LMX1B mutations with nails and kneecaps: a new paradigm?

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Nail–patella syndrome (NPS, OMIM 161200) is a rare pleotropic, genetic disease with both renal and extra-renal manifestations. The vast majority of affected individuals are recognizable by the presence of one or more of a number of characteristic physical findings—including the classical tetrad of absent or hypoplastic finger and toe nails, absent or hypoplastic patellae, elbow dysplasia (usually posterior subluxation of the radial head) and iliac horns. Renal involvement is common, although serious sequelae of NPS, such as nephrotic syndrome or renal failure, are not.

The molecular–genetic basis of clinical disease is inactivating mutations in the LIM-homeodomain transcription factor, LMX1B [1]. Missense, non-sense and frame-shift mutations in the LIM and homeodomain moieties of the gene, as well as partial or complete gene deletions, have been found in affected individuals [1, 2]. Co-transfection studies and the pathogenicity of complete gene deletion support haploinsufficiency as the mechanism of disease causation, rather than a dominant negative effect of the mutations [3]. With respect to the extra-renal findings in classic NPS, LMX1B activity is apparently needed only during fetal development, when it is important for dorsal–ventral pattern specification. After fetal life, LMX1B is not expressed in skeletal and other extra-renal tissues. Quite interestingly, LMX1B seems to play a quite different role in podocytes than it does in the rest of the body [4]. LMX1B starts being expressed in the glomerulus at the S-shaped body stage, when the process of podocyte differentiation starts [5]. At least in the mouse, LMX1B activity seems to be necessary postnatally for the proper expression of a number of podocyte-related genes, including CD2AP, NPHS2 [6] and COL4A3/COL4A4 (encoding the α3 and α4 chains of mature type IV collagen) [7]. In contrast, proteins for these putatively LMX1B-regulated genes are present in normal abundance in glomeruli from kidney biopsies of patients with NPS [8]. Understanding this species difference is complicated by the fact that the presence of the cardinal features of NPS requires the homozygous presence of the mutant gene in mice, while it is autosomal dominant in humans.
Direct evidence of transcriptional control of target genes by LMX1B using in vitro reporter systems has only been documented in a few studies [3, 6, 7, 9–11]. Sato et al. [10], for example, showed that—compared with the wild-type gene—plasmid constructs of two different human mutant LMX1B sequences resulted in negligible transcription (using a luciferase reporter) and no DNA binding (in EMSA) in transfected Cos-7 and HeLa cells, while showing no evidence of dominant negative effects. Partial DNA binding of the protein products of LMX1B sequences of known human mutations has been associated with partial transactivating function, especially in co-transfection studies with wild-type LMX1B sequences [3], the situation that obviously conforms best to the human situation of autosomal dominant disease expression. Varying findings in different in vitro test systems may reflect effects of the absence of necessary interacting transcriptional cofactors (e.g. Ldb1, the bHLH protein E47, PAX2, Pod1) in the various expression systems examined [9].

A number of molecular–genetic features of NPS continue to present conundrums. The difference in dosage sensitivity noted above may be responsible for the contrasting effects of gene mutation on the expression of LMX1B-regulated genes in mice and men. Although homozygous mutation is needed for the expression of the NPS phenotype in mice, the renal and extra-renal manifestations of the mutation are also more severe in that species. This is just another example of the limitations of murine renal manifestations of the mutation are also more severe in that species. The difference in dosage sensitivity noted above may be responsible for the contrasting effects of gene mutation on the expression of LMX1B-regulated genes in mice and men. Although homozygous mutation is needed for the expression of the NPS phenotype in mice, the renal and extra-renal manifestations of the mutation are also more severe in that species. This is just another example of the limitations of murine models in understanding human disease [12]. The ectopic presence of type III collagen in the glomerular basement membrane (GBM) of individuals with NPS is also surprising, given that LMX1B seems to influence transcription of the genes for the α3 and α4 chains of type IV collagen (which have LMX1B-targeted FLAT sequences in their common enhancer element). Perhaps, this reflects indirect effects of dysregulation of local collagen production. However, if so, why is type III collagen found in the GBM in NPS and not in Alport syndrome, since both diseases involve perturbations in type IV collagen expression? It is possible that, in human NPS, LMX1B normally represses Col III expression in the GBM. Perhaps, the gene dosage needed for this function exceeds that necessary for production of α3(IV) collagen, which is apparently intact in human NPS. Another puzzle is the presence of significant renal impairment in some individuals with NPS while other affected members of the same family—with the same LMX1B mutation—show no serious renal manifestations. It has been suggested that this may be due to otherwise phenotypically silent (e.g. heterozygous) mutations in the exons or regulatory sequences of podocyte-expressed genes the transcription of which is influenced by LMX1B, since these mutations would likely segregate independently of the LMX1B mutation [4].

A very small number of cases have been described in which characteristic renal pathologic findings of NPS occur in the absence of the typical extra-renal manifestations. This has led to the appellation of nail–patella-like renal disease (NPLRD). In this issue of NDT, Isojima et al. [13] describe a 6-year-old girl with microscopic hematuria and sub-nephrotic proteinuria, who showed the typical findings of NPS on renal biopsy, but lacked skeletal and other extra-renal manifestations of the disease. These authors demonstrated for the first time a presumably causal mutation in LMX1B in a patient with NPLRD. This novel mutation in LMX1B (R246Q) is predicted to have significant effects on gene action by both SIFT and PolyPhen2 analysis. The authors are to be complimented for undertaking a direct analysis of the molecular–genetic mechanism of this new mutation. When expressed in Cos-1 cells, this mutant form of LMX1B had diminished trans-activating potency on the rat insulin FLAT enhancer element, but no indication of a dominant negative effect on the action of co-transfected wild-type LMX1B. In biopsy tissue from the proband, only the expression of CD2AP in podocytes was altered, in apparent contrast to the findings of Heidet et al. [8], but similar to the situation in the murine model. The authors propose the terminology ‘LMX1B nephropathy’ to describe this new renal-limited form of disease in preference to NPLRD. Interestingly, a recent paper by Boyer et al. [14] has described three families with autosomal dominant focal and segmental glomerular sclerosis (FSGS) and mutations in LMX1B, but also without the extra-renal features of NPS. Two of these families had the same R246Q genotype as the subject in the paper of Isojima et al., while the other had a different mutation at the same residue (R246P). In contrast to the patient described by Isojima et al., the typical GBM changes of NPS were not verified pathologically in the Boyer study, although limited biopsy material seems to have been available and only one subject was examined by electron microscopy. The authors inferred decreased DNA binding by the homeodomain of the mutant LMX1B sequences from in silico homology modeling, but did not investigate this using in vitro reporter expression systems. LMX1B mutations thus accounted for ~4% of the families in a cohort of 74 unrelated families with autosomal dominant transmission of FSGS in the Boyer study.

As pointed out by Isojima et al., the phenotypic differences between NPS and LMX1B nephropathy may derive from the residual transcriptional activity present in the latter due to the R246Q mutation. However, some limited transactivating function has been found in in vitro studies of other LMX1B mutations (C142W) that cause classic NPS [3], so this effect may depend on whether the mutation affects the LIM or homeodomain moieties of the gene. Apparently, ‘haploinsufficiency’ may be a more complex phenomenon with respect to different LMX1B mutations than is reflected in transcriptional activity in a given reporter system. If so, we may need much more sensitive and complete in vitro assay systems to appreciate relevant differences in LMX1B action under the influence of various mutations. Even within the glomerulus of the patient with the R246Q mutation, protein level expression in the podocytes was mostly intact—CD2AP expression merely took on a granular appearance, which more likely suggests abnormal expression of a CD2AP-interacting protein than a direct effect of the mutation on CD2AP expression itself.

I do not suspect that a large number of patients with minor urinary abnormalities will end up having mutations in LMX1B, although the report of Boyer et al. [14] indicates that a number of cases of hereditary FSGS without extra-renal features of NPS may be due to mutations in the same residue (R246) of the LMX1B gene described by Isojima et al. Of course, the study of Boyer et al. may have been biased toward more severe cases as it involved families with known autosomal dominant transmission of FSGS. More such cases will
need to be detected and followed over time to see if the same 5–15% rate of nephrotic syndrome and renal failure develops with these specific mutations, with residual but diminished transcriptional activity, as develops with the LMX1B mutations associated with classic NPS. Additional mechanistic studies of LMX1B mutations are needed, using consistent and more complete in vitro test systems, to compare DNA binding and other molecular characteristics of various mutant LMX1B sequences with their transactivating potential as well as their interaction with protein cofactors—and then comparing these characteristics with the clinical behavior of human subjects with the same mutations.

One may speculate about the mechanism for the discordant renal and extra-renal effects of some LMX1B mutations (Figure 1). The specificity of DNA binding by the 60-amino acid homeodomain of LMX1B to its various gene regulatory targets presumably depends on the amino acid sequence (through its influence on the tertiary protein structure and on the ionic character of the residues within the homeodomain) as well as on the effects of interacting protein cofactors like Ldb1. In vitro reporter systems are often based on a single target DNA sequence (such as the FLAT element of the rat insulin promoter). It is likely, however, that the strength of attachment of LMX1B to different target DNA sequences is dependent on different amino acid residues in the HD region. It is possible that binding of LMX1B to regulatory sequences underlying skeletal dorsal–ventral specification is significantly less affected by the R246Q mutation, while binding to some or all of the podocyte regulatory DNA sequences is more severely affected by this mutation. Complete gene or homeodomain region deletions, on the other hand, would be expected to have both renal and extra-renal effects. Although LMX1B target sequences are known in the enhancer/promoter regions of some podocyte-expressed genes, it is not clear that we yet have a complete picture of the extent of podocyte-relevant genes that are under the control of LMX1B. For example, although COL4A3 and COL4A4 are apparently under transcriptional control of LMX1B, expression of type IV collagen is still found in NPS.

The R246Q mutation in LMX1B certainly expands the spectrum of LMX1B-related disease. The renal-limited effects of this novel mutation may ultimately be more closely related to the specificity of the transcriptional regulatory activity of the mutant gene product than to its degree of residual activity in a single reporter system. Why else should a slightly higher level of LMX1B transactivation be sufficient for dorsal–ventral specification in utero, but not adequate to maintain proper podocyte function and GBM structure? The paper by Isojima et al. is a good example of the beginnings of a transition toward molecular–genetic (i.e. mechanistic) rather than classical genetic (i.e. phenotypic) definitions of genetic and other renal diseases (which also includes seeing disease in a context of gene regulatory networks [15]). With whole genome sequencing and ChIP-seq technology, it should become feasible to search for target sequences of transcriptional regulators like LMX1B in the entire genome and to investigate the effects of different LMX1B mutations on the expression of the associated genes using expanded in vitro reporter systems. In order to make this transition, we also need to expand the spectrum of gene interactions we consider in understanding the pathogenesis of genetic renal disease—including a better appreciation of oligogenic inheritance patterns, in part explained by modules of interacting proteins [16], the effects of modifier genes (i.e. epistasis) and the interaction between LMX1B haploinsufficiency (in its various forms) and sequence variation in regulatory regions of target genes [4, 11].

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CONFLICT OF INTEREST

None declared.

(See related article by Isojima et al. LMX1B mutation with residual transcriptional activity as a cause of isolated glomerulopathy. *Nephrol Dial Transplant* 2014; 29: 81–88.)
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Do FGF23 levels change over time and if yes, what do such changes mean?

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FGF23 has emerged as a novel and exciting risk factor of adverse outcomes in patients with CKD, ESRD and kidney transplant [1–3]. FGF23 is a hormone produced in the bones.

FGF23, mortality risk, cardiovascular disease, chronic kidney disease

Its main physiologic roles are the enhancement of phosphaturia and the suppression of 1,25(OH)2 vitamin D levels [4]. To date, there is debate about the mechanisms whereby FGF23 may