

Genetic aspects of osteoporosis

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Abstract The multiple factors contributing to the pathogenesis of osteoporosis include genetic and environmental factors. Because decrease in bone mineral density (BMD) is the major clinical indicator and a useful quantitative trait, many association and linkage studies of BMD have been conducted. Although the series of studies showed apparently significant associations, the genes have not been found that can be utilized in clinical practice. Several genes identified in robust genome-wide association studies will be the new cutting edge in genetic studies of osteoporosis. Our recent reports of functional single nucleotide polymorphism in the tissue-nonspecific alkaline phosphatase gene and gamma-carboxylase gene are presented in this review to discuss the future prospects in the genetic research of osteoporosis from the point of view of genome–nutrition interaction.

Keywords Osteoporosis · Single nucleotide polymorphism · Bone mineral density · Genetics

Introduction

Osteoporosis brings about deterioration in activities of daily living (ADL) and quality of life (QOL) of the

affected patients. Although fragility fractures resulting from osteoporosis continue to increase in the current aging society, it is assumed that this disease is still undertreated [1]. In the 1990s, osteoporosis was defined as a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk [2]. This definition reflects the importance of bone mass and microarchitecture in determining bone strength. Because there have not been practical measures of microarchitecture, bone mass or bone mineral density (BMD) has been used as a quantitative trait in searching the genes for osteoporosis. It seems reasonable that a vast series of association and linkage studies have been conducted with BMD, but one should keep in mind that BMD is one of the complex traits of osteoporosis and one of the surrogate markers for bone fragility.

Recently, osteoporosis was redefined as a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture [3]. In the previous definition of osteoporosis, low BMD was not considered as a sole factor of osteoporosis, but the new definition declares more clearly that bone strength is determined not only by BMD but also by factors other than BMD [3]. According to the new definition, the genes of osteoporosis should be a group of genes contributing to the multiple aspects of pathogenesis. Although case–control studies by defining the case with the diagnostic criteria of this disease are suggested, the diagnosis of osteoporosis might not be suitable as a “phenotype” in genetic studies because the diagnosis contains biologically heterogeneous components. In this review, genetic aspects of osteoporosis are discussed mainly using BMD as one of the measurable phenotypes of osteoporosis.

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Genetic aspects of bone phenotypes

Predisposing factors of osteoporosis include both lifestyle factors and genetic factors. The first step in preventing osteoporosis should be the reduction of lifestyle-related risk factors. On the other hand, the genetic factors cannot be removed even when these are identified. However, it will be useful if one can learn that she or he has the genetically predisposing factor(s) and is thus motivated to avoid certain lifestyle risk factors.

Family history of fractures is included among the established risk factors for osteoporotic fractures [4], indicating the importance of the genetic background of osteoporosis. Twin studies also supported the heritability of BMD [5], which is the most valuable indicator of bone strength. On the other hand, it was reported that the possibility that the genetic determinants of BMD and those of fractures might be different [6]. Factors contributing to the variation in bone quality will be studied further from genetic aspects. For example, femoral neck cross-sectional geometry was successfully used as a clinical measure in quantitative trait locus analyses [7].

Approaches for the pathogenesis of diseases can be classified as deductive or inductive. Analyses about the roles of known substances or genes would be classified into deductive approaches and belong to the genetic approach. On the other hand, the recent availability of whole genome information has made the inductive approach possible, which is named a genome-wide association study (GWAS). In this mini-review, the recent genetic and genomic approaches for osteoporosis are reviewed, and our studies on the functional single-nucleotide polymorphisms (SNPs) related to osteoporosis are introduced.

Candidate gene approaches to the determinants of bone mineral density

Until now, BMD has been utilized most widely as a quantitative measure in genetic and genomic studies for osteoporosis. Quite a few association studies with BMD have been done with so-called candidate gene approaches [8]. Candidate genes have been chosen based on basic bone cell biology and clinical observations. In addition, the genome-wide linkage and association studies will show novel series of candidate genes that should be investigated further.

Association studies with the polymorphisms of these genes were done using genetic polymorphisms. Among the polymorphisms, SNPs were most commonly utilized. SNPs in the regulatory region (rSNPs) and those in coding regions (cSNPs) could be related to quantitative or qualitative variations of the gene expressions. In addition, other

SNPs, for example, those in introns, could affect the gene expression or could be markers for genomic study. Microsatellite polymorphisms (e.g., dinucleotide repeat or triple repeat) are other kinds of polymorphisms that have also been utilized in osteoporosis research.

Association studies with candidate gene polymorphisms have been published by many groups including ours [9–40]. If you search the database using the key words “gene polymorphisms and bone mineral density,” 1,000 and more articles will be hit. The genes analyzed are classified into nuclear receptors and related molecules, collagen and other matrix proteins, receptor activator of nuclear factor-kappa B ligand (RANKL)/RANK system, cytokines and related molecules, hormones and related molecules, enzymes, cell cycle-related molecules, lipoprotein receptor-related peptides (LRPs) and Wnt signals, cell-surface molecules, transcription factors, and others (Table 1). However, the contribution of most genes to determining BMD is small and the result is not always reproducible [41, 42]. Lifestyle-related factors as confounding factors against genetic factors should be managed in the association studies. In addition, ethnic factors have to be considered appropriately [43].

The vitamin D receptor gene has been studied most extensively, but the implications of vitamin D receptor gene polymorphisms have not been established [44]. Recent extensive meta-analyses [45] showed that the effects of the vitamin D receptor gene polymorphisms seem modest, although the significant effects of the polymorphisms on BMD and osteoporotic fractures were proved.

Searches for functional SNPs affecting variation in bone metabolism

When polymorphisms of genes were significantly and reproducibly associated with bone phenotypes, biological relevancy should be confirmed, and the methods of clinical application should be considered following that process. In other words, it would be a rational method in the genetic approach for osteoporosis to examine the association of functional polymorphisms with bone phenotypes. Although the contribution of each polymorphism to BMD would be small, the significant effects of each polymorphism supported by functional studies will be a clue suggesting that the gene should play important roles in the pathogenesis of osteoporosis.

We reported two functional SNPs in two genes that are related to the variation in BMD of the elderly. The first one was an SNP in the tissue-nonspecific alkaline phosphatase (TNSALP) gene [46]. TNSALP resides in the plasma membrane of osteoblasts and supplies phosphate to the

Table 1 Genes studied in candidate gene approaches for bone mineral density (BMD)

Nuclear receptors and related molecules
Vitamin D receptor
Estrogen receptor- α
Estrogen receptor- β
Androgen receptor
Glucocorticoid receptor
Peroxisome activator receptor- γ
Nuclear receptor co-activator-3
Er α co-factor retinoblastoma-interacting zinc finger protein
Collagen and other matrix protein
Type I collagen- α 1
Type I collagen- α 2
Osteocalcin
Matrix gla protein
Alpha 2-HS glycoprotein
RANKL/RANK system
RANKL
RANK
Osteonectin/SPARC
Cytokines and related molecules
Transforming growth factor- β 1
Insulin-like growth factor-1
Tissue necrosis factor- α
TNFRSF1B
TNFRSF11B
TNF receptor-associated factor-6
Bone morphogenetic protein-2
Bone morphogenetic protein-4
LTBP3
Interleukin-6
Interleukin-1
Interleukin-1 receptor antagonist
Interleukin-1 β
Interleukin-10
Tissue necrosis factor
Tissue necrosis factor receptor
Smad 6
TGF- β receptor-3
Adiponectin
Myostatin
Hormones and related molecules
Calcitonin
Calcitonin receptor
Thyroid hormone receptor
TSH receptor
Calcium-sensing receptor
PTH
PTH/PTHrP receptor
Dopamine receptor D4

Table 1 continued

Prepro-NPY
Growth hormone
Growth hormone receptor
POMC
Cannabinoid receptor type 2
Leptin receptor
β 3-adrenergic receptor
Vitamin D-binding protein
SHBG
Enzymes
Adenyl cyclase
Methylene tetrahydrofolate reductase
Methionine synthase
Catalase
Farnesyl diphosphate synthase
Farnesyl pyrophosphate synthase
FMS-related tyrosine kinase
Aromatase
p450
Carbon anhydrase
Angiotensin-converting enzyme
CYP1A1
CYP1B1
CYP3A7
CYP3A4*18
CYP17
CYT19
COMT
eNOS
GGCX
Urokinase
PAI-1
ALDH2
Pituitary glutamyl cyclase
Phosphodiesterase 40
Tissue-nonspecific alkaline phosphatase
CYP1A1
ALOX15
ALOX12
Lactase
Paraoxonase
Procollagen-lysine, 2-oxoglutarate 5-dioxygenase
Rho GTPase-Rho REF
WRN
Matrix metalloproteinase-1
Cathepsin K
Mature metalloproteinase-9
Delta-aminolevulinic dehydrogenase
Uridine diphosphate glucuronyl transferase 2B7

Table 1 continued

Cell cycle-related molecules
p57
Cdx-2
Cyclin D1
CD38
LRPs and Wnt signals
LRP5
LRP6
WISP 1
FZD 1
“multiple Wnt pathway genes”
SOST
Cell-surface molecules
Duffy antigen receptor
Toll-like receptor 4
GALR3 receptor
CC domain receptor 2
CD38
CD40
CLCN7 (chloride channel)
Osteoclast-associated receptor
Vascular proton pump
Purinergic P2RX7 receptor
Semaphorin 7
GALR3 receptor
Transcription factors
RUNX2/CBRA1
Microphthalmia-associated transcription factor
Forkhead box C2
Others
Perilipin

calcification site. We searched for nonsynonymous and functional SNPs in the exons of this gene. As a result, an SNP in exon 7 (787C > T), which replaces tyrosine at codon 246 to histidine, gives the biochemical differences between the products of each genotype. The K_m value of 787 His was smaller than that of 778 Tyr, which means that persons with 787 His may supply phosphate to the calcification site more efficiently. Elderly Japanese women with 787 His had higher radial BMD than those with other genotypes. This study demonstrated the importance of phosphate metabolism in bone metabolism in the elderly. Additional *in vitro* experiments supported the biochemical variations resulting from this polymorphism [47, 48]. Further studies are underway to examine the clinical meaning of this variation, for example, the effects of this genotype on the relationship between phosphate intake and hormones in calcium metabolism and aging.

Another gene is vitamin K-dependent gamma-glutamyl carboxylase (GGCX) [49]. GGCX carboxylates vitamin K-dependent proteins including bone Gla protein (osteocalcin) and matrix Gla protein. Functional polymorphisms in the GGCX gene, if any, might explain the variation in bone metabolism and BMD. Also in this case, polymorphisms in the exons were screened in Japanese elderly women and a nonsynonymous SNPs was found: about 8762 G > A (Arg325Gln). When the kinetic parameters of GGCX325-Gln and GGCX325-Arg were compared *in vitro*, V_{max}/K_m was significantly higher for GGCX325-Gln than for GGCX325-Arg. Association study of this polymorphism with radial BMD of Japanese postmenopausal women showed that the body mass index (BMI)-adjusted Z score in the subpopulation older than 75 years was higher in those with 325 Gln than those with 325 Arg/Gln or 325 Arg. In this study, we first reported the different activities of GGCX between the common genotypes and their association with BMD. Vitamin K deficiency is known as a nutritional risk factor for osteoporotic fractures, and a regimen of vitamin K₂ is utilized for osteoporosis treatment. The common allelic variation in the GGCX gene may explain the individual variation in the response to nutritional and/or pharmacological intervention with vitamin K. It would be rational to utilize the allele information in finding the level of vitamin K intake at which the effects of the genotype with lower enzymatic activity can be avoided. We have already reported that this GGCX gene polymorphism affects the correlation between the vitamin K status and gamma-carboxylation of osteocalcin in young males [50], and this kind of study is awaited in the group of elderly.

Implication from monogenic bone diseases

There are rare diseases involving bone that are caused by mutations of single genes and considered to be monogenic diseases. The causative genes of these diseases were identified by linkage analyses of the affected families. These genes would have important implications for the variations of bone phenotype also in the general population. A distinguished example is the gene for osteoporosis-pseudoglioma syndrome (OPPG) [51]. Positional cloning with the affected pedigrees showed that rare mutations in lipoprotein receptor-related peptide 5 (LRP5) gene cause the disease. In addition, another mutation in the same gene was demonstrated to cause a syndrome with high BMD [52]. It is also interesting that the LRP5 gene resides in the locus that has been among the loci related to BMD in the linkage studies [53]. Several groups including ours examined the relationship between the polymorphisms of LRP5 gene and BMD, and the results were reproducible [54–56].

Table 2 Candidate genes suggested by genome-wide association studies

Genes	Chromosome location
ADAMTS18 (ADAM metalloproteinase with thrombospondin type 1 motif, 18)	16q23
TGFBR3 (transforming growth factor-beta receptor III)	1p33–32
TNFRSF11B (tumor necrosis factor receptor superfamily, member 11b, osteoprotegerin)	8q24
LRP5 (lipoprotein receptor-related protein 5)	11q13.4
Receptor activator of nuclear factor-kappa B ligand (RANKL)	13q14
Osteoprotegerin (OPG)	8q24
Estrogen receptor-1 gene (ESR1)	6q25.1
Zinc finger and BTB domain containing 40 genes (ZBTB40)	1q36
Major histocompatibility complex region	6p21

In addition, the LRP5 gene was screened out by the recent GWAS, as mentioned below [57]. These results strongly suggest that variations in this gene would contribute to the variation of BMD in the general population.

Genes suggested by genome-wide association studies

Systematic search for the genes for osteoporosis has been done by genome-wide linkage studies with pedigrees, which have shown some hotspots linked to BMD, for example, those on chromosome 11 [53]. Further fine mappings were required to specify the genes contributing to the pathophysiology of osteoporosis and consequent analyses of their functions in bone biology. Recent advances in analyzing SNPs distributing to the whole genome area made it possible to conduct a GWAS (Table 2). One of the GWAS studies identified two SNPs, rs4355801 on chromosome 8 and rs3736228 on chromosome 11 [57]. The former is close to the osteoprotegerin gene and the latter nonsynonymous SNP is in the LRP5 gene, both of which are major components in bone biology. In the series of candidate gene approach, the significant correlation between the polymorphisms in LRP5 gene has been reproducible. The identification of the LRP5 gene in the GWAS study further strengthens the importance of this gene in the pathogenesis of osteoporosis. Osteoprotegerin was also identified to be correlated with BMD in another GWAS study [58].

Other examples of genes identified in GWAS studies are RANKL [58], estrogen receptor 1 (ESR1) [58], ADAM metalloproteinase with thrombospondin type 1 motif, 18 (ADAMTS18) [59], and transforming growth factor-beta receptor III (TGFBR3) [59].

Prevention of osteoporotic fractures is the major clinical goal of osteoporosis therapy, and the incidence of osteoporotic fractures should be an ideal phenotype used in the genetic studies searching the genes for osteoporosis.

Recently, Kung et al. [60] reported the association of the JAG1 gene with osteoporotic fractures as well as BMD with GWAS study. They also demonstrated the possible molecular mechanism with which the genetic variation of this gene affects bone metabolism [60].

Discussion

Selection of candidate genes for polymorphism studies of osteoporosis is rather arbitrary. This situation cannot be avoided because we do not know how many genes are involved in the pathogenesis of osteoporosis or in the determination of BMD. Recent genome-wide studies with a large population size are successfully overcoming this issue, and several genes were identified for osteoporosis. These genes include the novel series of candidate genes whose implications should be studied. So far the new list of genes contains “previous” candidate genes that are well known in the field of bone biology.

BMD is a surrogate marker for bone fragility, and one should not consider the genes for low BMD as immediately being those for osteoporosis. Although BMD is still a useful quantitative measure in genetic studies for osteoporosis, other phenotypes, particularly the incidence of fractures, should be kept in mind. Further studies are required to utilize the products of genetic studies for the advancement of osteoporosis practice.

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