

# The Genetic Determination of Osteoporosis

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## SUMMARY

Osteoporosis is a chronic progressive disease, whereby bone resorption prevails over bone formation, resulting in bone mass loss, a disorder in bone microarchitecture, and, subsequently, a decrease in bone density and increased risk of bone fracture. While it affects mostly postmenopausal women, elderly men are also at risk. Although osteoporosis is influenced by a variety of environmental factors (mainly diet and physical activity), analyses of results emerging from studies with different designs (population, family, as well as association studies) have shown that the risk of developing osteoporosis and suffering osteoporosis-related fractures is under significant genetic control. However, the association between genetics and osteoporosis is not yet fully understood. Nevertheless, results published to date clearly show that, although there are rare mutations of some genes causing osteoporosis, osteoporosis is primarily a polygenous disease, i.e., it is caused by common variants (polymorphisms) of several different genes. For example, it has been suggested that genes for vitamin D receptor, estrogen receptor  $\alpha$ , collagen type I  $\alpha 1$ , transforming growth factor  $\beta$ -1 and many of other genes play a role in the genetic determination of osteoporosis.

This paper provides a concise overview of our current knowledge about the genetic determination of osteoporosis.

**Key words:** heredity, polymorphisms, mutation, osteoporosis.

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## INTRODUCTION

The number of patients with osteoporosis in the Czech Republic is estimated to be 600,000 (1). The incidence of the disease rises with age, being equal in both sexes after the age of 80. In women the disease develops much earlier because of changes in bone metabolism as a result of reduced production of sex hormones after menopause (1).

Although it is evident that some environmental factors (e. g., physical activity, movement coordination, smoking, age, and dietary habits) (for reviews, see 2, 3) have an effect on the development of osteoporosis and osteoporosis-related fractures, a variety of studies have clearly shown that the important role in osteoporosis development is played by genetic predisposition (for review, see 4).

This paper provides an overview of the genetic variants potentially involved in the development of osteoporosis, which have been studied to date.

## CLINICAL FEATURES OF OSTEOPOROSIS

Osteoporosis is a chronic progressive disease whereby bone resorption prevails over bone formation, resulting in a decrease in bone mass and a disorder of bone microarchitecture and, subsequently, a decrease in bone strength and increased risk for fracture (5). It is a disease occurring asymptotically for years and manifesting by fracture caused by mild trauma that would normally not cause fracture. The fracture usually involves the distal forearm (Colles fracture), hip or vertebral fracture (compression). New-onset vertebral compression usually manifests itself as intense acute back pain, but it may also only manifest itself as sudden exacerbation of pre-existing chronic back pain - so it may not be clinically detected. Vertebral fractures, which may recur, contribute to chronic back pain, increased thoracic kyphosis and decrease of the patient's height. Osteoporotic fractures make the quality of life of the patient appreciably worse. In addition, hip fracture is associated with high costs of treatment and may have lethal consequences (1).

## DIAGNOSIS

At present, the techniques used to identify at-risk patients include determination of bone mineral density (BMD) using x-rays or ultrasound densitometry and determination of the rate of bone mass loss by measuring bone markers (e. g., osteocalcin, bone alkaline phosphatase, osteoclastic acid phosphatase, type I collagen C- or N-telopeptide, procollagen I N-terminal peptide and some other markers). However, these techniques and/or markers do not ensure adequate accuracy. Nevertheless, the current search for adequate genetic markers (6, 7) potentially permitting the identification of individuals at high risk of developing osteoporosis in time, can help in effective prevention of this disease.

### *Monogenous forms of osteoporosis*

Monogenous forms of osteoporosis (caused by a single mutation in a single gene), although clinically interesting, occur very rarely in the population (at rates usually lower than 1:1000), so they are virtually irrelevant for the epidemiology of osteoporosis. These include, e. g., mutations in collagen I alpha 1 and collagen I alpha 2 genes, which may cause osteogenesis imperfecta (8), a disease manifesting itself by frequent fractures and a variety of other symptoms of varying severity (deafness, blue sclera, damage to teeth, scoliosis, skeletal deformities up to severe neonatal malformations). Another example is the osteoporosis pseudoglioma syndrome due to a mutation of LDL receptor-related protein 5 gene (9).

### *Polygenous osteoporosis*

Two different approaches have been developed to the study of the heredity of polygenous diseases. The first approach investigates the genome (the complete genetic information) as a whole. Population and twin studies are designed to look for an association between the distribution of non-specific polymorphous markers in the genome and the disease. The studies are designed to identify sequences on chromosomes (and, later, using a detailed analysis, genes located in the sequences) responsible for osteoporosis (determination of BMD or other aspects of bone quality). The advantage of this approach is

that it may help identify even genes not fitting the current models of the pathophysiology of the disease. The problem is that the approach is both time-consuming and costly. Still, several sequences have been identified, which are believed to be related to osteoporosis. These sequences include those on chromosomes 1 (1p36 and 1q21-23), 2 (2p23-24 and 2p21), 3 (3q34), 4 (4q32-34), 5 (5q33-35), 6 (6p11-12), and 11 (11q12-13) (10, 11). However, the only gene identified at these intervals to date which has been clearly demonstrated to be related to osteoporosis is the LDL-receptor related protein 5 gene (see the Monogenous forms of osteoporosis Section). The gene has not yet been investigated in terms of its association to polygenous osteoporosis.

Another approach, which is used more frequently, is based on detailed analysis of a single, specific "candidate" gene assumed to be causally related, based on current knowledge, to the disease. Virtually all these candidate genes show polymorphisms, with common variants (with an incidence of at least 1% by definition) represented mainly by single nucleotide polymorphisms (SNP). However, polymorphisms are not necessarily a cause of the disease. They are only a manifestation of the genetic diversity of individuals and may ultimately predispose the development of some diseases.

Some polymorphisms may result in modulation of the protein in question or the amino acid composition necessary for protein function. Similarly, the function of proteins may be affected by the deletion or insertion of one or several nucleotides or, alternatively, some repeat sequences (repetitive polymorphisms).

Association studies are intended to demonstrate associations between the presence of a certain gene variant and development of the disease. Individual alleles may be either risk or protective factors. As regards osteoporosis, research has focused primarily on genes coding for bone proteins (collagen I  $\alpha$ 1, collagen I  $\alpha$ 2, osteocalcin) as well as for hormones, receptors and growth factors affecting bone metabolism (receptors for vitamin D, calcitonin, estrogens, transforming growth factor  $\beta$ -1 and others).

The principle of association studies consists of comparing two groups of individuals, e.g., osteoporotic and healthy ones. Some association studies follow changes in BMD or bone metabolism values in response to hormone replacement therapy or another type of therapy, depending on different genetic polymorphisms. A number of studies analyse the incidence of fractures in individuals with different genetic characteristics. However, the incidence of fractures is also affected by a variety of other environmental, non-genetic factors such as age, body weight, muscular weakness or poor vision, characteristics of fall resulting in fracture, or the number of layers of garment which could possibly protect against fracture (12).

Ideally, the study groups include hundreds to thousands of subjects and only differ in the presence or absence of the disease. However, ideal conditions are rarely obtained in practice; moreover, the population may be heterogeneous, which may lead to bias. Therefore, results of association studies are occasionally inconsistent and even contradictory in various populations. It is not easy to draw definitive conclusions from these studies, although meta-analyses (which summarize and evaluate data emerging from separate studies from different populations) may be of some benefit. The situation is made more complicated by considerable ethnic differences, since alleles commonly occurring in the Caucasian population may be rare (or even absent) in Asian or African populations.

Data published to date clearly show that osteoporosis is a polygenous disease, i.e., one affected by common variants of different, mutually independent genes.

Our current knowledge of candidate genes responsible for osteoporosis is not sufficient to fully understand the nature of the disease. Still, a number of polymorphisms have been identified in various

genes believed to be associated with osteoporosis - or their association to bone density or susceptibility to fracture has been reported.

#### ***Vitamin D receptor***

The gene encoding the vitamin D receptor (VDR) was one of the first genes studied in connection with genetic predetermination to osteoporosis and BMD.

Four polymorphisms of the gene have been identified, which are detectable using restriction enzymes ApaI, BsmI, FokI, and TaqI; three of these (ApaI, BsmI, and TaqI) have been investigated in some five dozen studies (for review, see 7); however, the results obtained are largely inconsistent. As a result, Cooper et al. (13) used the data obtained in 16 independent studies to conduct a meta-analysis of the association of three VDR polymorphisms, which did not reveal a consistent association to BMD or the risk for fractures.

A major role in this was played by the different designs of individual studies and ethnic differences. Moreover, Uitterlinden et al. (14) reported significant interaction between the effect of VDR polymorphism and polymorphism of the collagen  $\alpha$ -1 gene.

#### ***Collagen $\alpha$ type 1***

A promising genetic marker, which may play a major role in bone metabolism and bone quality, is Sp1 polymorphism (in the regulatory region of the collagen  $\alpha$  type 1 gene). Type I collagen comprises about 90% of all bone protein, and a change in the regulatory region of its gene could possibly have an effect on bone tissue characteristics and quality. Some studies have suggested an association between this polymorphism and bone density as well as fracture risk (15-17). The exact mechanism of the association between Sp1 polymorphism, bone mass and bone fragility is not fully understood. Mann et al. (18) has demonstrated that a role may be played by an imbalance between the numbers of  $\alpha$ -1 and  $\alpha$ -2 collagen fibres which results in impaired stability of the collagen fibre.

#### ***Transforming growth factor $\beta$ -1 (TGF $\beta$ -1)***

*In vivo*, TGF-1 has been shown (19) to be able to accelerate the transformation of mesenchymal stem cells into bone cells. Hence, TGF-1 affects bone development and, probably, it may have an effect on the development of osteoporosis.

Almost ten polymorphisms have been identified in the TGF-1 gene. Two of these polymorphisms, regulating cytokine levels (leucin10prolin and arginin25prolin), have been shown to be associated with bone density. However, data from different ethnic groups are inconsistent (20, 21), so any association between these polymorphisms and osteoporosis must be verified in future studies.

#### ***Calcitonin receptor***

Calcitonin, used for the treatment of osteoporosis, inhibits bone resorption via binding to the calcitonin receptors of osteoclasts. The results of several studies, summarized in a paper by Pondel (22), clearly show that the presence of proline in position 447, instead of leucin, is associated with a lower BMD, although not invariably with the development of osteoporosis or an increased risk of fracture.

#### ***Estrogen-receptor $\alpha$***

Estrogen-receptor  $\alpha$  is a receptor recognizing the steroid hormone estrogen, which plays a most important role in growth, development and maturation of bone cells.

Two polymorphisms have been identified in the gene for this receptor (detectable enzymes PvuII and XbaI). The relationship of these polymorphisms to osteoporosis has been studied in several Caucasian and Asian populations (23-25).

However, results published to date are conflicting, and future stud-

ies will be necessary to test the real effect of these polymorphisms. For example, an association between a lower BMD and the presence of the PvuII restriction locus was reported in a three-year cohort of pre- and postmenopausal women; however, the variant had no effect on hormone replacement therapy (26). By contrast, PvuII polymorphism in a five-year cohort of postmenopausal women (27) had no effect in actual BMD, but was associated with changes in BMD.

#### **Tumor necrosis factor $\alpha$**

Tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) stimulates osteoclast growth.

Among three studied polymorphisms (T-1031C, C-863A, and A-857T) in the regulatory region of the TNF- $\alpha$  gene, the presence of the C-1031C genotype was associated with increased BMD levels compared with that seen in T-1031 allele carriers among postmenopausal Japanese women (28). The other variants had no effect on BMD.

#### **The androgenic receptor**

Given the low BMD associated with hypogonadism, the androgenic receptor (AR) is also a candidate gene for the development of osteoporosis.

An association has been reported between the number of CAG triplet repeats in the AR gene on the one hand and BMD and the risk of osteoporosis on the other. Individuals with repetition numbers below 20 showed higher BMD and reduced risk of osteoporosis (29) as well as of osteoporosis-related fractures (30).

#### **Osteocalcin**

Osteocalcin (indispensable for bone tissue mineralization) is produced by mature osteoblasts and serves as a marker of their activity.

In a series of 97 healthy young women, the H allele of HindIII polymorphism has been showed to correlate with decreased humeral BMD (31). The same allele is responsible for an almost fivefold rise in the risk of osteopenia in Japanese postmenopausal women (32).

#### **Osteoprotegerin**

Osteoprotegerin (OPG) is an important regulator of bone tissue remodelling. The role of OPG in bone metabolism has been demonstrated by the creation of OPG knock-out mice having osteoporosis.

A total of 12 polymorphisms have been identified in the human OPG gene. In one study, the less frequent A163G and T245G allele variants occurred more often in patients sustaining vertebral fractures (33).

In another study, two variants (G209A and T245G) had a major effect on BMD in postmenopausal women with osteoporosis (34).

Results obtained in Caucasian populations were not confirmed in a Japanese study identifying only 6 variants in the OPG gene with none shown to be significantly related to osteoporosis (35).

#### **Interleukin-6**

Interleukin-6 (IL-6) is a pleotropic cytokine modulating osteoclast differentiation and function; compared with healthy controls, IL-6 expression in the bones of osteoporotic patients is significantly increased.

Bone mineral density in the lumbar spine region (36) and in the wrist (37) has been reported to be affected by (AT) $n$  and (AC) $n$  repetitive polymorphisms in the IL-6 gene.

Another polymorphism, G-174C, in the IL-6 gene promotor, affects gene resorption and, subsequently, the degree of bone resorption (38).

#### **Interleukin-1, interleukin-1 receptor associated kinase and interleukin-1 receptor autoantigen**

Interleukin-1 (IL-1) has a beneficial effect on bone resorption mainly by inhibiting osteoclast apoptosis. No polymorphism has been identified in the IL-1 gene.

However, polymorphisms have been shown in the gene (phenylalanin196serin and serin532leucin) for IL-1 receptor associated kinase, which is the basic "trigger" of the signal IL-1 cascade and, hence, necessary (along with IL-1) for adequate bone resorption, and in the gene for IL-1 receptor autoantigen (repetitive polymorphisms), a natural IL-1 inhibitor adversely affecting bone resorption.

An association of these variants to BMD has been reported (39, 40); however, further studies are necessary to verify the findings.

#### **Parathyroid hormone**

An association has been demonstrated for a BstB1 restriction enzyme-detectable polymorphism and BMD in 383 Japanese postmenopausal women (41). In healthy premenopausal women, the same polymorphism has been shown to have an effect on bone dimensions but not on BMD (42).

#### **Other genes**

Polymorphisms in many other genes have been studied in terms of their association to BMD or increased fracture risk, although it is not always clear in what way they could affect BMD or bone development. The data may be incidental, with beneficial results not confirmed in future, larger studies.

However, it cannot be ruled out that, although the polymorphisms identified to date do not play a role in the genetic predisposition to osteoporosis or modulation of bone metabolism, their alleles are in linkage disequilibrium with the alleles of other functional genes localized in the vicinity of the analyzed gene and involved in predisposition to osteoporosis.

Linkage disequilibrium is a status whereby certain alleles of one gene are preferentially linked to certain alleles of (an)other gene(s). In some cases, the association is as high as 100% and, quite often, over 90%. To determine the genetic characteristics, it is always necessary to identify only one of the several associated variants.

The genes shown to have been associated with BMD or fracture risk (although their role in bone metabolism is unclear) include the apolipoprotein E gene (43), playing a role primarily in lipid metabolism modulation, or the gene encoding methylene tetrahydrofolate reductase (the C677T variant affects the plasma enzyme levels) (44).

The above list of genes screened in relation to osteoporosis is by no means complete. There are dozens of other publications reporting associations between BMD, occurrence of osteoporosis or fracture risk, and variants of various genes. However, in most cases, these publications report individual findings, which must be confirmed by future studies.

## **CONCLUSION**

Data emerging from studies to date clearly show that osteoporosis is a multifactorial disease, with both genetic and environmental factors playing a role in its development. The development of osteoporosis is the result of interaction between a relatively large number of various genes and environmental factors. Genes affecting BMD and bone quality may be independent but may also interact.

The huge number of papers addressing genetic determination of osteoporosis suggests that it will not be easy to identify a combination of genetic markers carrying a risk of osteoporosis or one affording protection against the disease. One of the reasons is that some relatively common variants found to be important for the development of osteoporosis in one population are completely missing in other populations. This is mostly the case of Japanese and Caucasian populations. Still, results obtained to date are promising and suggest there is a solution to the problem.

Study of genetic variants in osteoporotic women may reveal important aspects of the onset and development of this disease and help expand our incomplete understanding of the genetic predisposition to osteoporosis. Early identification of individuals genetically predisposed to osteoporosis, if combined with adequate prevention, may help significantly reduce the incidence of complications of the disease, thus improving the social status of individuals with osteoporosis and reducing the cost of its treatment.

**List of abbreviations**

- AR – androgenic receptor
- BMD – bone mass density
- IL-1 – interleukin-1
- IL-6 – interleukin-6
- LDL – low-density lipoproteins
- OPG – osteoprotegerin
- SNP – single nucleotide polymorphism
- TGFβ-1 – transforming growth factor β-1
- TNF-α – tumor necrosis factor α
- VDR – vitamin D receptor

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## Comments upon the Article by Hubáček J. A. and Weichetová M.: “The Genetic Determination of Osteoporosis”

The first study on the high genetic association of bone mineral density in twins was published more than 30 years ago (1). It took 20 years for *Nature* to publish the paper by Morrison first reporting a specific gene variation associated with bone mineral density. The paper spurred hopes that several specific “bone genes” would soon be identified, shedding light on the genetic basis of the risk of fractures, particularly osteoporotic fractures (2). This was followed by an explosion in publications dealing with the genetic basis of vitamin D receptors and other topics - all of which actually made the situation more complicated instead of simpler. Generally, different mutations in the same gene may produce a series of subsequent abnormalities, and clusters of diseases are often caused by mutation of a component in the same path. Surprisingly, bone biology has shown numerous genetic changes in molecules that were not expected to be related directly to bone metabolism, based on data from biochemical and pathophysiological studies (3).

Large population-based association studies were gradually replaced by investigation of molecular mechanisms triggered by genetic alterations with simple Mendelian heredity and which produce relatively rare diseases. Naturally, this approach has only confirmed that there are at least dozens (if not hundreds) of gene-mediated factors affecting bone density, bone geometry, structural proteins of bone matrix and other components implicated in bone fragility. Considering also phenotype factors, it is now self-evident that it will be a long time before the genetic basis of osteoporosis and increased bone fragility has been fully explored. Still, it is worth mentioning some relatively new and potentially important and interesting discoveries.

Leaving aside the issue of developmental biology and genetic regulation of differentiation, development, and growth of individual bones in the body, one may take a closer look at cell differentiation and its regulation. Pluripotent mesenchymal stem cells usually differentiate into chondrocytes, and the resulting cartilaginous template is transformed, via a process called endochondral ossification, into a bone with osteoblasts and osteoclasts. At some sites, mesenchymal cells differentiate directly into osteoblasts (i. e., intramembranous ossification). Many genes encode the growth and transcription factors regulating these processes, though these are still poorly understood. *Runx2/Cbfa1* and *Osterix* have been shown to control osteoblast differentiation, whereas LRP5 (low-density lipoprotein receptor-related protein 5), occurring in almost all tissues, controls osteoblast proliferation. Many other positive and negative regulators still remain to be identified. We do have some more information about the gene-based control of osteoclast differentiation and proliferation. Use of particularly experimental gene deletion has helped identify a host of transcription factors involved in osteoclast proliferation (PU1, c-Fos, NF $\kappa$ B, NF- $\alpha$ Tcn, and Mitf). The gene and regulation associations of the OPG-RANK-RANKL system are generally well known (4).

Animal models have given researchers the opportunity to characterize and modulate the phenotype much more effectively than is the case with clinical trials. It was primarily studies in inbred mouse lines, which made it possible to investigate the QTL (quantitative trait loci) of genetic effects. To name but one, mention should be of the *Alox15* gene and its documented effect on bone mineral density in the proximal femur and biomechanical characteristics of the bone. The close association of this gene with the metabolism of arachidonic and linoleum acids and modulation of peroxisomes has led to the hypothesis that the high *Alox15* activity results in preferential differentiation of mesenchymal stem cells into the adipocyte line instead of the osteoblastic line (5). This hypothesis has paved the way for extensive research into hypothalamic control of bone mass. Leptin, an adipose tissue hormone, is a major mediator of bone formation and resorption, with its signal-transduction receptors located predominantly in hypothalamic neurons. Through its action on steroid hormone production, leptin thus performs the remarkable task of linking bone and adipose tissue metabolism with genetic and hormonal regulation.

Bone metabolism studies have long focused on calcium metabolism and its direct modulation. Recently, however, there has been an avalanche of information about the effects of yet other factors.

Phosphatonin, a circulating phosphaturic hormone, plays a major role in renal phosphate excretion. Phosphatonin has been shown to be identical with FGF-23 (fibroblast growth factor-23) and may cause severe syndromes involving loss of phosphates. Clinical evidence was first obtained in tumor-derived osteomalacia. However, phosphatonin has also been identified in the so-called *Hyp* murine strain, a murine model of hypophosphatemia, which is linked to the chromosome of X-autosomal dominant hypophosphatemic rickets and also associated with inadequately low serum 1,25-dihydroxyvitamin D<sub>3</sub> levels (6). The interactions between the metabolisms of phosphates and vitamin D, which are undoubtedly involved in the onset of osteoporosis, are fascinating.

Allelic polymorphism of the gene encoding methylenetetrahydrofolate reductase (MTHFR) has been incriminated in reduced bone density of postmenopausal females (TT genotype). This genotype is associated with slightly increased plasma homocysteine levels, which might control collagen maturation. The TT genotype goes hand in hand with more than a twofold relative risk of sustaining a fracture (7). As with the above-mentioned effect of leptin and its association, these facts may help unravel the increasingly intricate orchestration of gene-mediated processes involved in the metabolism of many tissues and organs, including the bone.

The time will soon come when we will have to determine how we can make use of the candidate gene polymorphisms, identified in asso-

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ciation studies, in clinical practice. The most important step will be to estimate the positive and negative predictive values of genetic markers for the risk and development of osteoporosis in a defined population. One of the most enticing avenues is a look “behind” bone mineral density, currently regarded as the main predictive factor of fracture risk, in search of definition and background information for assessing the basis for assessing the quality of bone tissue. Promising studies are searching for links between functional variants of the COL1A1 gene, resultant collagen quality, and the risk of fracture (8), or markers of bone resorption and functional interleukin-6 promoter alleles (9).

It seems that despite all the advances and scientific approaches, mankind will have to retain a certain humility in this respect: the latest findings also indicate that our concepts of simplistic causality are incomplete and imprecise. Data emerging from genetic studies to date are not sufficient for us to be able to define the biological implications in a deterministic and causal manner. There is still a long, yet exciting and stimulating road ahead of us.

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