



# Whole exome sequencing identifies causative mutations in the majority of consanguineous or familial cases with childhood-onset increased renal echogenicity

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Chronically increased echogenicity on renal ultrasound is a sensitive early finding of chronic kidney disease that can be detected before manifestation of other symptoms. Increased echogenicity, however, is not specific for a certain etiology of chronic kidney disease. Here, we performed whole exome sequencing in 79 consanguineous or familial cases of suspected nephronophthisis in order to determine the underlying molecular disease cause. In 50 cases, there was a causative mutation in a known monogenic disease gene. In 32 of these cases whole exome sequencing confirmed the diagnosis of a nephronophthisis-related ciliopathy. In 8 cases it revealed the diagnosis of a renal tubulopathy. The remaining 10 cases were identified as Alport syndrome (4), autosomal-recessive polycystic kidney disease (2), congenital anomalies of the kidney and urinary tract (3), and APECED syndrome (1). In 5 families, in whom mutations in known monogenic genes were excluded, we applied homozygosity mapping for variant filtering and identified 5 novel candidate genes

(*RBM48*, *FAM186B*, *PIAS1*, *INCENP*, and *RCOR1*) for renal ciliopathies. Thus, whole exome sequencing allows the detection of the causative mutation in 2/3 of affected individuals, thereby presenting the etiologic diagnosis, and allows identification of novel candidate genes.

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Renal ultrasound imaging (RUS) represents a simple and broadly accessible tool for the noninvasive early diagnosis of chronic kidney disease (CKD) in children and young adults. Often abnormal findings on RUS are detectable years before the kidney function deteriorates and before other symptoms develop. A typical abnormal finding in early stages of CKD is chronically increased echogenicity on RUS. It is frequently accompanied by loss of cortico-medullary differentiation and renal cysts. Increased echogenicity is easily detected as a degree of echogenicity that is equal to or more pronounced than the echogenicity of the liver. Unfortunately, chronically increased echogenicity is not specific to certain types of kidney disease.<sup>1–3</sup> Particularly in early stages, in which other symptoms are not yet present, a correct diagnosis can be challenging. In these cases, whole-exome

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sequencing (WES) provides a novel means of establishing an etiologic diagnosis. By revealing the causative monogenic mutation, it provides affected individuals and their families with an unequivocal, early diagnosis.<sup>1</sup> As a result, a targeted therapeutic regimen can be initiated early if available.

Chronically increased echogenicity on RUS is often found in the early stages of nephronophthisis-related ciliopathies (NPHP-RCs). NPHP-RCs represent a group of cystic and fibrotic kidney diseases with an autosomal recessive mode of inheritance that typically progress to end-stage renal failure within the first three decades of life.<sup>4,5</sup> Nephronophthisis can present as isolated renal disease (MIM #613550) or together with extrarenal symptoms such as retinal degeneration (Senior Loken syndrome MIM #266900), cerebellar vermis hypoplasia (Joubert syndrome MIM #213300), and hepatic fibrosis. The renal manifestation ranges from severe, early onset cystic kidney disease<sup>6</sup> to slowly progressive, fibrotic remodeling of the kidney with CKD starting in adolescence.<sup>7</sup> Interestingly, the genotype–phenotype correlation in NPHP-RC is dependent on the gene and the specific mutation involved, which can both give rise to a broad phenotypic disease spectrum. NPHP-RCs are a very heterogeneous disease group, as by now mutations in more than 90 genes have been identified as causative for renal ciliopathies in humans.<sup>4</sup> Mutations in some of these genes are very rare, accounting for only two<sup>8</sup> or three<sup>9</sup> families worldwide. WES with direct inspection of the coding regions of these genes therefore represents the most rational and currently, most cost-effective approach for mutation analysis in these patients.<sup>1,10–12</sup> So far, no more than 13 ciliopathy genes have been systematically studied in a larger patient cohort.<sup>13,14</sup>

Here, we performed WES combined with homozygosity mapping in an international cohort of 79 families with pediatric onset of CKD and suspected nephronophthisis based on renal ultrasound presentation with chronically increased echogenicity, loss of cortico-medullary differentiation, and/or  $\geq 2$  cysts. All individuals were born of consanguineous union, or represented familial cases of CKD, and were therefore at high risk for recessive, monogenic diseases. In summary, we were able to identify a causative mutation in a known monogenic disease gene in 50 families (63.3%). In 32 of these families (64%), WES identified mutations in NPHP-RC genes as the molecular disease cause and confirmed the suspected clinical diagnosis. However, in 18 families (36%), we discovered a molecular diagnosis of a monogenic kidney disease that was not NPHP-RC, specifically renal tubulopathies ( $n = 8$ , 16%), Alport syndrome ( $n = 4$ , 8%), congenital anomalies of the kidney and urinary tract (CAKUT) ( $n = 3$ , 6%), autosomal recessive polycystic kidney disease (ARPKD) ( $n = 2$ , 4%), and autoimmune nephropathy (APECED [autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy] syndrome) ( $n = 1$ , 2%). In five consanguineous families, in whom we excluded mutations in known monogenic disease genes, we identified five novel candidate genes for NPHP-RC (*RBM48*, *FAM168B*, *PLAS1*, *INCENP*, and *RCOR1*).

## RESULTS

### WES identifies the molecular disease cause in 63% of cases

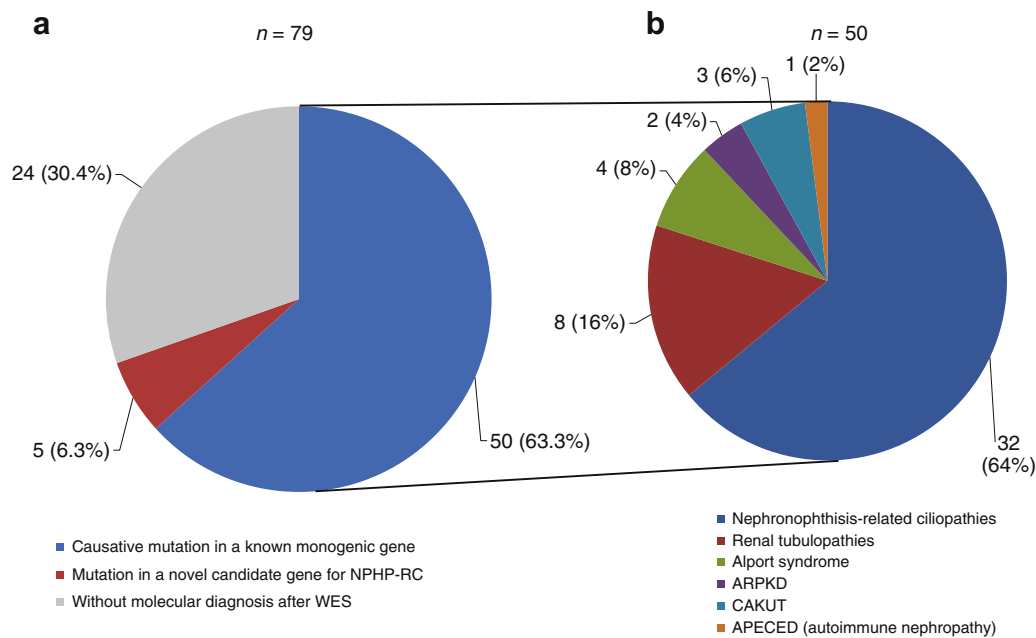
We performed WES in 79 families with suspected NPHP-RC based on renal ultrasound criteria (chronically increased echogenicity, loss of cortico-medullary differentiation, and/or  $\geq 2$  renal cysts). In 50/79 families (63.3%), we identified a mutation in a gene that is known to cause monogenic renal disease when mutated (Figure 1a, Supplementary Tables S1 and S2 online).

### NPHP-RCs account for 64% of molecularly diagnosed individuals

Thirty-two of the 50 families with established molecular diagnosis after WES (64.0%) harbored a mutation in a known NPHP-RC gene (Figure 1b). Of the 90 genes that are known to cause renal ciliopathies when mutated, which were systematically analyzed in this study, mutations in 18 genes contributed to this result. Mutations in the genes *NPHP3*, *NPHP4*, and *NPHP5* accounted for the majority of NPHP-RC cases (Table 1, Supplementary Tables S1 and S2 online). In addition to mutations in NPHP-RC genes, we detected causative mutations in monogenic genes of renal tubulopathies (8/50), Alport syndrome (4/50), CAKUT (3/50), and ARPKD (2/50). Furthermore, we established the molecular diagnosis of APECED in one previously undiagnosed individual (Figure 1b, Table 1, Supplementary Tables S1 and S2 online). In 10 individuals, the molecular diagnosis after WES was different from the previous clinical diagnosis. In particular, in one case of previously undiagnosed nephropathic cystinosis the molecular finding changed the therapeutic regimen (Supplementary Tables S1 and S2 online).

### Mutations in more than one recessive disease gene can be present in consanguineous families

In one example of a highly inbred family (412 Mb of cumulative homozygosity), we identified homozygous mutations in seven known monogenic disease genes. Four of them are known to cause diseases with renal involvement (Supplementary Table S4 online). The affected child showed a complex phenotype suggestive of a renal ciliopathy, including Caroli's disease with massive cystic dilation of intrahepatic bile ducts, CKD that progressed to end-stage renal failure within the third year of life, post-axial polydactyly, nystagmus, and rod cone dystrophy. In addition to chronically increased echogenicity as the classical symptom of NPHP-RC, the child showed congenital hydronephrosis due to uretero-pelvic junction obstruction that required corrective surgery, as well as renal tubular acidosis type 4 (Supplementary Table S4 online). An older brother, from whom no DNA sample was available for mutation analysis, deceased at 15 months of age due to end-stage renal and liver disease. In addition, he showed post-axial polydactyly and blindness at 5 months of age due to progressive retinal dystrophy. A younger brother presented with Caroli's disease and retinitis pigmentosa. Interestingly, no polydactyly was present, and the renal function was preserved at 3 years of age. The right kidney showed mild pelvicalyceal dilation.



**Figure 1 | Relative number of cases molecularly diagnosed by whole-exome sequencing (WES) in 79 families with childhood-onset chronically increased echogenicity or  $\geq 2$  cysts on renal ultrasound. (a)** We performed WES in 79 consanguineous or sibling cases with increased echogenicity and/or  $\geq 2$  cysts on renal ultrasound. In 50 families (63.3%), we identified a mutation in a known monogenic disease gene as causative. In 5 families (6.3%), we identified a mutation in a novel candidate gene for NPHP-RC, and 24 families (30.4%) remained without a molecular diagnosis after WES. **(b)** Fractional contribution of different disease entities to the molecular diagnosis of 50 families in whom a causative mutation in a known monogenic disease gene was detected (see Table 1 for the underlying monogenic causes in each disease group). APECED, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy; ARPKD, autosomal recessive polycystic kidney disease; CAKUT, congenital anomalies of the kidney and urinary tract; NPHP-RC, nephronophthisis-related ciliopathies.

### The percentage of molecularly solved cases in consanguineous families and familial cases of suspected NPHP-RC is comparable (~63%)

When performing WES in 79 families with suspected nephronophthisis based on RUS with childhood-onset chronically increased renal echogenicity and/or  $\geq 2$  renal cysts, we detected a causative mutation in a known monogenic disease gene in 38 of 60 consanguineous families (63.3%) (Figure 2). As postulated<sup>15,16</sup> in the vast majority of consanguineous families, the causative mutation was present in the homozygous state (37 of 38 families [97.4%]) (Figure 2). The only exception was a family in whom we identified a single heterozygous mutation in the dominant gene *HNF1B* as causative. In 12 of 19 non-consanguineous families with 2 or more affected children (63.2%), we identified a causative monogenic mutation, showing a comparable percentage of molecularly solved cases in both cohorts. However, as expected in non-consanguineous families,<sup>16</sup> only in 3 of 12 families (25.0%) the disease-causing mutation was present in the homozygous state. The remaining 9 of 12 families (75.0%) harbored two compound heterozygous disease-causing mutations (Figure 2).

### Identification of five novel candidate genes for NPHP-RC

By applying homozygous peak regions as a filter for WES data, we identified five novel candidate genes for monogenic, recessive NPHP-RC (Table 2). In these five consanguineous families, we had excluded mutations in known monogenic

disease genes with renal phenotypes through evaluation and direct inspection of the WES data. The five novel candidate genes (*RBM48*, *FAM168B*, *PIAS1*, *INCENP*, and *RCOR*) represented the most deleterious alleles within the homozygous regions (Supplementary Figure S2 online) after variant filtering as outlined in Supplementary Figure S1 online. In the example of family A2621 with 181 Mb of cumulative homozygosity (Supplementary Table S5 online), we started with 482,406 variants from normal reference sequence. Excluding common variants (minor allele frequency  $>1\%$  in healthy control cohorts), synonymous variants, and heterozygous variants reduced the number to 1699. Considering only variants that were positioned within homozygous peak regions reduced the number of remaining variants by 11-fold to 156. Exclusion of artifacts by direct inspection of the sequence alignment left us with three potentially disease-causing variants in this family (Supplementary Table S5 online). Subsequently, these variants were ranked based on their predicted likelihood to be deleterious for the function of the encoded protein following the criteria as outlined in Supplementary Figure S1 online. At the end of the filtering process, the gene *INCENP* (inner centromere protein) represented the strongest remaining variant in this family. All mutations in novel candidate genes were confirmed by Sanger sequencing in original patient DNA and segregated with the affected status.

Twenty-four families (30.4%) remained without a molecular diagnosis after WES. In these families, no convincing

**Table 1 | Diagnostic groups and distribution among specific genes with causative recessive mutations in 50 families with increased echogenicity and/or  $\geq 2$  cysts on renal ultrasound in whom the disease-causing mutation was detected by WES**

Disease group	Patients within the disease group	Gene with causative mutation (disease, OMIM <sup>a</sup> )
NPHP-RC	<b>32/50 (64%)</b>	
	2/50 (4%)	<i>NPHP1</i> (nephronophthisis type, juvenile, #256100)
	3/50 (6%)	<i>NPHP3</i> (nephronophthisis type 3, #604387)
	5/50 (10%)	<i>NPHP4</i> (nephronophthisis type 4, #606966)
	3/50 (6%)	<i>IQCB1</i> (Senior-Loken syndrome type 5, #609254)
	1/50 (2%)	<i>CEP290</i> (Senior-Loken syndrome 6, #610189)
	2/50 (4%)	<i>SDCCAG8</i> (Senior-Loken syndrome type 7, #613615)
	1/50 (2%)	<i>TMEM67</i> (nephronophthisis 11, #613550)
	2/50 (4%)	<i>TTC21B</i> (nephronophthisis 12, #613820)
	2/50 (4%)	<i>WDR19</i> (nephronophthisis 13, #614377)
	3/50 (6%)	<i>ANKS6</i> (nephronophthisis 16, #615382)
	1/50 (2%)	<i>TMEM138</i> (Joubert syndrome 16, #614465)
	1/50 (2%)	<i>TMEM231</i> (Joubert syndrome 20, #614970)
	1/50 (2%)	<i>ARL6</i> (Bardet-Biedl syndrome 3, #600151)
	1/50 (2%)	<i>BBS4</i> (Bardet-Biedl syndrome 4, #615982)
	1/50 (2%)	<i>MKKS</i> (Bardet-Biedl syndrome 6, #605231)
	1/50 (2%)	<i>BBS12</i> (Bardet-Biedl syndrome 12, #615989)
1/50 (2%)	<i>DYNC2H1</i> (short-rib thoracic dysplasia 3, #613091)	
1/50 (2%)	<i>IFT43</i> (cranioectodermal dysplasia 3, #614099)	
Renal tubulopathies	<b>8/50 (16%)</b>	
	3/50 (6%)	<i>CLDN16</i> (hypomagnesemia 3, renal, #248250)
	2/50 (4%)	<i>CLCNKB</i> (Bartter syndrome, type 3, #607364)
	1/50 (2%)	<i>ATP6V0A4</i> (renal tubular acidosis, distal, #602722)
	1/50 (2%)	<i>BCS1L</i> (mitochondrial complex III deficiency, #124000)
Alport syndrome	<b>4/50 (8%)</b>	
	1/50 (2%)	<i>CTNS</i> (cystinosis, nephropathic, #219800)
CAKUT	<b>4/50 (8%)</b>	
	2/50 (4%)	<i>COL4A3</i> (Alport syndrome, #203780)
	1/50 (2%)	<i>COL4A4</i> (Alport syndrome, #203780)
Autosomal recessive polycystic kidney disease	<b>3/50 (6%)</b>	
	1/50 (2%)	<i>COL4A5</i> (Alport syndrome, #301050)
APECED	<b>2/50 (4%)</b>	<i>HNF1B</i> (renal cysts and diabetes syndrome, #137920)
	<b>1/50 (2%)</b>	<i>FREM2</i> (Fraser syndrome, #219000)
	<b>1/50 (2%)</b>	<i>PKHD1</i> (polycystic kidney and hepatic disease, #263200)
	<b>1/50 (2%)</b>	<i>AIRE</i> (autoimmune polyendocrinopathy syndrome type I, #240300)

APECED, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy; CAKUT, congenital anomalies of the kidney and urinary tract; NPHP-RC, nephronophthisis-related ciliopathies.

<sup>a</sup>Online Mendelian Inheritance in Man (OMIM), <http://www.omim.org>.

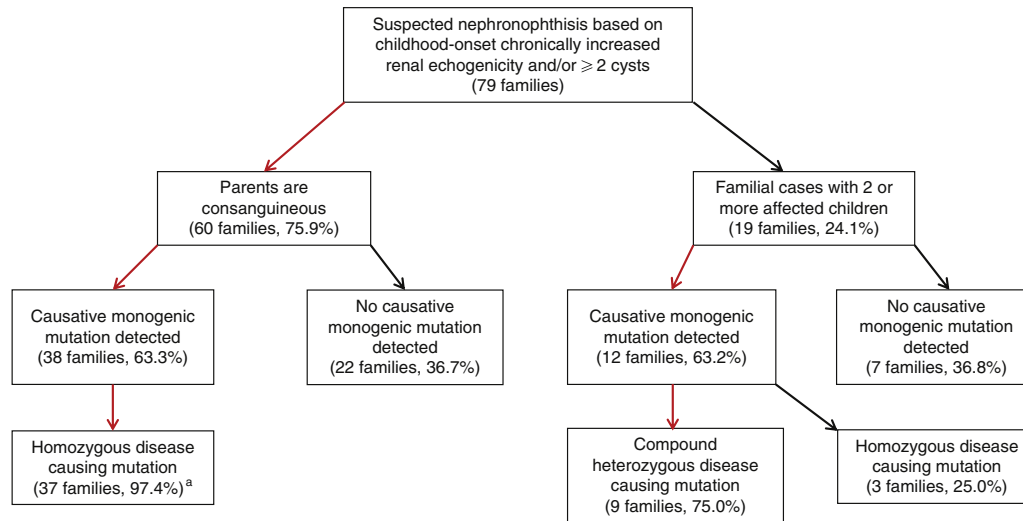
variants in known monogenic genes or candidate genes were identified that segregated with the affected status.

## DISCUSSION

We performed WES combined with homozygosity mapping in 79 consanguineous or familial cases of childhood-onset chronically increased renal echogenicity or the presence of  $\geq 2$  cysts on renal ultrasound. On the basis of renal ultrasound presentation, clinical presentation with childhood-onset of disease, and if available renal histology, an NPHP-RC was suspected as the primary disease cause. We show that, in this patient cohort, WES allows detecting the specific causative mutation in about two-thirds of affected individuals, thereby presenting the etiologic diagnosis. In addition, we identified five potential novel renal ciliopathy genes in consanguineous families in whom mutations in 90 known monogenic NPHP-RC genes had been excluded.

Chronically increased echogenicity on renal ultrasound represents a sensitive diagnostic criterion for early stages of CKD in children and young adults. Typically, this results in

the suspected diagnosis of nephronophthisis or related ciliopathies. However, we and others<sup>1–3</sup> have shown that other kidney diseases can phenocopy the presentation of NPHP-RC on renal ultrasound. Here, we study 79 consanguineous or familial cases with suspected NPHP-RC based on renal ultrasound presentation with childhood-onset increased renal echogenicity and/or more than one cyst, and perform WES in order to determine the percentage in which NPHP-RC and various other monogenic, recessive kidney diseases contribute to this phenotypic spectrum (Figure 2). As a second aspect, we aimed to systematically examine all 90 genes that are known monogenic, recessive causes of NPHP-RC or other CKD that can phenocopy the presentation of NPHP-RC in a large cohort of affected individuals. So far, this has not been done for more than 13 renal ciliopathy genes<sup>13,14</sup> at a time. As we identified the disease-causing mutation in  $\sim 2/3$  (50/79) of individuals with increased echogenicity on RUS, our data show that WES is an efficient tool for molecular diagnostics in this patient cohort. WES provides affected individuals and their families with an unequivocal diagnosis that avoids unnecessary diagnostic and therapeutic



**Figure 2 | Algorithm for molecular diagnostics in consanguineous or familial cases of suspected nephronophthisis based on renal ultrasound presentation.** Of the 79 families with childhood-onset increased renal echogenicity and/or  $\geq 2$  cysts on renal ultrasound imaging, 60 individuals were born of consanguineous unions and 19 families were nonconsanguineous with 2 or more affected children. In 63.3% of consanguineous families, we identified a mutation in a known monogenic disease gene as causative. The majority of these mutations were, as postulated, present in the homozygous state. In 63.2% of familial cases, we identified a causative mutation in a recessive, monogenic disease gene. Three-fourths of these mutations were compound heterozygous. <sup>a</sup>In one consanguineous family, a single heterozygous mutation in the dominant gene *HNF1B* was identified as the molecular disease cause.

interventions. WES is a noninvasive diagnostic tool with little risk, and, recently, the cost has dropped to a level that makes it accessible to a broader group of affected individuals in a clinical rather than a research setting. Considering the genetic heterogeneity of NPHP-RC, WES is the only available technique that can reliably establish the correct molecular diagnosis. The use of targeted sequencing panels as utilized for other disease entities in clinical genetics is technically not feasible and/or not economical for such a large number of potentially causative genes. However, WES yields a large number of variants from normal reference sequence, most of which are functionally irrelevant. Therefore, an efficient strategy for variant filtering is indispensable for the work with WES data. In addition, variant calling should follow an indication-driven strategy: After thorough clinical evaluation, a subset of potentially causative genes should be defined prior to WES, and the evaluation should primarily focus on

variants in these genes. For example, in a patient with increased echogenicity and/or  $\geq 2$  cysts on renal ultrasound, primarily variants in the  $\sim 90$  monogenic causes of renal ciliopathies, and genes that can phenocopy NPHP-RC, should be taken into consideration.

In our cohort, WES molecularly confirmed the clinically suspected diagnosis of NPHP-RC in 64% of cases. However, 18/50 individuals (36%) harbored a mutation in a gene that causes a monogenic kidney disease different from NPHP-RC. As already described in a smaller cohort,<sup>1</sup> the majority of these cases (8/18) were diagnosed as renal tubulopathies. In addition, we identified four individuals with Alport syndrome, three individuals with mutations in *CAKUT* genes, two individuals with *ARPKD*, and one individual with autoimmune nephropathy in the context of *APECED* syndrome. In 10 of these 18 cases, the molecular diagnosis differed from the initial clinical diagnosis. We therefore suggest that WES

**Table 2 | Novel candidate genes for NPHP-RC identified in 5 families with increased echogenicity and/or  $\geq 2$  cysts on renal ultrasound in whom causative mutations in known monogenic disease genes were excluded**

Family	Gene	Zygoty	Accession #	c.Position	p.Position	Continuously conserved to	Polyphen-2/ MutTast/SIFT	ExAC	EVS <sup>a</sup>	Clinical diagnosis
A2621	<i>INCENP</i>	Hom	NM_020238.2	C.2403G > C	p.Gln801His	<i>Danio rerio</i>	0.96/DC/del	0/337/120826	0/32/4267	NPHP
A1833	<i>RBM48</i>	Hom	NM_032120.2	c.835A>G	p.Thr279Ala	<i>Ciona intestinalis</i>	0.71/DC/del	0/5/120562	Not present	NPHP+JATD
A2275	<i>FAM186B</i>	Hom	NM_032130.2	c.506-2A>G	Obligatory splice site mutation			0/1/111410	Not present	NPHP
A2287	<i>PIAS 1</i>	Hom	NM_016166.1	c.317C>T	p.Ser106Leu	<i>Danio rerio</i>	0.77/DC/del	0/3/120766	Not present	NPHP
A1239	<i>RCOR1</i>	Hom	NM_015156.2	c.437-3C>T	Splice site mutation			0/3/121306	Not present	JBTS

DC, disease causing (MutationTaster); del, deleterious (SIFT); EVS, exome variant server; ExAC, Exome Aggregation Consortium; Hom, homozygous; JATD, Jeune asphyxiating thoracic dystrophy; JBTS, Joubert syndrome; MutTast, MutationTaster; NPHP, nephronophthisis; NPHP-RC, nephronophthisis-related ciliopathy; SIFT, sorting intolerant from tolerant. Each candidate gene represents the most deleterious mutation within the homozygous peak regions of the respective family.

<sup>a</sup>Genotypes in European Americans.

helps distinguish between NPHP-RC and other kidney diseases that phenocopy the presentation of NPHP-RC on renal ultrasound. This can be particularly advantageous in very early stages of disease progression in which additional characteristic symptoms might not yet be present, as well as for very rare genetic syndromes. This observation underlines an additional benefit of WES: The genetic diagnostic is not restricted to a limited subset of monogenic diseases that were already suspected based on the clinical presentation, but a broader spectrum of monogenic causes can be taken into consideration. In addition, the very high rate of detecting a causative mutation in genes that are known to be mutated in renal disorders with increased echogenicity confirms that increased renal echogenicity, even if performed in numerous centers worldwide, is a reproducible and reliable criterion for noninvasive diagnostic in pediatric kidney disease. Interestingly, the percentage of molecularly diagnosed individuals was not considerably different in consanguineous families as compared with outbred sibling cases. We therefore suggest that the relevance of our findings reaches beyond consanguineous cases.

As we and others have shown<sup>10,12,17</sup> combining WES and homozygosity mapping in consanguineous families represents a powerful tool for the identification of novel human disease genes. We applied this technique in five families in whom mutations in known human disease genes had been excluded, and we identified *RBM48*, *FAM168B*, *PIAS1*, *INCENP*, and *RCOR1* as novel candidate genes for renal ciliopathies. So far, mutation analysis has not yielded additional families with mutations in these genes. However, this rarity is not unexpected as for the majority of recently identified NPHP-RC genes less than 10 families with mutations have been described worldwide.<sup>9-12,18-20</sup> At this point, additional functional evidence to prove the pathogenicity of these mutations is lacking. Further genetic and experimental evidence in the future will help determine whether mutations in these newly identified candidate genes are indeed disease causing in humans with renal ciliopathies.

Twenty-four of 79 families in our cohort (30.4%) remained without a molecular diagnosis after WES. This is not unexpected as in recessive monogenic diseases only about 85% of all causative mutations are located within the coding sequence or the adjacent splice sites.<sup>21,22</sup> The remaining 15%, however, are complex deletion–insertion variants, copy-number variants, or reside within a promotor and other intronic region. As none of these variants can be detected by WES, this technical limitation explains why some cases cannot be solved by WES. Furthermore, WES might miss a subset of causative variants due to low coverage in the respective target region. Considering the fact that about 20% of all individuals with NPHP-RC harbor a homozygous deletion in the *NPHP1* gene, the percentage of solved cases in this study had been even higher than the reported 63% if these cases were included. As it has been postulated that only 85% of recessive mutations can be technically detected with currently available techniques,<sup>22</sup> we show that in the targeted

patient cohort virtually all cases with detectable recessive mutations are molecularly solved after WES.

We here present WES as a rapid and reliable tool for molecular diagnostics in consanguineous individuals or familial cases with clinically suspected NPHP-RC based on renal ultrasound that allows establishing an etiologic diagnosis in ~2/3 of affected individuals. This represents a major advance in a diagnostic finding that is frequently interpreted as ‘medical renal disease’ rather than leading to an etiologic diagnosis.

## MATERIALS AND METHODS

### Human subjects

The study was approved by the institutional review board of the University of Michigan and Boston Children’s Hospital. After obtaining informed consent, clinical data, and pedigree information, DNA samples were collected from individuals with suspected nephronophthisis based on clinical findings, renal ultrasound criteria, and, if present, renal histology. Subjects were either of consanguineous families or of sibling cases with multiple affected children in one family. A total of 107 individuals from 82 families were included in the study. Four individuals (from 3 families) were excluded for technical reasons, leading to a final cohort of 103 individuals from 79 families. Sixty families were consanguineous singletons, and 19 families were nonconsanguineous familial cases with more than one affected child (16 families with 2 affected children, 2 families with 3 affected children, and 1 family with 5 affected children) (Supplementary Table S3 online). In all families, a homozygous deletion in the *NPHP1* gene had been excluded prior to study inclusion. This was necessary as homozygous deletions in the *NPHP1* gene represent the most frequently observed mutation in individuals with nephronophthisis but cannot be detected by WES.

### Linkage analysis

For genome-wide homozygosity mapping, the GeneChip Human Mapping 250k *Sty* Array from Affymetrix (Santa Clara, CA) was used. Nonparametric LOD scores were calculated using a modified version of the program GENEHUNTER2.1<sup>23,24</sup> through stepwise use of a sliding window with sets of 110 single-nucleotide polymorphisms and the program ALLEGRO<sup>25</sup> in order to identify regions of homozygosity as described,<sup>15,26</sup> using a disease allele frequency of 0.0001 and Caucasian marker allele frequencies. The genetic mapping was based on multipoint analysis. A nonparametric LOD score of 2 was applied as the threshold for relevant homozygous peak regions and a nonparametric LOD cutoff of 0.5 as threshold for linkage in sibling cases.

### Whole human exome capture, next gen sequencing, sequence alignment, and variant calling

Genomic DNA was isolated from blood lymphocytes and subjected to whole-exome capture using a customized Agilent SureSelect All Exome Kit v2.0 (Agilent Technologies, Santa Clara, CA) according to the manufacturer’s protocol. The library was sequenced on an Illumina HiSeq sequencing platform. Image analysis and base calling were generated by the Illumina pipeline using default parameters. Sequence reads were mapped to the human reference genome assembly (NCBI build 3/hg19) using CLC Genomics Workbench (version 6.5.2) software (CLC bio, Aarhus, Denmark). In consanguineous cases, only variants with a common allele frequency of more than 80% were called for further evaluation in order to identify

homozygous variants. Trimmed sequence reads were mapped to the human reference genome (hg19) using the Map Reads to Reference program with the following settings: mismatch cost = 2, insertion cost = 3, deletion cost = 3, length fraction = 0.5, similarity fraction = 0.9, and map to nonspecific reads = 'randomly'. The nonspecific reads were then ignored for count and coverage. All variants with a minimum coverage of 2 were used. The variations in the samples were called using probabilistic variant detection using CLC bio. All the called variants were then annotated and evaluated using mutation calling criteria as outlined in [Supplementary Figure S1](#) online.

### Variant annotation and evaluation

After alignment to the human reference genome, variants were filtered as previously described<sup>1,27</sup> and as summarized in [Supplementary Figure S1](#) online. The ranking and calling of alleles as disease-causing mutations follow accepted standards in molecular diagnostics,<sup>28–31</sup> and, with regard to the composition of our cohort, we applied criteria that are even stricter than the ones typically used in the field. In the first step, variants with minor allele frequencies >1% in the dbSNP (version 135) or the 1000 genomes (1094 subjects of various ethnicities; May, 2011 data release) databases were excluded. In the second step, only homozygous and biallelic variants (in non-consanguineous cases) were kept, whereas single heterozygous variants were excluded from the further evaluation. Subsequently, in step 3, synonymous variants and intronic variants that were not located within splice site regions were excluded. In step 4, genetic mapping data (homozygosity mapping in consanguineous families and linkage analysis in sibling cases) were applied for variant filtering.<sup>15</sup> In the final step, remaining variants were ranked based on their probable impact on the function of the encoded protein. We considered only mutations as disease causing that fulfilled the following criteria: (a) protein-truncating, or (b) previously reported as disease causing in individuals with a similar phenotype (HGMD biobase), or (c) missense mutations were only included if they affected highly conserved amino acid residues (conserved in orthologs below vertebrate evolution) and were not reported with a minor variant frequency >0.5% in healthy control individuals, and were predicted to be deleterious for the protein function (PolyPhen-2 score<sup>32</sup> >0.7). Variants that were present in the homozygous state in any publicly available control cohort were excluded. Mutation calling was performed by geneticists/cell biologists, who had knowledge of the clinical phenotypes and pedigree structure, as well as experience with homozygosity mapping and exome evaluation. Remaining variants were confirmed in original patient DNA by Sanger sequencing as previously described.<sup>33</sup> Whenever parental DNA was available, segregation analysis was performed.

In a second evaluation process, variants in all 90 genes that are known monogenic, recessive causes of NPHP-RC or other CKD that can phenocopy the presentation of NPHP-RC were systematically evaluated.

### WEB RESOURCES

UCSC Genome Browser, <http://genome.ucsc.edu/cgi-bin/hgGateway>;  
1000 Genomes Browser, <http://browser.1000genomes.org>;  
Ensembl Genome Browser, <http://www.ensembl.org>;  
Exome Variant Server, <http://evs.gs.washington.edu/EVS>;  
Exome Aggregation Consortium, [exac.broadinstitute.org](http://exac.broadinstitute.org);  
Online Mendelian Inheritance in Man (OMIM), <http://www.omim.org>;  
Polyphen2, <http://genetics.bwh.harvard.edu/pph2>;

Sorting Intolerant From Tolerant (SIFT), <http://sift.jcvi.org>;  
MutationTaster <http://www.mutationtaster.org>.

### DISCLOSURE

All the authors declared no competing interests.

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### SUPPLEMENTARY MATERIAL

**Figure S1.** Variant filtering process for the identification of the causative mutations in consanguineous families using homozygous peak regions as candidate regions.

**Figure S2.** Homozygosity mapping data in families A2621, A1833, and A2275 in whom we identified novel candidate genes for renal ciliopathies.

**Table S1.** Genotypes of 38 consanguineous families of 50 families with increased echogenicity and/or  $\geq 2$  cysts on renal ultrasound in whom a causative mutation in a known monogenic disease gene was detected by WES.

**Table S2.** Genotypes of 12 non-consanguineous families of 50 families with increased echogenicity and/or  $\geq 2$  cysts on renal ultrasound, in whom a causative mutation in a known monogenic disease gene was detected by WES.

**Table S3.** Ethnicity, pedigree structure, and number of affected individuals in 38 consanguineous and 12 non-consanguineous families with increased echogenicity and/or  $\geq 2$  cysts on RUS, in whom a causative mutation in a known monogenic disease gene was detected by WES.

**Table S4.** Mutations in 7 known monogenic disease genes in an inbred family with three affected children.

**Table S5.** Variant filtering process in individual A2621 from a consanguineous kindred (180.9 Mb of cumulative homozygosity) using homozygous peak regions as candidate regions for the identification of the causative recessive gene.

Supplementary material is linked to the online version of the paper at [www.kidney-international.org](http://www.kidney-international.org).

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