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Review

Genetics of osteoporosis

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ABSTRACT

Osteoporosis is a skeletal disease characterized by low bone mineral density (BMD) and microarchitectural deterioration of bone tissue, which increases susceptibility to fractures. BMD is a complex quantitative trait with normal distribution and seems to be genetically controlled (in 50–90% of the cases), according to studies on twins and families. Over the last 20 years, candidate gene approach and genome-wide association studies (GWAS) have identified single-nucleotide polymorphisms (SNPs) that are associated with low BMD, osteoporosis, and osteoporotic fractures. These SNPs have been mapped close to or within genes including those encoding nuclear receptors and WNT-β-catenin signaling proteins. Understanding the genetics of osteoporosis will help identify novel candidates for diagnostic and therapeutic targets.

Contents

1. Introduction

Osteoporosis, a skeletal disease characterized by low bone mineral density (BMD) and microarchitectural deterioration of bone tissue, leads to decreased skeletal strength and increased susceptibility to fractures [1]. Osteoporosis and osteoporotic fractures are strongly associated with mortality and morbidity in developing as well as developed countries [2].

BMD is a complex quantitative trait with normal distribution and is thought to be genetically controlled (in 50–90% of the cases), according to twin and family studies [3–6]. Variations in BMD are associated with polymorphisms in several genes [6]. In this review, we have briefly summarized current literatures on genetic factors that are specifically associated with the pathogenesis of osteoporosis and fractures.

2. Polymorphisms in nuclear receptor genes and osteoporosis

Among the several candidate genes, the vitamin D receptor (VDR) gene encoding a nuclear hormone receptor was the first to be proposed as a major locus for its genetic control of BMD. In 1994, a single nucleotide polymorphism (SNP) in intron 8 (IVS8 + 284A > G, rs1544410) of the *VDR* gene was reported to be associated with BMD [7]. The VDR plays an important role in regulating calcium homoeostasis through the binding of the ligand $1\alpha,25(OH)_2D_3$, increasing the absorption of calcium [8]. Several SNPs within the *VDR* gene are associated with variations in BMD,

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and fractures [9]. An Australian study based on twins and the general population demonstrated that SNPs in intron 8 and the 3′-untranslated region (3′-UTR) were associated with BMD in a twin and general population study from Australia [7]. The presence of the homozygous G allele (bb genotype) characterized by the absence of the restriction site for *Bsm*I endonuclease is related to the highest BMD values, while the A allele (BB genotype) is typical in women with a BMD value below the threshold for risk of osteoporotic fractures. Three meta-analyses incorporating the results from major VDR studies have also confirmed the contribution of the IVS8 + 284A > G polymorphism (*Bsm*I) to the variation in BMD values [9–12].

Estrogen deficiency is another risk factor for postmenopausal osteoporosis [13]. Two estrogen receptors (ESRs), namely ESR1 (ERα) and ESR2 (ERβ), encoded by different genes, have been described in mammals [14–18]. ERα primarily mediates the action of estrogen in the bone [19–21]. Genetic screening of the *ESR1* gene locus revealed the existence of several polymorphic sites [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 *Pvu*II endonuclease) in the *ESR1* gene [23]. Subsequently, many other studies have also demonstrated the role of the TA VNTR of the *ESR1* promoter region, IVS1 − 397T > C SNP, and IVS1 − 351A > G SNP (rs9340799, detected by *Xba*I endonuclease), in BMD [21,24]. These two SNPs that lie in the introns of *ESR1* are in strong linkage disequilibrium. Those SNP alleles P and X (characterized by the absence of the restriction sites) as well as alleles p and x (with the presence of the 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, indicating that the disequilibrium is not complete. The IVS1 − 397T > C transition associated with the loss of the *Pvu*II site (P allele) results in a potential binding site for the transcription factor *myb*, followed by *in vitro* transcriptional changes, indicating that the presence of the P allele may amplify *ESR1* transcription [25]. VNTR polymorphisms in the vicinity of certain gene promoters can have a significant impact on transcriptional regulation [26]. Allelic variation arising from different TA repeat lengths can also affect promoter activity.

3. Genetic regulation of bone metabolism by WNT signaling genes

The WNT signaling pathway plays an important role in cell proliferation, differentiation, morphogenesis, and oncogenesis [27–29]. Studies using Drosophila, Xenopus, and mammalian models have established the canonical signaling pathway in which WNT proteins bind Frizzled (FZ) proteins and inhibit glycogen synthase kinase 3 (GSK3)-dependent phosphorylation and stabilization of β-catenin. Evidence from both genetic and biochemical experiments indicates that FZ proteins function as WNT receptors. In addition, low-density lipoprotein receptor-related proteins 5 and 6 (LRP5 and LRP6) also act as WNT co-receptors in the WNT-β-catenin signaling pathway (Fig. 1A). In 2001, Gong et al. reported that the WNT-β-catenin signaling pathway plays a pivotal role in regulating bone density through LRP5 [30]. The authors show that inactivating mutations in the human *LRP5* gene decrease the bone mass and cause an autosomal recessive disorder called osteoporosis-pseudoglioma syndrome (Fig. 1B). Moreover, activating mutations in *LRP5* lead to autosomal-dominant high bone mass traits (Fig. 1C) [31,32]. These data suggest that LRP5 controls the *in vivo* bone metabolism in humans. Additional studies have also reported that mutations in *LRP5* lead to osteoporosis-pseudoglioma syndrome and autosomal-dominant high bone mass traits (Fig. 2) [33,34].

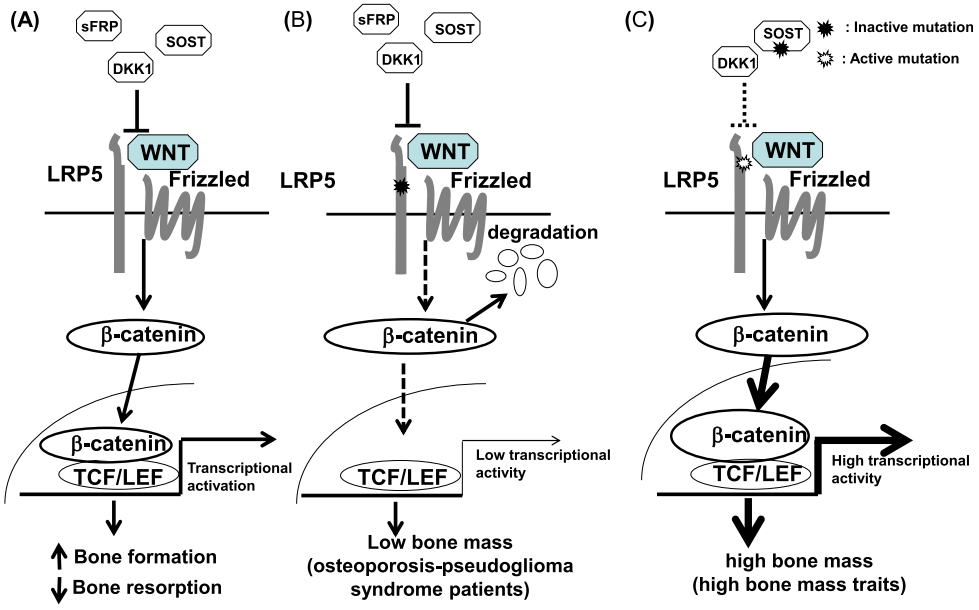

Fig. 1. Canonical WNT signaling pathway in the bone metabolism. (A) Binding of canonical Wnt ligands to a dual-receptor complex comprising the WNT co-receptor LRP5 and one of the seven transmembrane receptors of the Frizzled family initiates WNT-β-catenin signaling. The activation of WNT signaling inhibits the phosphorylation of β-catenin and its proteosomal degradation. β-Catenin accumulates in the cytoplasm and translocates into the nucleus, where it associates with members of the TCF/LEF transcription factors. Activation of the canonical WNT signaling pathway affects the entire osteoblastic lineage and increases bone formation. WNT-β-catenin signaling in osteoblasts and osteocytes indirectly represses osteoclast differentiation and bone resorption. Sclerostin (SOST), dickkopf 1 (DKK1), and secreted Frizzled-related protein (sFRP) act by inhibiting the interaction between the Frizzled family members, LRP5 and WNT. (B) In patients with osteoporosis-pseudoglioma syndrome, loss of function mutations in the *LRP5* gene lead to the destabilization of β-catenin and decrease in bone formation. (C) In high bone mass traits, gain of function mutations in the *LRP5* gene prevent the inhibition of WNT signaling by DKK1, resulting in increased WNT-β-catenin signaling and bone formation. Inactivating mutations of *SOST* also present with high bone mass traits. SOST: Sclerostin, DKK1: dickkopf 1, SFRP: secreted Frizzled-related protein.

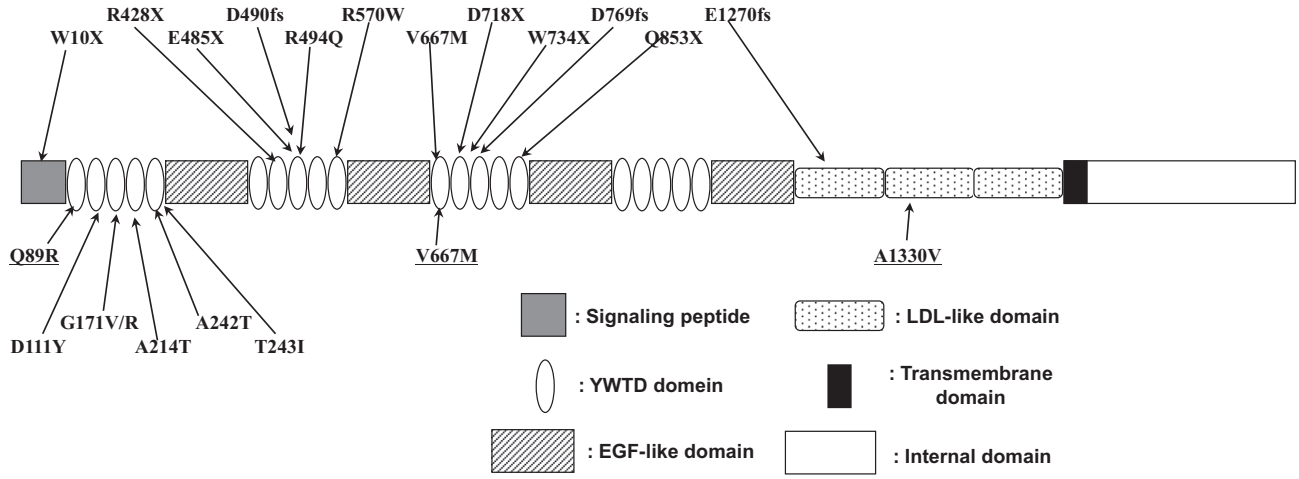

Fig. 2. Protein structure, mutations, and non-synonymous polymorphisms in the *LRP5* gene. The mutations found in patients with the osteoporosis-pseudoglioma syndrome are denoted above the peptide. The mutations (underlined) found in autosomal-dominant high bone mass traits are located below the peptide. The non-synonymous polymorphisms (underlined) that are significantly associated with osteoporotic phenotypes are located below the peptide.

Table 1
Association studies between SNPs and osteoporotic phenotypes.

Study group	Year	Journal	Participants	Parameters
(A) Association studies between the A1330V SNP in the LRP5 gene and bone mineral density (BMD)				
Urano et al. [35]	2004	J. Bone Miner. Metab.	Japanese postmenopausal women	Total body BMD
Mizuguchi et al. [37]	2004	J. Hum. Genet.	Japanese women (over 50 y.o.)	Lumbar BMD
Bollerslev et al. [38]	2005	Bone	Australian elderly women	Femoral neck and trochanter BMD
Ferrari et al. [39]	2005	Bone	Caucasian men	Femoral neck and trochanter BMD
vanMeurs et al. [40]	2006	J. Bone Miner. Res.	Caucasian men (over 55 y.o.)	Lumbar spine and femoral neck BMD
Saarinen et al. [41]	2007	Bone	Finnish young men	Femoral neck and trochanter BMD
Ezura et al. [36]	2007	Bone	Japanese women	Lumbar spine and radial BMD
Giroux et al. [42]	2007	Bone	French–Canadian women	Lumbar spine and femoral neck BMD
vanMeurs et al. [43]	2008	JAMA	Caucasian in Europe and North America	Femoral neck BMD and osteoporotic fractures
Richards et al. [44]	2008	Lancet	Caucasian (genome-wide association study)	BMD and osteoporotic fractures
Urano et al. [45]	2009	Endocr. J.	Japanese postmenopausal women	Total body BMD

Study group	Year	Journal	Participants in 1st screening	Participants in replication study	Identified genes
(B) Genome-wide association studies in bone mineral density and osteoporotic fracture					
FOS [66]	2007	BMC Med. Genet.	American Caucasian	–	
TwinsUK [44]	2008	Lancet	Caucasian twins	RS, British twins, British population	LRP5, RANKL
deCODE1 [67]	2008	N. Engl. J. Med.	Icelandic subjects	Icelandic, Danish, and Australian subjects	RANKL, OTB, ESR1, RANK
deCODE2 [68]	2009	Nat. Genet.	Icelandic subjects	European descent	SOST, MARK3, SP7, RANK
– [69]	2009	Am. J. Hum. Genet.	Caucasian in US	US white and Chinese, African and FOS data	ADAMTS18, TGFBR3
KARE [70]	2009	Nat. Genet.	Korean subjects	Korean subjects	sFrp4
GEFOS [71]	2009	Nat. Genet.	Meta analysis	Meta analysis	GPR177, MEF2C
HKOS [72]	2010	Am. J. Hum. Genet.	Asian in Hong Kong	Asian, Chinese and FOS and Twins UK data	JAG1
NCS [73]	2010	Bone	Japanese postmenopausal women	Japanese women	WDSOF1
– [74]	2010	PLoS One	Japanese subjects	Japanese subjects	FONG
NCS [75]	2012	J. Clin. Endocrinol. Metab.	Japanese postmenopausal women	Japanese women and FOS data	GPR98

FOS, Framingham Osteoporosis Study; KARE, Korean Association Resource study; ERF, Erasmus Rucphen Family Study; RS, Rotterdam Study; HKOS, Hong Kong Osteoporosis Study; NCS, Nagano Cohort Study.

In 2004, we first reported that a common SNP in the intron 17 of the *LRP5* gene affects BMD [35], and that the IVS17 − 1677C > A and the A1330V SNPs are in strong linkage disequilibrium (D' = 0.99, r^2 = 0.98) [36]. Thereafter, we, along with several other groups, demonstrated that a single SNP [caused by an amino acid change (3989C > T, A1330V)] located in the exon 18 of the *LRP5* gene, is associated with BMD and the incidence of fractures (Table 1A) [37–45].

In addition to the A1330V SNP, 2 non-synonymous variations, Q89R and V667M, are also association with bone metabolism. In the Caucasian population, the V667M polymorphism is strongly associated with BMD [46]. To our knowledge, V667M studies in the Asian population have never been reported, possibly due to the low minor allele frequency of this SNP in this population. On the other hand, the Q89R polymorphism has been studied only in the Asian population, most likely because of its low frequency in other ethnic groups. Q89R is associated with femoral neck BMD in young Korean men [47] and postmenopausal Chinese women [48]. Q89R is also associated with osteophyte formation in Japanese postmenopausal women according to our study [49]. In the Korean and Chinese population, strong linkage disequilibrium was observed between the Q89R and A1330V SNPs. In contrast, we did not observe linkage disequilibrium between these two SNPs in the Japanese population (D' = 0.65, r^2 = 0.08) [36].

Results from *in vitro* and *in vivo* experiments suggest that activation of the canonical WNT signaling pathway affects the entire osteoblastic lineage and increases bone formation. WNT-β-catenin signaling in osteoblasts and osteocytes indirectly represses osteoclast differentiation and bone resorption (Fig. 1A). These data suggest that the A1330V variation in the *LRP5* gene may affect the pathogenesis of osteoporosis by decreasing the WNT activity. To evaluate the functional significance of the alanine to valine

variation at codon 1330 in *LRP5*, we substituted the alanine at codon 1330 in the major-type *LRP5* (LRP5-A1330) with valine (LRP5-V1330) [45]. These *LRP5* constructs were then transfected in HEK293T cells and the TCF-Lef reporter activity was measured in response to the canonical WNT signaling. The WNT-induced TCF-Lef activity was significantly reduced in the cells containing the LRP5-V1330 compared to those containing the major-type allele. In the case of cells not treated with WNT, the TCF-Lef activity of cells transfected with LRP5-V1330 was not significantly changed compared to that of the LRP5-A1330 transfection. The A1330V polymorphism lies within the LDL repeat domain of *LRP5* (Fig. 2). Although the function of this region is still unclear, similar domains in the LDL receptors are known to be involved in ligand binding. Considering our data, it is possible that variations in the LDL repeat domain will alter the ligand binding and signaling of LRP5.

4. Methylenetetrahydrofolate reductase (MTHFR) polymorphisms in fractures

According to the definition of osteoporosis proposed by the National Institutes of Health, bone strength is determined by the BMD and bone quality [1]. Among these two components, BMD is a major determinant of the risk of future fractures. In fact, the measurement of BMD is utilized to diagnose osteoporosis [1,2]. In contrast, the assessment of bone quality is not applied in clinical practice, except for the measurement of bone turnover markers, which is employed to assess the risks of future fractures. Methylenetetrahydrofolate reductase (MTHFR), an important enzyme in the methionine cycle [50], is involved in the removal of circulating homocysteine and lies within a linkage region on chromosome 1p36 that is associated with the regulation of BMD [51,52]. A non-synonymous and functional polymorphism has been identified in exon 4 of the *MTHFR* gene that results in an alanine to valine change at codon 677 (C677T). The T-allele variant (valine type) has a lower MTHFR activity than the wild type (C-allele or alanine type) [50]. The T-allele variant is also associated with moderately elevated homocysteine levels [50], which has been recently recognized as a significant risk factor for fractures [53–55]. This risk for fractures is independent of age and BMD, because it is caused by the inhibition of collagen cross-links during bone calcification [56–58]. Nevertheless, clinical evidence regarding the role of MTHFR C677T polymorphism in BMD remains controversial. We previously reported that in Japanese postmenopausal women, MTHFR C677T polymorphism alone is a weaker risk factor for future fractures compared to the traditional risk factors [59,60]. However, patients bearing the TT genotype together with low BMD show higher risk and earlier occurrence of fractures than the other groups, indicating that the MTHFR TT genotype may act synergistically with the traditional risk factors that lead to fractures. A similar trend was reported in the Caucasian population, which showed a slight increase in the risk of hip fractures in the TT group compared with in the CC group. The patients bearing the TT genotype showed an increased risk of fractures when their plasma folate levels were low [54]. Lower plasma folate levels lead to an increase in the plasma levels of homocysteine, which is assumed to be a potent risk factor for hip fractures independent of BMD [53–55]. These results suggest a strong association of the effects of MTHFR C677T polymorphism on homocysteine and bone quality with the risk of fractures.

5. Genome-wide association studies related to low bone mineral density and fractures

Although the genetics of osteoporosis has been studied through various association studies, the major susceptibility genes and the molecular mechanisms responsible for this disease remain unclear. The identification of novel candidate genes that contribute to osteoporotic susceptibility will impact the diagnosis and treatment of this disorder. Rapid technological advances have facilitated the development of large-scale genome-wide association studies (GWAS) [61,62]. GWAS is an unbiased approach that involves scanning the entire genome to identify novel genes/genome regions with modest effects on complex diseases/traits [63–65]. A number of GWAS have uncovered novel SNPs associated with complex diseases/traits, including osteoporosis and BMD in Caucasian and Asian populations (Table 1B) [44,66–75].

GWAS for osteoporosis have identified the following candidate genes in the WNT-β-catenin signaling pathway [66,67,70–72,76]: catenin β1 (*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* [27–29,76]. Among these, WNT4, WNT5B, and WNT16 function as ligands while SOST, DKK1, and sFRP4 suppress the WNT-β-catenin signaling by inhibiting the interaction between the Frizzled family members, LRP5 and Wnt (Fig. 1A) [27–29,76]. MEF2C

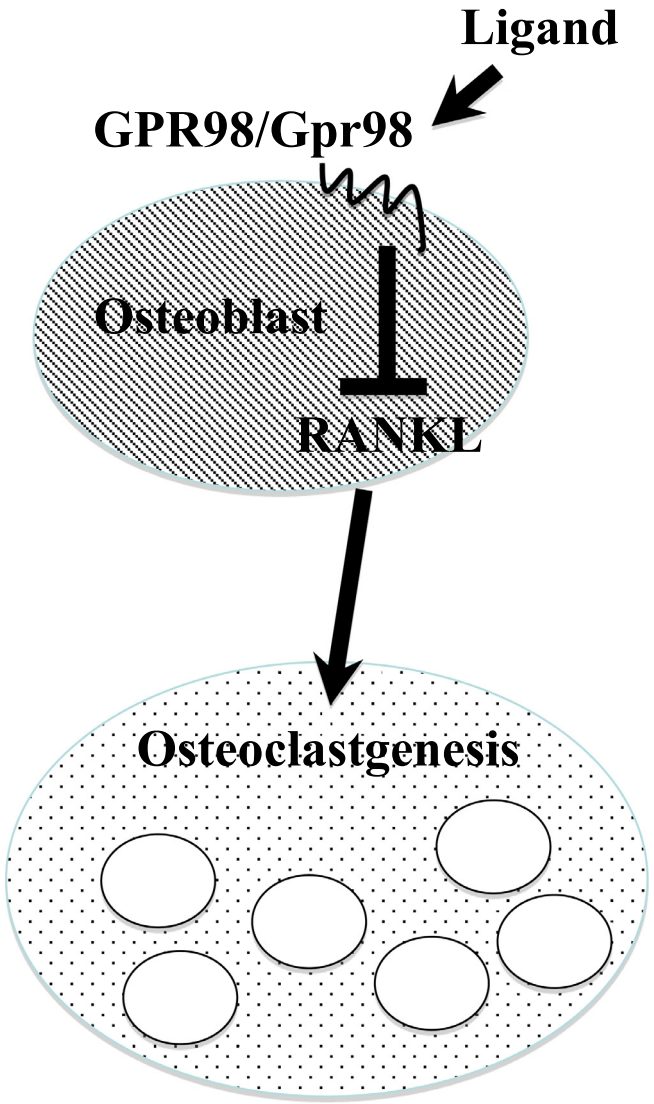

Fig. 3. A role of GPR98/Gpr98 in the bone metabolism. GPR98/Gpr98 is an orphan receptor, which belongs to the G-protein coupled receptor (GPCR) family. GPR98/Gpr98 could suppress the expression of RANKL in osteoblasts, leading to inhibition of the osteoclastgenesis.

potentiates SOST expression and JAG1 is a target of β-catenin transcriptional control [27–29,76]. GWAS for osteoporosis have also identified three important factors, namely, RANK, RANKL, and OPG in the bone resorption cycle [44,66,67,76]. Osteoblasts and osteocytes secrete a soluble factor called RANKL [77] 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 reported GWAS of BMD in Japanese postmenopausal women [73,75]. In these reports, we identified the association of the non-synonymous SNPs in the *WD repeats and SOF1 domain-containing* (*WDSOF1*) gene [73] and the SNP in 3′-flanking region of the *GPR98* gene [75] with BMD. We then explored 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 production in osteoblasts (Fig. 3) [75]. In addition to loss of BMD, loss of lean mass is a common disorder in the elderly. It is related to a series of diseases such as sarcopenia, osteoporosis, and frailty [78]. Thus, we expanded our study to GWAS of lean body mass in Japanese postmenopausal women [79]. We had discovered a significant association between a functional SNP in the *PRDM16* gene and low lean body mass. The PRDM16 plays an important role in the control of differentiation of the brown fat lineage from a progenitor that expresses myoblast markers [80]. This functional SNP can affect the transcriptional activity and protein–DNA interaction in the promoter/enhancer region of the *PRDM16* gene [79]. Together with previous reports, our results suggest that the amount of PRDM16 could regulate not only brown adipose metabolism but also white adipose and muscle cell metabolism.

Denosumab is a monoclonal anti-RANKL antibody that inhibits the binding of RANKL to RANK [81]. It decreases osteoclastogenesis and bone resorption of mature osteoclasts. Denosumab is currently used to treat osteoporosis and is a highly effective and safe parenteral therapy with good adherence for osteoporosis [81]. Inhibitors of SOST and DKK1 have been developed and seem to be promising agents for the treatment of osteoporosis [76,82]. GPR98/Gpr98 is a member of the G-protein coupled receptor (GPCR) family [75]. GPCR family represents one of the largest druggable families in the human genome [83]. The identification of the ligands of GPR98/Gpr98 may be an important step in developing the new drugs for osteoporosis. Thus, GWAS will be useful in the design of therapies as well as diagnosis for osteoporosis.

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