteoporotic Fracture in Postmenopausal Women. *Calcif Tissue Int* 2012; 91(15): 15–23. Available from DOI: <<http://dx.doi.org/10.1007/s00223-012-9602-9>>.
38. Koromila T, Georgoulas P, Dailiana Z et al. CER1 gene variations associated with bone mineral density, bone markers, and early menopause in postmenopausal women. *Human Genomic* 2013; 7(1): 21. Available from DOI: <<http://dx.doi.org/10.1186/1479-7364-7-21>>.
39. Mydlářová Blaščáková M, Petrejčíková E, Zigová M et al. Association of CER1 gene single nucleotide polymorphism rs 74434454 with osteoporosis in a cohort of postmenopausal women. VII Šrodokowo Europejski kongres osteoporozy i osteoartrozy 2019; 151–151.
40. Marozik P, Rudenka A, Kobets K et al. Vitamin D Status, Bone Mineral Density, and VDR Gene Polymorphism in a Cohort of Belarusian Postmenopausal Women. *Nutrients* 2021; 13(837). Available from DOI: <<https://doi.org/10.3390/nu13030837>>.
41. Boroňová I, Bernasovská J, Mačeková S et al. Association between vitamin D receptor gene polymorphisms (Fok I, Cdx-2) and bone mineral density in Slovak postmenopausal women. *Anthropol Anz* 2020; 77(3): 195–203. Available from DOI: <<http://dx.doi.org/10.1127/anthranz/2020/1048>>.
42. PRDM16 PR/SET domain 16. GeneCards 2019. Available from WWW: <<https://lnk.sk/mpg8>>.
43. Zhou B, Wang J, Lee SY et al. PRDM16 Suppresses MLL1r Leukemia via Intrinsic Histone Methyltransferase Activity. *Mol Cell* 2016; 62(2): 222–236. Available from DOI: <<http://dx.doi.org/10.1016/j.molcel.2016.03.010>>.
44. Anti-PRDM16 (N-term) antibody produced in rabbi. Merck 2020. Available from WWW: <<https://lnk.sk/m248>>.
45. Zeng HC, Bae Y, Dawson BC et al. MicroRNA miR-23a cluster promotes osteocytic differentiation by regulating TGF- β signalling in osteoblasts. *Nat Commun* 2017; 8: 15000. Available from DOI: <<http://dx.doi.org/10.1038/ncomms15000>>.
46. Martaityte I, Jorde R, Emaus N et al. Bone mineral density is associated with vitamin D related rs6013897 and estrogen receptor polymorphism rs4870044: The Tromsø study. *PLoS One* 2017; 12(3): 1–12. Available from DOI: <[10.1371/journal.pone.0173045](http://dx.doi.org/10.1371/journal.pone.0173045)>.
47. Montazeri-Najafabady N, Dabbaghmanesh MH, Mohammadian Amiri R et al. Influence of Estrogen Receptor Alpha Polymorphism on Bone Mineral Density in Iranian Children. *Hum Hered* 2019; 84(2): 82–89. Available from DOI: <[10.1159/000502230](http://dx.doi.org/10.1159/000502230)>.
48. Zhu H, Jiang J, Wang Q et al. Associations between ER α / β gene polymorphisms and osteoporosis susceptibility and bone mineral density in postmenopausal women: a systematic review and meta-analysis. *BMC Endocr Disord* 2018; 18(11): 1–16. Available from DOI: <[10.1186/s12902-018-0230-x](http://dx.doi.org/10.1186/s12902-018-0230-x)>.
49. Ferrari S. Human genetics of osteoporosis. *Best Pract Res Clin Endocrinol Metab* 2008; 22(5): 723–735. Available from DOI: <<http://dx.doi.org/10.1016/j.beem.2008.08.007>>.
50. Mondockovy V, Adamkovicova M, Lukacova M et al. The estrogen receptor 1 gene affects bone mineral density and osteoporosis treatment efficiency in Slovak postmenopausal women. In: *BMC Med genet* 2018; 19(1): 1–13. Available from DOI: <[10.1186/s12881-018-0684-8](http://dx.doi.org/10.1186/s12881-018-0684-8)>.
51. Xu X, Dong SS, Guo Y et al. Molecular genetic studies of gene identification for osteoporosis. *Endocr Rev* 2010; 31(4): 447–505. Available from DOI: <<http://dx.doi.org/10.1210/er.2009-0032>>.