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Chapter

Wnt Signaling and Genetic Bone Diseases

Yanqin Lu and Jinxiang Han

Abstract

The Wnt signal transduction plays a vital role in regulating development throughout the animal kingdom. The Wnt signal transduction is complex, including Wnt ligands, receptors, coreceptors, transducers, transcription factors, antagonists, agonists and their modulators, and target genes. It is classified into β-catenin-dependent canonical and independent non-canonical Wnt (mainly planar cell polarity and Wnt/Ca^{2+}) signaling pathways. Wnt signaling pathway is causative to multiple human diseases. Gene mutations from the components of WNT signaling machinery have been identified to relate with low or high bone mass diseases, such as osteogenesis imperfecta, Robinow syndrome, osteoporosis-pseudoglioma syndrome, and sclerosteosis. In this review, we provide an update of the Wnt signaling pathway and the bone diseases caused by the aberrant components of the pathways.

Keywords: Wnt, Wnt signaling pathway, genetic bone diseases

1. Introduction

The Wnt1 gene (originally named Int1) was identified in 1982 as a gene activated by integration of mouse mammary tumor virus (MMTV) proviral DNA in virally induced breast tumors [1]. The Int1 proto-oncogene is highly conserved in many species, the fly wingless (Wg) gene in Drosophila, functions in controlling segment polarity during larval development and also activated in cancer, was found to be a homolog of Wnt1 [2]. Later, McMahon and Moon found that ectopic expression of Int1 in Xenopus leads to dual axis formation, when mouse Int1 RNA was injected into Xenopus embryos. Duplication of axial structures was abolished by substitution of a single, conserved cysteine residue of Int1 [3]. Later, more and more Wnt family members were identified.

2. Wnt and its secretion

2.1 Wnt proteins and their structure

Till now, Wnt family currently includes 19 secreted lipid-modified glycoproteins in most mammalian genomes, including the human genome. They fall into 12 conserved Wnt subfamilies, of which at least 11 of these occur in the genome of a Cnidaria, highlighting the vital role of Wnt family members in the process of organismal patterning throughout the animal kingdom [4]. In humans, Wnt1 and Wnt10b are located adjacent to each other on chromosome 12, and they are

transcribed in opposite directions. Wnt6 and Wnt10a are located adjacent to each other on chromosome 2 and transcribed from the same strand of DNA. Other Wnt genes are prone to be clustered within the human genome also, including Wnt2 and Wnt16, Wnt3a and Wnt14, and Wnt3 and Wnt15 [5]. Wnt1-Wnt6-Wnt10 is an ancient cluster of Wnt genes in a common ancestor of vertebrates and arthropods and this cluster was duplicated leading to Wnt1-Wnt6-Wnt10a and Wnt1-Wnt6-Wnt10b cluster in vertebrates [5]. Based on their ability to induce transformation of the epithelial cell line C57MG, Wnt family are classified into highly transformation members, which includes Wnt1, Wnt2, Wnt3, and Wnt3a, and nontransformation members including Wnt4, Wnt5a, Wnt5b, and Wnt7b. High transformation members are related to Wnt/β-catenin canonical pathway and nontransformation members are related to noncanonical Wnt pathways. Wnt6 and Wnt7a are categorized as intermediate transformation members, leading to weak morphological changes [6].

Wnt genes encode proteins of ~350–400 residues in length, with molecular weight of about 40 kDa in size. Little is known about the structure of Wnts for their highly hydrophobic characteristics. In 2012, the 3D structure of Xenopus Wnt8 protein as bound to mouse Frizzled-8 cysteine-rich domain (CRD) was solved. XWnt8 is consist of an N-terminal α-helical domain (NTD) that includes the lipid-modified thumb and a C-terminal cysteine-rich region (CTD). They resemble the extended thumb and index fingers to project into a pocket in the opposite side of Fzd-CRD [7].

2.2 Posttranslational modifications of Wnts in the ER and Golgi apparatus

Wnt proteins share some features in common. They have an amino-terminal signal peptide that targets them to the ER and undergo a series of posttranslational modifications in the secretory pathway before transporting into the extracellular space. Wnts contain several charged residues and 23–25 cysteines on average, and some of them participate in inter- and intramolecular disulfide bonds, leading to Wnt folding and multimerization [7, 8]. All Wnt proteins (except Drosophila WntD) undergo posttranslational acylation and glycosylation [9]. There are two conserved residues of fatty acylation reported till now. The first acylation is palmitate attached to a conserved cysteine residue 77 in murine Wnt3a through a thioester linkage. The second lipid modification was identified at the position of serine 209 in murine Wnt3a protein. This conserved residue is modified by a monounsaturated fatty acid, palmitoleic acid [10–12]. This lipid posttranslational modification leads to extremely hydrophobicity of Wnts and restrict Wnt proteins to membranes by injecting into the lipid bilayer [9, 11]. Cys77 mutant leads to the loss of Wnt3a activity without affecting secretion, while Wnt3a Ser209Ala mutant is retained in the ER and secretion is blocked [10, 11]. Crystal structure of XWnt8 discovered that only conserved serine (corresponding to serine 209 in murine Wnt3a) is acylated. Cys77 is involved in the formation of disulfide bond with a second conserved cysteine [7]. Till now, *Drosophila* WntD is the only nonlipidated member of Wnt family [13]. Monoacylation is further corroborated by the lack of Cys77 palmitoylation study [14, 15]. This serine acylation is essential for Wnt binding to the coreceptor Frizzled, Wnt secretion and binding to the chaperone Wntless [7, 16, 17].

The attachment of palmitoleate to Wnt's conserved serine is mediated through substrate specificity by acyltransferase Porcupine, which is homologous to the superfamily of acyltransferase enzymes localized to the endoplasmic reticulum (ER). Mutation of Porcupine impeded Wnt acylation activity in vitro [18]. Wnt palmitoylation is reversible and it can be removed by Notum, the serine hydrolase, and this deacylase activity is specific for Wnt proteins [19, 20]. Hence, Notum's inhibitors have potential for treating degenerative diseases by targeting Wnt signaling [21].

N-Glycosylation is another common posttranslational modification of Wnt ligands, and nitrogen atom of multiple asparagine residues of Wnts is attached to oligosaccharide. This modification precedes palmitoylation and is independent of it [22, 23]. The number and position of N-glycosylation vary in different Wnt members [24]. The role of Wnt protein's N-glycosylation is unclear, but usually, it influences secretion, but not folding and structure [9]. For Wg protein, which has two known N-glycosylation, Asn103 and Asn414, Wg mutant can activate downstream signaling in both autocrine and paracrine signaling, despite reduced secretion ability. Loss of N-glycosylation of Wnt1 impairs paracrine signaling. For Wnt3a and Wnt5a, N-glycosylation is essential for secretion, but not for the activity of Wnt5a protein [23, 25]. Porcupine plays an important role in both lipid and glycosylated modifications of Wnts and its mutant displayed a decreased N-glycosylation activity [8–10, 26].

Besides acylation and N-glycosylation of Wnt proteins, several other modifications are included in the posttranslational modification. Posttranslational tyrosine sulfation of Wnt5a and Wnt11 is essential for the formation of Wnt5a/Wnt11 complexes, which induce the efficient signaling in the context of Xenopus axis formation [27]. Wnt1 is attached to glycosylphosphatidylinositol (GPI) anchor on the leaflet of the plasma membrane by the glycolipid tail. PGAP1 gene participates in this modification by creating a hydrophobic Wnt1 that is retained in the ER [28].

2.3 Secretion and release of Wnt proteins

After posttranslational palmitoylation and N-glycosylation, mature Wnt proteins are then transported from the Golgi to the plasma membrane for secretion by the conserved multipass transmembrane Wntless (Wls) receptor (known as GPR177 in mammals) [29]. Wnt secretion could not proceed with the absence of Wls, but other signaling proteins are not influenced by the removal of Wls [30–32]. Wls knockout mice exhibit impairment of body axis formation, and a phenotype mimics the deficiency of Wnt3. Wls is activated by β-catenin and LEF/TCF-dependent transcription and its mutants impede Wnt secretion and signaling [33]. Wls is essential for Wnt signaling, and tissue-specific knockouts of Wls impede varieties of processes including bone mass, skin homeostasis, peripheral lung differentiation, and pulmonary vascular development [34–36].

Endogenous Wls contains a carboxy-terminal ER-targeting signal, which directs Wls localizing predominantly in the ER, where it binds with acylated Wnt proteins [16, 37]. P24 protein family, which acts as cargo receptor for Wnt in the early secretory pathway, is essential for proper export of Wg from the ER [38–40]. Sec22 is packaged together with Wg and p24 during the early secretory phase of Wg and it functions as the vesicle SNARE (soluble NSF attachment protein receptor) [40].

The detailed mechanisms for Wnt secretion are not clear. Wnts-Wls complex transport from ER to plasma membrane is COPII vesicles dependent. Once arriving at the plasm membrane, Wnt is then released from plasm membrane and binds to lipoprotein particles or heparin sulfate proteoglycans (HSPGs) [41, 42]. The other theory supports that Wnt-Wls complex keeps together and internalizes at plasm membrane and dissociates from each other in endosomes. Then, Wnts is released through a recycling endosomal pathway and Wls is transported back to TGN through a retromer-dependent pathway [42–44]. Dpy23 and Vps35 are reported to regulate recycling of *C. elegans* Mig-14, which is the homolog of Wls. Wls is restricted to the plasma membrane with the Dyp23 mutant [45]. Retromer complex consists of Vps35, Vps29, Vps26, Vps10, Vps5, and Vps17 in yeast [46–48]. Vps35, Vps29, and Vps26 subcomplexes mediate cargo recognition and retrieve Vps10p from endosomes to

the Golgi [47]. Vps35 mutant has no influence on the transportation of Wls to plasm membrane and endocytosis, but the retromer-dependent shuttle to the Golgi is inaccessible, and endocytosed Wls progresses to MVBs and lysosomes for degradation [43, 44, 49]. Vps5 and Vps17 are membrane-bound subcomplexes of retromer, and they are sorting nexins (SNX) with a phosphoinositide-binding SNX-phox homology (SNX-PX) domain [50]. Nexins SNX1, SNX2, SNX5, and SNX6 are SNX-BAR coat complex that interact with cargo-selective Vps35-Vps29-Vps26 complex. They are needed for most of the retromer cargo proteins, but not for the process of Wls recycling [50, 51]. Wls recycling specifically relies on SNX3, the retromer without BAR domain [51, 52]. SNX3 cointeracts with Wls and Vps26 on early endosomes and helps the association of the cargo-selective complex to Wls [51]. Wls recycled in Golgi further retrogrades transport to ER, which is mediated by the conserved C terminal sequence of Wls targeting ER. This process is currently COPI dependent and requires ER-Golgi intermediate compartment ERGIC2, though retrieval mechanisms need further investigation [37, 53]. Recently, miR-307a is found to inhibit Wg secretion by targeting Wls, and its overexpression induces ER stress specifically in the Wg-expressing cells. KKVY motif of Wg is responsible for its retrieval and ER stress [53].

Wnts are classic morphogens, which play an important role in tissue patterning by activating their target genes in a concentration-dependent manner and act in short and long range way [14, 54]. Various carriers have been identified that associate with extracellular Wnts, which include exovesicles [55], exosomes [56, 57], lipoprotein [41, 58], cytonemes (filopodia-like protrusions) [59–61], and Swim (secreted Wnt-interacting molecule) belonging to lipocalin family of protein [62]. These secreted Wnts associate to specific receptors on target cells to activate either canonical Wnt/β-catenin pathway or noncanonical Wnt/Ca^{2+} pathway.

3. Wnt signaling pathway

3.1 The canonical Wnt signaling pathway

The Wnt signaling pathway serves many important functions in body axis patterning, embryonic development, cell proliferation, and differentiation. In the absence of Wnt signaling, β-catenin is phosphorylated and ubiquitinated to keep low level by forming β-catenin destruction complex. The complex includes β-catenin, axin, casein kinase-1 (CK1), glycogen synthase kinase-3β (GSK-3β), and the adenomatous polyposis coli (APC) [63, 64]. PP2A and HSP105 are also involved in this complex. HSP105 recruits the phosphatase PP2A to the degradation complex to antagonize the phosphorylation of β-catenin, thus keeping the balance of phosphorylation-dephosphorylation [65]. Maintaining a phosphostatus balance of the β-catenin protein leads to its accumulation or degradation based on the signaling cues. The complex binds and phosphorylates β-catenin, leading to the ubiquitination by β-transducin repeat-containing protein (β-TrCP) ubiquitin ligase and subsequent proteasomal degradation [66].

In the presence of Wnt ligands, Wnt ligands bind to the specific receptor including Frizzled (Fzd) family member and subsequent LRP5/6 coreceptor. Axin is dephosphorylated and sequestered at the membrane. The binding triggers the recruitment of phosphoprotein disheveled (Dsh/Dvl) to form the LRP/Fzd/Dsh complexes, inducing the phosphorylation of LRP by CK1ϒ and GSK3; as a consequence, axin is then dephosphorylated and sequestered at the membrane and destruction complex is inactivated. The signalosome composed of Fzd, LRP5/6, Dvl, axin, GSK3, and CK1 destroys the β-catenin destruction complex [67, 68].

Hence, cytosolic β-catenin accumulates and localizes to the nucleus, where it interacts with TCF/LEF family members and recruits other transcriptional coactivators, such as CBP, TBP, and BRG-1, to induce target gene expression [69, 70].

Axin is a scaffold protein and acts as an anchor for other four proteins in the complex. In addition, axin participates in the LRP6 phosphorylation on the PPPSPxS motifs, which in turn cause the accumulation of axin in the destruction complex and then lead to the initiation of β-catenin signaling [71]. Recently, axin was found to be fully phosphorylated in the state of Wnt-off and partly phosphorylated in the state of Wnt-on mediated by GSK-3β [72].

The role of APC in Wnt signaling is complex and multiple. APC acts as a carrier for GSK-3β and axin that promotes phosphorylation and consequent ubiquitin-dependent degradation of β-catenin [73]. It binds to β-catenin by 15 or 20-mer amino acid repeats. APC promotes export of β-catenin from nucleus, and hence the expression and transcriptional activity of nuclear β-catenin are reduced indirectly [74]. Meanwhile, APC downregulates the β-catenin/TCF transcription by directly interacting with transcriptional repressor C-terminal binding protein (CtBP) [75, 76]. APC may also serve as a positive regulator for Wnt signaling through downregulation of axin [77]. APC is vital for the phosphorylation of axin in both Wnt-off and Wnt-on states, the association with activated phospho-LRP6 and the rapid transition in axin activity [72]. Phosphorylated β-catenin requires APC for its targeting to ubiquitin ligase and protection from dephosphorylation mediated by protein phosphatase 2A (PP2A) [78]. Recently, APC was found to impede clathrin-dependent signalosome formation in the absence of ligand [79].

GSK3-β and CK1 are both serine/threonine kinases that phosphorylate the N-terminal portion of cytosolic β-catenin, and phosphorylation of β-catenin begins at Ser45 by CK1α and then phosphorylation of residues Thr41, Ser37, and Ser33 [80, 81]. Meanwhile, CK1, perhaps also GSK3β, phosphorylates APC on the 20-mer repeats. Phosphorylation of APC increases the binding affinity to β-catenin, and β-catenin disassociates from axin [63]. Phosphorylated β-catenin is then recognized by β-TrCP1, an F-box protein component of an Skp1/Cul1/F-box (SCF)-type ubiquitin ligase complex [82], followed by recruitment of E3 ubiquitin ligase and degraded by the 26S proteasome [83].

PP2A is a cellular heterotrimeric serine-threonine protein phosphatase consisting of a structural (A), a regulatory (B), and a catalytic subunit (C) [84]. PP2A has a dual opposite regulation role for Wnt signaling. PP2A is regarded as one of the members of β-catenin degradation complex [85]. PP2A dephosphorylates GSK3β through recruitment of DNAJB6 (DnaJ homolog subfamily B member 6) and HSPA8 (heat-shock cognate protein, HSC70) [86]. The B56 subunit of PP2A interacts with N-terminal of APC and decreases the amount of β-catenin and inhibits transcription of its target genes [87, 88]. Also, B56ε is required for Wnt/β-catenin signaling downstream of the Wnt ligand and upstream of Dsh [89]. PR61 β regulates Wnt signaling by inhibiting Dvl- and β-catenin–dependent T-cell factor activation, or suppressing the downstream target genes [90]. PR55α subunit of PP2A acts as the positive regulator for Wnt signaling. It interacts with β-catenin directly and controls dephosphorylation and degradation of β-catenin. Knockdown of PR55α increases β-catenin phosphorylation and decreases Wnt signaling, whereas is the same as PR55α overexpression [91, 92]. PP2C also upregulates Wnt signaling through the dephosphorylation of axin [93]. Meanwhile, many subunits of PP2A, such as PR55α, A, C, B56α, and PR61β and ϒ, are reported to interact with axin [87, 90, 91]. PR61ε subunit of PP2A is involved in the initiation of the Wnt pathway. PR61ε binds to Fzd receptor, and binding of Wnt ligands promote the interaction of LRP5/6-associated CK1ε and PR61ε. The latter dephosphatases CK1ε, leading to recruitment of Dvl-2 to the receptor complex and the initiation of the Wnt signaling [94].

3.2 The noncanonical Wnt pathway

Due to varieties of both Wnts and their receptors and coreceptors, Wnt pathways are multiple and complex. There are multiple branches of β-catenin–independent Wnt signaling pathways. One is the Wnt/Ca^{2+} pathway, modulating intracellular Ca^{2+} level. The second is the Wnt/planar cell polarity (PCP) pathway, utilizing small Rho-like GTPases [95].

3.2.1 Wnt/PCP pathway

Polarization is a global property of cells and tissues. In addition to the ubiquitous epithelial apical-basal axis, many multicellular tissues also have planar cell polarity, orthology to apico-basal polarity [96, 97]. Compared with canonical Wnt signaling, various cell surface receptors have been involved in PCP signaling. PCP is composed of core protein complexes and Fat/Dachsous (Ds)/Fj (four-jointed) group. The latter is reported to act upstream of PCP to provide a directional information [98, 99]. Core protein complexes are composed of Frizzled, Flamingo (Fmi/Celsr), Van Gogh (*Drosophila* Vang or Stb/mammalian Vang), disheveled (Dsh/Dvl), Diego, and Prickle (Pk) [100]. The core complex within puncta is predominately stable than elsewhere in the junctions and highly asymmetrically organized, while core protein stoichiometry in both puncta and nonpuncta region is similar. The core protein is assembled around a stoichiometric Fz-Fmi nucleus. The amount of Fz and Stb is maintained relative to their binding partners for normal asymmetry [101]. In many cancers, Wnt/PCP signaling is upregulated and it contributes to cancer malignancy by enhancing the proliferation and migration, priming metastasis niches, and causing resistance to therapy [102, 103].

3.2.2 Wnt/Ca^{2+} signaling pathway

Wnt5a is the most common ligand for noncanonical Wnt signal transducer. It activates calcium signaling pathway by binding to receptor Fz2, 3, 4, 5, and Fz6, as well as coreceptor Ror1/2, which is the membrane-bound receptor tyrosine kinase [104-107]. Dvl, axin, and GSK organize the complex and GSK phosphorylates Ror coreceptor [108]. Wnt/Fz/Ror then activates phospholipase C (PLC), leading to the generation of diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) from membrane-bound phospholipid phosphatidyl inositol 4,5-bisphosphate (PIP2). Recently, SEC1413/the Sec14-like protein acts as GTPase proteins to mediate specific Wnt-Fz-Dvl complex signals downstream to phospholipase C δ4a (PLCδ4a). The binding of SEC141 to Wnt-Fz-Dvl complexes induces its translocation of SEC1413 to the plasma membrane, and then further binds to and activates PLCδ4a. In turn, PLCδ4a acts as a GTPase-activating protein to promote the hydrolysis of Sec14l3-bound GTP to GDP [109]. IP3 promotes the concentration of intracellular Ca^{2+}, which activates calcineurin, phospho-serine/threonine specific protein phosphatase and calcium calmodulin-dependent protein kinase II (CaMKII). In turn, nuclear factor associated with T cells (NFAT) and regulatory proteins NFkB are activated. DAG activates protein kinase C (PKC), which further activates NFkB and CREB. Meanwhile, Wnt/Fz interaction may activate phosphodiesterase 6 (PDE6) in a calcium-dependent manner, leading to a decrease in cyclic guanosine monophosphate (cGMP) [110].

4. Wnt signaling in genetic bone diseases

Both bone modeling and remodeling are regulated by Wnt signaling, and mutation of Wnt signaling components is linked to various genetic bone diseases. **Table 1**

Phenotype	Phenotype MIM number	Inheritance	Gene	Gene MIM number	Reference
Wnt ligands					
Osteogenesis imperfecta, type XV	615220	AR	Wnt1	164820	Pyott et al. [125]
Osteoporosis, early-onset, susceptibility to, autosomal dominant	615221		Wnt1		Laine et al. [127]
Tetraamelia syndrome 1	273395	AR	Wnt3	165330	Niemann et al. [115]
Robinow syndrome, autosomal dominant 1	180700	AD	Wnt5a	164975	Person et al. [138]
Fuhrmann syndrome	228930	AR	Wnt7a	601570	Woods et al. [116]
Ulna and fibula, absence of, with severe limb deficiency	276820	AR	Wnt7a	601570	Woods et al. [116]
Odontoonychodermal dysplasia	257980	AR	Wnt10a	606268	Adaimy et al. [139]
Schopf-Schulz-Passarge syndrome	224750	AR	Wnt10a	606268	Bohring et al. [140]
Tooth agenesis, selective, 4	150400	AR, AD	Wnt10a	606268	Kantaputra and Sripathomsawat [111]
Split-hand/foot malformation 6	225300	AR	Wnt10b	601906	Ugur and Tolun [117]
Tooth agenesis, selective, 8	617073	AD	Wnt10b	601906	Yu et al. [112]
Receptor/coreceptor					
Robinow syndrome		AR	FZD2	600667	White et al. [141]
Nail disorder, nonsyndromic congenital, 10	614157	AR	FZD6	603409	Frojmark et al. [142]
Cenani-Lenz syndactyly syndrome	212780	AR	LRP4	604270	Li et al. [143]
Sclerosteosis 2	614305	AR, AD	LRP4	604270	Leupin et al. [132]
Osteopetrosis, autosomal dominant 1	607634	AD	LRP5	603506	Van Wesenbeeck et al. [122], Van Hul et al. [123]
Osteoporosis-pseudoglioma syndrome	259770	AR	LRP5	603506	Gong et al. [124]
Osteosclerosis	144750	AD	LRP5	603506	Van Wesenbeeck et al. [122]
Hyperostosis, endosteal	144750	AD	LRP5	603506	Van Wesenbeeck et al. [122]
Van Buchem disease, type 2	607636	AD	LRP5	603506	Van Wesenbeeck et al. [122], Little et al. [130]
Bone mineral density variability 1	601884	AD	LRP5	603506	Nguyen et al. [131]

Phenotype	Phenotype MIM number	Inheritance	Gene	Gene MIM number	Reference
Osteoporosis	166710	AD	LRP5	603506	Estrada et al. [121]
Tooth agenesis, selective, 7	616724	AD	LRP6	603507	Massink et al. [113]
Brachydactyly, type B1	113000	AD	ROR2	602337	Oldridge et al. [144]
Robinow syndrome, autosomal recessive	268310	AR	ROR2	602337	van Bokhoven et al. [145], Afzal et al. [146]
Simpson-Golabi-Behmel syndrome, type 1	312870	XLR	GPC3	300037	Pilia et al. [147]
Omodysplasia 1	258315	AR	GPC6	604404	Campos-Xavier et al. [148]
Fetal akinesia deformation sequence	208150	AR	MUSK	601296	Tan-Sindhunata et al. [149]
Antagonist					
Osteoarthritis susceptibility 1	165720	Mu	SFRP3	605083	Loughlin et al. [150]
Pyle disease	265900	AR	SFRP4	606570	Kiper et al. [129]
Craniodiaphyseal dysplasia, autosomal dominant	122860	AD	SOST	605740	Kim et al. [135]
Sclerosteosis 1	269500	AR	SOST	605740	Brunkow et al. [134]
Van Buchem disease	239100	AR	SOST	605740	Balemans et al. [133]
Agonists					
Robinow syndrome, autosomal dominant 2	616331	AD	DVL1	601365	White et al. [151]
Robinow syndrome, autosomal dominant 3	616894	AD	DVL3	601368	White et al. [152]
Bone mineral density, low, susceptibility to	615311		LGR4	606666	Styrkarsdottir et al. [128]
Palmoplantar hyperkeratosis with squamous cell carcinoma of skin and sex reversal	610644	AR	RSPO1	609595	Parma et al. [153]
Humerofemoral hypoplasia with radiotibial ray deficiency	618022		RSPO2	610575	Szenker-Ravi et al. [114]
Tetraamelia syndrome 2	618021		RSPO2	610575	Szenker-Ravi et al. [114]
Anonychia congenita	206800	AR	RSPO4	610573	Blaydon et al. [154]

Table 1.
Wnt signaling and human genetic bone diseases.

lists human genetic bone diseases caused by Wnt signaling disorders. Genotypic and phenotypic heterogeneity of genetic bone diseases–related Wnt signaling pathways is obvious. Tooth agenesis is caused by Wnt 10a, Wnt10b, and LRP6 by either autosomal dominant (AR) or autosomal recessive (AR) inheritance form [111–113]. Tetraamelia syndrome is skeletally characterized by limb agenesis or complete absence of limbs, bilateral cleft lip/palate, ankyloglossia, and mandibular hypoplasia with the pathogenic gene of Wnt3 and RSPO2 [114–115]. Other limb deficiency diseases in Wnt signaling includes Al-Awadi/Raas-Rothschild/Schinzel phocomelia syndrome (AARRS) (MIM 276820) and split-hand/foot malformation 6 (MIM 225300), with pathogenic gene of Wnt7a and Wnt10b, respectively [116, 117].

Robinow syndrome (RS) is characterized by facial features, orodental abnormalities, and hypoplastic genitalia [118]. All autosomal-dominant (DRS) and recessive (RRS) genes including Wnt5a, Dvl1, Dvl3, Fzd2, and ROR2 are involved in the Wnt/PCP pathways. This pathway plays an important role in the patterning and formation of the limb-bud outgrowth and growth plate in skeletal formation [119, 120].

Wnt signaling pathways are related to bone diseases with osteoporosis or high bone mass density (BMD) diseases. LRP5 gene is responsible for osteoporosis. Loss of function of LRP5 mutation causes osteoporosis (MIM 166710, 607634) and osteoporosis pseudoglioma syndrome (MIM 259770) [121–124]. Meanwhile, osteoporosis genes in Wnt signaling components include Wnt1, LGR4, and SFRP4. Wnt1 is the pathogenic gene for osteogenesis imperfect type XV (with bilateral mutations) and early onset osteoporosis (with heterozygous mutation) [125–127]. For LGR4, nonsense variation of c.376C-T is strongly associated with low bone mass density and osteoporotic fractures [128]. SFRP4 is the pathogenic gene for Pyle disease characterized by both osteoporosis and expanded trabecular metaphyses [129].

LRP5 is also the pathogenic gene for diseases with high BMD, Van Buchem syndrome type 2 (MIM 607636), bone mineral density variability (MIM 601884), osteosclerosis, and hyperostosis, endosteal (MIM 144750) [122, 130, 131]. LRP4 mutations lead to type I sclerosteosis (MIM 614305), which is also the disease with high BMD [132]. Sclerosteosis (SOST) gene mutation causes the high BMD diseases of Van Buchem syndrome (MIM 239100), sclerosteosis 1 (MIM 269500), and craniodiaphyseal dysplasia (MIM 122860) [133–135]. Sclerostin encoded by SOST gene is the endogenous Wnt signaling inhibitor, which interacts with LRP receptors [136]. Nowadays, monoclonal antibody of sclerostin is being tested in human clinical trials [137].

In all, the components of Wnt signaling including Wnt ligands, their receptors, coreceptors, antagonists, and agonists can cause different types of genetic bone diseases, which are related to both canonical and noncanonical Wnt signaling pathways. Study of Wnt signaling in genetic bone diseases and other human diseases provides promises for translational medicine.

5. Conclusions

We review the current status of Wnt signaling, including the secretion of Wnt ligands, and how Wnts binding to surface receptors trigger different intracellular response and transcription of different downstream target genes. However, the interactions among each components and the mechanisms of these interactions still need further study. Meanwhile, the cross talk network between canonical and noncanonical Wnt signaling, Wnt signaling, and other signaling pathways remains unsolved fully. Mutations in the components of Wnt signaling pathways lead to various genetic bone diseases and other genetic diseases. Genotypic and phenotypic

heterozygosis is common in these genetic bone diseases. For the vital role of Wnt signaling components in bone diseases, potential drugs based on Wnt signaling is useful for treating different bone diseases.

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Conflict of interest

No conflict of interest.

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