Polymorphisms associated with low bone mass and high risk of atraumatic fracture

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Genetics of osteoporosis

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Summary

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Osteoporosis is a serious disease characterized by high morbidity and mortality due to atraumatic fractures. In the pathogenesis of osteoporosis, except environment and internal factors, such as hormonal imbalance and genetic background, are also in play. In this study candidate genes for osteoporosis were classified according to metabolic or hormonal pathways, which regulate bone mineral density and bone quality (estrogen, RANKL/RANK/OPG axis, mevalonate, the canonical circuit and genes regulating the vitamin D system). COL1A1 and/or COL1A2 genes, which encode formation of the procollagen 1 molecule, were also studied. Mutations in these genes are well-known causes of the inborn disease ´osteogenesis impeerfecta´. In addition to this, polymorphisms in COL1A1 and/or COL1A2 have been found to be associated with parameters of bone quality in adult subjects. The authors discuss the perspectives for the practical utilization of pharmacogenetics (identification of single candidate genes using PCR) and pharmacogenomics (using genome wide association studies (GWAS) to choose optimal treatment for osteoporosis). Potential predictors of antiresorptive therapy efficacy include the following well established genes: ER, FDPS, Cyp19A1, VDR, Col1A1, and Col1A2, as well as the gene for the canonical (Wnt) pathway. Unfortunately, the positive outcomes seen in most association studies have not been confirmed by other researchers. The controversial results could be explained by the use of different methodological approaches in individual studies (different sample size, homogeneity of investigated groups, ethnic differences, or linkage disequilibrium between genes). The key pitfall of association studies is the low variability (7-10%) of bone phenotypes associated with the investigated genes. Nevertheless, the identification of new genes and the verification of their association with bone density and/or quality (using both

PCR and GWAS), remain a great challenge in the optimal prevention and treatment of osteoporosis.

Keywords: bone mineral density, osteoporosis, osteogenesis imperfecta, genome-wide association studies, bone remodeling, osteoporosis polymorphisms, estrogen, vitamin D

Introduction

Osteoporosis is a common serious disease with high morbidity and mortality. The early identification of at-risk individuals during the premorbid stage of the disease is a paramount precondition for the effective prevention of osteoporosis. Known causes of low bone density include hypoestrinism, nutritional deficiency (low intake of calcium, vitamins D and K), limited physical activity, „frailty" syndrome, nicotinism or excessive alcohol consumption. Part of the upward trend in the number of cases of osteoporosis is linked the rising incidence of autoimmune, rheumatic, and other diseases, the increasing number of patients with glucocorticoids-induced osteoporosis, and other immunosuppressive pharmaceuticals, and the use of anti-epileptics and anti-coagulant agents. Results from studies in affected families have confirmed that osteoporosis is a hereditary disease. It is known that the daughters or granddaughters of women with low bone mineral density develop osteoporosis more often than the daughters or granddaughters of women with normal bone density (Obermayer-Pietsch 2006).

Growing evidence indicates that osteoporosis involves multifactorial inheritance through a combination of genetic and environmental factors.

The degree of bone phenotype heritability (loss of bone density, biochemical markers of bone remodeling, structure and size of bones, and number of fractures) was measured in twin studies using the formula $H^2=2x$ (rMz-rDz), where rMz and rDz are correlations found in monozygotic and dizygotic twins. H^2 expresses the degree of genetically determined variations in bone phenotype (Bathum et al. 2004). It has been reported that the heritability of bone density is 60 to 80%, which means that 60-80% of bone mineral variation can be explained by genetic background. The heritability of bone density may depend on bone

location within the skeleton. It was found that H^2 in the lumbar spine is 0.89, while the H^2 of the femoral neck (where mechanical load is greater) is 0.77 (Ongphiphadanakul 2007). Twin studies have consistently shown that genetic factors account for approximatly 80 % of bone mineral density variabilityat all ages, indicating that genes are important in determining both peak bone mass and the rate of bone loss in later life (Young 2002)

Initially, chromosomal loci were identified, that determine bone mineral density (BMD) and its fragility, bone geometry and bone remodeling. Later, the allelic variants of a single nucleotide polymorphisms (SNPs), which controlled bone phenotypes, were identified (polymorphisms in the ERα gene located at chromosome 6q25.1, the vitamin D receptor located at 12q12-q14, the calcitonin receptor at 7q21.3 CALCR, or polymorphisms regulating the expression of TGFβ1, BMP-4, IGF-1, CYP17, the interleukin 1 receptor antagonist, interleukin 6 and the parathyroid hormone receptor). SNPs are identified using PCR, or a two-dimensional analysis of a wide range of genes using genome-wide association studies (GWAS) (Guo et al., 2010).

The majority of the genes associated with osteoporosis are, at present, fairly well established. However, to date no gene has been definitively identified as a major gene. Despite extensive research over the past two decades, little progress has been made in the identification of the genes that regulate bone remodeling, bone density, and fracture risk. Even GWAS datasets suffer from a number of limitations. An alternative approach was used by Faber (2013), in which he indentified novel interactions for genes having a known role in the regulation of bone mass. Array technologies are now capable of analyzing thousands of polymorphisms distributed throughout the genome. To date approximately 20 GWAS with osteoporosis phenotypes have beeb crried out. For example, GWAS identified SNPs in more

than 2000 women that were associated with bone mineral density (SNPs rs4355801 located on chromosome 8, close to the osteoprotegerin gene, and rs 3736228 polymorphism in the LRP5 gene on chromosome 11, associated with osteoporosis and/or atraumatic fractures (Richards et al., 2008).

Recently, a large a genome-wide association study performed on more than 5000 individuals of European origin found that a deletion located on chromosome 6p25.1 might predispose carriers to a higher risk of fracture (Oei at al., 2014). However, this association has not been observed in other geographically defined populations.

Our review offers overview of genes associated with osteoporosis and linked to bone-regulating hormonal circuits. Furthermore, it discusses possible roles of pharmacogenetics/pharmacogenomics with regard to response modulation associated wtith anti-osteoporotic pharmaceuticals.

Survey of fundamental genes for osteoporosis (see Tables 1 and 2)

Genes encoding the estrogen circuit

Bone remodeling is markedly regulated by ovarian estrogens, which inhibit bone resorption directly through the ER1 (ERα) and ER2 (ERβ) receptors in bone, or via the inhibition of osteoresorptive cytokines (interleukin-1, TNFα). The gene responsible for bone phenotype regulation is also the gene for the estrogen receptor (Xba1 and PvuII polymorphisms). A meta-analysis of 22 studies involving 5,000 women showed that the XX genotype of the XbaI polymorphism was associated with a high value of BMD in both the spine and the femoral neck, compared to carriers of the x allele. Within the Finnish population, the incidence of fractures was associated with the PvuII polymorphism (carriers

of the P allele have a lower incidence compared with carriers of the p allel). Another meta-analysis of European institution studies involving 19, 000 probands found an association between ERα and the risk of fractures, which did not always correlate with BMD (reviewed by Marini and Brandi., 2013; Riancho and Hernándeze, 2012). ERα studies in postmenopausal Italian women indicate that intragenic polymorphisms make a small, but statistically significant contribution to the heritability of bone mineral density. (Young, 2002). In the Caucasian population, an association between BMD and one SNP in the gene for ERβ was found in women, while this same phenotype was associated with three SNPs in the same gene in men (Ischikawa et al., 2005). This study shows a certain sexual differentiation in the relation between gene and bone phenotype.

The regulatory effect of estrogen on the skeleton probably has a wider sphere of influence. Genes for estrogen not only modulate bone density directly, but also through the precursors of sex steroids, or through binding proteins for sex steroids (SHBG). In a pilot study, the Xba1 polymorphism in ERα was associated with androstenedione levels in postmenopausal women (Žofková et al. 2002). The relationship to BMD has also been observed in the polymorphism of the SHBG gene (Riancho et al., 2008). Furthermore, CYP19A1 (located on the 15th chromosome) probably modulates BMD via aromatization of androgens to estrogen (Simpson, 2000).

RANK- RANKL-OPG (circuit - regulating bone remodeling)

RANKL is a protein in the tumor necrosis factor (TNF) familx; it is produced by osteoblasts and encoded by the TNFRSF11 (TNFRSF11A) gene. RANKL is the target molecule of osteoresorptive cytokines, thus it is not surprising that there is a relationship

between bone parameters and genes encoding cytokine production. Czerny et al. (2010) found an association between the 174 /C polymorphism in the gene for IL-1α and bone density, in a group of osteoporotic (but not healthy) postmenopausal women. Therefore, the regulatory effect of the IL-1α gene on BMD clearly depends on bone remodeling activity.

The candidate gene for osteoprotegerin (OPG) (the false soluble RANKL receptor, which blocks the binding of RANKL to RANK) is TNFRSF11B. In postmenopausal Chinese women a relationship between the G allele in the gene for OPG (the A163G and T245G polymorphims) and the risk of osteoporosis was found (Wang et al, 2009). Similarly, a relationship has been observed between the polymorphisms 1181G >C and 245T>G in the TNFRSF11B gene and BMD within the Caucasian population (Mencej-Bedrač et al., 2011). However, this association was not confirmed by a modified study in a large group of Australian women (Ueland et al., 2007). The differences between these studies could be related to different frequencies of allelic combinations in different populations.

Genes associated with the Wnt (canonical) circuit

Wnt signaling modulates cell differentiation through the non-canonical pathway (via calcium or cAMP signals) or through the canonical pathway, in which β-catenin plays a central role. The differentiation of osteoblasts is mostly under the control of canonical signaling. Wnt (βcatenin) binds to LRP receptor proteins (a low density lipoprotein-related protein) and FZDs (Frizzled receptors). Following the transfer of LRP and FZD into the nucleus of the pre-osteoblast and binding to transcription factor TCFS, proliferation and differentiation of this cell is induced (Whyte et al., 2004; Glass et al., 2005). Wnt inhibitors of canonical pathways, such as SFRP3 (encoded by the FRZB gene), DKK1-4 and sclerostin (encoded by the SOST gene) suppress bone formation. On the other hand, it was found that

antibodies against one of these inhibitors (e.g. anti-sclerostin antibodies) can increase BMD values in rats with hypoestrinisms (Li et al., 2009).

The canonical pathway is controlled by the LRP5 gene (Glass et al., 2005) (Table 1). The inactivation mutation in LRP5 manifests as juvenile osteoporosis in humans, on the contrary, activating mutation as osteopetrosis. A multicenter study of 37,534 participants from Europe and North America showed, that the V667M (in the exon 9) and A1330V (in the exon 18) polymorphisms of this gene were associated with a low BMD value and an increased incidence of fractures (van Meurs et al., 2008).

Gene for type I collagen

The majority of osteogenesis imperfecta (OI) cases are caused by mutations in one of the two genes, COL1A1 and COL1A2 encoding the two chains that trimerize to form the procollagen 1 molecule (Valadares et al., 2014). Alterations in gene expression and microRNAs (miRNAs) are responsible for the regulation of cell fate determination and may be involved in the OI phenotype. Uitterlinden et al. (1998) showed, that a combination of the alleles S and s in the Sp1 COL1A1 polymorphism was associated with a risk of fracture in the Caucasian population. The carriers of this combination had a 2.7 times higher riskof fracture than the carriers of the SS or ss genotypes. The relationship between COL1A1 and the risk of fractures was later confirmed by other authors (Ralston et al., 2006 – GENOMOS study; Jin et al., 2009). The genes that have been found to be associated with both fracture risk and low BMD values are summarized in Table 3 (Mitchell and Streeten, 2013).

Mutations in the COL1A1 and COL1A2 genes cause special forms of inherited osteoporosis, such as osteogenesis imperfecta (OI). OI is a brittle bone disease that is inheried

in an autosomal dominant manner, is often multisystemic, and affects whole families (Primorac et al., 2014). Most nonsense or missense mutations, insertions and partial gene deletions lead to a reduction in the amount of type I collagen (haplo-insuficiency) (reviewed by Kuklík et al. 2014). Mild cases of OI can result in a diagnosis of osteoporosis in adulthood or at an advanced age (O‘ Sullivan et al., 2014).. Recently, the genetic heterogeneity in OI has been confirmed through molecular genetics studies. At present, 17 genetic causes of OI have been identified (Van Dijk et al., 2014).The amino-acid glycine is the most obvious in the linear collagen sequence and correlate with the observed frequency of targeted mutations which were founded (reviewed by Kuklík et al. 2014). Nevertheless, the COL1A1 gene (located on the 17q21.31-q22 chromosome) also predicts bone quality in osteoporotic subjects without OI (Table 1, modified according to Marini Brandi, 2013; Brown et al. 2002).

Genes for vitamin D and calcitonin

The metabolite of vitamin D ($1,25(OH)_2D_3$) plays a key role in the regulation of bone metabolism. It activates specific receptors (VDR) on osteoblasts and stimulates bone formation. However, in high *in vitro* concentrations, it activates osteoclastic resorption. $1,25(OH)_2D_3$ via VDR also regulates calcium homeostasis *in vivo* outside of the bone by reducing calcium and phosphorus loss in the kidneys, while stimulating their absorption in the intestines, thereby ensuring adequate bone matrix mineralization (reviewed by Rincho and Hernández, 2012).

The gene for VDR was the first candidate gene, in which an association with bone mineral density was discovered by Morrison et al. (1994). The effect of the VDR genotype (the TaqI, BsmI and ApaI polymorphisms) on BMD has been found strongest in premenopausal women and decreases with age (Riggs et al., 1995). Later, however, only

isolated studies found an association between VDR and BMD values (Masi et al., 2002), while other studies (including those using the GWAS method had negative results. Instead, genes encoding the expression of protein-binding vitamin D (VDBP) and circulating 25(OH)D (CYP2R1) were identified, which predicted the risk of osteoporosis (reviewed by Riancho and Hernández, 2012).

A study of an intragenic polymorphism in VDR led to the suggestion that this gene, mutations in which cause vitamin D - dependend rickets type II, could account for as much as 75 % of the heritability of bone mineral density. (Young, 2002); although subsequent similar studies failed to confirm this observation.

Bone tissue is the primary target of calcitonin. An association between the calcitonin receptor (CTR) and bone mass has been found in several studies. In about 700 peri- and post-menopausal women, a relationship between CTR genotype (a combination of CC alleles) and bone mass was observed. However, binding was stronger in a sample of younger women (Braga et al., 2000). The relationship of CTR (the C1377T polymorphism) to BMD was observed in pilot studies of postmenopausal women (Masi et al., 2002; Zofková et al., 2003), howeve, other studies have not produced a consensus. Charopoulos et al.(2008) failed to document such an association. in young Greek men. Therefore, the effect of genes on bone mass appears to not only depend on age, but gender and ethnicity, as well. The final answer should come when larger homogeneous semples are analyzed using the GWAS method.

Additional genes with a potential effect on the bone metabolism

An insufficiency in the lactase enzyme, which splits lactose in the intestine, leads to a gastrointestinal intolerance of dairy products, as well as secondary calcium deficiency and osteopenia (osteoporosis). Therefore, the gene coding for the synthesis of lactase may have

predictive value for bone mass quality (Obermayer-Pietsch, 2006). However, relevant studies demonstrating the relationship of this gene to bone parameters are still lacking.

The gene for methyl tetrahydrofolate reductase (MTHFR) is responsible for the high levels of homocysteine, usually associated with some degenerative diseases (e.g.atherosclerosis). Interestingly, MTHFR also predicts the risk of osteoporotic fractures. In a senior population of twins, the association between this gene´s C677T polymorphism and risk of fractures was 1.5 times higher in the CTgenotype compared with the CC genotype, and 1.5 times higher in the CT genotype compared to the TT genotype. Thus, the risk allele for fractures is the T allele (Bathum et al., 2004). Similar results have been published by Villadsen et al. (2005), who demonstrated that the rare TT genotype of the C677T polymorphism was associated with an increased risk of osteoporotic fractures in women. Therefore, MTHFR seems to have a broader spectrum of body regulation, which includes bone quality.

Another gene associated with a risk of osteoporotic fractures is ALDH7A1. This gene is responsible for the detoxification of acetaldehyde, which is toxic to the skeleton. In Asian populations, an association between the rs 13182402 SNP in this gene, and osteoporotic fractures, was detected by GWAS (Guo et al., 2010). The relationship has not yet been described in the Caucasian population.

The risk of fractures is due not only to bone mass or its microstructure, but is also due to the geometric parameters of the skeleton. Protein phospholipase c-like 1 (PLCL1) regulates the response of bone to mechanical load, especially in the hip. GWAS was used to find the relationship of the PLCL1 gene to the size of the hip in a population of women (Liu et al., 2008). However, the relationship between the geometric parameters of the skeleton and the

PLCL1 gene could have an ethnic component. Additional genes related to the risk of fracture are shown in Table 3 (by Mitechell and Streeten, 2013).

Do candidate genes for BMD modulate osteotropic hormone expression?

Pilot association studies in a sample of 112 postmenopausal women found links between serum levels of androstenedione (a precursor of sex steroids) and the CTR gene (Zofkova et al., 2004) and/or the ER gene (Žofková et al., 2002). In the same group of women, associations were found between parathyroid hormone levels and the VDR gene (Zofkova et al., 2003; Laaksonen et al., 2009), as well as between FSH levels and LRP5 (Žofková et al., 2007).

On the other hand, bone density also seems to be modulated by the gene that has been primarily identified as a regulator of the extraskeletal system. In our group of postmenopausal women we found that carriers of the risk E4 allele in the gene for apolipoprotein (APOE, determining lipid metabolism) had lower bone mineral density in the spine than the carriers of the E1, E2 and E3 alleles (Zajíčková et al., 2003).

Altogether, these observations allow us to postulate, that gene pleiotropism, which most probably developed during evolution, integrates calcium into whole body homeostasis. This hypothesis, however, needs to be strengthned by additional studies on large samples using GWAS analysis.

Pharmacogenetics of osteoporosis

The aim of pharmacogenetics is the individual choice of pharmaceuticals that can be reasonably expected to be the most effective treatment with the lowest risk of side effects. To

date, the pharmacogenetics of antiresorptive agents (bisphosphonates and estrogen or SERMs) has been the focus of research..

Genes modulating the response of bone to bisphosphonates

Mevalonate is a target for the amino-bisphosphonates (alendronate, ibandronate, risedronate, zoledronate), which block the synthesis of cholesterol by the enzyme farnesyl diphosphate synthase (FDPS). The consequence of this blockade is the accumulation of nonprenylated proteins, which inhibit osteoclast function and accelerate their demise. The FDPS gene has a predictive role in BMD values and bisphosphonate treatment efficacy (reviewed by Riancho and Hernández, 2012). The rs2297480 and rs1126435 polymorphisms in the FDPS gene were associated with the response of bone to long-term bisphosphonate therapy, in postmenopausal Caucasian women. The carriers of the A allele had a better response on BMD and biochemical markers on alendronate or ibandronate treatments (Marini et al., 2008). Similarly, an association between the rs2297480 and rs1164359 polymorphisms in the FDPS gene, and response to treatment with alendronate or risedronate, has been shown in Spanish women. In the study, a 1% rise in BMD was observed in women with the AA genotype (the rs2297480 polymorphism), while the value of bone density in women carrying the CC genotype decreased by 1.6%. Improved skeletal response to bisphosphonates was also observed in women carrying the G allele in the rs1126435 polymorphism (Olmos et al., 2012). The risk allele (which may predict adverse effects of bisphosphonates on the skeleton) is the G allele in the 17024608 polymorphism of the RBMS3 gene. The relationship of the OPG gene relative to bone response to alendronate has been found in postmenopausal Chinese women (reviewed by Riancho and Hernández, 2012).

The Sp1 COL1A1 gene was also found associated with the response of bone mass to long-term bisphosphonate therapy. The etidronate-treated postmenopausal women with the SS genotype achieved a higher values of BMD compared to women with the Ss or ss. genotypes (Qureshi et al., 2002).

Furthermore, positive relationships between the BMD response to risedronate and V6677M (the rs4988321 polymorphism) and/or V1330V (the rs3736228 polymorphism) in the LRP5 gene, were observed in men (Kruk et al., 2009). Moreover, LRP5 is associated with the intestinal production of serotonin, which has a negative impact on bone metabolism. Therefore, the gene could be a potential predictor of the risk of osteoporosis in patients treated with selective serotonin reuptake inhibitors (SSRIs) (Wu et al., 2012).

Genes determining bone response to vitamin D administration

In a placebo-controlled study of girls undergoing long-term vitamin D supplementation, an association was found between BsmI and TaqI polymorphisms, and a percentage BMD increase in several parts of the skeleton (Arabi et al., 2009; reviewed by Riancho and Hernández, 2012). Finally, associations between the response of bone to estrogen or SERM and PvuII polymorphisms in ERα and/or A266G in LRP5 genes has been demonstrated (Kobayashi et al., 2002; Heilberg et al., 2005; reviewed by Riancho and Hernández, 2012). Nevertheless, these associations need to be confirmed by additional large studies, including those involving in men.

Pharmacogenetics may also predict the development of bisphosphonate adverse effects, such as osteonecrosis and/or fractures. An analysis of SPN in the VEGF and PPARγ genes, which regulate the transformation of precursor pluripotent cells into adipocytes or osteoblasts,

is currently in progress. Attention is also being devoted to genes regulating the activity of the RANK / RANKL / OPG system. However, the results of this study have yet to be published (Olmos et al., 2012).

Comments and future prospects

Osteoporosis is a polygenic disease, wherein each bone phenotype (density, quality, metabolic rate) is the result of interaction among many weak genes. The "essential gene", responsible for the manifestation of osteoporosis has not yet been identified, despide utilizing the most advanced methods. Some random observations indicate the broader impact of candidate genes for porosis of the skeleton. We mentioned the relationships between the ER or CTR genes and levels of precursor sex steroids, and the association between LRP5 and circulating FSH - the hormone, which activates bone resorption through interleukin 1-β and accelerates bone loss at high *in vitro* concentrations (Cannon et al., 2010). The regulatory effect of these genes on hormonal parameters needs to be further verified.

Genes are important in determining both peak bone mass and the rate of bone loss in later life. There is agreement that genetic factors are important, however, identifying the relevant genes have yielded inconsistent and conflicting results. Several genes are involved, some of which may be more important in particular ethnic groups than in others. (Young, 2002).

The genetics of osteoporosis has many pitfalls, that prevent application of information to practical osteology. An unanimous consensus has not been reached in the relationship between certain candidate genes and bone phenotypes. This may be due to inconsistent methodological approaches (e.g. the selection of probands, extent, and homogeneity of

investigated groups) or different allelic frequencies in the populations being compared. In addition, false positive results can be recorded in small groups, and are caused by linkage disequilibrium in candidate genes that are located in close proximity to another gene. A significant disturbing phenomenon is the effect of mutual interactions between genes (e.g. the interaction between ER and VDR) or between a gene and the environment (lifestyle, nutrition, physical activity). The effects of epigenetic factors must also considered, as they modulate the relationships between the reference gene and phenotype by methylation of cytosine in DNK, and by histone modifications and small RNK molecules (miRNK). One of the limitations of association studies is the relatively low variability of bone phenotypes associated with the candidate genes (ranging between 7 and 10%) (Marini and Brandi, 2013; Riancho and Hernández, 2012).

The GWAS method can partially overcome those pitfalls: it allows for the identaification of a range of candidate genes and an assessment of the impact of their mutual interactions on the appropriate phenotype in a single measurement (genomics). So far, about 20 GWAS studies have recorded the association of genes with bone density, fractures, or skeletal geometry (Riancho and Hernández, 2012). The analysis carried out on 47, 000 probands found 82 loci related to bone density, and 6 loci related to fractures (Estrada et al., 2011). We can assume that, in the future, the GWAS method will allow for the identification of genes (in the DNK and miRNK) encoding the binding of drugs to target receptors, as well as transport mechanisms, pharmacokinetics and pharmacodynamics (Rukov et al., 2011).

Discordant findings in GWAS and meta-analyses have been observed. However, the meta-analyses of GWAS data should be better at uncovering novel loci in homogeneous populations, and thus better able to identify true osteoporosis genes than could be obtained

through meta-analyses alone, particularly when the loss related to population heterogeneity is taken into consideration (Liu et al., 2013). From the point of further progress in genetics of osteoporosis, the polymorphic variations at multiple loci can be detected in the laboratory and subsequently analyzed mathematically with a computer using samples from very large numbers of affected individuals from multiplex families.

Despite all of the difficulties and numerous unanswered questions, the genetics (genomics) of osteoporosis remains a big challenge for further research in the field of pathogenesis, diagnosis and the personalized treatment of osteoporosis. Additionally, novel methods of the next generation, such as sequenations (both sides sequenation), multiple ligand probe analysis (MLPA), single strain conformation polymorphism analysis (SSCP), and fluorescent *in situ* hybridisation (FISH) are expected to expedite progress in this research (Zheng, 2013)..

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Table 1: Polymorphisms of candidate genes for bone mineral density (Marini and Brandi, 2013)

Candidate gene	Locus	SNP	
VDR	FokI	rs2228570	Exon 2
VDR	BsmI	rs1544410	Intron 8
VDR	TaqI	rs731236	Exon 9
ERα	PvuII	rs2344693	Intron 1
ERα	XbaI	rs9340799	Intron 1
ERα		rs3798575	Intron 6
ColIA1	Sp1	rs1800012	Intron 1
TNFRSF	OPG(A163G)	rs102735	Promoter region 5'UTR
TNFRSF	OPG (T245G)	rs134069	Promoter region 5'UTR
NFRSF	OPG (T950C)	rs2073617	Promoter region 5'UTR
FDPS		rs11264359	5'UTR
GGPS1		rs3840452	Promoter region 5'UTR
LRP5	C3893T		Exon 18

Table 2. Selection of genes associated with bone density of the femoral neck or spine identified using GWAS method (Riancho and Hernández, 2012).

Candidate gene	Locus	SNP
Wnt genes circuit		
LRP5	11q13.2	rs3736228
SOST	17q21.31	rs4792909
WNT16/FAM3C	7q31.31	rs3801387
WNT4	1p36.12	rs7521902
AXIN1	16p13.3	rs9921222
Genes for RANK-RANKL-OPG		
OPG(TNFRSF11B)	8q24.12	rs206237
RANK(TNFRSF11A)	18q2133	rs884205
RANKL(TNFRSF11)	13q14	rs9533090
The gene encoding sclerostin levels in the blood (found in black Caribbean population)		
SOST	17q11.2	rs851056, rs41455049, rs9909172
The genes for endochondral ossification		
CDKA1/SOX4	6p22.3	rs9466056
SOX6	11p15.2	rs7108738
SP7	12q13.13	rs2016266
SUPT3/RUNX2	6p21.1	rs11755164

Table 3: Selected candidate genes associated with bone density and with osteoporotic fractures (Mitchell and Streeten, 2013).

Candidate gene	Locus	SNP	Encoding
Wnt circuit			
CTNNB1	3p22.1	rs430727	β-catenin
LRP5	11q13.2	rs3736228	lipoprotein receptor related peptide 5
MBL2/DKK1	10q21.1	rs1373004	
SOST	17q21.31	rs4792909	sclerostin
WNT16/FAM3C	7q3131	rs3801367	
WNT4	1p36.12	rs7521902	Wingless proteins-type MMTV4
RANK-RANKL-OPG system			
TNFRSF11	13q14	rs9333090	receptor TNF 11 (RANKL)
TNFRSF11A	18q21.33	rs884205	receptor TNF 11a (RANKL)
TNFRSF11B	8q2412	rs2062377	receptor TNF 11b (OPG)
Gene for endochondral ossification			
MEPE/SPP1/IBSP	4q21.1	rs6532023	