Genetic and acquired factors influencing the effectiveness and toxicity of drug therapy in osteoporosis

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ABSTRACT

Introduction. Osteoporosis is a highly prevalent skeletal disorder characterized by compromised bone strength, usually related to decreased bone mass and microstructural alterations of bone tissue, predisposing a person to an increased risk of fracture. As other prevalent disorders, osteoporosis is the result of a complex interplay of genetic and acquired factors.

Areas covered. We provide an update of recent studies aimed to identify the clinical and genetic factors that influence the response of drugs used to treat osteoporosis, as well as those determining the risk of two intriguing adverse effects of antiresorptives: osteonecrosis of the jaw (ONJ) and atypical femoral fractures (AFF).

Expert opinion. Several clinical factors have been suggested to increase the risk of a poor drug response, such as advanced age and frailty. Candidate gene studies suggest that some common polymorphisms of the Wnt pathway and farnesyl diphosphate synthase (FDPS), the target enzyme for bisphosphonates, also influence the response to antiresorptives. However, they await for replication in large independent cohorts of patients. Similarly, some genetic and acquired factors may influence the risk of ONJ and AFF. Preliminary data suggest that the risk of suffering these adverse effects may have a polygenic basis.

Keywords: osteoporosis, fractures, pharmacogenetics, atypical femoral fractures, osteonecrosis of the jaw, bisphosphonates, denosumab.

1. Introduction

1.1. Osteoporosis: a disorder of skeletal strength

Osteoporosis is a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture [1]. It is usually related to decreased bone mass and microstructural alterations of bone tissue.

In the clinic, bone mass is most frequently determined by using dual-energy X-ray absorptiometry (DXA), which, by measuring the attenuation of x-rays, allows estimating the amount of calcified tissue. The results of DXA studies are usually given as "bone mineral density" (BMD). BMD is a good index of bone mass and it correlates with bone strength and the risk of fractures.

1.2. Determinants of bone strength

Common fractures related to bone fragility are those of the pelvis, the hip (proximal femur), the proximal humerus and the vertebral bodies. BMD, a surrogate of bone mass, is a major determinant of bone strength and consequently of the risk of fractures. Overall, each standard deviation decrease in BMD increases fracture risk by a factor between 1.4 and 2.1, depending on the age of the individual and the type of fracture [2].

DXA-measured BMD accounts for 60-70% of the variation in bone strength [3]. Other determinants of bone strength are depicted in figure 1. Bone geometry is also important to resist the forces induced by muscle contraction and those derived from traumatisms. One of the best-analyzed geometric characteristics in relation to fracture propensity is the hip length axis. The longer the axis, the higher the risk of hip fracture, an association that is independent of hip BMD [4,5]. The microarchitecture of bone tissue also influences bone strength. Among the likely important features are the porosity of the cortical bone and the thickness and connectivity of the trabeculae of the spongy bone [6].

Apart of the amount of bone tissue (this is, bone mass or bone density) and its spatial distribution, it seems likely that the quality of bone tissue also plays a role in determining bone strength and fracture risk [7,8]. "Quality" may have several components, which are likely to include collagen amount and polymerization, as well matrix mineralization. Unfortunately, the quality of bone tissue cannot be measured in clinical practice. Nevertheless, a recently introduced microindentation technique has provided some promising results in preliminary studies [9,10].

1.3. Bone remodeling

The mass, the microarchitecture and the qualitative properties of bone tissue are determined by bone remodeling. Remodeling is also critical for repairing microdamage to the skeleton, and to maintain the skeletal integrity by allowing the adaptation to changing mechanical loads [11].

The first phase of bone remodeling is bone resorption, mediated by osteoclasts. Following the recruitment and differentiation of osteoclast precursors, mature osteoclasts excavate a resorption cavity or lacuna on a trabecular surface (or a tunnel within the compact cortical bone). Bone removed by osteoclasts is later replaced by new bone formed by osteoblasts. Thus, small packets of bone tissue are being continuously removed and latter replaced by new bone at scattered foci throughout the skeleton. This tightly organized process is regulated by a complex interplay of systemic hormones and, particularly, local factors, including, among others, the RANKL-OPG system, and the Wnt signaling pathway [12-14]. It is evident that any alteration of bone remodeling with a predominance of bone resorption over bone formation will eventually result in a loss of bone mass and osteoporosis.

Some information about the status of bone remodeling may be obtained noninvasively by measuring the so-called bone turnover markers. Among the indices of bone formation, the bone isoenzyme of alkaline phosphatase, osteocalcin and PINP are the most widely used. Several collagen degradation products, such as crosslaps (telopeptide of type I collagen), and TRAP5b, an enzyme highly expressed by osteoclasts, are some of the best known markers of bone resorption [15].

2.   Drug therapy for osteoporosis

An adequate supply of nutrients, including calcium and vitamin D, is required for maintaining a balanced bone remodeling and bone mass. Therefore, it is important to prescribe supplements of these nutrients as needed for the prevention and treatment of osteoporosis.

Other drugs used for osteoporosis are classified either as antiresorptives or anabolics, depending on whether their main effect is to inhibit bone resorption or to stimulate bone formation (table 1). However, it is worthy noticing that, due to the existence of coupling mechanisms between osteoclasts and osteoblasts, in most instances the inhibition of bone resorption induced by antiresorptives drugs is followed by a secondary inhibition of bone formation. On the other hand, some increase in resorption usually follows the stimulation of bone formation by anabolic agents.

Estrogens have complex actions, and, through direct and indirect effects (reviewed in [16]), inhibit bone resorption and have a positive effect on bone mass. Although this may represent an additional benefit for patients taking estrogens for other reasons, given the adverse effects of estrogens on the vascular and breast tissues, they are not currently considered as antiosteoporotic agents of choice.

Selective modulators of estrogen receptors (SERMS, including raloxifene and bacedoxifene) retain part of the beneficial effects of estrogens on bone, but, different from natural estrogens, do not increase the risk of breast cancer. Therefore, they are approved for osteoporosis. In fact, they have been shown to increase BMD and decrease the risk of vertebral fractures. However, their anti-resorptive potency is limited and they do not reduce the risk of hip fractures [17,18] .

Aminobisphosphonates have a potent anti-osteoclastic effect that results in an inhibition of bone resorption, increased bone mass and decreased risk of both vertebral and peripheral fractures [17]. Alendronate and risedronate are the most commonly prescribed oral agents, usually administered once a week, whereas zoledronate is given intravenously once a year. Aminobisphosphonates impair osteoclast function and induce cell death by inhibiting the mevalonate pathway [19,20]. On the contrary, these drugs may have antiapoptotic actions on osteoblasts and osteocytes by a mechanism involving the phosphorylation of ERKs, which, in turn, tend to have some positive effect on bone formation [21]. However, bone formation is diminished in bisphosphonate-treated patients because that effect is over-ridden by the decrease in osteoblast activity that accompanies the inhibition of bone resorption by virtue of the coupling mechanisms between both arms of the skeletal remodeling.

Denosumab is a monoclonal antibody that blocks the receptor activator of nuclear factor kappa-B ligand (RANKL). RANKL is produced by osteocytes and other cells in the bone. It is present both in soluble and membrane-bound forms and, by binding to its receptor RANK, induces the differentiation of osteoclast precursors. Thus, denosumab has a potent antiresorptive effect, increases BMD and reduces the risk of vertebral and non-vertebral fractures [22].

Teriparatide, a molecule comprising the first 34 aminoacids of the intact parathyroid hormone, is the most commonly used anabolic agent. When administered as daily subcutaneous injections it stimulates bone formation by incompletely known mechanisms that appear to include increased expression of growth factors, such as IGFI, and activation of the Wnt pathway [23-26]. The latter is due, at least in part, to a reduced expression of sclerostin by osteocytes. Sclerostin, a peptide encoded by the SOST gene, inhibits the binding of Wnt ligands to their membrane receptors and consequently tends to inhibit the Wnt pathway [27-29].

Antibodies blocking sclerostin activity have induced marked increases in bone formation and BMD in clinical trials and are therefore a promising anabolic therapy [30]. Odanacatib inhibits cathepsin K, a protease involved in the degradation of bone matrix. This antiresorptive drug has increased BMD in several clinical trials [31,32]. However, there are some differences between odanacatib and other antiresorptive agents. In fact, osteoclast numbers is decreased by bisphosphonates, whereas it is increased in animals treated with odanacatib. In parallel with this, the expression of bone formation genes is decreased by bisphosphonates, whereas it is unaffected or increased by odanacatib [33]. These findings suggest that, besides it antiresorptive effect, odancatib could have a positive effect on bone formation.

3.   Clinical factors determining the effectiveness of drug therapy

The objective of treating osteoporotic patients is to reduce the risk of fracture. Although several antiosteoporotic medications have shown to be effective in reducing the risk of fracture in postmenopausal women and are recommended first-line therapies for patients with osteoporosis, they do not eliminate the possibility of fracture. In fact, with these medications the relative risk reduction is roughly 50-70% for vertebral fractures, 20% for nonvertebral fractures, and 40% for hip fractures, depending on the drug [17,18]. Consequently, an incident fracture sustained during treatment for osteoporosis does not necessarily represent treatment failure. Hence, several authors have tried to provide operative definitions of treatment failure. The patient response was initially classified as inadequate if there were two or more episodes of fracture despite adequate calcium and vitamin D supplementation and a good compliance to treatment for at least one year [34]. More recently, an operational definition of inadequate response to antiresorptives included the following circumstances: two or more incident fractures, or lack of reduction of bone turnover markers, or decrease of BMD below the least significant change [35].

As expected, good adherence is a critical factor to attain the expected therapeutic effect. A retrospective cohort study of patients with osteoporosis and rheumatoid arthritis concluded that non-adherence was the most powerful risk factor for bisphosphonate treatment failure [36]. Immobilization, high inflammatory activity and glucocorticoid use were other factors associated with a poor response.

Even among highly adherent users of oral BP therapy, a minority develop multiple fractures while on treatment. Improved and specific management strategies for fracture prevention are needed for this subgroup of patients. In line with this, several investigators tried to identify the factors associated with treatment failure in primary osteoporosis. An analysis of the Global Longitudinal Study of Osteoporosis in Women (GLOW) revealed three variables predictive of treatment failure after multivariable analysis: bad SF-36 vitality score, two or more falls in the past year and prior fracture [37]. Other factors associated with treatment failure in some studies are low vitamin D levels, smoking, alkaline phosphatase levels, number of concomitant treatments, older age, female sex, dementia, ulcer disease, and Parkinson's disease [37-39].

Although the consistency of the results across the studies is far from perfect, it seems that in general frail patients with advanced age, other comorbidities, prior fractures and propensity to fall are most likely to show an inadequate response.

4. Genetic factors influencing drug response

Many investigators have explored the association of genetic polymorphisms with the BMD response to antiosteoporotic drugs. A detailed description of those studies is out of the scope of this paper, but the interested reader is referred to several recent reviews [40-42] .

No large-scale, hypothesis-free, genome-wide pharmacogenetic studies of osteoporosis have been carried out. In fact, most published studies include a relatively small number of subjects and

explored just one or a few polymorphisms. Candidate genes have been usually selected on the basis of the current knowledge about bone biology and pharmacology. For example, the Wnt pathway is known to play a major role in the differentiation of osteoblast precursors and, as a consequence, stimulates bone formation. Moreover, Wnt activation increases the ratio of osteoprotegerin (OPG)/RANKL in the bone microenvironment. Since OPG is a decoy receptor for RANKL, it results in the inhibition of bone resorption. In line with this, several investigators have proposed that some polymorphisms of genes related to the Wnt pathway, such as the Wnt co-receptor LRP5, the intracellular signal transducer GSK, or the ligand inhibitor sclerostin, are associated with the response to bisphosphonates and raloxifene [43-46]. However, negative results were found in some studies [47] and a complete independent replication of those findings is still pending.

A more direct pharmacogenetic rationale underlies studies exploring the influence of genetic variants of the mevalonate pathway on bisphosphonate response. Farnesyl diphosphate synthase (FDPS) is the main target of the widely used aminobisphosphonates (alendronate, risedronate, zoledronic acid) [20]. Enzyme inhibition by these drugs impairs the prenylation of several proteins, which induce the apoptosis of osteoclasts. Several investigators found that a common polymorphism of FDPS is associated with changes in BMD and bone turnover markers following therapy with aminobisphosphonates in Caucasian [48,49], but not in Asian women [50,51]. Thus, European women with the less common CC genotype at rs2297480 had a blunted BMD response to oral bisphosphonates. Those studies suggest an ethnic interaction that needs to be confirmed in larger studies.

Vitamin D plays a critical role in skeletal homeostasis. Severe vitamin D deficiency impairs the mineralization of bone matrix and causes osteomalacia and rickets. Less severe vitamin D deficiency impairs calcium absorption may secondarily increase PTH secretion, which induces bone loss. Therefore, it is important to maintain adequate levels of vitamin D in patients being treated for osteoporosis. Nevertheless, the role of systematic vitamin D supplementation on bone mass and fracture risk is somewhat controversial [52-54]. Dozens of studies have been performed looking at the possible association of vitamin D receptor (VDR) polymorphisms with BMD and fractures, with discordant results. The controversy has not been solved after several meta-analyses [55-59].

Several investigators have studied the association of polymorphisms of vitamin D-related genes from nutrigenomic or pharmacogenomic perspectives. In those studies, they explored the relationship of vitamin D intake, sun exposure or vitamin D supplementation with the vitamin D nutritional status, usually assessed by the serum levels of 25-hydroxyvitamin D, the most abundant vitamin D metabolite. A genome-wide study revealed that allelic variants of several genes involved in vitamin D metabolism and transport are associated with serum levels of 25-hydroxyvitamin D [60]. Some, but not all, candidate gene studies also found associations between genetic variants and the response to vitamin D supplements, and more research is needed to clarify this issue [61-64].

Genome-wide association studies are appealing because they are less likely to show false positive results than candidate gene studies. Unfortunately, they are almost absent in the field of the pharmacogenomics of osteoporosis. Nevertheless, the preliminary results of a genome-wide study trying to identify genes associated with the response to teriparatide have recently been presented [65]. Although the study group included just 162 patients, a number of suggestive signals for association were found near several genes, including NLGN1 and WNT2B, among others.

In postmenopausal women, some synthesis of estrogens is maintained by the enzyme aromatase (encoded by the CYP19A1 gene), which converts androgenic precursors into estrogens [66]. Common polymorphisms of the CYP19A1 gene are associated with BMD and fractures in late postmenopausal women [67-70]. Interestingly, some studies suggest that those polymorphisms, as well as some SNPs in estrogen receptors, might also be associated with the beneficial effect of aromatase inhibitors in women with breast cancer, as well as with their adverse effect on bone mass [71-73].

5. Unique adverse effects of antiresorptive drugs: atypical fractures and osteonecrosis of the jaw

Most drugs used for treating osteoporosis have a good safety profile. However, antiresorptives have been associated with some rare adverse skeletal effects of intriguing clinical and pathogenetic characteristics: osteonecrosis of the jaw and atypical femoral fractures.

5.1. Osteonecrosis of the jaw (ONJ)

Until the year 2000, most ONJ cases occurred in patients with head and neck cancers treated with external beam radiotherapy [74]. In the early 2000s physicians started observing similar injuries in patients without a history of radiation exposure and realized that most of these patients had breast cancer metastatic to bone or myelomatous disease and were receiving different types of bisphosphonate therapy. This led the investigators to propose an association between bisphosphonate use and ONJ [75,76].

In 2007, criteria for the diagnosis of bisphosphonate-related ONJ were established: i) exposed bone in the maxillofacial region that remained unhealed for a minimum of 8 weeks, ii) exposure to bisphosphonates, and iii) no history of radiation therapy in the craniofacial region [77,78]. Pain, swelling, erythema, ulceration and loss of teeth may appear before the clinical detection of ONJ. The lesions occur more frequently in the mandible than in the maxilla (2:1 ratio). Patients with necrotic jawbones may present severe pain, alimentation difficulties, suppuration, sinusitis, soft-tissue abscesses, and extra-oral fistulae, all of which can seriously impair their quality of life [79].

The vast majority of ONJ cases occur in patients on intravenous high-dose aminobisphosphonates (especially zoledronic acid and pamidronate) for breast cancer and multiple myeloma. In fact, about 95% of ONJ cases appear in patients being treated with intravenous bisphosphonates, whereas only 5% are associated with oral bisphosphonates [80]. The differences in patient characteristics as well as in the doses used may explain the markedly different ONJ risk in both patient populations. However, the actual risk in patients with cancer is unclear, because the reported frequencies vary between 0.6 and 18% [81,82]. The incidence of ONJ in the osteoporosis patient population is much lower than in those with cancer. In a Japanese series, the absolute frequency of ONJ among patients with osteoporosis was 0.5-1% in those treated with oral bisphosphonates and 0.1-0.2% in those treated with other drugs (odds ratio 5; 95% confidence interval 2-13)[83]. In a recent review, the cumulative incidence of ONJ in the osteoporosis population has been estimated to be in the range of 1-90 per 100,000 patient-years of exposure to bisphosphonates [82].

Like many other complex disorders, ONJ results from a combination of genetic and environmental risk factors. Among environmental risks, there are some established risk factors, such as previous dental extraction and implants, periodontal disease, type and the route of bisphosphonate (higher risk with zoledronic acid and with the intravenous route). Other factors with less consistent influence are length/cumulative dose of bisphosphonate, other therapies such as thalidomide and glucocorticoids, radiation therapy, advanced age, diabetes, obesity and smoking [80,84-86].

As only a small percentage of people treated with BPs develop ONJ, it is likely that genetic variations confer susceptibility or resistance to developing this side effect. If confirmed, a genetic test capable of screening subjects for susceptibility to ONJ before starting treatment with bisphosphonates would be of great clinical utility to tailor the therapeutic schema. Several genome-wide association studies (GWAS) have been attempted with this goal in mind. Sarasquete first carried out a GWAS to identify single nucleotide polymorphisms (SNPs) associated with the development of ONJ in patients with multiple myeloma being treated with bisphosphonates [87]. The study explored 500,568 SNPs in patients with multiple myeloma, 22 with ONJ and 65 controls. Four SNPs (rs1934951, rs1934980, rs1341162, and rs17110453) mapped to the cytochrome P450-2C gene (*CYP2C8*) showed a different distribution between cases and controls. In particular, T alleles at the rs1934951 polymorphism were significantly associated with a higher risk of ONJ. Individuals homozygous for the T allele had an

increased likelihood of developing ONJ (odds ratio 12.7, 95% confidence interval 3.7-43.5). However, this association could not be replicated by Such et al. in another series of 79 patients with myeloma [88]. Similarly, English did not find a significant association of the CYP2C8 polymorphism and ONJ in a group of men with prostate cancer and bone metastases treated with bisphosphonates [89].

Another GWAS was carried out by Nicoletti, who genotyped 30 patients with bisphosphonate-related ONJ and 17 treatment-tolerant controls and subsequently expanded the control set to include other previously genotyped controls. A marker at the *RBMS3* gene, rs17024608, was associated with ONJ, with a p-value close to genome-wide statistical significance and an odds ratio of 5.8 [90].

Di Martino et al [91], studied 1,936 genetic variants of 225 genes in a case-control study of 19 patients with myeloma treated with zoledronic acid (9 with ONJ and 10 controls). Eight SNPs in four genes (PPARG, ABP1, CHST11 and CROT) were significantly associated with ONJ. The SNP rs1152003 in PPARG showed the strongest association, with an odds ratio of 31 for the CC genotype.

La Ferla et al[92] aimed to evaluate the association of some aromatase and estrogen receptor polymorphisms with ONJ. They studied 83 oncologic patients treated with zoledronic acid, 30 of which had ONJ. The TT genotype of the aromatase polymorphism 132810C>T was overrepresented among the cases (37 vs. 17% in controls; p <0.05) suggesting a role for this polymorphism in predicting ONJ risk.

Katz et al [93] performed a cohort study including 78 patients with myeloma on intravenous bisphosphonate therapy (12 of them developed ONJ). Besides clinical characteristics, they analyzed 10 SNPs from 7 candidate genes and concluded that smoking, type of bisphosphonate and the combined genotype score of COL1A1, RANK, MMP2, OPG and OPN were significantly associated with ONJ. Considering all five SNPs together, patients with genotype scores ≥ 5 had a ONJ event rate of 57%, whereas those with scores < 5 had a rate of 10%.

In order to determine whether a higher sensitivity to bisphosphonates could in part explain the development of ONJ, Marini evaluated a cohort of 68 Caucasian patients treated with zoledronic acid and studied the segregation of the A/C rs2297480 polymorphism of the FDPS gene. Their study concluded that the AA and CC genotypes were differently distributed among ONJ patients and controls, matched for sex and type of malignant disease. They found an association of the AA carrier status and the occurrence of ONJ after 18-24 months of treatment [94].

Bisphosphonates are not the only drugs involved in ONJ. Denosumab, and some anti-angiogenic drugs such as bevacizumab or sunitinib, alone or in combination with bisphosphonates, have also been associated with this side effect [95-97]. The combination of any of these along with bisphosphonates could increase the risk of developing ONJ over that posed by bisphosphonates alone [98]. The anti-RANKL antibody denosumab is used in patients with osteoporosis or metastatic cancer to the bones. According to a recent analysis of the US Food and Drug Administration's adverse event reporting system database, in cancer patients, the reporting odds ratios for zoledronate and denosumab were 125.2 and 4.9, respectively. In patients with osteoporosis, the odds ratios were much lower [97]. In a recent analysis of patients treated with denosumab for 5 years, the cumulative incidence rate of ONJ was 0.04 per 100 subject-years [99]. Thus, the denosumab associated risk of ONJ appears to be lower than the risk associated with bisphosphonates. Also, given the shorter duration of the anti-osteoclastic effect of denosumab, ONJ might resolve more rapidly after drug withdrawal in denosumab-related cases.

5.2. Atypical femoral fractures (AFF)

AFF have typical radiological characteristics. They occur in the subtrochanteric or diaphyseal regions of the femur and are typically transverse or slightly oblique, with absence or minimal comminution,

and with frequent evidence of periostal or endostal stress reactions. They occur with minor or no trauma and not infrequently are bilateral and simetric [100].

The pathogenesis of AFF is still unknown, but antiresorptive drugs have been reported to increase risk. The inhibition of bone remodeling can impair the reparation of microcracks and incomplete stress fractures, finally resulting in complete fractures. These fractures have been reported in patients taking bisphosphonates and denosumab, but they can also occur in patients not exposed to these drugs [100,101]. Odvina et al [102] were the first to propose a possible relation between prolonged use of bisphosphonates and atypical fractures. After describing 9 patients treated with alendronate who had developed fractures of the femoral shaft, proximal femur, sacrum, ischium, pubis and ribs they suggested that alendronate could potentially cause severely suppressed bone turnover resulting in increased susceptibility to nonspinal fractures that heal poorly. This complication appears to occur earlier when alendronate is co-administered with either glucocorticoids or estrogen but, unlike ONJ, atypical femoral fractures are not associated with high doses of bisphosphonates. The frequency of AFF is fortunately low. The incidence has not been clearly established, but several studies reported risks of 0.01 per 100 patient-years treated with denosumab and about 0.1 per 100 patients-years in osteoporotic women treated with oral bisphosphonates for at least 5 years [99,100].

Most authors consider AFF as a special type of fracture, with radiological and clinical features that do not exist in osteoporotic fractures, thus suggesting the involvement of different causal mechanisms and/or underlying peculiar risk factors present in those patients. In line with this concept, bisphosphonates have been associated with AFF in many, but not all studies. Whatever the pathogenetic mechanisms might be, it is still unknown why some patients on bisphosphonates develop AFFs while most of them do not.

Although several reports have tried to associate AFFs with some patients' characteristics, the pathogenetic factors involved in AFF have not been elucidated yet. Genetic factors might also participate in the pathogenesis of AFF. Several data are in line with this concept. For instance, Lo et al recently reported that AFF are much more common in women from Asian ethnicity than in Caucasians, with a hazard ratio of 6.6 after adjustment for potential confounders [103]. In addition, femoral hip geometry is associated with the propensity to AFF. In fact, increased varization of the femur is much more prevalent in women with AFF than in controls [104-106].

Although those studies are consistent with the concept that genetic factors influence the risk of AFF, the actual genes involved have not been elucidated. Isolated reports suggest that individuals with rare mutations of the alkaline phosphatase gene (ALPL) may have higher risk of developing AFF when treated with bisphosphonates [107]. However, ALPL mutations are very rare among patients with AFF, thus indicating that genetic variants of ALPL are not a major determinant of AFF risk [108,109]. There is very little information about the potential involvement of other genes in AFF. We recently explored the association of up to 300,000 genome-wide variants with AFF by using an exon array. Although the sample size was very small (13 AFF cases; 268 controls), patients tended to accumulate some rare variants, and consequently the number of risk variants was markedly different between patients and controls. These results suggest that AFF are polygenic in nature, associated with the accumulation of changes in the coding regions of several genes [108]. Most genes in the study did not have a well-established relation with bone metabolism. Therefore, the molecular and cellular mechanisms involved remain to be elucidated.

6. Conclusion

In summary, osteoporosis is a prevalent disorder of bone strength. Several drugs decrease the risk of fragility fractures, the relevant consequence of osteoporosis. Some clinical factors may influence the response to antiosteoporotic drugs, including frailty, but are incompletely defined. So far, poor

adherence is the main predictor of a poor response. Several pharmacogenetic studies using a candidate gene approach have suggested that polymorphisms of genes in the Wnt and estrogen pathways, as well as in enzymes that are targets for bisphosphonates may be associated with drug response. However, they need to be replicated in larger groups of patients. ONJ and AFF are rare peculiar side effects of antiresorptive drugs that may result from bone turnover suppression. Pharmacogenetic studies have given conflicting results when looking for genetic influences on ONJ. Genetic factors are also likely to play a role in AFF, but very limited pharmacogenetic data are available.

7. Expert opinion

Osteoporosis is a frequent disorder, particularly among postmenopausal women and elderly men. Indeed, osteoporotic fractures represent an important burden from the patient and the societal points of view. During the past two decades, a number of drugs have been marketed that effectively decrease fracture risk. However, they are far from perfect because the overall fracture risk reduction varies between 20 and 70%, depending on the drug and the site of fracture considered. These drugs are usually well tolerated, but they are not free from adverse effects, including some infrequent but potentially bone-related problems, such as ONJ and AFFs.

Given the high prevalence of osteoporosis, it would be desirable to have pharmacogenetic data allowing to tailor drug therapy to patient's characteristics. This would increase the likelihood of obtaining a good response (this is, increase bone strength and reduce fracture risk), while limiting the possibility of adverse effects. Unfortunately, most pharmacogenetic studies have used a candidate gene approach and included small numbers of patients. Additionally, very few of them have been replicated in two or more independent cohorts. Polymorphisms of the Wnt and mevalonate (including the FDPS enzyme) pathways have been associated with the response to bisphosphonates in several studies, but replication in larger cohorts is still pending.

An agnostic approach, such as that underlying genome-wide studies, could result in less biased data than the candidate gene approach. In addition, genome-wide studies could help to discover other unsuspected factors influencing drug response and could open the pathway to find new therapeutic targets. In this regard, including pharmacogenetic data in the clinical trials of osteoporosis appears to be a priority. These trials may allow finding significant associations between genetic variants and the reduction in fracture risk. However, getting meaningful results will take a long time, due to the prolonged follow-up needed in studies with fractures as the outcome. Alternative approaches to accelerate discoveries could include using surrogate markers of antifracture efficacy, such as changes in BMD (which may be relevant after 1-2 years of therapy) or in biochemical bone turnover markers (which can be detected after 2-6 months).

The stability of genetic markers allows the possibility of analyzing associations between genetic variants and drug responses not only in the new trials, but also in those already finished. If the practical, ethical and legal difficulties are worked out, valuable data can be obtained from those retrospective analyses in a relatively fast way.

Similarly, a hypothesis-free approach is needed to elucidate the factors determining the risk of ONJ and AFF. Although these are rare complications of antiresorptives (particularly in patients taking these drugs for osteoporosis), they can very difficult to cure. Hence, it would be very interesting to identify subjects at risk in order to prevent them from receiving antiresorptive drugs. Preliminary data from our laboratory suggest that the involved genes may be out of the pathways classically involved in the regulation of bone metabolism, thus emphasizing that genome-wide approaches are needed to advance in this field.

Investigators searching genetic variants associated with polygenic disorders, such as diabetes, hypertension or osteoporosis have been able to build multinational consortia that allowed them to

recruit very large numbers of patients. This, in turn, has resulted in the identification of a number of genetic variants associated with BMD and fracture risk [110,111] in genome-wide studies. This might mark the path for pharmacogenetic studies.

Besides DNA sequence variants, other genomic features may influence drug response and they worth to be explored. Among them, epigenetic marks are particularly appealing. In fact, epigenetic mechanisms are emerging as important determinants of the risk of prevalent disorders, such as osteoporosis, that result from the interaction between hereditary predisposition and environmental factors. Ideally, as it is the case for pharmacogenetic studies, epigenome-wide association studies would be preferable to those using candidate hypothesis-driven approaches. Among the epigenetic marks, DNA methylation and microRNAs could be particularly feasible to explore. However, unlike the genome, the epigenome is tissue-specific. This poses some additional difficulties, because differences in epigenetic marks present in target tissues (this is, bone in osteoporosis) may or may not be translated into blood or other fluids that can be obtained without invasive procedures.

Highlights

- Osteoporosis compromises bone strength and predispose to fragility fractures
- Both acquired and genetic factors influence the response to drug therapy
- Genetic variants of the Wnt and mevalonate pathways are appealing candidates to influence drug response
- Osteonecrosis of the jaw and atypical femoral fractures are intriguing adverse effects of antiresorptives likely to have a polygenic predisposition basis.
- Genome-wide studies including large number of patients are needed to identify the genetic mechanisms determining drug response and adverse effects.
- Obtaining pharmacogenetic data from osteoporosis clinical trials is essential to advance in this field.

ABBREVIATIONS
ABP1: Auxin Binding Protein 1
AFF: Atypical Femoral Fractures
BMC: Bone Mineral Content
BMD: Bone Mineral Density
BP: Bisphosphonate
BRONJ: Biphosphonate Related Osteonecrosis of the Jaw
CHST11: Carbohydrate (Chondroitin 4) Sulfotransferase 11
COL1A1: Collagen, type I, alpha 1
CROT: Carnitine O- octanoyltransferase
CYP2C8: Cytochrome P4502C8
DKK1:Dickkopf WNT signaling pathway inhibitor 1
DXA: Dual-energy X ray Absorptiometry
FDPS: Farnesyl diphosphate synthase
GLOW: Global Longitudinal Study of Osteoporosis in Women
GWAS: Genome-Wide Association Studies
MMP2: matrix metalloproteinase-2
ONJ: Osteonecrosis of the jaw
OPG: Osteoprotegerin
OPN: Osteopontin
PINP: Propeptide N-terminal of type I procollagen
PPARG: Peroxisome proliferator-activated receptor gamma
RANK: Receptor Activator of Nuclear Factor κ B
RANKL: RANK ligand
RBM53: RNA Binding Motif, single stranded interacting protein 3
SERMS: Selective modulators of Estrogen Receptors
SNP: Single Nucleotide Polymorphism
TBS: Trabecular Bone Score
TNF: Tumoral Necrosis Factor
Wnt: Wingless-type (a family of proteins and pathway)
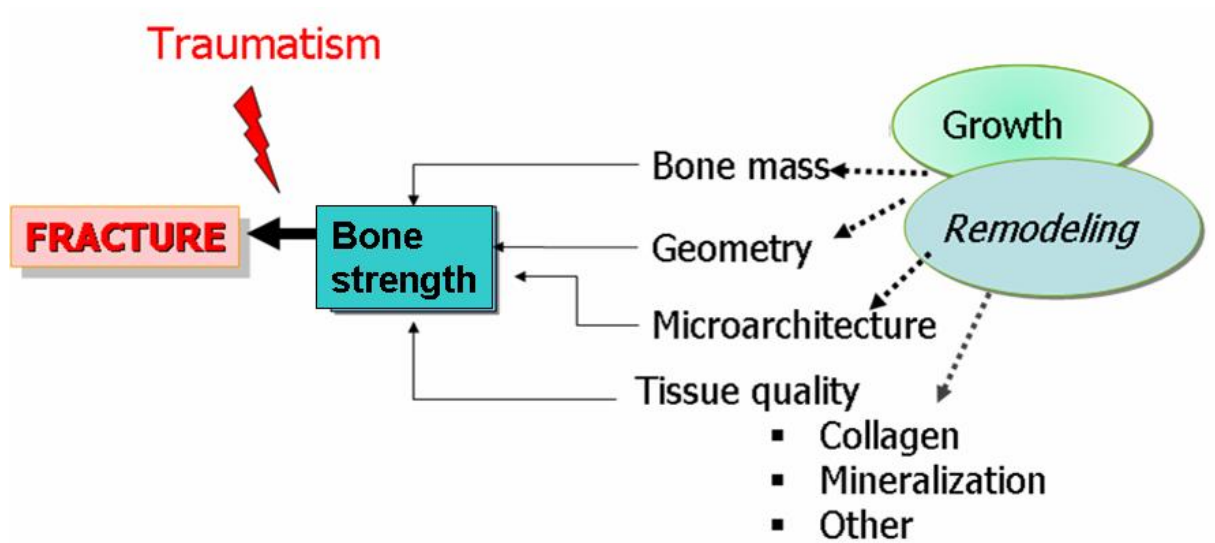
Figure 1. Determinants of bone strength.

Figure 2. Schematic view of factors determining effectiveness and adverse effects of antiosteoporotic drugs.
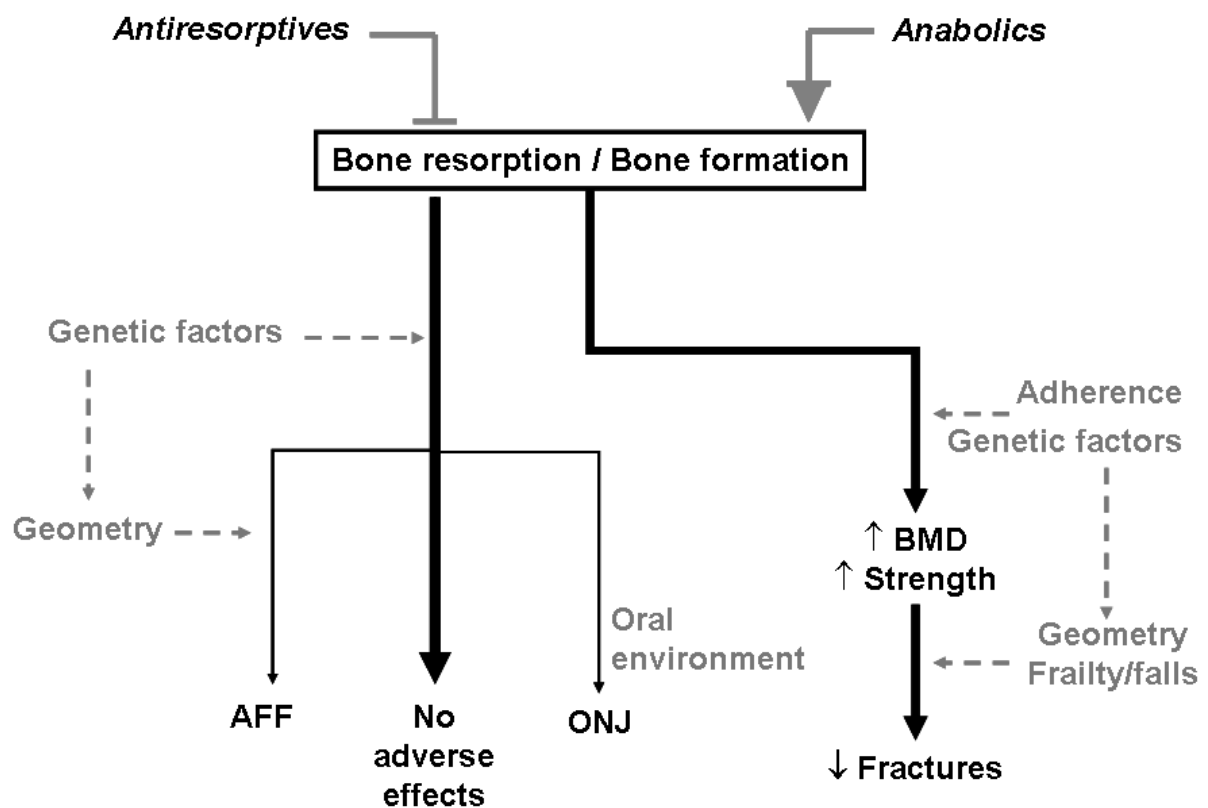
Table 1. Drug therapy of osteoporosis

Drug class	Approved	Under development
Antiresorptives	- Estrogen - SERMs (raloxifene, bacedoxifene) - Bisphosphonates (alendronate, risedronate, zoledronate, ibandronate, etc.) - Denosumab	- Cathepsin K inhibitors (odanacatib, etc) - Other bisphosphonates
Anabolics	- Teriparatide - PTH	- Anti-sclerostin - Anti-DKK1 - Abaloparatide
Other	- Strontium ranelate - Calcitonin - Calcium - Vitamin D	

Table 2. Genetic association studies of osteonecrosis of the jaw. BPs, bisphonates

Author (yr)	Type of study	Population	Genes explored	Main results
Sarasquete (2009)	Case-control	22 ONJ cases in 87 patients with multiple myeloma treated with BPs	GWAS	CYP2C8 (rs1934951) associated with ONJ
English (2010)	Case-control	17 ONJ cases in 100 men with prostate cancer treated with BPs	*CYP2C8* (rs1934951)	No association
Such (2011)	Case-control	9 cases of ONJ among 79 patients with multiple myeloma	CYP2C8 (rs1934951)	No association
DiMartino (2011)	Case - control	9 cases of ONJ among 19 patients with multiple myeloma treated with zoledronic acid	1936 SNPs in 225 genes (Affymetrix DMETTM plus platform)	PPARG (rs1152003) associated with ONJ (no multiple test correction)
Katz (2011)	Cohort	12 ONJ among 78 patients with myeloma treated with iv BPs	10 SNPs from 7 genes COLA1, RANK, MMP2, OPG, OPN, CYP2C8, TNF	Combined score including COL1A1 (rs1800012), RANK (rs12458117), MMP2 (rs243865), OPG (rs2073618) and OPN (rs11730582) associated with ONJ
Marini (2011)	Case-control	68 Caucasians with myeloma or bone metastases treated with zoledronic acid	FDPS (rs2297480)	FDPS (rs2297480) associated with ONJ
La Ferla (2012)	Case-control	30 ONJ among 83 cancer patients on zoledronic acid	aromatase; estrogen receptor polymorphisms (g.156705T>C and g.156751A>G)	The aromatase g.132810C>T polymorphism associated with ONJ
Nicoletti (2012)	Case-control	30 ONJ cases among 90 BP users	*GWAS*	*RBMS3* rs17024608 associated with ONJ

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