Genetic spectrum of Saudi Arabian patients with antenatal cystic kidney disease and ciliopathy phenotypes using a targeted renal gene panel

Mohamed H Al-Hamed,1 Wesam Kurdi, 2 Nada Alsahan, 2 Zainab Alabdullah, 3 Rania Abudraz, 2 Maha Tulfah, 2 Maha Alnemer, 2 Rubina Khan, 2 Haya Al-Jurayj, 1 Ahmed Alahmed, 1 Asma I Tahir, 1 Dania Khalil, 1 Noel Edwards, 4 Basma Al Abdulaziz, 5 Faisal S Binhuma, 1 Salma Majid, 1 Tariq Faquih, 5 Mohamed El-Kalioy, 5 Mohamed Abouelhoda, 1,5 Nada Altassan, 1,5 Dorota Monies, 1,5 Brian Meyer, 1,5 John A Sayer, 4 Mamdouh Albaqumi1,6

INTRODUCTION

Background Inherited cystic kidney disorders are a common cause of end-stage renal disease. Over 50 ciliopathy genes, which encode proteins that influence the structure and function of the primary cilia, are implicated in cystic kidney disease.

Methods To define the phenotype and genotype of cystic kidney disease in fetuses and neonates, we correlated antenatal ultrasound examination and postnatal renal ultrasound examination with targeted exon sequencing, using a renal gene panel. A cohort of 44 families in whom antenatal renal ultrasound scanning findings in affected cases included bilateral cystic kidney disease, echogenic kidneys or enlarged kidneys was investigated.

Results In this cohort, disease phenotypes were severe with 36 cases of stillbirth or perinatal death. Extra renal malformations, including encephalocele, polydactyly and heart malformations, consistent with ciliopathy phenotypes, were frequently detected. Renal gene panel testing identified causative mutations in 21 out of 34 families (62%), where patient and parental DNA was available. In the remaining 10 families, where only parental DNA was available, 7 inferred causative mutations were found. Together, mutations were found in 36 cases of stillbirth or perinatal death if the fetus carries two truncating mutations.12

Conclusions In families with ciliopathy phenotypes, mutational analysis using a targeted renal gene panel allows a rapid molecular diagnosis and provides important information for patients, parents and their physicians.

ABSTRACT

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Results In this cohort, disease phenotypes were severe with 36 cases of stillbirth or perinatal death. Extra renal malformations, including encephalocele, polydactyly and heart malformations, consistent with ciliopathy phenotypes, were frequently detected. Renal gene panel testing identified causative mutations in 21 out of 34 families (62%), where patient and parental DNA was available. In the remaining 10 families, where only parental DNA was available, 7 inferred causative mutations were found. Together, mutations were found in 12 different genes with a total of 13 novel pathogenic variants, including an inferred novel variant in NEK8. Mutations in CC2D2A were the most common cause of an antenatal cystic kidney disease and a suspected ciliopathy in our cohort.

Conclusions In families with ciliopathy phenotypes, mutational analysis using a targeted renal gene panel allows a rapid molecular diagnosis and provides important information for patients, parents and their physicians.

INTRODUCTION

The formation of cysts in kidney is a disease phenotype common to many inherited human diseases.1 Kidney cysts are fluid-filled epithelial lined structures arising from dilation in any part of the nephron or collecting duct. Cystic kidney disorders are a common cause of end-stage renal disease (ESRD). It is estimated that the prevalence of cystic kidney disease is 4.81% in the Arabian Gulf countries.2

Ciliopathy syndromes are inherited syndromes that are frequently associated with cystic kidneys and to date, mutations in over 50 genes have been identified.3 These include autosomal-dominant polycystic kidney disease (ADPKD), autosomal-recessive polycystic kidney disease (ARPKD), various forms of nephronophthisis (NPHP), Joubert syndrome (JBS), Meckel–Gruber syndrome (MKS), Bardet–Biedl syndrome (BBS) and many others.4 ADPKD is common and accounts for approximately 5–10% of the ESRD cases worldwide.5 Mutations in two genes, PKD1 (85% of patients with ADPKD) and PKD2 (15% of patients with ADPKD) underlie ADPKD.6 One to two per cent of patients with ADPKD may present as neonates with cystic kidneys.7 Biallelic mutations/variants in PKD1 and PKD2 have been described to give a severe neonatal onset of cystic kidney disease.8,9

ARPKD is a rarer condition affecting 1 in every 20 000 live births.10 It may be diagnosed in utero or prenatally by sonography showing bilateral large echogenic kidneys, and oligohydramnios in the most severe cases. Mutations in the polycystic kidney and hepatic disease 1 (PKHD1) gene are responsible for ARPKD, the severity of which depends on the type of mutations.11 The PKHD1 gene is located on chromosome 6p21 and encodes a fibrocytin protein that localises to the primary cilia of renal epithelial cells. There is a high risk of fetal presentation and neonatal death if the fetus carries two truncating mutations.12

Inherited ciliopathies may also cause multisystem pathology, which may be severe and result in early death for many patients. Aside from cystic kidney disease, other common clinical features of ciliopathies include hepatobiliary disease, laterality defects, polydactyly, agenesis of corpus callosum, retinal degeneration and occipital encephalocele.13 Ciliopathies with prominent renal phenotypes include NPHP, JBS and MKS.

NPHP is an autosomal-recessive disorder responsible for 6–10% of ESRD in children.14 NPHP is...
characterised by cysts that are typically restricted to the cortico-
medullary junction region of the kidney, and the kidney size is
normal or reduced. The disease is genetically heterogeneous.
Mutations in over 20 different recessive genes (including
NPHP1–NPHP19, AHI1 and XPNPEP3) have been identified in
about 50% of NPHP patients. Infantile NPHP is a disease that
progresses to ESRD usually before the age of 2 years and is
characterised by cortical microcysts associated with tubuloointer-
stitial lesions. Classically, it is linked to NPHP2/INVS gene
encoding inversin, but patients carrying NPHP3 mutations may also
develop the infantile phenotype frequently associated with
liver involvement.

JBTS is a neurodevelopmental disorder characterised by cere-
bellar vermis aplasia (CVA), a significant malformation of the
cerebellum that is linked to ataxia and may be seen on brain
MRI as ‘molar tooth sign’. JBTS follows an autosomal-
recessive inheritance pattern and there are currently over 26
known causative genes. For many of these ciliopathy syndromes, significant pheno-
typic variability has been observed even between members of
the same family, making clinical diagnosis, prediction of clinical
progression and genetic counselling a challenge.

Antenatal screening using ultrasound scanning (US) is a
means by which cystic kidney disease can be readily detected.
Serial ultrasound evaluation starting from 11 weeks of gestation
onwards can be used as a screening modality. Abnormal find-
ings that point towards a renal ciliopathy include increased size
of kidneys, a bright echotexture (hyperechogenicity) and a loss
of the normal cortico-medullary differentiation. Perinatal ultra-
sound appearance of kidneys can look similar in fetuses with
ARPKD, perinatal-onset ADPKD, MKS and some forms of
NPHP. In addition to renal anomalies, perinatal ultrasound can
detect other features of ciliopathies such as encephalopathy, polycy-
dactyly, situs inversus, agenesis of the corpus callosum, Dandy–
Walker malformation, fibrosis of the liver and structural heart
defects.

In this study, we have combined antenatal ultrasound exami-
nation of the fetus and targeted molecular genetic ‘panel testing’
for inherited renal disorders to characterise a cohort of Saudi
Arabian patients who presented antenatally with features of an
inherited renal ciliopathy.

MATERIALS AND METHODS

Study cohort

The cohort consists of 44 Saudi Arabian families where there
was evidence of antenatal US anomalies of the kidney, which
included cystic kidney disease, enlarged kidneys and echogenic
kidneys. Additional antenatal US findings including central
nervous system (CNS) anomalies (encephalocoele, CVA, ventricu-
loomegaly), cardiac defects (congenital heart malformation, peri-
cardial effusion) and skeletal defects (narrow thorax, polydactyly) were documented. Clinical phenotypes postnatally
were also reviewed, including postnatal renal US. For molecu-
lar genetic investigations, the cohort was divided into two
groups: Group A, where DNA was available from the affected
fetuses and their parents (n=34 families) and Group B, where
DNA was available from both parents but not the affected child
(n=10 families) (table 1). Following informed consent, DNA
was extracted from available chorionic villus sampling, amniotic
fluid, placental blood or peripheral blood cells using the Gentra
Systems PUREGENE DNA Isolation kit (Qiagen, Valencia,
California, USA). Ethical and study permissions were approved
by the Research Advisory Council of King Faisal Specialist
Hospital, Riyadh, Saudi Arabia (RAC#2050 043). We confirm
that all the diagnostic genetic work was performed in Saudi
Arabia with full ethical approval. The UK centre acted in an
advisory and strategic manner to direct the study.

Antenatal US examination

Prenatal anatomy US examination was performed at the
Obstetrics and Gynecology Department, King Faisal Specialist
Hospital and Research Centre, between weeks 18 and 22 of
pregnancy. For cases with a known family history of cystic
kidney disease/ciliopathy serial, antenatal US examinations started
between 12 and 22 weeks. For new referrals and
unknown family history, antenatal US started at the first visit.
Fetal anatomy was reported as either normal or abnormal with
explanations for features that includes cranium, cerebral ventri-
cles, posterior fossa, face, spine, chest, cardiac four-chamber
view, cardiac outflow tracts, heart axis, cardiac situs, stomach,
bowel, kidneys, bladder, abdominal cord insertion, number of
cord vessels, upper extremities and presence of hands, and lower
extremities and presence of feet. Published reference values for
renal length and volume for renal volumes based on three-
dimensional ultrasound were used. Fetal death was defined as
an intrauterine death greater than 10 weeks of gestation. A peri-
natal death is defined as a death within 7 days of birth, and an
infant death is defined as a death within 1 year of birth.

Maternal cell contamination and molecular karyotyping

In all fetal DNA samples, maternal cell contamination was
excluded by using the AmpFLSTR Identiﬁer PCR Ampliﬁcation
Kit as described by the manufacturer (Applied Biosystems, Life
Technologies, Paisley, UK). Where available fetal DNA was used
for molecular karyotyping (Affymetrix CytoScan HD Array Kit,
Santa Clara, California, USA) to exclude chromosomal aneu-
ploidy and to determine regions of homozygosity in the affected
patient.

Targeted renal genes panel and next generation sequencing

A customised 90 renal genes panel that includes ciliopathy genes (including 3 polycystic kidney disease genes, 10 NPHP
genes, 9 JBTS genes and 11 MKS genes) as well as and other
inherited renal disorders (see online supplementary table S1) was
prepared using Life Technologies proprietary AmpliSeq
multiplexing assay. This panel has previously undergone vali-
dation for its analytical sensitivity and speciﬁcity using 107 renal
patients and had 89% base reads on target with a read depth
(base coverage) of 840 after alignment and a of 98% coverage
of all genes. All samples were prepared within the Saudi
Human Genome Project Laboratories and loaded onto a Proton
I chip, and sequencing was performed on an Ion Proton system
(Ion Torrent—Life Technologies) as recommended by the manu-
facturer and as previously described.

NGS analysis pipeline

The analysis pipeline for processing the next generation sequen-
cing (NGS) reads went through several steps. Reads were exami-
ned for quality and parts of reads with low-quality value were
trimmed out. The reads were then aligned to the human refer-
ence genome GRCh37/Hg19 with the Torrent Mapping
| A or B | Family Consanguinity | Outcome | Renal phenotype | Oligohydramnios/antihydramnios | Encephalocele | Other CNS abnormalities | Skeletal/ growth malformations | Other defects | Number of other affected fetus/siblings | Segregation and unaffected sib | Gene | Mutation | Remarks and ExAC MAF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | FT-3 | Yes | Fetal death | Cystic | Yes | Oligohydramnios | Polydactyly | 0 | m,f (1x unaffected sib-het) | B9D1 | Homo c.508_510delCTC p.L170del | Novel |
| A | FT-1 | Yes | Fetal death | Enlarged, echogenic with cysts | Cystic | Polydactyly | Cystic hygroma | 2 | m,f | CC2D2A | Homo c.3084delG p.R1028Rfs*3 | Reported |
| A | FT-6 | Not known | Fetal death | Cystic | Yes | Oligohydramnios | CVA, dilated cisterna magna, corpus callosum agenesis | 0 | m,f (1x unaffected sib-het) | CC2D2A | Homo c.2364C>T p. P1122S | Reported |
| A | FT-8 | Yes | Fetal death | Enlarged, echogenic with cysts | Yes | Polydactyly | Corpus callosum agenesis and holoprosencephaly | 1 | m,f | CC2D2A | Homo c.4531T>C p. W1511R | Reported |
| A | FT-14 | Yes | Fetal death | Cystic | Yes | Spina bifida | Spina bifida | 2 | m,f | CC2D2A | Homo c.4531T>C p. W1511R | Reported |
| A | FT-15 | Yes | Fetal death | Cystic | Yes | Polydactyly | CVA, dilated cisterna magna, corpus callosum agenesis | 2 | m,f | CC2D2A | Homo c.3084delG p.R1028Rfs*3 | Reported |
| A | FT-21 | Yes | Fetal death | Cystic | Yes | Polydactyly | Clubfoot | 0 | m,f | CC2D2A | Homo c.3084delG p.R1028Rfs*3 | Reported |
| A | FT-26 | Yes | Fetal death | Cystic | Yes | Polydactyly | Ascites | 2 | m | CC2D2A | Homo c.4437 +1G>A | Novel |
| A | FT-7 | Not known | Alive at 6 m | Cystic | Yes | Polydactyly | CVA | 0 | m,f | CEP290 | Homo c.5668G>T p. G1890* | Reported |
| A | FT-9 | Yes | Perinatal death | Enlarged echogenic | Yes | Polydactyly | Ventricleomegaly | 1 | m,f (unaffected sib –het, unaffected sib –wt) | INVS | Homo c.1760delA p.Q587Rfs*2 | Novel |
| A | FT-27 | Yes | Fetal death | Cystic | Yes | Polydactyly | Clubfoot | 1 | m | MKS1 | Homo c.417 +1G>A | Novel |
| A | FT-5 | Yes | Fetal death | Cystic | Yes | Polydactyly | Congenital heart malformations, lung hypoplasia | 3 | m,f | MKS1 | Homo c.1066C>T p.Q356* | Novel |
| A | FT-13 | Yes | Fetal death | Cystic | Yes | Polydactyly | Congenital heart malformations, lung hypoplasia | 3 | m | MKS1 | Homo c.1066C>T p.Q356* | Novel |
| A | FT-31 | Yes | Infant death (8 mo) | Cystic | Yes | Polydactyly | Congenital heart malformations, lung hypoplasia | 0 | m,f | PKHD1 | Homo c.4870C>T p. R1624W | Reported |
| A | FT-33 | Yes | Alive at 12 mo | Cystic | Yes | Polydactyly | Hepatic cysts | 0 | m,f | PKHD1 | Homo c.4870C>T p. R1624W | Reported |

Continued
Table 1  Continued

| A or B | Family | Consanguinity | Outcome | Renal phenotype | Oligohydramnios/ anhydramnios | Encephalocele | Other CNS abnormalities | Skeletal/ growth malformations | Other defects | Number of other affected fetus/ siblings | Segregation and unaffected sib | Gene | Mutation | Remarks and ExAC MAF |
|--------|--------|---------------|---------|-----------------|-------------------------------|--------------|------------------------|-----------------------------|----------------|-----------------------------|------------------------|------|----------|-------------------|
| A      | FT-34  | Yes           | Alive at 14 mo | Cystic          | Yes                           | Yes          | Micrognathia           | 0 m,f                       | PKHD1 | Homo c.4870C>T p. R1624W | Reported (MAF=0.0001812) |
| A      | FT-19  | Yes           | Fetal death   | Cystic          | Yes                           | Yes          | Narrow thorax, dolichocephaly | 3 m,f (1× unaffected sib-het) | RPGRIP1 | Het c.640G>A p. V214I Het c.685G>A p. A2297 | Reported (V214I MAF=0.0005292) |
| A      | FT-20  | Not known     | Perinatal death | Enlarged echogenic | Yes                            | Yes          | Yes                    | 0 m,f                       | TCTN2 | Homo c.1852C>T p.Q618* | Novel |
| A      | FT-10  | Yes           | Fetal death   | Enlarged, echogenic with cysts | Yes          | Narrow thorax, dolichocephaly | 0 m,f                       | TMEM67 | Homo c.457T>G p.C153G | Novel |
| A      | FT-22  | Yes           | Fetal death   | Enlarged cystic | Yes                           | Yes          | CVA, hydrocephalus     | 2 m,f                       | TMEM67 | Homo c.1413-2A>G | Novel |
| A      | FT-18  | Yes           | Perinatal death | Cystic          | Yes                            | Yes          | Corpus callosum agenesis | Clubfoot | Hepatic cysts | 1 m,f | TMEM231 | Homo c.751G>A p.V251I | Reported |
| A      | FT-23  | Yes           | Fetal death   | Increased echogenicity | Yes                      | Yes          | CVA, dilated cisterna magna, Dandy–Walker malformation | Pericardial effusion | 1 | Unsolved |
| A      | FT-28  | Yes           | Fetal death   | Increased echogenicity | Yes                      | Yes          | Dandy-Walker malformation | Polyactyly | 2 | Unsolved |
| A      | FT-4   | Yes           | Fetal death   | Cystic          | Yes                            | Yes          | Narrow thorax, dilated cisterna magna | Pericardial effusion | 1 | Unsolved |
| A      | FT-12  | Yes           | Fetal death   | Increased echogenicity | Yes                      | Yes          | Dolichocephaly | 2 | Unsolved |
| A      | FT-11  | Yes           | Perinatal death | Cystic          | Yes                            | Yes          |                      | 0 | Unsolved |
| A      | FT-17  | No            | Alive at 36 mo | Cystic          | Yes                            | Yes          | CVA, dilated cisterna magna | Narrow thorax | 0 | Unsolved |
| A      | FT-24  | Yes           | Fetal death   | Cystic          | Yes                            | Yes          | CVA, dilated cisterna magna | Narrow thorax | 0 | Unsolved |
| A      | FT-25  | Yes           | Fetal death   | Cystic          | Yes                            | Yes          | Narrow thorax | 0 | Unsolved |
| A      | FT-29  | No            | Fetal death   | Cystic          | Yes                            | Yes          | Narrow thorax | 0 | Unsolved |
| A      | FT-30  | Yes           | Fetal death   | Cystic          | Yes                            | Yes          | Ventriculomegaly | 1 | Unsolved |
| A      | FT-16  | Not known     | Fetal death   | Enlarged kidneys | Yes                            | Yes          |                      | 0 | Unsolved |

Al-Hamed MH, et al. J Med Genet 2016;53:338–347. doi:10.1136/jmedgenet-2015-103469 341
| Family | Consanguinity | Phenotype | Oligohydramnios/Anhydramnios | Encephalocele | Other CNS Abnormalities | Skeletal/Growth Malformations | Other Defects | Number of Other Affected Fetus/Siblings | Segregation and Unaffected Sib | Gene | Mutation | Remarks and ExAC MAF |
|--------|---------------|-----------|-----------------------------|--------------|------------------------|-----------------------------|----------------|---------------------------------|-------------------------------|------|---------|---------------------|
| A      | FT-2          | Yes       | Cystic                      | Yes          | CVA, dilated cisterna magna |                            |                | 3                               | Unsolved                      |      |         |                     |
| A      | FT-32         | Yes       | Alive at 20 mo              | Cystic       | Yes                    |                            |                | 0                               | Unsolved                      |      |         |                     |
| B      | FT-35         | Yes       | Fetal death                | Cystic       | Yes                    | CVA, dilated cisterna magna |                | 3                               | m,f                           | CC2D2A | Presumed homo c.3084delG p.R1028Rfs*3 | Reported (MAF=0.00002548) |
| B      | FT-40         | Yes       | Fetal death                | Cystic       |                       |                            |                | 1                               | m,f                           | CEP290 | Presumed homo c.3772-3778delAG p.R1259Sfs*16 | Novel |
| B      | FT-43         | Yes       | Fetal death                | Cystic       | Yes                    |                            |                | 2                               | m,f                           | MKS1  | Presumed homo c.1066C>T p.Q356*     | Novel (MAF=0.00008237) |
| B      | FT-36         | Yes       | Fetal death                | Cystic       | Yes                    | CVA, dilated cisterna magna | Bilateral bowed femurs | 0                               | m,f                           | NEK8  | Presumed homo c.1401G>A p.W467*     | Novel (MAF=0.0003553) |
| B      | FT-41         | Yes       | Fetal death                | Cystic       |                       | Congenital heart malformation |                | 1                               | m,f                           | NPHP3 | Presumed homo c.2694_1_2delAG | Novel |
| B      | FT-45         | Yes       | Fetal death                | Cystic       | Yes                    | Hepatomegaly               |                | 1                               | m,f                           | PKHD1 | Presumed homo c.3539G>A p.G1180E | Novel |
| B      | FT-42         | Yes       | Fetal death                | Cystic       |                       |                            |                | 2                               | m,f                           | TCTN2 | Presumed homo c.252_253delTG | Novel |
| B      | FT-37         | Yes       | Fetal death                | Cystic       |                       |                            |                | 1                               |                              | Unsolved |                      |                     |
| B      | FT-38         | Yes       | Fetal death                | Cystic       |                       |                            |                | 0                               | Unsolved                      |      |         |                     |
| B      | FT-44         | Yes       | Fetal death                | Cystic       |                       |                            |                | 1                               | Unsolved                      |      |         |                     |

Novel mutations are in bold.
A: samples where DNA from affected and parent(s) was available. B: Samples where maternal and paternal DNA was available and mutation is presumed (with a 25% chance) to be causative.
CVA, cerebellar vermis aplasia; CNS, central nervous system; f, father; het, heterozygous; homo, homozygous; m, mother; MAF, minor allele frequency; mo, month; sib, sibling.
Alignment Program (TMAP) Aligner software. Once the reads were aligned, the variants were called using the Torrent Variant Caller (TVC) program. The TMAP and the TVC programs are distributed as part of the Torrent Suite (https://github.com/iontorrent/TS) package. The resulting variant files were stored in variant call format (VCF) files. The VCF file generated for each sample was processed through an annotation pipeline against databases such as OMIM, GenBank, dbSNP, 1000 genome project, Human Gene Mutation Database, and a local database (SGP737) of 550 patients containing Arab-specific variants. Variants with a minor allele frequency (MAF) >1% were discounted.

In addition to allele frequency, annotation provides pathogenicity scores, homozygosity/heterozygosity, read quality scores and other parameters used to identify candidate causative variants. All NGS and targeted sequencing and bioinformatics analysis were performed at the Saudi Human Genome Project Laboratories at KFSHRC and KACST.

For predicting the damaging effect of the reported mutation, four in silico prediction tools were used: PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2), Provean (http://provean.jcvi.org/index.php), MutationTaster (http://www.mutationtaster.org/) and Human Splicing Finder (http://www.umd.be/HSF/). Reported allele frequency of all putative pathogenic variants was determined using the ExAC database (http://exac.broadinstitute.org), and evolutionary conservation was determined from sequence alignments using MutationTaster and UCSC (https://genome.ucsc.edu).

### Sanger sequencing

Direct sequencing of PCR amplicons was carried out to confirm positive gene panel results. PCR was performed using Qiaquick (Manchester, UK) master mix kit. Oligonucleotide primers for PCR amplification of targeted genomic DNA were designed using Primer3 software (http://frodo.wi.mit.edu/) and synthesised by Metabion International AG (Munich, Germany). Primer sequences are available on request. Following treatment with the Agencourt AMPure PCR purification system (Agencourt Bioscience, Beverly, Massachusetts, USA), products were sequenced using BigDye Terminator Cycle Sequencing kit (PE Applied Biosystems, Massachusetts, USA) and run on an ABI 3730xl capillary sequencer. Sequences were analysed using Mutation Surveyor software V3.24 (SoftGenetics LLC, State College, Pennsylvania, USA).

### RESULTS AND DISCUSSION

A cohort of 44 families were analysed, where 38 (86%) were known to be consanguineous and 26 (59%) had more than one affected fetus. The antenatal renal USS findings included either bilateral cystic kidney disease, echogenic kidneys or enlarged kidneys in all cases (table 1). Antenatal USS also detected extra-renal malformations at a high rate (figure 1): 25 (57%) had oligohydramnios or anhydramnios, 14 (32%) had encephalocele and 9 (20%) had CVA. Other anomalies included limb defects including polydactyly and structural cardiac defects. The phenotype of this cohort was extremely severe with 38 (86%) cases dying as stillborn infants or perinatally. Only six cases survived the perinatal period (table 1).

Where patient DNA was available (group A, n=34), none of the cases had evidence of chromosomal aneuploidy (data not shown) and therefore malformation syndromes associated with renal cysts, such as trisomy 13 (Patau), trisomy 18 (Edward) and trisomy 21 (Down), were excluded.

Using the renal gene panel in this cohort, 96.98% coverage of target genes was achieved, with an average base coverage of 194x. Where patient DNA was available (group A, n=34), none of the cases had evidence of chromosomal aneuploidy (data not shown) and therefore malformation syndromes associated with renal cysts, such as trisomy 13 (Patau), trisomy 18 (Edward) and trisomy 21 (Down), were excluded.

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Sequencing. Mutations in genes can be identified (including screening unaffected siblings) using Sanger sequencing in both parents allowing a genetic diagnosis to be inferred (see online supplementary figure S1). All mutations identified were confirmed and segregation analysis was performed (including screening unaffected siblings) using Sanger sequencing. Mutations in genes B9D1, CC2D2A, CEP290, INVS, MKS1, NEK8, PKHD1, RPGRIP1L, TCTN2, TMEM67 and TMEM231 were identified (table 1) with a total of 13 novel variants detected in this study (table 2 and figure 2). Pathogenic rare (<1% MAF) sequence variants were not detected in the other renal panel genes, in particular digenic or oligogenic changes in renal ciliopathy genes were not seen. A total of 13 novel variants detected in this study (table 2 and figure 2).

**Pathogenic rare (<1% MAF)** sequence variants were not detected in the other renal panel genes, in particular digenic or oligogenic changes in renal ciliopathy genes were not seen. A common RPGRIP1L missense variant in its heterozygous state was identified as a third allele in two cases and is discussed below. Consistent with the lethal phenotypes seen in this cohort, mutations in B9D1, CC2D2A, CEP290, MKS1, RPGRIP1L, TCTN2, TMEM67 and TMEM231 are all known to cause a MKS phenotype. Mutations in INVS and NEK8 have been reported in severe neonatal forms of NPHP with numerous extrarenal features. Phenotypes in some of the patients with mutations with PKHD1 mutations were comparatively less severe, accounting for three of the cases which survived beyond the perinatal period.

In group A (34 families), homozygous mutations were detected in 20 families with just one family with compound heterozygous mutations (FT-19), in keeping with the known high rates of consanguinity. In group B (10 families), homozygous mutations were inferred by finding identical heterozygous variants in both parents in seven cases, consistent with the known parental consanguinity. These mutations were presumed to be found in their homozygous state in the affected patient. Unfortunately, direct sequencing of patient DNA or any unaffected siblings was not available in these cases. All mutations detected were either previously reported (and known to be pathogenic) or novel and predicted to be pathogenic by using in silico scores (table 2). Novel mutations were all homozygous (or inferred to be homozygous from parental samples) and included predicted missense, frameshift, nonsense and splicing defects. The types of mutations detected in this cohort seem to correlate closely with the phenotypes observed. Most mutations detected were truncating, frameshift and splice site mutations. These mutations were often lethal, causing fetal death or perinatal death. In this study, six missense mutations resulted in fetal or perinatal death.

Mutations in CC2D2A gene were the most common cause of antenatally detected cystic kidney disease in our cohort, accounting for eight cases. All patients with CC2D2A mutations had severe CNS abnormalities; six had evidence of an encephalocoele indicative of a MKS phenotype and two had evidence of severe CNS abnormalities. The chances of the affected child inheriting both these alleles was 25%. The identified rare alleles identified in these parental samples are listed in online supplementary table S2. In each of the families, there is only a single rare heterogeneous change that was identified and confirmed using Sanger sequencing in both parents allowing a genetic diagnosis to be inferred (see online supplementary figure S1). All mutations identified were confirmed and segregation analysis was performed (including screening unaffected siblings) using Sanger sequencing. Mutations in genes B9D1, CC2D2A, CEP290, INVS, MKS1, NEK8, PKHD1, RPGRIP1L, TCTN2, TMEM67 and TMEM231 were identified (table 1) with a total of 13 novel variants detected in this study (table 2 and figure 2).

### Table 2: in silico analysis of novel mutations

| Gene   | Mutation | Reference sequence | Mutation type | Provean | PolyPhen-2 | Mutation Taster | Human Splicing Finder | ExAC database | Evolutionary conservation |
|--------|----------|--------------------|---------------|---------|------------|-----------------|-----------------------|---------------|--------------------------|
| B9D1   | c.508_510delCT (p.L170del) | NM_015681 | Indel | Deleterious (−7.568) | N/A | Disease causing (0.989) | Absent | Caenorhabditis elegans |
| CC2D2A | c.4437+1G>A | NM_001080522  | Splice site | N/A | N/A | Donor site broken | Absent | Perkinsus marinus |
| CEP290 | c.3777_3778delAG (p.R1259del*16) | NM_025114 | Deletion, frameshift | N/A | N/A | Disease causing (1.000) | Absent | Danio rerio |
| INVS   | c.1760delA (p.Q587fs*2) | NM_014425 | Deletion, frameshift | N/A | N/A | Disease causing (1.000) | Absent | Xenopus tropicalis |
| MKS1   | c.417+1G>A | NM_017777 | Splice site | N/A | N/A | Donor site broken | Absent | D. rerio |
| NEK8   | c.1066C>T (p.Q356*) | NM_017777 | Nonsense | N/A | N/A | Disease causing (1.000) | Absent | D. rerio |
| NPHP3  | c.2694_1_2delAG | NM_153240 | Deletion, frameshift | N/A | N/A | Disease causing (1.000) | Absent | D. rerio |
| PKHD1  | c.3539G>A (p.G1110E) | NM_138894 | Missense | Deleterious (−6.296) | N/A | Disease causing (0.761) | Absent | Mus musculus |
| TCTN2  | c.252_253delCT (p.V85del*24) | NM_024809 | Deletion, frameshift | N/A | N/A | Disease causing (1.000) | Absent | X. tropicalis |
| TMEM67 | c.4577>G (p.C153G) | NM_153704 | Missense | Deleterious (−7.289) | N/A | Disease causing (0.999) | Absent | C. elegans |
| TMEM67 | c.1413-2A>G | NM_153704 | Splice site | N/A | N/A | Acceptor site broken | Absent | D. rerio |

Evolutionary conservation at the protein level for non-synonymous changes was analysed by comparing the wild-type amino acid in the human with other orthologues in lower species. The lowest species where exact conservation of amino acid was preserved is shown.

Het, heterozygous; MAF, minor allele frequency; N/A, not applicable.
CV A with a dilated cisterna magna, in keeping with a JBTS phenotype. Typical MKS phenotypes were seen in three patients with MKS1 mutations, which included two novel changes (table 2). The novel nonsense MKS1 mutation (p.Q356*) was present in two families (FT-5 and FT-13), suggesting that these families were related. In family FT-18, the TMEM231 mutation (p.V251I) led to perinatal death in two fetuses. The mutation is predicted to cause a splicing defect (table 2), due to its position as the last nucleotide in exon 4 of the TMEM231, although it appears to be a missense change.

PKHD1 gene mutations were found in four families. One case (FT-31) had associated lung hypoplasia and cardiac malformations and another (FT-33) had evidence of intrahepatic cysts (table 1). It has been reported that truncating mutations in PKHD1 gene may be lethal.12 In this study, mutations detected in PKHD1 gene were homozygous missense mutations rather than truncating mutations, one of which was novel (c.3539G>A; p.G1180E) and led to a perinatal death in the proband and a sibling (FT-45). Three families shared the c.4870C>T (p.R1624W) mutation, which has been reported previously (also in its homozygous state) in a Saudi Arabian patient, with a ‘later-onset’ ARPKD phenotype.30 Despite known consanguinity, compound heterozygous variants in RPGRIP1L have previously been associated with MKS.35 However, this gene remains a rare cause of renal ciliopathies. The fetus in this case presented with posterior encephalocele and bilaterally enlarged multicystic dysplastic kidneys and bilateral clubfeet (but not polydactyly). An additional disease allele in CEP290 identified in the fetus may have modified the phenotype.36 The B9D1 protein has structural similarities to MKS1 and similar severe phenotypes would be predicted. More recently, mutations in B9D1 have been described in two unrelated patients (aged 7 and 9 years) and with JBTS and a neurological limited phenotype, suggesting a wider phenotypic spectrum.37

Mutations in NEK8 are also a rare cause of a renal ciliopathy. Previously, homozygous mutations in NEK8 have been described in a Kurdish child with kidney microcysts and likely NPHP, reaching ESRD at 14 years of age (c.1273C>T, p.H425Y)38 and in three stillborn fetuses with enlarged cystic kidneys and cystic changes in the liver and pancreas (c.1795C>T, p.R599*).35 Some fetuses had additional features including heterotaxy, truncus arteriosus and other structural heart defects, hypoplastic lungs and skeletal anomalies (bowed femurs). Here, we identified a single stillborn fetus (FT-36) with a novel nonsense change in NEK8 (c.1401G>A, p.W467*) who had cystic kidneys, oligohydramnios, CVA and bilateral bowing of the femurs. This and the variant has been shown to compromise the interaction of RPGRIP1L with RPGR. To determine the frequency of this allele in our patient cohort, Sanger sequencing of RPGRIP1L exon 6 was performed. The rs16747071 variant was also present heterozygously in affected patients from FT-8 (with a homozygous CC2D2A missense mutation) and FT-10 (with a homozygous TMEM67 missense mutation). The additional pathogenicity of this allele in these patients is unknown.

 Compound heterozygous mutations in B9D1 have previously been associated with MKS.36 but this gene remains a rare cause of renal ciliopathies. The fetus in this case presented with posterior encephalocele and bilaterally enlarged multicystic dysplastic kidneys and bilateral clubfeet (but not polydactyly). An additional disease allele in CEP290 identified in the fetus may have modified the phenotype.36 The B9D1 protein has structural similarities to MKS1 and similar severe phenotypes would be predicted. More recently, mutations in B9D1 have been described in two unrelated patients (aged 7 and 9 years) and with JBTS and a neurological limited phenotype, suggesting a wider phenotypic spectrum.37

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nonsense mutation is predicted to disrupt the highly conserved regulator of chromatin condensation 1 (RCC1) domain and is in proximity to the murine jck mutation (p.G448V).39

Antenatal presentations of cystic kidney disease are often associated with severe phenotypes and poor outcomes. These can include early presentation of ADPKD, or more commonly in consanguineous families, a presentation of an autosomal-recessive renal ciliopathy disease, as we have seen in this cohort. Extrarenal manifestations on the antenatal USS such as encephalocele and CVA may suggest MKS or JBTS phenotypes, respectively. Other features such as polydactyly and thoracic cage abnormalities may point towards other ciliopathies such as BBS40 or skeletal dysplasias such as Jeune syndrome.41

Screening for ciliopathy genes in the diagnostic setting, especially in the perinatal period, is challenging. While whole exome sequencing (WES) is one possible approach, targeted gene panel exome sequencing may be preferable in diagnostic laboratories for specificity, deliverability and low cost. A disease-specific gene panel approach avoids the common difficulty of reporting secondary genetic findings that often occurs following WES. However, any predesigned NGS gene panel will be limited to known genes directed towards specific phenotypes and will not allow for recently discovered ciliopathy genes to be screened. Our gene panel contained 90 genes and included the 3 known polycystic kidney disease genes (PKD1, PKD2 and PKHD1) and 11 of the 12 known MKS genes (see online supplementary table S1). However, it included only 10 of the 21 genes known to cause NPHP and 9 of the 26 JBTS genes. Therefore, our panel was biased towards (and very effective at) diagnosing MKS in this cohort with very severe disease phenotypes, but the precise molecular genetic diagnosis remained unknown in others. Indeed, in the 14 cases whom had antenatal USS evidence of an encephalocele suggestive of a MKS phenotype, all except 2 had a molecular genetic diagnosis. A recent study confirmed the strong cystic kidney disease phenotype in MKS patients, where cystic kidneys were found in (97.7%) of MKS cases.22

To improve diagnostic yield, unsolved samples via our panel gene testing could be subjected to WES, especially in cases where there are DNA samples available from more than one affected in each family. However, this approach is more costly and can be more time consuming, when compared with a targeted panel approach. We hope to develop an updated renal gene panel in the near future, as the NGS sequencing platform we have developed will allow for additional genes (and their amplicons) to be analysed. In this study cohort of antenatal cases, the mutation detection rate was higher than reported by others36 42 who used PCR exon sequencing alone. More recently, a combination of WES and targeted resequencing of a ciliopathy gene panel was successfully used in a cohort of patients with Jeune asphyxiating thoracic dystrophy.43

In summary, using a cohort of patients with antenatal evidence of kidney disease and associated ciliopathy syndromes, we have performed targeted genetic panel testing using patient and/ or parental DNA samples to reveal the molecular genetic diagnosis in 64% of patients. Our high detection rate of homozygous disease-causing alleles reflects a high underlying rate of consanguinity. We would predict a reduction in diagnostic yield in less consanguineous populations. The genetic spectrum remains wide and certainly we have not identified a reason to narrow our diagnostic panel, rather it should be expanded to capture more recently reported genetic causes of developmental renal disease. It is interesting to note that CC2D2A mutations were the commonest cause of an antenatal ciliopathy in our cohort, but the genetic heterogeneity of inherited cystic kidney disease is also borne out by our study. In our population, renal gene panel testing provided diagnostic information that was valuable to clinicians, genetic counsellors and families. A molecular genetic diagnosis provides an accurate diagnosis, which is hugely valuable when there are such severe phenotypes affecting one or more family members and can be used to predict recurrence rates and allow planning, including preimplantation genetic diagnosis for future pregnancies.

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