BRIEF COMMUNICATION

Could the interaction between *LMX1B* and *PAX2* influence the severity of renal symptoms?

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Abstract

Nail Patella syndrome (NPS) is a rare autosomal dominant disease characterized by varying degrees of patella, nail, and elbows dysplasia and also ocular and renal congenital abnormalities. The renal involvement, ranging from hematuria and proteinuria to end-stage renal disease, is present in 22–60% of NPS cases. Heterozygous variants in *LMX1B* are known to be responsible of NPS and it has been hypothesized that the variable expressivity is due to the interaction of *LMX1B* with other developmental genes. We reported a case of co-presence of *LMX1B* and *PAX2* variants in a child with extrarenal manifestation of NPS and end-stage renal disease but congenital bilateral renal hypodysplasia and vesicoureteral reflux. The *LMX1B* variant was de novo, whereas the *PAX2* variant was inherited from the mother that had bilateral renal hypoplasia although in presence of only a mild chronic kidney disease. The molecular interaction between *LMX1B* and *PAX2* has been already reported in vitro and this finding suggest that the worst renal NPS phenotype of our patient could be due to the defective expression of these two genes during nephrogenesis. In conclusion, our finding suggests that *PAX2* may act as modifier gene in Nail Patella phenotype.

Introduction

LMX1B (OMIM #602575) encodes a LIM-homeobox transcription factor that has multiple roles during embryonic development, in particular it is involved in limb, eye, and kidney development, accounting for the pleiotropic manifestations of the Nail Patella syndrome (NPS, OMIM #161200)—a rare autosomal dominant disease. NPS common features are varying degrees of patella, nail and elbows dysplasia, ocular and renal anomalies. The renal

involvement is present in 22–60% of NPS cases and is the most serious manifestation of the disease ranging from hematuria and proteinuria to end-stage renal disease [1]. High inter-individual and intra-familial variability has been showed in NPS patient. More recently *LMX1B* heterozygous variants have been associated also to nephrotic syndrome without extra-renal manifestations [2, 3]. It has been speculated that the variable expressivity could be due to the *LMX1B* interaction with other developmental genes. Some authors have hypothesized the presence of genetic

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Fig. 1 **a** Output of HRM analysis showed a different melting curve for *PAX2* exon 4. **b** Sequences aligned and their codons translation. There are two stars in the variants of electropherograms.

heterogeneity [1]. Furthermore, looking for modifier genes, Marini et al. demonstrated the *LMX1B*/*PAX2* interaction by co-immunoprecipitation and yeast two-hybrid assays [4]. *PAX2* (OMIM #167409) encodes a paired-box transcription factor involved in multiple embryonic organs development and it is known to be essential in the first steps of nephrogenesis, in particular during ureteric bud induction and its branching morphogenesis [5]. *PAX2* haploinsufficiency is causative of Papillorenal syndrome (OMIM #120330) and heterozygous variants in this gene are also reported in congenital anomalies of the kidney and urinary tract with or without ocular abnormalities (e.g., renal hypo/dysplasia, vesicoureteral reflux) and adult onset focal segmental glomerulosclerosis (FSGS7, OMIM #616002) [6, 7].

Here, we reported the first case of co-presence of *LMX1B* and *PAX2* variants in a NPS patient with end-stage renal disease, congenital bilateral renal hypodysplasia, and bilateral vesicoureteral reflux. The patient inherited the *PAX2* variant from the mother that had bilateral renal hypoplasia, but presented only mild chronic kidney disease, whereas the *LMX1B* variant was de novo. Although, the synergic interaction between these developmental genes has been already shown in vitro [4], to our knowledge, this study represents the first report of such interaction in a clinical case.

Patients and methods

Patients

We considered a child with NPS, severe kidney phenotype, and positive family history for chronic kidney disease.

The proband is a female born at 35 gestational age, with nail dysplasia and bilateral patella hypoplasia, bilateral clinodactyly, and bilateral coxa valga. She had neonatal renal failure with serum creatinine concentration of 2.3 mg/dl. She had ultrasonographic evidence of bilateral small kidneys (<2 SD compared to the expected mean) associated both with hyperechogenicity and lack of cortical-medullary differentiation, compatible with renal hypodysplasia diagnosis. Furthermore, voiding cystourethrography showed the presence of bilateral vesicoureteral reflux. At the 7 years of age, the patient underwent renal transplantation. The diagnosis was NPS and the genetic analysis confirmed the presence of the previously described splicing variant *LMX1B*: c.742-2A>G (HGMD® Public Version: CS042548; Genbank: NG_017039.1: NM_002316.3) [8]. The proband's mother had bilateral renal hypodysplasia and at 40 years of age showed only mild chronic kidney disease with onset during pregnancy, but not nail and skeletal abnormalities. Assuming NPS variable expressivity, the inheritance was checked in the mother, but she was not the child's *LMX1B* variant carrier. Both mother and child did not have optic disc or nerve head anomalies. The father, who did not have any renal or extrarenal finding, was not a carrier of this pathogenic variant.

Nucleotide sequence analysis

After proper written informed consent, the patients' blood samples were collected in EDTA tube. Genomic DNA was obtained, using a DNA extraction kit, according to manufacturer instructions (QIAamp® DNA Blood Mini Kit, QIAGEN, Hilden, Germany).

Fig. 2 PyMOL paired-box domain output of PDB: 1K78A 3D structure. **a** The picture highlighted the amino acid Arginine 140 (Arg140) in the alpha helix motif of PAX transcriptional factor and their molecular interaction with the major groove of DNA. **b** A higher

magnification of interaction site: Arg140 links the guanine nucleotide (DG-4) in the DNA helix. Arg140 also bounds the Asparagine 136 (Asn136) in the same protein alpha helix.

PAX2 coding sequence analysis was performed by PCR and High-Resolution Melt Analysis in 0.1 ml tubes on the RotorGeneTM6000 (Corbett Research, Sydney, Australia) with the ready to use mix 5X HOT-FIREPol[®] EvaGreen[®]

HRM-MIX (Solis BioDyne OÜ, Estonia). Direct sequencing of both strands was performed using Big Dye Terminator-v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, MA, USA) and analyzed on an ABI PRISM[®]

3100 Genetic Analyzer (Thermo Fisher Scientific, MA, USA). The primers used in our analysis were previously published [6]. The reference cDNA sequences and DNA sequence for exon numbering for *PAX2* (NM_003987.3; NG_008680.2) are available through the NCBI website. The nomenclature system for the description of the changes in DNA is based on the Human Genome Variations Society recommendations. Nucleotide +1 corresponds to the A of the ATG translation initiation codon of the respective reference sequence.

The disease-causing potential evaluation of the sequence alterations was assessed using MutationTaster2 (<http://www.mutationtaster.org/>) software [9]. The 3D structure variations were predicted by automated homology modeling program EsyPred3D (<http://www.unamur.be/sciences/biologie/urbm/bioinfo/esypred/>) and molecular visualization was done by PyMOL software (<https://www.pymol.org/>) [10, 11].

The variant was submitted to the Leiden Open Variation Database (#0004132 <https://grenada.lumc.nl/LOVD2/PAX2>).

Results

High-Resolution Melt analysis showed a different melting curve for *PAX2* exon 4 (Fig. 1a) and Sanger sequencing confirmed the presence of a new missense variant (Fig. 1b) in renal hypodysplasia mother's DNA (NM_003987.3; NG_008680.2 Genbank), that is c.418C>T. Sanger sequence analysis have shown that c.418C>T was inherited by the NPS proband. This variant is not present in the healthy father, in dbSNP142, in the 1000 genome project and in ExAC browser.

The c.418C>T substitution would result in the p.(Arg140Trp) protein change at the high conserved paired-box N-terminus domain; the MutationTaster2 software classified this variant as disease causing. Viewing by PyMOL the paired-box domain of PDB: 1K78A 3D structure has shown that the amino acid replaced in our patient would have direct contact with the DNA (Fig. 2).

Discussion

We report the case of a NPS patient with complete syndrome phenotype and with a family history of renal phenotype only from the mother's side. Our patient carried a known splicing variant on *LMX1B*, and this variant was not inherited from the mother. Two could be the most accredited hypotheses of this discrepancy between mother and child phenotypes: (a) the presence of a mosaicism in the mother, which, however, did not show any other sign of the NPS; (b) the maternal phenotype was due to abnormalities of other kidney developmental genes. Focusing on the

second possibility, we started to screen the patient's mother for *PAX2* sequence variant. Indeed it is known that *PAX2* variants are one of the most common genetic causes of the renal hypodysplasia (up to 10% of cases) [12]. We found that she carried a missense variant in the paired-box domain, predicted to be disease causing. Furthermore, we confirmed that this *PAX2* affects function variant was inherited by our patient.

The proband showed NPS features due to *LMX1B* variant, in addition she had a worse renal condition compared to the mother. Based on the experimental data already published [4], we concluded that the most severe proband renal phenotype is due to the interaction between the two genes variants during kidney development. Our result highlights the possibility that *PAX2* could act as modifier gene in the 5% of NPS cases reported in literature to have phenotype with ESRD [13].

Nephrogenesis is due to a time-spaced-regulated genes network that includes molecules interacting among each other (e.g., transcriptional factors, growth factors, and signal transduction proteins). More than 20 genes are known to be associated to congenital anomalies of the kidney and urinary tract such as renal hypodysplasia [12].

Oligogenic inheritance, such as the one described in our work, could be underestimated in developmental kidney disease, when the aim is to focus only on one gene [14, 15]. To date, next-generation sequencing contributes to increase the discovery of modifier alleles in different nephrogenetic diseases, including manifestations that were not previously associated to their original phenotypic spectrum disease [16–18]. In conclusion, in this new scientific scenario, our classic mutational study sustains the importance of considering oligogenic inheritance to explain the NPS phenotype variability. Furthermore, we highly recommend *PAX2* genetic analysis in NPS patients with renal hypodysplasia phenotype in order to define a better genetic diagnosis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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