REVIEW

Recent genetic discoveries in osteoporosis, sarcopenia and obesity

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Abstract. Osteoporosis is a skeletal disorder characterized by low bone mineral density (BMD) and an increased susceptibility to fractures. Evidence from genetic studies indicates that BMD, a complex quantitative trait with a normal distribution, is genetically controlled. Genome-wide association studies (GWAS) as well as studies using candidate gene approaches have identified single-nucleotide polymorphisms (SNPs) that are associated with BMD, osteoporosis and osteoporotic fractures. These SNPs have been mapped close to or within genes including those encoding WNT/ β -catenin signaling proteins. Understanding the genetics of osteoporosis will help to identify novel candidates for diagnostic and therapeutic targets. Genetic factors are also important for the development of sarcopenia, which is characterized by a loss of lean body mass, and obesity, which is characterized by high fat mass. Hence, in this review, we discuss the genetic factors, identified by genetic studies, which regulate the body components related to osteoporosis, sarcopenia, and obesity.

Key words: Single nucleotide polymorphism (SNP), Osteoporosis, Sarcopenia, Obesity, GPR98

OSTEOPOROSIS is a skeletal disease characterized by low bone mineral density (BMD) and microarchitectural deterioration of bone tissue, predisposing those with the disease to an increased risk of fractures [1]. Many of these osteoporotic fractures are associated with significant morbidity and mortality not only in developing countries, but also in developed countries [2]. BMD, the primary surrogate marker of osteoporosis and a major predictor of fracture risk, is a complex quantitative trait with a normal distribution in humans. Several twin and family studies have consistently shown that genetic factors account for approximately 50–90% of the interindividual variation in BMD [3–5]. Modest associations have been reported between BMD variations and genetic polymorphisms in or near some genes [6, 7].

In addition to osteoporosis, the loss of skeletal muscle is a common disorder in the elderly [8–10]. This symptom is related to diseases such as sarcopenia, a major skeletal disease characterized by low lean

body mass, leading to decreased skeletal strength and increased susceptibility to falls and fractures [9, 10]. As with low BMD, low lean body mass has a strong genetic component, with heritability ranging over 50% [11, 12]. However, the specific genes underlying the variation in low lean body mass are largely unknown.

The prevalence of obesity has increased in recent years [13]. Obesity has become one of the most serious health problems, and its occurrence is attributed to the interplay between environmental and genetic factors and obesity-associated genes [14–16]. Over 40% of the variation in obesity-related phenotypes is estimated to be heritable [14–16]. Genetic studies and knockout mouse models have uncovered new obesity-associated genes [16, 17].

In this review, we briefly summarize the current knowledge about the genetic factors that are related to the pathogenesis of osteoporosis, sarcopenia, and obesity.

Genetic variations in estrogen receptor genes and osteoporosis

Estrogen deficiency is an important risk factor for postmenopausal osteoporosis [18]. The physiological functions of estrogen are modulated largely by two

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estrogen receptors (ESRs), namely ESR1 (ER α) and ESR2 (ER β), encoded by two different genes [19]. ER α mainly mediates the action of estrogen in the bone [20, 21]. In 1995 and 1996, we reported the correlation between BMD and the TA variable number of tandem repeats (VNTR) within the *ESR1* promoter region [22] and also between BMD and the IVS1 - 397T > C SNP (rs2234693, detected by *PvuII*) in *ESR1* [23]. Thereafter, many other studies also demonstrated the association of the TA VNTR of the *ESR1* promoter region, IVS1 - 397T > C SNP, and IVS1 - 351A > G SNP (rs9340799, detected by *XbaI*) with BMD [21, 24].

rs2234693 and rs9340799 lie within the introns of *ESR1* and are in strong linkage disequilibrium. The SNP alleles P and X (characterized by the absence of both restriction sites) as well as alleles p and x (indicating the presence of both restriction sites) are strongly associated with each other. Although haplotype pX was not detected in most of the studies, the haplotype Px was detected at a low frequency, suggesting that linkage disequilibrium is not complete. The T > C change at rs2234693 resulted in a potential binding site for the transcription factor myb, followed by *in vitro* transcriptional changes, indicating that the T > C change may alter *ESR1* transcription [25].

VNTR polymorphisms in the vicinity of certain gene promoters can have a significant effect on transcriptional regulation [26]. Genetic variation arising from different TA repeat lengths can also affect promoter activity in *ESR1*. Recently, the SNPs in *ESR1* have been shown to be related to susceptibility to osteoporosis not only in studies using a candidate gene approach but also in several GWAS and meta-analyses [27-31].

Regulation of bone metabolism by variations in the *LRP5*

The WNT signaling pathway plays an important role in several cellular functions [32]. Studies using *Drosophila*, *Xenopus*, and mammalian models have indicated that the WNT signaling pathway is subdivided into three branches: the WNT/ β -catenin pathway, also called the canonical WNT pathway; the non-canonical WNT-planar cell polarity pathway; and the WNT-calcium pathway. In the canonical WNT pathway, WNT proteins bind Frizzled (FZ) proteins and either LRP5 or LRP6. This results in the inhibition of glycogen synthase kinase 3 (GSK3)-dependent phosphorylation of β -catenin, followed by the stabilization

of β -catenin. Both genetic and biochemical results have provided evidence indicating that FZ proteins and either LRP5 or LRP6 function as Wnt receptors.

Fourteen years ago, the WNT/ β -catenin pathway was shown to regulate bone density through LRP5 [33-36]. In 2001, Gong *et al.* reported that inactivating mutations in the human *LRP5* decreased bone mass and caused an autosomal recessive disorder, osteoporosis-pseudoglioma syndrome [33]. Subsequently, in 2002, Little *et al.* and Boyden *et al.* reported that activating mutations in *LRP5* led to autosomal dominant high bone mass traits [34, 35]. Taken together, these data indicate that LRP5 controls bone metabolism *in vivo* in humans.

In 2004, we first reported that a common SNP in intron 17 of *LRP5* was significantly associated with BMD in Japanese postmenopausal women [37]. We have also found that the IVS17 - 1677C > A SNP and the nonsynonymous SNP in exon 18, caused by an amino acid change of alanine to valine (3989C > T, A1330V), were in strong linkage disequilibrium ($D' = 0.99$, $r^2 = 0.98$) [38]. We and several other groups have since shown that the A1330V SNP is associated with BMD and incidence of fractures (Table 1) [39-51]. These data suggest that the amino acid change at codon 1330 in *LRP5* may affect the pathogenesis of osteoporosis through decreasing activity of the WNT/ β -catenin pathway.

To evaluate the functional significance of the A1330V SNP, we substituted the alanine at codon 1330 in the major-type LRP5 (LRP5-A1330) to valine (LRP5-V1330) [50]. We transfected HEK293T cells with these LRP5 constructs and measured TCF-Lef reporter activity in response to canonical Wnt signaling. The Wnt-accelerated TCF-Lef activity was significantly reduced in the cells containing LRP5-V1330 compared to those containing the major-type allele. These data suggest that the nonsynonymous SNP at codon 1330 may alter the activity of the WNT/ β -catenin pathway and affect BMD in humans.

Since our first report in 2004, there have been several reports on the association between nonsynonymous *LRP5* SNPs and different bone phenotypes, including BMD and osteoporotic fracture [7, 52-55]. These SNPs included 3 nonsynonymous SNPs: Q89R, V667M, and A1330V. In Caucasian populations, the V667M SNP was found to be significantly associated with BMD [52]. To our knowledge, no association studies between the V667M SNP and bone phenotypes

Table 1 Association studies between the SNPs in the LRP5 gene and bone mineral density (BMD).

Study group	Year	Journal	Participants	Parameters
Urano <i>et al.</i> [37]	2004	<i>J Bone Miner Metab</i>	Japanese postmenopausal women	Total body BMD
Mizuguchi <i>et al.</i> [39]	2004	<i>J Hum Genet</i>	Japanese women (over 50 y.o.)	Lumbar BMD
Koay <i>et al.</i> [40]	2004	<i>J Bone Miner Res</i>	Osteoporotic probands and their families	lumbar spine, femoral neck, and hip BMD
Bollerslev <i>et al.</i> [41]	2005	<i>Bone</i>	Australian elderly women	Femoral neck and trochanter BMD
Ferrari <i>et al.</i> [42]	2005	<i>Bone</i>	Caucasian men	Femoral neck and trochanter BMD
vanMeurs <i>et al.</i> [43]	2006	<i>J Bone Miner Res</i>	Caucasian men (over 55 y.o.)	Lumbar spine and femoral neck BMD
Saarinen <i>et al.</i> [44]	2007	<i>Bone</i>	Finnish young men	Femoral neck and trochanter BMD
Xiong <i>et al.</i> [45]	2007	<i>J Bone Miner Res</i>	Chinese and Caucasian subjects	spine, hip, and ultradistal radius BMD
Ezura <i>et al.</i> [38]	2007	<i>Bone</i>	Japanese women	Lumbar spine and radial BMD
Giroux <i>et al.</i> [46]	2007	<i>Bone</i>	French-Canadian women	Lumbar spine and femoral neck BMD
vanMeurs <i>et al.</i> [47]	2008	<i>JAMA</i>	Caucasian in Europe and North America	Femoral neck BMD and osteoporotic fractures
Richards <i>et al.</i> [48]	2008	<i>Lancet</i>	Caucasian (Genome-wide association study)	BMD and osteoporotic fractures
Agueda L <i>et al.</i> [49]	2008	<i>J Bone Miner Res</i>	Spanish postmenopausal women	Lumbar spine, femoral neck BMD and osteoporotic fracture
Urano <i>et al.</i> [50]	2009	<i>Endocr J</i>	Japanese postmenopausal women	Total body BMD
Lee <i>et al.</i> [51]	2009	<i>Rheumatol Int</i>	Meta-analysis	Meta-analysis

Fig. 1 The metabolism of homocysteine. Homocystinuria is a rare autosomal recessive disease in which the metabolism of homocysteine is severely impaired leading to excessive accumulation of homocysteine in the blood and urine. A common cause of homocystinuria is cystathionine-β-synthase deficiency. THF, tetrahydrofolate.

have been reported in Asian populations. This could be due to the low allele frequency of the V667M SNP in Asian populations. The Q89R SNP has been studied only in Asian populations, most likely because of its low frequency in other ethnic populations. Significant associations between the Q89R SNP and femoral neck BMD were reported in young Korean men [53] and Chinese women [54]. Q89R was also associated with lumbar osteophyte formation in Japanese postmenopausal women [55]. In Korean and Chinese populations, strong linkage disequilibrium was observed between Q89R and A1330V [53, 54]. In our previous study, Q89R and A1330V SNPs were not in significant linkage disequilibrium ($D' = 0.65$, $r^2 = 0.08$) [38].

SNPs in the homocysteine and folate-related genes and fractures

Several cohort reports indicated that B-vitamins could play protective roles in bone health [56]. Homocystinuria is a rare autosomal recessive disease in which the metabolism of homocysteine is severely impaired, leading to excessive accumulation of homocysteine in the blood and urine (Fig. 1). A common cause of homocystinuria is a cystathionine-β-synthase deficiency. Homocystinuria is characterized by precocious osteoporosis. The major determinant of homocysteine concentration is the biomarker status of the metabolically related folate, vitamin B12, and vita-

min B6 (Fig. 1). Methylene tetrahydrofolate reductase (MTHFR), an important enzyme in the methionine cycle [57], is involved in the removal of circulating homocysteine. *MTHFR* lies within a linkage region on chromosome 1p36 that is associated with the regulation of BMD [58, 59]. A C677T nonsynonymous and functional polymorphism has been identified in exon 4 of *MTHFR* that results in an alanine to valine change. The T-allele variant (valine type) has a lower MTHFR activity than the wild type (C-allele or alanine type) and is also associated with moderately elevated homocysteine levels [57]. An elevated homocysteine level has been recognized as a significant risk factor for fractures [60-62], independent of age and BMD, because it is caused by abnormal collagen cross-links during bone calcification [63-65]. Clinical evidence regarding the role of the *MTHFR* C677T polymorphism in BMD remains controversial [66].

We previously reported that in Japanese postmenopausal women, the *MTHFR* C677T polymorphism alone is a weaker risk factor for future fractures compared to traditional risk factors [67]. However, 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. Another report in the Caucasian population showed that the risk of hip fractures increased in the TT group compared with the CC group. Patients bearing the TT genotype showed an increased risk of fractures when their plasma folate levels were low [61]. Lower plasma folate levels led to an increase in the plasma levels of homocysteine. These results suggest a strong association of the effects of the *MTHFR* C677T polymorphism on homocysteine and bone quality with the risk of fractures.

Folate is known to have an effect on homocysteine metabolism (Fig. 1). We recently analyzed the association between plasma folate levels and fractures [68]. Postmenopausal Japanese women with the lower plasma folate levels had a significantly increased risk of fracture. These data are consistent with previous reports. Folate metabolism is distributed between the cytosolic and mitochondrial compartments. *SLC25A32* is a transporter that shuttles folate from the cytoplasm into mitochondria [69, 70]. Recently, we identified SNPs in *SLC25A32* that are associated with plasma folate levels [68]. We also found that the SNPs in this gene are associated with the incidence of frac-

tures. Interestingly, these SNPs were not associated with BMD in this population; however, they may affect the bone quality. Thus, genetic analyses revealed that *SLC25A32* is a novel candidate gene associated with folate metabolism and bone fragility.

GWAS related to osteoporosis and osteoporotic fractures

Although the genetics of osteoporosis have been studied through various association studies by candidate gene approaches, the major susceptibility genes and the molecular mechanisms responsible for osteoporosis and osteoporotic fracture remain unclear. In contrast to the candidate gene approach, GWAS is an unbiased approach that involves scanning the entire genome to identify novel genes with modest effects on complex diseases/traits [6-7]. Over the past eight years, a number of GWAS have uncovered novel SNPs associated with complex diseases/traits, including osteoporosis and BMD in Caucasian and Asian populations (Table 2) [30, 31, 48, 71-82].

GWAS for osteoporosis and osteoporotic fractures have identified the following candidate genes in the WNT/ β -catenin signaling pathway [27, 31, 48, 73, 75, 76, 80-83]: catenin β 1 (*CTNNB1*), sclerostin (*SOST*), *LRP4*, *LRP5*, *GPR177*, *WNT4*, *WNT5B*, *WNT16*, dickkopf1 (*DKK1*), gene for secreted Frizzled-related protein 4 (*sFRP4*), Jagged 1 (*JAG1*), *MEF2C*, and *AXIN1*. GWAS for osteoporosis have also identified three important factors, namely, RANK, RANKL, and OPG in the bone resorption cycle [48, 72, 73]. Osteoblasts and osteocytes secrete a soluble factor called RANKL [84] that interacts with RANK located on the surface of osteoclast precursor cells, leading to the differentiation and fusion of osteoclastic lineage cells. Osteoblasts and osteocytes also produce a decoy receptor for RANKL called OPG, which inhibits its binding to RANK, thereby impeding the bone resorption cycle.

We also analyzed GWAS of BMD in Japanese postmenopausal women [77, 79]. We showed the association of nonsynonymous SNPs in the WD repeats and SOF1 domain-containing gene (*WDSOF1*) with BMD [77]. We also identified the association of the SNP in the 3'-flanking region of *GPR98* with BMD and fractures. We then showed the phenotype of *Gpr98* knockout mice and revealed that the GPR98/Gpr98 signaling pathway could be critical in the regulation of BMD and bone fragility through the modulation of RANKL pro-

Table 2 Genome-wide association studies in bone mineral density and osteoporotic fracture.

Study group	Year	Journal	Participants in 1 st screening	Participants in replication study	Identified genes
Kiel <i>et al.</i> [71]	2007	<i>BMC Med Genet</i>	American Caucasian	-	
Richards <i>et al.</i> [48]	2008	<i>Lancet</i>	Caucasian twins	RS, British twins, British population	LRP5, OPG
Styrkarsdottir <i>et al.</i> [72]	2008	<i>N Engl J Med</i>	Icelandic subjects	Icelandic, Danish, and Australian subjects	RANKL, OTB, ESR1, RANK
Styrkarsdottir <i>et al.</i> [73]	2009	<i>Nat Genet</i>	Icelandic subjects	European descent	SOST, MARK3, SP7, RANK
Xiong <i>et al.</i> [74]	2009	<i>Am J Hum Genet</i>	Caucasian in US	US white and Chinese, African and FOS data	ADAMTS18, TGFB3
Cho <i>et al.</i> [75]	2009	<i>Nat Genet</i>	Korean subjects	Korean subjects	sFrp4
Rivadeneira <i>et al.</i> [27]	2009	<i>Nat Genet</i>	Meta analysis	Meta analysis	ESR1, GPR177, MEF2C
Kung <i>et al.</i> [76]	2010	<i>Am J Hum Genet</i>	Asian in Hong Kong	Asian, Chinese and FOS and Twins UK data	JAG1
Urano <i>et al.</i> [77]	2010	<i>Bone</i>	Japanese postmenopausal women	Japanese women	WDSOF1
Kou <i>et al.</i> [78]	2010	<i>PLoS One</i>	Japanese subjects	Japanese subjects	FONG
Urano <i>et al.</i> [79]	2012	<i>J Clin Endocrinol Metab</i>	Japanese postmenopausal women	Japanese women and FOS data	GPR98
Estrada <i>et al.</i> [80]	2012	<i>Nat Genet</i>	Meta analysis	Meta analysis	LRP5, FAM210A, SLC25A13, DKK1
Medina-Gomez <i>et al.</i> [81]	2012	<i>PLoS Genet</i>	Meta analysis	Meta analysis	Wnt16
Zheng <i>et al.</i> [82]	2012	<i>PLoS Genet</i>	Meta analysis	Meta analysis	Wnt16
Zhang <i>et al.</i> [30]	2014	<i>Hum Mol Genet</i>	Meta analysis	Meta analysis	ESR1, SMOC1, CLDN14
Moayyeri <i>et al.</i> [31]	2014	<i>Hum Mol Genet</i>	Meta analysis	Meta analysis	ESR1, Wnt16, DKK1, TMEM135

duction in osteoblasts [79].

Denosumab is a monoclonal anti-RANKL antibody that inhibits the binding of RANKL to RANK, decreasing osteoclastogenesis and bone resorption of mature osteoclasts. Denosumab is currently used to treat osteoporosis and is a highly effective treatment for osteoporosis [85]. Inhibitors of SOST and DKK1 have been developed and seem to be promising drugs for the treatment of osteoporosis [86]. GPR98/Gpr98 is a member of the G-protein coupled receptor (GPCR) family [79]. The GPCR family represents one of the largest drug-gable target families in the human genome [87]. The identification of the ligands of GPR98/Gpr98 may be an important step in developing new drugs for osteoporosis. Thus, GWAS will be useful in the design of therapies for as well as the diagnosis of osteoporosis.

GWAS related to sarcopenia and lean mass

Loss of skeletal muscle function and mass is a major disorder in geriatric medicine and is related to diseases such as sarcopenia, osteoporotic fracture, and frailty

[8-10]. Sarcopenia is a common skeletal disease characterized by low muscle mass, leading to decreased skeletal strength and increased susceptibility to falls and osteoporotic fractures [9, 10]. Measuring lean body mass by DXA is a good index for the quantity and quality of skeletal muscles [88]. Low lean body mass has a strong genetic component, with heritability ranging over 50% [11, 12]. However, the specific genes underlying the variation in low lean body mass are largely unknown. In 2009, Liu *et al.* reported a GWAS on lean body mass variation, using Affymetrix 500K SNP arrays [89]. In this report, two SNPs within the thyrotropin-releasing hormone receptor gene (*TRHR*) were significantly associated with low lean body mass. These associations were replicated in three independent samples. Subsequently, several reports have identified the SNPs and genes that are related to the low lean body mass phenotype (Table 3) [89-93].

In 2014, we reported a large-scale association study for low lean body mass using Affymetrix 250K SNP arrays in Japanese postmenopausal women [93]. The association of an SNP (rs12409277) located in the

Table 3 Genome-wide association studies in lean body mass.

Study group	Year	Journal	Participants	Parameters	Identified genes
Liu <i>et al.</i> [89]	2009	<i>Am J Hum Genet</i>	Caucasian	Total body lean mass measured by DXA	TRHR
Hai <i>et al.</i> [90]	2012	<i>J Hum Genet</i>	Chinese population	Total body lean mass measured by DXA	gremlin1
Guo <i>et al.</i> [91]	2013	<i>Hum Genet</i>	Han Chinese adults and US Caucasians	Appendicular lean mass measured by DXA	GLYAT
Ran <i>et al.</i> [92]	2014	<i>PLoS One</i>	Chinese population	Appendicular lean mass measured by DXA	GIMAP1, SERHL
Urano <i>et al.</i> [93]	2014	<i>Aging Cell</i>	Japanese postmenopausal women	Total body lean mass measured by DXA	PRDM16

5'-flanking region of the PRD1-BF-1-RIZ1 homologous domain containing protein 16 gene (*PRDM16*) was further confirmed in replication study with postmenopausal Japanese women comprised of 1081 subjects. *PRDM16* plays an important role in the control of differentiation of the brown fat lineage from a progenitor that expresses myoblast markers [94]. This functional SNP can affect the transcriptional activity and protein-DNA interaction in the promoter/enhancer region of *PRDM16* [93]. Together with previous reports, our results suggest that the amount of *PRDM16* could regulate not only brown adipose tissue metabolism, but also white adipose tissue and muscle cell metabolism.

GWAS related to obesity and fat mass

Obesity is a key feature of metabolic diseases such as type 2 diabetes and cardiovascular disease [13]. Twin and adoption studies have shown that genetic factors play a role in the obesity phenotype [14, 16]. With the development of high-throughput genotyping techniques and the implementation of GWAS, common variants have been associated with obesity and body mass index (BMI). In 2007, three independent studies identified a strong association between genetic variance within fat mass and obesity-associated gene (*FTO*) and human obesity [95-97]. Subsequently, replication studies and meta-analysis suggested a positive association between *FTO* gene polymorphisms and overweight/obesity risk among children, adolescents, and the general population [98]. Moreover, studies in mice and cellular models revealed that the perturbation of *FTO* enzymatic activity dysregulated genes related to energy metabolism, causing the disruption in the energy and adipose tissue homeostasis [98]. In addition to *FTO*, GWAS have identified at least 75 obesity

susceptibility loci [99-104].

Given the increasing prevalence of obesity and the aging of the population, understanding the factors associated with obesity in old age is critical. Recently, we performed a search for SNPs associated with body fat percentage using GWAS and undertook a concurrent replication study and animal study [105]. Baseline examinations were conducted in 251 (first-stage analysis), 499 (second-stage analysis), and 732 (additional analyses) Japanese postmenopausal women (mean age $\pm$ standard deviation: 66.5 ± 9.4 years). In this report, we focused on an SNP of *SLC25A24* that showed the lowest significant *P* value obtained from multiple comparison tests among Japanese postmenopausal women. A significant association was also confirmed between *SLC25A24* SNPs and body mass index in the 1482 Japanese postmenopausal women. Compared with wild-type mice, *Slc25a24*-knockout mice had significantly lower body weights, liver weights and white adipose tissue weights. Adipocyte differentiation and lipid accumulation was inhibited in *Slc25a24*-knockout adipose tissues and *Slc25a24*-knockdown adipocytes. These data indicated the importance of *SLC25A24*/*Slc25a24* in the regulation of body fat mass and adipogenesis, in both humans and mice [105].

Disclosure statements

All authors have nothing to declare.

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