2

Pharmacogenetics of osteoporosis

Francesca Marini, PhD, Research Fellow, Molecular Biologist 1,
Maria Luisa Brandi, MD, PhD, Full Professor of Endocrinology

Metabolic Bone Unit, Department of Surgery and Translational Medicine, University of Florence, Florence, Italy

Keywords:
pharmacogenetics
gene polymorphism
osteoporosis
anti-fracturative drugs
drug response

The challenge of personalized medicine is to move away from the traditional ‘one-size-fits-all’ pharmacology to genotype-based individualized therapies. As an individual’s response to drugs is under the control of genes, personal genetic profiles could help clinicians to predict individual drug response and prescribe the right drug and dose, thereby optimising efficacy and avoiding risk of adverse effects. Currently, the concrete application of pharmacogenetics into clinical practice is limited to a few drugs, and the genetic prediction of drug response is far from clear for many of the principal complex disorders. This is even more evident in the field of osteoporosis and metabolic bone disorders, for which few pharmacogenetic studies have been conducted, and no conclusive results are available. In this chapter, we review recent research on pharmacogenetics of osteoporosis, evaluate criticisms, and offer possible suggestions for improvements in this field and for possible future applications into clinical practice.

© 2014 Published by Elsevier Ltd.

Introduction

The rise in life expectancy has strongly increased the incidence of chronic and degenerative complex diseases. These disorders require long-term pharmacological treatments; therefore, it is important that prescribed drugs are effective and do not induce severe adverse events so that people taking prescribed medication are granted a better quality of life, and drug-related morbidity, mortality, and costs of drug-induced hospitalization are reduced.

* Corresponding author. Metabolic Bone Unit, Department of Surgery and Translational Medicine, University of Florence, Largo Palagi, 1, 50139 Florence, Italy. Tel.: +39 055 7946304; Fax: +39 055 7946302.
E-mail addresses: f.marini@dm.unifi.it (F. Marini), marialuisa.brandi@unifi.it (M.L. Brandi).

1 Metabolic Bone Unit, Department of Surgery and Translational Medicine, University of Florence, Viale Pieraccini, 6, 50139 Florence, Italy. Tel.: +39 055 4271507; Fax: +39 0554271506.
For many common multifactorial disorders, various drugs with different molecular mechanisms of action are available. This offers clinicians the opportunity to select the most effective drug for any single patient based on personal variables that can control drug response (e.g., gender, age, ethnicity, clinical family history, renal, hepatic function, or both, concomitant pathologies and medications, diet, alcohol intake, and smoking). For most of the currently available drugs, real effectiveness, and the possible development of secondary adverse effects, cannot be predicted before drug administration. Indeed, today, most therapeutic plans are generally standardized, and they can be modified for each patient only after ascertaining their clinical response to drug administration.

The challenge of personalized medicine is to move away from the traditional ‘one-size-fits-all’ pharmacology to genotype-based individualized therapies. Personalized medicine assumes that drug response is related to personal features (e.g., sex, age, ethnicity, renal, hepatic function, or both), environmental influences (e.g., diet, concomitant therapies, alcohol assumption, smoke), and mainly to changes in drug pharmacokinetics and pharmacodynamics, which are dependent on an individual’s genome and epigenome.

Pharmacokinetics and pharmacodynamics regulate the availability, activity and metabolism of drugs within the organism, and are responsible for personal drug response, and are both under the control of genes. Therefore, a person’s genotype is strongly responsible for their response to drugs, both in terms of efficacy and safety. Genetic deficiencies (or over-activity of one or more drug-metabolising enzymes), and genetic alterations in drug absorption and distribution proteins, can strongly influence elimination and accumulation profiles of a drug, or both. They can also influence the availability of a drug to its specific tissue and cell target, as this is responsible for an individual’s response to drugs. This dictates the need for personal selection of drug type, dose, or both, based on a person’s personal characteristics and variables, and also on their specific genotype. Pharmacogenetics and pharmacogenomics aim to individuate the gene variations responsible for the regulation of pharmacokinetics, pharmacodynamics and mechanisms of action of drugs; the main goal of these novel disciplines is to predict the response to drugs before their administration based on a patient’s genotype [1].

In the future, the expectation is that patients could present a chip containing their personal genetic profiles, thus, enabling clinicians to prescribe the right drug at the right dose, and to optimize clinical efficacy and avoid any risk of adverse effects.

The role of genetic markers in predicting drug response is currently unclear. A number of pharmacogenetic tests have been approved by regulatory agencies, are commercially available, and are used in clinical practice.


The Pharmaceutical Medicines and Devices Agency (PMDA) in Japan approved the label of about 14% of all drugs with pharmacogenetic information. Most drugs included in the pharmacogenetic lists of FDA, EMEA and PMDA are represented by drug-metabolising enzymes, but none of them are administered in the pharmacotherapy of osteoporosis or other metabolic bone disorders.

Pharmacotherapy of osteoporosis

Bone is an active and dynamic tissue, characterized by a lifetime-spanning complex process of renewal and remodelling. Bone remodelling is a fine process, consisting of an osteoclast resorption
phase of old bone tissue followed by an osteoblast synthesis phase of new bone tissue. In normal adults, the activity of osteoclasts is balanced by the activity of osteoblasts to grant the correct homeostasis of the skeleton. All the phases of bone turnover process are finely regulated by the high interaction between several hormonal, genetic, nutritional, and environmental factors. Deregulations and malfunctions in the bone remodelling process are responsible for numerous metabolic bone disorders, the most common of which is age-related osteoporosis.

Osteoporosis is a common pathological condition among elderly people, characterized by bone demineralisation, loss of bone mass, and alteration of normal bone micro-architecture. This is all caused by increased activity of bone resorption and a reduced activity of new bone formation. Bone tissue, mainly trabecular, progressively loses its mineral content, its peculiar structure, and its strength, leading to fragility fractures, the main clinical manifestation of the disease. These fractures occur mainly in the wrist, spine and femur. Osteoporosis fractures are estimated to be over 9 million a year worldwide (one out of three women and one out of five men over the age of 50 years), and represent a serious public health, clinical, social, and economic problem [2]. Currently, in developed countries, the mortality rate resulting from hip fractures is about 30% during the first year and up to 40% during the second year after fracture occurrence [3]. Fragility fractures also account for nearly 1% of all worldwide disability [4].

Pharmacotherapy for osteoporosis principally aims at preventing the occurrence and recurrence of fragility fractures by reducing the rate of bone resorption, or stimulating new bone formation. Anti-osteoporosis drugs are usually taken in association with vitamin D or calcium supplementation, which are both effective in preventing fractures [5-11]. Therefore, the challenge faced by clinicians is to choose between the different options available, and to prescribe the ‘best drug’ for each individual with osteoporosis. Currently, the specialist selects the anti-fracturative drug on the basis of an individual’s clinical, personal, familial and lifestyle profile.

Anti-fracturative drugs present different molecular targets and mechanisms of action. Any pharmacological molecule has been specifically designed to interfere with one or more peculiar molecular pathways involved in the regulation of bone remodelling, mineral metabolism, or both. The aim is to restore the correct balance between osteoclast and osteoblast activity, and to maintain the right homeostasis of calcium and phosphorus, thereby preventing bone loss, micro-architecture deterioration, and occurrence of fragility fractures.

Selective oestrogen receptor modulators (SERMs) mimic oestrogen action in bone tissue through agonistic binding to oestrogen receptors alpha and beta (ERα and ERβ). They are designed to reap the benefits of oestrogens but without the potential hormonal side-effects on the lining of the uterus and on the breast glands. Like oestrogens, SERMs inhibit bone resorption through direct and indirect action on osteoclasts. This is achieved by regulating their apoptosis positively, and by modulating the cytokine production in the bone microenvironment that regulates osteoclast differentiation and activity (i.e. oestrogens block the osteoblasts’ synthesis of interleukin 6, a potent stimulator of bone resorption, and also antagonize the interleukin 6 receptors) [12].

Clinical trials have shown that raloxifene, the only approved second-generation SERM for female osteoporosis, increases bone mineral density (BMD) by 1.8% and 2.1%, respectively, in the lumbar spine and hip after 2 years of treatment, and it is effective in preventing osteoporosis and in treating established disease [13]. Also, novel third-generation SERMs, bazedoxifene and lasofoxifene, have been shown to increase BMD at femur and lumbar spine, and reduce vertebral and non-vertebral fractures in postmenopausal women [14,15]. Selective oestrogen receptor modulators are prescribed to postmenopausal women who experience side-effects to bisphosphonates, such as acid reflux or gastrointestinal intolerance. They present some side-effects because of their anti-oestrogenic actions (i.e. hot flashes) or their oestrogen-like activities (i.e. deep vein thrombosis and pulmonary embolism). They should be avoided in women who are at risk of blood clots or in elderly women who spend much time in bed.

Bisphosphonates are synthetic analogues of pyrophosphates, presenting a strong chemical affinity for bone hydroxyapatite and a specific inhibitory activity on osteoclasts. On the basis of their biochemical structure and molecular mechanism of action, they can be classified as not-aminobisphosphonates and amino-bisphosphonates (NBPs). Not-aminobisphosphonates are metabolized by osteoclasts to adenosine triphosphate (ATP) analogues, accumulate in the cytosol, and induce cell
osteoblasts with a pick of BMD gain 6. The natural inhibitor of the osteoblast-activating Wnt pathway. In this way, PTH recruits and activates increase of gastrointestinal calcium absorption, and in an increase of kidney calcium resorption. Administration of PTH or teriparatide results in bone anabolic stimulus on osteoblast activity, in an intermittent between osteoblasts and osteoclasts, the activation of osteoblasts leads to osteoclast bone resorption after 6 months of treatment, with a pick of resorption at 12 months. Therefore, the intermittent administration of PTH or teriparatide results in bone anabolic stimulus on osteoblast activity, in an increase of gastrointestinal calcium absorption, and in an increase of kidney calcium resorption.

All the approved bisphosphonates are powerful anti-resorptive molecules able to reduce fragility fractures by 40–70% in postmenopausal women with osteoporosis and men with osteoporosis. These drugs are inadvisable for people with acid reflux or gastrointestinal problems. Oral absorption is low, and they should be taken on an empty stomach standing upright. They are, therefore, not indicated for the treatment of elderly or sick people who must spend long periods of time in bed. Side-effects of bisphosphonate treatment have been reported in 1% of people; however, irritation of the oesophagus occurs in 10% of people, and is associated with oral administration of alendronate, ibandronate and risedronate. Moreover, difficulty and pain in swallowing, stomach pain, constipation, diarrhoea, headache, and rash, have been reported in a few cases of oral bisphosphonate treatment. Acute phase reaction (e.g. transient fever, flu, myalgias and arthalgias, for about 2–3 days), after the first administration of intravenous bisphosphonate, has been described in about 25% of people. Atrial fibrillation, heartburn, muscle, bone or joint pain, orbital inflammation, iritis, scleritis, episcleritis, and conjunctivitis have also been described in up to 1% of people.

Two of the most severe adverse reactions to long-term bisphosphonate treatment are the development of bisphosphate-related osteonecrosis of the jaw (BRONJ), with an estimated incidence rate of 0.028–4.3% for intravenous bisphosphonate administration in osteoporosis, and the occurrence of atypical subtrochanteric femoral fractures, with an estimated number of one case every 1000 people treated annually. Bisphosphonate-related osteonecrosis of the jaw is most often seen in people who have received bisphosphonates through intravenous treatment, but some cases have also been reported of people taking oral bisphosphonates, with the risk of BRONJ strongly dependent on dosage and length of treatment.

The risk of BRONJ can be substantially reduced by replacing intravenous with oral administration, and by avoiding any dental or periodontal surgical procedure or dental implant placement during the treatment with bisphosphonates, together with constant and good oral hygiene, and periodical dental examinations. In recent years, an association has been reported in several clinical case reports and case studies of an association between atypical low-trauma subtrochanteric or femoral shaft fractures and people treated with bisphosphonates for 3–5 years. Major features of bisphosphonate-associated atypical femoral fractures are that they occur as a consequence of minimal or even no trauma; they are bilateral in some people, simultaneous or sequential. On radiography, the fracture line seems to be transversal or shortly oblique compared with the more common osteoporosis-related fractures, which have a characteristic spiral feature. Moreover, these atypical fractures are commonly associated with prodromic pain for several weeks or months before the fracture occurs.

To avoid occurrence of this atypical fracture, it has been suggested that bisphosphonates are given for 3–5 years, and then replaced after 5 years with another treatment in elderly postmenopausal women who have been post-menopausal for over 10 years, have a T-score within the osteoporotic range, and a family history of fractures. Bisphosphonate treatment can be continued for 5–10 years, and then replaced with another anti-fracturative drug for 3–5 years in women who have been post-menopausal for over 10 years with an initial T-score in the osteoporotic range, but no family history of fracture and a personal moderate risk of fracture.

Parathyroid hormone (PTH) and its derived recombinant peptide teriparatide act on bone and kidneys, regulating calcium and phosphorus homeostasis. They represent the only anabolic drug for the treatment of osteoporosis. Parathyroid hormone activates multiple pathways within osteoblasts by activating specific target genes such as RUNX2, TGFβ, RANKL and, at the same time, it inhibits sclerostin, the natural inhibitor of the osteoblast-activating Wnt pathway. In this way, PTH recruits and activates osteoblasts with a pick of BMD gain 6–9 months after starting treatment. Because of the coupling between osteoblasts and osteoclasts, the activation of osteoblasts leads to osteoclast bone resorption after 6 months of treatment, with a pick of resorption at 12 months. Therefore, the intermittent administration of PTH or teriparatide results in bone anabolic stimulus on osteoblast activity, in an increase of gastrointestinal calcium absorption, and in an increase of kidney calcium resorption.
Conversely, continuous administration results in osteoclast function stimulation, associated with increase bone mass loss [24].

The US FDA has approved teriparatide for the treatment of osteoporosis only for 2 years, as studies in rat models have shown an association between 24 months or more of teriparatide treatment and the development of osteosarcoma. No cases of osteosarcoma have been reported in humans, but teriparatide should not be given to people with a history of cancer or those affected by Paget’s disease of bone. Side-effects of PTH 1–84 are hypercalciuria, hypercalcaemia, headache, nausea, dizziness, and leg cramps.

Calcitonin is a 32-amino acid polypeptide hormone that decreases bone resorption by direct, rapid, transient, and reversible inhibition of osteoclast activity, through specific binding to calcitonin receptor on osteoclast surface. Prolonged administration of intravenous calcitonin can prevent postmenopausal bone loss or increase trabecular bone mass in people with established osteoporosis. Nasal spray administration, however, is easier to be maintained in the long run, and is much better tolerated; calcitonin 200 IU nasal spray has been shown to stabilize or increase BMD and reduce spine fracture risk by 33% [25]. Calcitonin also has an analgesic short-term effect on acute bone pain relief in people with vertebral fractures. Recently, the use of nasal calcitonin has been associated with higher risk of cancer [26].

Strontium ranelate is a synthetic molecule that combines an antiresorptive effect with an anabolic action. It stimulates pre-osteoblast differentiation and osteoblast synthesis of bone matrix proteins and, at the same time, inhibits osteoclast differentiation and activity; the exact molecular mechanism of action is still unknown. The result is a combined reduction of bone resorption and promotion of bone formation that improves bone architecture, mass and strength, and reduces risk of fractures in postmenopausal women with osteoporosis [10]. The treatment is approved for men and postmenopausal women with severe established osteoporosis and high risk of fracture. In phase III controlled clinical trials, the treatment has been associated with an increased incidence of pulmonary embolism, venous thromboembolism, and serious heart problems, including myocardial infarction; therefore, strontium ranelate should be strongly avoided in individuals with a history of, or currently experiencing, heart or circulatory problems.

The receptor activator of nuclear factor-kappB ligand (RANKL), its membrane receptor RANK, and its soluble antagonist osteoprotegerin (OPG), are key effectors of osteoclastic activation and function; therefore, they are ideal targets for therapeutic interventions in metabolic bone diseases. Anti-RANKL human monoclonal antibody (Denosumab) is a recent anti-resorptive drug that binds RANKL and inhibits its positive action on osteoclasts, reducing bone resorption. It has shown to improve BMD in postmenopausal women with osteoporosis. The treatment has been approved for established osteoporosis but not yet for the prevention of the disease. Six cases of osteonecrosis of the jaw and one case of bilateral atypical femoral fractures have been reported in the 6 years of phase III clinical trials among all treated participants [18,27]. Major side-effects of denosumab include respiratory and urinary tracts infections, constipation, cataracts, joint pain, eczema, and rashes.

New treatments, interfering with molecular pathways regulating the osteoblast, or osteoclast differentiation and activity, are currently under investigation in pre-clinical studies and phase 1 clinical trials, such as sclerostin and dkk1 inhibitors, integrin antagonist and cathepsin-K inhibitors.

Pharmacogenetics of osteoporosis: the state of the art

A small percentage of people taking anti-fracturative drugs do not respond well (i.e. gain in bone mass may be small, bone resorption rate may reduce, or new fractures may occur). Anti-fracturative drugs are also associated with adverse events, ranging from minor side-effects to incapacitating, acute or chronic, collateral clinical damages, as shown in clinical trials, phase III extension trials, and in clinical practice. Anti-fracturative drugs act differently within target cells, interfering with different molecular pathways that are regulated by various genes. Therefore, the genetic profile of a patient can be the main cause of an individual’s variable response to a drug, both in terms of efficacy and safety. Pharmacogenetics of osteoporosis could assist clinicians and bone specialists in choosing the most effective available anti-fracturative drug, and can also help them to design an individualized therapeutic plan for each patient, based on their genetic profile.
The investigation of putative genes and polymorphisms involved in modulation of response to anti-osteoporotic drugs should consider all genes related to bone metabolism, together with specific genes involved in pharmacodynamics, pharmacokinetics and target pathways of any specific pharmacological molecule.

Some candidate genetic factors have already been investigated in pharmacogenetics studies of raloxifene, bisphosphonate and hormone replacement therapy (HRT), even if the latter is no longer prescribed as an anti-osteoporotic therapy. Only one small negative study has been conducted on pharmacogenetics of teriparatide [28]. No pharmacogenetics studies are available on calcitonin, prescribed as an anti-osteoporotic therapy. Only one small negative study has been conducted on raloxifene, bisphosphonate and hormone replacement therapy (HRT), even if the latter is no longer prescribed.

Most genes are related to bone metabolism, such as receptors for key systemic regulators of skeletal and mineral metabolism homeostasis (vitamin D receptor [VDR] and oestrogen receptors alpha and beta [ERα and ERβ]). Others include genes encoding for proteins of bone matrix (collagen type 1 [COL1A1]), genes involved in osteoblast and osteoclast-regulating pathways (lipoprotein receptor-related protein 5 and 6 [LRP5 and LRP6]), genes of the Wnt pathway, and osteoprotegerin (TNFRSF11B) [29–45].

The specific targets of NBP therapy have been investigated in four recent pharmacogenetic studies examining genes encoding for key enzymes of the mevalonate pathway within the osteoclasts farnesyl diphosphate synthase (FDPS) and geranylgeranyl diphosphate synthase [GGPS1] [46–49]. Two studies [46,47] of white postmenopausal women reported an association between the AA genotype (and the A allele) of rs2297480 polymorphism of FDPS and improved response to alendronate or ibandronate treatments through better urinary CrossLaps variation after 24 months, but not after 12 or 18 months of treatment [46]. Bone mineral density increased by 1% a year in the AA genotype of rs2297480 compared with a loss of 1.6% in BMD in the opposite CC genotype after an average period of 2.5 years of treatment with alendronate or risedronate, but not with raloxifene [47]. This second study also associated the GG genotype of rs11264359 polymorphism of FDPS with improved gain of hip BMD [47]. Other studies [48,49] in Asia and China have failed to find an association between BMD gain after use of alendronate and risedronate and different FDPS genotypes [48,49]. One study reported an association between CC genotype of rs2297480 polymorphism and a lower increase of serum alkaline phosphatase level after 3 and 12 months of treatment [49]. The CC genotype of rs2297480 has previously been associated with a lower BMD in elderly women [50], and three of these pharmacogenetic studies also seem to be associated with a lower response to NBP therapy. The less responsive CC genotype is rare among white women (about 3%) [51] and common among Asian women (about 40–50%) [52,53]. These contrasting ethnic-related genotype frequencies could strongly explain the ethnic difference in response to NBP therapy between white women receiving treatment and Asian women with osteoporosis. One of these studies [48] extended the pharmacogenetic analysis to the GGPS1 gene, and showed that women with two deletion alleles of 8188 A ins/del (rs3840452) polymorphism had a significantly higher femoral neck BMD at baseline compared with one or no deletion allele, but had a lower response (28.6%) to NBP therapy compared with women with one (81.4%) or no deletion allele (75.0%).

Characteristics and main results of all pharmacogenetic studies are presented in Table S1.

Recently, three studies [54–56] have reported an association between polymorphisms of three genes (ABCB1, SLCO1B1 and UGT1A1) involved in the pharmacodynamics and pharmacokinetics of raloxifene, and the level of available drug and the bone pharmacological response in individuals with osteoporosis. The c.3435C > T polymorphism of ABCB1 gene was associated with higher serum concentrations of raloxifene and significantly higher increase in hip BMD in TT genotype compared with the CC genotype [54]. The same research group showed that serum concentration of raloxifene was higher in individuals with the G allele of SLCO1B1 c.388A > G polymorphism, together with improvements in femoral neck BMD after 1 year of raloxifene treatment [55]. The third study [56] showed that individuals homozygous for the *28 allele of UGT1A1 gene had a two-fold higher serum concentration of raloxifene compared with the heterozygous and homozygous wild type allele. These individuals also presented a significantly greater increase in hip BMD [56].

Almost all pharmacogenetic studies have focused on female postmenopausal primary osteoporosis, apart from one small negative study on male osteoporosis [40]. No studies have been published on...
pharmacogenetics of secondary (to another disease or to long-term bone metabolism-interfering pharmacological treatment) osteoporosis. Moreover, most of these studies investigated the association between genetic polymorphisms and response to treatment only in terms of BMD gain, decrease in biochemical markers of bone turnover, or both, compared with baseline. These parameters are imperfect surrogates for real anti-fracturative efficacy, making interpretation of these pharmacogenetic studies difficult and approximate. According to the working group from the Committee of Scientific Advisors of the International Osteoporosis Foundation, response to anti-fracturative treatment should be assessed using all three outcomes: occurrence of incident fractures during treatment, percentage of change in BMD, and percentage of variations in turnover markers compared with baseline values.

No pharmacogenetic studies have considered an association between genotypes and the side-effects known to be associated with anti-fracturative drugs. Different adverse reactions have been reported in clinical trials for all the anti-fracturative drugs and, anecdotally, in clinical practice; however, the variability and the rarity of these events has made it difficult to recruit a sufficient number of people to participate in pharmacogenetic studies, and multicentre and international studies will surely be required to bypass this limitation.

Pharmacogenetics of BRONJ development, in response to intravenous NBP therapy, has been investigated only in patients affected by bone metastases for which this complication affects about 3–10% of treated patients. Conversely, in patients treated for osteoporosis, BRONJ manifests in only about 0.1%, possibly because of the lower dosage of intravenous NBP. No pharmacogenetic studies have been conducted owing to the few available cases of osteoporosis-related BRONJ. Bisphosphonate-related osteonecrosis of the jaw is a multifactorial clinical complication of NBP therapy; however, all these studies indicate the importance of genetic profile in the risk of developing this disorder, and have suggested a future possibility of using pharmacogenetics for predicting individuals at higher risk before NBP administration.

Commentary and future perspectives

The positive association between genetic profile and response to anti-fracturative drugs reported in all published pharmacogenetic studies have not been consistently replicated or supported by functional studies to confirm their validity and application in clinical practice. Negative associations and contradictory results may be due to ethnic difference, the small population analysed, use of different drugs, doses, or both, use of retrospective clinical trials, use of concomitant drugs, no supplementation with calcium or vitamin D, or both, and differences in factors influencing lifestyle, environmental factors, and dietary habits of the analysed population. To assess the real utility of genetic variations on response to anti-fracturative drugs, and to permit their validation and future clinical application, the first step should be the design of prospective controlled clinical trials, in which patients' inclusion and exclusion criteria, drug posology, and outcome parameters are decided before participants are enrolled and genetic analyses are carried out. Strict monitoring of patients' adherence to treatment must also be considered, and finally, long-term follow up patients (at least 2 years) will allow late benefits or resultant adverse events of treatment to be evaluated. Positive results should be replicated and, in general, studies should be repeated in different ethnic groups to assess the role of ethnicity in different genotype distributions in response to different drugs. Multicentre studies, involving different countries, are needed to enrol larger populations to increase the strength of statistical associations, and to be able to recruit adequate numbers of participants for investigation. The pharmacogenetics of side-effects associated with anti-fracturative therapies should also be investigated. Moreover, all the genetic markers identified by pharmacogenetic association studies need to be functionally validated before any possible application in clinical practice. Validation should include global gene expression profile and proteomic analyses; in vitro functional studies (preferably using human cell lines from subjects bearing different genotypes); and studies on animal models.

Bone homeostasis involves numerous molecular pathways, and osteoporosis is a complex disease caused by the synergic contribution of several genes, exerting major and minor effects on bone turnover and mineral metabolism. The effect of a single gene, however, is relatively small (except for
rare cases of osteoporosis-like mendelian genetic syndromes); therefore it would be restrictive to conduct pharmacogenetic studies for the analysis of single genes and single polymorphisms.

Today novel high-throughput technologies are available that allow simultaneous screening of hundreds of polymorphisms through the entire genome (genome-wide scan analyses; GWA) or the sequencing of the entire genome, exome, transcriptome, and miRNome in only one run (next-generation sequencing [NGS]) methodologies. Genome-wide scan analyses has been successfully applied in the identification of novel susceptibility genes for several complex multigenic disorders. In the field of bone, a recent meta-analysis of 17 different GWA studies reported an association of 56 genetic loci (32 previously unknown) with BMD and 14 also with fragility fractures. Many of these loci were associated with Wnt and RANKL-RANK-OPG pathways or with osteoblast differentiation and bone mineralisation, but the application of GWA also allowed the identification of causative polymorphisms in genes not previously known to exert an effect on bone and mineral metabolism [61].

Two recent studies [57,62] applied GWA technology to the study of pharmacogenetics of BRONJ in people affected by bone metastases, and reported an association between four polymorphisms of P450-CYP2C8 gene [57] and one polymorphism of RBMS3 gene with the risk of developing this disorder [62]. These preliminary results prompt the future application of GWA, and even more of the higher throughput and more flexible NGS technologies, in the area of pharmacogenetics for the discovery of suspected and unknown genetic markers and also for the design of pharmacogenetic screening platforms.

Today, evidence is growing that genes encoding drug transporters, receptors, metabolising enzymes and molecular targets are controlled by epigenetic factors, such as DNA methylation of cytosines in gene promoters and regulating regions, histone modifications (i.e. methylation, acetylation and ubiquitination) and regulatory small non-coding RNAs (i.e microRNAs [miRNAs]). Epigenetics modulates gene and protein expression independently by heritable variations in the DNA genome sequence, in a reversible, dynamic, age tissue-specific and environmental manner, and also in a non-genetic-influenced manner; each individual may have multiple and variable epigenomes during their life in relation to non-genetic exogenous influences. Epigenetic factors can control, together with inherited genetic variants, the pharmacokinetics and pharmacodynamics of drugs influencing personal drug response, thereby representing the link between genome and environment and explaining the controversial results of pharmacogenetic studies limited to the analysis of polymorphisms. This evidence has supported the growth of a novel discipline, the pharmacoeigenetics, and its whole genome application pharmacoeigenomics, which aims to investigate the role of epigenetic factors in the response to drugs. Pharmacoeigenetic studies should take into account the role of DNA methylation and histone modifications of genes related to pharmacokinetics, pharmacodynamics, and mechanism of action of a drug. Moreover, individual post-transcriptional expression regulation, by miRNAs, of genes encoding drug transporters, drug metabolising enzymes, and drug biological targets, could be one of the main actors of personal drug response, and miRNAs represent important players to be studied to comprehend the intricate regulation of individual drug response. A different drug response can occur as a result of individual, temporary specific miRNA expression, or both. A personal higher expression or lower expression of those miRNAs that post-transcriptionally down-regulate the expression of proteins necessary for drug biological efficacy, can result, respectively, in an ineffective or an excessive drug action. Conversely, a higher or lower expression of miRNAs that regulate expression of genes which proteins inhibit drug function can result, respectively, in a reduced or increased drug effect. Moreover, a role of polymorphisms in miRNA genes and in their target genes in the modulation of personal drug response has recently been suggested. A polymorphism in the three prime untranslated region of a mRNA target could affect its binding to, and its regulation by, miRNAs and resolve in the deregulation of one or few downstream molecular pathways involved in drug pharmacokinetics, pharmacodynamics, or both. Conversely, a polymorphism in genes encoding miRNA or in genes encoding proteins that regulate miRNA biogenesis and maturation, may potentially affect the expression of several target genes involved in numerous molecular pathways regulating drug absorption, transport, metabolism and function. Therefore, the emerging and complex field of pharmacoeigenomics should also be considered in the future of pharmacogenetic studies of osteoporosis.
Summary

Pharmacogenetics aims to give clinicians information about the efficacy and safety of drugs based on patient genotype before drug administration, enabling them to prescribe the right drug and dose, to optimize efficacy, and to avoid risks of side-effects.

Few pharmacogenetic drug labels and even fewer pharmacogenetic tests have been validated and applied in clinical practice, and none of them in the field of osteoporosis.

Osteoporosis pharmacotherapy aims to prevent the occurrence, recurrence, or both, of fragility fractures. Different effective anti-fracturative drugs, with different molecular targets and mechanisms of action, are today available. A variable percentage of patients receiving treatment respond poorly or not at all to these drugs, or develop various side-effects. At the moment, neither non-response nor development of adverse reactions can be predicted before drug administration.

Few pharmacogenetic studies have been conducted on osteoporosis therapies with hormone replacement therapies, raloxifene and bisphosphonates. No study is available on parathyroid hormone, calcitonin, strontium ranelate, or denosumab. Results are still inconclusive; many single studies need to be replicated. No global gene expression, proteomic, or functional analyses are available yet.

Pharmacogenetics of osteoporosis needs to be improved by [1] designing prospective controlled trials on larger well-characterized populations, preferentially from multicentre consortia [2]; applying GWA, NGS techniques, or both [3]; carrying out global gene expression and proteomic analyses [4]; carrying out in vitro and in animal model functional studies; and [5] evaluating the role of epigenetic factors and of the possible interaction between genes and exogenous factors.

Practice points

- An anti-fracturative treatment is strongly recommended for elderly women and men as age accelerates bone loss and the risk of fracture is high (one of three women and one of five men over the age of 50 years).
- Menopausal women should be prescribe anti-fracturative therapy to prevent the drastic lost of bone mass during the first 5 years after the menopause.
- Deficiency of calcium and vitamin D are endemic in elderly people; therefore supplementation is strongly recommended to maintain correct mineral metabolism and bone homeostasis.
- Selection of the most appropriate drug is currently based on the patient's personal, familial and clinical features, and treatment is usually adjusted after administration and clinical response.

Research agenda

- Design of prospective pharmacogenetic clinical trials on large, well-characterized populations, preferentially from multicentre studies.
- Discover novel unsuspected pharmacogenetic biomarkers by GWA and NGS technologies.
- Carry out global gene expression and proteomic validations.
- Validate pharmacogenetic associations by functional studies in cell lines and animal models.
- Conduct pharmacoepigenetic studies.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.beem.2014.07.004.
References


