Molecular Biology Reports (2005) 32: 67–77
DOI 10.1007/s11033-004-7657-z
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# LIM-homeodomain genes in mammalian development and human disease

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Accepted 6 December 2004

*Key words:* differentiation, mouse, rat, transcription factor

**Abstract**

The human and mouse genomes each contain at least 12 genes encoding LIM homeodomain (LIM-HD) transcription factors. These gene regulatory proteins feature two LIM domains in their amino termini and a characteristic DNA binding homeodomain. Studies of mouse models and human patients have established that the LIM-HD factors are critical for the development of specialized cells in multiple tissue types, including the nervous system, skeletal muscle, the heart, the kidneys, and endocrine organs such as the pituitary gland and the pancreas. In this article, we review the roles of the LIM-HD proteins in mammalian development and their involvement in human diseases.

*Abbreviations:* bHLH – basic helix–loop–helix; BMP – bone morphogenetic protein; CNS – central nervous system; CPHD – combined pituitary hormone deficiency; FSH – follicle-stimulating hormone; GABA – gamma aminobutyric acid; GAP – GTPase-activating protein; HD – homeodomain; ISL – islet; LHX – LIM homeobox gene; LIM-HD – LIM homeodomain; LMX – LIM homeobox gene; MGE – medial ganglionic eminence; NIDDM – non-insulin-dependent diabetes mellitus; NPS – nail-patella syndrome; RNAi – RNA interference; SH3 – Src homology 3; SNP – single nucleotide polymorphism.

## Introduction

LIM proteins are found in invertebrate and vertebrate eukaryotes and have diverse biochemical functions (reviewed in [1–4]). Originally named for the LIM domain-containing transcription factors Lin11, Isl1, and Mec3, the LIM protein superfamily includes cytoskeleton-associated proteins that contribute to cellular architecture, intracellular signaling proteins such as protein kinases and GTPase-activating factors, transcription factors, and transcriptional coactivators. At least one hundred LIM proteins are presently annotated as gene products of the human genome (NCBI, http://www.ncbi.nlm.nih.gov/). The LIM domain is a multifunctional protein/protein interaction domain of approximately 50–60 amino acids. Each cysteine-rich LIM domain binds two zinc ions to form a finger-like structure [5–7]. LIM proteins can contain single or multiple LIM domains that are sometimes found in association with other domains, including homeodomains, PDZ domains, kinase domains, SH3 domains, and GAP domains (Pfam, http://www.sanger.ac.uk/Software/Pfam/).

Mammalian genomes such as those of mice, rats, and humans contain at least 12 LIM homeodomain (LIM-HD) genes that encode key regulators of developmental pathways (Table 1). LIM-HD proteins are transcription factors that form a subfamily of LIM proteins featuring two LIM domains in their amino termini and a centrally located HD that is used to interact with specific DNA elements in target genes. Alignments of the primary amino acid sequences of the LIM-HD proteins indicate evolutionary relationships between the LIM-HD genes (Figure 1) and the

*Table 1*. Genetic loci and diseases associations of mouse, rat and human LIM-HD genes. OMIM, Online Mendelian Inheritance in Man database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM)

| Mouse/rat gene | Alternate names | Human gene | Mouse chromosomal location | Rat chromosomal location | Human chromosomal location | OMIM number | Human disease |
|---|---|---|---|---|---|---|---|
| *Lhx1* | *Lim1* | *LHX1* | 11 (48.0 cM) | 10q26 | 17q11.2-q12 | 601999 | None known |
| *Lhx2* | *LH-2*, *LH-2A*, (*apterous*) | *LHX2* | 2 | 3q11 | 9q33-9q34.1 | 603759 | Expression associated with (not the cause of) chronic myelogenous leukemia |
| *Lhx3* | *Lim3*, *P-Lim* | *LHX3* | 2 (16.0 cM) | 3 | 9q34.3 | 600577 | Pituitary hormone deficiency and rigid cervical spine. |
| *Lhx4* | *Gsh-4* | *LHX4* | 1 (82.0 cM) | 13q21 | 1q25.2 | 602146 | Pituitary hormone deficiency, ectopic posterior pituitary, cerebellar defects, and sella turcica abnormalities. *LHX4* gene also involved in chromosomal translocations found in some leukemias. |
| *Lhx5* | *Lim2*, *Lim5* | *LHX5* | 5 (64.0 cM) | 12q16 | 12q24.31-q24.32 | 605992 | None known |
| *Lhx6* | *Lhx6.1* | *LHX6* | 2 | 3p11 | 9q33.2 | 608215 | None known |
| *Lhx7/Lhx8* | *L3* | *LHX7/ LHX8* | 3 | 2q45 | 1p31.1 | 604425 | None known |
| *Lhx9* | *LH-2B* | *LHX9* | 1 | 13q13 | 1q31-q32 | 606066 | None known |
| *Isl1* | *Islet-1* | *ISL1* | 13 | 2q15 | 5q11.2 | 600366 | Potential association with type II diabetes |
| *Isl2* | *Islet-2* | *ISL2* | 9 | 8q24 | 15q23 | – | None known |
| *Lmx1a* | *Lmx1.1*, *dreher*, *shaker short-tail* (*sst*) | *LMX1a* | 1 (88.2 cM) | 13q24 | 1q22-q23 | 600298 | None known |
| *Lmx1b* | *Lmx1.2* | *LMX1b* | 2 (21.0 cM) | 3p11 | 9q34 | 602575 | Nail-patella syndrome; abnormal kidney development |

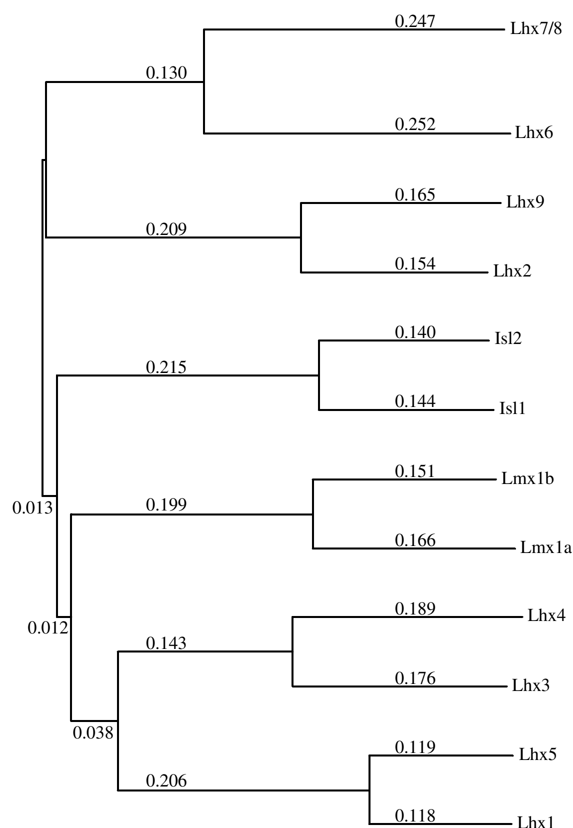


*Figure 1.* Phylogenetic tree comparing the complete amino acid sequences of mouse LIM-HD proteins. Multiple alignments were performed using ClustalX 1.83 and the resulting tree was visualized using NJplot [132]. Numbers reflect the number of substitutions/site/sequence. Similar groupings were obtained by Failli and colleagues [69] in a broader alignment including other vertebrate and invertebrate species.

known expression patterns and functional properties of the LIM-HD factors are generally consistent with these groupings (see below).

The actions of LIM-HD proteins are modulated by their interactions with partner proteins (reviewed in [1]). These LIM domain-interacting proteins include NLI/LDB/CLIM/CHIP, MRG1, SLB, and RLIM, an ubiquitin protein ligase (reviewed in [1] and see [8–10]). For example, NLI factors are nuclear proteins that can mediate homo- and heterodimerization of LIM-HD proteins. NLI family members also may act to control the activities of LIM-HD proteins by modulating their ability to regulate target genes (reviewed in [1]). Further, LIM-HD proteins and their regulatory partners, such as NLI, appear to regulate the transcription of target genes by acting in tandem with other transcription factors. Examples include synergistic regulation of anterior pituitary genes by the LHX3 LIM-HD factor and the PIT-1 POU-domain protein (reviewed in [11]) and combinatorial control of tissue-specific genes in the developing nervous system and pancreas by LIM-HD proteins and basic helix–loop–helix transcription factors (e.g. [12–14]). In addition to mediating interactions with other proteins, the LIM domains of LIM-HD factors can confer *trans*-activation functions (e.g. [8, 15]) and, in selective cases, can modulate the DNA binding properties of the proteins (reviewed in Hobert [15–17]). In this article, we summarize the functions of the mammalian LIM-HD proteins in development and their association with human diseases.

## Functions of LIM-HD factor genes

### *Lhx1*

During embryogenesis in the mouse, *Lhx1* (also known as *Lim1* – see Table 1) is expressed early in mesodermal tissue, then later during urogenital, kidney, liver, and nervous system development [18–20]. In the adult, expression is restricted to the kidney and brain. A targeted deletion of the entire *Lhx1* gene in mouse embryos causes an anencephalic phenotype, with the animals lacking anterior head structures, kidneys, and gonads, but with normally developed trunk and tail morphology [21]. Disruption of the *Lhx1* LIM domain encoding sequences is alone enough to phenocopy the "headless" phenotype of the complete *Lhx1* knockout [22]. In the developing nervous system, Lhx1 is required, along with the Lmx1b LIM-HD protein, to direct the trajectories of motor axons in the limb [23]. *Lhx1* is expressed in the developing mouse Müllerian ducts, and *Lhx1* null female mice lack Müllerian-derived structures, including the oviducts and uterus [24]. These observations suggest that *Lhx1* is required for regulating the vertebrate head organizer, the nervous system, and female reproductive tract development.

The human LHX1 protein shares 87% amino acid sequence identity with its murine counterpart. *LHX1* expression has been observed in human adult brain, tonsil, thymus, and in a human

leukemia cell line [19]. To date, there have been no reports of mutations in the human *LHX1* gene.

*Lhx2*

In the mouse, *Lhx2* expression is detected in the fetal liver, the diencephalic and telencephalic regions of the developing brain, the nerve cords, the eyes, the olfactory organs, the limbs, and in B and T lymphoid cell lines [25, 26]. Some of these sites of expression are observed for a *Drosophila* homolog, *apterous,* suggesting similarity of functions across species [25]. Roberson et al. [27] described the expression of mouse *Lhx2* in the anterior pituitary gonadotrope and thyrotrope cell lineages where it is involved in trophic hormone gene regulation.

Animal models have indicated that *Lhx2* plays important roles in eye, cerebral cortex, limb, and erythrocyte development [28, 29]. In *Lhx2* null embryos, eye development arrests after formation of the optic vesicle, the cerebral cortex is smaller because of a decrease in proliferation of neural precursor cells, and erythropoiesis is incomplete [29]. These animals typically die a few days prior to birth due to severe anemia. *Lhx2* gene knockout mice also exhibit impaired patterning of the cortical hem and the telencephalon of the developing brain [30], and a lack of development in olfactory structures, including the olfactory bulb and the olfactory neurons [31]. The Lhx2 protein has been shown to bind to the mouse *M71* olfactory receptor promoter [31].

The human LHX2 amino acid sequence is 90% identical to the mouse primary sequence [32]. No disease-associated mutations in the human *LHX2* gene have been discovered to date. However, *LHX2* expression may be an important marker in chronic myelogenous leukemia: high levels of *LHX2* are detected in all tested cases, likely as a result of hypomethylation at the gene locus [32].

*Lhx3*

During mouse embryogenesis, the *Lhx3* gene is expressed in the ventral spinal cord, the pons, the medulla oblongata, and the pineal gland of the developing nervous system, and later transcripts are found in the emergent pituitary gland [33–35]. The *Lhx3* genes of rodents and humans produce at least two mRNAs from which three distinct protein products (LHX3a, LHX3b, and M2-LHX3 in humans) are translated [15, 35–37]. The human LHX3 protein isoforms exhibit different DNA binding and gene activation capacities suggesting that they exert individual functions *in vivo* [15, 36].

Homozygous *Lhx3* knockout mice are stillborn or die within 24 h of birth [38]. These mice exhibit incomplete pituitary development featuring a lack of the intermediate lobe and an absence of four of the five anterior lobe hormone-secreting cell types [38, 39]. Consistent with this observation, Lhx3 proteins have been demonstrated to directly bind to the promoters of several pituitary hormone gene promoters (reviewed in [11]). *Lhx3* null mice also have defective development of spinal cord motor neurons [40] and it appears that Lhx3 functions in concert with other transcription factors to specify interneuron and motor neuron fates during development (e.g. [14]).

Two mutations in the human *LHX3* gene have been associated with combined pituitary hormone deficiency (CPHD) disease [41]. These patients are deficient in all anterior pituitary hormones except adrenocorticotropin, and have a rigid cervical spine with limited head rotation [41]. Molecular studies have shown that these mutations likely cause the expression of aberrant proteins with reduced capacities to activate pituitary hormone genes [42, 43].

*Lhx4*

*Lhx4* is expressed in the developing mouse hindbrain, cerebral cortex, pituitary gland, and spinal cord [44, 45]. The mouse Lhx4 protein closely resembles the fellow LIM-HD family member Lhx3, with 77% and 86% identity in the first and second LIM domains respectively and 95% identity in the HD ([46] and Figure 1). Mice homozygous for a *Lhx4* gene disruption die shortly after birth from lung defects, a phenotype resembling respiratory distress syndrome in humans [44]. This mouse model has also revealed a role for *Lhx4* in pituitary gland development: *Lhx4* is required in conjunction with *Lhx3* to form a definitive Rathke's pouch, the primordial structure in pituitary organogenesis. In mice lacking both *Lhx3* and *Lhx4*, pituitary development stops at a rudimentary pouch stage, a more severe phenotype than that of the single gene ablations [39]. Other studies have begun to elucidate the genetic hierarchies that

govern *Lhx4* function during pituitary organogenesis. For example, *Lhx4* is required for proper activation of *Lhx3* and cell survival [47].

The human LHX4 protein shares 99% homology with its mouse counterpart, differing in only 3 amino acid residues [48]. Analysis of a consanguineous family with members exhibiting CPHD, short stature, and hypoplastic anterior pituitaries revealed a heterozygous mutation in the *LHX4* gene, suggesting a dominant action of the mutant allele [48]. Other studies indicate that inappropriate expression of *LHX4* resulting from chromosomal translocations involving the gene can be a contributing factor in some leukemias [49, 50].

### *Lhx5*

The mouse *Lhx5* LIM-HD transcription factor gene is expressed in the developing cerebral cortex, basal ganglia, thalamus, hypothalamus, hindbrain, and the spinal cord [51]. The mouse Lhx5 protein is closely related to the Lhx1 LIM-HD factor (Figure 1) and complementary or overlapping roles of these two regulatory proteins have been suggested [51]. Further, the genes encoding these two mouse proteins have similar organizations [52]. The expression of *Lhx5* in the anterior portion of the mouse neural tube suggests a role in patterning of the forebrain [51]. Whereas mice that are heterozygous for a *Lhx5* gene disruption appear normal, most homozygous mice die a few days after birth [53]. The affected mice have hippocampal defects, yet have grossly normal forebrain and hindbrain structure, perhaps due to compensatory actions of *Lhx1* in these regions. Further characterization of the hippocampal phenotype of the surviving homozygous mice reveals behavioral and cognitive deficits consistent with the role of the hippocampus as a center for learning and memory [54].

At the amino acid level, human LHX5 is 98.8% homologous to its mouse counterpart [55]. As with mouse *Lhx5*, there is expression of human *LHX5* in the adult CNS, including the spinal cord, thalamus, and the cerebellum [55]. To date, there have been no documented cases of human mutations of the *LHX5* gene, perhaps due to an essential role in brain development or compensatory actions of *LHX1*.

### *Lhx6*

*Lhx6* expression is seen at high levels in several regions of the embryonic mouse CNS, including the telencephalon and hypothalamus, and the first branchial arch [56]. *Lhx6* is also a marker for the medial ganglionic eminence (MGE), which houses neurons that migrate to give rise to the cerebral cortex [57]. Like the *Lhx3* gene, *Lhx6* produces mRNAs that are predicted to encode distinct protein isoforms: the Lhx6.1a and Lhx6.1b proteins have different carboxyl termini [58]. *Lhx6* is proposed to have a role in patterning of the mandible and maxilla, and in signaling during odontogenesis [56, 59]. In brain sections, RNAi-mediated knockdown of *Lhx6* blocks the normal migration of neurons to the cortex, although gene silencing has no effect on GABA neurotransmitter expression in dissociated MGE cell cultures [60].

Human LHX6 shares over 95% homology to the mouse protein at the amino acid level and also has two protein isoforms [58]. No mutations in human *LHX6* have been reported thus far.

### *Lhx7/Lhx8*

During mouse development, *Lhx8* is expressed in the ventral forebrain, jaw mesenchyme, and during tooth morphogenesis [59, 61, 62]. As development continues, levels of *Lhx8* expression decrease: by postnatal day 1, only weak expression is found in cholinergic rich MGE-derived tissue and in jaw mesenchyme [62].

*Lhx8* knockdown results in decreased expression of other factors known to be involved in tooth morphogenesis, including *Lhx6* [59]. Targeted disruption of exons 4–6 (that encode the LIM2 domain, part of LIM1 and the HD) within the mouse *Lhx8* gene results in a complete cleft of the secondary palate leading to death shortly after birth in most homozygous mice [63]. Heterozygous mice lack a cleft secondary palate, as do homozygous mice that survived past weaning. Further examination of homozygous *Lhx8* mutant mice has revealed roles for *Lhx8* in the development of cholinergic neurons in the telencephalon [64]. These mutants fail to form some of the cholinergic neurons of the forebrain, and completely lack the nucleus basalis, a cellular cluster that provides major cholinergic input to the cerebral cortex. An alternate disruption of *Lhx8* in exon 7 (that

encodes the central portion of the HD) yielded similar phenotypes to that of the exon 4–6 disruption, although there was a higher incidence of the cleft palate phenotype and larger reduction of cholinergic neurons as compared to the exon 4–6 disruption [65].

Primary sequence alignments indicate that the mouse Lhx8 protein is most similar to the Lhx6 protein within the LIM-HD family (Figure 1). Human LHX8 shares 84% identity to mouse sequences at the amino acid level ([66] and Hunter and Rhodes, unpublished observations). To date, there have been no documented examples of mutations in the human *LHX8* gene.

### *Lhx9*

Upon its initial cloning, the *Lhx9* LIM-HD gene was found to be expressed in several regions of the developing mouse brain (diencephalon, telencephalon, and dorsal mesencephalon), the spinal cord, the pancreas, in limb mesenchyme, and in the urogenital region [67, 68]. Phylogenetic analyses suggest an evolutionary relationship between *Lhx2* and *Lhx9*, placing their encoded proteins in one of six total groupings of the LIM-HD family of transcription factors (Figure 1 and [69]). By alternative splicing after exon 4, a truncated protein isoform of Lhx9, termed Lhx9$\alpha$, can be generated [69]. This isoform lacks the third recognition helix of the HD, but retains LIM domains. Lhx9$\alpha$ does not bind to DNA but rather may serve a role as an endogenous dominant-negative factor [69]. Homozygous mice lacking functional *Lhx9* alleles exhibit apparently normal CNS, limb, and pancreatic development, but display numerous urogenital defects [70]. *Lhx9* null mice are phenotypically female, even those that are genotypically male. All mice display gonadal agenesis, infertility, and undetectable levels of testosterone and estradiol coupled with high FSH levels. *Lhx9* null mice have reduced levels of the Sf1 nuclear receptor that is required for gonadogenesis, suggesting a role for *Lhx9* in early gonadal development. Consistent with this model, recent studies have shown that Lhx9 is able to activate the *Sf1/FtzF1* gene [71].

Human LHX9 shares 98% amino acid identity with murine Lhx9. To test the hypothesis that LHX9 may play role in human reproductive development, Ottolenghi et al. [72] cloned human *LHX9* and screened patients with 46XY gonadal agenesis and dysgenesis for mutations in the *LHX9* gene; however, no mutations were observed.

### *Isl1*

The Isl1 transcription factor was initially cloned from pancreatic insulin-producing cells where it is able to bind the *insulin* gene enhancer [73]. *Isl1* is also expressed in the thyroid, pituitary, kidney, spinal cord, arcuate nucleus of the hypothalamus, diencephalon, telencephalon, inner ear, and during tooth morphogenesis [73–77]. Rat Isl1 also co-localizes and interacts with estrogen receptor alpha within the arcuate nucleus of the hypothalamus where it is able to modulate target gene activation [78]. A protein variant of Isl1, Isl1$\beta$, is a truncated product found in the pancreatic islet cells [79]. In comparison to the full-length isoform (or Isl1$\alpha$), Isl1$\beta$ has unique expression patterns within the pancreas, distinct target gene activation properties, and different post-translational modification potential [79].

Mice deficient in *Isl1* fail to form the dorsal exocrine pancreas and islet cells fail to differentiate [80]. *Isl1* is also required outside the pancreas: the hearts of *Isl1* null mice are missing the outflow tract, right ventricle, and atria [81]. These results indicate that *Isl1* is an important gene for a population of cardiac progenitor cells. Additional studies place *Isl1* upstream of the heart transcription factor, *Mef2c* [82]. Here, Isl1 is able to bind the enhancer of *Mef2c* to promote the development of the anterior heart field.

In addition to its roles in the pancreas and heart, *Isl1* functions as an early marker in motor neuron differentiation within the embryonic spinal cord [83]. Observations using a chick model system have established that Isl1 works in combination with other LIM-HD factors to direct motor neuron subtype specification [84]. Similarly, *Isl1* null mouse embryos that have arrested during development lack motor neurons and a subpopulation of interneurons [85]. These data suggest a central role for Isl1 in the correct differentiation of the many neuronal subtypes within the developing spinal cord. Combinatorial codes of gene regulatory proteins including Isl1, Isl2, Lhx3, and NLI appear to specify the differentiation of neuronal progenitors into either interneurons or motor neurons [14, 86].

The human *ISL1* gene locus has been examined for associations with a number of metabolic/obesity-related parameters including body mass index, leptin levels, and non-insulin-dependent (or type II) diabetes mellitus (NIDDM) (e.g. [87, 88]). Studies of a simple sequence repeat within the gene revealed multiple alleles within populations of both normal and NIDDM patients but no correlation was found with allelic differences between disease and non-diseased groups [89]. A heterozygous mutation in human *ISL1* was found within in a Japanese NIDDM patient with a strong familial history of NIDDM [90]. The mutant ISL1 protein (Q310X) retains only 50% activity of the wild type protein [90]. A study of *ISL1* in a population of obese French patients suggests a possible association with NIDDM [91]. Within this group, 3 single nucleotide polymorphisms (SNPs) were found, although no allele distribution differences between obese and control groups were observed. Further study is needed to elucidate any association of *ISL1* SNPs with NIDDM.

*Isl2*

*Isl2* cDNAs were originally cloned from Chinook salmon pituitary [92]. Across species, Isl2 is highly conserved at the amino acid level, with highest identity within the LIMs and HD [93]. Mouse *Isl2* is expressed in the retinal ganglion cells and the developing spinal cord where it plays a role in motor neuron development [84, 94, 95]. Within the CNS, *Isl2* is expressed in motor neurons after the initial markers *Isl1* and *Hb9* [95]. Mice with null mutations in *Isl2* exhibit deficiencies in motor neuron specification within the thoracic spinal cord, with defective specification of visceral and somatic motor neurons [95]. In the hindbrain, the *Pax* family gene, *Pax6*, plays an important role in axonal growth and motor neuron specification. In *Pax6*-deficient rats, abducent and hypoglossal nerves are missing and *Isl2* expression is lost [96]. Little is known about the role of *Isl2* in the pancreas, but as for Isl1, Isl2 may be able to bind the *insulin* gene enhancer to promote gene activation. Axolotl *Isl2* is expressed in the pancreas of this amphibian, and both axolotl and rat Isl2 can activate a rat *insulin* reporter gene [93].

Human ISL2 shares over 97% amino acid identity with the mouse Isl2 protein (Hunter and Rhodes, unpublished observations and [93]). Little is known about the expression pattern of *ISL2* in humans and, to date, no mutations of human *ISL2* have been reported.

*Lmx1a*

Mouse *Lmx1a* is expressed in multiple tissues, including the roof plate of the neural tube, the developing brain, the otic vesicles, the notochord, and the pancreas [12, 97]. Within pancreatic cells, the Lmx1a protein interacts synergistically with the bHLH transcription factor E47 to activate the *insulin* gene enhancer/promoter [12, 13]. Roles for *Lmx1a* in the developing nervous system have been revealed by studies of the spontaneously occurring mutant mouse, *dreher*. The *dreher* strain is derived from wild mice found in a factory in Detmold, Germany [98]. Homozygous *dreher* mice exhibit circling and head-tossing behaviors, deafness, and hyperactivity [98]. In the *dreher* mouse, mutations in *Lmx1a* result in failure of the roof plate to develop [99]. The roof plate functions as a signaling center to coordinate development of the dorsal CNS and its loss results in abnormal differentiation of the dI1 interneurons [100]. *Lmx1a* may act upstream of other roof plate markers such as *MafB*, *Gdf7*, *Bmp6*, and *Bmp7*. BMP signaling is necessary for proper expression of *Lmx1a* and roof plate formation [101]. Further characterization of these mice reveals numerous defects including disorganized cerebellum, hippocampus, and cortex; altered pigmentation; female sterility; skeletal defects; and behavioral abnormalities ([99, 102–104] and reviewed in [105]).

Human *LMX1a* is expressed in numerous tissues including pancreas, skeletal muscle, adipose tissue, developing brain, mammary glands, and pituitary [106]. Because of its ability to activate *insulin* expression and its locus linkage to NIDDM, *LMX1a* has been considered a candidate gene for this disease. Studies on a Pima Indian population revealed several SNP variants and two isoforms of *LMX1a*, but no direct link to NIDDM [106]. No mutations of human *LMX1a* have been reported.

*Lmx1b*

Together with *Lmx1a*, *Lmx1b* is part of a related subfamily of LIM-HD genes ([107] and Figure 1).

*Lmx1b* is expressed is expressed in multiple murine tissues, including the developing limbs and eyes, the kidneys, the brain, and in cranial mesenchyme [23, 107–112]. Using a targeted disruption of the mouse *Lmx1b* gene, multiple functions of this factor have been uncovered. Interestingly, the *Lmx1b* null mouse model mimics the human disease associated with *LMX1b* mutations, Nail-Patella Syndrome (NPS). In mice with homozygous disruption of exons 3–7 (encoding LIM2, the HD, and the carboxyl terminus) of *Lmx1b* there are kidney and limb defects [107]. The mice display dorsal-to-ventral conversion of the limbs and a lack of the patella and nails. Normal dorsoventral asymmetry is lost, and several downstream *Lmx1b* targets (*sFrp2*, *Six1*, *Six2*) thought to play a role in asymmetry have altered expression patterns [113]. Within the kidneys, abnormalities in the basement membrane of the glomerulus are observed, possibly leading to the proteinuria phenotype found in NPS. Further characterization of the glomerulus has shown numerous problems including poor capillary network formation and altered podocyte morphology [114]. Within the brain, *Lmx1b* is important for generation of mesencephalic dopamine neurons [112] and the differentiation of serotonergic neurons [115]. In the mouse eye, *Lmx1b* regulates anterior segment (cornea, iris, ciliary body, trabecular meshwork, and lens) development: mice lacking functional *Lmx1b* exhibit numerous abnormalities, including a lack of ciliary body, iris stroma, and corneal dysplasia [110].

First characterized in 1897, NPS is an autosomal dominant disorder with patients presenting with nail dysplasia, hypoplastic or missing patella, elbow dysplasia, and, often, neuropathy [116]. NPS has been linked to chromosome 9q34, where *LMX1b* has been mapped [117, 118]. *LMX1b* is expressed in multiple tissues including testis, thyroid, duodenum, skeletal muscle, and pancreatic islet cells [117]. Patients with mutations in *LMX1b* have similar phenotypes to the *Lmx1b* null mouse model ([119–121] and reviewed in [122]). Nail, patella, elbow, and iliac skeletal phenotypes are reported in ~70–90% of NPS cases [123]. Kidney glomerular abnormalities have been reported in about 40% of NPS cases [124–126], and open angle glaucoma has been found in about 10% of NPS patients [122, 127]. There are at least 137 mutations within the *LMX1b* coding region [128].

## Conclusions

It is clear that mammalian LIM-HD genes encode transcription factors with important roles in the development of many tissues and organs. Analyses of mice with engineered mutations of LIM-HD genes and molecular diagnoses of human patients with defective LIM-HD genes have furthered our understanding of the unique and overlapping functions of LIM-HD gene products and their interactions with other genes.

At the protein level, much remains to be understood about the biochemical mechanisms that underlie LIM-HD factor actions. Interactions with partner proteins may influence the subcellular locations of LIM-HD proteins and complex signals may mediate their nuclear localization (reviewed in [1, 129]). Within the nucleus, LIM-HD proteins may exert their actions in complex ways. For example, some LIM-HD proteins have been demonstrated to possess features of architectural transcription factors: they associate with the nuclear matrix, recognize AT-rich DNA sequences, and can change local DNA topology, consistent with their promoting (or repressing) the activities of other transcription factors (e.g. [17, 129]).

The completion of the draft human genome led to estimates of a lower total number of genes than had been expected (reviewed in [130]). However, the inherent capacity of the genome can be increased by the transcription of multiple mRNAs from single genes (using alternate promoters and RNA splicing), by the translation of multiple proteins from individual mRNAs, by the post-translational modification of proteins, and by interactions of proteins with regulatory cofactors. These mechanisms may be especially important when utilized by regulatory genes (such as the LIM-HD genes) that can subsequently impact the transcription of many target genes. Several LIM-HD genes have been shown to encode multiple protein isoforms (e.g. [15, 36, 58, 69, 79]). Studies indicate that these isoforms may have different expression patterns and unique properties (e.g. [15, 69]). Recent studies also indicate that some LIM-HD proteins can be modified by post-translational modification such as phosphorylation, perhaps allowing them to respond rapidly to intracellular signaling pathways [79, 131]. A better comprehension of the biochemical and genetic mecha-

nisms by which the LIM-HD transcription factors exert their actions will facilitate improved future diagnoses and treatments for diseases involving aberrant actions of the LIM-HD genes.

### Acknowledgements

We thank Dr. John Watson for expert advice on sequence alignments and Marin Garcia and Jesse Savage for constructive comments on the manuscript. SJR is grateful for grant support from the National Institutes of Health, the National Science Foundation, and the Indiana 21st Century Research and Technology Fund. We apologize to colleagues whose work may not have been directly cited due to space constraints.

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