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# LRP Receptor Family Member Associated Bone Disease

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## Abstract

A dozen years ago the identification of causal mutations in the low-density lipoprotein receptor-related protein 5 (LRP5) gene involved in two rare bone disorders propelled research in the bone field in totally new directions. Since then, there have been an explosion in the number of reports that highlight the role of the Wnt/β-catenin pathway in the regulation of bone homeostasis. In this review we discuss some of the most recent reports (in the past 2 years) highlighting the involvement of the members of the LRP family (LRP5, LRP6, LRP4, and more recently LRP8) in the maintenance of bone and their implications in bone diseases. These reports include records of new single nucleotides polymorphisms (SNPs) and haplotypes that suggest variants in these genes can contribute to subtle variation in bone traits to mutations that give rise to extreme bone phenotypes. All of these serve to further support and reinforce the importance of this tightly regulated pathway in bone. Furthermore, we discuss provocative reports suggesting novel approaches through inhibitors of this pathway to treat rarer diseases such as Osteoporosis-Pseudoglioma Syndrome (OPPG), Osteogenesis Imperfecta (OI), and Sclerosteosis/Van Buchem disease. It is hoped that by understanding the role of each component of the pathway and their involvement in bone diseases that this knowledge will allow us to develop new, more effective therapeutic approaches for more common diseases such as post-menopausal osteoporosis, osteoarthritis, and rheumatoid arthritis as well as these rarer bone diseases.

### Keywords

LRP; Wnt/β-catenin; bone related disease

## Introduction

In the mid-1990's two independent groups, working on the seemingly unrelated low bone mass disease, Osteoporosis Pseudoglioma Syndrome (OPPG) [1] and the High Bone Mass (HBM) kindred [2] identified a common linkage region harboring the causal gene/mutations, which raised the possibility that these were conditions were allelic variants of each other [1, 2]. A few years later, both groups were able to identify the low-density lipoprotein receptor related-protein 5 (*LRP5*) gene and the mutations responsible for these bone associated traits: presumed loss-of-function mutations in *LRP5* were causative for OPPG [3] and a single

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amino acid change in the first β-propeller module of LRP5 was causal for the HBM trait [4]. At the time these mutations were identified little was known about the function of LRP5. Other studies [5] however demonstrated that LRP5 was a co-receptor for Wnt ligands regulating the Wnt/β-catenin signaling pathway. After publication of these two groundbreaking LRP5 papers the field of Wnt signaling in bone related diseases has grown exponentially [6-14]. Nowadays, LRP5 is not the only LDL receptor family members that is known to play an important role in bone homeostasis, more and more data has been collected that demonstrate an equally important role for Lrp6 [15-17] and recently, Lrp4 and Lrp8 have emerged as others receptors from the family that play important roles in bone [18-21].

Members of this family regulate the Wnt/β-catenin signaling pathway. Hence, manipulating this pathway has become a major target for developing new therapeutics to treat patients with post-menopausal osteoporosis [22-28] and other bone related diseases (for review see [29, 30]). In this review we will present the most recent reports describing the involvement of members of the LRP family in bone homeostasis, discuss newly discovered mutations in these proteins that are causative of various bone diseases, as well as address the new discoveries further illustrating the importance of these molecules in the treatment of bone diseases. We will not discuss the growing literature involving targeting of Wnt signaling pathways that do not involve LRP family members (i.e. the non-canonical pathways) or other diseases outside of bone in which modulation of this pathway may have clinical applications, such as cancer.

## The LRP family of proteins

The low-density lipoprotein receptor (LDLR) family consists of several members. Proteins in this family have characteristic features: in the extracellular domains they contain ligand binding repeats, β-propeller motifs and epidermal growth factor-like repeats. In the intracellular domains they have several domains that are responsible for downstream signaling events by interacting with cytoplasmic adaptors and scaffolds (for review see [30]). LRP5 and LRP6 are structurally related proteins and share around 71% homology at the nucleotide level [31]. LRP5 and LRP6 are type I transmembrane receptors (C-terminus in cytosol); at the extracellular domain they have four YWTD β-propellers, four EGF-like domains, and LDLR type A domains and at the intracellular domain they have five PPPSP motifs. The structural organization of LRP4 and LRP8 is markedly different from Lrp5/6 [30]. Lrp4 is a type II transmembrane receptor (N-terminus in cytosol) and it belongs to the LRP subfamily III along with LRP5 and LRP6 [32]. Similar to LRP 5/6 it has four β-propeller motifs and four epidermal growth factor-like repeats; unlike LRP 5/6 proteins, LRP4 has a NPxY motif in the cytosolic domain. LRP8 is also known as apolipoprotein E receptor 2, it belongs to the LRP subfamily I along with LDLR and VLDLR. The difference between LRP5/6 is that LRP8 only contains one β-propeller, and like LRP4, it has one NPxY motif in the intracellular domain [30].

Binding of the Wnts, ligands for these receptors, to LRP5 or LRP6 (LRP5/6) and their participating co-receptors, the frizzled (Fz) family of 7 transmembrane spanning proteins, results in a series of downstream intracelullar events [5], in particular the inhibition by

subsequent phosphorylation of GSK-3β [33]. GSK-3 β is critical for the intracellular fate of β-catenin. Normally β-catenin is held in a "degradation complex" that includes a scaffolding protein, Axin, and dozens of other proteins including GSK-3β [34]. When GSK-3β is active it phosphorylates β–catenin, which leads to its ubiquitination and degradation by the 26S proteasome [35, 36]. Binding of Wnt ligand leads to both the inhibition of GSK-3β by phosphorylation and the collapse of the degradation complex, which allows free β-catenin to accumulate in the cytoplasm allowing it to translocate to the nucleus and activate targeted genes (for review see [37]).

There are several extracellular inhibitors to the pathway including Sclerostin (the product of the *SOST* gene) [38], the Dickkopf family of proteins (Dkk1-4) [39-41] along with Kremen 1 and 2 [42, 41, 43], and the secreted frizzled related proteins (sFRPs) [44, 45]. In addition the Wise protein modulates the pathway in various contexts, mainly during development [46-48]. Sclerostin and Dkk-1 have a similar mode of inhibiting activation of the pathway; both bind to β-propeller motifs inhibiting the formation of Wnt/LRP/Fz trimolecular complex. The difference is the binding site: sclerostin binds to the first or second β-propeller whereas Dkk-1 binds to the third β-propeller. Kremen acts with high affinity for Dkk and forms a ternary complex with Dkk and LRP5/6 inducing endocytosis of this formed complex and resulting of the removal of LRP5/6 from plasma membrane [49, 41, 40]. sFRP-1 and sFRP-4 inhibit the pathway by binding Wnt ligand and preventing its binding to the Lrp5/6-frizzled co-receptor complex [44].

While the involvement of LRP5 and LRP6 in bone homeostasis has been established for the past dozen years, more recently MEGF4/LRP4 and LRP8 have been shown to play a role [50, 19, 20, 18, 21, 51] Global deletion of Lrp5 ($Lrp5^{-/-}$) in mice resembles the phenotypes observed in patients with OPPG. These mice present low bone mass phenotype and a persistent embryonic eye vascularization through their adult life [52]. Kato and colleagues determined that the reason for the low bone mass phenotype in this animal model was due to a decreased osteoblast proliferation and a decrease in bone matrix deposition. They also determined that the persistent embryonic eye vascularization was due to a reduced level of macrophage-induced endothelial cell apoptosis [52]. A mouse model for the HBM trait was originally created by Babij and colleagues in 2003 [53]. These transgenic mice carry the point mutation (G171V) (HMB) in the human LRP5 cDNA under the expression of the 3.6-kb rat type I collagen promoter. Recently Warman and colleagues have generated knock-in mutations in the mouse germline harboring the original G171V HBM mutation and the A214V mutation [54]. All of these HBM mouse lines resemble the human phenotype of increase bone mass. Opposite to the $Lrp5^{-/-}$ mouse model, the HBM mice had decreased osteoblast apoptosis and no difference in the osteoclast number. The fact that Lrp5 might play a role in osteoblast proliferation and apoptosis in osteoblasts cells has recently supported by Javaheri et al (2013) [55]. In their paper they observed a sex difference in the proliferating state of the cells *in vitro*: osteoblasts-like cells from HBM female proliferated faster and presented lower apoptosis than littermate controls; and osteoblasts-like cells from $Lrp5^{-/-}$ females proliferated slower with higher apoptosis rates than littermate controls. The authors also reported that the expected increase in cell number in response to high levels of strain (3,400με) was no different between genotypes [55]. Mutation of Lrp4 caused skeletal

abnormalities. Lrp4$^{-/-}$ mice are smaller, with retardation in growth and delay in ossification; in addition, digits are fused and shortened [21]. LRP8 plays a role in Wnt3a-induced osteoblastogenesis [51].

## Involvement of the different LRPs in bone homeostasis

The specific role of LRP5 and LRP6 in bone is not entirely clear; however recent studies have begun to tease out the subtle aspects of bone regulation controlled by these co-receptors. Even though both receptors are needed for normal bone development, they have distinct roles as well. In the past year it has been established that Lrp5 and Lrp6 control osteoblast differentiation at different stages. Lrp6 is needed for early stages of differentiation, whereas Lrp5 is required for late stages of differentiation. Riddle et al (2013) studied the deletion of Lrp5 and Lrp6 in osteoblasts [17]. In mice where Lrp5 was deleted specifically in osteoblasts (using an Osteocalcin Cre model) bones appeared normal up to 8 weeks of age (equal number of osteoblasts and osteoclasts). Bone accrual after this age started to present with decreased mineralizing surface per bone surface, decreases in bone formation and an increase in mineralization lag time. The authors concluded that Lrp5 function can be compensated during the period of rapid bone growth but it is required for proper osteoblast function later in postnatal life. Deleting Lrp6 in osteoblast had an impact on the trabecular compartment as at 4 month of age these mice did not reach peak trabecular bone mass. Both Lrp5 and Lrp6 mutants had decreased cortical bone area, cortical thickness and polar moment of inertia by 24 weeks of age. Deletion of both co-receptors led to an early death with mice suffering osteopenic phenotype. Osteoblasts isolated from calvaria differentiated into chondrocytes in *in vitro* osteoblast differentiation studies [17]. Conditional deletion of Lrp5 in osteocytes resulted in smaller and weaker bones with a compromised quality of cortical bone. It also resulted in diminished, but not abolished, new bone formation in response to mechanical loading [56]. Recently Lrp8 has been shown to play a role in Wnt3a-induced osteoblast differentiation (*in vitro* studies only), but no human related bone diseases have been reported at this point in time [51].

## LRP mutations in bone related diseases

### LRP and Osteoporosis

Ever since its original description [3, 4] various research laboratories around the world have attempted to identify *LRP5* variants that can predict the incident of osteoporosis [6, 9-11, 13, 14]. Recently, Falcón-Ramirez et al (2013) studied the allelic and genotypic frequencies of four polymorphic markers (single nucleotide polymorphisms (SNPs) (C/T rs3736228, G/A rs4988321, T/C rs627174 and T/C rs901824) in the *LRP5* gene in 100 post-menopausal osteoporotic Mexican women. When genotype frequencies of these four SNPs were studied only the G/A polymorphism (rs4988321, Val667Met) showed differences between the osteoporotic and the control subjects. This led them to conclude that this SNP has a significant association with osteoporosis in this population. An equally interesting finding was that when the authors analyzed the haplotypes of these polymorphisms, there was a clear association between osteoporosis in two haplotypes: the CGTT haplotype was related with lower risk of osteoporosis; and the TACT haplotype was significantly associated with higher risk of osteoporosis [57].

In a study by Sassi et al. (2014) the incidence of Ala1330Val (rs3736228) and Val667Met (rs4988321) polymorphisms in Tunisian women with osteoporosis was examined. They found that Ala1330Val could be potentially associated with lower spine density in post-menopausal Tunisian women. They discussed that this was aligned with other researchers finding where they determined association of A1330V with lumbar spine BMD in Asians. However, in Caucasian populations the association was observed in both spine and femoral neck BMD [58]. Park SE et al (2014) found an association with LRP5 rs599083 and femoral neck BMD in Korean men [59]. Campos-Obando N. (2014) reported a single case of a 27-year old patient who during her 7-month of pregnancy who obtained a mid-thoracic pain after lifting an object. DNA analysis determined two compound heterozygous missense mutations in the LRP5 gene [60]. This raises the question that although fractures and osteoporosis during pregnancy are not common, should patients with a family history of osteoporosis be genetically screened before conception? Recently Boudin and colleagues reported that mutation in the LRP4 was associated with osteoporosis. They found an association between rs2306029 and rs6485702 and BMD at all sites except in the lumbar spine [50].

## Lrp5 and Osteoarthritis

Several genome-wide scans for osteoarthritis-susceptibility genes have shown that there is a linkage to chromosome 11q12-13 in the region where the LRP5 gene locus is located. This strongly suggested that LRP5 might be involved in the pathogenesis of osteoarthritis (OA) [61-63]. After that report several researchers have found mutations in *LRP5* in patients with OA. In 2005 Smith et al reported that a common haplotype in the Lrp5 gene was associated with a 1.6 fold increase risk of knee OA in two Japanese cohorts [64]. In 2007 Urano *et al* reported a single polymorphism in the Lrp5 (Q89R) associated with spinal osteoarthritis in Japanese women. Yerges-Armstrong et al (2014) reported that the rs3736228 variant in the LRP5 gene (along with other 3 SNPs in other genes) was associated with increased risk of radiographic knee osteoarthritis in the Osteoarthritis Initiative and the Johnston County Osteoarthritis Project [65]. Lrp6 might be also playing an important role in OA progression. Joiner D et al (2013) presented data suggesting that deletion of one allele of Lrp6 can aggravate the progression of the condition [16]. Whether any of these *LRP5/6* polymorphisms are causally related to the development of OA is unclear at this time. Global deletion of LRP5 (where LRP6 is still present) results in an increase in cartilage degradation in the destabilization of the medial meniscus (DMM) model. It is well known that $LRP5^{-/-}$ mutants have lower bone mass [66]. So results in this study were unexpected since in humans it has been reported that there is an inverse relationship between osteoarthritis and osteoporosis [67].

## LRP5 in Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic autoimmune disease in which the etiology is still unknown. Recently the involvement of the canonical Wnt/β-catenin signaling has been described in the pathology of RA (for review see [68]). Diarra and collegues reported that Dkk-1 was involved in the bone-destructive pattern observed in a mouse model of rheumatoid arthritis. Furthermore, they identified tumor necrosis factor-α (TNFα) as a key regulator of the Wnt pathway. In a search for variants in genes encoding DKK1, Sclerostin,

Kremen-1 and Lrp-5, de Rooy and colleagues screened 600 patients with RA in cohorts from Groningen (NL), Sheffield (UK) and Lund (Sweden) searching for single nucleotide polymorphisms (SNPs). In the Groningen (NL) cohort they found six DKK-1, three SOST, one Kremen-1 and ten LRP-5 SNPs were significantly associated with radiological progression of joint destruction. After performing a meta-analysis, three DKK-1 SNPs (rs1896368, rs1896367 and rs1528873) were associated with progression of joint damage and two Sost SNPs trended to significance (rs4792909 and rs6503475). They determined that RA patients bearing mutations in the DKK-1 gene had higher levels of DKK-1 and this was correlated with progression of the disease [69].

## LRP5 and the Treatment of Bone Disease

Understanding the molecular aspects of the Wnt/β-catenin signaling pathway, and its regulation, has allowed scientists to develop new pharmaceutical approaches for treatment of low bone mass related diseases. Blockage of the natural inhibitors of the Wnt/β-catenin signaling pathway is receiving considerable attention and pharmaceutical developmental effort as a therapeutic option for the treatment for osteoporosis. Anti-sclerostin antibodies are the most advanced in the drug development pipeline and are being developed to increase bone formation in post-menopausal osteoporotic and osteopenic women [23, 26, 70]. In addition to the development of anti-sclerostin antibodies as a therapeutic, small molecule inhibitors that target sclerostin are being developed to circumvent the potential immunological reactions observed in some patients treated with the anti-sclerostin antibodies [30].

Arguably one of the more novel advancements in the field of pharmacological treatment of bone diseases by targeting the LRP5 signaling pathway in the past year has been attempts in animal models to treat diseases such as Osteoporosis-Pseudoglioma Syndrome (OPPG) and Osteogenesis Imperfecta (OI) with the anti-sclerostin antibody. OPPG is a genetic disease and it is well established that mutation in the LRP5 gene leads to this condition [1, 32, 4, 10, 14, 13, 7-9]. It is characterized by visual loss and severe juvenile osteoporosis. However, two independent groups have proposed new strategies for OPPG treatment [71, 72]. Kedlaya and colleagues (2013) showed that by crossing $Lrp5^{-/-}$ mouse (a model for OPPG) with the $Sost^{-/-}$ mouse (a model of sclerosteosis) they were able to improve bone density and quality of the bones in the $Lrp5^{-/-}$ mice. They also treated $Lrp5^{-/-}$ mice with an anti-sclerostin antibody for 3 weeks. Interestingly, the $Lrp5^{-/-}$ responded positively to the antibody treatment with increased bone mineral density, content and bone formation rates, which the authors attributed to a redundant level of function associated with the presence of Lrp6 in this animal model. The approach of regulating sclerostin function has further implications as a potential strategy for treating other sclerosing bone diseases.

Other groups have studied the effect of LRP5 manipulation treatment in Osteogenesis Imperfecta (OI)[73, 74]. OI is a genetic disorder in which a defective collagen is produced that ultimately leads to brittle bones. There are several types (degrees) of OI. Current lines of treatment include bisphosphonate, monoclonal RANKL antibody treatment, and rPTH (1-34) (teriparatide) (for review see [75]). Children with moderate to severe phenotype are currently treated with bisphosphonates. Clinicians normally follow this treatment since this

class of drugs inhibits osteoclast activity, increases bone mass, and therefore decreases bone fracture. The unfortunate aspect of bisphosphonate therapy in children is the risk of developing bisphosphonate-related osteonecrosis of the jaw [76, 77]. In addition, inhibiting osteoclast activity only means that the osteoclasts are not removing bone; while osteoblasts are still making defective collagen. In the past year, a new approach for treatment has been tested and it was determined that activation of LRP5 signaling pathway can overcome mild malfunctions of collagen assembly [73, 78, 79]. Sinder and colleagues [78] injected anti-sclerostin antibodies for two weeks in 8-week old mice that were heterozygous for the col1a1 G349C point mutation (Brtl/+ mice). They reported that this short treatment led to increased osteocalcin levels in serum, increased periosteal bone formation rates, increased mineral apposition rates, and a slight increase in bone mass (measured by microCT). However local micromechanical properties (measured by nanoindentation) were not significantly different among groups, suggesting that sclerostin antibody treatment does not improve the quality of the bone [78]. In 2014, the same group reported that treatment of 6 month old Brtl/+ mice with anti-sclerostin antibody (for 5 weeks) produced an anabolic effect and the bones were stronger [79]. Jacobsen CM. *et. al.* (2014) also tested the same concept but used a different approach. In their study two mouse models were crossed: an OI mice model ($Col1a2^{+/p.G610C}$) and a HBM mouse model ($Lrp5^{+/p.A214V}$). By doing this cross the bone properties of OI mice were highly improved. Furthermore, when OI mice were treated with anti-sclerostin antibody bone mass and strength were improved significantly [73]. However, the anti-sclerostin antibody treatment didn't have the same effect on another mouse model that had advanced (high severity) disease [74]. The difference might be due to the mouse model used as well as the length, frequency of the treatment and the source of the antibody. Roschger and colleagues used the $Col1a1^{rt/+}$ mice and they used the anti-sclerostin antibody from Novartis (Sost-ab BPS804) (100mg/kg body weight) once per week for 4 weeks. Whereas Jacobsen et al used the anti-sclerostin antibody from Amgen (Scl-AbIII) (25mg/kg) twice a week per 6 weeks. Snider et al injected the anti-sclerostin antibody from Amgen (Scl-AbVI) (25mg/kg) two times a week for 5 weeks (in the adult mice studies).

## Conclusions and Prospectives

The increasing reports describing mutations of the members of the LRP family and their relation to bone reinforces the important role of these genes/proteins in the pathogenesis of devastating diseases such as osteoporosis, osteoarthritis, rheumatoid arthritis, Osteoporosis Pseudoglioma Syndrome and Osteogenesis Imperfecta. Figure 1 aims to summarize the important role of LRP family members in bone related disease; mutations in these proteins can lead to increased or decreased bone mass as well as the points of possible pharmaceutical interventions for treatment of diseases. The field is advancing considerably but more research is needed to fully understand the LRPs/Wnt/β-catenin pathway in order to develop new therapeutic approaches to successfully improve the lives of patients afflicted with these diseases. Treatment with anti-sclerostin antibody is a promising approach on the near horizon; however, it is important to keep in mind that development of new drugs to control this pathway need to be bone tissue specific as the Wnt/LRPs/β-catenin is present and tightly regulated in nearly all cell types in the body. The anti-sclerostin antibody shows

great promise in this regard owing to the expression of sclerostin mainly in bone, which confers a high degree of bone cell targeting of this particular pharmaceutical agent. The past dozen years since the original description of the LRP5 mutations causal for OPPG and HBM has seen significant advance in our understanding of how bone mass is (mis)regulated and brought us to the doorstep of a new era of bone anabolic agents. Hopefully the next dozen years will bring us full circle to complete treatment for many bone diseases through targeting of the LRP receptor family mediated pathway.

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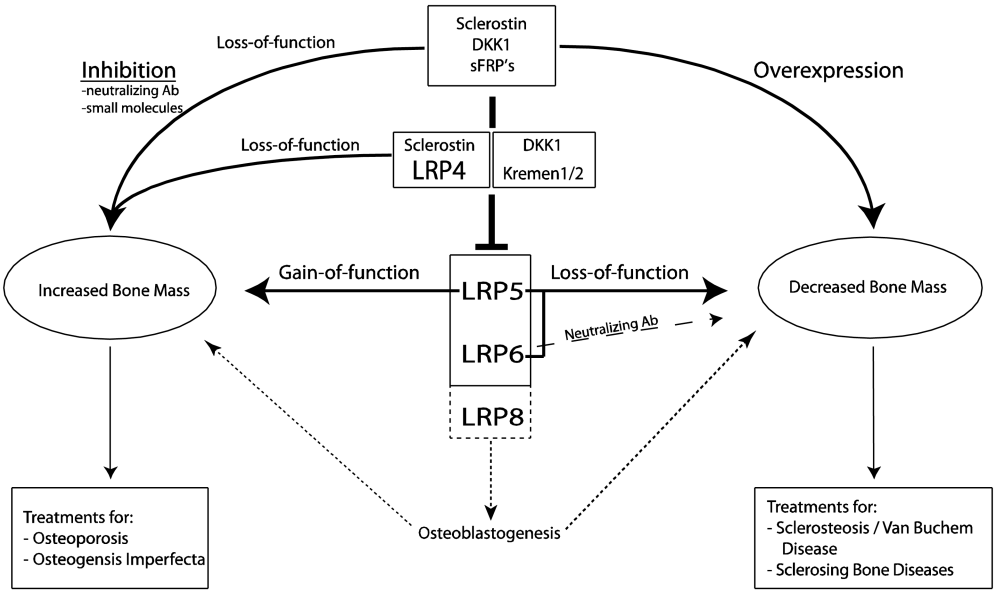


**Figure 1.**

The roles of various key members of the LRP receptor family; LRP4, LRP5, LRP6 and LRP8 in bone related traits and bone mass regulation. Gain-of-function mutations in LRP5 give rise to high bone mass phenotypes in humans and mice, whereas loss-of-function mutations in LRP5 and LRP6 give rise to decreased bone mass. Knockout or loss of function mutations of the inhibitors of the Wnt/β-catenin signaling pathway such as sclerostin, DKK1 and the sFRPs give rise to increased bone mass and overexpression of these proteins results in decreased bone mass. Sclerostin inhibition of LRP5/6 appears to be LRP4 dependent. Loss-of-function mutations in LRP4 lead to increased bone mass. Likewise, Dkk1 inhibitory function is mediated through Kremen1/2 and loss of either of these binding partners results in increased bone mass. At present it is unclear whether LRP8 is inhibited by sclerostin or Dkk1. Recent studies have shown that neutralizing antibodies to LRP6 can reduce bone mass. Treatment with neutralizing antibodies to sclerostin or Dkk1 as well as small molecule inhibitors results in increased bone mass. LRP5/6 and LRP8 appear to have roles in regulating osteoblastogenesis and therefore have the potential through this axis to either increase or decrease bone mass. Diseases such as Osteoporosis and Osteogenesis Imperfecta are envisioned as candidates diseases for treatment with agents that lead to increased bone mass, whereas diseases such as Sclerosteosis/Van Buchem's and potentially other sclerosing bone diseases are candidates for approaches that lead to decreased bone mass. See text for references