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PHARMACOGENETICS OF OSTEOPOROSIS: TOWARDS NOVEL THERANOSTICS FOR PERSONALIZED MEDICINE?

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ABSTRACT

Osteoporosis is a complex multi-factorial bone disorder with a strong genetic basis. It is the most common, severe, progressive skeletal illness that has been increasing particularly in developed countries. Osteoporosis will no doubt constitute a serious clinical burden in healthcare management in the coming decades. The genetics of osteoporosis should be analyzed from both the disease susceptibility and the pharmacogenetic treatment perspectives. The former has been widely studied and discussed, while the latter still requires much more information and research. This paper provides a synthesis of the literature on the genetics of osteoporosis and an update on progress made in pharmacogenetics of osteoporosis in recent years, specifically regarding the new molecular targets for antiresorptive drugs. In-depth translation of osteoporosis pharmacogenetics approaches to clinical practice demands a new vision grounded on the concept of "theranostics". That is, the integration of diagnostics for both disease susceptibility testing as well as for prediction of health intervention outcomes. In essence, theranostics signals a broadening in the scope of inquiry in diagnostics medicine. The upcoming wave of theranostics medicine also suggests more distributed forms of science and knowledge production, both by experts and end-users of scientific products. Both the diagnosis and personalized treatment of osteoporosis could conceivably benefit from the emerging postgenomics field of theranostics.

INTRODUCTION

Osteoporosis is a systemic skeletal disease characterized by low bone mass and deterioration in the microstructure of bone tissue, which causes bone fragility and the consequent increase in fracture risk (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, 2001). The disability resulting from osteoporosis in Europe is greater than the combined disability of all non-lung cancers (Lenise A., 2010). The lifetime fracture risk of a patient with osteoporosis is as high as 40%, and fractures most commonly occur in the hip, spine or wrist (Rachner TD., 2011). Hip fracture (HF) is the most threatening because of its high mortality rate which can reach 30% the year after the fracture (Franzo A., 2005).

Osteoporosis is a multifactorial and polygenic disease (Ralston SH., 2002), so the individual likelihood of having a fracture depends on the combination of several risk factors, such as low bone mineral density (BMD) and genetic predisposition (Cummings SR., 1995; Rojo K., 2010).

The consequences of osteoporotic fractures generate significant direct and indirect economic impact on the level of healthcare needed. It is important to note that the impact of osteoporotic fractures on the economy has not been extensively studied and evaluated in hospitals. Estimations of the costs associated with hip fractures vary depending on the parameters analyzed. Hospital costs have demonstrated expenses that range from 9.000 (Pike C., 2010) to approximately 40,000 Euros (year/patient) (The Economic Cost of Hip Fracture in the UK, 2000). The studies do not take into account expenses such as hospitalization, ambulance, and primary care. Currently, these cost-effectiveness analyses are starting to become increasingly important for global hospital management (Leslie WD., 2011; Blume SW., 2011).

One of the best and most widely-used clinical determinants of the bone status of an individual is the BMD measurement (Kanis JA., 2002), which is considered a valid parameter for diagnosing osteoporosis and predicting fracture risk (Cummings SR., 2002). Although many external factors play fundamental roles in determining BMD, it has been estimated that over 50% of women and 70% of men who have suffered fractures did not previously have osteoporotic BMD values determined (Nguyen ND., 2007). Furthermore, in studies of osteoporosis therapy, increases in BMD were not linearly proportional to fracture risk reductions. The change in BMD induced by antiresorptive drugs explains approximately 15% of the reduction in fracture risk (Delmas PD., 2004). The International Osteoporosis Foundation projects that measure BMD using Dual-Energy X-ray Absorptiometry (DEXA) are believed to overestimate BMD by 20% to 50% (Johnell O., 2006) and are poor predictors of fracture in individuals. Advances in imaging techniques of factors such as cortical porosity with high-resolution peripheral CT may allow volumetric bone-density data to better predict bone strength, and thus fracture risk (Zebaze RM., 2010).

Decision-making methods such as the fracture-risk assessment tool (FRAX) have integrated clinical risk factors with DEXA-based BMD to predict an individual's 10-year risk of sustaining a hip fracture, as well as the 10-year probability of having a major osteoporotic fracture, clinically-defined as spine, forearm, hip, or shoulder fracture. Each country adjusts this algorithmic tool to the data based on geographic variation Version 3.0 of the FRAX has shown to give lower probability estimates for major osteoporotic fracture and hip fracture than version 2.0, but has had little impact on rank order of risk. This tool bridges the gap between the parameters for diagnosing osteoporosis and identifying individuals with structurally compromised bone that causes an increased risk of fracture. However, as it is a recently-developed scale, it has

limitations because it does not contain some important risk factors associated with falls and fractures. These risk factors may include high consumption of specific drugs like inhibitors of the proton pump, and benzodiazepines.

It is necessary to consider that discovering genes that affect osteoporosis comprise two main areas: genetics of disease susceptibility and pharmacogenetics of drug response. This review will approach pharmacogenetics of drug response and its implications to current clinical practice including a review of the validation process, which allows the development of specific guidelines. Future perspectives of pharmacogenetic research should be supported by validation processes, which would involve the control of different parameters related to accuracy, precision, and repeatability of the genotyping methods. We will first give a brief overview of the genetics of osteoporosis, and then proceed to studies in pharmacogenetics of osteoporosis.

GENETICS OF OSTEOPOROSIS

The genetics of osteoporosis has revealed promising associations between many polymorphisms in candidate genes and bone traits, both quantitative and qualitative. The association of many candidate genes with BMD and osteoporotic fracture risk has become controversial, owing to the failure of independent replication, possibly due to insufficient statistical power and false-positive results. Genes involved in common bone formation/destruction pathways have been described as being related to the risk of osteoporosis, the risk of hip and vertebral fractures, and to BMD values. Therefore, these gene variants could affect homeostasis and bone structure, and as a result, BMD values.

Given the available studies from different reviews and the clinical importance of osteoporosis, this section will focus on those pathways and genes most frequently studied in relation to BMD and osteoporosis fracture risk.

In this context, one of the active pathways is the osteoclastogenesis pathway, which is related to the process of bone remodeling through the activity of *OPG, RANK* and *RANK‑L* loci (Hofbauer LC., 2000). *RANK* and *RANK-L* play a significant role in the signaling enhancement pathway of the number, survival and activity of osteoclast. *OPG* acts as a competitor of *RANK* in binding to the *RANK-L* receptor, thus inhibiting the activity of osteoclasts (Jones HD., 2002; Pearce SH., 1997).

The Wnt signaling pathway participates in the process of bone formation and resorption by including transmembrane proteins such as *LRP5*, *LRP6* (Tamai K., 2000) and *ITGB3*. The Wnt signaling pathway is essential for the maturation of osteoclasts, and thus for bone resorption (Teitelbaum SL., 2000).

Likewise, the active metabolite of vitamin D (1α,25(OH)2D3) plays a fundamental role in bone metabolism by binding to its *VDR* receptor. *VDR* regulates calcium homeostasis through the binding and nuclear translocation of the 1α,25[OH]2D3, in order to regulate bone turnover and increase gut calcium absorption (Wesley P., 2002).

Synthesis of estrogens is essential for the acquisition and maintenance of bone mass, predominantly in women (Ichikawa S., 2005). The physiological functions of estrogens are performed when they bind to the α‑ and β‑receptors (*ESR‑α*, *ESR‑β*), the final biological impact being expressed in both osteoblasts and osteoclasts (Bord S., 2001).

Moreover, collagen is an important component of the body's structural proteins. *COL1A1* is the largest and most abundant constituent of all bone tissue proteins, and

mutations in its structure or regulation are associated with osteoporosis (Byers PH., 1990).

In homeostasis, the calcium-phosphorus pathway is the Calcium Sensing Receptor (*CaSR*), which belongs to the G-protein coupled receptor super family, and serves as a sensor of the extracellular calcium levels in different tissues (Pearce SH., 1997). It is expressed in bone cells, and recent data indicate that this receptor can be involved in the regulation of osteoclastic bone resorption (Kameda T., 1998).

The key enzymes in the mevalonate pathway are *FDPS* and *GGPS1*. These enzymes are targets in the isoprenoid biosynthesis. This pathway has been demonstrated to be involved in the regulation of mechanisms by which bisphosphonates induce apoptosis of osteoclasts (Marini F., 2008; Choi HJ., 2010).

The genetic analysis in osteoporosis includes the use of high throughput whole genome expression microarray applications (a technology that allows researchers to study the expression of many genes at once). This technology has found specific up-regulated or down-regulated genes, and has discovered putative clinical biomarkers. This analysis describes the functional studies of expression, but the results are controversial. The results of osteoporosis-related genes based on microarray identification have been obtained at the cellular level in primary cultures of human osteoblasts (Trost Z., 2010).

A compilation of the main genetic factors described through potential candidate gene, genome-wide association studies (GWAS) and meta-analyses related (Hsu Y., 2010; Richards JB., 2009; Rivadeneira F., 2009; Styrkarsdottir U., 2009; Ralston SH., 2010; 31:629-62; Ralston SH., 2010; 1192:181-9; Mencej-Bedrac S., 2009; Reppe S., 2010; Uitterlinden AG., 2006; Ji GR., 2010; Fang Y., 2006; Macdonald H., 2006; Mann V., 2003; Yazdanpanah N., 2007; Kapur K., 2010; Tsukamoto K., 2000; Harding B.,

2006; Levy ME., 2007) with osteoporosis, BMD and osteoporotic fractures are summarized in Table 1, which identifies the main genes with their respective signalling pathways, and genetic or genomic screening technologies and/or approaches.

The conflicting results compiled for each pathway are possibly owing to the complexity of the osteoporosis phenotype itself, added to the limitations in the molecular tools available. These conflicts should be approached by using improved screening, risk assessment, diagnosis and treatment initiation (MacLaughlin E., 2010). In this regard, an important contribution would be the complementary use of validated pharmacogenetic tests in clinical practice (Lamberts S., 2009; Van Straaten T., 2010).

PHARMACOGENETICS OF OSTEOPOROSIS

Osteoporosis Pharmacotherapy:

The therapeutic breakthroughs that have emerged for the treatment of osteoporosis may improve bone quality (the constellation of bone architecture, bone turnover, and damage accumulation and mineralization) and bone quantity (integration of bone mass, estimated by BMD). These breakthroughs are within a range of pharmacological alternatives, which are used for prevention of osteoporotic fractures (Delmas PD., 2005).

The treatments used in this disease fall into two classes: antiresorptive drugs and anabolic drugs (Gates BJ., 2009). Antiresorptive drugs, such as bisphosphonates (BP) (alendronate, risedronate, etidronate, ibandronate, zoledronate), raloxifene, and estrogen, slow down bone resorption. Anabolic drugs stimulate bone formation, and include teriparatide (parathyroid hormone) and possibly strontium ranelate which has been suggested to induce a combination of modest effects on bone formation and resorption. Widely accepted clinical guidelines have concluded that all these drugs have

been shown to reduce the risk of osteoporotic fractures to a greater or lesser extent, along with concomitant increases in bone density and decreases in high bone turnover.

Due to advances in genomic and proteomic studies which revolutionized drug discovery and validation processes, new prospects have emerged for the identification of novel therapeutics against skeletal diseases (Rachner TD., 2011). According to new pharmacology treatment, there are drugs that have been developed based on monoclonal antibody actions and small molecules mimicking similar designs established for anticancer molecular-directed therapy: Denosumab, Odanacatib, Saracatinib, and antibodies against the proteins sclerostin and dickkopf-1 (two endogenous inhibitors of bone formation) (Rachner TD., 2011; Lewiecki EM., 2011). Of these molecular drugs, only Denosumab has been approved by the FDA and EMEA for osteoporosis treatment.

Pharmacogenetics of Osteoporosis Treatment

The great majority of association studies have investigated only those genes which affect BMD, bone turnover marker variation, and fracture risk; they could be independent from genes affecting drug responses (adverse affect and efficacy limitations).

The field of pharmacogenetics, which represents the use of individual genetic data to predict the outcome of a drug treatment in relation to efficacy and adverse effects, could be a valuable avenue of study (Gervasini G., 2010)

The variability in osteoporosis drug response is much more complicated than simple variability in BMD, bone turnover markers or fracture risk becauseit is necessary to consider other intrinsic characteristics of the patient (morbidity, enviromental factors) that impact the kinetics and dynamics of the drug, and therefore the end-of-treatment response. Thus, it will be very important in future work to define the phenotypes of

osteoporosis drug response (disease, quality of life, food, drugs) and to enlarge pharmacogenetic studies to include genes involved in drug-specific pharmacokinetics and pharmacodynamics.

Due to the controversial results of the studies of expression and to potential concern to clinicians and patients in non-responders to osteoporosis therapy (Lewiecki EM., 2003) the emerging field of pharmacogenetics is very useful for refining and optimizing osteoporosis drug treatment. Pharmacogenetics could potentially allow the identification of the most effective drug and dose for each patient in terms of beneficial and adverse effects, based on the single genotype expression. In order to develop this area, the study of pharmacogenetics of osteoporosis should include the understanding of molecular mechanisms of drug action, the identification of drug response candidate genes and their variants, and the expansion towards clinical trials that include patients' genetic profiles. These approaches could provide useful tools to tailor decisions about osteoporosis drug treatments in order to maximize the health and well-being of osteoporotic patients.

To date, no more than 20 studies of the pharmacogenetics of osteoporosis have been published. The great majority of these studies have demonstrated modest effects in response to anti-resorptive drugs. Primarily, major osteoporosis candidate genes, such as those encoding the *VDR* (Palomba S., 2003; Marc J.,1999; Palomba S., 2005) *ER-α/ER-β* (Heilberg IP., 2005; Arko B., 2002) and *COL1A1* (Qureshi AM., 2002), have demonstrated a different effect on lumbar BMD and hip BMD depending on the genotype of the patients. Some interesting studies observed that in the *VDR* polymorphism (BsmI genotype: BB, Bb, bb) the major effectiveness (increase on DMO) was in patients' homozygous BB when they were treated by Raloxifene (Wang C., 2009) or Etidronato (Creatsa M., 2011) alone. In other cases (Rizzoli R., 2011), in

heterozygous Bb and homozygous BB patients, the combination of Alendronato (ALN) with Hormone Replacement Therapy (HRT) and the association of Raloxifene plus ALN had a stronger effect on BMD compared with either HRT or Raloxifene treatment alone, but no more effective than ALN alone. In the cases of *ER* gene polymorphisms, it was observed that patients' homozygous PP for the PvuII polymorphism (genotype: PP, Pp, pp) and patients' homozygous xx for the XbaI polymorphism (genotype: XX, Xx, xx) exhibited better lumbar spine BMD response values when treated with antiresorptive Raloxifene (Siris ES., 2009). Patients with genotype Rr for the RsaI SNP (genotype: RR, Rr, rr) showed a smaller increase in BMD, but did not show a significant responsive to ALN therapy. For *COL1A1* gene polymorphism (Sp1 genotype: SS, Ss, ss), it was shown that site-specific heterogeneity exists in the response of BMD to cyclical Etidronate therapy (Kennel KA., 2009). In patients' homozygous SS, the response of femoral neck BMD increased significantly in comparison to the rest of the genotype.

As noted in the studies above, research in pharmacogenetics of osteoporosis has been conducted mainly with antiresorptive drugs. This may be because important clinical guidelines have recommended the use of these drugs for primary and secondary prevention of osteoporosis and osteoporotic fractures. As described in the analyzed studies, the effect of antiresorptive drugs will depend primarily on the genotype expressed in the patient. It is possible that the difference in the response of each drug in relation to genetic polymorphisms is related to the mechanism of action.

Recent studies on Pharmacogenetics of antiresorptives:

In order to update the studies in pharmacogenetics of antirresorptive drugs, this section will analyze in detail the studies performed in the last four years regarding the

response of these drugs depending on the genotypic characteristics of the patients. Table 2 summarizes the studies based on pharmacogenetics of antiresorptives.

Six novel studies specifically related to pharmacogenetic osteoporosis treatment response merit special consideration. One of these studies, (2008), evaluated the effects of the rs1800012 (G>T) SNP of the *COL1A1* gene on BMD response to at least 3 years of low-dose hormone replacement therapy in 111 postmenopausal Turkish women (Simsek M., 2008) and demonstrated that the increase in lumbar BMD and femoral BMD were higher in women with the homozygous GG genotype compared to those with the heterozygous genotype.

In the same year, a study of 234 osteoporotic Danish women associated the rs2297480 (A>C) SNP of *FDPS*, the molecular target of amino-bisphosphonates in osteoclasts, with the response to 2-year amino bisphosphonate treatment (Ralston SH., 2010). They found that subjects with the homozygous CC genotype showed a decreased response by urinary Crosslaps after two years, but not after one year of amino-bisphosphonate therapy (Alendronate or Ibandronate) when compared to the heterozygous AC and to the homozygous AA genotypes. However, *FDPS* polymorphism did not show any relationship to baseline spinal and femoral BMD.

In 2009, a study analyzed the influence of the rs3736228 (C>T) and rs4988321 (G>A) polymorphisms of the *LRP5* gene in a cohort of 249 osteoporotic or osteopenic men (Kruk M., 2009). The results showed that the rs3736228 (C>T) polymorphism was associated with hip BMD in osteoporotic men before treatment. However, there was no association between these polymorphisms and BMD and bone turnover response after 2 years with risedronate treatment.

In the same year, 80 postmenopausal women were studied to determine if the A163G (genotype: AA, AG, GG) and T245G (genotype: TT, GT, GG) polymorphisms

of the OPG gene are associated with the change of hip BMD and lumbar spine BMD after alendronate therapy (Wang C., 2009). This study observed that after 12 months of treatment, the percentage of BMD change at the femoral neck was higher in genotype AA, while at site T245G, the percentage of BMD change at inter-troche and total hip were higher in genotype TT, concluding that both genotypes show a better therapeutic effect to alendronate.

In 2010, a study of 144 osteoporotic Korean women (Mencej-Bedrac S., 2009), investigated whether genetic polymorphisms of *FDPS* (rs2297480 and rs11264361) or *GGPS* (the rs3840452 and rs3841735) genes were associated with the response to alendronate or risedronate in terms of changes in lumbar spine and femoral neck BMD. After 1 year of treatment, it was found that women with two deleted alleles of *GGPS1* -8188A ins/del (rs3840452) had significantly higher femoral neck BMD at the baseline compared with those with one or no deleted alleles. The response rate of women with two deleted alleles of *GGPS1* -8188A ins/del was significantly lower than the rate of women with one or no deleted alleles. Moreover, women with two deleted alleles of *GGPS1* -8188A ins/del had a 7-fold increased risk of non-response to bisphosphonate therapy compared to women with other genotypes in *GGPS1* -8188, after adjusting for baseline BMD. No polymorphisms of the FDPS gene were associated with lumbar spine BMD or femoral neck BMD.

A recent pharmacogenetics of osteoporosis study evaluated the effect of the *BsmI* (G>A) polymorphism of the *VDR* gene in forty-two postmenopausal women receiving alendronate or teriparatide during 1 year (Creatsa M., 2011). The results showed the effectiveness on BMD only with alendronate treatment. Carrier patients of the b (A) allele (Bb/AG, bb/AA) were more responsive to treatment compared to patients with the BB (GG) genotype BsmI polymorphism.

Global results from these studies suggest that patient genotyping could be useful to target subjects most likely to respond to osteoporosis drug treatments in terms of BMD and bone turnover marker changes. However, association studies can have some limitations, such as inadequate sample size or sampling errors, genetic differences between ethnic groups, the presence of gene-gene and/or gene-environment interactions acting as confounding factors, the complexity of genome and gene regulation (epigenetic factors, somatic mutations, microRNAs), frequent accidental statistical association (not due to a real association between genotype and phenotype), and the lack of independently replicated studies. For all these reasons, no definite gene variations have conclusively been shown to be responsible for the regulation of any anti-osteoporosis drug responses. This research has contributed to the understanding of this disease and has demonstrated the influence of genetic polymorphisms in response to antiresorptive treatment. For a proper and efficient application to clinical practice, it is essential to consider the key issues that will be discussed below.

Adverse drug reactions associated with genetic factors

Drugs do not always reach their therapeutic target because they are not equally effective in all patients, and may lead to a variety of adverse effects.

Although osteoporosis drugs are effective in the majority of cases, most are associated with adverse effects (Rizzoli R., 2011) that render long-term administration and adherence problematic (Siris ES., 2009). Several genes have been evaluated that could be related to these adverse effects. Table 3 shows the main side effects of treatments for osteoporosis (antiresorptive and anabolic drugs).

Table 3 shows adverse effects of antiresorptive treatment, particularly bisphosphonates (esophageal irritation, thromboembolic disease). The most threatening

side effect of bisphosphonates is the development of osteonecrosis of the jaw (ONJ), which is the only side effect associated with genetic factors (Kennel KA., 2009). The incidence of ONJ in patients treated for osteoporosis is low (0.1%) while the incidence in cancer patients treated with high doses of intravenous drugs is higher (3-10%) (Reid IR., 2009; Franken AA., 2001). During the last 4 years, 6 genes have been proposed to be involved in the risk of developing ONJ (Sarasquete ME., 2008; Sarasquete ME., 2009; Lehrer S., 2009; Katz J., 2011; Marini F., 2011) and the most relevant of these studies are summarized in Table 4.

One GWAS_determined the role of genes in the pathogenesis of ONJ after 2 years of intravenous BP treatment (pamidronate or zaledronate), carried out in 2 groups of patients with multiple myeloma (22 with ONJ and 65 without) (Pietschmann P., 2009). Homozygosis for the T allele of the *CYP2C8* rs1934951 (C>T) was associated with an increased risk of developing ONJ. Among genetic factors, *CYP2C8* polymorphisms are arising as a promising risk factor, and the bisphosphonate-related ONJ can be predicted by an association of genetic and environmental risk factors (Pietschmann P., 2009; Patsch JM., 2011). However, this conclusion has not been confirmed by studies on an independent series of patients, so it remains to be seen if that association is a consequence of the genetic background and/or environmental factors unique to the population studied. Despite the interesting contribution of this preliminary study through a GWAS, variability in the genes encoding *CYP2C8* would not play a role in the metabolism of B, because these drugs do not undergo any physical-chemical modification (Gong L., 2011).

Another study (Haney E., 2008) proposed the matrix metalloproteinase 2 (*MMP2*) protein as the candidate gene for bisphosphonate-induced ONJ. The study determined whether any abnormalities in serum bone markers are related to

bisphosphonate-induced ONJ in patients (2 metastatic breast cancer, 3 osteoporosis, 1 prostate cancer, and 1 Gaucher`s disease) treated with intravenous or oral BPs (pamidronate, zoledronate or alendronate). This protein was selected on the basis of its potential involvement in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodelling, as well as in disease processes, such as arthritis and metastasis. Although the values of bone turnover markers in these patients were normal, the study proposed that *MMP2* is a candidate gene for bisphosphonate-induced ONJ for 3 reasons: 1) *MMP2* is associated with bone abnormalities which could be related to ONJ, 2) *MMP2* is the only gene known to be associated with bone abnormalities and atrial fibrillation, and 3) a network of disorders and diseases, genetically linked by known disorder-gene associations, indicates that cardiovascular diseases and bone diseases are related. This suggests that a single drug such as a bisphosphonate, acting on a single gene, *MMP2*, could have both bone and cardiovascular side effects different from the osteoclast inhibition that is characteristic of bisphosphonates. Nevertheless, further studies on patients without ONJ are needed.

A third study (Dagdelen S., 2007) explored the possible genetic variability of seven genes associated with drugs, risk of osteoporosis and bone metabolisms (*CYP2C8, COL1A1*, *RANK, OPN, MMP2, OPG* and *TNF*) as the predictive risk factor for the development of bisphosphonate-induced ONJ. This study was performed on a cohort of 78 patients with multiple myeloma (12 with ONJ) who received intravenous BP (pamidronate or zaledronate) over 1 year. The results indicated that the risk of developing ONJ is 4 times higher in patients with a history of smoking than those without a history of smoking (p=0.048). The authors also found that the type of BP treatment associated with ONJ in patients treated with pamidronate, resulted in patients

having 4 times higher risks of developing bisphosphonate induced-ONJ compared with those on zaledronate. Moreover, it was observed that the carriers of individual SNPs for *COL1A1* (rs1800012), *RANK* (rs12458117), *MMP2* (rs243865), *OPG* (rs2073618) and *OPN* (rs11730582) had a trend towards higher odds ratio for developing ONJ in multiple myeloma patients undergoing intravenous BP therapy. The probability of this trend increased 11-fold for the combined genotype score of the five SNPs listed above.

Finally, a recent study (Mani A., 2007) makes an interesting analysis of the influence of rs2297480 (A/C) SNP on the target *FDPS* gene (an enzyme involved in the mechanism of action of the BP), in a cohort of 68 Caucasian patients with multiple myeloma, metastatic mammary and prostate cancer treated with an intravenous BP (zoledronic acid). The results of this case-controlled study demonstrated significant differences in the AA and CC genotype frequencies of this SNP between ONJ cases, and controls with a positive correlation between AA carrier status and disease expression (p=0.033). It also showed differences between CC carrier status and the absence of the ONJ complications after 18-24 months of intravenous zaledronic acid treatment (p=0.045). These results are in agreement with the study previously described (Ralston SH., 2010) in which higher responsiveness of the AC and AA genotypes to oral treatment with amino-BP was found when compared to CC genotype. It could be assumed that the A allele segregates with the ONJ complications through a positive modulation of the response to a potent amino-BP, like zoledronic acid. However, the authors determined no direct causative relationship between ONJ and *FDPS* gene polymorphisms. They propose to confirm this study in other patient cohorts as well as the validation of this genetic marker for ONJ.

Therefore, it remains to be determined whether the BPs are the cause of ONJ development. The possibility of environmental and/or genetic variation between

individuals may confer susceptibility or resistance to developing ONJ. No pharmacogenetic studies related to other side effects have been reported at this time.

Other factors associated with osteoporosis treatment response.

Many risk factors contribute to the pathogenesis of osteoporosis. Aging affects bone density variations that are also mainly determined by genetic factors. Nevertheless, not only genetic factors have an observable effect. In addition, lifestyle factors such as weight-bearing physical activity; nutrition (insufficient calcium and vitamin D intake, excessive alcohol consumption); nicotine abuse; illnesses that affect the bone (kidney failure, hypothyroidism); or the intake of medications with a negative impact on bone metabolism (such as diuretics or inhibitors of the proton pump) are risk factors for osteoporosis and can affect drug responses (Weinshilboum R., 2003).

Genetic variations in a population create an impressive spectrum of phenotypic diversity, particularly when changes in diet or environment are superimposed on the population. GWAS have become powerful tools for linking sequence variants with overlying systems level phenotypes, but they do not provide insight into the mechanisms through which genetic variation drives phenotypic variation (Voy BH., 2011). Systems genetics is an emerging discipline that provides a means to fill this knowledge gap by assembling a hierarchy of interactions among genes, proteins, and other intermediate phenotypes that manifest themselves as phenotypic variations.

On the other hand, no conclusive results have determined whether there is a difference in the response to the treatments used for osteoporosis, taking into account age, gender, and environmental factors (disease, calcium intake, exercises, among others) or if none of these factors are associated with or influenced by genetics. However, it is necessary to consider some aspects that will be described below.

Osteoporosis is an age-related disease with several gender-specific differences, more prevalent in women than men; however, once a hip fracture has occurred, mortality is higher in men (Rojo K., 2010). Differences in sex hormone production, especially the abrupt decline of estrogens in women, are responsible for inter-gender differences in the pathophysiology of osteoporosis.

The treatment of osteoporosis also differs by gender; therapy options have been studied only in women (Pietschmann P., 2009). Men have no pharmacological protection from the disease (Patsch JM., 2011). Therefore, the treatment in men is understudied and the magnitude of the impact of male osteoporosis has been also underestimated (Haney E., 2008).

Another important aspect in the response to the treatment of osteoporosis is that some studies have shown the influence of certain diseases. In a retrospective, matched case-control study, the efficacy of alendronate in postmenopausal osteoporotic women with type 2 diabetes mellitus (DM) was evaluated by the change in BMD. Patients with type 2 DM proved resistant to long-term BP treatment, especially in hip, femoral neck, and forearm regions of t BMD (Dagdelen S., 2007). Metabolic syndrome diseases such as DM are genetically linked to a missense mutation in *LRP6* (Wnt signaling), one of the genes related to osteoporosis (Mani A., 2007).

It is well analyzed that Vitamin D deficiency has been related to osteoporosis. Patients with low BMD and an initial deficiency of Vitamin D were tested with alondronate and raloxifene, and their response showed that basal status does not affect BMD response to BPs when they were co-administered with Vitamin D and calcium (Antoniucci DM., 2009; Antoniucci DM., 2005). On the contrary, a study performed in women with postmenopausal osteoporosis showed that optimal vitamin D status seems to be necessary in order to maximize the response to antiresorptives (alendronate,

risedronate and raloxifene) in terms of both BMD changes and anti-fracture efficacy (Adami S., 2009).

In addition, a decrease in calcium absorption could contribute to the pathogenesis of osteoporosis. A recently randomized, double-blind, placebo-controlled and multicenter clinical trial (Shapses SA., 2001) evaluated whether alendronate increased the fractional calcium absorption (FCA) in postmenopausal women with low BMD and Vitamin D ≤ 25 ng/ml. Patients who were treated during 5 weekly doses of alendronate 70 mg + Vitamin D(3) 2800 IU (ALN+D) showed an increase in FCA compared with those who received the placebo.

In general, external factors such as age and gender, as well as modifiable factors such as intake of calcium and vitamin D, not only have implications as risk factors for osteoporosis, but probably in response to treatment.

Translation of pharmacogenetic findings to clinical practice

The translation of pharmacogenetic findings of osteoporosis-related bone fractures to clinical practice should be addressed in both a general context, and with parallel advancements in other pharmacogenetic treatment approaches.

In order to accelerate the translation and transition based on scientific results found in screening drug effects over a different inter-individual patient population, the following recommendations are proposed.

Additional Research

1) Analytical performance characteristics of biomarkers, stratification, identification of responders, or tests to avoid prescribing to either biomarker positive or biomarker negative subjects should be established (Tesch G., 2010). 2) All

polymorphisms in genes that can directly or indirectly affect these markers should be studied for their potential as putative genomic biomarkers. However, there is no evidence of clinical use of multiple pharmacogenetic variable genes, due to the controversial result of association which has been described throughout this review. It is well known that classical therapeutic approaches in osteoporosis have been established for controlling marker turnover, bone formation, and bone resorption that are now commercially available for decreasing fracture rate as the clinical end-point. 3) Global analysis of variability in bone gene pathway profiles of patients could help solve the contradictory findings from gene-isolated SNP studies (Karasik D., 2012). 4) The use of a putative biomarker in several populations (Zhang LS., 2011) should be verified and replicated by performing similar pharmacogenetic studies on the general population to contribute data to serve as validation. The validation process would involve the control of different parameters related with accuracy, precision, and repeatability of the genotyping methods. These important steps could become a reality through the use of high throughput technologies. 5) Create an international consortium which evaluates the results of studies retrieved from various clinical and research teams done on different populations by genotyping candidate genes and GWAS data. To date, 20 genome-wide association studies (GWASs) for osteoporosis and related traits have been conducted, identifying dozens of genes (Zheng HF., 2011).

Government and External Regulation

6) Genetic findings must be supervised and approved by the appropriate public entity. They should allow the implementation of specific and reliable tests for those valid biomarkers that have demonstrated association with the safety and efficacy of therapy. 7) The provision of specific recommendations and guidelines from an expert consortium

on pharmacogenetics (Becquemont LM., 2011) would quickly help to introduce biomarkers in clinical practice. 8) Expert government expert agencies must be responsible for regulating and determining the status of a specific biomarker and also the initiatives pursued until their mandatory use in hospitals or clinics. There are consortiums of experts such as Joint Voluntary Genomic Data and Submission (VGDS) from the FDA/EMA that process this type of information (U.S. Food and Drug Administration). 9) Guidelines of anti-osteoporosis drugs necessary for pharmaceutical care should be elaborated (Drieling RL., 2011). These guidelines could also include recommendations of specific pharmacogenetic testing (Dell R., 2010).

Clinical Application

10) Important investments and coordinated macro studies will be necessary to improve the quality of life of the elderly patient population, because fractures related to osteoporosis will continue to be a substantial and growing public health problem. 11) An algorithm including a flow-chart of decision-making priorities for demonstrated genotypes should be developed, that could impact bone patient status, even if this task was considered highly difficult and controversial It could help to establish the basis for future application. 12) Clinical evidence of the effectiveness of very recently-reported molecules should be provided, which could lead to better practice of pharmacotherapy because no specific guidelines have been designed for them (National Institute for Health and Clinical Excellence (NICE): Technology appraisal guidance 160, 2010; National Institute for Health and Clinical Excellence (NICE): Technology appraisal guidance 161, 2010). In that regard, the weakness of the pharmacogenetics of osteoporosis and its pharmacological treatments is that therapeutic decisions are not harmonized due to the complexity and heterogeneous manifestation of the disease.

Other important considerations must be taken into account before the translation of pharmacogenetic achievements can be used in routine clinical practice, such as molecular biology pharmacogenetic formation, ethical-legal issues, quality management in genetic test laboratories, ISO norms, and cost-effective successful strategies.

Clinicians must be properly informed for the administration of and application of the requirements for genetic tests. Moreover they must have the proper infrastructure required for analysing the sample and interpretation of results, and coordinating themselves. Thus, training at the undergraduate and graduate level must be enhanced.

Currently, specific formation programs for pharmacogenetics are scarce and limited to a select number of schools of pharmacy and medicine (Murphy JE., 2010; Maize DF., 2010). Moreover, a general lack of knowledge of molecular genetics constitutes the first barrier to implement pharmacogenetic tests in routine clinical practice. Previous experiences described in pharmacogenetic literature can help to establish models for effective advancement in pharmacogenetic applications (Gurwitz D., 2010).

Further limitations to the translation of validated pharmacogenetic biomarkers and biomarkers under research in clinical applications include cost-effectiveness, economic incentives, reimbursement issues, and the difficulty of making real evaluations of these limitations. Today, there are few studies evaluating the economic aspect of osteoporosis management that could decrease the hip fracture rate by 25-50% and be cost-effective at the same time (Dell R., 2010). Thus, to overcome the first step of implementation of pharmacogenetics as a useful tool in a hospital context, more osteoporosis therapy studies must be conducted.

Future perspectives

This paper provided a synthesis of the literature on the genetics of osteoporosis and an update on progress made in pharmacogenetics of osteoporosis in recent years, specifically regarding the new molecular targets for antiresorptive drugs. In-depth translation of osteoporosis pharmacogenetics approaches to clinical practice demands a new vision grounded on the concept of "theranostics". That is, the integration of diagnostics for both disease susceptibility testing as well as for prediction of health intervention outcomes. The exponential progress and technological advances since the elucidation of the human genome provide opportunities for gaining a better understanding of complex diseases, including osteoporosis as seen through the lens of theranostics. The success in finding osteoporosis genes rests on the collection of large number of cohorts of clinically well-characterized individuals. It is expected that with such statistically robust large-scale studies, many of the genes that contribute to inter-individual variation in therapeutic responses influenced by osteoporosis phenotypes will likely be identified. Ultimately, theranostics of osteoporosis is an essential emerging approach to diagnostic medicine whereby the genomics components of both the disease and its treatment are considered inseparable, and thus requiring an integrative vision in both disease biology and clinical therapeutics.

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Table 1: Updated signalling pathways and putative genes involved in osteoporosis and osteoporotic fractures.

Name of Pathways	Name Genes (Alternative symbols)	Genetic and genomic screening technologies/ approaches	References
Osteoclastogenesis	Osteoprotegerine (OPG), Receptor activator of NF-$_k$B Ligand (RANK-L), Receptor activator of NF-$_k$B (RANK).	GWAS Candidate gene Meta-analysis	Hsu Yi-Hsiang, 2010; Richards JB., 2009; Rivadeneira F.,2009; Styrkarsdottir U., 2009; Ralston SH., 2010, 31:629-62; Ralston SH., 2010; 1192:181-9;
Wingless-type MMTV intregration site (Wnt) signalling	Low density lipoprotein receptor-related protein 5 and 6 (LRP4, LRP-5, LRP-6), Integrin-β (ITGB), Integrin-α, Dickkopf-1 (Dkk-1), Sclerostin (SOST), secreted frizzled-related protein (Sfrp).	GWAS Candidate gene Meta-analysis	Richards JB., 2009; Rivadeneira F.,2009; Styrkarsdottir U., 2009; Ralston SH., 2010, 31:629-62; Ralston SH., 2010, 1192:181-9; Mencej-Bedrac S., 2009; Reppe S., 2010;
Other Main Signalling Proliferation/Inhibition Growth Cellular:	secreted phosphoprotein 1 (SPP1), spectrin beta non-erythrocytic 1 (SPTBN1), G protein-coupled receptor 177	GWAS Candidate gene Meta-analysis	Hsu Yi-Hsiang., 2010; Richards JB., 2009; Rivadeneira F., 2009;

- Canonical Wnt **- TGF-B signalling** **-Beta-catenin phosphorilation** **- MAPK pathway**	(GPR177), catenin (cadherin-associated protein) beta 1 (CTNNB1), myocyte enhancer factor 2C (MEF2C), SRY (sex determining region Y)-box 6 (SOX6), histone deacetylase 5 (HDAC5), corticotropin releasing hormone receptor 1 (CRHR1), zinc finger and BTB domain-containing protein 40 (ZBTB40), Forkhead box protein L1 (FOXL1), Osterix (OS7).		Ralston SH., 2010, 31:629-62.; Ralston SH., 2010, 1192:181-9; Mencej-Bedrac S., 2009; Uitterlinden AG., 2006;
Vitamin D	Vitamin D receptor (VDR), Vitamin D receptor binding protein (DBP).	GWAS Candidate gene Meta-analysis	Ralston SH., 2010, 31:629-62; Ralston SH., 2010, 1192:181-9; Uitterlinden AG., 2006; Ji GR., 2010; Fang Y., 2006; Macdonald H., 2006;
Estrogens	Estrogen receptor-α (ESR-α), Estrogen receptor- β (ESR-β).	GWAS Candidate gene	Richards JB., 2009; Ralston SH., 2010, 31:629-62; Ralston SH., 2010, 1192:181-9;
Collagen	Collagen type 1 A (COL1A1)	GWAS Candidate gene Meta-analysis	Ralston SH., 2010, 31:629-62; Ralston SH.,

			2010, 1192:181-9; Mann V., 2003; Yazdanpanah N., 2007;
Homeostasis calcium phosphorus	Calcium sensing Receptor (CaSR)	GWAS Candidate gene Metaanalysis	Hsu Yi-Hsiang, 2010; Kapur K., Año; Tsukamoto K., 2000; Harding B., 2006;
Mevalonate	Farnesyl diphosphate synthase (FDPS), geranylgeranyl diphosphate synthase 1 (GGPS1)	GWAS Candidate gene	Marini F., 2008; Choi HJ., 2010; Levy ME. 2007

Table 2: Summary of relevant pharmacogenetic studies on antirresorptive drug response

Gene	Variant (alleles)	Osteoporotic Drug	Phenotype	Drug Efficacy	Reference
VDR	rs1544410-BsmI (G>A)	Raloxifene	BMD	The increase in lumbar BMD was higher in patients homozygous GG compared to those with the heterozygous genotype.	Palomba, et al. 2003
		Etidronate	BMD	The increase in lumbar BMD was higher in patents homozygous GG compared to those with the heterozygous genotype.	Marc J. et al 2009
		Alendronate + HRT Alendronate + Raloxifene Alendronate alone	BMD	The increase in lumbar BMD was higher in patients heterozygous AG and homozygous GG.	Palomba, et al. 2003
		Alendronate or Teriparatide	BMD	Effectiveness in BMD only with alendronate treatment. Carriers patients of the A allele (AG, AA) presented more responsive to treatment compared to patients with the GG genotype BsmI polymorphism.	
ER	rs2234693-PvuII (T>C) rs9340799-XbaI (C>G)	Raloxifene	BMD	The increase in lumbar BMD was higher in patients homozygous PP (TT) for the PvuII polymorphism and patients homozygous xx (GG) for the XbaI polymorphism.	Heilberg IP. et al 2005.
	rs1256049-RsaI (A>G)	Alendronate	BMD	A smaller increase on lumbar BMD, but no significant different, was showed in patients with heterozygous GA.	Arko B. et al 2002
COL1A1	rs1800012-Sp1 (G>T)	Etidronate	BMD	The increase in femoral neck BMD was higher in patients homozygous GG (SS) in comparison with the rest of the genotype	Qureshi AM, et al. 2002
		Hormone replacement therapy (HRT)	BMD	The increase in lumbar BMD and femoral BMD were higher in patients homozygous GG compared to those with heterozygous genotype.	Simsek M, et al. 2008
FDPS	rs2297480 (A>C)	Alendronate or ibandronate	BMD	Any relationship was found with spinal and femoral BMD.	Marini F., et al 2008
	rs2297480 (A>C) rs11264361 (G>T)	Alendronate or Risedronate	BMD	Any polymorphisms were associated with lumbar spine BMD or femoral neck BMD.	Choi HJ., et al 2010
LRP5	rs3736228 (C>T) rs4988321 (G>A/C)	Risedronate	BMD	Any relationship was found with BMD at any site.	Kruk M., et al. 2009
OPG	rs3102735 (A>G) rs3134069 (T>G)	Alendronate	BMD	At site A163G, the increase in femoral neck BMD was higher in patients homozygous AA, while at site T245G, the percentage of BMD change at inter-troche and total hip were higher in genotype TT.	Wang C., et al. 2009
GGPS1	rs3840452 (8188T ins /	Alendronate or	BMD	Patients with two deleted allele of GGPS1 -8188A ins/del was	Choi HJ., et al.

	del)	Risedronate		significantly lower than the rate of patients with one or no deleted allele. Patients with two deleted allele had 7-fold higher risk of non-response to bisphosphonate therapy compared with women with other genotypes in GGPS1 -8188, after adjusting for baseline BMD.	2010

BMD: bone mineral density. HRT: Hormone replacement therapy. Ref.: reference.

Table 3: Side-effect of established treatments for osteoporosis.

Type of therapy	Drugs	Doses/interval/route	Side effects	Predisposing factors	Dose-response
Antiresorptives	Bisphosphonates Alendronate Risedronate Ibandronate Zolendronate	70mg/weekly/Oral 35mg/weekly/Oral 150mg/monthly/Oral 5mg/yearly/IV	Osteonecrosis of the jaw	Patients with cancer, IV administration and odontological invasive surgical procedures	+
			Subtrocanteric fractures	Long term therapy	+
			Esophageal irritation	Biphosphonates orally administered	+
			Hypocalcemia	Rapid iv administration	+
			Potential renal toxic effects	Rapid iv administration	+
	SERM Raloxifene Bazedoxifene	60mg/daily/Oral 20mg/daily/Oral	Thromboembolic disease	Immobilization Hipercoagulability	+
			Hot flushes and Leg cramps	First 6 months of treatment	+
	Denosumab	60mg/6 months/SC	Infections (urinary tract, skin, GI tract, hear)	Skin wounds Peripheral vascular and venous stasis disorders	-
			Osteonecrosis of the jaw	Cancer	-
			Cancer	Unknown	-
Anabolics	Strontium ranelate*	2g/daily/Oral	Thromboembolic disease	Unknown	-
			Drugs rash with eosinophilia systemic syndrome	Hypersensitivity	-
	Teriparatide PTH (1-84)**	20µg/daily/SC 100µg/daily/SC	Hypercalcaemia	Unknown	+
			Nausea, headache, dizziness	Unknown	+
			Diarrhea	Unknown	+

IV: Intravenenous. SC: Subcutaneous. * Approved in more 70 countries. ** Approved in European but no in USA.

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Table 4: Summary of pharmacogenetic studies of adverse events with bisphosphonates.

Gene	Variant	Osteoporotic Drug	Adverse drug reactions	Results	Ref.
CYP2C8	rs1934951 (C>T)	Bisphosphonates (pamidronate or zaledronate)	ONJ	Homozygosity for the T allele was associated with increased risk of developing ONJ.	Sarasquete ME., et al 2008, Sarasquete ME., et al 2009.
MMP2	No defined	Bisphosphonates (pamidronate or zaledronate or alendronate	ONJ	This protein was selected on the basis of its potential involvement in the breakdown of extracellular matrix in normal physiological processes, such as tissue remodelling, as well as in disease processes, such as arthritis and metastasis.	Lehrer S., et al 2009
COL1A1	rs1800012 (G>T)	Bisphosphonates (pamidronate or zaledronate)	ONJ	A trend towards higher odds ratio for developing ONJ in patients with multiple myeloma undergoing intravenous BP therapy was observed for the combined genotype score together with other gene SNPs (RANK, MMP2, OPG, OPN)	Katz J., et al 2011.
RANK	rs12458117 (A>G)	Bisphosphonates (pamidronate or zaledronate)	ONJ	A trend towards higher odds ratio for developing ONJ in patients with multiple myeloma undergoing intravenous BP therapy was observed for the combined genotype score together with other gene SNPs (COL1A1, MMP2, OPG, OPN)	Katz J., et al 2011.
MMP2	rs243865 (C>T)	Bisphosphonates (pamidronate or zaledronate)	ONJ	A trend towards higher odds ratio for developing ONJ in patients with multiple myeloma undergoing intravenous BP therapy was observed for the combined genotype score together with other gene SNPs (COL1A1, RANK, OPG, OPN,)	Katz J., et al 2011.
OPG	rs2073618 (G>C)	Bisphosphonates (pamidronate or zaledronate)	ONJ	A trend towards higher odds ratio for developing ONJ in patients with multiple myeloma undergoing intravenous BP therapy was observed for the combined genotype score together with other gene SNPs (COL1A1,RANK, MMP2, OPN)	Katz J., et al 2011.
OPN	rs11730582 (C>T)	Bisphosphonates (pamidronate or zaledronate)	ONJ	A trend towards higher odds ratio for developing ONJ in patients with multiple myeloma undergoing intravenous BP therapy was observed for the combined genotype score together with other gene SNPs (COL1A1, RANK, MMP2, OPG)	Katz J., et al 2011.
FDPS	rs2297480 (A>C)	zoledronic acid	ONJ	Positive correlation between AA carriers status and disease	Marini F., et al.

				expression, as well as between CC carrier's status and the absence of the ONJ complications.	2011

ONJ: osteonecrosis of the jaw.