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/ALCI 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 hydronephrotic 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

### 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 hydroureter), 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 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 the 2q11.2 microduplication syndromes caused by microdeletion or microduplication of chromosome region 22q11.2, suggesting that in certain genes both a loss-of-function and a gain-of-function may lead to CAKUT [2–4].

A number of developmental genes, such as *EYA1* and *SIX1* causing autosomal dominant (AD) branchio-oto-renal syndrome [5, 6], *HNF1B/TCF2* associated with AD renal cysts and diabetes syndrome [7], and *PAX2* causing AD renal-coloboma syndrome [8], have been implicated in the pathogenesis of CAKUT [9].

To screen for novel chromosomal regions and genes associated with CAKUT pathogenesis, we recently performed genome-wide array-based comparative genomic hybridisation in 30 children with various CAKUT phenotypes and extrarenal anomalies [10]. In a patient presenting with renal hypoplasia, 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, Iswi, 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 megareter/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 hypoplastic 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 megareters. 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 unrelated cryptorchidism and subglottic stenosis, presumed secondary to repeated and prolonged mechanical ventilation. Renal failure 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). Ultrasound revealed bilateral hypoplasia of the kidneys. No VUR was detected by MCUG. Exorbrenal 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 of the CHD1L gene was aligned with the reference sequence and 5'- and 3'-UTR were screened for mutations in 61 CAKUT patients by direct sequencing using the Big-Dye Terminator v1.1 Sequencing Kit (Applied Biosystems Deutschland GmbH, Darmstadt, 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 Zuzana Horejsi and Simon J. 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), Ile765Met (exon 19) and Ile827Val (exon 21), were introduced into wild-type CHD1L by PCR mutagenesis with primers (Metabon, Martinsried, Germany) containing the following sequences (CHD1L-forward: 5'-GGAGGAGACTCATAGAGG-3', CHD1L-reverse: 5'-CAGGTTGTCTTCCCAACTTGC-3'). The myc-tagged full-length human wild-type CHD1L construct was generated by amplification of the provided untagged wild-type CHD1L plasmid with primers containing the appropriate restriction sites and lacking the stop codon (CHD1L_KmI_forward: 5'-GGTACCGACATGGAGCGG-3', CHD1L_KmI_reverse: 5'-CAGGTTGTCTTCCCAACTTGC-3').

The myc-tagged full-length human wild-type CHD1L construct was generated by amplification of the provided untagged wild-type CHD1L plasmid with primers containing the appropriate restriction sites and lacking the stop codon (CHD1L_KmI_forward: 5'-GGTACCGACATGGAGCGG-3', CHD1L_KmI_reverse: 5'-CAGGTTGTCTTCCCAACTTGC-3').
3' and CHD1L_3EcoRV_reverse: 5'-ctggatctctctgctcaGGCACCAGCTGTCTGAGAAG-3' and in each case introduced as a 620-bp PsuMIEcoRV restriction fragment into the PsuMIEcoRV 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 microlitre) 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, 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 60 Ruby; GE Healthcare, Freiburg, Germany). Proteins were blotted on a polyvinylidine difluoride membrane (Amersham, USA) using a semi-dry transfer unit (TE77 ECL; GE Healthcare). The myc-tagged wild-type and mutant CHD1L proteins were detected using a mouse anti-c-myc monoclonal antibody (Santa Cruz Biotechnology Inc.), PARP1 using a rabbit anti-PARP1 polyclonal antibody (Chemicon, USA) 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.

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 (1:750; Atlas Antibodies) and a secondary goat anti-rabbit Alexa Fluor 568 labelled antibody (1:350; Invitrogen), Alexa Fluor 488 phallolidin (1:40; Invitrogen), which specifically detects F-actin, was used to visualise actin filaments within the cytoplasm. Nuclei were visualised with 4',6-diamidino-2-phenylindole counterstain. Fluorescent images were captured using a Leica DMRXA2 epifluorescence microscope.

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. Testis 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 glomerulus 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).

**Fig. 1.** Identification of CHD1L variants in CAKUT patients. Pedigrees and electropherograms of the three patients with the CHD1L missense variants: CHD1L,c.2098G>A;p.Gly700Arg (exon 18), CHD1L,c.2295A>G;p.Ile765Met (exon 19) and CHD1L,c.2479A>G;p.Ile827Val (exon 21). The affected nucleotide positions in the electropherograms are marked by arrows (A). Genomic location of the three missense variants within the CHD1L gene: CHD1L,c.2098G>A;p.Gly700Arg (exon 18), CHD1L,c.2295A>G;p.Ile765Met (exon 19) and CHD1L,c.2479A>G;p.Ile827Val (exon 21) relative to the functional Snf2 family N-terminal domain (SNF2_N), the helicase superfamily C-terminal domain (Helic_C) and the macro domain (Macro) of the CHD1L protein. Note that all variants are localized close to or within the macro domain (B).
CHD1L was also immunolocalized to tubular cells throughout the nephron. Although Patient 2 was older at nephrectomy material, 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 hypodysplastic kidneys or hydronephrosis due to obstructive megaureters. 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 obstructive megaureters, a malformation primarily of the ureter, 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. Interestingly, in the tubular cells and urothelium of the hydropnephrotic kidney and hydroureter 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 terminal nephron differentiation described in CAKUT. In the developing human kidney and the malformed kidney and ureter, 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 development in mammalian cells to orchestrate spatiotemporally distinct gene expression programmes necessary for cellular 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.
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 remodeller 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
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 such reduced interaction 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. (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.

**Conflict of interest statement.** None declared.

**References**


**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.

**Acknowledgements.** We express our gratitude to the children and their families for kindly participating in this study. We thank Dr Elke Wühl, Heidelberg, Germany and Dr M. Schröder, Frankfurt, Germany for providing patient data, Prof. Dr Roland Möll, Marburg, Germany and Dr Ulrich Hammer, Rostock, Germany for providing tissue samples and gratefully acknowledge embryonal samples supplied by the MRC/Wellcome-funded Human Developmental Biology Resource at the UCL Institute of Child Health, London, UK. Support for this study was obtained from the Else Kröner-Fresenius-Stiftung (2010_A97). M.K.-J. and P.W. were supported by grant funding from Kidney Research UK and Kids Kidney Research.


Received for publication: 4.2.11; Accepted in revised form: 6.10.11