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A LOOK BEHIND THE SCENES: THE RISKS AND PATHOGENESIS OF OSTEOPOROSIS

Hendrickx Gretl[1*], Boudin Eveline[1*], Van Hul Wim[1]

[1]Department of Medical Genetics, University of Antwerp, Belgium

[*]These authors contributed equally to this work

Gretl.Hendrickx@uantwerpen.be; Eveline.Boudin@uantwerpen.be; wim.vanhul@uantwerpen.be

Corresponding author: Wim Van Hul, Department of Medical Genetics, University of Antwerp, Prins Boudewijnlaan 43B, 2650 Edegem, Belgium. Tel +32 (0)3 275 9761 Fax +32 (0)3 275 9722
E-mail: **wim.vanhul@uantwerpen.be**

KEY POINTS

- The pathogenesis of osteoporosis is complex and influenced by both environmental and genetic factors.
- Oxidative stress, apoptosis, sex steroid deficiency and macroautophagy are age-related risk factors contributing to the pathogenesis of osteoporosis.
- Lifestyle factors such as calcium and vitamin D intake, physical activity, smoking and excessive alcohol use are important risk factors for osteoporosis.
- Mutations in several genes can cause different monogenic disorders characterized by decreased bone mineral density and increased bone fragility.
- The genetic impact on multifactorial osteoporosis is determined by variation in many genes with small effects.

ABSTRACT

Osteoporosis is a common and multifactorial disorder affecting hundreds of millions of people worldwide. Many factors, both heritable and non-heritable, contribute to its pathogenesis. As for non-heritable factors, age-related mechanisms like sex steroid deficiency and an increase in oxidative stress are known risk factors for osteoporosis. Here, age is an unbeatable risk factor, but the influence of a person's lifestyle is adaptable to some extent. A person's diet and physical activity can indeed be advantageous for both bone structure and density. On the other hand, medication use and pathologies can adversely influence a person's risk for secondary osteoporosis, which is beyond the scope of this review. Next to these non-heritable factors, heritable factors influencing bone fragility can be either monogenic or multifactorial. Here, we discuss Osteogenesis Imperfecta, juvenile osteoporosis and syndromes with a decreased bone density as monogenic disorders characterized by increased bone fragility. So far, the role of genetic factors in multifactorial osteoporosis is mainly investigated by performing genome wide association studies. However, epigenetic mechanisms also contribute to this heritable character of multifactorial osteoporosis. The knowledge of all these heritable and non-heritable risk factors has already led to the discovery of therapeutic targets against osteoporosis, emphasizing the importance of research on the pathogenic mechanism of osteoporosis.

INTRODUCTION

Osteoporosis is a skeletal disorder affecting hundreds of millions of people through an increment in bone fragility and susceptibility to fracture. Currently, one in three women and one in five men above the age of fifty will experience osteoporotic fractures, which makes osteoporosis a defining health problem of our aging society. These osteoporotic fractures predominantly occur at the level of the forearm, hip and lumbar spine bone and are associated with a substantial morbidity and mortality. Hip fractures, for example, are responsible for a mortality rate up to 36% in the first year after the fracture occurred[1, 2]. Moreover, hospitalization, follow-up and treatment of patients are expensive and illustrate an accompanying financial burden to our society. With a view to the future, these healthcare costs will only increase because of the aging of our population. The costs to health services in the European Union were estimated at €31.7 billion in the year 2000 and are expected to increase to €76.7 billion in 2050, considering the changes in the European demography[3]. This makes osteoporosis an important target for research, in order to generate a better understanding, prevention and cost-effective treatment of this common skeletal disorder.

Osteoporotic fractures are the consequences of a decrease in bone mineral density (BMD) in combination with a deteriorated micro-architecture of the bone tissue. The risk for osteoporotic fractures is strongly dependent on the peak bone mass (pBMD), obtained around the age of 25 (Figure 1). The lower this pBMD, the higher the risk for more fragile bones later on in life[4]. After pBMD is reached, BMD remains more or less stable until the age of 55 years when age related bone loss starts (Figure 1). After bones have completed their longitudinal growth, almost 10% of the bone is remodeled each year[5]. Bone remodeling is a physiological process whereby old bone is removed by osteoclasts and new bone is formed by osteoblasts. In this way, micro-damage can be repaired and bone strength and mineral homeostasis are maintained. In order to maintain a stable bone mass, it is important that the activity of the osteoclasts and osteoblasts is well balanced. Therefore, the bone remodeling process is strictly regulated by numerous signaling pathways. Important coordinators of bone remodeling are osteocytes, terminally differentiated osteoblasts located in the mineralized bone tissue. Osteocytes can detect microcracks, mechanical strain and changes in the hormonal environment of the bone and subsequently initiate bone remodeling when necessary[5, 6]. Although the process of bone remodeling is strictly regulated, bone loss occurs during life as a result of hormonal changes and aging. The rate of bone loss throughout life is dependent on a person's lifestyle and genetic background. In addition to pBMD and bone loss the risk for osteoporosis and related fractures is also influenced by bone quality which is defined by different parameters including properties of the proteinaceous matrix and the mineral phase. For example the degree of collagen

crosslinking and posttranslational modifications as well as the collagen fibril diameter has an effect on bone strength[7]. Also noncollagenous proteins such as proteoglycans, alkaline phosphatase, osteonectin and periostin,… do have diverse effects on bone composition and mineralization[8, 9]. Finally bone strength is also influenced by the architecture of the bone, interpreted by geometrical parameters, is subjected to genetic and non-genetic influences as well[10]. In this way, osteoporosis is a textbook example of a common, multifactorial disorder.

The complexity of a multifaceted skeletal disorder like osteoporosis is a challenge for the medical community. Prevention, diagnosis and treatment would be favored by an individual approach. Here, a better understanding of all different hereditary and non-hereditary factors contributing to the development of fragile bones is essential to improve the capability in treating osteoporosis patients more efficiently in the future. Therefore, this review will summarize the main factors playing a role in the pathogenesis of osteoporosis, including both non-heritable and heritable factors. Non-heritable factors include typical age-related and life-style influences on the density and quality of the bones. To review the genetic character of osteoporosis, also monogenic conditions associated with decreased bone mass are discussed, as well as known epigenetic mechanisms.

NON-HERITABLE FACTORS IMPLICATING THE ONSET OF OSTEOPOROSIS

1. Age-related factors

Since osteoporosis is mainly affecting people above the age of fifty, age is a definite risk factor (Figure 1). Actually, age here stands for the gradual or sudden disturbance of several mechanisms throughout life. Among the plethora of mechanisms that influence osteoporosis oxidative stress, apoptotic mechanisms, sex steroid deficiency and macroautophagy can be discussed separately but are actually all concomitant in the aging individual (Figure 2).

1.1. Oxidative stress

Both men and women experience a progressive decline in BMD that starts as soon as pBMD is reached and which is initially independent of sex steroid levels (Figure 1). This is due to other aging-associated mechanisms, like oxidative stress[11]. Throughout life, the normal intracellular metabolism generates, as a byproduct, reactive oxygen species (ROS) such as superoxide (O_2^-) anions, hydroxyl radicals (HO^-) and hydrogen peroxide (H_2O_2). Oxidative stress arises when ROS excessively accumulate, which occurs when ROS scavenging mechanisms (like superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione (GSH) and catalase (CAT)) are reduced, the membrane-associated NADPH oxidase (NOX) activity is increased or by mitochondrial respiratory chain leakage

(Figure 2). Subsequently, oxidative stress can damage macromolecules such as lipids of the cell membrane, nuclear and mitochondrial DNA and transcription factors[12]. Increased oxidative stress levels and accumulation of oxidative damage are associated with aging and subsequently, with aging-associated disorders. Murine models of premature aging due to oxidative damage, exhibit an osteoporosis phenotype, supporting a deleterious role of oxidative stress in bone as well[13, 14]. Also, mice deficient in cytoplasmic Sod (*Sod1*$^{-/-}$) have higher levels of oxidative stress and show a decreased BMD and lower osteoblast and osteoclast numbers[15, 16].

The forkhead box O (FoxO) transcription factors are essential in both oxidative stress defense and bone homeostasis (Figure 2). ROS lead to retention of these FoxOs in the nucleus, which in turn activates transcription of target genes for DNA repair (*Gadd45*), ROS detoxification (*SOD2*, *CAT*), cell cycle arrest (*CyclinG2*, *p21*, *p27*) and apoptosis (*Fas*-L, *Bim*-1)[11]. A murine model with combined deletion of *Foxo1, 3* and *4* showed higher oxidative stress levels in bone and bone loss in both cancellous and cortical bone. This was due to a decrease in bone formation, resulting from a decreased osteoblast number[17]. Moreover, β-catenin is an essential co-activator of FoxOs, implying a crosstalk between FoxOs and WNT/β-catenin signaling, a key pathway in bone homeostasis. Since oxidative stress increases with age, FoxOs will sequester more and more β-catenin, at the expense of WNT/β-catenin signaling activity. Therefore, this could contribute to the decreased bone formation and loss of bone mass associated with aging and the pathogenesis of osteoporosis[18-20].

At last, the p53-p66^{Shc} signaling pathway is an important regulator of the intracellular redox status and is involved in age-related skeletal involution[21, 22]. P53 is able to increase ROS levels by increasing p66^{Shc} protein abundance (Figure 2). This was confirmed by a *p66*^{Shc} knockout (*p66*$^{Shc-/-}$) mouse model, which showed a general decrease in oxidative stress level, a lower intracellular ROS concentration in bone and a higher bone mass[21-25]. Also, compared to their wildtype littermates, the *p66*$^{Shc\ -/-}$ mice had a 30% increase in lifespan, which reinforces the notion that oxidative stress is strongly associated with aging. Moreover, a hyperactive p53 mutant mouse model displays premature aging and an osteoporosis phenotype, while *p53* null mice have a high bone mass phenotype, due to an increased osteoblast number and bone formation rate[14, 26, 27].

1.2. Apoptosis

Apoptosis is an essential mechanism during all stages of an organism's development and in eliminating poorly functioning cells and possible cancerous cells later on in life. Aging, however, has a detrimental effect on the regulation of apoptosis, hereby contributing to the development of disorders like osteoporosis (Figure 2). Mainly the effect of aging on the death of osteocytes, the most abundant and long-lived cell type in bone tissue, significantly contributes to bone fragility at older

age. Moreover, osteocyte apoptosis is linked to an increased local bone resorption, due to RANKL production by dying osteocytes[28]. This was seen in a mouse model deficient for *Bax* and *Bak*, the pro-apoptotic members of the Bcl-2 protein family, selectively in osteocytes and osteoblasts. These mice had an increased trabecular bone mass, due to prolongation of the lifespan of short-lived osteoblasts. Conversely, there was an increase in pore formation in the cortex, an increased RANKL-expression and numerous osteoclasts within the pores. This is due to an exaggeration of the adverse effects of aging on long-lived cortical osteocytes, hereby confirming the age-related influence on osteocyte apoptosis[29]. Sex steroids, on the other hand, have an anti-apoptotic effect on osteocytes, and consequently post-menopausal sex steroid deficiency increases osteocyte apoptosis. This was seen in the ovariectomized rat and mouse model of estrogen loss, where osteocyte apoptosis was remarkably increased[19, 30].

A phenomenon associated with osteocyte apoptosis is micropetrosis or hypermineralization of the osteocyte lacunae. Here, osteocyte apoptosis is followed by hypermineralization of perilacunar bone and later with filling of canaliculae with mineralized connective tissue[31]. Busse and colleagues found that aged bone showed more hypermineralized occlusions, while Carpentier and colleagues found an increased percentage of hypermineralized lacunae specifically in osteoporosis patients[32, 33] (Figure 2). Overall, osteocyte apoptosis and micropetrosis cause a decrease in canalicular fluid flow and a deteriorated detection of micro-damage, with deleterious consequences for the bone's structural integrity. In this way, hypermineralization of the lacunae will contribute to bone brittleness and fragility[32, 33].

In addition to osteocyte apoptosis, osteoblasts suffer from apoptosis due to increased oxidative stress levels associated with aging. Here, the $p66^{Shc}$ redox protein translates oxidative signals in osteoblasts into osteoblast apoptosis, which is demonstrated by the $p66^{Shc-/-}$ mice that have lower ROS levels in bone and a high bone mass phenotype[25]. Moreover, the murine model in which *Foxo1, 3* and *4* are deleted, shows bone loss as a result of a decreased bone formation rate and osteoblast number, which is due to increased osteoblast apoptosis[17]. This is why a possible mechanism through which $p66^{Shc}$ regulates apoptosis is by inhibiting the FoxO transcription factors.

1.3. Sex steroid deficiency

Sex steroids play an indispensable role in bone growth, the attainment of peak bone mass during the first decades of life and skeletal homeostasis during adulthood[34]. Here, differences in androgen and estrogen levels in boys and girls contribute to gender differences in bone size and strength. This is due to the fact that periosteal bone formation is restrained by estrogens and stimulated by

androgens, resulting in bigger and stronger bones in males[35]. Furthermore, this initial difference in bone growth and pBMD is already part of the reason why osteoporosis is more prevalent in women.

Androgens and estrogens modulate bone homeostasis differentially during adulthood. Mouse models with cell-specific deletion of the estrogen receptor (ERα) and the androgen receptor (AR), together with studies of hormone replacement identified dissimilar actions of both sex steroids on the different bone cell types[34]. Estrogens exert bone-protective effects through mediating bone resorption of trabecular bone by direct effects on osteoclasts and have indirect effects on osteoblast progenitors to attenuate resorption of cortical bone[36, 37]. The AR in male mice, on the other hand, has protective effects on trabecular bone through osteoblasts and osteocytes, but not through osteoclasts[38-40].

Overall, sex steroids are of major importance to maintain the balance between bone formation and resorption and for slowing down the rate of bone turnover[41]. Later on in life, a decline in sex steroid levels contributes to bone fragility and osteoporotic fractures in men and women[12, 42] (Figure 1). In women, the effects of sex steroid deficiency are accelerated during menopause and therefore cause a rather sudden drop in BMD. In general, age-related bone loss is characterized by decreased bone remodeling in the trabecular bone compartment but increased bone remodeling in the cortical compartment, resulting in increased cortical porosity[43-45]. This difference could be explained by the fact that estrogens regulate trabecular and cortical bone through different cell types, as previously mentioned, and that both cell types respond differently to deviating estrogen levels at older age[34].

Finally, sex steroids have antiosteoporotic effects through their ability to protect against excessive oxidative stress levels[12, 46] (Figure 2). This is demonstrated in 5-month-old gonadectomized female and male mice which show the same increase in ROS levels and p53 and $p66^{Shc}$ activity that is observed with advancing age. This increase in oxidative stress levels could be reversed by replacement with estrogens or a non-aromatizable androgen or by the administration of the antioxidant N-acetylcysteine (NAC). Furthermore, the subsequent increase in osteoblast and osteocyte apoptosis could also be prevented in this way[47]. In addition, an anti-apoptotic effect of estrogens on osteocytes and osteoblasts was also reported by many others[30, 48-52]. So, loss of estrogens during menopause will therefore result in an increase in osteoblast and osteocyte apoptosis and contribute to the development of post-menopausal osteoporosis.

1.4. Macroautophagy

The past decade, knowledge increased about macroautophagy, an evolutionary conserved process of recycling damaged organelles and misfolded proteins in order to prolong cell survival. It is therefore

an important mechanism to sustain cell function, but also to respond to environmental or internal stressors, like hypoxia or increased oxidative stress[53, 54]. A genome-wide association study identified a link between genetic variation in macroautophagy-related genes and BMD[55]. Also, recent studies showed that macroautophagy declines in efficiency with age, with an effect on bone homeostasis[56]. Further *in vivo* evidence for a role of macroautophagy in the development of osteoporosis has been gained by deletion of S6 kinase 1 (S6K1) in mice. Female *S6K1*$^{-/-}$ mice exhibit protection from age-related bone loss, even though they had a 20% increase in lifespan[57]. Here, S6K1 is a ribosomal protein that limits excessive overactivation of macroautophagy under nutrient deprived conditions, suggesting that the *S6K1*$^{-/-}$ mice were less prone to the age-related decline in macroautophagy[57, 58]. Furthermore, murine models with specific deletion of macroautophagy-related genes (*Atg7*, *Atg5* and *FIP200*) in osteoclasts, osteoblast and osteocytes recently all reinforced the notion that macroautophagy in all three bone cell types is essential for bone homeostasis[59-61].

2. Lifestyle factors

Age is an unbeatable risk factor for osteoporosis, but the influence of a person's lifestyle should not be underestimated and is adaptable to some extent. The composition of a person's diet, physical activity, cigarette smoking and alcohol consumption can have a considerable advantageous or deleterious effect on the susceptibility to osteoporotic fractures (Figure 3).

First, a diet partly contains nutrients that are endogenously present in the body, but still need to be taken up through nutrition or the environment, such as vitamin D and calcium (Figure 3). Together with parathyroid hormone (PTH), vitamin D is part of an endogenous and homeostatic mechanism to regulate calcium and phosphate levels, the building blocks of strong and mineralized bone tissue. Insufficient supply in calcium, phosphate and vitamin D impairs bone formation and mineralization and increases the resorption of bone. As a result, bone mass and strength will decrease and fracture risk will increase. Furthermore, undernutrition of these nutrients will decrease muscular mass and strength, neuromuscular function and balance, thereby increasing the risk of falls and fractures. Therefore, sufficient intake of calcium and vitamin D as well as sun exposure, which is essential to produce vitamin D, are strongly recommended, certainly for people at risk for osteoporosis[62-64].

As previously mentioned, oxidative stress has detrimental aging-associated effects on bone homeostasis. Including fruits and dietary phytochemicals in a person's diet therefore might help in the defense against excessive ROS levels[65, 66]. Several studies demonstrated a beneficial effect of frequent fruit intake on bone health[67-70]. Specific advantageous effects on bone health of antioxidants in tomatoes, dried plum, citrus fruits, berry fruits, grapes and apples in human, animal

and cellular studies were recently reviewed elsewhere[66]. Besides antioxidants, dietary protein intake has effects on bone health as well. Protein is identified as being both detrimental and beneficial to bone mass depending on several factors, like the amount of protein and its source, calcium intake and the acid-base balance of the diet[71]. Overall, higher protein diets are associated with greater bone mass and fewer fractures, as long as calcium intake is adequate[71, 72]. The acid-base balance is negatively influenced by a relatively higher consumption of meat, eggs, fish and cereal (net acid load), while consumption of fruit and vegetables results in a potential positive effect (net base load)[71]. So, besides positively affecting the redox balance, consumption of fruit and vegetables generates a state of metabolic alkalosis, which is favorable for bone growth and calcium balance, prevents bone loss and reduces the risk for osteoporotic fractures[73, 74].

The last decade, physical activity became more and more important in light of osteoporosis and possible prevention (Figure 3). Physical activity stands for mechanical pressure and loads on the complete musculoskeletal system. As mentioned previously, osteocytes form a tight and extensive communication network that can sense mechanical strain during physical exercise. By doing so, osteocytes react by emitting specific signals to cells at the bone surface and in this way they can initiate the bone remodeling process[5]. For example, in reaction to mechanical forces osteocytes decrease the secretion of sclerostin, an inhibitor of the WNT/β-catenin signaling pathway. The WNT signaling pathway is activated when WNT ligands bind to the receptor complex formed by LRP5/6 coreceptors and Frizzled (Fz) receptors. Activation of the pathway by WNT results in stabilization of β-catenin and activation of several target genes involved in osteogenesis. Since it is demonstrated that different genetic mutations in *LRP5* can lead to monogenic bone disorders with either an increased or decreased bone mass, several *in vitro* and *in vivo* experiments have confirmed that this pathway is one of the most important pathways in the regulation of osteoblast proliferation, differentiation and function[75, 76]. Consequently, this pathway is a key regulator of bone formation and is considered to be an interesting target for osteoporosis treatment. Sclerostin is encoded by the *SOST* gene in which mutations were found in two monogenic sclerosing bone dysplasias (Van Buchem disease and sclerosteosis)[77, 78]. Later it turned out that sclerostin is an extracellular inhibitor of WNT/β-catenin signaling[79, 80]. Therefore, a decreased secretion of sclerostin by osteocytes as reaction to mechanical loading, will results in a higher activation of the WNT/β-catenin signaling pathway which results in increased bone formation, BMD and bone strength. The purpose of this mechano-sensing mechanism is indeed to trigger bone remodeling[81, 82]. In addition to its effects on osteocytes, the levels of oxidative stress are beneficially modulated by exercise as well[83].

Alcohol consumption is part of a person's lifestyle as well and affects the bone metabolism depending on the consumption quantity (Figure 3). Chronic, excessive intake of alcohol is associated

with a decrease in BMD and bone mineral content, thereby increasing the susceptibility to fractures. These effects are the results of both direct and indirect effects on bone metabolism. Furthermore, it was shown that excessive alcohol intake results in inhibition of bone formation as well as increased bone resorption via several mechanisms[84]. Even though negative effects are ascribed to alcohol consumption, there is evidence that moderate alcohol consumption is beneficial for bone. So, here again, moderation is everything and defines the eventual effect on bone tissue[84, 85].

Several studies showed a clear association between cigarette smoking and a dose-dependent deficit in BMD at several sites in the body of smokers compared to non-smokers (Figure 3). The mechanisms underlying this bone loss are less clear. This is mainly due to the fact that smoking affects many tissues and regulatory pathways so that the effect seen on bone is probably a combination of many direct and indirect actions. Anyhow, smoking cessation is strongly recommended, since the deleterious effects on bone are, at least partially, reversible[86].

HERITABLE FACTORS IMPLICATING THE RISK FOR OSTEOPOROSIS

As previously mentioned, the most common form of osteoporosis is multifactorial and caused by both genetic and environmental factors. In case of multifactorial osteoporosis, the genetic impact is multigenic and defined both by a large number of polymorphisms with small effect sizes and by epigenetic factors. However, osteoporosis can also be caused by one mutation in one gene. Monogenic conditions associated with reduced bone mass have a low prevalence and are mostly detected in children or adolescents. Based on the genetic cause and the presence of additional symptoms, this group of monogenic disorders can be subdivided. Here, we discuss the genetic cause of Osteogenesis Imperfecta, juvenile osteoporosis and of some other syndromes characterized by osteoporosis. Studying the genetic cause of these rare Mendelian disorders has been a valuable strategy resulting in the identification of genetic determinants contributing also to multifactorial osteoporosis.

1. Conditions with decreased bone mineral density and increased bone fragility

1.1. Osteogenesis Imperfecta

Osteogenesis Imperfecta (OI) is a clinically and genetically heterogeneous group of connective tissue disorders which are commonly referred to as brittle bone disease[87]. In patients with OI, low bone mass and fractures are associated with extraosseus connective tissue symptoms such as blue sclera and dentinogenesis imperfecta. Classification of this heterogeneous group of disorders changed throughout the years. Van Dijk et al. recently reviewed the clinical phenotype, nomenclature and severity of the different OI types and grouped them in five major OI types (I-V)[88]. At this moment, 16 causative genes are identified and this number will probably increase in the coming years with the use of next generation sequencing technologies (Table 1)[88, 89]. Despite this increasing number of disease causing genes, 90 % of patients have a mutation in *COL1A1* or *COL1A2*[90]. In these patients, OI is inherited in a dominant manner and depending on the type and location, mutations in *COL1A1*/*2* can cause OI type I, II, III or IV. OI type V is also inherited in an autosomal dominant manner, but only one disease causing gene*, interferon-induced trans membrane protein 5 (IFITM5)*, has been identified, at least to date (Table 1)[87, 89, 91].

Around 10% of the OI cases are inherited in a recessive manner and, so far, 13 causative genes are identified. Mutations in some of these genes (*CRTAP*, *PPIB* and *LEPRE1*) can cause different types of OI, similar as reported for *COL1A1/2.*

The majority of these genes are involved in the collagen type 1 biosynthesis [87, 88, 92]. However, one study demonstrated that the expression of TGFβ receptors is increased in osteoblast of OI patients[93]. Furthermore, in several mouse models with OI altered TGFβ signaling is observed and suggested as the primary mechanism in the pathogenesis of OI. Consequently, targeting TGFβ signaling could be a promising target for OI treatment[94]. Besides the genes involved in collagen type 1 biosynthesis and in TGFβ signaling, some of the genes have another role in bone homeostasis (Table 1). *Sp7* encodes for osterix, a well-known osteoblast-specific transcription factor regulating osteoblast differentiation[95]. *SERPINF1*, encodes pigment epithelium-derived factor (EPDF) which is required for osteoid mineralization and consequently, is important for bone formation and bone remodeling[92]. Finally, WNT1 is a known activator of the WNT/β-catenin signaling pathway, a well-established pathway regulating bone formation as described. Mutations in *WNT1* can not only cause OI type III but are also found in several patients with juvenile osteoporosis. In the latter group of patients, osteoporosis is dominantly inherited[96, 97].

1.2. Juvenile osteoporosis

Juvenile osteoporosis (JO) is a group of heritable disorders presenting during childhood and characterized by a low BMD and bone fragility without the extraskeletal features found in patients with OI. The low bone mass in JO patients is typically the result of decreased osteoblast activity and mainly affects cancellous bone[98, 99]. As mentioned previously, heterozygous mutations in *WNT1* are found in several patients with JO. Functional studies demonstrated that the mutated WNT1 has an impaired capacity to activate WNT/β-catenin signaling[96, 97]. Besides *WNT1*, a co-receptor of the WNT/β-catenin signaling, namely low density lipoprotein receptor related protein 5 (LRP5), is shown to cause JO. Here, heterozygous mutations in *LRP5* result in impaired activation of the WNT/β-catenin signaling[98-100].

Finally, a third disease-causing gene for juvenile osteoporosis, Plastin 3 (PLS3), was recently discovered by van Dijk and colleagues[101]. Pathogenic variants in the *PLS3* gene are found in several families with X-linked osteoporosis. Expression studies demonstrated that PLS3 is ubiquitously expressed in solid tissue, however, the function of PLS3 in bone remains unclear. It is known that PLS3 can bind actin and that it is part of a protein family that plays a role in the dynamic assembly and disassembly of the actin cytoskeleton by interacting with monomeric or globular and filamentous actin. More studies are needed to clarify the role of PLS3 in the regulation of bone mass and in the pathogenesis of osteoporosis[101, 102].

1.3. Syndromes with decreased bone density

The final group of monogenic disorders discussed here, are several syndromes characterized by osteoporosis and additional features (Table 2)[88, 103]. Osteoporosis pseudoglioma (OPPG) and Bruck syndrome are caused by genes linked to JO or OI. OPPG is an autosomal recessive disorder marked by severe osteoporosis and early-onset blindness, caused by homozygous or compound heterozygous mutations in *LRP5*[98, 99, 104, 105]. The importance of LRP5 in the regulation of bone formation is demonstrated by the fact that mutations in *LRP5* can cause different disorders characterized by either a decreased bone density (OPPG and JO) or an increased bone density (High bone mass phenotype or Worth disease), depending on the type or location of the mutation[75, 106].

Bruck syndrome (BS) type 1 and 2 are autosomal recessive inherited syndromes caused by mutations in respectively, *FKBP10* and *PLOD2*. Mutations in both these genes are also reported to cause OI type III which again demonstrates the importance of these proteins in the regulation of bone mass (Table 1 and 2). In addition to osteoporosis, patients with BS also have contractures of the large joints and pterygia[88, 103]. BS and OI type III are both recessive disorders and it has been reported that the same mutation in one family can result in both disorders, which is probably due to the influence of additional genetic modifiers[107-109].

2. The role of genetic factors in multifactorial osteoporosis

Despite the fact that fractures are the most important clinical outcome, at this moment BMD measurements serve as the most widely used phenotype to diagnose osteoporosis since it is an important predictor of fracture. Several twin and family studies have shown that 50-85% of the variance in BMD is genetically determined[110]. Although BMD is the best predictor of fractures, it is demonstrated that the heritability of fractures (25-35%) is partially independent of BMD. Furthermore, a large number of individuals with osteoporotic fractures do not have osteoporosis according to BMD criteria[111]. This confirms that, besides BMD, additional parameters such as geometrical bone parameters, bone quality and muscle strength are important in determining the risk for osteoporotic fractures[112, 113].

In the last years, several genome wide association studies have been performed in populations with increasing sample sizes. These studies highlighted the extreme polygenic nature of the variance in BMD[114-121]. Many SNPs (over 50 loci) are already reported to be associated with BMD; however, they can only explain a small fraction (5.8%) of the total genetic variance in BMD[115]. Several of the associated SNPs are located in or nearby genes with a known function in the regulation of bone homeostasis. A large number of these genes are involved in WNT signaling (*LRP5, SOST, WNT1*, *LRP4*, …), enchondral ossification (*RUNX2*, *SOX9*,…), osteoclastogenesis (*RANK*/*RANKL*/*OPG*) or were

previously reported to be causative for rare monogenic bone disorders (Figure 4). In the same study, Estrada and colleagues not only demonstrated that the genetic influence on BMD is highly polygenic but they also showed that several SNPs associated with BMD are also associated with fracture risk in a smaller and consequently less powerful study population[115].

Since only a small fraction of the genetic impact is yet identified, more studies are needed with different study designs and different phenotypes (fracture risk, heel BMD, …) in order to identify more variants and to explain the missing heritability[122-124]. Populations with increasing sample size will increase the power of the studies. In addition large sequencing efforts will make it possible to identify also rare variants influencing osteoporosis risk. Furthermore, part of the genetic impact on osteoporosis and bone fragility can probably also be explained by structural variations or epigenetic mechanisms.

Copy number variations represent a form of structural variation in which the number of copies of a DNA fragment varies in individuals. Only few genome-wide studies investigating the effect of CNVs on osteoporosis and BMD are performed. Two independent studies identified respectively *VPS13B* and *UGT2B17* as susceptibility genes for osteoporosis, however, the results of *UGT2B17* could not be replicated in an independent candidate gene study[125-127]. Recently, a rare deletion in an intergenic region on the 6p25.1 locus was reported to be associated with osteoporotic fractures. It is yet to be determined how the deletion of this region influences fracture risk. It is possible that the region contains regulatory elements which affect the transcription of target genes or that a yet unidentified gene is disrupted by the deletion[124].

3. Epigenetic mechanisms influencing the risk for multifactorial osteoporosis

Epigenetics refers to stable and heritable changes in phenotype or gene expression due to mechanisms other than the changes in the underlying DNA sequence. These mechanisms are dependent in the interaction between the environment and the genome. There are three epigenetic mechanisms described; posttranslational histone modifications, microRNAs (miRNA) and DNA methylation[128-130]. The role of epigenetic regulation in bone homeostasis and osteoporosis risk is not yet extensively studied, but several studies already demonstrated that differentiation and activity of osteoblasts and osteoclasts can be regulated by all three epigenetic mechanisms (for review see[129]).

Of these mechanisms, posttranslational histone modifications are probably the most complex mechanism. They are dynamic and regulated by enzymes which can both promote or reverse specific modifications[129, 130]. An example of an enzyme that regulates bone remodeling by regulating posttranslational histone modifications is Sirtuin1 (Sirt1). Sirt1 is a histone deacetyltransferase that

can repress the expression of *SOST* by deacetylating the *SOST* promotor[131]. Decreased expression of *SOST* results in increased WNT/β-catenin signaling activity and bone formation[75]. In addition to Sirt1, there are other enzymes that can regulate bone remodeling by regulating osteoblast and osteoclast differentiation which are recently reviewed by Vrtacnik and colleagues[129].

MiRNAs are small non-coding RNA molecules that are incorporated in the RNA-induced silencing complex (RISC complex) and bind to target mRNA[129, 130]. The RISC complex is a multiprotein complex that is activated upon binding of the miRNA to the complementary mRNA which results in degradation or translational repression of the target mRNAs[132]. Most miRNAs are able to bind several mRNAs and most mRNAs can be targeted by several miRNAs. There are already numerous miRNAs reported that regulate the differentiation of osteoblast and osteoclasts and are associated with osteoporosis. The role of miRNAs in the regulation of osteogenesis and osteoporosis was recently reviewed elsewhere[133, 134].

The third epigenetic mechanism is DNA methylation, a reversible modification of a cytosine residue located 5' to a guanosin residue (CpG). DNA methylation is primarily associated with repression of gene expression by either inhibiting or facilitating the binding between proteins and DNA[129, 130]. Several studies already demonstrated that expression of some important modulators of bone remodeling are regulated by methylation[129]. It is widely recognized that expression of *SOST* is mainly found in the osteocytes embedded in the mineralized bone. More recently, Delgado-Calle and colleagues demonstrated that the differentiation of osteoblasts to osteocytes is accompanied by a decreased methylation of the *SOST* promotor which facilitates the osteocyte specific expression of *SOST*[135]. Another important regulatory pathway of bone remodeling is the RANKL/OPG/RANK pathway. RANKL and OPG are both expressed in the osteoblasts and it is demonstrated that the RANKL/OPG ratio is at least partially regulated by (de)methylation of a CpG island downstream of RANKL and a CpG island upstream of OPG[136]. Both studies clearly show the importance of DNA methylation as coregulator of bone homeostasis, however, more studies are needed to further elucidate this.

CONCLUSION

Osteoporosis presents itself in most cases as a typical disease of the elderly implicating that prevention and treatment of this condition deserves attention in our aging population. The underlying reduced bone mass and quality, resulting in an increased risk for fracturing, have a complex etiology as being influenced by endogenous, environmental and genetic factors. To some extend the disease seems to be an unavoidable part of the human ageing process. Many processes that are involved in aging of other parts of the body, contribute also to the observed deterioration of the bony parts of our skeleton. Especially oxidative stress within cells increases over age due to a decrease in antioxidative mechanisms (including the effect of sex steroids) and increase in mitochondrial respiratory chain leakage. Partially secondary to the increased oxidative stress, the mechanisms of apoptosis and macroautophagy are also deregulated during aging. All these interconnected processes have an effect on osteoblasts, osteocytes and osteoclasts resulting in unbalanced bone remodeling and a decrease in bone mass over age.

In addition, some environmental factors contribute to the risk for osteoporosis, implicating that one can influence its own individual risk to some extent. Vitamin D, calcium, a diet rich in antioxidants as well as physical activity work protective while smoking and overconsumption of alcohol have a detrimental effect on bone. However, a major part of the risk to develop osteoporosis is explained by genetic factors. In a very small minority of cases this is due to a genetic defect in one gene. A lot of these disease causing genes have been identified in the last two decennia resulting in important novel insights in the mechanisms of bone metabolism and homeostasis. With the recent implementation of high throughput next generation DNA sequencing technologies, a number of additional gene identifications can be expected in the near future broadening our current knowledge and understanding. However, in most cases with a diagnosis of osteoporosis, the genetic architecture behind this phenotype is complex with a lot of genetic factors involved each of them with a small effect size. The large efforts performed in huge consortia have resulted in the identification of an extended number of risk variants within genes. For the moment, the relevance of the generated data lies more in the nature of these genes and pathways rather than providing us a tool to explain or a test for the genetic risk for osteoporosis in an individual. The majority of the genetic risk variance for osteoporosis cannot be explained yet. In future, this could partially be solved by all the huge sequencing efforts that are currently ongoing. By sequencing the exomes or even whole genomes of large groups of individuals, the identification of rare variants that have a larger effect on bone mineral density than the common variants currently studied in genetic association studies can be

expected. However, it is rather questionable whether even these novel data a genetic test will be established with a predictive value that comes close to for example BMD measurements.

Finally, it is clear that all the scientific breakthroughs realized in recent years are impacting on the future prevention and treatment of osteoporosis. Several pathways identified turned out to have high therapeutic potential. On the bone resorption site, a RANKL antibody (Denosumab) turned out to be very effective and results from ongoing clinical studies indicate that Odanacatib, an antibody against the lysosomal protease Cathepsin K, might also have high potential[137, 138]. Maybe even more important is the fact that on the anabolic site, alternatives to teriparatide (a recombinant form of PTH) are expecting to make their way to the market. Phase 2 clinical trials for Romosozumab and Blozozumab, both monoclonal antibodies to the Wnt-signaling inhibitor sclerostin, are very promising. With these, a significant increase in bone formation can be generated in a short period of time[137, 138]. Long term follow-up studies will be needed to evaluate whether this increase in bone mass can be kept over time, potentially using a combined treatment with an antiresorptive agent.

In conclusion, a lot of factors involved in multifaceted osteoporosis have been unraveled in the last two decades not only resulting in a better understanding of its pathogenesis but also with therapeutic implications for the current and future patient. With no doubt, this will help the medical community to treat a patient with osteoporosis in a more individual and efficient manner.

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REVIEW CRITERIA

PubMed was searched for articles reviews between February 2014 and May 2014 with particular attention to original papers and reviews published in the past 10 years. The database was searched using these keywords or using the combination of the keywords: “osteoporosis”, “bone loss”, “bone fragility”, “bone quality”, “pathogenesis”, “age related”, “genetics”, “bone mineral density or BMD”, “fracture risk”, “association studies”, “meta-analysis”, “Osteogenesis Imperfecta”, “juvenile”, “idiopathic”, “X-linked”, “post-menopausal”, “sex steroid deficiency”, “lifestyle”, “oxidative stress”,

"apoptosis", "macroautophagy", "copy number variations or CNV", "epigenetic" and "miRNAs". If appropriate, reference lists were screened for additional publications.

AUTHOR DETAILS

Gretl Hendrickx, PhD student, graduated in molecular and cellular biomedical sciences at the University of Antwerp, Belgium. Currently, she is completing her PhD at the Department of Medical Genetics of the University of Antwerp in Wim Van Hul's lab. Her main research interests are the genetics of osteoporosis and sclerosing bone disorders, with a specific focus on genes of the Wnt signaling pathway.

Eveline Boudin, PhD, graduated in biomedical science and obtained a PhD in biomedical science from the University of Antwerp, Belgium. Currently, she is working as postdoctoral researcher at the Department of Medical Genetics of the University of Antwerp. Her research interests and research focuses on the genetics of bone disorders with a specific focus on monogenic sclerosing bone disorders.

Wim Van Hul, PhD, is full professor of molecular biology and genetics at the University of Antwerp, Belgium. He is principal investigator at the department of medical genetics and the aims of his research team are to characterize the genetic causes of monogenic skeletal dysplasias as well as to contribute to the unraveling of genetic mechanisms underlying osteoporosis and obesity.

TABLES

Table 1: Overview of the different types of osteogenesis imperfecta, causative genes and function of the proteins in bone metabolism

** Classification based on Van Dijck et al 2014*

OI Type *	Gene	Inheritance	Protein product	Function
Type 1 ***Non-deforming OI with blue sclerae***	*Col1A1*	AD	Collagen type I alpha 1	Collagen type 1 biosynthesis
	Col1A2	AD	Collagen type I alpha 2	Collagen type 1 biosynthesis
Type2 ***Perinatally lethal OI***	*Col1A1*	AD	Collagen type I alpha 1	Collagen type 1 biosynthesis
	Col1A2	AD	Collagen type I alpha 2	Collagen type 1 biosynthesis
	CRTAP	AR	Cartilage-associated protein	Collagen type 1 biosynthesis
	LEPRE1	AR	Leucine proline-enriched proteoglycan (leprecan) 1, prolyl 3-hydroxylase 1	Collagen type 1 biosynthesis
	PPIB	AR	PPIB peptidylprolyl isomerase B (cyclophilin B)	Collagen type 1 biosynthesis
Type 3 **P*rogressively deforming***	*Col1A1*	AD	Collagen type I alpha 1	Collagen type 1 biosynthesis
	Col1A2	AD	Collagen type I alpha 2	Collagen type 1 biosynthesis
	BMP1	AR	Procollagen type I C-propeptidase	Collagen type 1 biosynthesis
	CRTAP	AR	Cartilage-associated protein	Collagen type 1 biosynthesis
	FKBP10	AR	FKBP-type peptidyl-prolyl cis/trans isomerase	Collagen type 1 biosynthesis
	LEPRE1	AR	Leucine proline-enriched proteoglycan (leprecan) 1, prolyl 3-hydroxylase 1	Collagen type 1 biosynthesis
	PLOD2	AR	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2	Collagen type 1 biosynthesis
	PPIB	AR	PPIB peptidylprolyl isomerase B (cyclophilin B)	Collagen type 1 biosynthesis
	SERPINF1	AR	Serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	Osteoid mineralization
	SERPINH1	AR	Serpin peptidase inhibitor, clade H (heat shock protein 47), member 1	Collagen type 1 biosynthesis
	TMEM38B	AR	Trimeric intracellular cation channel N (TRIC-B)	?
	WNT1	AR	Wingless-type MMTV integration site family, member 1	Bone formation, osteoblast activity
	CREB3L1	AR	OASIS (cAMP responsive element binding protein 3-like 1)	Collagen type 1 biosynthesis
Type4 ***Common variable OI with normal sclerae***	*Col1A1*	AD	Collagen type I alpha 1	Collagen type 1 biosynthesis
	Col1A2	AD	Collagen type I alpha 2	Collagen type 1 biosynthesis
	CRTAP	AR	Cartilage-associated protein	Collagen type 1 biosynthesis
	PPIB	AR	PPIB peptidylprolyl isomerase B (cyclophilin B)	Collagen type 1 biosynthesis
	SP7	AR	Osterix	Osteoblast-differentiation
Type5 ***OI with calcification in interosseous membranes***	*IFITM5*	AD	Interferon-induced transmembrane protein 5	?

Table 2: Overview of syndromes with low bone mass and other features, causative genes and encoded proteins.

*mutations in this gene are also linked to OI or JO

Disorder	**Inheritance**	**Gene**	**Protein**	**Additional clinical features**
Osteoporosis pseudoglioma syndrome	AR	*LRP5**	Low density lipoprotein receptor (LDLR) related protein 5	Severe blindness
Bruck syndrome type I	AR	*FKBP10**	FKBP-type peptidyl-prolyl cis/trans isomerase	Joint contractures, pterygia
Bruck syndrome type II	AR	*PLOD2**	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2	Joint contractures, pterygia
Hajdu-Cheney syndrome	AD	*NOTCH2*	Notch 2	Facial abnormalities, acro-osteolysis, hearing loss, renal cysts
Gnathodiaphyseal dysplasia	AD	*ANO5*	Anoctamin 5	Purulent osteomyelitis of the jaws in adulthood
Geroderma osteodysplasticum	AR	*SCYL1BP1*	SCYL1-binding protein 1	Wrinkly skin
Spondylocular syndrome	AR	/	/	Platyspondyly, cataract, retinal detachment, short trunk, facial dysmorphism, immobile spine, kyphosis
Cole-carpenter dysplasia	/	/	/	Craniosynostosis, ocular proptosis, distinctive facial features, hydrocephalus,
Calvarial doughnut lesions with bone fragility	AD	/	/	Lumps on the head, dental caries, increased serum ALP levels
Cleidocranial dysplasia	AD	*RUNX2*	Runt-related transcription factor 2	Persistent fontanels, hypoplasia/aplasia of clavicles, scoliosis, wide pubic symphysis, dental and digital anomalies,
ADCAD2	AD	*LRP6*	LDLR related protein 6	Early-onset coronary disease

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