Identification of a Novel Heterozygous De Novo 7-bp Frameshift Deletion in PBX1 by Whole-Exome Sequencing Causing a Multi-Organ Syndrome Including Bilateral Dysplastic Kidneys and Hypoplastic Clavicles

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Introduction: Congenital anomalies of the kidney and urinary tract (CAKUT) represent the primary cause of chronic kidney disease in children. Many genes have been attributed to the genesis of this disorder. Recently, haploinsufficiency of PBX1 caused by microdeletions has been shown to result in bilateral renal hypoplasia and other organ malformations.

Materials and methods: Here, we report on a 14-year-old male patient with congenital bilateral dysplastic kidneys, cryptorchidism, hypoplastic clavicles, developmental delay, impaired intelligence, and minor dysmorphic features. Presuming a syndromic origin, we performed SNP array analysis to scan for large copy number variations (CNVs) followed by whole-exome sequencing (WES). Sanger sequencing was done to confirm the variant’s de novo status.

Results: SNP array analysis did not reveal any microdeletions or -duplications larger than 50 or 100 kb, respectively. WES identified a novel heterozygous 7-bp frameshift deletion in PBX1 (c.413_419del, p.Gly138Valfs*40) resulting in a loss-of-function. The de novo status could be confirmed by Sanger sequencing.

Discussion: By WES, we identified a novel heterozygous de novo 7-bp frameshift deletion in PBX1. Our findings expand the spectrum of causative variants in PBX1-related CAKUT. In this case, WES proved to be the apt technique to detect the variant responsible for the patient’s phenotype, as single gene testing is not feasible given the multitude of genes involved in CAKUT and SNP array analysis misses rare single-nucleotide variants and small Indels.

Keywords: CAKUT, PBX1, dysplastic kidneys, hypoplastic clavicles, developmental delay
INTRODUCTION

Congenital anomalies of the kidney and urinary tract (CAKUT) represent the primary cause of chronic kidney disease in children. CAKUT is the collective term for many different renal and urinary tract malformations. In recent years, a multitude of monogenic disease-causing genes has been discovered (1). Disruption of the normal nephrogenesis by pathogenic variants in genes involved in kidney development is a basic principle of CAKUT (2).

When it comes to heterogeneous diseases like CAKUT, with many different genes involved, large-scale next-generation sequencing has become an extremely useful tool for unbiased detection of pathogenic variants (3). In whole-exome sequencing (WES), the coding regions (the exome) of the human genome are enriched and sequenced. This has proven to be both an economic, as the exome only comprises 1% of the genome, and a pragmatic approach, as about 85% of disease-related variants can be found in the exome (4).

PBX1 encodes a transcription factor that has already been linked to nephrogenesis as shown by Pbx1-deficient mice (5). Additionally, earlier mouse models revealed its role in bone formation (6). In 2017, microdeletions comprising PBX1 as a minimal common region could be identified by microarray analysis in eight patients with syndromic CAKUT with predominantly renal hypoplasia. In the same publication, it was shown that PBX1 is strongly expressed in the fetal kidney and brain (7). Here, we report on a 14-year-old male patient presenting to the pediatric nephrology department with the predominant clinical features of bilateral dysplastic kidneys, hypoplastic clavicles, and developmental delay.

MATERIALS AND METHODS

This study was approved by the local Ethics Committee of the Technical University of Munich and performed according to the standard of the Helsinki Declaration of 1975. Written informed consent was obtained from the parents of the participant for publication of this case report. Blood samples were collected after written informed consent.

DNA was extracted from peripheral blood using the Gentra Puregene Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA sample of the patient was analyzed by using the SNP Array Affymetrix® CytoScanTM 750 K Array (Affymetrix® Inc., Santa Clara, CA, USA) with an average space between two oligonucleotides of 4 kb. Scanning was performed by the Affymetrix® GeneChip Scanner 3000 7G (resolution 0.51–2.5 µm). The data analysis was conducted using the Affymetrix® Chromosome Analysis Suite Software (ChAS), version 3.0, hg19.

Exome sequencing was performed using a Sure Select Human All Exon 60 Mb V6 Kit (Agilent) and a HiSeq4000 (Illumina) as previously described (8, 9). Reads were aligned to the UCSC human reference assembly (hg19) with BWA v0.5.8. More than 98% of the exome was covered at least 20×. PBX1 was covered >20× to 100%. Single-nucleotide variants and small insertions and deletions were detected with SAMtools v.0.1.7. Variant prioritization was performed based on an autosomal recessive pattern of inheritance (homozygous or putative compound heterozygous variants with a minor allele frequency <0.1%) as well as an autosomal dominant pattern of inheritance (heterozygous variants with a minor allele frequency <0.001%).

Sanger sequencing was used to confirm the identified variant and to test the patient’s parents. Oligonucleotide primer sequences are available upon request.

CASE REPORT AND RESULTS

The 14-year-old boy is the first child of healthy parents (Figure 1). He has one younger healthy brother and one younger healthy half-brother. The patient was born at a gestational age of 38 weeks [birth weight: 2,840 g (25th–50th percentile), birth length: 48 cm (10th–25th percentile)]. There were no obvious malformations noted at birth. During the neonatal period, a slender thorax and short clavicles were identified clinically. Body height was on the third percentile during infancy and early childhood (Figure 2) and a global developmental delay (including motor and speech delay) was diagnosed on regular pediatric screening examinations.

An ultrasound of the kidneys at 3 months of age revealed small kidneys with hyperechogenic parenchyma. The boy was then regularly seen by a pediatric nephrologist. By the age of six, the right kidney had a length of 6.2 cm (<1st percentile, see Figure 3), the left kidney had a length of 5.8 cm (<1st percentile, see Figure 4). Kidney function ranged between eGFR 59 mL/min/1.73 m2 at 4 years of age, 90 mL/min/1.73 m2 at 8 years of age, and 69 mL/min/1.73 m2 at 13 years of age (Schwartz estimate, CKD II). Moreover, the patient had bilateral cryptorchidism for which he received hormonal therapy by the age of 2 years.

Psychological intelligence testing by the age of 13 revealed a below average speech comprehension (IQ 81), a reduced information processing speed (IQ 74) and an impaired auditory working memory (IQ 77).

On orthopedic examination the patient had a slim shoulder profile, impaired abduction of the arms, and a hunched back. Thoracic X-ray revealed hypoplastic clavicles (Figure 5, only left clavicle visible). He also had some dysmorphic features (wide nasal bridge, short neck, bilateral overfolding of the helix, and bilateral clinoactyly).

In this child, we presumed a syndromic origin and initially performed SNP array analysis. However, no microdeletions or microduplications were detected, suggesting a microdeletion of the PBX1 gene. Further genomic analysis was performed using SNP array analysis (CytoScanTM 244K Array, Affymetrix) as well as whole-exome sequencing (SureSelect Human All Exon 60 Mb V6) and targeted sequencing of the PBX1 gene (Illumina TruSight Exome) using an Illumina HiSeq4000. A novel missense variant in the spalt-like transcription factor 1 gene (SALL1) was identified (NM_181972). This variant was confirmed by Sanger sequencing and cosegregated with the phenotype. SALL1 encodes a transcription factor that has already been shown to be essential for kidney development (10). Therefore, we propose a renal phenotype in association with the SALL1 variant.

Figure 1 | Pedigree of the family. Solid symbol, affected individual; circles, females; squares, males.
duplications (copy number variations, CNVs) larger than 50 or 100 kb, respectively, could be detected. As CAKUT have been associated with a large number of genes, we then performed WES. By WES, we could identify a novel heterozygous 7-bp deletion in PBX1 leading to a frameshift (c.413_419delGGGCAGG, p.Gly138Valfs*40), resulting in either nonsense-mediated decay of the mRNA or a truncated protein lacking the DNA-binding domain. The variant is not listed in 60,000 control individuals of the Exome Aggregation Consortium (ExAC) browser. The ExAC browser does not list any high-confidence loss-of-function variants in PBX1 indicating that PBX1 is intolerant to loss-of-function variants. To verify the de novo status of the variant, we performed Sanger sequencing in the patient and his parents. The variant could not be detected in the blood DNA of both parents (Figure 6).

**DISCUSSION**

CAKUT represent the primary cause of chronic kidney disease in children and many genes have been attributed to the genesis of this disorder with both dominant and recessive modes of inheritance.
Figure 6: Partial nucleotide sequence of exon 3 of PBX1 of the patient and his parents showing the heterozygous de novo variant c.413_419delGGGCAGG, p.Gly138Valfs*40. Shown reference sequence: TTCTGGAGGGGCAGG. Genomic position of the variant: chr1:164761876–164761882 (hg19, transcript NM_002585.3).

Table 1: Summary of genetic changes in PBX1 and clinical features reported so far.

| Patient | Kidney/urinary tract phenotype | Kidney function | Extrarenal phenotype                                                                 | Genetic change | Protein change | Mode of inheritance |
|---------|--------------------------------|----------------|-------------------------------------------------------------------------------------|----------------|-----------------|-------------------|
| This report | Bilateral dysplasia, hyperechogenicity | eGFR = 59–90 mL/min/1.73 m² (at 21 years) | Bilateral cryptorchidism, hypoplastic clavicles, postnatal growth retardation (height third percentile), global developmental delay (including motor and speech delay), impaired intelligence, dysmorphic features (wide nasal bridge, short neck, bilateral overfolding of the helix, bilateral clinodactyly) | c.[413_419delGGGCAGG];[=] p.[Gly138Valfs*40];[=] | de novo |
| K175 | Bilateral hypoplasia | eGFR = 40 mL/min/1.73 m² (at 11 years) | Deafness, scoliosis | c.[428delA];[=] | p.[Asn143Thrfs*37];[=] | de novo |
| K179 | Bilateral cystic hypodysplasia | eGFR = 51 mL/min/1.73 m² (at 11 years) | Dysmorphic features, developmental delay | c.[550C>T];[=] | p.[Arg184*];[=] | de novo |
| K186 | Bilateral hypoplasia with oligonephronia | Oligohydramnios | No | c.[511-2A>G];[=] (exon 4, splice acceptor site variant) | de novo |
| K181 | Hypoplastic horseshoe kidney, absence of corticomedullar differentiation | eGFR = 40 mL/min/1.73 m² (at 39 years) | Deafness | 2.5-Mb deletion (encompassing 8 genes) | de novo |
| K136 | Unilateral agenesis/small hyperechogenic kidney | Normal renal function (at 18 months) | Dysmorphic features, impaired intelligence | 9.1-Mb deletion (encompassing 131 genes) | de novo |
| PT1 | Normal kidney phenotype, bifid right ureter, bilateral pelvis dilatation, bilateral VUR, small urethral valve | Not available | Sacral pit, postnatal growth retardation (weight and height < 3rd percentile), severe global developmental delay, neonatal hypotonia, bilateral perceptive hearing loss, dysmorphic features (low-set ears, anteverted nares, prominent philtrum, thick lips, uvula bifid), seizures in infancy | 6.0-Mb deletion (chr1:161650414–167622545, encompassing 36 genes) | de novo |

(Continued)
Identification of a Novel PBX1 Variant

Patients K175, K179, K186, K181, K136 see Ref. (11); patients PT1-8 see Ref. (7). Reference genome for deletion coordinates: hg19; ASD, atrial septal defect; eGFR, estimated glomerular filtration rate; VSD, ventricular septal defects.

| Patient | Kidney/urinary tract phenotype | Kidney function | Extrarenal phenotype | Genetic change | Protein change | Mode of inheritance |
|---------|-------------------------------|-----------------|----------------------|----------------|----------------|---------------------|
| PT2     | Bilateral renal hypoplasia, nephrocalcinosis | eGFR = 40 mL/min/1.73 m² (at 1.5 months) | VSD, ductus arteriosus, severe kyphoscoliosis, postnatal growth retardation (weight and height < 3rd percentile), microcephaly (<3rd percentile), global developmental delay (including motor delay), deep sound hearing loss, dysmorphic features (antimongoloid eyesplit, frontal bossing, anterior fontanelle closed prematurely, low-set, pointed ears), hypermetropism | 9.2-Mb deletion (chr1:162703368–171908659, encompassing 62 genes) | de novo |
| PT3     | Bilateral renal hypoplasia, right renal ectopia, hyperechogenicity, dedifferentiation | eGFR = 66 mL/min/1.73 m² (at 5 years) | VSD, ductus arteriosus, sacral pit, mild global developmental delay (including motor and speech delay), dysmorphic features (hypoplastic lobes, left-sided ear pit, long, narrow face, wide nasal bridge, broad nasal tip, mild retrogнатia) | 2.8-Mb deletion (chr1:163193466–166058476, encompassing 11 genes) | de novo |
| PT4     | Bilateral renal hypoplasia, hyperechogenicity | Normal, eGFR = 106 mL/min/1.73 m² (at 2 years) | Bilateral cryptorchidism, multiple skeletal malformations (shoulder blade, acromioclavicular joint, skull basis, vertebral defects, hip dislocation), postnatal growth retardation (weight 3rd to 10th percentile), motor delay, hypotonia, dysmorphic features (small, low-set, posteriorly rotated ears, abnormal folding of the helix, divergent strabismus, short nose, antverted nares, prominent philtrum, short neck) | 1.5-Mb deletion (chr1:163574086–165092429, only encompassing PBX1) | de novo |
| PT5     | Bilateral renal hypoplasia | CKD stage 3 (at 3 years) | Corpus callosum hypoplasia, sacral pit, anal malposition, difficult neonatal adaptation, unilateral vocal fold paralysis, postnatal growth retardation (weight 3rd to 10th, height <3rd percentile), global developmental delay, neonatal hypotonia, hearing loss, dysmorphic features (prominent metopic ridge, broad nasal bridge, abnormally formed ears, hypertelorism, epicanthus, divergent strabismus, thin upper lip, long hands and feet, unilateral fifth finger clinodactyly), encopresia, enuresia | 3.6-Mb deletion (chr1:163811431–167385298, encompassing 16 genes) | de novo |
| PT6     | Bilateral renal hypoplasia | CKD stage 5 (transplant at 10 months) | Unilateral cryptorchidism, mitral regurgitation, left ventricular hypertrophy, spina bifida occulta, unilateral inguinal hernia, mild global developmental delay (including motor and speech delay), autism spectrum disorder | 0.9-Mb deletion (chr1:164330973–165207097, encompassing 2 genes) | unknown |
| PT7     | Horseshoe kidney | Not available | VSD, ASD, ductus arteriosus, cleft of the posterior arch of L5, anal malposition, postnatal growth retardation (weight 3rd to 10th, height 3–10th percentile), microcephaly (<3rd percentile), global developmental delay (including motor and speech delay), hearing loss, dysmorphic features (thin, low-set hair, antverted, low-set ears, crumpled, thick helix, thick lower lip, prognathism, mandibular hypermobility, dental malocclusion, bilateral clinodactyly, wide-based gait) | 6.9-Mb deletion (chr1:164501003–171424595, encompassing 51 genes) | de novo |
| PT8     | Bilateral renal hypoplasia, right renal ectopia, right renal dysplasia | Normal, eGFR = 130 mL/min/1.73 m² (at 4.2 years) | Right cryptorchidism, mild global developmental delay (including motor and speech delay), autism spectrum disorder, bilateral conductive hearing loss, dysmorphic features (bilateral ear hypoplasia), joint laxity | 0.3-Mb deletion (chr1:164523918–164799811, only encompassing PBX1) | unknown |
CAKUT mainly occur as part of (multi-organ) syndromes but there are also isolated cases described in the literature (1, 2, 10). Just recently, haploinsufficiency of PBX1 caused by microdeletions was shown to result in bilateral renal hypoplasia and other organ malformations (7). Furthermore, a targeted exome sequencing of 330 genes in 204 unrelated CAKUT patients could identify five novel de novo heterozygous loss-of-function variants/deletions in PBX1 (11).

PBX1 encodes a transcription factor which promotes protein–protein interaction and is important for organogenesis (12). Pbx1−/− mice die at an embryonic age and show extensive organ malformations including hypoplastic kidneys with unilateral agenesis (5). In a further publication, Pbx1-deficient mice exhibited—besides multiple organ malformations—a pronounced skeletal phenotype with a slender thorax, hunched posture, and axial malformations. PBX1 is highly expressed in proliferating chondrocytes (6). Additionally, there is strong PBX1 expression in the fetal brain (7), and it regulates patterning of the cerebral cortex in progenitor neurons in mice (13). To date, two publications reported CAKUT phenotypes related to pathogenic PBX1 variants/microdeletions; however, only two of the eight patients published by Le Tanno et al. had heterozygous microdeletions only encompassing PBX1 (7). The phenotype of these patients involved, among other things, bilateral renal hypoplasia with hyperechogenic parenchyma, cryptorchidism, skeletal malformations, and developmental delay. The other patients in this publication had larger deletions involving a more extensive set of genes contributing to the phenotype. The five patients with novel loss-of-function variants/deletions in PBX1 identified in a targeted exome sequencing study (11) lacked a detailed genotype–phenotype correlation, as only limited information on the extrarenal phenotype was provided. See Table 1 for a detailed summary of the genetic changes in PBX1 and clinical features described so far.

The patient in our report displayed a complex clinical picture with kidney and skeletal malformations and a neuronal phenotype with developmental delay and impairment of intelligence. In addition to the previously published data from PBX1 mouse models, microdeletions and loss-of-function variants/deletions mentioned above, we provide a detailed description of the phenotype and make the case for an improved diagnostic approach in CAKUT: in this patient our diagnostic algorithm involved SNP array analysis which did not yield a positive result. We then performed WES and identified a novel heterozygous de novo 7-bp frameshift deletion in PBX1 (c.413_419del, p.Gly138Valfs*40). For future CAKUT cases, we recommend directly employing whole-exome or whole-genome sequencing, as these are the apt techniques to identify new pathogenic variants/CNVs in this genetically heterogeneous syndrome. This is especially true for syndromal and familial CAKUT. In patients with isolated CAKUT, however, diagnostic yield is probably rather low, as less than 10% carry variants in 20 known CAKUT genes (2).

**ETHICS STATEMENT**

This study was carried out in accordance with the recommendations of the Ethics Committee of the Technical University of Munich with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee of the Technical University of Munich.

**AUTHOR CONTRIBUTIONS**

KR, MW, TM, and JH were responsible for writing and revision of the manuscript. CS and CM cared for the patient and provided the clinical data. KR, BA, RK-N, and MW did the exome analysis.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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