CHD1L: a new candidate gene for congenital anomalies of the kidneys and urinary tract (CAKUT)

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Abstract

Background. Recently, we identified a microduplication in chromosomal band 1q21.1 encompassing the CHD1L/ALC1 gene encoding a chromatin-remodelling enzyme in congenital anomalies of the kidneys and urinary tract (CAKUT) patient.

Methods. To explore the role of CHD1L in CAKUT, we screened 85 CAKUT patients for mutations in the CHD1L gene and performed functional analyses of the three heterozygous missense variants detected. In addition, we quantitatively determined CHD1L expression in multiple human fetal and adult tissues and analysed expression of CHD1L protein in human embryonal, adult and hydronephrotic kidney sections.

Results. Two of three novel heterozygous missense variants identified in three patients were not found in >400 control chromosomes. All variants lead to amino acid substitutions in or near the CHD1L macro domain, a poly-
ADP-ribose (PAR)-binding module interacting with PAR polymerase 1 (PARP1), and showed decreased interaction with PARP1 by pull-down assay of transfected cell lysates. Quantitative messenger RNA analysis demonstrated high CHD1L expression in human fetal kidneys, and levels were four times higher than in adult kidneys. In the human embryo at 7–11 weeks gestation, CHD1L immunolocalized in the early ureteric bud and the S- and comma-shaped bodies, critical stages of kidney development. In normal postnatal sections, CHD1L was expressed in the cytoplasm of tubular cells in all tubule segments. CHD1L expression appeared higher in the hydrenephrotic kidney of one patient with a hypofunctional CHD1L variant than in normal kidneys, recapitulating high fetal levels.

**Conclusion.** Our data suggest that CHD1L plays a role in kidney development and may be a new candidate gene for CAKUT.

**Keywords:** CAKUT; CHD1L; expression pattern; hypofunctional variant; kidney development

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**Introduction**

Renal tract malformations can occur at the level of the kidney (e.g. aplasia, hypoplasia, dysplasia with and without cysts or duplex ureter), collecting system (e.g. hydronephrosis or hydro-ureter), bladder (e.g. vesicoureteral reflux (VUR)) or urethra (e.g. posterior urethral valves) and are subsumed by the term congenital anomalies of the kidneys and urinary tract (CAKUT). CAKUT comprise ~15% of all congenital anomalies detected prenatally and are found in >250 syndromes and in more than one-third of chromosome aberrations [1]. Among these are disorders like the chromosome 22q11.2 deletion and thra (e.g. posterior urethral valves) and are subsumed by the droureter), bladder [e.g. vesicoureteral reflux (VUR)] or ure-ralis, proximal ureteric stenosis and additional anomalies, we detected a duplication of 2.73 Mb in 1q21.1 [10]. Among the genes duplicated was CHD1L (syn.: ALC1, amplified in liver cancer 1), which encodes the chromodomain helicase DNA-binding 1-like protein. CHD1L belongs to the Snf2 family of helicase-related ATP-hydrolyzing proteins and contains a helicase-like region, which is similar to that of other members of the Snf2-like group, such as Snf2, Islw1, Chd1 and CHD7 [11]. ATPases of this family often combine a helicase domain with motifs that mediate selective recognition of protein modifications. In CHD1L, this is a macro domain, which is an ADP-ribose/poly-ADP-ribose (PAR)-binding module [12]. CHD1L has been implicated as an oncogene with a major impact in hepatocellular carcinoma development [13–15] and has been identified as a chromatin-remodelling enzyme that interacts with PAR and catalyses PAR polymerase 1 (PARP1)-stimulated nucleosome sliding [16, 17].

Chromatin-remodelling and -modifying enzymes are predicted to play key roles in differentiation, development and tumour pathogenesis via effects on chromatin structure and accessibility [18, 19]. Mutations in CHD7, a gene structurally related to CHD1L, cause CHARGE syndrome, which includes renal developmental anomalies [20–22]. Therefore, in this study, we screened 85 CAKUT patients for mutations in the CHD1L gene and performed functional analyses of the three heterozygous missense variants identified. In addition, we quantitatively determined CHD1L expression in multiple human fetal and adult tissues and analysed expression of CHD1L protein in human embryonal, adult and hydrenephrotic kidney sections. Our data provide evidence for a role of CHD1L in kidney development and for CHD1L mutations in the anomalies of the renal tract.

**Materials and methods**

**Patients**

The mutation analysis was approved by the ethics committee of the Medical Faculty of the University of Heidelberg, Germany, and informed assent and/or consent was obtained from the patients and/or parents as appropriate. Eighty-five patients presenting with different CAKUT phenotypes, defined by clinical and renal sonographic assessment, were screened for CHD1L mutations. The patients presented with one or more of the following CAKUT phenotypes: kidney agenesis (6 patients), kidney hypoplasia (17 patients), dysplastic kidneys (39 patients), medullary cystic kidney disease (3 patients) and duplex kidney (11 patients). Nine patients showed ureteral anomalies: proximal ureteral stenosis (1 patient) and megaureter/hydronephrosis (8 patients). Twenty-four patients presented with VUR. Posterior urethral valves were identified in eight patients. In three patients, heterozygous missense variants were detected in the CHD1L gene.

**Patient 1.** The boy was the first child of non-consanguineous healthy parents. Renal abnormalities are not known in the family. While the kidney ultrasound of the mother during pregnancy did not disclose any abnormality, the father was not available for examination by renal ultrasound. The antenatal story of the patient was uneventful, and prenatal ultrasound did not disclose any abnormality. The boy was born at term with weight (3750 g) and length (50 cm) within the normal range and without dysmorphic features. A febrile urinary tract infection (UTI) occurred at age 4 months, which led to the diagnosis of a hypodysplastic right kidney with right-sided grade III–IV and left-sided grade II VUR demonstrated by renal ultrasound and micturating cysto-urethrography (MCUG). While no further UTI occurred, growth of the right kidney was impaired. At the age of 6 years, the kidney was small for age (volume 34 mL, <3rd percentile), whereas the contralateral kidney showed compensatory hypertrophy (volume 100 mL, >97th percentile). Blood pressure and urine analysis were normal, in particular no leukocyturia or erythrocyturia, no glucosuria and no pathological protein excretion were detected. The child exhibited normal psychomotor and somatic development.

**Patient 2.** The boy is Albanian and was born in Kosovo after an uneventful pregnancy. The parents were unrelated and healthy with normal renal morphology on the ultrasound scan. During the first year of life, the patient developed repeated episodes of febrile UTI. Clinical workup revealed a severe CAKUT phenotype with bilateral massive hydronephrosis due to obstructive megaureters. He received bilateral pyelostomy at 18 months of age. The further clinical course was complicated by repeated UTIs leading to nephrectomy of the left kidney at age 2.5 years. The ureteropelvic drainage of the right kidney was switched to a cutaneous ureterostomy at the age of 5 years when the family moved to Germany. At that time, advanced chronic
renal failure [estimated glomerular filtration rate (GFR) 23 mL/min/1.73m²], uraemic bone disease and growth failure (height 14 cm, <3rd percentile) were diagnosed. Further findings were unilateral cryptorchidism and subglottic stenosis, presumed secondary to repeated and prolonged mechanical ventilation. Renal function gradually progressed and renal replacement therapy was initiated at the age of 13 years. Right-sided nephrectomy and excision of the hydroureter were performed at the time of renal transplantation at the age of 16 years. Histopathological workup showed atrophy of the renal parenchyma, chronic tubulointerstitial inflammation and chronic ureteritis.

**Patient 3.** The boy was referred for clinical examination at 3 weeks of age with severe renal insufficiency (estimated GFR 17 mL/min/1.73m²). Ultrasonography revealed bilateral hydropsplasia of the kidneys. No VUR was detected by VCUG. Extrarenal organ malformations were not observed. At the age of 12 months, cystoscopy revealed a posterior urethral valve with membranous stenosis of the urethra and a trabeculated bladder. The posterior urethral valve was subsequently excised. During the following 8 years, renal function declined to a GFR <10 mL/min/1.73m² and peritoneal dialysis was started at 9 years of age. At the age of 10 years, the patient received a cadaveric kidney transplant with immediate graft function.

**Materials**

All immunohistochemistry was performed on formalin-fixed paraffin-embedded sections. Samples included (i) phenotypically normal human kidney samples from chemically induced terminations of pregnancy between 7 and 11 weeks of gestation (n = 7), collected by the Wellcome Trust and Medical Research Council-funded Human Developmental Biology Resource at the UCL Institute of Child Health, London, UK. Informed consent to analyse these samples was obtained from the mothers involved, and use was approved by the Joint University College London/University College Hospital Committee on the Ethics of Human Research. (ii) Normal postnatal kidneys (n = 4) from autopsies of children who had died at a mean age of 3.5 years (range: 20 months to 8 years) from causes not associated with kidney disease, provided by the Department of Forensic Medicine of the University of Rostock, Germany. Use of these samples was approved by the ethics committee of the University of Rostock. (iii) Kidney and ureteric specimens from Patient 2, who was nephrectomized at time of transplantation, was provided by the Pathology Department of the University of Marburg, Germany.

**Sequence analysis of CAKUT patients**

Genomic DNA samples were obtained from peripheral blood of patients and blood donors (controls). Twenty-four primer pairs were designed to amplify 23 coding exons and all adjacent splice sites, the 5′-untranslated region (5′-UTR) and the 3′-untranslated region (3′-UTR) of the **CHD1L** gene by standard polymerase chain reaction (PCR). The entire coding region, including the corresponding splice sites at the exon–intron boundaries and the 5′- and 3′-UTR was screened for mutations in 61 CAKUT patients by direct sequencing using the Big-Dye Terminator v1.1 Sequencing Kit (Applied Biosystems Deutschland GmbH), and comparative TaqMan Gene Expression Assay for the **CHD1L** gene (Applied Biosystems Deutschland GmbH). Each sample was normalized to the TaqMan Endogenous Control Beta-2-Microglobulin (Applied Biosystems Deutschland GmbH), and comparative Cq quantification software (ΔΔCq) was applied. The expression of the **CHD1L** gene in the other tissues was calculated relative to the expression in the fetal kidney, which was arbitrarily defined as 1. Each sample was assayed in triplicate.

**Immunohistochemistry of renal sections**

Immunohistochemical analysis of tissue samples (5 μm sections mounted on SuperFrost® slides) was performed essentially as described previously [23, 24]. Briefly, sections were deparaffinized in histoclar and xylene and dehydrated through a series of graded alcohols prior to blocking endogenous peroxidase. For antigen retrieval, slides were boiled in 0.01 M citrate buffer (pH 6.0) using a microwave (1 × 10 min or 3 × 8 min, 450 W). Unspecific binding sites and endogenous biotin were saturated [10% fetal calf serum (FCS)] by incubation (4°C, overnight) with the specific antibody [CHD1L, rabbit polyclonal IgG purchased from Atlas Antibodies (Catalog Number HPA 028 670; diluted 1:300 for postnatal and 1:500 for embryonal sections), Sweden; megalin (proximal tubule), rabbit polyclonal antisera (1:100; Santa Cruz Biotechnology Inc., Heidelberg, Germany); Tamm–Horsfall protein (THP, thick ascendin limb of Henle’s loop and distal tubule), rabbit polyclonal antisera (1:500; Santa Cruz Biotechnology); aquaporin-2 (collecting duct), rabbit polyclonal antisera (1:100; Santa Cruz Biotechnology). Subsequently, slides were treated with a biotin-labelled secondary antibody (Vector Laboratories) prior to incubation with peroxidase-conjugated avidin–biotin complexes (Vectastain Elite ABC kit; Vector Laboratories) and diaminobenidze (Merck KG, Darmstadt, Germany; tablets of 10 mg). Counterstaining was carried out with haematoxylin. Slides derived from renal cell carcinoma were used as positive controls for CHD1L expression. For negative controls, the specific antibody was replaced by an unrelated antibody of the same subclass (isotype control). Slides were examined with a Zeiss Axiosphill 40 (Jena, Germany) or a Leica DMI 4000 microscope (Wetzlar, Germany) equipped with a digital camera (DFC 320 R2; Leica).

**CHD1L expression constructs**

The generation of the full-length human wild-type **CHD1L** expression construct has been described elsewhere, and this construct was kindly provided by Zuzanna Horejsi and Simon I. Boulton from the DNA Damage Response Laboratory, Clare Hall, London Research Institute, South Mimms, EN6 3LD, UK [16].

The three identified missense variants, Gly700Arg (exon 18), Ile827Met (exon 19) and Ile827Val (exon 21), were introduced into wild-type **CHD1L** by PCR mutagenesis with primers (Metabion, Martinsried, Germany) containing the corresponding mutation (5′-CGGAGGAGACTCATAGAGG-3′, CHD1L-Gly700Arg-forward: 5′-CGCAGATTCTGAAATAGGAAGAGACG-3′, CHD1L-Ile827Val-forward: 5′-GAGAGCTGCTGAA-3′, CHD1L-Ile827Met-forward: 5′-CCAAGAGAATGAAATAGGAAGAGACG-3′, CHD1L-Ile827Met-reverse: 5′-GCCAGATGGGAAGAGAGACG-3′, CHD1L-Ile827Val-reverse: 5′-GAGAGCTGCTGAA-3′). PCR products were gel-purified and propagated in E. coli (DH5α strain) and sequenced (ABI 3130xl Genetic Analyzer, Applied Biosystems). The wild-type and mutant constructs were introduced into the mammalian expression vector pCMV6-XL4 (Invitrogen) containing a CMV promoter and a poly-His tag at the 3′end of the coding sequence.

**Tissue-specific messenger RNA expression analysis**

To test for tissue-specific expression of the **CHD1L** gene, we used the Human Multiple Tissue cDNA (MTC) Panels I and II and the Human Digestive System MTC panel for fetal tissues (Clontech—Takara Bio Europe, Saint Germain-en-Laye, France). In the MTC panels, fetal cDNAs were pooled from 13 to 59 spontaneously aborted Caucasian fetuses aged 16–37 weeks. Relative quantifications in real-time experiments were performed on the ABI Prism 7900HT Fast Real-Time PCR System (Applied Biosystems Deutschland GmbH) using the inventoried TaqMan Gene Expression Assay for the **CHD1L** gene (Applied Biosystems Deutschland GmbH). Each sample was normalized to the TaqMan Endogenous Control Beta-2-Microglobulin (Applied Biosystems Deutschland GmbH), and comparative Cq quantification software (ΔΔCq) was applied. The expression of the **CHD1L** gene in the other tissues was calculated relative to the expression in the fetal kidney, which was arbitrarily defined as 1. Each sample was assayed in triplicate.
3’ and CHD1L_EcoRV_reverse: 5’-ctgatttagctgccagcGACGCT GTCTTGAGGAGG-3’ and in each case introduced as a 620-bp PvuMI/ EcoRV restriction fragment into the PvuMI/EcoRV digested pcDNA3.1 wild-type CHD1L myc-tagged construct. All constructs were verified by sequencing.

**Cell culture and transfection**

Human embryonic kidney (HEK) 293T cells were grown in Dubecco’s modified Eagle’s medium (Gibco/Invitrogen) with 10% FCS and seeded 1 day prior to transfection. Cells were transiently transfected with 750 ng (100 μL Optimem, 2.5 μL Fugene) and 4 μg (400 μL Optimem, 12 μL Fugene) of myc-tagged wild-type and mutant CHD1L constructs, respectively, using Fugene® HD Transfection Reagent according to the manufacturer’s instructions (Roche Diagnostics, Grenzach, Germany). Twenty-four hours after transfection, cells were lysed with 0.05 M HEPES supplemented with 1 mM dithiothreitol (DTT).

**Immunoprecipitation of myc-tagged CHD1L proteins**

For immunoprecipitation (IP) with anti-c-myc-conjugated agarose beads (Sigma Aldrich, St. Louis, MO), the ratio of protein lysate (in microgram) to anti-c-myc agarose (in micro litre) was 1:5. For each cell lysate, a suspension of anti-c-myc-conjugated agarose was settled in a microcentrifuge tube by a short spin (30 s at 8000 g), the supernatant was removed and the resin was washed three times with 150 μL PBS. Subsequently, the respective cell lysate was added to the resin, and the final volume was brought to at least 200 μL with 1× PBS. The suspension of anti-c-myc agarose and cell lysate was incubated for 2.5 h on an orbital shaker at 4°C. Afterwards, the resin was pelleted by centrifugation and was washed twice with 150 μL 1× PBS. Finally, the supernatant was aspirated, except –10 μL which were left above the agarose. For western blot analysis, 0.05 M HEPES buffer containing 1 mM DTT and 2× sodium dodecyl sulphate (SDS) sample buffer containing 400 mM DTT were added in a ratio of 1:1, and the samples were denatured for 5 min at 95°C.

**Western blot analysis**

Samples were used in equal amounts for SDS–polyacrylamide gel electrophoresis (SDS–PAGE; SE 600 Ruby; GE Healthcare, Freiburg, Germany). Proteins were blotted on a polyvinylidine difluoride membrane (Amersham Healthcare) using a semi-dry transfer unit (TE77 ECL; GE Healthcare). Western blot analysis of CHD1L proteins was performed with a chemiluminescent detection kit (Applichem, Darmstadt, Germany). Blots were developed using a chemiluminescent detection kit (Roche Diagnostics, Grenzach, Germany).

**Immunofluorescence**

HEK 293T cells, which were transiently transfected with empty pcDNA3.1/myc-His expression vector (mock) or wild-type or mutant CHD1L constructs, were seeded on coverslips before fixation with 4% wt/vol paraformaldehyde, permeabilization with 0.1% Triton X-100 and blocking with 5% fat-free milk powder/0.1% Triton X-100/1% goat serum. CHD1L was detected via a primary rabbit anti-CHD1L polyclonal antibody (Abcam, Cambridge, UK) and β-actin using a rabbit anti-β-actin monoclonal antibody (Sigma Aldrich), each followed by incubation with horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit antibodies (Santa Cruz Biotechnology Inc.). Blots were developed using a chemiluminescent detection kit (Amersham, Darmstadt, Germany). Western blot bands were quantified using the NIH ImageJ software.

**Results**

**CHD1L mutation analysis in CAKUT patients**

Sequencing of the entire coding region of the CHD1L gene in 61 CAKUT patients and of the CHD1L exons 18, 19 and 21 in 24 additional CAKUT patients revealed three different heterozygous missense variants. The variant found in Patient 1 (whose parents were not available for genetic testing) was a guanine-to-adenine transition at nucleotide position 2098 in exon 18 leading to a glycine-to-arginine substitution (c.2098G>A;p.Gly700Arg). The Gly700Arg variant was not found in 440 control chromosomes from central European individuals and was predicted to be probably damaging using the web-based PolyPhen software. The variant found in Albanian Patient 2 (inherited from his mother, who had no kidney or urinary tract anomalies on ultrasound) was an adenine-to-guanine transition at nucleotide position 2295 in exon 19 leading to an isoleucine-to-methionine substitution (c.2295A>G;p.Ile765Met). The Ile765Met variant was found in one of 430 control chromosomes from central European blood donors in whom a subtle CAKUT phenotype was not excluded, but not in 136 control chromosomes from Albanian individuals, and was predicted to be possibly damaging. The variant found in Patient 3 (inherited from his father, who was not available for examination by renal ultrasound) was an adenine-to-guanine transition at nucleotide position 2479 in exon 21 leading to an isoleucine-to-valine substitution (c.2479A>G;p.Ile827Val). The Ile827Val variant was not found in 402 control chromosomes from central European individuals and was predicted to be benign (Figure 1). All affected amino acids are conserved (p.Ile765) or highly conserved (p.Gly700 and p.Ile827) in higher animals.

**CHD1L messenger RNA expression in different human tissues**

To further investigate whether CHD1L is associated with the CAKUT phenotype, we quantified the messenger RNA (mRNA) levels of CHD1L in various fetal and adult tissues including the kidney (Figure 2). CHD1L was expressed in all analysed tissues. In the fetus, CHD1L expression was highest in brain followed by kidney and then by muscle, liver, thymus, lung, heart and spleen. Testicos showed the highest CHD1L mRNA expression level of all adult tissues (data not shown). Fourfold less expression was measured in adult brain, followed by liver, muscle, pancreas, small intestine, ovary, kidney, colon, prostate, placenta, heart, lung, spleen and leukocytes. The CHD1L mRNA levels in fetal kidney were approximately four times higher than in adult kidney. Thus, among all corresponding fetal and adult tissues investigated, the fetal to adult expression ratio was highest in the human kidney.

**Localization of the CHD1L protein in the normal human developing kidney**

At 7 weeks gestation, the developing metanephric kidney consists of the central epithelial ureteric bud, with numerous peripheral branches that will give rise to the adult collecting ducts and collecting system and two types of mesenchyme: loose mesenchyme that will form stroma of the mature organ and condensing or condensed mesenchyme adjacent to the bud tips which undergoes mesenchymal-to-epithelial transformation to form the remainder of the nephrons from glo merulus to distal tubules. During the latter process, this ‘induced’ mesenchyme goes through vesicle, comma-
S-shaped body stages before the first identifiable glomeruli and tubule segments can be discerned. The first site with identifiable CHD1L immunoreactivity was the ureteric bud, where protein was detected in rare cells in early cortical bud branches, although not in the more mature medullary segments. At later stages from 8 to 11 weeks, sporadic staining of individual cells was detected in loose mesenchyme, but strongly immunoreactive CHD1L was detected as mesenchyme condensed, with clear expression in early nephron precursors. Occasional positive cells were still detected in the ureteric bud, predominantly in the outer cortex where the bud was in intimate contact with the condensing mesenchyme. Subcellular localization was mainly nuclear, although a cytoplasmic signal could not be ruled out in some cells. Positive cells were also observed in some more mature structures, such as fully formed glomeruli or tubular structures (Figure 3). By immunofluorescence, CHD1L was exclusively localized to the nucleus of HEK cells (Figure 3). The subcellular localization of mutant CHD1L in HEK cells transfected with constructs expressing variant CHD1L was nuclear and thus not different from endogenous CHD1L or after wild-type CHD1L transfection (data not shown).

Localization of the CHD1L protein in the normal human postnatal kidney

In the normal kidney from a 5-year-old boy (autopsy material), CHD1L was immunolocalized to tubular cells in all segments of the tubule system. This was verified by staining of parallel sections with established markers of the tubular system, i.e. megalin for the proximal tubule, THP for the Henle loop and aquaporin-2 for the distal tubule and collecting duct. CHD1L staining was mainly detected in the cytoplasm and to a lesser extent in the nuclei of normal tubule cells (Figure 4).
CHD1L protein expression in the kidney and hydroureter from Patient 2

In the hydronephrotic kidney removed from Patient 2 at age 16 years prior to transplantation (nephrectomy material), CHD1L was also immunolocalized to tubular cells throughout the nephron. Although Patient 2 was older at nephrectomy than our healthy control, the intensity of CHD1L staining in the tubules appeared to be higher in the hydronephrotic kidney compared to the normal kidney. CHD1L staining was preferentially nuclear in the hydronephrotic kidney and in the corresponding hydroureter, where nuclear CHD1L expression was seen throughout the urothelium (Figure 4).

Interaction of mutant CHD1L protein with PARP1

To elucidate the functional effect of the three CHD1L variants with respect to their interaction with PARP1, we transfected HEK293T cells with c-myc-tagged wild-type and mutant CHD1L constructs. After cell lysis, IP of c-myc-tagged CHD1L protein was performed with anti-c-myc-conjugated agarose beads, followed by protein separation using SDS–PAGE. Western blot analysis with an anti-β-actin antibody demonstrated successful IP: β-actin signals were detectable in the input but not after IP (data not shown). PARP1 was immunoprecipitated together with CHD1L wild-type and mutant proteins and detected with an anti-PARP1-antibody, while immunoprecipitated c-myc-tagged wild-type and mutant CHD1L was detected with an anti-c-myc antibody. Visual inspection of western blot bands after IP showed that the signals for PARP1 in relation to the c-myc signals were clearly decreased in all mutants compared to wild-type CHD1L (Figure 5A).

Western blot bands were quantified using the NIH ImageJ software. The PARP1 and the respective c-myc Western blot signals after IP were measured, and the ratio of both signals was calculated to compare the interaction of PARP1 with wild-type and mutant CHD1L (Figure 5B). When averaging the results from three independent experiments, CHD1L and PARP1 interaction was diminished ~1.9-fold in variant Gly700Arg, ~2.3-fold in variant Ile765Met and ~2.2-fold in variant Ile827Val as compared to wild-type CHD1L.

Discussion

In this study, we describe heterozygous missense variants in the CHD1L gene in three male patients with a CAKUT phenotype characterized by uni- or bilateral hypoplasia of kidneys or hydronephrosis due to obstructive megareters. Both patients with bilateral CAKUT had severe kidney failure requiring renal replacement therapy from age 9 or 13 years, respectively. The patient with unilateral hypoplasia had a compensatory hypertrophy of the other kidney so that renal function was normal when he was last examined at 6 years of age. Two of the three variants in CHD1L were inherited from a parent, one of whom had a normal renal tract on ultrasound and the other being unavailable for sonographic examination. The parents of the third patient were not available for genetic testing, so inheritance or de novo occurrence could not be ascertained. Reduced penetrance and variable expressivity are not unusual in CAKUT; aberrations in the PAX2, EYA1, SIX1 and HNF1B/TCF2 genes cause highly variable and even missing renal phenotypes [9, 25].

There are various lines of evidence suggesting that the detected missense variants in CHD1L could be associated with the CAKUT phenotype in the patients of the present study. Firstly, two variants were not detected in at least 400 control chromosomes indicating a frequency of much <1% of these variants in the general population. The Albanian patient’s missense variant was not found in 136 control chromosomes from Albanians, but in 1 of 430 control chromosomes from central Europeans, in whom a subtle CAKUT phenotype cannot be excluded because renal ultrasound was not routinely performed. Secondly, using the web-based PolyPhen software, two of the three variants were predicted to be probably or possibly damaging, while only one of the variants was predicted to be benign. This software tool predicts the possible impact of an amino acid substitution on the structure and function of a human protein using physical and comparative considerations but does not invariably have to be correct.

Further evidence for a role of CHD1L in kidney development came from quantitative mRNA analysis. The CHD1L gene was strongly expressed in the fetal human kidney, and the renal fetal to adult expression ratio (4:1) was highest compared to all other tissues tested, suggesting that CHD1L expression is of particular importance in the developing kidney. By immunohistochemistry of human embryonal sections with a CHD1L-specific antibody, we demonstrated that CHD1L immunolocalized in the early ureteric bud and in early nephron precursors from 7 through 11 weeks of gestation. For normal kidney development, mutual induction from the tips of the ureteric bud to the adjacent metanephric mesenchyme is essential, the sites in which CHD1L immunoreactivity was strongest. The bud lineage develops into the collecting system while induced mesenchyme undergoes comma- and S-shaped body morphological stages en route to forming the nephrons from glomerulus to distal tubule [26, 27]. The fact that CHD1L is expressed in both the
ureteric bud and the metanephric mesenchyme may explain
the different CAKUT phenotypes observed in patients
with CHD1L variants, i.e. hydronephrosis secondary to ob-
structive megaureters, a malformation primarily of the ure-
ter, and renal hypodysplasia, an anomaly primarily of the
nephron.

While in human embryonal kidney cells, CHD1L was
mainly detectable in the nucleus, in the kidneys from a 5-
year-old child, CHD1L immunolocalized preferentially in
the cytoplasm and only rarely to the nuclei of tubular cells
in all parts of the mesenchyme-derived nephron. Interest-
ingly, in the tubular cells and urothelium of the hydro-
nephrotic kidney and hydrourerter removed at the age of 16
years from Patient 2, the CHD1L expression seemed to be
higher than in the normal kidney of the younger child
recapitulating fetal levels, compatible with a lack of ter-
minal nephron differentiation described in CAKUT. In the
developing human kidney and the malformed kidney and
urter, CHD1L expression was predominantly nuclear, in
line with recent reports that CHD1L is a chromatin-
remodelling enzyme that catalyses nucleosome sliding
and can act as a DNA damage response protein rapidly
recruited to DNA damage sites in the nucleus [16, 17].
Chromatin remodelling is required for normal develop-
ment in mammalian cells to orchestrate spatiotemporally
distinct gene expression programmes necessary for cellu-
lar differentiation [18, 19]. Thus, the CHD1L variants
identified here may induce deficits in differentiation in
tubular cells of the kidney and in the urothelium due to
impaired chromatin remodelling.

Fig. 3. Expression of CHD1L in developing human kidneys. Immunohistochemistry for CHD1L in 8–11 weeks human embryonal kidneys (A–D). All
sections were counterstained with haematoxylin; positive immunohistochemical signal is brown; in control samples, 10% FCS was substituted for the
primary antibody. Control panel from 8-week-old embryo demonstrating lack of background staining and illustrating early renal development with
ureteric bud (u) and stages of nephron formation from comma shaped bodies through developing glomeruli (A). Nearby section demonstrating positive
nuclear staining in the nephrogenic zone around the periphery of the kidney, where new nephrons are being formed (B). Control higher power view from
11 weeks showing ureteric bud, ureteric bud tip (t) and developing glomeruli (g), with loose mesenchyme (dotted arrow) and condensed mesenchyme
(solid arrow) (C). Nuclear CHD1L was predominantly detected in condensed mesenchyme, although occasional positive cells were detected at the very
tips of the ureteric bud (i.e. adjacent to the condensing mesenchyme) and in loose mesenchyme; fully formed glomeruli and deeper, more mature tubule
segments were negative (not shown) (D). Immunofluorescence for CHD1L in HEK cells (E–H). CHD1L was stained for using a primary rabbit anti-
CHD1L polyclonal antibody and a secondary goat anti-rabbit Alexa Fluor 568 labelled antibody (E). 4',6-diamidino-2-phenylindole was used as
counterstain to delineate the nucleus (F), filamentous actin was visualized in the cytoplasm and at the cell membrane using Alexa488-phalloidin (G).
The merged image clearly demonstrates the nuclear localization of CHD1L in embryonal cells (H).
Impaired chromatin remodelling could alter the expression of key factors in renal system development such as glial-derived neurotrophic factor (GDNF), which is secreted by the metanephric mesenchyme and mediates ureteric bud induction from the nephric duct, and the RET receptor tyrosine kinase expressed in the ureteric bud to induce branching [28, 29]. In particular, the transcriptional fine tuning of factors regulating GDNF levels and spatial expression [30, 31] and the factors regulating RET [32–34] could be compromised by impaired chromatin remodelling due to mutated \( CHD1L \). The dysregulated expression of the genes important for renal system development could cause aberrant interactions between the ureteric bud and the metanephric mesenchyme, which is known to cause renal hypodysplasia [27].

Strong evidence that mutations in a chromatin-remodelling enzyme can play a role in human renal tract anomalies comes from the \( CHD7 \) gene, an ATP-dependent chromatin remodeler with structural homologies to \( CHD1L \) [11]. Mutations in \( CHD7 \) cause CHARGE syndrome [20], which is associated with renal tract anomalies, such as horseshoe kidneys, renal agenesis, VUR and renal cysts, in ~20% of patients carrying \( CHD7 \) mutations [22]. Likewise, in an animal model, a heterozygous mutation in Chd2, another member of the Snf2-like group of ATPases that function in chromatin remodelling, results in a complex renal phenotype consisting of glomerulopathy, proteinuria and significantly impaired kidney function in ~85% of mice [35, 36].

The hypothesis that the \( CHD1L \) mutations identified in CAKUT patients may impair chromatin remodelling is further substantiated by the fact that all variants resulted in amino acid substitutions within or close to the macro domain of the \( CHD1L \) protein. The intact C-terminal macro domain binds PAR and interacts with chromatin-associated PARP1 in vitro [16, 17]. PARP1 localizes to a large fraction of active promoters with a distinct role in determining gene expression [37] and strongly activates \( CHD1L \) ATPase- and chromatin-remodelling activities [17]. By pull-down assay of transfected cell lysates, we

**Fig. 4.** Detection of megalin, THP and aquaporin-2 in normal human postnatal kidney (left panel) and of \( CHD1L \) in healthy and diseased specimens (right panel). Sections were probed with mAbs specific for megalin (A), THP (B), aquaporin-2 (C) and \( CHD1L \) (D–F) [normal kidney of 5-year-old (D); hydronephrotic kidney of 16-year-old Patient 2 with hypofunctional \( CHD1L \) variant (E); hydronephrotic kidney from same patient (F)]. Bound antibody was visualized using biotin-labelled secondary antibody in combination with peroxidase-conjugated avidin–biotin complex and 3,3'-diaminobenzidine as substrate. Magnification: ×100 (bar: 200 μm) (A–E), ×25 (bar: 1 mm) (F) and ×400 (bar: 50 μm) (inserts in D–F). In normal postnatal sections, \( CHD1L \) was expressed in the cytoplasm of tubule epithelial cells in all segments of the tubule system. \( CHD1L \) expression appeared higher in the dysplastic tubules of Patient 2 with a hypofunctional \( CHD1L \) variant than in normal kidneys, recapitulating high fetal levels, and was predominantly nuclear in tubular cells and urothelium of hydronephrotic kidney and hydroneurter.
found that all three CHD1L variants detected in our CAKUT patients showed decreased PARP1 interaction compared to wild-type CHD1L. These data suggest that the CHD1L variants identified may be hypofunctional and that the reduced interaction with PARP1 may compromise CHD1L ATPase- and chromatin-remodelling activities. This has recently been shown for CHD1L variants identified may be hypofunctional and in the cytoplasm of tubule cells in the normal postnatal kidney. (ii) Mutant CHD1L was hypofunctional with respect to interaction with PARP1. (v) The hydronephrotic kidney from a 16-year-old CAKUT patient with a hypofunctional CHD1L variant showed high nuclear CHD1L expression in dysplastic tubule cells mimicking the embryonal situation, compatible with a lack of terminal nephron differentiation described in CAKUT.

Conclusion

In summary, our study provides evidence that the ATP-dependent chromatin-remodelling enzyme CHD1L may play a role in renal development and in congenital anomalies of the kidneys and the urinary tract when altered. These conclusions are based on the following novel findings: (i) CHD1L expression was high in fetal kidneys and was four times higher in fetal compared to adult kidney. (ii) CHD1L immunolocalized in the early ureteric bud and early nephron precursors, critical stages of kidney development, in which CHD1L expression was predominantly nuclear and in the cytoplasm of tubule cells in the normal postnatal kidney. (iii) Heterozygous missense variants in CHD1L were detected in 3 of 85 CAKUT patients analysed, all leading to amino acid substitutions within or near the macro domain necessary for interaction with PAR and PARP1. (iv) Mutant CHD1L was hypofunctional with respect to interaction with PARP1. (v) The hydronephrotic kidney from a 16-year-old CAKUT patient with a hypofunctional CHD1L variant showed high nuclear CHD1L expression in dysplastic tubule cells mimicking the embryonal situation, compatible with a lack of terminal nephron differentiation described in CAKUT.

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Conflict of interest statement. None declared.

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