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REVIEW

Harnessing low-density lipoprotein receptor protein 6 (LRP6) genetic variation and Wnt signaling for innovative diagnostics in complex diseases

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Wnt signaling regulates a broad variety of processes in both embryonic development and various diseases. Recent studies indicated that some genetic variants in Wnt signaling pathway may serve as predictors of diseases. Low-density lipoprotein receptor protein 6 (LRP6) is a Wnt co-receptor with essential functions in the Wnt/β-catenin pathway, and mutations in LRP6 gene are linked to many complex human diseases, including metabolic syndrome, cancer, Alzheimer's disease and osteoporosis. Therefore, we focus on the role of LRP6 genetic polymorphisms and Wnt signaling in complex diseases, and the mechanisms from mouse models and cell lines. It is also highly anticipated that LRP6 variants will be applied clinically in the future. The brief review provided here could be a useful resource for future research and may contribute to a more accurate diagnosis in complex diseases.

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INTRODUCTION

The *Wnt1* gene was identified in 1982. Ensuing studies in *Drosophila* and *Xenopus* unveiled a highly conserved Wnt/β-catenin pathway, namely, canonical Wnt signaling. With the identification of Wnt transcription factors, receptors and co-receptors, the Wnt/β-catenin pathway has become a hot topic in molecular biology studies. Considering its essential role in certain physiological process, including embryonic development, insulin secretion, stem cell homeostasis, bone formation and neurogenesis, abnormal signaling through this pathway has a serious effect on cell growth and function, leading to a plethora of debilitating and life-threatening disorders. Low-density lipoprotein receptor-related protein 6 (LRP6) acts as an essential co-receptor of Wnt/β-catenin signaling and has recently become 'a star molecule'.

In recent years, the question of whether and how LRP6 variants influence the development and progression of various diseases has attracted much interest. Many researchers have demonstrated that in different populations, specific mutations in LRP6 and Wnt pathway-related genes are associated with increased risk of disease. In addition to clinical evidence, results from genetically engineered transgenic mouse models and cell lines support this conclusion. In this review, we present the most recent evidences regarding the role of LRP6 and Wnt signaling in various human diseases, discuss the newly discovered mutations, and address the potential of innovative diagnostics for complex diseases.

OVERVIEW OF LRP6

LRP6 structure

The low-density lipoprotein receptor (LDLR) family comprises cell surface receptors, and several members are involved in numerous signaling pathways and expressed in various target organs.[1] LDLR-related proteins 5/6 (LRP5/6) belong to this large family and function as co-receptors of the Wnt/β-catenin pathway. These proteins are structurally related, with ~71% homology at the nucleotide level,[2] and are broadly expressed in humans, including in the hippocampus, rental tubular cells, hepatocytes, intestinal epithelial cells, osteoblasts and osteoclasts. Nonetheless, previous studies highlighted that LRP5 and LRP6 mediated distinct actions due to differences in tissue distribution and affinity for individual Wnt ligands.[3] In contrast, murine models suggested incomplete functional redundancy between the two receptors.[4] Although both have a crucial role in the regulation of bone mass,[5] LRP6 is closely related to glucose and lipid metabolism signaling pathways[6–8] and plays a more important role than LRP5 in developmental processes.[9]

The human LRP6 gene (hLRP6), which is located on chromosome 12p13.2, is 150 kb in length and contains 23 exons. hLRP6 is highly conserved evolutionarily, with almost no species differences;[7,10] for instance, hLRP6 is highly conserved among *Drosophila*, *Xenopus* and *Mus*. The protein encoded by hLRP6 is 1613 amino acids and is a type I signal transmembrane protein that contains a signal peptide and a transmembrane domain. The LRP6 receptor possesses extracellular and intracellular domains, and the structure of the former is essential for regulating interaction with Wnt ligand, Dickkopf-related protein 1 (Dkk1), and Sclerostin (Sost)[11] (Figure 1). The extracellular domain contains four YWTD (Tyr-Trp-Thr-Asp)-type β propellers, four epidermal growth factor (EGF)-like domains, and three LDLR type A domains; the intracellular domain harbors PPPSP (Pro-Pro-Pro-Ser-Pro) motifs. According to the crystal structure of an LDLR that contains a single propeller, the YWTD β propeller comprises six YWTD repeats that form a six-bladed β-propeller structure.[12] An EGF-like

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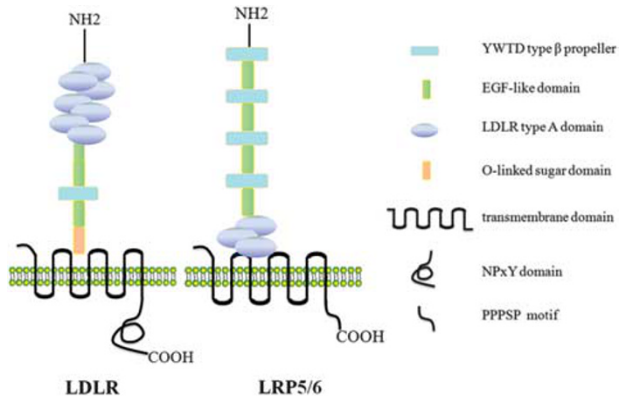

Figure 1. Structure of low-density lipoprotein receptor (LDLR) and LDLR-related 5 and 6 (LRP5/6). LDLR is an extraordinarily multifunctional subgroup, which includes LRP5/6. Both of LDLR and LRP6 share the common domains structurally, including LDLR type A domain, EGF-like domain, YWTD-type β propeller, transmembrane domain and a cytoplasmic domain. There is a NPxY motif in the cytoplasmic domain of LDLR, while a PPPSP motif is in the cytoplasmic domain of LRP6. EGF, epidermal growth factor; LDLR, low-density lipoprotein receptor; NPxY, Asn-Pro-any amino acid (x)-Tyr; PPPSP, Pro-Pro-Pro-Ser-Pro; YWTD, Tyr-Trp-Thr-Asp.

domain of ~40 bases follows every YWTD-type β propeller motif.[7] Although LRP6 belongs to the LDLR family, it differs from other LDLR family members; for example, the cytoplasmic C-terminal domain of LRP6 contains at least one copy of a PPPSP motif in place of the NPxY (Asn-Pro-any amino acid (x)-Tyr) domain[7] (Figure 1). Some variants of LRP6 might have altered protein function, so understanding the structure of LRP6 is essential for studying signal transduction and the discovery of novel therapeutic targets.

Overview of Wnt/β-catenin signaling

The Wnt/β-catenin pathway mainly consists of Wnt, Frizzled (Fz), LRP5/6, disheveled (Dvl), β-catenin, glycogen synthase kinase-3β (GSK-3β), axis inhibition protein 1 (Axin), adenomatous polyposis coli (APC), casein kinase 1 (CK1) and nuclear T-cell transcription factor/lymphatic enhancement factor (TCF/LEF; Figure 2). In the absence of upstream signal, the Wnt/β-catenin pathway is inactive, and with the Wnt ligands, the pathway is active (Figure 2). In addition to Wnt/β-catenin pathway, Wnt can also signal through protein kinase C, Rho or c-Jun N-terminal kinase, namely non-canonical Wnt signaling pathway.[13] A considerable number of basic and clinical studies confirm that Wnt ligands, APC, LRP6 and TCF7L2 play crucial roles in the synthesis and secretion of triglyceride (TG), LDL and very low-density lipoprotein.[14,15] Moreover, these genes are shown to be related in cancer,[16,17] AD[18] and osteoporosis.[19]

LRP6 GENETIC POLYMORPHISMS AND METABOLIC SYNDROME

Metabolic syndrome is a comprehensive disease typical of metabolic disorder that comprises dysglycemia, dyslipidemia, hypertension and the procoagulant state.[20] According to the surveys, the morbidity of metabolic syndrome among American and Chinese is 34 and 23.3%, respectively.[21,22] Indeed, numerous reports confirmed that mutants of LRP6 and TCF7L2 caused atherosclerosis,[23] diabetes,[24] hyperlipidemia[23,25] and hypertension.[26] Recently, attenuation of Wnt-mediated transcription resulting from mutations in LRP6 has been genetically linked to coronary artery disease (CAD) as well as several features of metabolic syndrome including hyperlipidemia, hypertension and diabetes but not obesity.[13] In an effort to clarify the correlation of LRP6 gene polymorphisms and metabolic syndrome, we provide a complete and up-to-date summary of the reported LRP6 genetic mutations in metabolic disorders and the regulatory mechanisms in Table 1.

Type 2 diabetes mellitus (T2DM)

T2DM is characterized by insulin resistance or insulin secretion deficiency. However, the etiology of T2DM is complicated, with involvement of genetic background, lifestyle and the environment.[27] In 2007, Mani *et al.*[10] found a missense mutation of a cysteine for arginine substitution at a highly conserved position of an EGF-like domain of LRP6 that impaired Wnt signaling *in vitro*. The article attracted the attention of investigators to the interrelationship between LRP6 variants and various human disorders. Furthermore, a number of investigators have confirmed that LRP6 is associated with the insulin pathway. For example, Masako *et al.*[28] indicated that three single-nucleotide polymorphisms (SNPs) of LRP6 (rs7136900, rs10743980 and rs2417086) were associated with T2DM in Japanese patients in the initial study, but this association was not confirmed in the replication panel. Thereafter, Singh *et al.*[29] reported that the LRP6 R611C mutation impaired glucose tolerance and activity of the insulin pathway; the study also found that LRP6 enhanced glucose metabolism by promoting TCF7L2-dependent insulin receptor expression and the mTOR pathway.[29]

Hyperlipidemia

Hyperlipidemia, which is characterized by a state in which blood has abnormally high levels of lipids, including cholesterol and TGs, is a major risk factor for CAD, the leading cause of death in the world.[30,31] It is reported that the risk of early CAD, hyperlipidemia, hypertension and diabetes in LRP6 R611C crowd is markedly higher than the normal.[10] Singh *et al.*[32] found three new missense mutations (that is, 1418G>A, 1079G>A and 1298T>C) of LRP6 in the Caucasian subjects, and all mutation carriers had hyperlipidemia, diabetes, or impaired glucose tolerance and hypertension. In addition, Wang *et al.*[33] demonstrated that LRP6 rs11054731 was associated with the risk of CAD ($P=0.001$) in the Chinese population.

Based on the fact that there is an association between LRP6 variants and hyperlipidemia or CAD, many researchers are taking an effort to explain this phenomenon. On the one hand, some studies implicated that LRP6 influenced activity of the mTOR pathway via Wnt/β-catenin signaling, and that overactivation of mTOR may up-regulate the lipid synthesis process.[29,34] Moreover, Go *et al.*[35] highlighted the effect of LRP6 on lipid synthesis in the liver, resulting in increased serum TC and TGs through insulin-like growth factor 1, mTOR and sterol regulatory element-binding protein 1/2 (SREBP1/2), namely, the IGF1-mTOR-SREBP1/2 pathway.[35] On the other hand, Wang *et al.*[36] found increased activity of non-canonical Wnt signaling in homozygous $LRP6^{R611C}$ ($LRP6^{mut/mut}$) mice, with both steatohepatitis and steatofibrosis. Besides that, some studies also reported that LRP6 was essential for normal LDL clearance and that gain- or loss-of-function mutations of LRP6 may induce hyperlipidemia or other diseases.[37,38] Xu *et al.*[39] showed that LRP6 regulated lipid metabolism mainly through human umbilical vein endothelial cell proliferation and migration. In conclusion, it is possible that LRP6 is a regulatory factor in lipid metabolism via multiple pathways.

Atherosclerosis

Atherosclerosis refers to a vascular inflammatory disorder characterized by shrinking of the blood vessel lumen due to the accumulation of lipids, inflammatory cells, vascular smooth muscle cells and platelets.[40–42] In humans, expression of LRP6 in

atherosclerotic coronary arteries is markedly increased, and the protein colocalizes with platelet-derived growth factor receptor β.[43] Further investigation showed that wild-type LRP6 inhibited, but LRP6 R611C promoted, vascular smooth muscle cell proliferation in response to platelet-derived growth factor.[43] In the clinical, Sarzani *et al.*[23] demonstrated that LRP6 I1062V (rs2302685)

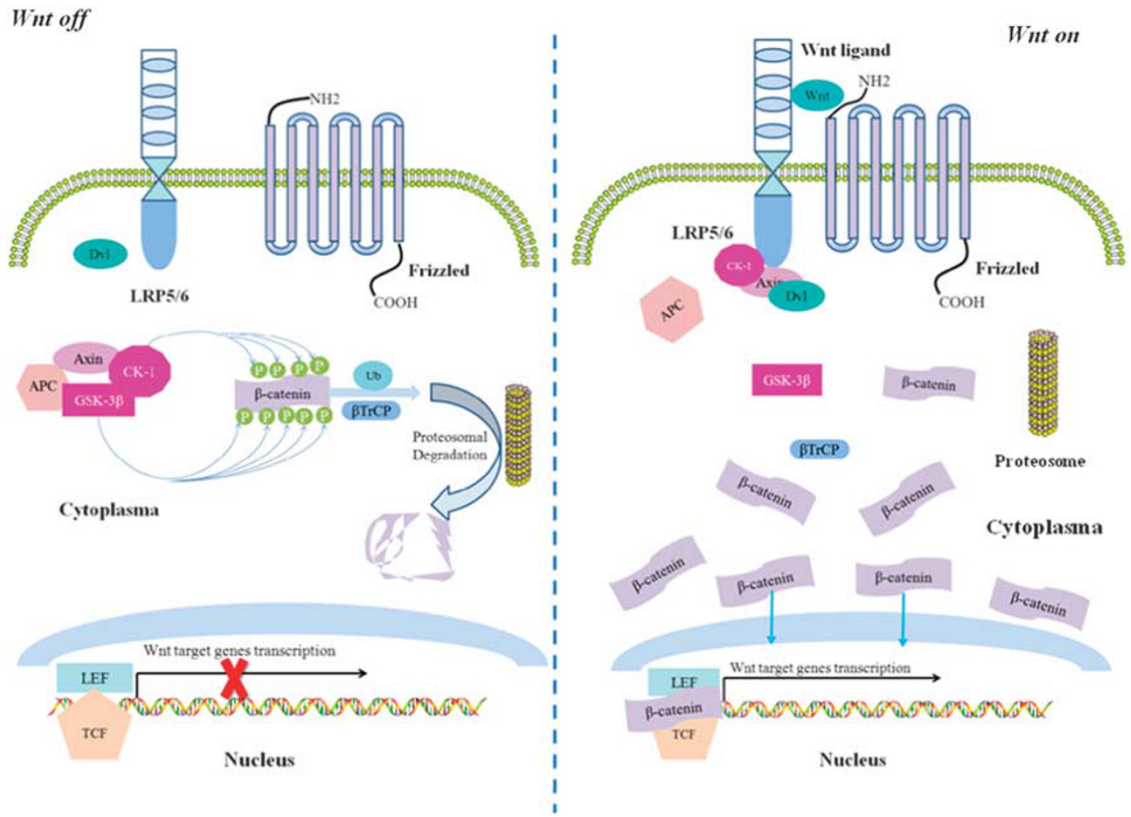

Figure 2. Canonical Wnt pathway (namely Wnt/β-catenin siganling pathway). Inactivate pathway: the absence of Wnt ligand results in the cytoplasmic pool of β-catenin being recruited into a multiprotein destruction complex that includes APC, CK1, Axin (axis inhibition protein 1) and GSK-3β. This destruction complex facilitates the GSK-3β-dependent phosphorylation of β-catenin and subsequent ubiquitinylation, leading to the degradation of β-catenin via the proteasome (the left panel). Active pathway: Wnt ligands combine with Fz (Frizzled) and LRP5/6, forming a Wnt-Fz-LRP5/6 ternary complex, which accelerates phosphorylation of LRP5/6 intracelluar region, attracting Axin complex, generating the inhibition of β-catenin, while the LRP5/6 phosphorylation protein inhibits the phosphorylation and degradation of β-catenin from GSK-3β, so that β-catenin in the cytoplasm is stable freely, and β-catenin combines with TCF/LEF (nuclear T-cell transcription factor/ lymphatic enhancement factor) in the nucleus with activation of downstream target genes (the right panel). APC, adenomatous polyposis coli; CK1, casein kinase 1; Dvl, disheveled; GSK-3β, glycogen synthase kinase-3β; Ub, ubiquitylation; βTrCP, β-transducin-repeat-containing protein.

Table 1. Summary of reported LRP6 genetic mutations and regulatory mechanism in metabolic disorders

Mutation	*Diseases*	*Phenotype*	*Regulatory pathway*	*Ref.*
LRP6 R611C	T2DM, hypertension, CAD	LDL, TG↑, blood pressure↑,blood glucose↑	Wnt-LRP6-β-catenin pathway	10
	T2DM	Insulin response↓,plasma insulin↑, blood glucose↑	LRP6-TCF7L2-mTOR pathway	29,34
	Hypercholesterolemia	Nonalcoholic fatty liver↑	LRP6-TCF7L2-mTOR pathway/ non-canonical Wnt pathway	29,34,36
		TC, TG↑,plasma LDL level↑,hepatic lipid synthesis↑,VLDL secretion in liver↑	LRP6-IGF1-mTORC-SREBP1/2 pathway	35
	Atherosclerosis	Formation of atherosclerotic plaques↑	PDGF pathway	43
LRP6 rs2302685	CAA	Incidence of CAA plagues↑	ND	23
LRP6(1418G > A,1079G > A, 1298T > C)	CAD	Blood glucose↑, blood pressure↑	HUVECs proliferation and migration pathway	32,39
LRP6 rs11054731	CAD	TG and LDL↑	Wnt-LRP6 -TCF/LEF pathway	33

Abbreviations: CAA, carotid artery atherosclerosis; CAD, coronary artery disease; HUVECs, human umbilical vein endothelial cells; LDL, low-density lipoprotein; LEF, lymphatic enhancement factor; ND, not determined; PDGF, platelet-derived growth factor; T2DM, type 2 diabetes mellitus; TC, serum total cholesterol; TG, triglyceride; TCF, T-cell transcription factor; VLDL, very low-density lipoprotein.

was associated with carotid artery atherosclerosis and speculated that LRP6 rs2302685 was likely to be an independent risk factor of atherosclerotic plaque formation through retrospective analysis of 334 hypertensive patients. In addition, Cheng *et al.*[44] studied the role of LRP6 in atherosclerosis in the SM22-Cre;LRP6(fl/fl); $LDLR^{-/-}$ animal model, conditionally depleting LRP6 in the VSM lineage in male LDLR-null mice; the authors discovered that the conditional knockout of LRP6 enhanced aortic calcification and vessel stiffening. One explanation may be that non-canonical Wnt signals promoted mature tissue calcification, whereas LRP6 restrained this pathway in part by sequestering certain Fz receptors.[45] Thus, LRP6 might play a uniquely important role in bone-vascular interactions.[46] In conclusion, changes in Wnt/LRP6/β-catenin signaling pathway activity may be a main cause of atherosclerotic plaque formation, and LRP6 genetic polymorphism is possibly to be a predictor of atherosclerosis.

LRP6 GENETIC POLYMORPHISMS AND CANCERS

The Wnt/β-catenin signaling pathway is a key modulator of cellular proliferation due to its regulation of stem cell homeostasis, and candidate gene approaches have investigated associations between genetic variants in Wnt pathway genes and susceptibility to cancers.[47] As an indispensable Wnt co-receptor, LRP6 is over-expressed in several types of cancer and malignant tissues,[48,49] and altered LRP6 expression leads to abnormal Wnt protein activation, cell proliferation and tumorigenesis.

Recently, Pierzynski *et al.* conducted a study including a cohort of 803 bladder cancer cases and an equal number of healthy controls, and showed that LRP6 rs10743980 was associated with a decreased risk of bladder cancer (OR = 0.76, 95% CI = 0.58–0.99, $P = 0.039$), with the findings validated in a genome-wide association study using a bladder microarray.[50] A recent study involving 500 non-small cell lung cancer (NSCLC) patients and 500 healthy controls implicated that LRP6 rs10845498 contributed to a reduced risk of lung squamous cell carcinoma and that the LRP6 rs6488507 increased the risk of NSCLC in tobacco smokers.[51] LRP6 was overexpressed in human breast tumors,[49] and down-regulation of LRP6 inhibited breast cancer tumorigenesis.[52] Based on the known genetic predisposition for some diseases, Richarda *et al.* identified that LRP6 rs141458215 as a novel candidate risk factor for early-onset colorectal cancer using whole-exome sequencing.[53] Lemieux *et al.*[54] found that Wnt/β-catenin pathway showed increased activity through LRP6 in colorectal cancer through KARS signaling. In addition to its role in the development of the cancers mentioned above, LRP6 is also necessary for the proliferation of prostate cancer cells.[55]

Over the past decade, mutations in LRP6 have been linked to a wide variety of cancers, and its critical role in Wnt signaling pathway has made LRP6 a hot topic of research. It has been confirmed that the LRP6 variants are related to the risk of cancer and tumor progression; accordingly, this gene may be used as a biomarker to predict the risk of cancer.

LRP6 GENETIC POLYMORPHISMS AND AD

AD, the most common form of age-associated dementia affecting a growing population of elderly individuals, is a progressive neurodegenerative disorder characterized by a deficit in cognitive processes that manifest as alterations in memory, judgment and reasoning.[56] Understanding the exact pathogenesis of AD and finding more effective biomarkers and accurate gene variants would contribute to therapy for this disorder and benefit patients. It is well established that Wnt signaling activity is linked to the pathogenesis of AD, leading researchers to examine whether the LRP6 gene, is associated with this disease.

Genome-wide linkage studies involving a multicenter case–control series as well as a large family-based series defined a broad susceptibility region for late-onset AD on chromosome 12, a region that contains the LRP6 gene.[57] Moreover, De Ferrari *et al.*[57] found a significant association between a synonymous SNP in exon 18 (18e, rs1012672, C→T) and AD in combined multiple samples (Zurich/U.K/U.S series). Individuals carrying the minor T allele had 69–80% greater risk of developing AD compared with CC homozygote individuals.[57] In addition, haplotype tagging of SNPs identified a putative risk haplotype that included a SNP in LRP6 14e (rs2302685; T→C). Interestingly, the LRP6 I1062V caused reduced activation of a β-catenin-responsive reporter gene in HEK293T/STF recombinant cells, suggesting that reduced signaling through the canonical pathway may predispose people toward AD.[57,58] Except the common SNPs of genes, altered posttranscriptional metabolism has recently become a hot topic. To date, several key genes of the Wnt/β-catenin pathway have been demonstrated to be alternatively spliced, such as TCF7L2 in colorectal, gastric and endometrial carcinomas,[59] β-catenin, axin and the homologous LRP5 in human colorectal tissue.[60] A recent study evaluated posttranscriptional metabolism of the LRP6 messenger RNA by sequentially scanning 23 exons in human tissues and reported that a novel LRP6 isoform that completely skipped exon 3 was present in all tissues. In addition, the messenger RNA level was significantly increased in AD brains compared with controls (1.6-fold; $P = 0.037$) and other pathological samples (2-fold; $P = 0.007$).[61] As shown in these clinical studies, LRP6 is linked to the risk of AD, and plays a pivotal role in AD development and progression.

To confirm that LRP6 deficiency may lead to AD and to elucidate the mechanism by which LRP6 regulates levels of Aβ, researchers conditionally deleted the LRP6 gene in neurons, generating LRP6 conditional knockout (LRP6 CKO) and corresponding littermate control mice.[62] This study showed that neuronal LRP6 deficiency contributed to significant memory impairment, impaired long-term potentiation induction, correlating with learning and memory, and increased expression of glial fibrillary acidic protein and ionized calcium-binding adaptor molecule 1 (Iba1), markers for astrocyte and microglia activation, respectively.[62] Furthermore, the study reported that LRP6 deficiency significantly increased mouse endogenous Aβ40 and Aβ42 levels in the brain compared with control mice.[62] Despite evidence demonstrating the relevance between LRP6 and Aβ levels, the researchers designed *in vitro* experiments in human neuroblastoma SH-SY5Y cells overexpressing human APP (SHSY5Y-APP) to confirm the *in vivo* results. They found that LRP6 regulated APP trafficking and processing to Aβ[62] and also reported that LRP6 levels and Wnt signaling were down-regulated in AD brains in comparison with controls.[62] All these studies showed that LRP6 deficiency contributed to the development of AD.[62]

LRP6 GENETIC POLYMORPHISMS AND OSTEOPOROSIS

Osteoporosis is a major health problem in western societies, affecting up to half of the elderly female population. The condition is characterized by low body bone mineral density (BMD) and bone fragility and fractures.[63] Heritability studies show that genetic factors may account for 50–80% of the variance in BMD.[64,65] The canonical Wnt pathway is critical for skeletal development and maintenance, though the precise roles of Wnt co-receptors LRP5/6 are not entirely clear. Approximately a decade ago, the identification of causal mutations in LRP5 involved in two rare bone disorders propelled research in the bone area into completely new directions.[66]

Mani *et al.*[10] found that individuals with LRP6 mutations developed many abnormalities including diabetes, hypertension, CAD and osteoporosis. Moreover, van Meurs *et al.*[67] discovered an association of LRP6 rs2302685 with height ($P = 0.04$) and lumber spine bone area ($P = 0.01$) in healthy white individuals. The authors

Table 2. Summary of the relevance between currently known LRP6 genetic variants and complex diseases

Disease	*SNP*	*Case*	*Control*	*Ethnicity*	*Case/control*	*OR (95% CI)*	P-*value*	*Ref.*
		Allele/frequency (%)	*Allele/frequency (%)*					
CAA	rs2302685	T (63.37) C (36.63)	T (77.33) C (22.67)	Italian hypertensive	167/167	2.08 (1.27–3.41)	0.005	23
T2DM	rs7136900	G (92.00) A (8.00)	G (90.00) A (10.00)	Japanese	608/366	0.72 (0.52–0.99)	0.042	28
	rs10743980	C (81.00) T (19.00)	C (77.00) T (23.00)	Japanese	608/366	0.77 (0.61–0.96)	0.019	28
	rs2417086	A (81.00) G (19.00)	A (76.00) G (24.00)	Japanese	608/366	0.74 (0.59–0.93)	0.008	28
CAD	rs11054731	C (72.00) T (28.00)	C (66.00) T (34.00)	Chinese	766/806	1.29 (1.10–1.50)	0.001	33
Bladder cancer	rs10743980	C (59.05) T (40.95)	C (55.92) T (44.08)	American white	803/803	0.76 (0.58–0.99)	0.039	50
NSCLC	rs10845498	A (80.2) G (19.8)	A (82.8) G (17.2)	Chinese	500/500	0.69 (0.48–1.00)	0.04	51
AD	rs2302685	T (84.9) C (15.1)	T (81.3) C (18.7)	Zurich/U.K./U.S. series	398/339	1.30 (0.97–1.71)	0.075	57
	rs1012672	C (91.1) T (8.9)	C (94.1) T (5.9)	Zurich/U.K./U.S. series	398/339	1.54 (1.03–2.31)	0.037	57
Ileal CD	rs2302685	T (79.44) C (20.56)	T (80.30) C (19.70)	European	377/736	4.093 (1.981–8.455)	0.00004	80

Abbreviations: AD, Alzheimer's disease; CAA, carotid artery atherosclerosis; CAD, coronary artery disease; CI, confidence interval; Ileal CD, the early ileal Crohn's disease; LRP6, lipoprotein receptor-related protein 6; NSCLC, non-small cell lung cancer; OR, odds ratio; SNP, single-nucleotide polymorphism; T2DM, type 2 diabetes mellitus.

went on to analyze fracture risk and found that LRP6 I1062V carriers also had a 60% increased risk for fragility fractures.[67] In analyses of the interaction between polymorphisms in LRP5 and LRP6, the researchers observed that men carrying both LRP5 A1330V and LRP6 I1062V risk alleles had a 90% higher risk of vertebral fracture ($P = 0.08$) compared to all other individuals.[67] Furthermore, LRP6 rs1181334 was also associated with decreased total hip BMD ($P = 0.014$) in 425 postmenopausal woman of an urban Mexican population.[68] These data support previously reported associations of LRP6 with BMD.[69]

In the past years, it has been established that LRP6 controls osteoblast differentiation in mice models. A study more than a dozen years ago reported genetic enhancement of a Wnt mutant phenotype in mice lacking one functional copy of LRP6 and found that this type of mouse displayed defects in both limb and axial development.[70] Numerous studies in mice also showed that a point mutation in LRP6 contributed to abnormal formation of the axial skeleton and a low bone mass phenotype.[5,71] Furthermore, a mouse in which LRP6 was selectively disrupted in the osteoblast was generated, with a significant reduction in BMD, and deficiencies in bone structure were evident much earlier in the development.[72] Moreover, the LRP6 mutant mice failed to attain peak trabecular bone volume, accompanied by a profound decrease in osteoblasts and significant reductions in mineral apposition and bone formation rates.[72] Another group generated mice carrying a collagen2a1-Cre-mediated deletion of LRP6 to examine the function of this gene in both developing and adult mouse skeletons; they found that mutation in LRP6 within the osteochondral lineage caused reductions in bone mass.[73] These studies proved that LRP6 had a pivotal role in the modulation of BMD and influenced skeletal development in mice.

Several lines of evidence suggest that LRP6 may be a key determinant of bone mass. Indeed, loss-of-function mutations in LRP6 may increase the risk of osteoporosis. Gaining insight into the relationship between LRP6 mutations and BMD will provide insight into utilizing LRP6 as a therapeutic target for osteoporosis and other bone-related diseases. A summary of the relevance between currently known LRP6 genetic variants and complex diseases is listed in Table 2.

WNT SIGNALING AND DIAGNOSTICS

Besides the LRP6 genetic polymorphisms, many other genetic variants in Wnt signaling pathway may also serve as predictors of some complex diseases. Kanazawa *et al.*[74] found a SNP in the Wnt5B gene to be strongly associated with T2DM in the Japanese population. A meta-analysis by Tong *et al.*[75] implicated IVS3C>T and IVS4G>T of TCF7L2 as risk factors for T2DM. Stewart DJ *et al.*[76] showed that Wnt inhibitory factor 1 rs10878232 may be related to NSCLC survival. Wnt16 rs2536182 was found to influence NSCLC recurrence, and Fz4 rs10898563 gene was found to be associated with both recurrence-free and overall survival in Caucasian patients.[77] Many researchers focused on the complex associations between mutations in Wnt pathway genes and bladder cancer, such as secreted frizzled-related protein 1 rs3242.[78] Wang JY *et al.*[79] discovered that Dkk1 rs2241529 correlated to baseline BMD. As emerging molecular biomarkers, some variants of Wnt siganaling pathway genes are in the process of translation to the clinic to help clinical diagnosis and treatment. And we discriminate the phase of the translational phase and the clinical annotation levels of evidence according to the pharmgkb website. A summary of mutations in Wnt pathway-related genes and their predicting functions in various diseases is shown in Table 3. And the location of known LRP6 SNPs in various diseases is shown in Figure 3.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

It is clear that the Wnt pathway has a functional role in metabolic regulation, cell proliferation, synaptic maintenance and function and bone mass regulation. Accordingly, Wnt pathway genes are good candidate genes for susceptibility to various diseases. As an indispensable co-receptor of the Wnt/β-catenin pathway, LRP6 has recently gained much attention. Such strong attention to the gene

Table 3. Summary of mutations in Wnt pathway-related genes and their predicting functions in various diseases

Gene	*SNP*	*Diseases*	*Population*	*Predicting function*	*Clinical annotation levels of evidence*	*Ref.*
Wnt5B	rs2270031	T2DM	Japanese	Susceptibility	Low level	74
TCF7L2	rs7903146	T2DM	Caucasian/ North European	Susceptibility	Moderate level	75
WIF1	rs10878232	NSCLC	American	Survival	Low level	76
Wnt16	rs2536182	NSCLC	Caucasian	Recurrence	Early discovery	77
Fz4	rs10898563	NSCLC	Caucasian	Recurrence-free & overall survival	Early discovery	77
SFRP1	rs3242	Bladder cancer	Caucasian	Susceptibility	Early discovery	78
Dkk1	rs2241529	osteoporosis	Chinese	Susceptibility	Early discovery	79

Abbreviations: Dkk1, Dickkopf-related protein 1; NSCLC, non-small cell lung cancer; SFRP1, secreted frizzled-related protein 1; SNP, single-nucleotide polymorphism; T2DM, type 2 diabetes mellitus; WIF1, Wnt inhibitory factor 1 .

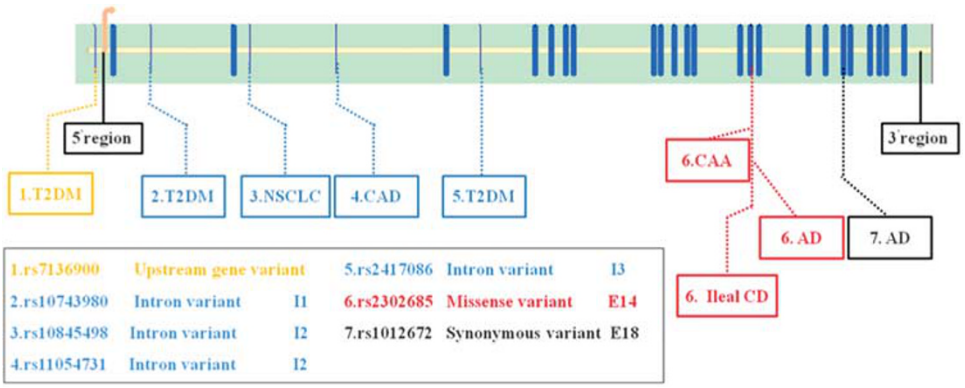

Figure 3. Diagram of known LRP6 SNPs in various diseases. Orange arrow represents transcriptional direction, and the blue columns are representative of exons. The LRP6 variants and possibly related diseases are noted in the genecard. AD, Alzheimer's disease; CAA, carotid artery atherosclerosis; CAD, coronary artery disease; Ileal CD, the early ileal Crohn's disease; I1: intron1; I2: intron 2; I3: intron 3; E14: exon 14; E18: exon 18; LRP6, lipoprotein receptor-related protein 6; NSCLC, non-small cell lung cancer; SNP, single-nucleotide polymorphism; T2DM, type 2 diabetes mellitus.

network of LRP6 has provided an opportunity to illustrate the importance of LRP6 mutations in the prediction of various diseases. Here, we summarized that variations in LRP6 and Wnt genes may affect the formation or progression of metabolic diseases, cancers, AD and osteoporosis.

Based on the fact that the majority of the published data were preliminary, further investigations are deserved to validate the currently reported biomarkers in LRP6 and Wnt pathway genes. However, it is highly anticipated that LRP6 variants will be applied clinically in the future. For instance, LRP6 rs2302685 has the potential to become one of the most useful and powerful biomarkers of accelerated atherosclerosis. Thus, hypertensive patients with this mutation may need some clinical checkment to detect subclinical atherosclerosis.[23] Besides, LRP6 rs2302685, predisposing people toward early-onset ileal Crohn's disease, may also turn into an attractive therapeutic target as an alternative to the current anti-inflammatory approaches in the therapy of Crohn' diseases.[80] Another variant, LRP6 rs6488507, may be associated with the increased risk of NSCLC in tobacco smokers, and thus the clinical diagnostic testing may be necessary for carriers with smoking history.[51] Hence, it is expected that further research in this field means a lot to the prediction or treatment of complex diseases.

Although previous studies have shown that LRP6 is associated with a variety of diseases, there are many problems demanding prompt resolution. (1) The existing results are frequently conflicting because of racial differences. Thus, expanding sample sizes in different ethnic populations needs to be addressed. (2) Many aspects of LRP6 and the Wnt pathway remain eclusive (for example, knowledge about the contributions of LRP6 in different cancer types). Undoubtedly, such knowledge will be crucial to support more accurate diagnosis. (3) There are important issues in translational science, namely, how to translate findings from bench to clinical application. A possible solution to this problem is to begin an investigation into the regulation of LRP6 in various cells and tissues to broaden our knowledge of the roles of genes. Such an approach will contribute to address the unmet demands of molecular development, both diagnostics and therapeutic targets, to improve the clinical management of these diseases' diagnosis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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