Genetics of the bone response to bisphosphonate treatments

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Summary

Bone mineral density (BMD) is the best established marker for bone health. Over the last years a large number of studies have pointed to the variability in many target genes and their relation with bone mass and with other determinants of fracture risk such as ultrasound bone properties, skeletal geometry and bone turnover markers. The importance of genetic factors in the bone quality is substantial, but no consensus exists yet on the genes that are involved. Furthermore, there are many differences of clinical outcomes during bone-active treatments in the population-based studies. Heterogeneity in drug response may reflect varying responsiveness to bone-active treatments due to allele variation in the polymorphic target genes. In this regard, polymorphisms of vitamin D receptor and estrogen receptor loci appear genetic determinants of their corresponding hormonal treatment response such as vitamin D and estrogens. The present review focuses on the genetic determinants involved in the clinical response to bisphosphonate treatments for bone disorders. Knowledge of the molecular and functional consequences of the target genes is crucial to fully appreciate their significance and understand their potential clinical implications.

KEY WORDS: bisphosphonate, collagen type 1, genetics, interleukine 1, polymorphism, vitamin D receptor.

Introduction

While medicinal use has been relatively recent, bisphosphonates (BPs) were first synthesized over a century ago by Von Baeyer & Hoffmann (1). However, commercial application for these compounds did not take place until 1960 when Blazer & Worms (2) reported their use for dental detergent solutions as complexing agents for calcium and magnesium. Then, cyclical etidronate (ETN) was one of the first bisphosphonates to be used for osteoporosis treatment (3) and clinical experience has shown it to be a safe and effective drug for the prevention and treatment of vertebral osteoporosis (4, 5). Today, BPs are a widely utilized class of compounds for the prevention and treatment of a variety of bone diseases (6-10).

BPs are compounds with a molecular structure analogous to inorganic pyrophosphate (Fig. 1), the simplest of polyphosphates, which is able to inhibit the aggregation and the dissolution of calcium phosphate crystals *in vitro*. *In vivo*, pyrophosphate physiologically prevents the calcification of soft tissues (11) and could play a role in the processes of bone mineralisation. By substituting an oxygen atom (P–O–P) with a carbon atom (P–C–P) in the pyrophosphate molecule (Fig. 2), it is possible to obtain a class of compounds which are resistant to pyrophosphatase while maintaining the chemical-physical properties of pyrophosphate. These compounds can be considered stable analogues of pyrophosphate, resistant to hydrolysis. Due to the presence of a double link with phosphate by a single carbon atom these molecules are called “bisphosphonates”. Like pyrophosphate, BPs inhibit the formation, aggregation and dissolution of calcium phosphate crystals. Furthermore, they have a high affinity for bone mineralised matrix and are able to inhibit bone resorption processes increasing bone mineral density (BMD), their most important biological effect. It

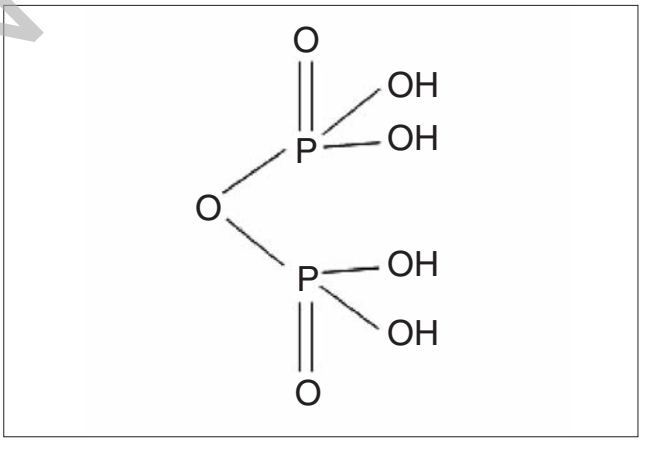

Figure 1 - Chemical structure of pyrophosphate.

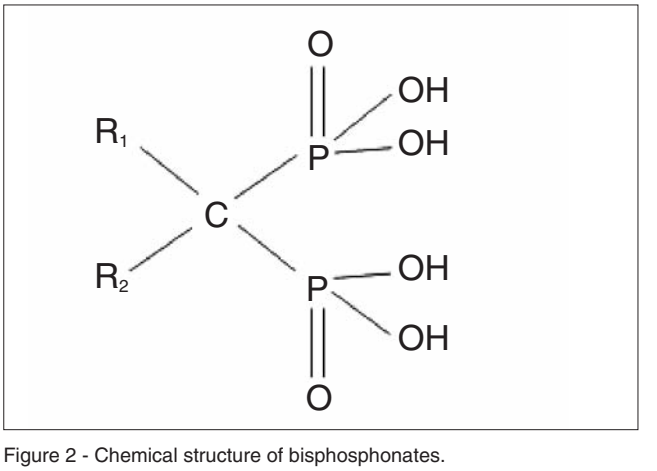

Figure 2 - Chemical structure of bisphosphonates.

is generally accepted that BPs inhibit bone resorption by preventing osteoclast formation, restraining the bone-resorbing activity of osteoclasts and acting indirectly on stromal and hemopoietic cells (12). The BPs are classified according to the molecular mechanism of action. Non-nitrogen-containing BPs are metabolized by osteoclast to cytotoxic ATP analogues that are accumulated within the cell, whereas nitrogen-containing bisphosphonates inhibit the farnesyl-diphosphonate synthase, an enzyme in the mevalonate pathway (13). In spite of these different mechanisms, both classes of BPs ultimately lead to apoptosis by activation of caspases (14). This explains why these compounds are used, besides in osteoporosis, in all the pathological conditions characterised by increased bone resorption, such as Paget disease, malignant hypocalcaemia during myeloma, osteolytic bone metastasis and fibrous dysplasia of bone.

To date, interest of most scientists and clinicians working in genetics, is to recognize the markers useful in the diagnosis and in the patient management. In this view, genetics not only offer possibility to precociously recognize patients at risk to develop bone disorders, but also to foresee the individual response to drugs. Pharmacogenetics has the potential to allow early specific and efficacious treatments, with consequent better chances for the patient health and reduced economic loss for the patient and the society.

The present review focuses on the available molecular data of BP treatments regarding predictor markers for their clinical drug response. Many clinical clues suggest human genetic backgrounds play major role determining treatment effectiveness. Clinical response to BP treatments seems to be affected by specific genotypes of target genes such as vitamin D receptor (*VDR*), collagen type 1 (*COLIA1*) and interleukin-1β (*IL1B*). In our opinion, this review could offer argument of pharmacological data reanalysis and/or new future health strategies.

Vitamin D receptor gene

That the gene encoding for VDR is the major genetic locus of bone mass has been well established since 1990s (15). The *VDR* gene is located on the long arm of chromosome 12 (12q12-14) and is composed by 10 exons, the first of which is not transcribed, and 8 introns (16). The 9 coding exons are transcribed into the *VDR* messenger RNA (mRNA), which in turn is translated into the functional VDR protein.

Nearly 300 polymorphisms of the human *VDR* gene have been reported (17), and especially in the regulatory region (18), leading to a precise haplotype map of the *VDR* gene. Several restriction fragment length polymorphisms (RFLPs) in the human *VDR* locus have been used in population-based studies (19). The respective restriction endonuclease sites have been conventionally indicated with lowercase letter (*t*, *a*, *b* or *f*, respectively for *Taq*I, *Apa*I, *Bsm*I and *Fok*I restriction endonucleases), while uppercase letter (*T*, *A*, *B* or *F*) indicates the absence of the restriction site. The *Bsm*I and *Apa*I polymorphisms lie in a *VDR* untranslated region (intron 8) and probably do not confer any functional diversity per se (19). Similarly, the silent nucleotide substitution in exon 9 that creates the *Taq*I polymorphism does not affect the amino acid composition of VDR protein (15). Because of their next sites, these *VDR* 3'end polymorphisms (*i.e. Bsm*I, *Taq*I and *Apa*I *RFLP*s) are in linkage disequilibrium such that *A* and *B* alleles are strongly associated with *t* allele, while *a* and *b* alleles with the absence of *Taq*I restriction site (*T* allele) (19).

The *VDR Bsm*I genotype has been reported to be involved in the individual response to antiosteoporotic BP drugs, such as ETN (20) and alendronate (ALN) (21, 22). In 1999, Marc et al. (20) studied 24 late postmenopausal women with osteoporosis during ETN treatment. In their limited series, the lumbar spine BMD increased significantly faster in the *BB* and *Bb* groups (7.3% and 7.0%, respectively) compared with the *bb* group (2.5%) during 1 year of 400 mg/day ETN therapy and 1000 mg/day calcium supplementation (20). The biochemical marker of bone resorption (urinary hydroxyproline excretion) as well as the bone formation marker (serum levels of osteocalcin) decreased during ETN treatment (20). With respect to *VDR* genotype, a significantly higher decrease in osteocalcin level was observed in *bb* as compared with *BB* subjects (20).

In the Palomba et al. (21) study, the influence on BMD gain due to the *VDR Bsm*I genotype during various antiresorptive treatments was evaluated in 1,100 postmenopausal Caucasian women. In line with two previous uncontrolled prospective studies (22, 23), the effect of 10 mg/day ALN and 60 mg/day raloxifene (RLX) varied according to *VDR Bsm*I genotype (21). The 1-yr administration of ALN and RLX treatments induced a significantly greater improvement in BMD and bone turnover markers in *VDR bb* and in *VDR BB* genotypes, respectively (21). Moreover, *VDR Bb* heterozygotes had an intermediate percentage change in lumbar BMD, serum osteocalcin and urinary deoxypyridinoline levels, which was not significantly different from that seen in *BB* and *bb* homozygotes (21). Furthermore, in *VDR bb* homozygotes and in *Bb* heterozygotes, ALN plus hormonal replacement therapy (HRT) (0.625 mg/day conjugated equine estrogens plus 2.5 mg/day medroxyprogesterone acetate) and ALN plus RLX associations induced a greater influence on BMD compared with HRT alone or RLX alone, respectively, but were not more effective than 1-yr ALN alone (21). Finally, in *VDR BB* homozygotes, ALN plus RLX induced a greater BMD gain than ALN plus HRT or ALN alone or RLX alone (21). From these results, it is conceivable that ALN and HRT, administrated alone or together, have a weak influence in women with the *VDR BB* genotype (or the linked *TT* genotype) (22, 24). However, these findings did not support the remarkable BMD response to ALN plus HRT reported in other Caucasian studies (25, 26), being the *VDR BB* genotype most frequent in Caucasians (27). On the contrary, RLX segregated with a considerable bone gain in the *VDR bb* genotype while no *VDR* genotype effect is detected after ALN plus RLX withdrawal (21).

In conclusion, at the moment, there are scarce clinical and experimental data on molecular mechanism by which *VDR* genotypes may influence the bone gain during BP-based treatments.

Collagen type 1 gene

The collagen type 1 is an important component of bone matrix and previous work has identified a G-T substitution affecting a Sp1 binding site in the transcriptional control region of the *COLIA1* gene. Alleles in which a G-base is present at the Sp1 binding are designed *S*, whereas alleles in which a T-base is present at this site are designed as the *s* allelic variant. Clinical studies have shown that T containing *s* allele is associated with reduced BMD and osteoporotic fracture in several populations (28-32).

The first intron of the *COLIA1* gene has been shown to be of importance in the regulation of collagen transcription (33-35) and there is good evidence to suggest that *COLIA1* Sp1 alleles influence gene regulation. A previous study (32) has shown that the *S* allele has increased binding affinity for the Sp1 protein in gel shift assays as compared with the *S* allele. Studies of allele-specific transcription showed an increased abundance of primary RNA transcripts derived from the *s* al-

lele compared with the *S* allele in bone samples from *Ss* heterozygotes. Cultured osteoblasts from *Ss* heterozygotes also produced increased amounts of collagen α1(1) chain, relative to the α2(1) chain. Finally, the yield strength of bone samples derived from *Ss* heterozygotes was found to be significantly reduced independently from differences in bone density when compared with bone from *SS* individuals (32, 33). This findings emphasize the importance of *COLIA1* Sp1 alleles as determinant of bone mass and of bone quality.

Though femoral neck BMD has also been found to increase in patients who have been ETN-treated, the response at this site is less marked than at the spine (36). The poor response of femoral BMD to ETN has generally been attributed to the lower rate of bone turnover in cortical bone which predominates in the femoral neck (37). Qureshi et al. (38) reported that there is also a significant heterogeneity in response of femoral BMD to ETN (400 mg daily for 14 days), which is related to *COLIA1* Sp1 genotype in 52 early postmenopausal women. Though individual with *SS* genotype (n = 32) responded reasonably well to ETN therapy with a 2.36% increase in femoral BMD after 2 years, those with the osteoporosis-associated *Ss* or *ss* (n = 19) genotypes responded poorly, such that BMD fell by –0.62% (38). This difference in response in BMD was observed throughout the treatment period and also during a treatment-free follow-up period of 1 year (38). The genotype-related differences in response of BMD could not be attributed to confounding factors such as anthropomorphic criteria or baseline BMD since *COLIA1* genotype was an independent predictor of femoral BMD response in a multiple regression analysis.

The mechanism by which the *COLIA1* Sp1 polymorphism predicts the response of femoral neck BMD, but not spine BMD to etidronate (38) remains unclear and will require further investigation. There is some evidence to suggest that the unfavorable *s* allele may act as a marker for increased age-related bone loss (31, 39, 40), although this has not been observed in all studies (41). Similarly, the *s* allele has been associated in some studies with reduced collagen production, as reflected by serum collagen propeptide levels (28) and with increased bone resorption, as reflected by urinary pyridinoline cross link excretion (30), although this has not been observed in other studies (42). In this regard, deoxypyridinoline values were significantly higher at baseline in the *SS* genotype compared with the *Ss+ss* group (38). Values of deoxypyridinoline/creatinine ratio and of pyridinoline/creatinine ratio fell to a similar extent in both genotypes in response to ETN, so differences in inhibition of bone resorption are unlikely to have been responsible for the differing response between genotypes at the hip (38).

Finally, it is possible that the poor response of femoral BMD in the *Ss+ss* genotype group may be a reflection of an impaired osteoblastic response or abnormalities in collagen synthesis. In keeping with this hypothesis, recent work has been shown that osteoblasts cultured from patients who carry the *s* allele produce an abnormally increased ratio of the collagen type 1 α1 chain, relative to the collagen type 1 α2 chain (32).

Whatever the underlying mechanism, the data by Qureshi et al. (38) have potential clinical implications in identifying a subgroup of patients whose femoral BMD response poorly to ETN therapy. While the results of their study must be treated with caution in view of the limited sample size (n = 52), the consistency of the response during ETN treatment and in the follow-up phase suggests that the genotype-specific differences in response to femoral BMD is a real phenomenon. If their data can be confirmed by other studies, *COLIA1* genotyping may be of clinical value in targeting BP therapy to those most likely to respond, with potential advantages in term of cost and clinical outcome.

Interleukin-1β gene

It is well known that cytokines are involved in the regulation of bone remodeling (43) and are also associated with several bone diseases (44). In particular, interleukin-1β (IL-1β) is a potent osteoclast-activating factor that promotes bone resorption both *in vitro* and *in vivo* (45, 46) and is antagonized by IL-1 receptor antagonist (IL-1Ra) (47). Well-estrablished evidence indicates that osteoclasts are the direct target cells of IL-1 which prolongs the viability of purified osteoclasts (48). *In situ* hybridization experiments revealed mRNA expression of IL-1 type 1 (*IL-1R1*) and type 2 receptors (*IL-1R2*) in murine and rat osteoclasts in normal bone and in inflammatory bone tissues (49). IL-1 increases mature osteoclast survival by inducing the activation of nuclear factor κB (NFκB) (50) and the expression of the receptor activator of NFκB ligand (RANKL) in osteoblasts (51). These results suggest that the direct effect of IL-1 on osteoclasts is an important mechanism by which IL-1 mediates physiological and pathological bone resorption.

The IL1 gene family is located on chromosome 2q (52) and encodes nine proteins, including IL-1α, IL-1β, IL-1Ra and IL-1R1 which are coded by *IL1A*, *IL1B*, *IL1RN* and *IL1R1* gene respectively (53). IL-1Ra competes with IL-1β for the IL-1R1 receptor, and it is a potent inhibitor of IL-1 activity (54, 55).

Cytokine expression levels are partially associated with genetic polymorphisms located mainly in the promoter and coding sequences of the genes that encode for these proteins. The *IL1B* gene has at least two biallelic polymorphisms, at positions –511 in the promoter region (56) and +3953 within exon 5 (57) both of which are related to changes in the production of the cytokine (58, 59). Five alleles have been described, corresponding to 2 to 6 copies of the repetitive sequence which forms part of the variable number of tandem repeats (VNTR) located within intron 2 of the *IL1RN* gene, but only the 4-repeat (*IL1RN*1*) and the 2-repeat (*IL1RN*2*) alleles are commonly found. In vitro studies have shown that the *IL1RN*2* allele is associated with higher IL1RN production (60, 61), and healthy carriers of the *IL1RN*2* allele have significantly higher plasma levels of IL1RN than non-carriers (62). A polymorphism (G to A) in position –1622 in the promoter region of the *IL1R1* gene has been described. Individuals that carry the wild-type genotype have higher IL-1R1 plasma levels than those with the mutant genotype (63).

Due to the fact that interleukins are involved in bone remodeling, displays over-expression in osteoclasts and is increased after BP treatment, Corral-Gudino et al. (64) hypothesized that variations in genes of the IL-1 family could be associated with clinical outcome of Paget disease to BP treatments. Interestingly, they found that the –511C/T polymorphism of the *IL1B* gene is associated with resistance to BP treatments (e.g. ETN, clodronate, tiludronate and risedronate-based) (64). It has been reported that the C/T polymorphism at position –511 in the human *IL1B* gene is associated with variations in IL-1, plasma levels. The allele T has been related to increased serum levels of IL-1β (58, 59). Bearing in mind the pro-resorptive effects of IL-1β, one would expect more severe presentation of Paget disease and poorer response to treatment with higher levels of IL-1α. Nevertheless, carriers of allele T (n = 96) show a better response to treatment with BPs when compared with homozygous carriers of allele C (n = 69) (64).

The mechanisms underlying resistance to BP-based treatments are poorly understood (65). It is known that resistance to one BP may be followed by response to another BP drug (66, 67). Since IL-1β prolongs the life span of osteoclasts (68), it seems unlikely that the poorer response to BPs observed in homozygous carriers of allele C, that relates to lower levels of IL-1β, could be attributed to a mechanism related to osteoclast apoptosis (69). It is well documented that both BP classes in-

duce macrophage apoptosis and decrease the production of IL-1β (70-72). Thus, we could expect a higher promotion of osteoclast differentiation in carriers of the T allele because of higher levels of IL-1β. In this situation, more osteoclasts are susceptible to BPs, whereas macrophage induced osteoclastogenesis slows down. Therefore, the osteoclast precursor pool would be more efficiently depleted in carriers of the T allele than in carriers of the C allele. However, this hypothesis needs to be confirmed in an appropriate experimental model.

On the other hand, It might be possible that the reported data by (64) were the consequence of linkage disequilibrium between the *IL1B* locus and another potential locus encoding for a protein involved in BP metabolism. However, polymorphisms in exon 5 of the *IL1B* gene, intron 2 of the *IL1RN* gene and promoter region of *IL1R1* gene, all located close to *IL1B* locus, are not associated with response to BP treatment in 165 patients (64), suggesting a direct relationship between *IL1B* gene polymorphism and response to BP treatment at least in patients with Paget disease.

Although the study limits mainly regarding the small sample size series (64), –511 C/T *IL1B* polymorphism is proposed as response marker to BP treatment. Thus, –511C/T *IL1B* polymorphism could be used to select the more convenient BP compound prescribing the more active drug for homozygous carriers of allele C. The above data prompt future pharmacogenetic studies with BPs not only in Paget disease but in other metabolic bone diseases such as osteoporosis.

Conclusions

The potential implication of pharmacogenomics in clinical research and clinical medicine is that disease could be treated according to genetic and specific individual markers, selecting medications and dosages that are optimized for individual patients ("*the right drug into the right patient*") (19). The possibility of defining patient populations genetically may improve outcomes by predicting individual responses to drugs, and could improve therapy safety and efficacy. This personalizing of medicines has been the holy grail of pharmacogenomics since sequencing the human genome was conceptualized (73).

The available data suggest that none of the allelic variations in the proposed target genes could completely value the pharmacogenetics of the antiosteoporotic BP drugs. One limitation is represented by the ethnic-dependent allelic distribution of gene loci. Therefore, population analyses should encompass large homogeneous ethnic cohorts. A second level of complexity is represented by the need to analyze simultaneously all the functional gene variants within a individual background. In this regard, the application of genomic technologies such as gene sequencing, statistical genetics and gene expression analysis to drug development, holds great promise for the future of medicine.

Future studies and preventive strategies to management bone disorders need to take in account individual genetic backgrounds.

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