

## Review

# LRP5 and Wnt Signaling: A Union Made for Bone

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### INTRODUCTION

THE IDENTIFICATION OF mutations in the human low-density lipoprotein (LDL) receptor-related protein 5 (*LRP5*) gene that give rise to conditions of low bone mass<sup>(1,2)</sup> and increased bone mass<sup>(3-5)</sup> has brought what just a few years ago were two seemingly unrelated fields crashing together, namely bone biology and Wnt signaling. It is now clear that the Wnt signaling pathway is another key pathway involved in the regulation of bone mass. This is not to diminish the importance of any other pathway that is known to play a role; rather, this discovery only highlights how little we probably really know about bone mass regulation. Certainly, a big challenge we now face will be to integrate the Wnt signaling pathway with all of its complexities and subtleties into the already complex nature of the other pathways that have been studied, with the ultimate goal of developing a composite understanding of their collective roles and interactions.

In this article, we will summarize the main points of our current understanding of *LRP5*, Wnt's and Wnt signaling, and the role they may play in bone. This is by no means comprehensive, and there are more unanswered questions than answers.

### LDL RECEPTOR FAMILY

There are currently at least 13 known members of the LDL receptor family that serve a variety of cargo (transport) and cell signaling functions in various tissues and/or cell types.<sup>(6)</sup> All members of the family are single-pass transmembrane proteins that contain highly conserved motifs; most notably, all contain copies of a complement-like cysteine-rich repeat motif (the LDL repeat). In addition, most contain a tyrosine-tryptophan-threonine-aspartate (YWTD) amino acid repeat that forms a  $\beta$ -propeller structure critical for proper function (ligand binding). Another common feature of most members is the presence of an epidermal growth factor (EGF)-like repeat motif and an

NPxY sequence in the cytoplasmic tail that serves as a signal sequence for endocytosis of the receptor. The parental member of the family, the LDL receptor, has been well studied and characterized because of its central role in the pathophysiology of familial hypercholesterolemia.<sup>(7)</sup> The elucidation of the pathway through which the LDL receptor regulates the binding, endocytosis, and degradation of cholesterol-rich lipoproteins is a classic discovery in the fields of cell and molecular biology and has been reviewed extensively.<sup>(8)</sup> Blacklow and colleagues have extensively studied the structure of the LDL receptor in terms of the functional properties of the various motifs in the extracellular domain of the protein and how mutations observed in familial hypercholesterolemia alter receptor folding and function.<sup>(9-12)</sup> His group has also reported the crystal structure of the YWTD-EGF domain pair.<sup>(13)</sup>

### *LRP5/6* GENES AND PROTEINS

*LRP5* was cloned independently by three different groups in 1998.<sup>(14-16)</sup> In a screen for loci genetically linked to type I diabetes, evidence was obtained that the IDDM4 locus on chromosome 11q13 contained a major susceptibility gene.<sup>(17,18)</sup> Hey et al.<sup>(16)</sup> subsequently identified a novel member of the LDL receptor family, which they termed *LRP5*, as a candidate gene for the IDDM4 locus (although it now seems that mutations in *LRP5* do not contribute to the IDDM phenotype). They cloned both the human and mouse *LRP5* gene. Later this group identified a closely homologous gene to *LRP5* in human and mouse that they called *LRP6*.<sup>(19)</sup> *LRP6* is located on chromosome 12p11.2-p13.3.<sup>(19)</sup> The genomic structure of *LRP5* has been described.<sup>(20)</sup> The genomic structure contains 23 exons and spans a region of 160 kb. Kim et al.<sup>(14)</sup> and Dong et al.<sup>(15)</sup> also independently cloned *LRP5* in 1998. Kim et al.<sup>(14)</sup> identified the human and rabbit *LRP5* gene as a result of their screen for new receptors that might play a role in apo-E containing lipoproteins clearance. Dong et al.<sup>(15)</sup> identified a gene they called *LR3* from a screen of a human osteoblast cDNA library. Subsequent sequence analysis

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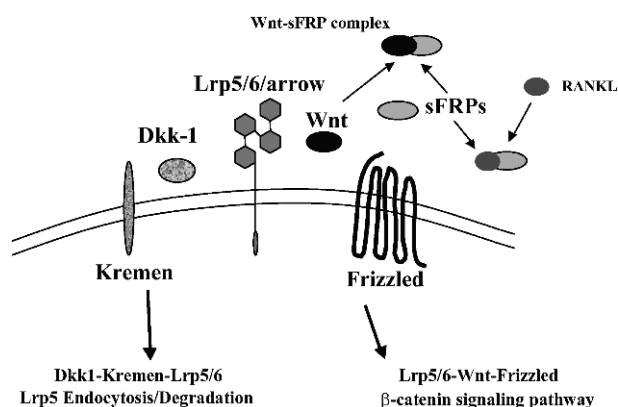
showed that their *LR3* gene was the same as the *LRP5* gene cloned by Hey et al.<sup>(16)</sup> and Kim et al.<sup>(14)</sup>

LRP5 and 6 are homologous to the *Drosophila* protein, Arrow.<sup>(21)</sup> LRP5 and 6 are unique with respect to other members of the LDL receptor family in that they do not contain the internalization signal sequence for endocytosis. The LRP5 protein contains 1615 amino acids, whereas LRP6 contains 1613 amino acids. Overall homology between LRP5 and 6 at the nucleotide level is 64%, whereas coding sequence homology is 71%.<sup>(19)</sup> The extracellular portion consists of four EGF-like repeats, each separated by six YWTD spacer domains. These EGF-like repeats are implicated in protein–protein interactions because they contribute to ligand binding in some LRPs.<sup>(22)</sup> In addition, there are three LDLR type A repeats between the EGF-like repeats and the membrane. LDLR repeats are required for binding of the LDL particle by the LDL receptor and so may also contribute to ligand–receptor interactions.<sup>(22)</sup> The crystal structure of the EGF domain pair in the LDL receptor indicates that the YWTD repeats form a six-bladed  $\beta$ -propeller structure.<sup>(13)</sup> A recent study of the nidogen  $\beta$ -propeller domain that binds laminin by Takagi et al.<sup>(23)</sup> has revealed that the  $\beta$ -propeller domain of LRP5 closely resembles the nidogen binding site for LE4 and suggests a structural paradigm for high affinity ligand-binding to the YWTD  $\beta$ -propeller 1 of LRP5. As will be discussed later, all of the mutations in *LRP5* that give rise to conditions of increased bone mass<sup>(3–5)</sup> are found in this first YWTD  $\beta$ -propeller. Strickland et al.<sup>(6)</sup> have recently published a model showing and comparing the domain structures for all members of the LDL receptor family, including LRP5/6 in the review.

### Wnt PROTEINS, Wnt SIGNALING, AND Wnt FUNCTIONS

The Wnt proteins are secreted from cells, but in a lipid-modified and very hydrophobic form that can only be solubilized in detergent.<sup>(24,25)</sup> The lipid modification consists of a covalent attachment of a palmitate to a conserved cysteine near the amino terminus of the Wnt protein. Enzymatic removal of the palmitate results in loss of activity, and together with mutational analysis of the modified cysteine, indicates that the palmitoylation is important for Wnt signaling. There is genetic evidence for a dedicated enzyme, encoded by the *Drosophila* gene *porcupine*, catalyzing Wnt palmitoylation.<sup>(26)</sup> It is not known whether secreted forms of Wnt's are bound to carrier molecules, but it is possible that such carriers exist, in particular, molecules that may bind to the lipid to generate a complex that is more water soluble and released from cells. In addition to lipidation, Wnt's are also modified by glycosylation, but this modification is not essential for activity.<sup>(27)</sup>

Given the general importance of the LDL receptor family for the uptake of lipids and other macromolecules, the relationship between LRPs and the response of cells to Wnt's is intriguing. Because Wnt's are lipid-modified and dependent on the lipid for signaling activity, it is tempting to speculate that LRPs specifically interact with the lipid on the Wnt and thereby promote signaling. While this is cer-



**FIG. 1.** The multiple levels of extracellular regulation of LRP5/6/arrow and modulation of Wnt signaling. The binding of Wnt ligand to the *frizzled* and LRP5/6/arrow receptor leads to activation of the  $\beta$ -catenin signaling pathway (see Fig. 2). The binding of the Wnt antagonist Dickkopf (Dkk-1) results in the formation of a ternary complex with the single pass transmembrane protein, kremen, which results in LRP5/6/arrow internalization and degradation, thus removing LRP5/6/arrow from being able to interact with Wnt and frizzled. Secreted frizzled-related proteins (sFRPs) are also capable of binding Wnt and thus preventing it from binding to the LRP5/6/arrow-frizzled receptor. sFRP has also been shown to bind to RANKL and prevent its interaction with RANK, thereby preventing osteoclastogenesis.

tainly an attractive possibility, there is yet no evidence supporting it, and it is equally likely that the interaction is indirect and involves an apoprotein that is bound to the lipid on the Wnt molecule. Further biochemical experiments are necessary to distinguish between these mechanisms.

The extracellular portion of LRP5/6 bind several different molecules, principally the Wnt's and Dkk's, and the co-receptor protein, Frizzled (Fz; Fig. 1). The Fz transmembrane receptor<sup>(28,29)</sup> consists of an extracellular cysteine rich domain (CRD) that serves as a ligand-binding unit,<sup>(30,31)</sup> and a 7 transmembrane signaling portion. Using a deletion construct approach, the binding of Dkk-1 to LRP6 has been shown to occur through interaction with the third and fourth EGF-like repeat domains, and binding to Fz occurs with the EGF domain 1 and 2 of the receptor in a Wnt-dependent manner.<sup>(32,33)</sup>

Wnt signaling is required for a diversity of developmental events including mesoderm induction, organogenesis, CNS organization and limb patterning.<sup>(34–37)</sup> In addition, a number of Wnt's have been implicated in vertebrate skeletal development. For example, there is evidence that Wnt3a, Wnt4, Wnt5a, Wnt5b, and Wnt7a all have important roles in chondrogenesis.<sup>(37–41)</sup> Another member of the Wnt family, Wnt9A (formerly Wnt14), can induce morphological and molecular signs of joint formation when inappropriately expressed, indicating that Wnt9A plays a crucial role in the initiation of synovial joint development.<sup>(42)</sup> Wnt9A expression can also lead to the arrests and reversal of chondrogenic differentiation *in vitro*.<sup>(42)</sup> Thus, Wnt's have various functions during the formation and the patterning of the skeleton. Recent evidence (O MacDougald, personal communication, 2004) has suggested an important role for Wnt10b in both bone and adipose tissue. Notably, activation

of the canonical Wnt pathway by Wnt10b blocks adipogenesis in vitro and adipose tissue development in mice and increases osteoblastogenesis in vitro and trabecular bone mass in mice. Also, Wnt10b<sup>-/-</sup> mice have decreased trabecular mass. However, the natural ligand for LRP5/6 in bone remains unproven at this time and is a major unanswered question.

Wnt's also bind to the secreted frizzled related proteins (sFRPs),<sup>(43-45)</sup> which can modulate Wnt activity (Fig. 1). These proteins contain a cysteine rich domain (CRD) that is highly homologous to the CRD in the Fz receptors, but lack the transmembrane domain and are secreted as extracellular proteins. Hausler et al. have shown that sFRP-1 also binds to RANKL, which raises the possibility that analogous to the binding of RANKL by osteoprotegerin (OPG),<sup>(46)</sup> sFRP-1 may regulate osteoclastogenesis.

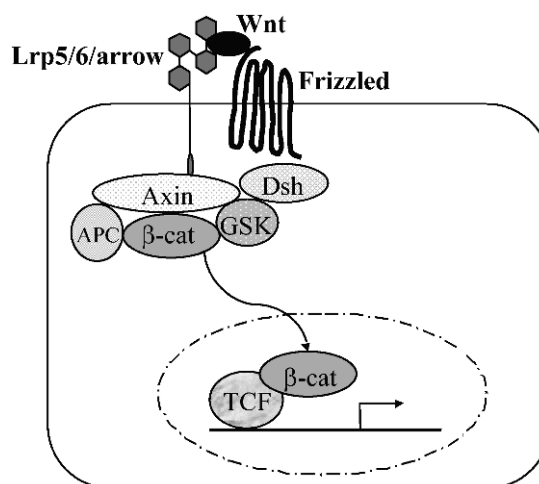
### A SPECIFIC FUNCTION FOR LRP5/6/ARROW IN Wnt SIGNALING

In general, members of the LDL receptor family have roles in the endocytic pathway and mediate the uptake of macromolecules. Examples are apolipoproteins endocytosed through clathrin-coated pits.<sup>(22,47)</sup> Mice carrying mutations in members of the LDL receptor family (the VLDL and ApoER2 receptors) have phenotypes similar to mouse mutant for *reelin*, indicating that LDL receptors can participate in specific signaling pathways.<sup>(48)</sup>

Initially, genetic evidence implicated the *Drosophila arrow* gene and its mammalian homolog *LRP6* as having phenotypes very similar to Wnt phenotypes.<sup>(21,49)</sup> Genetic epistasis experiments in *Drosophila* have placed the requirement for Arrow/LRP5/6 downstream of the Wnt gene *wingless* and upstream of cytoplasmic signaling components, implying Arrow/LRP5/6 functions in parallel to or downstream of Fz.<sup>(21)</sup>

The intracellular region of the LRPs is highly enriched in proline, indicating that the cytoplasmic tail may serve as a docking site for SH3-domain containing proteins, although such proteins have not been identified. These proteins also contain putative internalization sequences within their intracellular domains. While genetic evidence has implicated LRP6 in Wnt signaling, analysis of different mammalian cell lines indicates that LRP5 is also involved in the canonical Wnt signal transduction pathway.<sup>(1,50)</sup> Other cell culture experiments using a Lef/TCF-dependent Luciferase assay show that Arrow/LRP5/6 functions cooperatively with Fz to stimulate the  $\beta$ -catenin pathway in response to different Wnt proteins.<sup>(51)</sup>

Biochemical analysis of human LRP6 shows that the extracellular portion binds to mouse Fz8 in a Wnt1-dependent manner.<sup>(32)</sup> These data invite the idea that Wnt may initiate signal transduction by simply bringing LRP5/6 and Fz into close apposition. Furthermore, FRET analysis has shown a Wnt-dependent interaction between LRP5 and Axin.<sup>(33)</sup> More recently, Tamai et al.<sup>(52)</sup> have shown that a PPPSP motif, which is reiterated five times in the LRP5/6/Arrow intracellular domain, is necessary for Wnt signaling. In addition, Wnt signaling leads to phosphorylation of the PPPSP motif, which then generates an inducible binding



**FIG. 2.** The binding of Wnt to the LRP5/6/arrow-frizzled receptor leads to recruitment of Axin to the cytoplasmic tail of LRP and possibly to a complex between Axin and Dishevelled (Dsh). Dishevelled inhibits glycogen synthase kinase-3 $\beta$  (GSK3 $\beta$ ) from phosphorylating  $\beta$ -catenin. The combination of Axin binding to LRP5/6/arrow and inhibition of GSK3 $\beta$  causes the release of  $\beta$ -catenin from the Axin-APC-GSK3 $\beta$  complex, on which  $\beta$ -catenin is stabilized to enter the nucleus and interact with TCF.

site for Axin (Fig. 2). The observed Axin recruitment to LRP5/6 provides the first evidence of a distinct connection between extracellular receptor activation and intracellular signaling events. In view of the evidence that Fz can bind Dsh and that Dsh and Axin can directly contact each other through their DIX domains,<sup>(53)</sup> it is tempting to postulate that Wnt signaling leads to a higher order complex at the membrane, containing the two receptors plus Axin and Dsh<sup>(54-56)</sup> (Fig. 2).

The current model of the Wnt canonical signaling pathway is that activation of cells by Wnt signaling initiates a cascade of intracellular events triggered by the cytoplasmic protein Dishevelled (Dsh) interacting directly with the Fz receptor<sup>(54,56)</sup> (Fig. 2). In the absence of a Wnt signal,  $\beta$ -catenin is localized to the cytoplasmic  $\beta$ -catenin destruction complex, which includes adenomatous polyposis coli (APC), glycogen synthase kinase (GSK)-3 $\beta$ , and Axin proteins. APC and Axin function as critical scaffold proteins that allow GSK3 $\beta$  to bind and phosphorylate  $\beta$ -catenin, thus tagging the protein for degradation through the ubiquitin/proteasome pathway.<sup>(55,57-59)</sup> On Wnt binding to the co-receptor complex, Dsh activation leads to the phosphorylation of GSK3 $\beta$ , thereby inhibiting the phosphorylation of  $\beta$ -catenin. The cytoplasmic tail of the Lrp5/6 binds to Axin, which facilitates dissociation of the destruction complex and leads to increased intracellular levels of  $\beta$ -catenin.<sup>(50)</sup> This results in  $\beta$ -catenin stabilization and subsequent nuclear translocation (Fig. 2). Once  $\beta$ -catenin is localized to the nucleus, it interacts with Lef1/TCF transcription factors to initiate target gene activation.<sup>(57)</sup>

The specificity of LRP5/6/arrow in the Wnt pathway is further illustrated by the identification, both in mice and flies, of genes that are required as ER chaperones for transport and folding for LRPs. These chaperone genes, *Boca*

and *Mesd*, have phenotypes very similar to Wnt pathway components.<sup>(60,61)</sup> In addition to its role in Wnt reception at the membrane, LRP5/6 is also involved in negative regulation of the pathway. The secreted Wnt modulator, Wise, interacts with LRP,<sup>(62)</sup> although it is not known what the consequence of this binding is at this time. It is interesting to note that Wise and SOST bear homology to each other (see below).<sup>(62)</sup> Both suppression and enhancement of Wnt signaling as the consequences of Wise activity have been reported.<sup>(62)</sup>

### DICKKOPF-LRP INTERACTIONS

In the context of LRP and bone growth, the interactions between LRPs and the secreted Wnt antagonist, Dickkopf (Dkk)<sup>(63)</sup> are of special interest. Dkk interferes with canonical Wnt signaling in vertebrates by binding directly to LRP5/6.<sup>(33,64,65)</sup> At the same time, Dkk interacts with another transmembrane protein, Kremen (Krm).<sup>(66)</sup> This complex is internalized, thus removing LRP from the cell surface and making LRP unavailable for Wnt signaling.<sup>(65,66)</sup> The point mutation in the extracellular YWTD motif of LRP5, identified in human patients exhibiting a high bone mass defect,<sup>(3,4)</sup> prevents binding of Dkk, suggesting that the high bone density in these patients is caused by unrestrained Wnt signaling, not counteracted by Dkk activity.<sup>(4)</sup> These are important findings that suggest that interfering with Wnt signaling may contribute to the clinical management of bone disease.

### LRP5/6 FUNCTION IN BONE

The finding that LRP5 mutations lead to abnormalities in bone growth in humans has led to attempts to generate animal models for bone defects. Two groups have made mice lacking LRP5 function. Fujino et al.<sup>(67)</sup> have provided evidence that *Lrp5* deficiency leads to increased plasma cholesterol levels in mice that are fed on a high-fat diet, implying that *Lrp5* is also required for normal cholesterol and glucose metabolism. Using a different *Lrp5* mutant mouse, however, Kato et al. emphasize that *Lrp5* is required for normal proliferation and function of osteoblasts. They also found that *Lrp5*-deficient mice display persistent embryonic eye vascularization because of a failure of macrophage-induced endothelial cell apoptosis.<sup>(68)</sup> The role of LRP5 in bone mass accrual suggests that Wnt proteins have a role beyond embryonic skeletal patterning in post-natal bone maintenance. It is not clear whether the seemingly contrasting phenotypes described by these two groups can be reconciled.

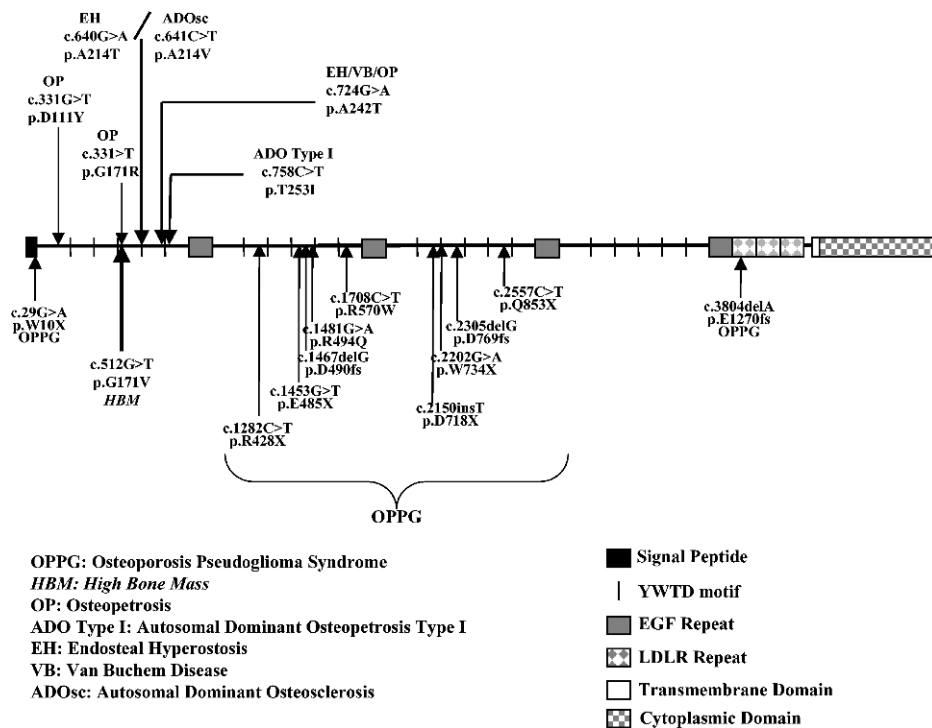
Mouse embryos homozygous for a nonfunctional allele of *Lrp6* die at birth and exhibit various developmental abnormalities, including a truncation of the axial skeleton, deletion of the caudal midbrain, and limb defects.<sup>(49)</sup> The defects in *Lrp6* mutant mice are a striking composite of those caused by mutations in individual Wnt genes, suggesting a broad role for *Lrp6* in the transduction of multiple Wnt signals.<sup>(49)</sup> However, not all known Wnt phenotypes are present in LRP6 mutants. Because the closely related *Lrp5* gene is expressed widely in mid-gestation embryos, genetic redundancy is possible, but remains to be tested.

The gain of function phenotype associated with a point mutation in the extracellular YWTD motif of LRP5, identified in human patients with high bone mass, is recapitulated in transgenic mice expressing this very same mutant version of LRP5.<sup>(69)</sup> These LRP5 G171V transgenic mice have increased BMD, trabecular thickness, trabecular number, strength, and resistance to fracture. Babij et al.<sup>(69)</sup> also reported decreased osteoblast and osteocyte apoptosis, which is consistent with increased activity of the Wnt signaling pathway in other cell/tissue systems.

Recent work from Johnson and colleagues at Creighton, in collaboration with colleagues at Wyeth Research (Collegeville, PA, USA and Cambridge, MA, USA) and Genome Therapeutics (Waltham, MA, USA), has shown a key role for LRP5/Wnt signaling in mechanosensation in bone (M Chatterjee-Kishore, PJ Yaworsky, W Zhao, C Li, Y Kharonde, JA Robinson, E Fortier, L Sauter, M Cain, B Bhat, M Wasko, P Babij, R Bhat, PVN Bodine, EL Brown, ML Johnson, MP Akhter, DM Cullen, RR Recker, and FJ Bex, unpublished data, 2004). These studies have studied bone formation in response to loading with a four-point tibia bending model.<sup>(70)</sup> They have shown that tibias from the LRP5 G171V transgenic mice have a more robust bone formation response to loading, and that the threshold level of strain required to induce a bone formation response is lower in the LRP5 G171V transgenic mice. Gene expression profiling studies have shown that, in response to mechanical loading, the expression of downstream gene targets of the Wnt signaling pathway are significantly elevated in the LRP5 G171V transgenic mouse tibias above the increases observed in wildtype control mice tibias. An additional gain-of-function associated with the LRP5 G171V mutation was observed in that osteoprotegerin (OPG) mRNA levels were also significantly elevated in these mice tibias without any change in RANKL levels in response to loading. An increased OPG:RANKL ratio, if translated to a functional protein level change, would result in reduced osteoclastogenesis. Thus, the high bone mass achieved and maintained in the human kindreds and the transgenic mice with the LRP5 G171V mutation could be the result of multiple effects on different cells and involvement of several different mechanisms and/or the interaction of multiple bone cell regulatory pathways. The net balance of these effects favoring increased bone formation and maintenance of the formed bone. Obviously, there is much that needs to be understood about the role of LRP5 in the regulation of bone mass, mechanosensation, and cell function.

### LRP5 POLYMORPHISMS AND BONE MASS VARIATION

In 1996, Gong et al.<sup>(71)</sup> described a number of patients with the autosomal recessive condition, osteoporosis pseudoglioma syndrome (OPPG). This disease is characterized by severe, juvenile-onset osteoporosis and progressive blindness. The following year, Johnson et al.<sup>(72)</sup> described an extended pedigree including a large number of individuals with a very significantly increased spinal BMD as the only phenotype. This so-called "high bone mass" (HBM) phenotype was inherited as an autosomal dominant trait,



**FIG. 3.** *LRP5* mutations and associated phenotypes. Model of the *LRP5* protein with the various motifs found in the protein represented as shown. The various known mutations in *LRP5* and the associated phenotypic conditions for each are given with both the nucleotide mutation (based on the coding or cDNA derived sequence) and the corresponding amino acid change.

with the “affecteds” being clinically asymptomatic. Both of these traits were mapped by linkage analysis to chromosome 11q12–13, and Johnson et al.<sup>(72)</sup> hypothesized that perhaps they were allelic variants of each other. By a large positional cloning effort, both groups were finally able to identify mutations in *LRP5* that cause the OPPG<sup>(1)</sup> and HBM<sup>(3)</sup> phenotypes. Another report of *LRP5* mutations giving rise to OPPG has recently appeared.<sup>(2)</sup> The G171V mutation found in the HBM family was also detected in another family with an autosomal dominant increased BMD phenotype.<sup>(4)</sup> Interestingly, in this family, in addition to the increased BMD, a wide and deep mandible and a torus palatinus were detected in the individuals carrying the *LRP5* mutation.

The HBM phenotype is to be classified among the sclerosing bone dysplasias. Increased bone mass can be caused by a variety of causative factors, including infection and metabolic or neoplastic abnormalities. On the other hand, a large number of conditions have been recognized in which solely genetic factors cause the sclerosing bone phenotype. The genes involved have a function in the bone resorption process by osteoclasts, as seen in the different forms of osteopetrosis, can increase the bone formation rate, or can disturb the coupling between bone resorption and bone formation.<sup>(73)</sup> Clinically, these conditions can range from very mild, even asymptomatic, to very severe, sometimes causing early death. In general, all these conditions are very rare. Therefore, and because there is a lot of clinical and radiological overlap between some of the conditions, a diagnosis in a patient with a sclerosing bone phenotype is not always easy to make for clinicians.

In the last few years, major breakthroughs have been made in the identification of genes mutated in some of these

conditions, including the finding of the *LRP5* missense mutation in the HBM phenotype.<sup>(3,4)</sup> These newly generated molecular findings provide very useful tools in the making or confirming of diagnoses initially based on clinical and radiological characteristics. Moreover, they often indicate the need to re-evaluate the classification of conditions either by grouping previously separated conditions, or in the other direction, by differentiating conditions that were previously assumed to be identical.

The evaluation of the role of the *LRP5* gene in different sclerosing bone dysplasias nicely shows such a possible impact on diagnostics and nosology of conditions by gaining molecular insights. Recently, a number of missense *LRP5* mutations have been described in a number of families initially diagnosed with endosteal hyperostosis, autosomal dominant osteosclerosis, Van Buchem disease, and autosomal dominant osteopetrosis type I.<sup>(5)</sup> All six novel missense mutations were located within the first propeller domain of *LRP5*. It is likely that these mutations will have a similar effect on the functioning of the co-receptor, similar to the one reported for the G171V mutation in *LRP5*.<sup>(4)</sup> All of the currently published mutations in *LRP5* are shown in Fig. 3.

Based on these molecular data, *LRP5* seems to unite conditions previously differentiated from each other. However, focusing on the radiological aspects of all these conditions, there is a clear overlap, making differentiation between the conditions very difficult. Therefore, it might also be that different names are currently being used for the same condition. The latter is supported by the fact that the A242T mutation in *LRP5* was found in cases diagnosed with endosteal hyperostosis, Van Buchem disease, and autosomal dominant osteopetrosis type I.<sup>(5)</sup> The most significant hall-

TABLE 1. CONDITIONS OF INCREASED BONE MASS

<i>Condition</i>	<i>Mode of inheritance</i>	<i>Gene</i>	<i>Molecular mechanism</i>	<i>Reference</i>
Worth disease	AD	<i>LRP5</i>	Missense mutation	76
Endosteal hyperostosis	AD	<i>LRP5</i>	Missense mutation	97, 98
Autosomal dominant osteosclerosis	AD	<i>LRP5</i>	Missense mutation	99
Autosomal dominant osteopetrosis type I	AD	<i>LRP5</i>	Missense mutation	80, 81
Idiopathic osteosclerosis	AD	?		100
Van Buchem disease	AR	<i>SOST</i>	Suppression of expression	74
Sclerosteosis	AR	<i>SOST</i>	Loss of function mutation	101, 102
Generalized cortical hyperostosis	AR	?		103

Various known conditions of increased bone mass that could be considered as “cranio-tubular hyperostoses.” The mode of inheritance is shown as either autosomal dominant (AD) or autosomal recessive (AR). The molecular underlying mechanisms are given when known.

mark in all conditions is the increased cortical thickness of all bones. Van Buchem et al., in 1955, first used the term “hyperostosis corticalis generalisata familiaris” to describe two sibs with increased cortical thickening affecting the skull, and most prominently, the mandible.<sup>(74)</sup> Later, this condition, meanwhile called Van Buchem disease, was classified within the endosteal hyperostoses together with sclerosteosis and Worth disease, the former being a condition clinically very similar to Van Buchem disease but somewhat more severe and sometimes associated with syndactyly.<sup>(75)</sup> Worth disease was first described by Worth and Wollin<sup>(76)</sup> in a large Canadian family. The use of the term “endosteal hyperostosis,” including two autosomal recessive forms (Van Buchem disease and sclerosteosis) and one autosomal dominant form (Worth disease), was based on the impression that the observed cortical thickness was caused by increased apposition of endosteal bone, causing narrowing of the medullary cavity combined with an unchanged periosteal diameter of the long bones. However, it has been a general impression that this is not really representing the reality, and recently, this was confirmed for Van Buchem disease by showing that the periosteal diameter is highly increased in metacarpals of Van Buchem patients,<sup>(77)</sup> but the same goes for the endosteal diameter, giving an increased size of the medullary cavity that increases with age.<sup>(78)</sup> Therefore, the name endosteal hyperostosis does not seem appropriate.

Autosomal dominant osteopetrosis type I was differentiated from the other form of autosomal dominant osteopetrosis initially described by Albers-Schönberg<sup>(79)</sup> and currently is known as type II based on both clinical and radiological features.<sup>(80,81)</sup> Type I is radiologically very similar to cases previously diagnosed with autosomal dominant endosteal hyperostosis (Worth disease) but was suggested to be caused by a bone resorption defect mainly based on the decreased number of osteoclasts found in the extensively studied Danish patients.<sup>(80)</sup> The presence of an *LRP5* mutation in these patients was recently shown.<sup>(5)</sup> Because this mutation (*T253I*) also affects the first propeller domain of *LRP5*, this suggests an increased bone formation rate rather than the presumed decreased bone resorption rate. However, the mutation found is, thus far, specific for these Danish patients; therefore, functional studies are ongoing to evaluate a possible effect of this mutation on osteoclast differentiation. An alternative explanation for the

reduced number of osteoclasts would obviously be an increased OPG:RANK ratio as has been found in loading studies with *LRP5 G171V* transgenic mice (ML Johnson, personal communication, 2004).

In conclusion, a number of names are currently being used for conditions showing a very similar radiological phenotype. The explanation for this is the clinical variety that can be observed by comparing cases with the same mutation as already shown by the clinical differences found in patients of the two families with the *G171V* mutation. The most likely explanation for these differences is the effects of modifier genes that influence the phenotypic consequences. By taking into account the molecular findings, we could in the end come to a simplification of the nomenclature. All conditions mentioned could be considered as “cranio-tubular hyperostoses,” the latter representing the increased cortical thickness, and the former representing the sites of affection being the skull and tubular bones. An autosomal dominant form would then include conditions such as the HBM phenotype, endosteal hyperostosis Worth type, autosomal dominant osteosclerosis, autosomal dominant osteopetrosis type I, idiopathic osteosclerosis (Table 1). An autosomal recessive form would include Van Buchem disease and sclerosteosis. Remarkably, one family was referred to us with a diagnosis of Van Buchem disease based on clinical and radiological findings.<sup>(82)</sup> However, when we re-evaluated the pedigree, it turned out to be an autosomal dominant phenotype, not in line with a diagnosis of Van Buchem, which was later confirmed by the finding of a missense *LRP5* mutation in this family. This shows that the molecular findings are in line with the differences in mode of inheritance because the first conditions are caused by missense mutations in *LRP5*, activating Wnt signaling, whereas Van Buchem disease and sclerosteosis are caused by a loss of function or suppression of expression of sclerostin encoded by the *SOST* gene.<sup>(83–85)</sup> This gene is currently suggested to be an osteocyte-expressed extracellular antagonist of bone morphogenetic protein (BMP) signaling.<sup>(86)</sup> Interestingly, a functional link between sclerostin and the Wnt signaling pathway can definitely not be excluded, because we previously mentioned that Wise, a homolog of the *SOST* gene, interacts with *LRP6*.<sup>(62)</sup> Finding such a functional link would obviously explain the clinical and radiological similarities between the dominant and recessive conditions. It would definitely not be the end of the story,

because currently, there are a number of patients and families that have similar clinical and radiological findings but without a mutation in either the *LRP5* or the *SOST* gene. Obviously other participants in this putative *SOST-LRP5/Wnt* pathway would be strong candidate genes for causing these phenotypes.

### CONCLUSION

The past decade has seen considerable interest and effort put forth in trying to identify genes that regulate normal human variation in bone mass and other bone phenotypes. Several groups have been involved in human whole genome scans,<sup>(87–90)</sup> whereas others have used animal models<sup>(91–94)</sup> in an attempt to dissect the genetic basis of osteoporosis. Recently Klein et al.<sup>(95)</sup> identified the *Alox15* gene that encodes a 12/15 lipoxygenase as a negative regulator of peak bone mass in mice. This identification came about through their efforts to identify quantitative trait loci (QTLs) controlling bone mass and is another illustration of the potential surprises in store for us and how little we really know about the genes controlling bone mass. Ultimately, one has to ask the questions: what do these revealing family studies tell us about bone biology, and do the underlying causal genes in these extreme conditions have anything to do with normal bone mass variation? Clearly, these single gene trait studies can reveal new pathways that are important in bone and were never suspected based on existing knowledge at the time of their discovery. As far as what contribution they may make to normal variation in BMD in the population, the jury is still out. In the case of *LRP5*, a recent study by Choudhury et al. suggests that normal polymorphisms may contribute to some variation in BMD,<sup>(96)</sup> but more studies are needed. If the lessons of the past few years have shown anything, clearly we have much to learn, and the next several years promise to be exciting and challenging.

*Note added in proof:* A paper was recently published describing mutations in *LRP5* that give rise to the human eye disease, familial exudative vitreoretinopathy (FEVR). (Tomes et al., 2004 Mutations in *LRP5* or *F2D4* underlie the common familial exudative vitreoretinopathy locus on chromosome 11q. *Am J Hum Genet* **74**:721–730.)

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