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Genetic factors, osteoporosis and bone fractures

Czynniki genetyczne, osteoporoza i złamania kości

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S u m m a r y

Bone fracture risk is influenced by a number of factors, including bone mineral density (BMD), bone quality parameters and non-skeletal factors affecting the risk of falls. Each of these factors is itself under at least partial genetic control. Several studies suggested that between 50 and 85% of the variance in peak bone mass was genetically determined. It was also presumed that genes contributed significantly to variability in age-related bone loss and other determinants of fracture risk. Candidate genes for osteoporosis were classified according to metabolic or hormonal pathways. It was found that polymorphisms of genes encoding and/or regulating the Wnt/β-catenin signaling pathway, the RANK-RANKL-OPG pathway, vitamin D receptor (VDR) and vitamin D binding protein (DBP), procollagen 1 molecule, and the estrogen receptor α influenced BMD and bone fracture risk.

S t r e s z c z e n i e

Ryzyko złamań kości zależne jest od wielu czynników, takich jak gęstość mineralna (BMD) i jakość kości oraz od czynników pozaszkieletowych wpływających na ryzyko wystąpienia upadków. Każdy z tych czynników znajduje się, przynajmniej częściowo, pod kontrolą oddziaływań genetycznych. Wyniki przeprowadzonych badań sugerują, że 50-85% zmienności szczytowej masy kostnej jest uwarunkowane genetycznie. Przypuszcza się, że geny odgrywają również istotną rolę w regulacji utraty masy kostnej związanej z wiekiem oraz innych determinantów ryzyka złamań. Geny wiązane z rozwojem osteoporozy są klasyfikowane zgodnie z ich wpływem na metaboliczne lub hormonalne szlaki sygnałowe. Wykazano, że polimorfizm genów kodujących lub regulujących: szlaki sygnałowe Wnt/β-katenina, RANK-RANKL-OPG, receptor witaminy D (VDR) i białko wiążące witaminę D (DBP) oraz prokolagen typu 1 i receptor estrogenowy α wywiera istotny wpływ na BMD oraz ryzyko złamań kości.

INTRODUCTION

Current definition determines osteoporosis as a systemic skeletal disorder characterized by low bone mass and microarchitectural deterioration of bone tissue that result in reduced bone strength, bone fragility, and increased risk of fracture. The main clinical end points as well as complications of osteoporosis are skeletal fractures. The most common fracture sites are hip, spine and forearm although any bone can be affected (1).

Fracture risk is influenced by a number of factors, including bone mineral density (BMD), bone quality parameters and non-skeletal factors such as muscle strength, balance, cognition and cardiovascular function affecting the risk of falls (2). Each of these factors is itself under at least partial genetic control. BMD can be determined by dual energy X-ray absorptiometry (DXA) usually performed at the lumbar spine and hip. It was estimated that each standard deviation decrease in BMD from the age-adjusted mean was associated with a 2.3-fold increase in incidence of vertebral fractures and a 2.6-fold increase in hip fractures. Bone quality can be assessed by three-dimensional imaging modalities, such as peripheral computed tomography and high resolution magnetic resonance imaging, that allow to determine geometric parameters of the skeleton and to assess its microstructure (1).

GENETIC CONTROL OF BONE MINERAL DENSITY AND BONE FRACTURES

Growing evidence indicates that fracture risk is influenced by a combination of genetic and environmental factors. Several twin and family studies suggest that between 50 and 85% of the variance in peak bone mass is genetically determined, depending on skeletal site and the age of the subjects studied (3, 4). It is also presumed that genes contribute significantly to variability in aging-related bone loss and other determinants of fracture risk, including femoral neck geometry, muscle strength, bone turnover, body mass index and age at menopause (1).

It is probably a multi-gene relation and no single gene was found to prevail over others in the determination of BMD and fracture risk. Studies of post-menopausal women and their first-degree relatives as well as twin studies (5) showed that the heritability of forearm fractures was about 25-50%, of hip fractures approximately 50% and of vertebral fractures 24% (6-8). In contrast, heritability study of elderly twins from Finland showed little evidence to suggest that fractures were heritable (9). These divergent results may be explained by the fact that the heritability of fracture decreases with age as environmental factors become more important. It was demonstrated in a large study of Swedish twins that the heritability of hip fractures was as high as 68% among persons under the age of 65 but dropped off rapidly with age to reach a value of almost zero by the eighth decade (6). It has been also shown that genetic component of low muscle mass, increasing the susceptibility to falls, was over 50% (4).

THE METHODS OF GENETIC STUDIES

Early efforts to identify specific genes related to variation in BMD and fracture risk focused on identifying biologically motivated candidate genes and testing specific genotyped variants for association with BMD and/or fractures. The vitamin D receptor gene (VDR), the collagen type I alpha 1 gene (COLIA1) and estrogen receptor gene (ER) alpha have been most widely investigated and found to play a role in regulating BMD, but the effects were modest and together probably accounted for less than 5% of the heritable contribution to BMD. Candidate gene association studies are relatively easy to perform and a well validated method for the identification of genes responsible for monogenic diseases. However, they have low statistical power to detect genes having modest effects on BMD and fractures, and hence require family samples of several thousand people.

Demonstration of an association between a candidate gene and BMD or fractures does not necessarily mean that the gene is causally responsible for the effect observed, as there may be linkage disequilibrium with a nearby causal gene. Linkage disequilibrium refers to the phenomenon whereby genes lying close together tend to be inherited together (10).

With advances in genomic technology genome-wide association studies (GWAS) have been published. GWAS is an approach that involves scanning of the entire genome to identify novel genes with modest effects on complex diseases or traits. Array GWAS technologies are capable of analyzing thousands of polymorphisms distributed throughout the genome. Through the use of dense genotyping, large study samples, and replication studies to confirm results, these studies have led to the discovery of many genetic variants that have robust statistical evidence for association with various diseases. It has become possible to perform association studies on a genome-wide basis by analyzing a large number of closely spaced single-nucleotide polymorphisms (SNPs) spread randomly across the genome (11). The GWAS studies published to date disclosed more than 82 loci significantly associated with BMD, of which at least 16 were found to be associated also with fractures. The effect sizes of these loci are small, each accounting for less than 1% of the total variation in BMD (1, 12). Finding genes for fracture risk is likely to be more difficult than for BMD due to the complexity of the fracture phenotype. The vast majority of SNPs that have been associated with fracture have odds ratios for fracture of 1.11 or lower (13).

CANDIDATE GENES FOR LOW BMD AND FRACTURES

Candidate genes for osteoporosis were classified according to metabolic or hormonal pathways, which regulate BMD and bone quality, however, to date no gene has been definitively identified as a major gene.

A large collaborative study of more than 19,000 men and women identified 241 SNPs from 9 genes, which were significantly associated with lumbar spine BMD (230 SNPs), femoral neck BMD (100 SNPs), or both (89 SNPs). Among them 60 SNPs from 4 genes were also significantly associated with risk for fracture. The effect of these SNPs on fracture rate ranged between an odds ratio (OR) of 1.13 and 1.43 for the allele that was associated with decreased BMD. These results confirmed the highly polygenic nature underlying BMD variation and the role of several biological pathways influencing osteoporosis and fracture susceptibility (12, 14).

The Wnt/β-catenin signaling pathway, also called the canonical Wnt pathway, is crucially important for a variety of processes, including bone cell differentiation, proliferation, and apoptosis. Interactions of Wnt proteins with their receptors cause an accumulation of β-catenin in the cytoplasm and then in the nucleus where it participates in gene transcription. In the canonical Wnt pathway Wnt proteins bind Frizzled proteins and either lipoprotein receptor-related proteins 5 or 6 (LRP5 or LRP6). This results in the inhibition of glycogen synthase kinase 3-dependent phosphorylation of β-catenin, followed by the stabilization

of β-catenin (4, 10). Following the transfer of LRP and Frizzled proteins into the nucleus of the pre-osteoblast and binding to transcription factor TCFS, proliferation and differentiation of this cell is induced. The LRP5 pathway was discovered to be a key regulator of bone mass mainly by influence on osteoblast proliferation and bone matrix deposition (15, 16).

The Rotterdam Study, that included 2995 participants, revealed that SNP of the gene involved in osteoblast differentiation through activation of Wnt/β-catenin signals localized on chromosome 16q24, was significantly associated with an increased risk of vertebral fractures evident on the spinal radiographs. Compared to non-carriers, the heterozygous carriers of the minor allele (C) had the OR = 1.7 for vertebral fractures, and the homozygous carriers OR = 5.8. The vertebral fracture SNP was not associated with either lumbar spine or femoral neck BMD (17).

LRP5 and LRP6 genes have been implicated to play a role in bone metabolism, and LRP5 was thought to be important for the establishment of peak bone mass (15, 18). A study of 7983 inhabitants of Rotterdam, aged > 55 years, showed that in men, the Ala-1330Val polymorphism in the LRP5 gene was associated with significantly decreased BMD at the lumbar spine and femoral neck, reduced vertebral body size and femoral neck width, and a 60% increased risk for fragility fractures. Carriers of two risk alleles LRP5 1330Val and LRP6 1062Val had a 2.4 and 1.9 times higher risk for fragility and vertebral fractures, respectively, compared with men not carrying a risk allele. It was suggested that both SNPs account for one-tenth of the fracture cases in men, while in women carrying those risk alleles only nonsignificant trend for 30% higher risk for both fragility fractures and vertebral fractures was found (19).

Large multicenter study including more than 37,000 participants from Europe and North America showed, that the Val667Met (in the exon 9) and Ala1330Val (in the exon 18) polymorphisms of LRP5 gene were associated with an increased incidence of vertebral fractures by 26 and 12% respectively and an increased incidence of all fractures by 14 and 6% respectively. These data suggest that SNPs in LRP5 and LRP6 may affect the pathogenesis of osteoporosis through decreasing activity of the WNT/β-catenin pathway (20).

The RANK-RANKL-OPG pathway is an important regulator of bone resorption. It involves receptor activator of nuclear factor-κB (RANK) – expressed by osteoclasts and their precursors, its ligand (RANKL) produced by osteoblasts and encoded by the TNFRS-F11A gene, as well as osteoprotegerin (OPG) – the false soluble RANKL receptor encoded by TNFRSF11B gene. It was found that the presence of G allele due to 163A > G and 245T > G polymorphisms in the OPG gene was related to increased risk of osteoporosis in postmenopausal Chinese women (21). Similar relationship has been observed between the polymorphisms 1181G > C and 245T > G in the TNFRSF11B gene and BMD within the Caucasian population (22).

Vitamin D plays a crucial role in bone metabolism. Its active metabolite 1,25(OH)$_2$ vitamin D (calcitriol) interacts with specific receptors (VDR) to play an important role in calcium homeostasis by regulation of intestinal calcium absorption, renal calcium and phosphorus reabsorption, and parathyroid hormone secretion. Calcitriol stimulates differentiation of osteoblasts and stimulates synthesis of type I collagen.

Mutations in VDR gene, localized in chromosome 12q12-14, result in vitamin D-resistant rickets characterized by hypocalcemia, hypophosphatemia, and severe rickets resistant to treatment with vitamin D and its active metabolites. The results of studies on VDR in relation to bone mass have been conflicting but they suggested that polymorphism in VDR gene could account for as much as 75% of the heritability of bone mineral density (15). Some of the studies, however, showed no influence of the VDR genotype upon the BMD (23, 24). Special attention has been focused on polymorphisms of 4 loci within the VDR gene situated near the 3' flank of VDR recognized by the restriction enzymes BsmI, ApaI, TaqI and FokI. The study that included premature Mexican girls revealed the effect of the VDR gene on BMD of the lumbar spine (25). It was found that the effect of the TaqI, BsmI and ApaI polymorphisms of the VDR gene on BMD was strongest in premenopausal women and decreased with age (26). Evaluation of Polish population showed prevailing allele T of TaqI polymorphism in patients with osteoporosis (27). It was noted that patients with allele T had 2-3-fold higher risk of spinal fractures (28).

A retrospective meta-analysis concluded that association between spine BMD and the BsmI polymorphism is an equivalent to approximately 0.15 Z-score units between the BB genotype and the other genotypes (29). It was found that BB and B-containing genotypes of BsmI polymorphisms were associated with a 8.8 and 5.3-fold increase in risk of stress fractures compared to the bb genotype, respectively. The multivariate analysis revealed that the presence of homozygous ff (OR = 10.2), heterozygous Ff (OR = 3.7) and f-containing (OR = 4.1) genotypes of FokI polymorphisms remained independent risk factors of stress fractures. Genotype frequencies of TaqI and ApaI polymorphisms were found to be similar between healthy controls and patients with stress fractures (30). An analysis of Polish population showed that females with osteopenia and genotype AA in ApaI polymorphism of 283G > A manifest lower values of BMD in the lumbar spine (23).

A polymorphism in the promoter region of VDR at a binding site for the transcription factor Cdx-2 was found to be associated with BMD and with fractures (31, 32). It was documented that haplotype alleles in the promoter region and 3' untranslated region (UTR) were associated with an increased risk of

fracture, probably through an effect on bone geometry. For a subgroup of individuals who carried risk alleles at both sites, the fracture risk was significantly increased by 48% when compared with control subjects (33).

The classical function of vitamin D binding protein (DBP) is to store and prolong the half-life of circulating vitamin D metabolites. It was determined that DBP bound 88 and 85% of serum 25(OH)D and 1,25(OH)$_2$D, respectively and transported them to liver, kidney, bone, and other target tissues. The DBP variations were identified by two polymorphisms in exon 11: a substitution at codon 416G > A, leading to a Glu/Asp amino acid change, and a substitution at codon 420C > A, leading to a Thr/Lys amino acid change (34). In a prospective The Rotterdam Study that included more than 6,000 Caucasian individuals aged > 55 years these two SNPs in the DBP gene were genotyped. Haplotypes of the DBP SNPs corresponded to protein variations referred to as Gc1s, Gc2, and Gc1f haplotypes. It was found that Gc1s haplotype was associated with increased serum levels of 25(OH) vitamin D$_3$ (by 7.4 nmol/L) and 1,25(OH)$_2$ vitamin D$_3$ (by 8.4 pmol/L) while Gc2 haplotype was associated with decreased serum levels of 25(OH) vitamin D$_3$ (by 15.7 nmol/L) and 1,25(OH)$_2$D$_3$ (by 7.3 pmol/L). The DBP genotype was not associated with fracture risk in the entire study population but in the DBP haplotype Gc1s-carrier group, subjects of homozygous risk allele in the 3' untranslated region of the VDR gene had 33% increased fracture risk compared to noncarriers of this allele. In a subgroup of individuals with low dietary calcium intake (< 1.09 g/day) persons homozygous for DBP Gc1s haplotype had 47% increased risk of clinical fracture compared to noncarriers. It was concluded that genetic effect of the DBP gene on fracture risk was apparent if other genetic and environmental cofactors were present (33, 34).

Type I collagen is the major protein of bone. Mutations in the genes encoding the two chains that trimerize to form the procollagen 1 molecule: COL1A1 and COL1A2 cause the inborn disease 'osteogenesis imperfecta' (OI) which is a heterogeneous group of at least 17 monogenic disorders characterized by reduced BMD and multiple bone fractures (15). From clinical point of view the polymorphism of COL1A1 and/or COL1A2 is associated mainly with parameters of bone quality and interacts with BMD to enhance fracture prediction (10).

It was found that the COL1A1 gene predicted bone quality in osteoporotic subjects without OI. A polymorphism affecting the transcription factor Sp1 binding site in the first intron of *COLIA1* has been associated with decreased BMD, bone geometry, and fragility fractures. Individuals homozygous for this variant were found to have a 2-4-fold increased risk of fracture (3, 35, 36).

A prospective meta-analysis of over 20,000 participants of the GENOMOS study, as well as retrospective meta-analyses of published data have concluded that carriage of the T allele of the Sp1 polymorphism was associated with reduced BMD at the lumbar spine and femoral neck and increased risk of vertebral fractures (37-39). It was also shown that the carriers of combination of the alleles S and s in the Sp1 COL1A1 polymorphism in the Caucasian population had a 2.7 times higher risk of fracture than the carriers of the SS or ss genotypes (40).

The estrogen receptor α, one of the key mediators of hormonal response in estrogen-sensitive tissues, is encoded by the ESR1 gene located on chromosome 6q25. It was found that estrogens inhibited bone resorption through the ER1 (ERα) and ER2 (ERβ) receptors in bone, or via the inhibition of osteoresorptive cytokines such as interleukin-1 and TNFα. Most of studies that have looked for evidence of an association between ESR1 alleles and BMD and bone fractures, have been focusing on polymorphisms recognized by the XbaI and PvuII restriction enzymes, and on a TA repeat in the promoter (28, 41).

A meta-analysis of 22 studies involving 5,000 women revealed that the XX genotype of the XbaI polymorphism was associated with a high values of BMD at the spine and the femoral neck, compared to carriers of the x allele (15, 42). Another meta-analysis of published results showed evidence of an association between the XbaI polymorphisms and both BMD and fractures, with higher BMD values and a reduced risk of fractures in "XX" homozygotes (43). An assessment of data from over 18,000 subjects in the GENOMOS study confirmed that "XX" homozygotes had a reduced risk of fracture which did not always correlate with BMD, indicating that ESR1 may influence bone fracture risk independent of an effect on BMD (15, 44). A meta-analysis that investigated the associations between both common ESR1 polymorphisms PvuII 693C > T and XbaI 799A > G and hip fracture, including a total of 1,838 hip fracture cases and 14,972 healthy controls revealed that the PvuII T allele is a highly significant risk factor for hip fractures susceptibility in Caucasian, but not in Asian populations (15).

Apolipoprotein E (apoE) is a surface component of chylomicrons, very-low-density lipoproteins, and some subclasses of high-density lipoproteins. Recently the importance of apoE as a regulator of bone metabolism has been underlined. The APOE gene is polymorphic, consisting of three common alleles: E2, E3, and E4.

ApoE (E2) allele has been identified as a potential genetic risk factor for low trabecular bone mass and vertebral fractures in humans (45-47). A hospital-based study conducted in 3,000 patients with fractures and 3,000 age- and gender-matched healthy controls revealed that patients with fractures had a significantly higher frequency of apoE E2/E2 genotype with OR = 2.0 than healthy controls. When stratifying by fracture type, it was found that patients with vertebral

fractures had a significantly higher frequency of apoE E2/E2 genotype with OR = 2.86, while no significant differences were found in nonvertebral fractures. Studies of Japanese older adults found that the APOE E4 allele was associated with a low bone mass and increased risk for hip fractures (48, 49).

Members of the TGF-β superfamily control critical cellular functions. Transforming growth factor β1 (TGF-β1), which is encoded by the TGF-β1 gene, has been shown to have effects on both osteoblast and osteoclast function *in vitro* (50). Polymorphisms within intron 4 of the TGF-β1 gene were associated with severe osteoporosis. An association between a different polymorphisms in intron 5, in the promoter and first exon of TGF-β1 and BMD were also reported (3, 51, 52).

Polymorphic variation in bone morphogenic proteins (BMPs), that are part of the TGF-β superfamily of molecules, may be involved in regulation of bone mass and susceptibility to osteoporosis. A coding polymorphism identified at codon 37 of the BMP2 protein, resulting in a substitution of alanine for a serine residue, was found to be overrepresented in Icelandic and Danish patients with osteoporosis (53). It was also reported that common polymorphism, which causes an alanine-to-valine substitution at position 152 in the BMP4 protein, was associated with low BMD values (54).

An elevated homocysteine level, related to folate, vitamin B_{12}, and vitamin B_6 status, has been recognized as a significant risk factor for fractures, independent of age and BMD, as it is connected with abnormal collagen cross-linking during bone formation (4).

Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme involved in the removal of circulating homocysteine. The gene for MTHFR lies within a linkage region on chromosome 1p36 that is associated with the regulation of BMD (55, 56). A 677C > T polymorphism has been identified in exon 4 of MTHFR but its role in BMD remains controversial (4). In Japanese postmenopausal women, the MTHFR 677C > T polymorphism alone was found to be a weaker risk factor for future fractures compared to traditional risk factors but patients bearing the TT genotype together with low BMD show a higher risk and earlier occurrence of fractures than other groups, indicating that the MTHFR TT genotype may act synergistically with traditional risk factors that lead to fractures (57). In a senior population of twins, the association between 677C > T polymorphism and risk of fractures was 1.5 times higher in the CT genotype compared with the CC genotype, and 1.5 times higher in the TT genotype compared to the CT genotype, indicating that risk allele for fractures is the T allele (58). Similar results have been published by Villadsen et al., who demonstrated that the rare TT genotype was associated with an increased risk of osteoporotic fractures in women (59). Another report in the Caucasian popula-

tion showed that the risk of hip fractures in patients bearing the TT genotype was additionally increased when their plasma folate levels were low (60).

Paraoxonase 1 (PON1), a product of the PON1 gene, is a glycoprotein closely associated with high-density lipoproteins (HDL) in serum that contributes to the antioxidant effect of HDL, prevents the oxidation of low-density lipoprotein (LDL), and metabolizes biologically active phospholipids in oxidized LDLs (61).

Increased lipid oxidation causes oxidative stress and reduces Wnt signaling, thereby decreasing the differentiation and survival of osteoblasts. Oxidized lipids increase adipogenesis of marrow stromal cells at the expense of their osteogenic differentiation and induce osteoclastic differentiation via a cAMP-mediated pathway. In a population of Korean postmenopausal women analysis of PON1 gene polymorphisms identified 26 SNPs. None of the polymorphisms was associated with BMD, but polymorphism 5989A > G exerted a highly protective effect against non-vertebral fractures (OR = 0.59), while the minor allele of 26080T > C was associated with increased susceptibility to vertebral fractures (OR = 1.73). The exact mechanism by which the SNPs influence bone fracture risk independently of BMD in postmenopausal women is not clear. It was suggested that these polymorphisms may be markers that lie close to or in linkage disequilibrium with other functional genes (62).

CONCLUSIONS

Susceptibility to osteoporosis is mediated, in all likelihood, by multiple genes each having small effect. Osteoporosis is a polygenic disease, wherein each bone phenotype, such as density, quality, and metabolic rate is the result of interaction among many weak genes. The "essential gene" responsible for the manifestation of osteoporosis has not been identified, despite utilizing the most advanced methods. Additionally, novel methods, such as sequenations (both sides sequenation), multiple ligand probe analysis (MLPA), single strain conformation polymorphism analysis (SSCP), and fluorescent in situ hybridization (FISH) are expected to expedite progress in this research (15).

The effects of epigenetic factors should also be considered, as they modulate the relationships between the reference gene and phenotype. Epigenetic factors do not modify the DNA sequence itself, but rather gene expression. The most known epigenetic mechanisms are DNA methylation and histone modifications, which act at the level of gene transcription, and microRNAs, which act at the post-transcriptional level. Recent evidence suggests that methylation-dependent mechanisms may influence the transcription of RANKL and OPG expression (1).

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