Review

Quantitative trait loci, genes, and polymorphisms that regulate bone mineral density in mouse

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Abstract

This is an in silico analysis of data available from genome-wide scans. Through analysis of QTL, genes and polymorphisms that regulate BMD, we identified 82 BMD QTL, 191 BMD-associated (BMDA) genes, and 83 genes containing known BMD-associated polymorphisms (BMDAP). The catalogue of all BMDA/BMDAP genes and relevant literatures are provided. In total, there are substantially more BMDA/BMDAP genes in regions of the genome where QTL have been identified than in non-QTL regions. Among 191 BMDA genes and 83 BMDAP genes, 133 and 58 are localized in QTL regions, respectively. The difference was still noticeable for the chromosome distribution of these genes between QTL and non-QTL regions. These results have allowed us to generate an integrative profile of QTL, genes, polymorphisms that determine BMD. These data could facilitate more rapid and comprehensive identification of causal genes underlying the determination of BMD in mouse and provide new insights into how BMD is regulated in humans.

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Introduction

Osteoporosis is a multifactorial bone disease affecting millions of people around the world. One of the major determinants of risk for bone fracture in individuals with osteoporosis is bone mineral density (BMD). BMD is a quantitative trait regulated by complex interactions of genetic and environmental factors [1]. BMD is largely inheritable with twin and family studies showing that genetic factors may account for 50–80% of the variance in BMD [2–4]. Accumulating evidence indicated that BMD is under the control of multiple genes, each with modest effects [3–5]. In order to determine the genetic components underlying BMD variation, many investigators have performed breeding studies to identify quantitative trait loci (QTL). Using mouse models they have established the location of a large number of QTL. However, the identification of the genes responsible for these QTL remains a major challenge [6]. Since standard QTL mapping results in identification of large regions which may include up to hundreds of genes, it has proven difficult to establish which genes are responsible for the QTL from such large segments of chromosomes (Chr). One approach to this problem is to generate congenic sublines to reduce the size of the linked region [7]. Because this is an expensive and time consuming approach, only few genes have been identified as causal genes of BMD QTL [8–11].

Decades of research on molecular biology and genetics has accumulated tremendous amount of data related to gene function. The completion of the mouse genome sequence and advances in genome annotation has shown that many genes are associated with BMD in a variety of populations or species. However, it is still a challenge to integrate those data from gene function analysis into a comprehensive understanding of how genes are related to BMD. In this review, we systematically examine the possible involvement of every known gene in BMD regulation over the whole mouse genome according to the literature information from PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) and Online Mendelian Inheritance in Man (OMIM, http://www.ncbi.nlm.nih.gov/omim/), as well as the information from Mammalian Phenotype Ontology (http://www.informatics.jax.org/searches/MP_form.shtml) and Gene Ontology (http://www.informatics.jax.org/searches/GO_form.shtml). We have established the distribution of BMD-associated (BMDA) genes and those genes containing known BMD-associated polymorphisms (BMDAP) within regions of the chromosome where QTL have been identified. Those data permits an alternative approach to determination of the genes which are the most important candidates for regulating BMD and the association of these genes with BMD QTL. Our aim was to bridge the gap between QTL analysis and genome annotation, and generate an integrative profile of QTL genes and polymorphisms that regulate BMD over whole mouse genome. These data will be a valuable source for BMD QTL studies, and will facilitate more rapid and comprehensive identification of genes underlying BMD QTL.

Materials and methods

Identification of QTL

A literature search using PubMed was conducted with key words “Bone” and “QTL” for every publication up to January 2008. A total of 82 BMD QTL were identified (Table 1). For well-defined QTL, the flanking markers provided by the authors were used to establish the limits of the QTL region. For other QTL, the 2-LOD support interval, the chromosomal region in which the QTL is located with a confidence of ~95%, was used to establish the limits of the QTL region. For those QTL in which neither flank markers nor information of 2-LOD support interval was not available, a genomic region containing 20 mega base pairs (Mbp) on each side of the peak marker was considered as the QTL region. We assumed that 20 Mbp on each side of the peak marker would be adequate to encompass the underlying genes or polymorphisms responsible for the QTL. Several QTL have been dissected into multiple linked sub-loci. For the purposes of our analysis, we list those sub-loci as independent QTL to allow investigators to better compare and evaluate them. It is possible that our search method was insufficient to collect every relevant publication or QTL.

Identification of genes

We systematically evaluated the potential involvement of every gene in BMD variation over the whole mouse genome with all available reports in PubMed, OMIM, Mammalian Phenotype Ontology, and Gene Ontology. To accomplish this, we obtained the genes for every chromosome and QTL from the Ensembl database (Release 48); then we searched PubMed and OMIM to get a preliminary list of candidate genes associated with BMD. The search terms were the combination of the symbol of the gene and BMD/bone mineral density. We performed the searching using PGMapper, software which we have recently developed [12]. Next, we obtained another preliminary list of candidate genes by searching Mammalian Phenotype Ontology and Gene Ontology with key words “abnormal bone mineralization” and “bone mineralization”, respectively. Finally we hand curated the associated literature to determine the actual connection between those preliminary candidate genes and BMD. We considered a gene to be a BMDA gene if it was associated with BMD by at least one of the following criteria: 1) established by functional studies such as knockouts, transgenics, mutagenesis, RNA interference, etc.; 2) identified in association studies; and 3) identified in clinical studies. Many BMDA genes were identified in human studies. We included the mouse homologues of these human genes in
<table>
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<tr>
<th>QTL</th>
<th>Chr</th>
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our analyses since we assumed that the homologs would have similar functions in mice.

### Distribution of BMDA genes between QTL regions and non-QTL regions

We identified 191 BMDA genes within the whole mouse genome. Among them, 133 (approximately 70%) were located in regions known to contain QTL for BMD. The catalogue of all BMDA genes and relevant literature establishing their candidacy can be found in Supplemental Table 1. In total, there are substantially more BMDA genes in QTL regions than in non-QTL regions. To investigate if this is also true at the chromosomal level, we examined the distribution of the BMDA genes for every chromosome. Fig. 1 shows the distribution of these genes between QTL and non-QTL regions for each chromosome. We found that for most chromosomes there were substantially more BMDA genes in QTL regions compared to non-QTL regions confirming our whole chromosome analysis. The QTL regions included all known BMDA genes on Chr2, Chr4, Chr11, Chr12, and Chr18. In addition there were more BMDA genes in QTL than in non-QTL regions on 10 chromosomes, including Chr1, Chr5, Chr6, Chr7, Chr10, Chr13, Chr14, Chr15, Chr16, and Chr17. There was no difference for Chr3 and Chr9. No QTL have been identified on Chr8 and ChrY. Only two chromosomes, namely Chr19 and ChrX, included fewer BMDA genes in QTL regions as compared to non-QTL regions.

### Distribution of BMDAP genes between QTL regions and non-QTL regions

We identified 83 genes containing BMD-associated polymorphisms (BMDAP) either in coding or regulatory regions, most of which fell within regions of the genome containing known QTL. Among 83 BMDAP genes, 58 (approximately 70%) were located in QTL regions, a proportion similar to that of BMDA genes. We also examined the distribution of these genes between QTL and non-QTL regions for each chromosome (Fig. 2). The list of all BMDAP genes and the references establishing their candidacy can be found in Supplemental Table 1. Consistent with the results for BMDA genes, there are substantially more BMDAP genes in QTL regions than in non-QTL regions on most chromosomes. On Chr1, Chr2, Chr4, Chr11, Chr12, Chr15, and Chr16, all BMDAP genes are located within QTL regions. More BMDAP genes are found in QTL regions than in non-QTL regions on Chr5, Chr6, Chr9,
Hwang et al. [28] These investigators selected 10 polymorphisms in PLXNA2 within lower than in individuals bearing other genotypes. Polymorphisms for association studies in postmenopausal women. The results PLXNA2 +34863G N individuals bearing a minor homozygous genotype (A/A of with the BMD of the lumbar spine (LS). The levels of BMD in strain, namely C3H/HeJ (C3H), Edderkouki et al. [8] found that the six (B6) and high BMD CAST/EiJ (CAST) mice with another high BMD phenotype exhibited by CAST and C3H mice. Further functional suggesting that one or more of these SNPs could contribute to the high SNPs in the coding region were conserved in both CAST and C3H, chromosomes that have no known BMD QTL, only four chromosomes, namely Chr3, Chr8, Chr19, and ChrX, have fewer BMDAP genes in QTL regions compared to non-QTL regions.

Genes containing known BMD-associated polymorphisms

**Chromosome 1**

Chromosome 1 contains 15 BMD QTL (Table 1) [13,14,21,26,27,29–32], of which Bmd5 has been later dissected into several sub-loci by two independent investigations [26,29]. Five genes containing polymorphisms associated with BMD are located on this chromosome: (1) Duffy antigen receptor for chemokines (Darc), (2) receptor activator of NF-kappa-B (Rank, also named Tnfrsf11a), (3) plexin A2 (Plxna2), (4) parathyroid hormone type 1 receptor (Pthr1), and (5) interleukin 10 (Il10). Most QTL harbor an important BMDAP gene, Darc, which has been identified as a QTL gene underlying QTL BMD1–2. By comparing the Darc gene sequence of low BMD C57BL/6j (B6) and high BMD CAST/Ej (CAST) mice with another high BMD strain, namely C3H/HeJ (C3H), Edderkouki et al. [8] found that the six SNPs in the coding region were conserved in both CAST and C3H, suggesting that one or more of these SNPs could contribute to the high BMD phenotype exhibited by CAST and C3H mice. Further functional analysis indicated that Darc is a negative regulator of osteoclasts and that bone resorption is reduced in the absence of Darc, resulting in increased BMD. Another two BMDAP genes within QTL regions are Rank and Plxna2. In a large-scale study of postmenopausal women, Koh et al. [15] found that the +34863G>A and +35928insdelC polymorphisms in intron 6 of RANK gene were significantly associated with the BMD of the lumbar spine (LS). The levels of BMD in individuals bearing a minor homozygous genotype (A/A of +34863G>A and del/del of +35928Cinsdel) were significantly lower than in individuals bearing other genotypes. Polymorphisms within PLXNA2 have also been associated with BMD abnormalities by Hwang et al. [28] These investigators selected 10 polymorphisms in PLXNA2 for association studies in postmenopausal women. The results revealed that the subjects carrying the minor homozygote genotype (AA) of +14G>A tended to have higher LS BMD compared with those carrying the major homozygote alleles or heterozygote alleles, while the subjects carrying the minor homozygote genotype (TT) of +183429C>T tended to have lower LS BMD.

Two BMDAP genes, Pthr1 and Il10, are located outside of QTL region. PTHR1 has been associated with BMD by Scillitani et al. [33] who demonstrated that the subjects bearing at least one (AAAC)6 allele in the P3 promoter of PTHR1 gene have a higher femoral neck (FN) BMD than those without, suggesting the variation in promoter activity of the PTHR1 gene may exert a relevant genetic influence on BMD. IL10 has been found to be associated with BMD by Park et al. [16]. The investigators studied the possible associations of genetic variants in five-candidate genes with spinal BMD. Among them, IL10 −592A>C and/or IL10 ht2 were associated with decreased bone mass. The levels of spinal BMD in individuals bearing the IL10 −592CC genotype were lower than those in others, and the BMD of IL10 ht2 bearing individuals were also lower than those in others.

**Chromosome 2**

Eleven BMD QTL have been identified on Chromosome 2 (Table 1) [21,30–32,42,46]. A total of six BMDAP genes were found: (1) bone morphogenetic protein 2 (Bmp2), (2) tumour necrosis factor receptor-associated factor 6 (Traf6), (3) matrix metalloproteinase-9 (Mmp9), (4) pyroglutamylated RFamide peptide (Qrfp), (5) Catalase (Cat), and (6) nuclear receptor coactivator-3 (Ncoa3). All of the BMDAP genes on this chromosome are contained within QTL regions. BMP2 has also been implicated to be a major risk factor for osteoporosis as well as low BMD in an association analysis [50]. Three variants of BMP2 gene, a missense polymorphism and two anonymous single nucleotide polymorphism haplotypes, have been associated with osteoporosis in the Icelandic patients. In another cross-sectional study, significant associations were observed between BMP2 c.584G>A, c.893T>A, c.893T>G, and c.893T>C polymorphisms in BMD for males at the calcaneus as well as females at the distal radius. Men with BMP2 c.893 AA genotype had a 16% higher BMD at the calcaneus, whereas women with this genotype had a 7% lower BMD at the distal radius than the other
genotypes. In addition, the AAGA haplotype of BMDP2 was significantly associated with low bone mass in female distal radius [49].

Yamada et al. [40] examined the association of BMD with a −1562C→T polymorphism in the promoter of the MMP9. BMD at various sites was significantly lower in the combined group of men with the CT or TT genotypes or in men with the CT genotype than in those with the CC genotype. However, no significant differences in BMD among MMP9 genotypes were observed in premenopausal or postmenopausal women. These data may indicate −1562C→T polymorphism influences on BMD variation in a sex-specific fashion.

**Chromosome 3**

Two BMD QTL are located on Chromosome 3 (Table 1) [13,46]. Only one BMDAP gene in non-QTL region was found. C/A polymorphism (rs2257480) in the promoter of Farnesyl diphosphate synthase (FDPS) has been associated with BMD in postmenopausal elderly women. The presence of C allele contributes to significant reductions in bone mineral density. The majority of skeletal sites showed the lowest BMD with the CC and CA genotypes and the highest BMD with the AA genotype. In silico analysis of this polymorphism reveals that the A allele may create a binding site for Runx1, which may decrease osteoclast activity by inhibiting FDPS transcription [182].

**Chromosome 4**

Chromosome 4 contains five BMD QTL (Table 1) [13,21,30,31,72]. We identified 7 BMDAP genes and all of them are located within QTL regions, including: (1) methylenetetrahydrofolate reductase (Mthfr), (2) tumor necrosis factor receptor superfamily member 1B (Tnfrsf1b), (3) procollagen-lsine, 2-oxoglutarate 5-dioxogenase 1 (Plod1), (4) brain natriuretic peptide (Bnp, also named Nppb), (5) cannabinoid receptor 2 (Cnr2), (6) gap junction protein alpha-4 (Gja4), and (7) leptin receptor (Lepr). A common MTHFR G677T polymorphism has been associated with BMD in postmenopausal Danish women [60]. The less common genotype TT was associated with significantly lower BMD at the femoral neck, total hip and spine. This polymorphism is also associated with low BMD in Japanese women [61]. Two studies have shown evidence of an association between BMD and haplotypes defined by polymorphisms at positions 593, 598 and 620 in TNRFSF1B 3′ untranslated region. In one study, an association was observed between LS BMD and the A593-G598-T620 (AGT) haplotype [64], whereas another larger study showed an association between FN BMD and the A593-T598-C620 (ATC) haplotype [62]. The PLOD1 gene is a strong functional candidate for BMD regulation as it encodes Procollagen-lysine, 2-oxoglutarate, 5-dioxogenase, which catalyses the hydroxylation of lysine residues during the posttranslational modification of type I collagen. In a population-based study of women from the UK, Tasker et al. [65] reported a significant association between LS BMD and the G386A polymorphism, which results in an alanine–threonine amino acid change at codon 99. Heterozygotes for G386A had significantly reduced LS BMD when compared with the other genotype groups. Association analysis between nucleotide variations of the BNP gene and radial BMD in 378 Japanese postmenopausal women revealed a significant association of the −381T/C SNP in the promoter region of BNP gene with radial BMD. BMD was lowest in T/T homozygotes, intermediate among heterozygotes, and highest among C/C homozygotes in the test population. Accelerated bone loss also correlated with the −381 T allele in a 5-year follow-up study, suggesting that variation of BNP may be an important determinant of postmenopausal osteoporosis [66].

**Chromosome 5**

BMD2 is the only BMD QTL mapped at Chromosome 5 (Table 1) [13,14]. Seven BMDAP genes are found and BMD2 includes four of them: (1) Kit, (2) Cd38, (3) aldehyde dehydrogenase (Aldh2), and (4) sulfotransferase 1E1 (Sult1e1). Kit plays an important role in the differentiation of osteoclasts. Kim et al. [73] examined the associations between Kit gene polymorphisms and BMD in postmenopausal Korean women. Haplotype analyses showed that the h3 haplotype (−1694T–+41894A–+49512G) was significantly associated with lower BMD at femoral neck. Drummond et al. [74] demonstrated that the CD38-PvuII polymorphism was significantly associated with LS BMD in pre- and postmenopausal women. The CD38-PvuII polymorphism was also significantly associated with FN BMD in the premenopausal cohort. The G allele at this locus appears to be a risk allele for low BMD. CD38 knockout mice also displayed significantly decreased BMD at all skeletal sites at 3 months of age compared to those wild-type mice. ALDH2 Glu487Lys polymorphism has been strongly associated with osteoporosis. The risk of reduced bone mass was significantly higher in the group having the Lys/Lys genotype than in the group having the Glu487Lys or Glu/Glu genotype. This suggests that possession of Glu allele may be protective against osteoporosis [75]. In a community-based cross-sectional study conducted on 397 Korean women, Lee et al. [76] found that BMD in the calcaneus was influenced by the genetic polymorphism of SULT1E1*959 G→A and phytostrogen consumption. In addition, the association between phytostrogen consumption and calcaneal BMD might be modified by genetic polymorphism of SULT1E1. Women with the SULT1E1*959 GG genotype had a lower BMD at the distal radius and calcaneus compared to those with the AA genotype, especially at the calcaneus in premenopausal women.

Three BMDAP genes are located outside of QTL region. Interleukin 6 (IL6) is a well-known factor affecting BMD. In 470 Japanese subjects, Oku et al. [83] found a correlation between the presence of the G allele of a C/G polymorphism at nucleotide −634 in the promoter region and decreased BMD. BMD was lowest among the GG homozygotes, highest among the CC homozygotes, and intermediate among the heterozygotes. Chung et al. [84] also described a SNP 1694G→C in the IL6 promoter region that showed significant association with BMD. The C allele was associated with increased BMD in a gene-dose-dependent manner in premenopausal women. Kawano et al. [185] examined the relationship between the three common SNPs of klotho (KL) gene and BMD in white women and Japanese postmenopausal women. In the white population, one in the promoter region (G→939A) and one in exon 4 (C→1818T) and their haplotypes were significantly associated with bone density in aged postmenopausal women (≥5 years), but not in premenopausal or younger postmenopausal women. These associations were also seen in Japanese postmenopausal women. These results indicate that the KL gene may be involved in the pathophysiology of bone loss with aging in humans. In a population-based prospective cohort study of aging and age-related diseases, Yamada et al. [186] confirmed the association of −395G→A polymorphism with BMD in Japanese women. LS BMD was significantly lower in subjects with the GG genotype than in those with the AA genotype. Koh et al. [187] found that the minor allele of FLRT3 +13348C→T, an intronic polymorphism in FMS-related tyrosine kinase 3 (FLRT3), was significantly associated with low LS BMD and FN BMD. Haplotype analysis revealed that FLRT3–ht2 (TTCT) containing the rare allele in the +13348 position also showed significant association with low BMD at both sites.

**Chromosome 6**

Chromosome 6 contains three BMD QTL (Table 1) [13,21,27,46]. Seven BMDAP genes are identified. Among them, four are within QTL region, which are microphthalmia-associated transcription factor (Mif), peroxisome proliferator-activated receptor gamma (Pparg), vitamin-K-dependent gamma-glutamyl carboxylase (Ggcx), and matrix Gla protein gene (Mgp). MIF plays a critical role in osteoclast development and thus is an important candidate gene affecting BMD. Koh et al. [83] found that two polymorphisms, MIF+227719C→T,
MITF + 228953A→G and one haplotype, MITF-ht3, were significantly associated with the BMD of the proximal femur in postmenopausal women. The MITF + 227719C→T polymorphism was significantly associated with low BMD of the trochanter and total femur. The effects of MITF + 227719C→T on the BMD of the trochanter were gene-dose dependent; the highest BMD being found in homozygotes for the common allele, intermediate BMD in heterozygotes, and the lowest BMD in homozygotes for the rare allele. Moreover, the MITF + 228953A→G polymorphism was also associated with low BMD of the femoral shaft. Homozygotes for the G allele had lower BMD at the femoral shaft compared with those without either G allele. The MITF-ht3 haplotype was associated with low BMD of the trochanter and total femur. These results suggest that MITF variants may play a role in the decreased BMD of the proximal femur in postmenopausal women.

Three BMDAP genes outside of QTL region are collagen type I alpha2 (Col1a2), neuropeptide Y (Npy), and calcitonin receptor (CalcR).

Chromosome 7

Chromosome 7 contains five BMD QTL (Table 1) [21,27,32,42,46] and six BMDAP genes. Among them, transforming growth factor beta 1 gene (Tgfβ1), periplin (Plin), and apolipoprotein E (ApoE) are within QTL region. The 1243C→T polymorphism of PLVN has been associated with BMD for the total body, lumbar spine, femoral neck, and trochanter in men, with the C allele being related to reduced BMD [92].

Three BMDAP genes in non-QTL region are: (1) osteoclast-associated receptor (Oscar), (2) dopamine receptor D4 gene (Drd4), and (3) parathyroid hormone (Pth). A polymorphism OSCAR −2322 A→G in the 5′ flanking region of OSCAR gene might be one of genetic determinants of BMD. OSCAR −2322 A→G was associated with BMD at both the lumbar spine and femoral neck. At the lumbar spine, the genetic effects of OSCAR −2322 A→G on BMD were gene-dose dependent, and the highest BMD was found in homozygotes for the common allele. However, at the femoral neck, the OSCAR −2322 A→G showed dominant effect on BMD values; a higher BMD was found in individuals bearing the GG genotype than in others (AA and AG genotypes). The genetic effects of OSCAR −2322 A→G on BMD were also detected at other femoral sites [188]. DRD4 may be a candidate locus for reduced BMD in men. The −521C→T SNP of DRD4 has been significantly associated with BMD at various sites in Japanese men. The C allele appears to be acting as a risk allele for low BMD [189].

Chromosome 8

There is no BMD QTL mapped into Chromosome 8. One BMDAP gene is identified. The −512C→T polymorphism of forkhead box C2 (FoxC2) has been associated with BMD for the distal and proximal radius in men and in premenopausal women as well as with BMD for the distal radius and total body in postmenopausal women, with the T allele representing a risk factor for reduced BMD [92].

Chromosome 9

Three BMD QTL are located on Chromosome 9. We identified seven BMDAP genes for this chromosome. Among them, four are within QTL region, including: (1) cytochrome P450, family1, subfamily A, polypeptide 1 (Cyp1a1), (2) semaphorin 7a (Sema7a), (3) lipase, hepatic (Lipc), and (4) intercellular adhesion molecule 1 (ICAM1). Estrogen metabolism is an important determinant of bone mass [101]. The C4887A polymorphism of CYP1A1 gene, one of the key enzymes that metabolize estrogen, has been associated with BMD. C→A transversion, which results in an amino acid change from threonine to asparagin at codon 461, was identified as a possible genetic risk factor for low BMD. The reduced BMD as well as consequently the risk of osteoporosis associated with this polymorphism may be a result of accelerated estrogen catabolism and increased bone resorption [100]. In addition, several polymorphisms of cytochrome P450, family19, subfamily A, polypeptide 1 (CYP19A1) gene, an enzyme involved in estrogen synthesis, have also been associated with BMD [190,191]. Among them, a polymorphism Ar01 (rs4775936), located in a promoter region of CYP19A1, has been associated with LS BMD in postmenopausal women. Homozygotes AA exhibited significantly higher LS BMD, compared with GG or GA individuals [190].

The (AAAG)n polymorphism in the P3 promoter of the parathyroid hormone type 1 receptor (PTHR1) gene has been associated with BMD in Caucasian women. The subjects bearing at least one (AAAG)6 allele have a higher FN BMD than those without, suggesting the variation in promoter activity of the PTHR1 gene may exert a relevant genetic influence on BMD [33]. In addition, the 190G→A (Val64Ile) polymorphism of CC chemokine receptor-2 (CCR2) has been associated with BMD at various sites in community-dwelling, middle-aged Japanese men and postmenopausal women, and that the AA genotype represents a contributing factor to increased bone mass [192].

Chromosome 10

Chromosome 10 contains two BMD QTL (Table 1) [27,107] and two BMDAP genes. Among them, insulin-like growth factor 1 (Igf1) is located in QTL region. In a large population-based study of elderly men and women, Rivadeneira et al. reported that the absence of the wild-type (192-bp) allele in a (CA)n repeat polymorphism in the promoter region of the IGFI gene is associated with lower BMD levels and higher rates of bone loss at the different femoral sites in women. However, no associations were observed in men at any femoral site of BMD measurement [108].

Estrogen and the estrogen receptor 1 (ESR1, also named ER) play a central role in bone metabolism. The relationship between the polymorphisms associated with the ESR1 gene and BMD and osteoporotic fracture has been extensively investigated. The (TA)n dinucleotide repeat polymorphism at the 5′ end of the ESR1 gene has been associated with BMD. Subjects with a low number of repeats (TA<15) showing the lowest BMD values and increased fracture risk [193,194]. In addition, a newly identified CA repeat polymorphism of ESR1 has been associated with BMD variation. The number of CA repeats was linearly related to hip BMD in postmenopausal women. Postmenopausal women with CA repeats <18 had higher risks of having osteoporosis and low trauma fractures than those with ≥18. The operator/◦＝was changed to ≥18. Please check appropriate≥18 repeats. Perimenopausal women with <18 CA repeats had significantly greater bone loss in 18 months at the hip than those with ≥18 repeats [195].

Chromosome 11

Seven BMD QTL are mapped on Chromosome 11 (Table 1) [13,21,30–32,42,131]. A total of 8 BMDAP genes are identified: (1) arachidonate 15-lipoxygenase (Alox15), (2) arachidonate 12-lipoxygenase (Alox12), (3) PDZ-LIM domain protein 4 (Pdlim4, also named Ril), (4) growth hormone (Gh, also named Gh1), (5) sex hormone-binding globulin (Shbg), (6) collagen type 1 alpha 1 (Col1a1), (7) angiotensin converting enzyme (Ace), and (8) sclerostosis/van Buchem disease gene (Sost). All of these genes are located within QTL region. Through combined genetic and genomic approaches, Klein et al. [9] identified Alox15 as a negative regulator of peak bone mineral density in mice. This gene was also considered as the causal gene underlying a BMD QTL peaked at D11Mit349. There are three lipoxygenases in humans, ALOX15, ALOX15B, and ALOX12, that correspond to 12/15-lipoxygenase in mice. A SNP −5299G/A in ALOX15 5′-flanking region, has been associated with BMD in postmenopausal Japanese women. Subjects with the A allele had significantly lower LS BMD and total body BMD [114]. Interestingly, Ichikawa et al. [115] tested genetic association of ALOX12 and ALOX15 with BMD variation in a large cohort of healthy American white men.
and women, they observed that polymorphisms in ALOX12, but not ALOX15, are significantly associated with spine BMD in white men and women. In adult Japanese women, Omasu et al. [117] found an association between the T allele in the −3333T−C polymorphism in the 5′-prime flanking region of the PDLIM4 gene to low bone mineral density (BMD) in an allele-dosage-related manner. This variation may also be an important determinant of osteoporosis. Polymorphisms at COL1A1 and TGFβ1 and haplotypes at COL1A1 and ESRI were found to be associated with BMD in a cohort of postmenopausal Spanish women. Moreover, COL1A1 polymorphisms showed significant interactions among them and with the VDR 3′ polymorphisms [93]. Pérez-Castrillón et al. [123] assessed the relationship between bone mineral density and insertion/deletion (I/D) ACE polymorphism in hypertensive postmenopausal women. They found that women with II genotype showed a higher intact parathyroid hormone without a decrease in calcium, and higher bone mineral density than women with ID and homozygous deletion genotype, suggesting the ACE polymorphism could be one of the factors causing bone mass variations. Two variants, SRP3 and SRP9, in the SOST region have been associated with BMD variation. SRP3 was associated with decreased BMD in women at the LS and FN, with evidence of an allele-dose effect in the oldest age group. Similarly, a G variant SRP9 was associated with increased BMD in men at the LS and FN [124].

Chromosome 12

Chromosome 12 contains two BMD QTL (Table 1) [21,31]. Two BMDAP genes are identified. Both of these are located within QTL region. Several SNPs of estrogen receptor beta (ESR2) gene have been associated with BMD. The C allele of T-1213C was associated with lower BMD and a 2−3-fold increased risk of osteoporosis in both men and women, while the G allele of A110732G was associated with higher BMD and a 40% reduction in risk of osteoporosis at the spine in postmenopausal women. Besides, C-1018T was associated with significant reduction in risk of osteoporosis at the hip in premenopausal women. Among all SNPs, T-1213C was the most significant predictor of BMD, risk of osteoporosis and osteoblastic fractures [132]. Thyroid stimulating hormone (TSH) inhibits, through the TSH receptor (TSHR), both osteoclastic bone resorption and osteoblastic bone formation. TSHR knockout mice display high-turnover osteoporosis [135]. In the Rotterdam Study, van der Deure et al. [134] found that a common polymorphism TSHR-Asp727Glu in TSHR gene was dose-dependently associated with higher FN BMD. Carriers of the Glu allele had a higher FN BMD than noncarriers.

Chromosome 13

Chromosome 13 contains four BMD QTL (Table 1) [13,14,21,42,131] and five BMDAP genes. The BMDAP genes are: (1) secreted frizzled-related protein (Sfrp4), (2) hemochromatosis gene (Hfe), (3) receptor tyrosine kinase-like orphan receptor 2 (Ror2), (4) integralalpha1 (Itga1), and (5) phosphodiesterase 4D (Pde4d). Among them, Sfrp4, Hfe, and Ror2 are within QTL region. Sfrp4 has been identified as the responsible gene of QTL Pbd2 affecting peak BMD in SAMP6 mice [10]. The syntenic region of this locus corresponds to human-7p14, which has also been detected as a QTL for BMD [136,137]. These evidences suggest the possibility that a common susceptibility gene for human and mouse peak BMD is present in this location, and a polymorphism of Sfrp4 may contribute to the variation in human peak BMD.

Lee et al. [196] analyzed eight SNPs in integralalpha1 (Itga1) region for their potential involvement in osteoporosis in postmenopausal women. The SNPs, +73187C>T and +76969T>C, and their haplotypes BL_ths were associated with BMD at various femur sites. Moreover, +159174A>C and its haplotype BL3_ht1 showed a highly significant association with risk of non-vertebral fracture and the minor allele of +159174A>C showed a protective effect. These results are suggestive of the association of ITGA1 with osteoporosis and related risk in postmenopausal women.

Chromosome 14

Five BMD QTL are located on Chromosome 14 (Table 1) [13,21,31,32,72]. Two BMDAP genes are receptor activator of NF-kappa B ligand (Rankl, also named Tnfsf11) and bone morphogenetic protein 4 (Bmp4). Only Tnfsf11 is within QTL region. Three key genes in a bone remodeling pathway, RANKL, receptor activator of NF-kappa B (RANK, also named Tnfrsf11a), and osteoprotegerin (OPG, also named Tnfrsf11b) were assessed for their genetic contribution to BMD variation. Significantly positive associations were found for A163G polymorphisms in the promoter regions of the OPG gene, a missense substitution in exon 7 (Ala192-Val) of the RANK gene and rs9594782 SNP in the 5′ UTR of the RANKL gene with BMD in men only. Men with TC/CC genotypes of the rs9594782 SNP had a 2.1 times higher risk of extremely low hip BMD, and lower whole body BMD. Subjects with the TC genotype of the Ala192Val polymorphism had a 40% reduced risk of having extremely low hip BMD, and higher whole body BMD. Subjects with the GG genotype of the A163G polymorphism had a 70% reduced risk of having extremely low hip BMD, and higher whole body BMD. Significant gene−gene interactions were also observed among the OPG, RANK and RANKL genes. These findings suggest that genetic variation in genes involved in the RANKL/RANK/OPG bone remodeling pathway are strongly associated with BMD at different skeletal sites in adult men, but not in women [142]. Another report also indicated that the −290C>T polymorphism in the Tnfsf11 gene promoter could contribute to the genetic regulation of BMD [143].

A polymorphism 6007C>T in the BMP4 gene has been associated with hip bone density in postmenopausal women. This polymorphism codes for a nonsynonymous amino acid change with the T allele coding for valine, while the C allele codes for alanine. BMD was lower in the 32% of subjects homozygous for the C allele. In addition, a major haplotype defined by G−C−T alleles in SNPs 5826G>A, 3564C>T and 6007C>T respectively, showed association with high bone mass [197].

Chromosome 15

Chromosome 15 contains six BMD QTL (Table 1) [13,14,31,46,107]. Three BMDAP genes were found and QTL region covers all of them. Studies on the role of polymorphisms in the vitamin D receptor (VDR) gene in the determination of bone mineral density have been conflicting. Among a group of prepubertal American girls of Mexican descent, Sainz et al. [158] found that girls with the aa and bb genotypes had 2 to 3% higher femoral bone density and an 8 to 10% higher vertebral bone density than girls with AA and BB genotypes. Horst-Sikorska et al. [159] also observed a statistically significant association of the VDR polymorphisms and haplotypes with the BMD and with the occurrence of fractures. However, several other studies found no association between VDR genotype and BMD [160,161]. A novel heterozygous acceptor splice site mutation of exostoses 1 (EXT1) results in hereditary multiple exostosis (HME) that is associated with a low peak bone mass, indicating a possible additional role for EXT1 in bone biology and in regulating BMD [153]. In addition, the A163G polymorphisms in the promoter regions of the Tnfrsf11b gene have also been associated with BMD [142].

Chromosome 16

Four BMD QTL were identified on Chromosome 16 (Table 1) [21,32,42]. QTL region covers all three BMDAP genes including calcium-sensing receptor (Casr), cathelin-O-methyltransferase (Comt), and alpha2-HS glycoprotein (Alsg). Tsukamoto et al. [163] investigated the association between the CA-repeat polymorphism at the human CASR gene locus and BMD of radial bone in
postmenopausal Japanese women. Participants with A3 allele had significantly lower adjusted BMD than the participants who did not carry an allele of that size. This result suggests that genetic variation at the CASR gene locus is associated with some determinants for BMD in postmenopausal women. In addition, a functional polymorphism val158met in COMT gene has been associated with peak BMD in men [166]. AHSG gene polymorphisms are also associated with BMD in Caucasian nuclear families [164].

**Chromosome 17**

Two BMD QTL are located on Chromosome 17 (Table 1) [31]. We found four BMDAP genes, among them, tumor necrosis factor (TNF), runt related transcription factor 2 (Runx2), and chloride channel 7 (Clcn7) are within QTL region. Two polymorphisms TNF-alpha-863C/A and the -1031T/C of the TNF gene have shown linkage with the LS BMD in early postmenopausal Japanese women. There was a significantly higher prevalence of the alleles TNF-alpha-863A and TNF-alpha-1031C in women with the low BMD than women with the high BMD [168]. RUNX2 is a master regulator of osteoblast function. RUNX2 contains a glutamine–alanine repeat. Two common variants were detected within the alanine repeat: an 18-bp deletion and a synonymous alanine codon polymorphism with alleles GCA and GCG (noted as A and G alleles, respectively). In addition, rare mutations that may be related to low BMD were observed within the glutamine repeat. In 495 randomly selected women of the Geelong Osteoporosis Study (GOS), the A allele was associated with higher BMD at all sites tested. The effect was maximal at the ultrradial radius. In a separate fracture study, the A allele was significantly protective against Colles' fracture in elderly women but not spine and hip fracture. The A allele was associated with increased BMD and was protective against a common form of osteoporotic fracture, suggesting that RUNX2 variants may be related to genetic effects on BMD and osteoporosis [170]. In addition, -1025 T/C polymorphism in the promoter 2 of RUNX2 gene has been associated with FN BMD in Spanish post-menopausal women [171].

QPCT is located outside of QTL region. However, multiple SNPs in QPCT revealed significant association with forearm aBMD among adult women from the general population in Japan. Most of these variations were potentially functional, specifically a nonsynonymous coding SNP, R54W (rs2255991), and several SNPs that seemed to be in promoter sequences [198]. The human QPCT gene lies on chromosome 2p22.3, within the region where a QTL for forearm BMD has been identified in the Chinese population [199] and near a QTL for spinal BMD identified among whites [200]. Huang et al. performed a gene-wide and tag single nucleotide polymorphism (SNP)-based association study of four positional and functional candidate genes. The rs3770748 within the QPCT gene showed a significant association with spine BMD in both singlemarker and haplotype association analyses. Subgroup analysis revealed that the effect was primarily driven by an association in the postmenopausal women, presumably suggesting that the rs3770748 affects postmenopausal bone loss rather than peak bone mass. These results suggest that QPCT may be the QTL gene at chromosome 2p for spine BMD variation in the Chinese population [11].

**Chromosome 18**

Although three BMD QTL were mapped on Chromosome 18 (Table 1) [13,21,31,32], no BMDAP gene was found. Some genes responsible for BMD variation may not yet be identified on this chromosome.

**Chromosome 19**

One QTL was mapped on Chromosome 19 (Table 1) [32]. Five BMDAP genes were identified. Only one BMDAP gene, cytochrome P450, family 17, subfamily A, polypeptide 1 (Cyp17a1) is located in QTL region. Yamada et al. [179] examined the associations of BMD with three polymorphisms, the −34T→C polymorphism of CYP17A1, the −493G→T polymorphism of microsomal triglyceride transfer protein gene (MTTP), and a CGG repeat polymorphism of the very low density lipoprotein receptor gene (VLDLR). The −34T→C polymorphism of CYP17A1 and the CGG repeat polymorphism of VLDLR have been associated with BMD in postmenopausal women and in men, respectively.

Multiple investigations have reported the associations between BMD and polymorphisms and/or haplotypes in the low-density lipoprotein receptor-related protein 5 (LRP5) gene. Mizuguchi et al. [201] performed an association study between BMD and 9 candidate genes in Japanese women. They found that only LRP5 showed a significant association with BMD. A follow-up case-control study revealed a significant difference in allelic frequency of the LRP5 c.2220C→T SNP. The T allele was more frequently deposited in patients than in normal control women. The authors suggested that LRP5 is a BMD determinant and contributes to a risk of osteoporosis. The results from other studies also supported this standpoint [202–204]. Allelic variation at the G–1102A polymorphism in the promoter of T cell immune regulator 1 (TCIRG1) gene has been associated with BMD in premenopausal women of Scottish descent. This polymorphism is situated at a consensus recognition sequence for the transcription factor API. In the presence of the G-nucleotide, a consensus API site is present on the reverse strand (TCACGGC) whereas in the presence of the A nucleotide, the consensus sequence is altered (TCATGGC). Homozygotes for A allele had BMD values significantly higher than individuals who carried the A allele [205]. A statistically significant association between LS BMD of white premenopausal women and a regulatory variant in the estrogen-related receptor alpha (ESRRA) promoter has been observed. Women with long variants showed a higher LS BMD than those with common short variants. The same trend was observed for FN BMD. These results support the genetic influence of this ESRRA regulatory variant on BMD [206].

**Chromosome X**

Chromosome X carries only one QTL (Table 1) [181]. Two BMDAP genes are found. However, neither of them is located in QTL region. Androgen receptor (AR) gene was reported to be a determinant of BMD in premenopausal Japanese women, with the (CAG) (n≥23) allele of a CAG repeat polymorphism representing a risk factor for reduced BMD [186]. The haplotype 196F/532S constructed from two amino acid-substituting SNPs in the interleukin-1–associated kinase 1 (IRAK1) gene has been significantly associated with decreased radial BMD. Radial bone mineral density was lowest among 196F/532S homozygotes, highest among 196S/532L homozygotes, and intermediate among heterozygotes. Accelerated bone loss also correlated with the 196F/532S haplotype in a 5-year follow-up. These results suggest that variation of IRAK1 may be an important determinant of postmenopausal osteoporosis, in part through the mechanism of accelerated postmenopausal bone loss [207].

**Chromosome Y**

Neither BMD QTL nor BMDAP gene are identified on Chromosome Y.

**Conclusions**

We performed a whole genome scan to identify QTL genes, polymorphisms that determine BMD. We found that a large number of BMD QTL have been identified in mouse models. However, direct determination of causal genetic factors (i.e. genes) within the BMD...
QTL regions has been slow and remains a major bottleneck in fully understanding the genetic mechanism underlying BMD variation. To our knowledge, only four genes were identified as responsible for BMD QTL [8–11]. With advances in genetics and genomics, an enormous amount of data related to gene function has been accumulated in some large publicly accessible databases. By genome-wide analysis of genes and polymorphisms involved in regulation of BMD using the currently available data in PubMed, OMIM, Mammalian Phenotype Ontology, and Gene Ontology, we found that many genes have been shown to be associated with BMD, most of which were identified from association studies in human subjects. These genes are important candidates that may be responsible for the QTL effects and should be carefully investigated in direct experiments to precisely establish their functional roles. Evidence from functional assessment (e.g. from knockouts or transgenics), gene expression profiling, and SNP analyses will be necessary to confirm the actual involvement of these genes in BMD regulation and measure the degree of their contribution to BMD.

Based on our in silico analysis, most QTL include more than one BMDA/BMDAP gene. One possibility is that these QTL include several linked sub-loci and therefore are caused by a series of genes, each with a small effect. This possibility is demonstrated by QTL Bmd5, which has been subdivided into three linked loci by two independent investigations [26,29]. Of course, we cannot rule out that some QTL may be caused by a major gene with large effect. It is also possible that some BMDA/BMDAP genes identified in human population may not be a functional contributor to BMD variation in mouse. Another possibility is that some BMDA/BMDAP genes may have no actual contribution to BMD variation under the conditions for the measurement of BMD QTL since we didn't evaluate the QTL or genes in a site-, gender-, age-, strain- or population-specific manner. Convincing evidences have shown that the QTL or genes which regulate BMD have site-, gender-, age-, strain-, or population-specific effects [21,26,137], accordingly, those candidate genes should be evaluated and tested separately. Although our data provide a starting point for such a test, experiments would have to be conducted to test those candidate genes.

There were no QTL detected on Chromosome 8 although the obvious candidate genes exist. In addition, for some QTL no BMDA/BMDAP gene was identified, and some chromosomes include fewer BMDA/BMDAP genes in QTL regions than in non-QTL regions. This complexity may be explained by several possibilities: 1) methods adopted for QTL mapping cannot detect all QTL especially some small-effect QTL because of small sample size, small phenotypic variance, sparse marker coverage, etc; 2) genome annotation is not complete. some unknown BMDA/BMDAP genes may exist in QTL regions and/or non-QTL regions since direct effects on BMD regulation may not yet be recognized for many genes; 3) some BMDA/BMDAP genes identified from human or other species may have no effect on BMD regulation in mouse because the same gene or polymorphism may have different influences on the same phenotype in different species or ethnic groups; 4) Among B3 BMDAP genes, only 4 came from mouse studies. Most of BMDAP genes were selected based on association studies in humans linking a polymorphism with lower (or higher) BMD. Few of these polymorphisms have been confirmed as causal. It is thus possible that it is not the gene named, but an adjacent gene that contains the causal polymorphism. Of course, there is a good chance that adjacent genes are syntenic in mouse. Finally, our methods will not identify new genes.

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Appendix A. Supplementary data


References

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