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INVITED REVIEW

Nail-patella syndrome

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Abstract The pathognomonic symptoms of patients with nail-patella syndrome are their small or absent patellae and dysplastic or absent finger- and toenails. Many of the patients suffer from renal symptoms which also affect their prognosis. In 1998, mutations in the gene encoding the transcription factor LMX1B were identified as underlying this autosomal-dominant disease. The *LMX1B* gene is expressed in a variety of tissues, and the symptoms are reflected nicely by its expression pattern. LMX1B is essential for dorso-ventral pattern formation in the limbs, for differentiation of the anterior portions of the eyes, for development of certain neuron populations in the central nervous system, and for the differentiation and maintenance of podocytes. Accordingly, kidney biopsies of patients with nail-patella syndrome show an altered podocyte structure and defects in the glomerular basement membrane. Recent evidence suggests that LMX1B regulates genes which encode proteins associated with the actin cytoskeleton.

Keywords Nail-patella syndrome · LMX1B · LDB1 · Actin cytoskeleton · Podocytes · Glomerular basement membrane · Podocin

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Introduction

The first description of patients suffering from the disease which was later called nail-patella syndrome was published in the scientific literature in the year 1897 [45]. The hallmarks of nail-patella syndrome, i.e., malformed or absent kneecaps and finger- or toenails, were already highlighted in the original report. Other presentations of patients with nail-patella syndrome were provided by additional authors, and therefore, the disease was first known also as Österreicher syndrome [59], Turner syndrome [76], Turner-Kieser syndrome [41], Fong disease [21], arthro-onychodysplasia [8], and hereditary osteo-onycho-dysplasia [65]. It was not until the 1950s that the hereditary disorder was named nail-patella syndrome.

The incidence of nail-patella syndrome is frequently quoted as 1 in 50,000, but I am not aware of any published study supporting this figure. It is inherited in an autosomal-dominant fashion, and the spectrum of symptoms can be quite diverse even between family members. Although nail-patella syndrome was one of the first genetic diseases for which linkage was established [63], it took until 1998 before mutations in the *LMX1B* gene were reported to be responsible for the disease [15, 51, 79]. This readily explains the skeletal abnormalities in the patients, because LMX1B is responsible for establishing dorso-ventral polarity in the extremities [64, 77]. Nevertheless, we are still far away from understanding how symptoms in the various affected organs develop.

Extra-renal symptoms

Skeletal system and nails

The diagnosis of nail-patella syndrome can be easily established by careful physical examination of the patients.

In 74–93% of the patients, the patellae are underdeveloped or absent [2, 3, 11, 74], and in 95–100% of the patients, various dysplasias of the finger- and toenails have been described with the thumb typically showing the most severe symptoms [2, 3, 11, 74]. The triangular appearance of the lunulae is pathognomonic for nail-patella syndrome, but hypoplastic and split nails or a complete absence of nails have been described as well. An additional characteristic sign of nail-patella syndrome are iliac horns, exostoses extending from the dorsal side of the iliac portion of the pelvis. In many instances, iliac horns can already be palpated, but in others, they can only be detected by X-ray imaging; they are present in 68–81% of the patients [2, 3, 11, 74].

Ocular symptoms

Whereas the symptoms just described are of only cosmetic significance or cause minor discomfort for the patients, the ocular symptoms are clinically more relevant. Ten to 12% percent of the patients will develop glaucoma and another 4–7% suffer from ocular hypertension [2, 3, 74]. During ocular development, the *LMX1B* gene is expressed in the anterior portions of the eye where it controls the differentiation of the trabecular meshwork and of Schlemm's canal [60]. It is therefore not surprising that glaucoma represents part of the symptoms, but it is not understood why not even one out of every five patients develops ocular hypertension or glaucoma. One other common ocular finding has been called Lester's sign, a hyperpigmentation of the central part of the iris assuming the shape of a clover leaf [44]; it was seen in 46–54% of the patients [11, 74]

Other extra-renal symptoms

Additional extra-renal symptoms such as other skeletal abnormalities, hearing, neurological, and dental problems shall only be mentioned cursorily [3, 74]. Some of them are poorly understood and will not be discussed here further.

Renal symptoms

The prognosis of patients with nail-patella syndrome is determined by their renal symptoms. In ~40% of the patients, the kidneys are affected; the symptoms range from mild proteinuria until end-stage renal failure [3]. The involvement of the kidneys in nail-patella syndrome was first described in 1950 [26]. Typically, renal symptoms take (many) years to develop and the severity of symptoms varies considerably between families and even within a family. Since the diagnostic criteria usually are obvious (patellae, nails, iliac horns), kidney biopsies are not required and our knowledge of the pathogenetic events in the kidneys is rather limited. As far as ultrastructural data are available, the electron microscopic alterations are again pathognomonic [1, 13] (Fig. 1). The glomerular basement membrane is thickened and contains both electron lucent areas and fibrillar deposits whose periodicity has been measured at 64 to 66 nm which is consistent with that of fibrillar collagen [55]. Later studies have indeed shown the existence of type III collagen by immunofluorescence [27]. In addition, the loss of podocyte foot processes has been noted which represents the rather uniform response of this cell type to various kinds of injuries. Despite the fact that nail-patella syndrome is inherited in an autosomal-dominant fashion, only a portion of the podocytes loses their foot processes.

Isolated glomerular sclerosis and nail-patella-like renal disease

Over the years, rare patients have been identified without the extra-renal hallmarks of nail-patella syndrome, i.e., those patients had normal patellae and nails and no iliac horns were detected [14, 33, 68, 82]. By electron microscopy, however, the characteristic lesions described in patients with nail-patella syndrome were observed: electron lucent areas and deposits of fibrillar collagen in the glomerular basement membrane. Therefore, this entity was called nail-patella-like renal disease. Its etiology was unknown until two publications reported mutations in the *LMX1B* gene in the affected families [7, 34]. The same amino acid, arginine at position 246, was substituted either by a glutamine or by a proline residue. This arginine is located in the homeodomain of LMX1B and is crucially important for binding of the protein to DNA. Indeed the authors in one of those publications showed a reduced yet still detectable transcriptional activity of the mutated LMX1B protein [34].

Characteristics of the LMX1B protein

The LMX1B protein belongs to the family of LIM-homeodomain transcription factors, because in addition to a homeodomain, it also contains LIM motifs at its NH_2-terminus. Zinc-binding domains have been identified in a multitude of proteins, and accordingly, they serve very diverse functions. In contrast to the Cys_2His_2 and the Cys_4 zinc finger motifs which represent classical DNA binding domains, the LIM domains (consensus sequence $CysX_2CysX_{16-23}HisX_2CysX_2CysX_2CysX_{16-21}$ $CysX_2Cys/His/Asp$) [22, 39] mediate protein-protein interactions. The LMX1B protein contains two LIM domains at its NH_2-terminus which are preceded by 55 additional amino acids of unknown function. Both LIM domains are separated by a spacer of eight amino acids (Fig. 2). Soon after the cloning of the LMX1B cDNA, two interaction partners were identified which bind to the LIM domains of LMX1B. The E47 protein is encoded by the *E2A*

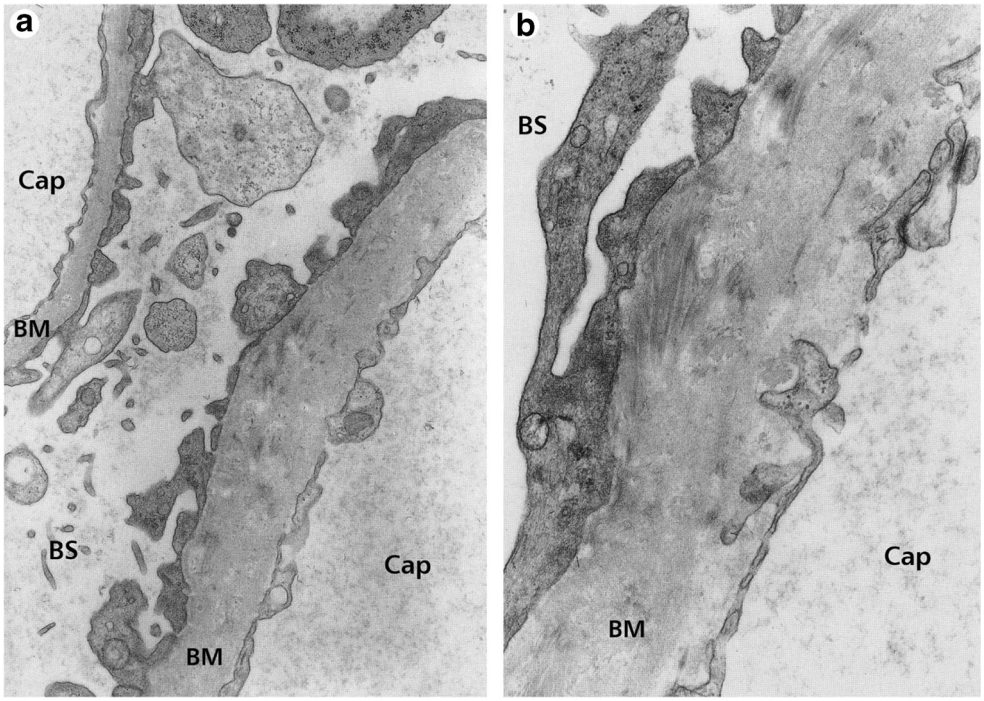

Fig. 1 Ultrastructural changes in glomeruli of patients with nail-patella syndrome. **a** Normal and altered capillary loops can exist in the same glomerulus. In comparison to the normal capillary loop in the *upper left-hand corner*, the glomerular basement membrane in the *lower right-hand corner* is thickened, contains fibrillar inclusions, and shows the typical moth-eaten appearance arising from the electron-lucent areas. No drastic changes can be seen in the podocyte foot processes. **b** At the higher magnification, the ultrastructural changes can be better appreciated. *BM* glomerular basement membrane, *BS* Bowman's space, *Cap* capillary lumen (from T. Taguchi et al., Nephropathy of nail-patella syndrome. Ultrastruct Pathol 12: 175–183, 1988)

gene which is transcribed into an alternatively spliced messenger RNA (mRNA) (the E12 protein being the protein product of the other splice variant). Whereas the E47 protein strongly increases the transcriptional activity of LMX1B [16, 35], the other interaction partner LDB1 shows the opposite effect [16, 47]. The actual situation, however, may be more complex when looking at the interaction between the closely related protein LMX1A with E47 and LDB1. Again, LDB1 downregulated the transcriptional activity of LMX1A but this effect was only observed when E47 was present. In its absence, no effect of LDB1 was noticed [36]. LDB1 in turn interacts with the ubiquitin ligase RLIM and with SSDP1 (single-stranded DNA-binding protein 1). RLIM leads to the proteasome-dependent degradation of LDB1 [28, 58], and SSDP1 enhances the transcriptional activation by LIM-homeodomain transcription factors [57]. Furthermore, evidence has been presented that LMX1B interacts with the transcription factor PAX2 [48] and with PSPC1 (paraspeckle protein 1) [29]. The relevance of these interactions in podocytes is not clear at present.

The homeodomain was originally identified in transcription factors of the fly *Drosophila melanogaster* which were involved in homeotic transformations [50, 71], i.e., the transformation of one body part into another (the term homeobox refers to the DNA sequence encoding the homeodomain). In the subsequent years, it became clear that homeodomain proteins are not restricted to *Drosophila* but rather exert essential functions in many organisms. The homeodomain represents a well-characterized DNA binding domain, and the elucidation of its three-dimensional structure has helped to identify amino acids essential for the interaction between the protein and its target sequence in the genomic DNA. For LMX1A, it has been demonstrated that the homeodomain binds to AT-rich sequences, so-called FLAT elements [24], and in light of the

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  1  MDIATGPESL ERCFPRGQTD CAKMLDGIKM EEHALRPGPA TLGVLLGSDC
 51  PHPAVCEGCQ RPISDRFLMR VNESSWHEEC LQCAACQQAL TTSCYFRDRK
                                          LIM-A
101  LYCKQDYQQL FAAKCSGCME KIAPTEFVMR ALECVYHLGC FCCCVCERQL
                                          LIM-B
151  RKGDEFVLKE GQLLCKGDYE KEKDLLSSVS PDESDSVKSE DEDGDMKPAK
201  GQGSQSKGSG DDGKDPRRPK RPRTILTTQQ RRAFKASFEV SSKPCRKVRE
                                   Homeodomain
251  TLAAETGLSV RVVQVWFQNQ RAKMKKLARR HQQQQEQQNS QRLGQEVLSS
301  RMEGMMASYT PLAPPQQQIV AMEQSPYGSS DPFQQGLTPP QMPGDHMNPY
351  GNDSIFHDID SDTSLTSLSD CFLGSSDVGS LQARVGNPID RLYSMQSSYF
401  AS
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Fig. 2 Sequence of the human LMX1B protein. Shown is the isoform with 402 amino acids in length. The zinc-coordinating residues in the two LIM domains are *circled*, and the seven amino acids which are inserted or deleted by alternative splicing are *boxed*

fact that the homeodomain of LMX1B is identical to that of LMX1A, it was expected that this holds up for LMX1B as well. Meanwhile, this assumption has been experimentally verified (see below) but why LMX1B binds to only certain FLAT elements and not to others which occur thousands of times in the mammalian genome is not understood at present; possibly, the local chromatin environment plays an important role in determining the accessibility of distinct FLAT elements. Once bound to the regulatory region of its target gene, LMX1B is believed to act as a transcriptional activator. This assumption is based on the finding that deletion of the region beyond the homeodomain leads to a drastic reduction of its transcriptional activity in reporter assays [16, 35].

Genetics

Due to the fact that different portions of the skeletal system and a variety of organs are affected in patients with nail-patella syndrome, it was not immediately clear whether only one gene was mutated in those patients. Nevertheless, nail-patella syndrome was one of the first genetic diseases for which linkage was established (to the AB0 blood groups) [63], and another more than 40 years later, three publications reported the first mutations in the *LMX1B* gene [15, 51, 79]. The gene is located on human chromosome 9q34 and consists of eight exons [79] which give rise to a ~7-kb mRNA [15]. In 1998, the year in which the sequence of human LMX1B was published for the first time, two different forms were reported, one of 372 amino acids in length [15] and one of 379 amino acids in length [79]. This discrepancy was explained shortly thereafter when alternative splicing was described for the last 21 nucleotides of exon 7 [72]. As a matter of fact, the human LMX1B protein is even 23 amino acids longer because a start codon further upstream of the original one was identified several years later so that we are dealing with isoforms of 395 and 402 amino acids, respectively [17]. The first LIM domain is encoded by exon 2, the second LIM domain by exon 3, and the homeodomain by exons 4 to 6 [79].

The vast majority of mutations have been located in exons 2 to 6 [3, 17, 49] (the Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff, http://www.hgmd.org/) and therefore affect the function of the LIM domains and of the homeodomain, either by the missense mutations which lead to the substitution of amino acids essential for the binding of zinc (in the case of the LIM domains) or by the substitution of amino acids which are essential for the binding of LMX1B to DNA (in the case of the homeodomain). Alternatively, nonsense and frame-shifting mutations will lead to the loss of LMX1B protein sequence beyond the mutation. It is surprising that almost no mutations have been identified in the region of the *LMX1B* gene downstream of the homeobox [17]. This either indicates that this portion of LMX1B is not very important for its function or that the substitution of only one amino acid has no major effects.

While nonsense and frameshift mutations will lead to the synthesis of a truncated protein, the pathogenetic effect of missense mutations is much less clear. The few missense mutations whose function has been investigated show a complete or partial loss of function. So far, it has not been demonstrated that the mutant LMX1B proteins interfere with the function of the wild-type LMX1B protein and therefore it is generally assumed that haploinsufficiency causes nail-patella syndrome [16, 34, 69]. This hypothesis is supported by the fact that whole deletions of the *LMX1B* gene have been reported in patients with nail-patella syndrome [4, 49, 70].

The modular structure of LMX1B naturally leads to the question whether the severity of the symptoms in patients with nail-patella syndrome depends on the site of the mutation. Such a hypothesis is supported by the finding that preferentially, the second LIM domain and not the first one interacts with E47 [35], and that is also probably true for LDB1 which associates more strongly with the second LIM domain of the highly similar LMX1A protein [36]. Efforts to establish a genotype-phenotype relationship have yielded only limited evidence that the nature of the mutations affects the clinical picture [3, 18]. Only one publication has suggested that mutations affecting the homeodomain have a stronger impact on kidney function [3]. In light of the fact that even within the same family patients present with quite variable symptoms, the influence of modifying genes seems to be more severe than the nature of the underlying mutation.

Mouse models and molecular pathogenesis

Development of the metanephric kidney

The human kidney (and that of other mammals) develops in three stages. It begins with the pronephros, then the mesonephros appears, and finally—between the fifth and the 34th week of gestation—the metanephric kidney forms. The metanephric kidney consists of two ontogenetically different portions, the nephrons and the collecting ducts. Collecting ducts originate from the ureteric bud which grows out from the Wolffian duct through the interaction between the ureteric bud and the surrounding metanephric mesenchyme. The ureteric bud branches repeatedly and thus gives rise to the collecting duct system, whereas the metanephric mesenchyme differentiates into the nephrons with its glomerular, proximal tubular, intermediate tubular, and distal tubular portions. Podocytes represent the visceral epithelial cells sitting on the outside of the glomerular capillaries where they are anchored through their highly interdigitating foot processes. During earlier stages of differentiation, however, podocytes assume a cuboidal shape and they are connected by classical cell-cell

contacts. Only later do podocytes elaborate primary processes which branch and finally form foot processes. These lie ~40 nm apart and are connected by slit diaphragms. Together with the glomerular endothelium and the glomerular basement membrane, the slit diaphragms make up the glomerular filtration barrier. The importance of the slit diaphragm is emphasized by the fact that a number of hereditary kidney diseases are due to mutations in genes encoding slit diaphragm proteins, such as nephrin, podocin, and CD2AP (see the following section). On the intracellular side, slit diaphragm proteins are connected to the actin cytoskeleton whose importance again is emphasized by its involvement in hereditary kidney diseases (see the following section). Obviously, the differentiation of podocytes must be regulated by a genetic program of which we know very little but it is clear that LMX1B plays an essential role.

The conventional *Lmx1b* knockout mouse

The transcription factor LMX1B first gained prominence because of its involvement in dorso-ventral axis formation during limb development [64, 77]. In the mesenchymal cells on the dorsal side of the limb buds, LMX1B orchestrates a genetic program which prevents their ventralization. This readily explains some of the cardinal symptoms of nail-patella syndrome, such as the hypoplastic or absent patellae, the malformed or absent nails, and the iliac horns. Indeed, the constitutive inactivation of the *Lmx1b* gene in conventional knockout mice reproduces many features of the human disease but there are also important differences. Homozygous *Lmx1b* knockout mice die on the day of birth, and they lack patellae [12]. Emphasizing the role of LMX1B in dorso-ventral axis formation the mice form foot pads not only on the ventral but also on the dorsal side of their paws [12]. Furthermore, the *Lmx1b* knockout mice also suffer from structural defects in the anterior portions of the eyes [60]. In contrast to the human disease with its dominant pattern of inheritance, heterozygous *Lmx1b* knockout mice show no phenotype up to 12 months after birth [66].

Most relevant in this context, podocyte differentiation is severely affected upon the inactivation of *Lmx1b*. The renal phenotype of homozygous *Lmx1b* knockout mice is already evident from the small size of the kidneys. By light microscopy, the glomerular tuft appears smaller [66] and proteinaceous material can be found in the tubules, thus pointing to a leaky glomerular filtration barrier [12]. This assumption is confirmed on an ultrastructural level where it can be seen that podocyte development is arrested in the cuboidal stage and no foot processes and slit diaphragms are elaborated [53, 66]. Furthermore, the glomerular basement membrane which usually forms by fusion of the basement membranes underlying the podocytes and the glomerular endothelium is still split in some capillary loops [66]. Finally, the glomerular endothelial cells form less fenestrations in the homozygous *Lmx1b* knockout mouse than is usually the case [66].

Obviously, the reduced expression of genes which are mutated in hereditary glomerulopathies could be an explanation of the podocyte phenotype. Patients suffering from congenital nephrotic syndrome of the Finnish type carry mutations in the *NPHS1* gene [40]. The protein product of *NPHS1*, nephrin, has been localized to the slit diaphragm [30, 31, 67], and the inactivation of *Nphs1* in homozygous knockout mice results in the absence of slit diaphragms [61]. Somewhat surprisingly, nephrin could still be detected in glomeruli of homozygous *Lmx1b* knockout kidneys and therefore the absence of slit diaphragms upon the inactivation of *Lmx1b* cannot be explained by the reduced expression of *Nphs1* [53, 66]. The *Nphs2* gene is mutated in patients with steroid-resistant nephrotic syndrome: it encodes the slit diaphragm-associated protein podocin [5]. The inactivation of *Lmx1b* leads to the reduced expression of the *Nphs2* gene both on the mRNA and protein level which made podocin a promising candidate to explain the lack of slit diaphragms and *NPHS2* an attractive target gene to be directly regulated by LMX1B [53, 66]. By electrophoretic mobility shift assay, LMX1B was demonstrated to bind to a FLAT element in the promoter region of *NPHS2* [53, 66], and a construct with four concatemerized FLAT elements of the *NPHS2* promoter was activated ~twofold by LMX1B [53]. However, we were not able to demonstrate the activation of a 4.4-kbp fragment of the *NPHS2* promoter by LMX1B, and we also were not able to show that the endogenous *NPHS2* gene in stably transfected HeLa cells was activated by LMX1B (this of course can be due to the absence of necessary co-factors which are usually present in podocytes) [66].

Conflicting evidence has been published for the expression of another slit diaphragm-associated protein, CD2AP. Mutations in the *CD2AP* gene have been detected in patients with focal segmental glomerulosclerosis, and therefore, it represents another attractive target for the regulation by LMX1B [42, 46]. Whereas another group reported the downregulation of *Cd2ap* after the inactivation of *Lmx1b* [53], we could not detect a difference in its expression levels [66]. The reason for this conflicting result is not clear, but it is known that the genetic background in different mouse strains plays an important role in determining glomerular disease susceptibility.

Similar to other basement membranes, the glomerular basement membrane contains a meshwork of collagen IV. It is special in that respect that early in glomerular development, collagen IV is built up of α1 and α2 chains which are later substituted by α3, α4, and α5 chains [37, 52]. Since mutations in collagen IV result in Alport syndrome [75], alterations in the collagen IV network could readily explain the structural abnormalities in patients with nail-patella syndrome. In the conventional *Lmx1b* knockout mouse, the α3 and α4 chains of collagen IV were not detected in the glomerular basement

membrane. Furthermore, it has been shown that LMX1B binds to FLAT elements in the first intron of the *COL4A4* gene which encodes the α4 chain of collagen IV, and that LMX1B activates a reporter construct containing six concatemerized binding sites from the *COL4A4* intron [54].

The formation of fenestrations in the glomerular endothelium critically depends on the secretion of vascular endothelial growth factor (VEGF)-A. Both too high and too low, a level of VEGF-A results in glomerular damage [19] and it is therefore clear that the levels of secreted VEGF-A have to be carefully balanced. In the constitutive *Lmx1b* knockout mouse, markedly reduced levels of VEGF-A were detected which correlates well with the reduced frequency of fenestrations in the glomerular endothelial cells [66].

Podocyte-specific inactivation of *Lmx1b*, *E2a*, and *Ldb1*

Although the constitutive inactivation of *Lmx1b* in knockout mice has unequivocally demonstrated the importance of LMX1B for glomerular development, these mice at the same time suffer from the severe drawback that they die on the day of the birth and that the heterozygous knockout mice do not develop a podocyte phenotype. Certain genetic techniques allow the inactivation of a gene of interest specifically in podocytes, and therefore, this approach was taken to investigate the role of LMX1B and its interacting partners E47 and LDB1 in podocytes. Since E47 and LDB1 are produced in many different cell types, the significance of the interaction with LMX1B in podocytes was not clear.

The podocyte-specific inactivation of *Lmx1b* resulted in the death of the homozygous knockout mice ~2 weeks after birth. Already 5 days after birth, the mice suffered from severe albuminuria which was reflected by the presence of protein casts in tubular profiles on histological sections (Fig. 3). By transmission electron microscopy, the loss of foot processes and slit diaphragms as well as a thickened glomerular basement membrane were observed. Surprisingly nephrin, podocin, CD2AP, and the α3 and α4 chains of collagen IV were all still present after the onset of proteinuria which made it unlikely that these proteins play a role in the pathogenesis of the glomerular phenotype [73].

The importance of LDB1 for podocyte development was unclear, because conventional *Ldb1* knockout mice die in utero before the onset of renal development [56]. Conventional *E2a* knockout mice, on the other hand, are viable which argues against severe kidney damage [81]. The constitutive podocyte-specific inactivation of the *E2a* and *Ldb1* genes resulted in drastically different phenotypes. Whereas the inactivation of *E2a* in podocytes had no effect, the inactivation of *Ldb1* resulted in albuminuria and the death of most mice at ~1 month of age. Since proteinuria developed later than in the podocyte-specific *Lmx1b* knockout mice and the mice also died later, the loss of *Ldb1* can be compensated longer then the loss of *Lmx1b* but nevertheless, it is an essential gene in podocytes [73]. Therefore, at least in podocytes, the interaction between LMX1B and LDB1 is more important than that between LMX1B and E47.

The data from the conventional and of the constitutive podocyte-specific *Lmx1b* knockout mice have shown without any doubt that LMX1B is essential for the differentiation of podocytes. In the latter mouse model, it appeared as if the podocytes first formed foot processes and slit diaphragms and lost them thereafter. Therefore, the question came up whether LMX1B is also necessary to maintain the differentiation status of podocytes in the adult organism. The inactivation of *Lmx1b* in mice several months after birth resulted in pronounced albuminuria (Fig. 4), thus demonstrating that LMX1B also is necessary after podocytes have reached the full differentiation status [10]. This is consistent with the finding that LMX1B has been detected in the glomeruli of adult mice. Similar to the situation in the constitutive podocyte-specific *Lmx1b* knockout mice, the slit diaphragm proteins nephrin, podocin, and CD2AP were not downregulated at the time of proteinuria (Fig. 4) [10].

The actin cytoskeleton is of crucial importance for podocyte function. Certain forms of hereditary focal-segmental glomerulosclerosis are caused by mutations in the genes encoding α-actinin-4 [38] and the inverted formin INF2 [6, 9]; furthermore, the loss of the actin-associated proteins synaptopodin [32] and cofilin-1 [23] makes podocytes more sensitive to certain types of injury. Indeed, a time-resolved DNA array analysis after the inactivation of *Lmx1b* in adult mice led to the identification of three genes which code for actin-associated proteins [10]. They encode (i) transgelin, an actin-binding protein that stabilizes actin fibers; (ii) the actin-binding and Rho-activating protein ABRA which has been suggested to enhance the formation of actin filaments and/or to stabilize actin fibers; and (iii) the monomeric GTPase ARL4C which may play a role in the establishment of focal contacts. For the *ABRA* and *ARL4C* genes, it was also possible to demonstrate a direct binding of LMX1B to FLAT elements in their promoter regions using chromatin immunoprecipitation and electrophoretic mobility shift assays [10]. Finally, the downregulation of homologous genes in zebrafish corroborates the genetic interaction between LMX1B and ABRA for the proper function of the pronephros [10]. Therefore, it seems at least possible, if not likely, that the podocyte pathogenesis in nail-patella syndrome results from a dysregulation of the actin cytoskeleton.

Perspective and open questions

In the almost 20 years which have passed since the first mutations in patients with nail-patella syndrome were reported, important insight has been gained. We now know for sure that the *LMX1B* gene is expressed in podocytes and that LMX1B

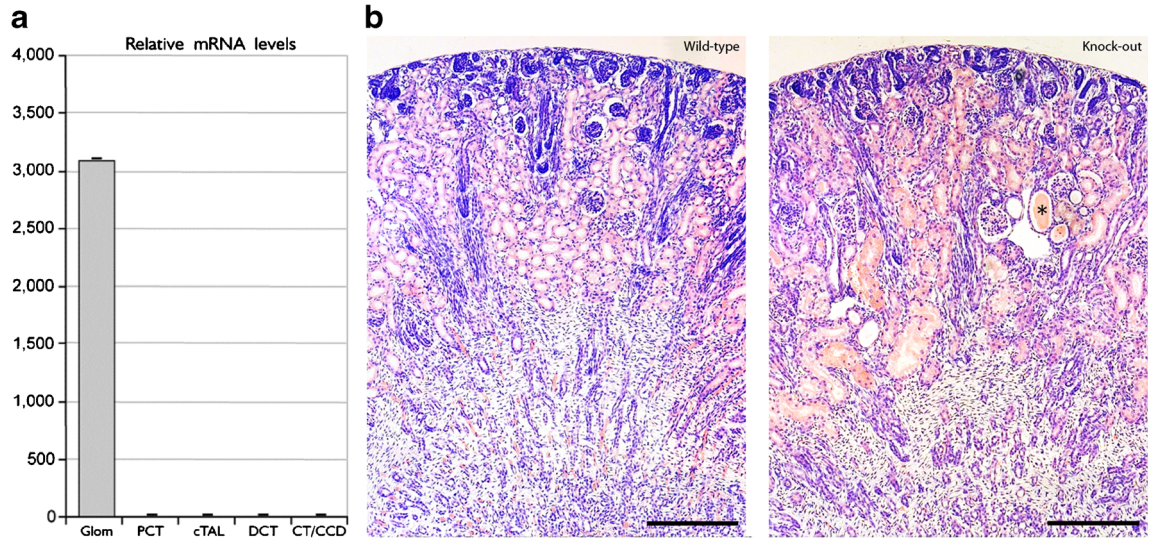

Fig. 3 Constitutive podocyte-specific inactivation of *Lmx1b* causes proteinuria and perinatal death. **a** Messenger RNA (mRNA) was isolated from murine microdissected nephron segments (*Glom* glomeruli, *PCT* proximal convoluted tubules, *cTAL* cortical thick ascending limbs, *DCT* distal convoluted tubules, CT/CCD connecting tubules/cortical collecting ducts) and subjected to quantitative polymerase chain reaction. The *Lmx1b* mRNA is exclusively detected in glomeruli. **b** When the *Lmx1b* gene was constitutively inactivated in podocytes, most mice died ~2 weeks after birth. A hematoxylin and eosin-stained paraffin kidney section of 5-day-old mice shows the presence of some tubular profiles (*asterisk*) with proteinaceous material in their lumen but no gross abnormalities. *Bar*, 100 μm (from H. Suleiman et al., The podocyte-specific inactivation of *Lmx1b*, *Ldb1* and *E2a* yields new insight into a transcriptional network in podoctes. Dev Biol 304: 701–712, 2007)

is essential both for the development of podocytes and for the maintenance of their differentiation status. We also know that only one of the interactions between LMX1B and other proteins reported in the literature is essential in podocytes, that between LMX1B and LDB1. On the other side, there is still a lot we have to learn about the molecular pathogenesis of nail-patella syndrome. It is highly unlikely that LMX1B interacts with only one other protein in podocytes; there have to be other interaction partners waiting to be identified. Looking at the variability of the symptoms between patients, the genes encoding these interaction partners will be good candidates as modifier genes for disease severity.

The genes encoding podocin, CD2AP, and the α3 and α4 chains of collagen IV seem like natural candidates as target genes of LMX1B. One should not forget, however, that the assays which have been employed to demonstrate a regulation

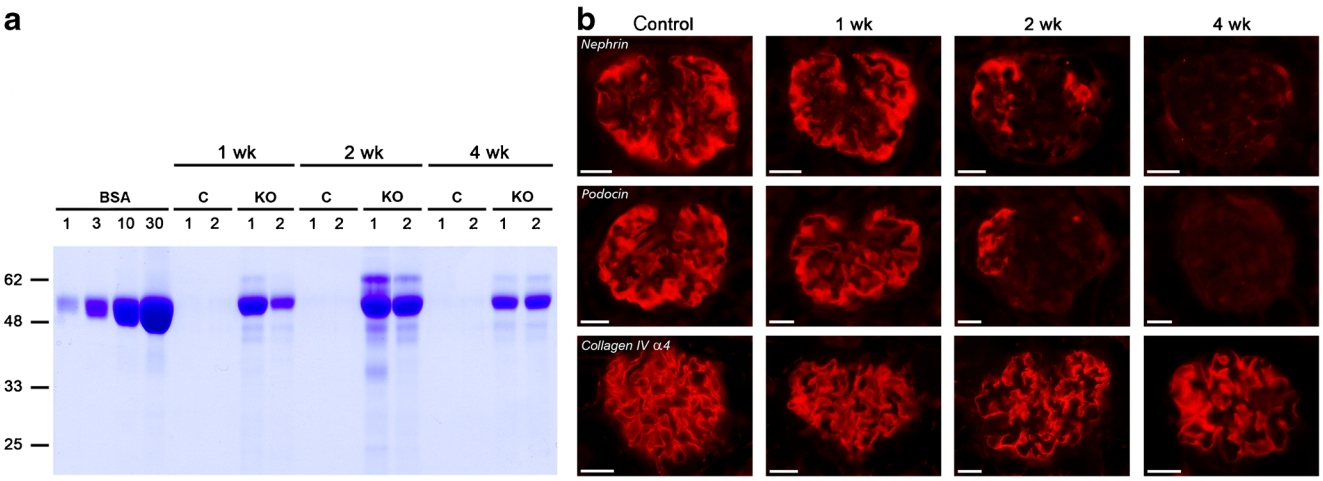

Fig. 4 The inactivation of *Lmx1b* in adult mice leads to pronounced albuminuria even in the presence of nephrin, podocin, and the α4 chain of collagen IV. **a** When the *Lmx1b* gene was inactivated in adult mice, albuminuria was observed already 1 week later as determined by protein gel electrophoresis [One, 3, 10, and 30 μg of bovine serum albumin (BSA) were run as a size comparison and to allow for an estimate of albuminuria.]. Two mice each were analyzed at 1, 2, and 4 weeks into the inactivation of *Lmx1b*. *Numbers on the left* indicate the molecular masses of the size standard. *C* control mice, *KO* knockout mice. **b** Immunofluorescence staining for nephrin, podocin, and the α4 chain of collagen IV demonstrates the presence of these proteins at the onset of albuminuria. *Bar*, 20 μm (from T. Burghardt et al., LMX1B is essential for the maintenance of differentiated podocytes in adult kidneys. J Am Soc Nephrol 24: 1830–1848, 2013)

of these potential target genes by LMX1B are somewhat artificial, because they rely on concatemerized binding sites and have not been carried out in the natural environment of LMX1B. Furthermore, studies using biopsies from patients with nail-patella syndrome have failed to demonstrate an absence of podocin, CD2AP, and of the α3 and α4 chains of collagen IV in glomeruli [27]. This is in concordance with the aforementioned findings that the podocyte-specific inactivation of *Lmx1b*, be it constitutive or inducible, leads to albuminuria even in the presence of these proteins. A dysregulation of the actin cytoskeleton in podocytes therefore opens a new door towards a better understanding what is going wrong in the podocytes of those patients. It is possible that the molecular pathogenesis in podocytes of nail-patella syndrome patients is similar to that resulting from mutations in the gene encoding α-actinin-4 where it is believed that a stiffer actin cytoskeleton forms [78, 80]. Another screen for LMX1B target genes has led to the identification of NF-κB target genes in stably transfected cell lines [62]. In addition, DNA array screens have been carried out with limb buds of wild-type and *Lmx1b* knockout mice [20, 25, 43]. Since there is only very little overlap between those three screens (and with the DNA array data from the inducible podocyte-specific *Lmx1b* knockout mice), it is difficult to interpret them at present.

One other both puzzling and pressing issue concerns the fact that nail-patella syndrome is inherited in a dominant fashion but that heterozygous knockout mice do not develop a phenotype [66]. We have to keep in mind that in the knockout mice, we are dealing with null mutants of *Lmx1b* whereas quite a few of the mutations in patients with nail-patella syndrome are missense mutations. Although so far no dominant-negative effect or a gain of function was demonstrated for the few LMX1B mutant proteins examined, so far such phenomena can still not be ruled out. The generation of mouse mutants which mimic the mutations found in patients will hopefully help us determine whether we are dealing with haploinsufficiency or with other effects.

References

1. Ben-Bassat M, Cohen L, Rosenfeld J (1971) The glomerular basement membrane in the nail-patella syndrome. Arch Pathol 92:350–355
2. Bongers EMHF, Gubler M-C, Knoers NVAM (2002) Nail-patella syndrome. Overview on clinical and molecular findings. Pediatr Nephrol 17:703–712
3. Bongers EMHF, Huysmans FT, Levtchenko E, de Rooy JW, Blickman JG, Admiraal RJC, Huygen PLM, Cruysberg JRM, Toolens PAMP, Prins JB, Krabbe PFM, Borm GF, Schoots J, van Bokhoven H, van Remortele AMF, Hoefsloot LH, van Kampen A, Knoers NVAM (2005) Genotype-phenotype studies in nail-patella syndrome show that *LMX1B* mutation location is involved in the risk of developing nephropathy. Eur J Hum Genet 13:935–946
4. Bongers EMHF, de Wijs IJ, Marcelis C, Hoefsloot LH, Knoers NVAM (2008) Identification of entire *LMX1B* gene deletions in nail patella syndrome: evidence for haploinsufficiency as the main pathogenic mechanism underlying dominant inheritance in man. Eur J Hum Genet 16:1240–1244
5. Boute N, Gribouval O, Roselli S, Benessy F, Lee H, Fuchshuber A, Dahan K, Gubler M-C, Niaudet P, Antignac C (2000) *NPHS2*, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. Nat Genet 24:349–354
6. Boyer O, Benoit G, Gribouval O, Nevo F, Tête M-J, Dantal J, Gilbert-Dussardier B, Touchard G, Karras A, Presne C, Grunfeld J-P, Legendre C, Joly D, Rieu P, Mohsin N, Hannedouche T, Moal V, Gubler M-C, Broutin I, Mollet G, Antignac C (2011) Mutations in *INF2* are a major cause of autosomal dominant focal segmental glomerulosclerosis. J Am Soc Nephrol 22:239–245
7. Boyer O, Woerner S, Yang F, Oakeley EJ, Linghu B, Gribouval O et al (2013) *LMX1B* mutations cause hereditary FSGS without extrarenal involvement. J Am Soc Nephrol 24:1216–1222
8. Brixey AM Jr, Burke RM (1950) Arthro-onychodysplasia. Hereditary syndrome involving deformity of head of radius, absence of patellas, posterior iliac spurs, dystrophy of finger nails. Am J Med 8:738–744
9. Brown EJ, Schlöndorff JS, Becker DJ, Tsukaguchi H, Uscinski AL, Higgs HN, Henderson JM, Pollak MR (2010) Mutations in the formin gene *INF2* cause focal segmental glomerulosclerosis. Nat Genet 42:72–76
10. Burghardt T, Kastner J, Suleiman H, Rivera-Milla E, Stepanova N, Lottaz C, Kubitza M, Böger CA, Schmidt S, Gorski M, de Vries U, Schmidt H, Hertting I, Kopp J, Rascle A, Moser M, Heid IM, Warth R, Spang R, Wegener J, Mierke CT, Englert C, Witzgall R (2013) LMX1B is essential for the maintenance of differentiated podocytes in adult kidneys. J Am Soc Nephrol 24:1830–1848
11. Carbonara P, Alpert M (1964) Hereditary osteo-onycho-dysplasia (HOOD). Am J Med Sci 248:139–151
12. Chen H, Lun Y, Ovchinnikov D, Kokubo H, Oberg KC, Pepicelli CV, Gan L, Lee B, Johnson RL (1998) Limb and kidney defects in *Lmx1b* mutant mice suggest an involvement of *LMX1B* in human nail patella syndrome. Nat Genet 19:51–55
13. del Pozo E, Lapp H (1970) Ultrastructure of the kidney in the nephropathy of the nail-patella syndrome. Am J Clin Pathol 54:845–851
14. Dombros NK, A. (1982) Nail patella-like renal lesions in the absence of skeletal abnormalities. Am J Kidney Dis 1:237–240
15. Dreyer SD, Zhou G, Baldini A, Winterpacht A, Zabel B, Cole W, Johnson RL, Lee B (1998) Mutations in *LMX1B* cause abnormal skeletal patterning and renal dysplasia in nail patella syndrome. Nat Genet 19:47–50
16. Dreyer SD, Morello R, German MS, Zabel B, Winterpacht A, Lunstrum GP, Horton WA, Oberg KC, Lee B (2000) *LMX1B* transactivation and expression in nail-patella syndrome. Hum Mol Genet 9:1067–1074
17. Dunston JA, Hamlington JD, Zaveri J, Sweeney E, Sibbring J, Tran C, Malbroux M, O'Neill JP, Mountford R, McIntosh I (2004) The human LMX1B gene: transcription unit, promoter, and pathogenic mutations. Genomics 84:565–576
18. Dunston JA, Lin S, Park JW, Malbroux M, McIntosh I (2005) Phenotype severity and genetic variation at the disease locus: an investigation of nail dysplasia in the nail patella syndrome. Ann Hum Genet 69:1–8
19. Eremina V, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, Gerber H-P, Kikkawa Y, Miner JH, Quaggin SE (2003) Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. J Clin Invest 111:707–716
20. Feenstra JM, Kanaya K, Pira CU, Hoffmann SE, Eppey RJ, Oberg KC (2012) Detection of genes regulated by Lmx1b during limb dorsalization. Develop Growth Differ 54:451–462
21. Fong EE (1946) "Iliac horns" (symmetrical bilateral central posterior iliac processes). Radiology 47:517–518

22. Freyd G, Kim SK, Horvitz HR (1990) Novel cysteine-rich motif and homeodomain in the product of the *Caenorhabditis elegans* cell lineage gene *lin-11*. Nature 344:876–879
23. Garg P, Verma R, Cook L, Soofi A, Venkatareddy M, George B, Mizuno K, Gurniak C, Witke W, Holzman LB (2010) Actin-depolymerizing factor cofilin-1 is necessary in maintaining mature podocyte architecture. J Biol Chem 285:22676–22688
24. German MS, Wang J, Chadwick RB, Rutter WJ (1992) Synergistic activation of the insulin gene by a LIM-homeodomain protein and a basic helix-loop-helix protein: building a functional insulin minienhancer complex. Genes Dev 6:2165–2176
25. Gu WXW, Kania A (2010) Identification of genes controlled by LMX1B in E13.5 limbs. Dev Dyn 239:2246–2255
26. Hawkins CF, Smith OE (1950) Renal dysplasia in a family with multiple hereditary abnormalities including iliac horns. Lancet 255: 803–808
27. Heidet L, Bongers EMHF, Sich M, Zhang S-Y, Loirat C, Meyrier A, Broyer M, Landthaler G, Faller B, Sado Y, Knoers NVAM, Gubler M-C (2003) In vivo expression of putative *LMX1B* targets in nail-patella syndrome kidneys. Am J Pathol 163:145–155
28. Hiratani I, Yamamoto N, Mochizuki T, Ohmori S-Y, Taira M (2003) Selective degradation of excess Ldb1 by Rnf12/RLIM confers proper Ldb1 expression levels and Xlim-1/Ldb1 stoichiometry in *Xenopus* organizer functions. Development 130:4161–4175
29. Hoekstra EJ, Mesman S, de Munnik WA, Smidt MP (2013) LMX1B is part of a transcriptional complex with PSPC1 and PSF. PLoS One 8:e53122
30. Holthöfer H, Ahola H, Solin M-L, Wang S, Palmen T, Luimula P, Miettinen A, Kerjaschki D (1999) Nephrin localizes at the podocyte filtration slit area and is characteristically spliced in the human kidney. Am J Pathol 155:1681–1687
31. Holzman LB, St. John PL, Kovari IA, Verma R, Holthofer H, Abrahamson DR (1999) Nephrin localizes to the slit pore of the glomerular epithelial cell. Kidney Int 56:1481–1491
32. Huber TB, Kwoh C, Wu H, Asanuma K, Gödel M, Hartleben B, Blumer KJ, Miner JH, Mundel P, Shaw AS (2006) Bigenic mouse models of focal segmental glomerulosclerosis involving pairwise interaction of CD2AP, Fyn, and synaptopodin. J Clin Invest 116: 1337–1345
33. Ikeda K, Yokoyama H, Tomosugi N, Kida H, Ooshima A, Kobayashi K (1990) Primary glomerular fibrosis: a new nephropathy caused by diffuse intra-glomerular increase in atypical type III collagen fibers. Clin Nephrol 33:155–159
34. Isojima T, Harita Y, Furuyama M, Sugawara N, Ishizuka K, Horita S et al (2014) *LMX1B* mutation with residual transcriptional activity as a cause of isolated glomerulopathy. Nephrol Dial Transplant 29: 81–88
35. Johnson JD, Zhang W, Rudnick A, Rutter WJ, German MS (1997) Transcriptional synergy between LIM-homeodomain proteins and basic helix-loop-helix proteins: the LIM2 domain determines specificity. Mol Cell Biol 17:3488–3496
36. Jurata LW, Gill GN (1997) Functional analysis of the nuclear LIM domain interactor NLI. Mol Cell Biol 17:5688–5698
37. Kalluri R, Shield CF III, Todd P, Hudson BG, Neilson EG (1997) Isoform switching of type IV collagen is developmentally arrested in X-linked Alport syndrome leading to increased susceptibility of renal basement membranes to endoproteolysis. J Clin Invest 99: 2470–2478
38. Kaplan JM, Kim SH, North KN, Rennke H, Correia LA, Tong H-Q, Mathis BJ, Rodríguez-Pérez J-C, Allen PG, Beggs AH, Pollak MR (2000) Mutations in *ACTN4*, encoding a-actinin-4, cause familial focal segmental glomerulosclerosis. Nat Genet 24:251–256
39. Karlsson O, Thor S, Norberg T, Ohlsson H, Edlund T (1990) Insulin gene enhancer binding protein Isl-1 is a member of a novel class of proteins containing both a homeo- and a Cys-His domain. Nature 344:879–882
40. Kestilä M, Lenkkeri U, Männikkö M, Lamerdin J, McCready P, Putaala H, Ruotsalainen V, Morita T, Nissinen M, Herva R, Kashtan CE, Peltonen L, Holmberg C, Olsen A, Tryggvason K (1998) Positionally cloned gene for a novel glomerular protein—nephrin—is mutated in congenital nephrotic syndrome. Mol Cell 1: 575–582
41. Kieser W (1939) Flughaut beim Menschen. Ihre Beziehung zum Status dysraphicus und ihre Erblichkeit Z menschl Vererb Konstitutionslehre 23:594–619
42. Kim JM, Wu H, Green G, Winkler CA, Kopp JB, Miner JH, Unanue ER, Shaw AS (2003) CD2-associated protein haploinsufficiency is linked to glomerular disease susceptibility. Science 300:1298–1300
43. Krawchuk D, Kania A (2008) Identification of genes controlled by LMX1B in the developing mouse limb bud. Dev Dyn 237:1183–1192
44. Lester AM (1936) A familial dyschondroplasia associated with anonychia and other deformities. Lancet 228:1519–1521
45. Little EM (1897) Congenital absence or delayed development of the patella. Lancet 150:781–784
46. Löwik MM, Groenen PJTA, Pronk I, Lilien MR, Goldschmeding R, Dijkman HB, Levtchenko EN, Monnens LA, van den Heuvel LP (2007) Focal segmental glomerulosclerosis in a patient homozygous for a CD2AP mutation. Kidney Int 72:1198–1203
47. Marini M, Bongers EMHF, Cusano R, di Duca M, Seri M, Knoers NVAM, Ravazzolo R (2003) Confirmation of CLIM2/LMX1B interaction by yeast two-hybrid screening and analysis of its involvement in nail-patella syndrome. Int J Mol Med 12:79–82
48. Marini M, Giacopelli F, Seri M, Ravazzolo R (2005) Interaction of the *LMX1B* and *PAX2* gene products suggests possible molecular basis of differential phenotypes in nail-patella syndrome. Eur J Hum Genet 13:789–792
49. Marini M, Bocciardi R, Gimelli S, di Duca M, Divizia MT, Baban A et al (2010) A spectrum of *LMX1B* mutations in nail-patella syndrome: new point mutations, deletion, and evidence of mosaicism in unaffected patients. Genet Med 12:431–439
50. McGinnis W, Levine MS, Hafen E, Kuroiwa A, Gehring WJ (1984) A conserved DNA sequence in homeotic genes of the *Drosophila* Antennapedia and bithorax complexes. Nature 308:428–433
51. McIntosh I, Dreyer SD, Clough MV, Dunston JA, Eyaid W, Roig CM, Montgomery T, Ala-Mello S, Kaitila I, Winterpacht A, Zabel B, Frydman M, Cole WG, Francomano CA, Lee B (1998) Mutation analysis of *LMX1B* gene in nail-patella syndrome patients. Am J Hum Genet 63:1651–1658
52. Miner JH, Sanes JR (1994) Collagen IV a3, a4, and a5 chains in rodent basal laminae: sequence, distribution, association with laminins, and developmental switches. J Cell Biol 127:879–891
53. Miner JH, Morello R, Andrews KL, Li C, Antignac C, Shaw AS, Lee B (2002) Transcriptional induction of slit diaphragm genes by Lmx1b is required in podocyte differentiation. J Clin Invest 109: 1065–1072
54. Morello R, Zhou G, Dreyer SD, Harvey SJ, Ninomiya Y, Thorner PS, Miner JH, Cole W, Winterpacht A, Zabel B, Oberg KC, Lee B (2001) Regulation of glomerular basement membrane collagen expression by *LMX1B* contributes to renal disease in nail patella syndrome. Nat Genet 27:205–208
55. Morita T, Laughlin LO, Kawano K, Kimmelstiel P, Suzuki Y, Churg J (1973) Nail-patella syndrome. Light and electron microscopic studies of the kidney. Arch Intern Med 131:271–277
56. Mukhopadhyay M, Teufel A, Yamashita T, Agulnick AD, Chen L, Downs KM, Schindler A, Grinberg A, Huang S-P, Dorward D, Westphal H (2003) Functional ablation of the mouse *Ldb1* gene results in severe patterning defects during gastrulation. Development 130:495–505
57. Nishioka N, Nagano S, Nakayama R, Kiyonari H, Ijiri T, Taniguchi K, Shawlot W, Hayashizaki Y, Westphal H, Behringer RR, Matsuda

Y, Sakoda S, Kondoh H, Sasaki H (2005) Ssdp1 regulates head morphogenesis of mouse embryos by activating the Lim1-Ldb1 complex. Development 132:2535–2546
58. Ostendorff HP, Peirano RI, Peters MA, Schlüter A, Bossenz M, Scheffner M, Bach I (2002) Ubiquitination-dependent cofactor exchange on LIM homeodomain transcription factors. Nature 416: 99–103
59. Österreicher W (1930) Gemeinsame Vererbung von Anonychie bzw. Onychatrophie, Patellardefekt und Luxatio radii. Dominantes Auftreten in 5 Generationen. Z Konstitutionslehre 15:465–476
60. Pressman CL, Chen H, Johnson RL (2000) *Lmx1b*, a LIM homeodomain class transcription factor, is necessary for normal development of multiple tissues in the anterior segment of the murine eye. Genesis 26:15–25
61. Putaala H, Soininen R, Kilpeläinen P, Wartiovaara J, Tryggvason K (2001) The murine nephrin gene is specifically expressed in kidney, brain and pancreas: inactivation of the gene leads to massive proteinuria and neonatal death. Hum Mol Genet 10:1–8
62. Rascle A, Neumann T, Raschta A-S, Neumann A, Heining E, Kastner J et al (2009) The LIM-homeodomain transcription factor LMX1B regulates expression of NF-kappa B target genes. Exp Cell Res 315:76–96
63. Renwick JH, Lawler SD (1955) Genetical linkage between the ABO and nail-patella loci. Ann Hum Genet 19:312–331
64. Riddle RD, Ensini M, Nelson C, Tsuchida T, Jessell TM, Tabin C (1995) Induction of the LIM homeobox gene *Lmx1* by WNT7a establishes dorsoventral pattern in the vertebrate limb. Cell 83: 631–640
65. Roeckerath W (1951) Hereditäre Osteo-onycho-dysplasie. Fortschr Geb Rontgenstr 75:700–712
66. Rohr C, Prestel J, Heidet L, Hosser H, Kriz W, Johnson RL, Antignac C, Witzgall R (2002) The LIM-homeodomain transcription factor Lmx1b plays a crucial role in podocytes. J Clin Invest 109:1073–1082
67. Ruotsalainen V, Ljungberg P, Wartiovaara J, Lenkkeri U, Kestilä M, Jalanko H, Holmberg C, Tryggvason K (1999) Nephrin is specifically located at the slit diaphragm of glomerular podocytes. Proc Natl Acad Sci U S A 96:7962–7967
68. Salcedo JR (1984) An autosomal recessive disorder with glomerular basement membrane abnormalities similar to those seen in the nail patella syndrome: report of a kindred. Am J Med Genet 19: 579–584
69. Sato U, Kitanaka S, Sekine T, Takahashi S, Ashida A, Igarashi T (2005) Functional characterization of LMX1B mutations associated with nail-patella syndrome. Pediatr Res 57:783–788
70. Schlaubitz S, Yatsenko SA, Smith LD, Keller KL, Vissers LE, Scott DA et al (2007) Ovotestes and XY sex reversal in a female with an interstitial *9q33.3-q34.1* deletion encompassing *NR5A1* and *LMX1B* causing features of genitopatellar syndrome. Am J Med Genet 143A:1071–1081
71. Scott MP, Weiner AJ (1984) Structural relationships among genes that control development: sequence homology between the Antennapedia, Ultrabithorax, and fushi tarazu loci of *Drosophila*. Proc Natl Acad Sci U S A 81:4115–4119
72. Seri M, Melchionda S, Dreyer S, Marini M, Carella M, Cusano R, Piemontese MR, Caroli F, Silengo M, Zelante L, Romeo G, Ravazzolo R, Gasparini P, Lee B (1999) Identification of *LMX1B* gene point mutations in Italian patients affected with nail-patella syndrome. Int J Mol Med 4:285–290
73. Suleiman H, Heudobler D, Raschta A-S, Zhao Y, Zhao Q, Hertting I, Vitzthum H, Moeller MJ, Holzman LB, Rachel R, Johnson R, Westphal H, Rascle A, Witzgall R (2007) The podocyte-specific inactivation of *Lmx1b*, *Ldb1* and *E2a* yields new insight into a transcriptional network in podocytes. Dev Biol 304:701–712
74. Sweeney E, Fryer A, Mountford R, Green A, McIntosh I (2003) Nail patella syndrome: a review of the phenotype aided by developmental biology. J Med Genet 40:153–162
75. Tryggvason K (2001) Mutations in type IV collagen genes and Alport phenotypes. Contrib Nephrol 117:154–171
76. Turner JW (1933) An hereditary arthrodysplasia associated with hereditary dystrophy of the nails. J Am Med Assoc 100:882–884
77. Vogel A, Rodriguez C, Warnken W, Izpisúa Belmonte JC (1995) Dorsal cell fate specified by chick *Lmx1* during vertebrate limb development. Nature 378:716–720
78. Volkmer Ward SM, Weins A, Pollak MR, Weitz DA (2008) Dynamic viscoelasticity of actin cross-linked with wild-type and disease-causing mutant alpha-actinin-4. Biophys J 95:4915–4923
79. Vollrath D, Jaramillo-Babb VL, Clough MV, McIntosh I, Scott KM, Lichter PR, Richards JE (1998) Loss-of-function mutations in the LIM-homeodomain gene, *LMX1B*, in nail-patella syndrome. Hum Mol Genet 7:1091–1098
80. Weins A, Kenlan P, Herbert S, Le TC, Villegas I, Kaplan BS, Appel GB, Pollak MR (2005) Mutational and biological analysis of α-actinin-4 in focal segmental glomerulosclerosis. J Am Soc Nephrol 16:3694–3701
81. Zhuang Y, Soriano P, Weintraub H (1994) The helix-loop-helix gene E2A is required for B cell formation. Cell 79:875–884
82. Zuppan CW, Weeks DA, Cutler D (2003) Nail-patella glomerulopathy without associated constitutional abnormalities. Ultrastruct Pathol 27:357–361