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Identification of 99 novel mutations in a worldwide cohort of 1,056 patients with a nephronophthisis-related ciliopathy

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Abstract

Nephronophthisis-related ciliopathies (NPHP-RC) are autosomal-recessive cystic kidney diseases. More than 13 genes are implicated in its pathogenesis to date, accounting for only 40 % of all cases. High-throughput mutation screenings of large patient cohorts represent a powerful tool for diagnostics and identification of novel *NPHP* genes. We here performed a new high-throughput mutation analysis method to study 13 established *NPHP* genes (*NPHP1–NPHP13*) in a worldwide cohort of 1,056 patients diagnosed with NPHP-RC. We first applied multiplexed PCR-based amplification using Fluidigm Access-Array™ technology followed by barcoding and next-generation resequencing on an Illumina platform. As a result, we established the molecular diagnosis in 127/1,056 independent individuals (12.0 %) and identified a single heterozygous truncating mutation in an additional 31 individuals (2.9 %). Altogether, we detected 159 different mutations in 11 out of 13 different *NPHP* genes, 99 of which were novel. Phenotypically most remarkable were two patients with truncating mutations in *INVS/NPHP2* who did not present as infants and did not exhibit extrarenal manifestations. In addition, we present the first case of Caroli disease due to mutations in *WDR19/NPHP13* and the second case ever with a recessive mutation in *GLIS2/NPHP7*. This study represents the most comprehensive mutation analysis in NPHP-RC patients, identifying the largest number of novel mutations in a single study worldwide.

Introduction

The term nephronophthisis-related ciliopathies (NPHP-RC) describes a group of rare autosomal-recessive cystic kidney diseases, characterized by a broad genetic and clinical heterogeneity and accounting for the majority of genetic causes of end-stage renal disease (ESRD) during childhood (Hildebrandt et al. 2009; Hildebrandt and Otto 2005; Wolf and Hildebrandt 2011). NPHP-RC includes isolated nephronophthisis (NPHP), Senior-Loken syndrome (SLS), Joubert syndrome (JBTS), and Meckel Gruber syndrome (MKS). In renal histology, the most prominent features of NPHP are tubular atrophy, basement membrane disintegration, interstitial fibrosis, and cyst formation. The most common extrarenal manifestation observed in NPHP is progressive retinal dystrophy defined as SLS. The hallmark of JBTS is mid-hindbrain malformation and cerebellar vermis hypoplasia or aplasia, descriptively designated as “molar tooth sign” on a cranial MRI. This results in various neurological features including developmental delay, intellectual disability, muscle hypotonia, ataxia, oculomotor apraxia, nystagmus, and respiratory distress (Parisi 2009). MKS, a perinatally lethal ciliopathy, represents the most severe manifestation of the NPHP-RC clinical spectrum. It is characterized by central nervous system malformations, bilateral postaxial hexadactyly, hepatobiliary ductal plate malformation, and multicystic dysplastic kidneys (Johnson et al. 2003). As the phenotype of NPHP-RC shows a vast and partially overlapping spectrum, the genotype is also broadly heterogeneous, with more than 13 *NPHP*

genes implicated to date (Table 1), accounting for only about 40 % of all cases: *NPHP1, INVS/NPHP2, NPHP3, NPHP4, IQCB1/NPHP5, CEP290/NPHP6, GLIS2/NPHP7, RPGRIP1L/NPHP8, NEK8/NPHP9, SDCCAG8/NPHP10, TMEM67/NPHP11, TTC21B/NPHP12 and WDR19/NPHP13* (Hildebrandt et al. 1997; Olbrich et al. 2003; Otto et al. 2002, 2003, 2005, 2008b, 2009b, 2010; Mollet et al. 2002; Sayer et al. 2006; Attanasio et al. 2007; Delous et al. 2007; Davis et al. 2011; Bredrup et al. 2011). In addition, JBTS or MKS results from mutations in a subset of these genes or from any of at least 20 additional disease genes (*MKS1, B9D1, B9D2, AHI1, INPP5E, ARL13B, TMEM216, CC2D2A, KIF7, TCTN1, TCTN2, TCTN3, ATXN10, CEP41, OFD1, TMEM138, C5ORF42, ZNF423, TMEM231 and TMEM237*), most of which have been identified only recently (Kyttälä et al. 2006; Hopp et al. 2011; Dowdle et al. 2011; Ferland et al. 2004; Bielas et al. 2009; Cantagrel et al. 2008; Valente et al. 2010; Gorden et al. 2008; Dafinger et al. 2011; Garcia-Gonzalo et al. 2011; Sang et al. 2011; Huang et al. 2011; Lee et al. 2012a, b; Coene et al. 2009; Srour et al. 2012a, b; Chaki et al. 2012; Thomas et al. 2012).

The common feature of proteins encoded by genes mutated in NPHP-RC is their localization to primary cilia, basal body or centrosomes, which results in defects of the respective cell organelle. The discovery of the crucial role of primary cilia led to the general term “ciliopathy” (Hildebrandt et al. 2011).

Since 60 % of NPHP-RC cases harbor mutations in genes that are yet to be identified, the detection of novel, disease causing *NPHP* genes remains a major challenge. In order to address this issue, mutation analysis of established genes is a necessity in way of a priori exclusion. Due to an increasing number of *NPHP* genes, comprehensive mutation analysis by Sanger sequencing becomes more tedious and costly. However, technical advances in next-generation resequencing (NGS) and development of commercially available high-throughput polymerase chain reaction (PCR)-based resequencing platforms facilitate and accelerate mutation analysis. One of those platforms is the 48.48 Access Array™ microfluidic system from Fluidigm (South San Francisco, CA), which enables amplification of 48 DNA samples in combination with each of 48 target-specific primer pairs, resulting in 2,304 individual PCRs in parallel. Applying a tenfold primer pooling strategy, we recently were able to successfully scale up the Fluidigm/NGS approach to about 23,000 parallel PCRs (Halbritter et al. 2012). This pilot project was conducted in 192 patients and showed high efficiency at a low cost with a sensitivity of 90 % and specificity of 87 %. In the present study, we describe a streamlined screening approach using the Fluidigm platform to amplify all coding exons of 13 known *NPHP* genes by multiplexed-PCR and barcoded consecutive NGS in a comprehensive cohort of 1,056 individuals with NPHP-RC. The most frequent mutation in patients with NPHP-RC, a homozygous *NPHP1* deletion, has been excluded in all affected individuals prior to inclusion in the present study.

Materials and methods

Human subjects

We obtained blood samples, pedigrees, and clinical information after receiving informed consent (http://www.renalgenes.org). Approval for experiments on humans was obtained from the University of Michigan Institutional Review Board. The diagnosis of NPHP-RC

was based on published clinical criteria (Chaki et al. 2011). The total cohort of 1,056 patients with NPHP-RC included 447 patients with isolated NPHP versus 609 patients with additional extrarenal manifestations mainly in patients with Joubert syndrome (109), Senior-Loken syndrome (103), Meckel–Gruber syndrome (9), and Jeune syndrome (5). Frequent extrarenal manifestations seen in our cohort were retinal dystrophy (157), cerebellar vermis hypoplasia (109), liver fibrosis/hepatomegaly (94), early blindness/Leber congenital amaurosis (49), heart anomalies (30), oculomotor apraxia (30), deafness (18), polydactyly (17), microcephaly (15), situs inversus (14), facial dysmorphic features (11), retina coloboma (10), cone-shaped epiphysis (9), hydrocephalus (6), pancreatic cysts (6), and microophthalmia (2). Our total cohort consisted of 159 families with multiple affected cases vs. 897 single affected cases. Consanguinity was known to be present in 190 (18 %) families. As a first diagnostic step, homozygous deletions of *NPHP1* were excluded in all patients by applying a multiplex PCR-based deletion analysis described elsewhere (Otto et al. 2008a).

Primer design and evaluation for the Fluidigm Access Array IFC system

We designed 345 target-specific primer pairs to cover all 316 coding exons and intron/exon boundaries of the genes *NPHP1–NPHP13* (Suppl. Table 1). The maximum amplicon size was chosen as 300 bp, anticipating subsequent NGS bidirectional sequence reads of 150 bases from each side. Universal primer sequences 5′-ACACTGACGACA TGGTTCTACA-[target-specific forward]-3′ and 5′-TAC GGTAGCAGAGACTTGGTCT-[target-specific reverse]-3′ were added at the 5′ end to all target-specific forward and reverse primers, respectively.

Target DNA enrichment by Fluidigm 48.48 Access Array™ IFC system

Primers were pooled to generate 7-plex NPHP primer pools per PCR with a final concentration of 1 μM per primer. Every sample master mix solution contained 50 ng genomic DNA, 1× FastStart High Fidelity Reaction Buffer with $MgCl_2$, 5 % DMSO, dNTP (200 μM each), 1× Access Array™ loading reagent, and FastStart High Fidelity Enzyme Blend (Roche, Indianapolis, IN, USA). In one microfluidic 48.48 Access Array™, 48 different DNA samples and 48 different primer pools were applied. Subsequently, thermal cycling on a Fluidigm FC1 Cycler was performed. PCR products were then harvested as 48 separate amplicon pools using the IFC controller AX and transferred to a 96-well plate. In a separate PCR on a standard thermocycler, Illumina sequence-specific adaptors and sample barcodes were attached. Altogether, we processed 22 different Fluidigm 48.48-Access Arrays™ and divided all of the 1,056 patient-derived amplicon pools into batches of 144 unique barcodes/indices. The primer sequences required for bidirectional amplicon tagging (requiring 2 separate PCRs) compatible with Illumina NGS were as follows: PE1-CS1, 5′-AATGATACGGCGACCA CCGAGATCTACACTGACGACATGGTTCTACA-3′ and PE2-BC-CS2, 5′-CAAGCAGAAGACGGCATACGAGA T-[BARCODE]-TACGGTAGCAGAGACTTGGTCT-3′ as well as PE1-CS2, 5′AATGATACGGCGACCACCGA GATCTTACGGTAGCAGAGACTTGGTCT-3′, and PE2-BC-CS1, 5′-CAAGCAGAAGACGGCATACGAGAT-[BARCODE]-ACACTGACGACATGGTTCTACA-3′. Subsequently, 7 × 144 indexed samples and 1 × 48

indexed samples were pooled in order to allocate all 1,056 samples to 8 lanes of an Illumina next-generation sequencing instrument.

Next-generation resequencing on an Illumina GAIIx platform

Pooled and indexed PCR products were sequenced on 8 lanes of an Illumina GAIIx instrument as one 150 base run (v2.5 reagents) following standard Illumina protocols with the following modifications. In order to sequence the Fluidigm specific barcodes, we substituted the Illumina-specific index sequencing primer with a mixture of two custom Fluidigm-specific index primers (CS1rc, 5′-T+GT+AG+AACCATGTCGTCAGTGT-3′ and CS2rc, 5′-A+GAC+CA+AGTCTCTGCTACCGTA-3′). Modified oligos were ordered from Exiqon company (http://www.exiqon.com, Vedbaek, Denmark) with nucleotides preceded by a "+" representing LNA® nucleotides. To decipher the full Fluidigm barcodes, we extended the index read length to 10 cycles. Finally, for a single 150 base Illumina sequence run, we equally mixed and applied Fluidigm custom primer CS1 (5′-A+CA+CTG +ACGACATGGTTCTACA-3′) and CS2 (5′-T+AC+GGT+AGCAGAGACTTGGTCT-3′).

Bioinformatics pipeline

Sequence reads were separated according to their barcodes using the CASAVA v1.7 demultiplex.dp script (Illumina) resulting in 30–40 million bases per barcode. Sequence reads were aligned for each barcode (patient) using CLC Genomics Workbench™ software (CLC-bio, Aarhus, Denmark) to a single reference sequence containing the concatenated genomic sequences of all 13 *NPHP* target genes (*NPHP1–NPHP13*). We annotated all donor and acceptor splice sites of all exons within that reference sequence. Variant calls were obtained using the following filter parameters: minimum central base quality = 20, minimum average quality = 15, variant frequency ≥ 20 %. A minimum variant count of 2 was applied for potential truncating mutations (nonsense, frameshift, and obligatory splice-site mutations). More stringent parameters were applied to non-synonymous missense variants with a minimum count of 10 and a PolyPhen2 score above 0.9. The rationale for choosing the variant frequency and count parameters has been previously described in detail (Halbritter et al. 2012). Synonymous variants and common dbSNP (v135) with a population allele frequency above 1 % were excluded. Variants were ranked by the criteria of whether mutations were likely to truncate the conceptual reading frame (nonsense, frameshift, and obligatory splice mutations). Missense variants were ranked by evolutionary conservation and using web-based programs (PolyPhen2, Mutation Taster, SIFT), predicting the impact on the encoded protein.

Sanger sequencing confirmation and segregation analysis

Variants/mutations detected by NGS and predicted to be detrimental were subsequently confirmed by Sanger sequencing using original DNA samples from the respective patients as PCR template. Whenever parental DNA was available, we performed segregation analysis. Polymerase chain reaction was performed using a touchdown protocol described previously (Otto et al. 2011). Sequencing was performed using BigDye® Terminator v3.1 Cycle Sequencing Kit on an ABI 3730 XL sequencer (Applied Biosystems). Sequence traces

were analyzed using Sequencher (version 4.8) software (Gene Codes Corporation, Ann Arbor, MI, USA).

Next-generation sequencing using the Illumina MiSeq Personal Sequencer

In patients with only a single confirmed heterozygous truncating or obligatory splice-site mutation, a standard PCR-amplification of all coding exons of the respective gene was performed. After barcoding the various patient-derived PCR-products, all samples were pooled and sequenced on an Illumina MiSeq Personal Sequencer instrument in one 2 × 151 bases paired-end run following the standard Illumina protocol with the following modifications. In order to sequence the Fluidigm-specific barcodes, we used the chemically modified CS1rc oligo (5′-T+GT+AG+AACCATGTCGTCAGTGT-3′). To sequence the forward and reverse paired-end reads, we used custom oligo CS1 (A+CA+CTG +ACGACATGGTTCTACA) and CS2 (T+AC+GGT+AGCAGAGACTTGGTCT), respectively. These oligos contain Locked Nucleic Acid® (LNA®) oligonucleotides, indicated with a plus sign in front of the modified base, and provide superior hybridization characteristics and enhanced biostability compared to conventional oligos. The LNA® oligos were purchased from Exiqon (Vedbaek, Denmark).

Results

Illumina NGS and mapping statistics

We performed sequencing on 8 lanes of a GAIIx instrument after targeted amplification of 316 coding exons (345 amplicons) in 1,056 different indexed patients using the Fluidigm platform. The total output (8 lanes) was 204 million reads of 150 bases (25.5 million reads per lane) yielding an average of 193,000 reads per DNA sample. Using CLC Genomics Workbench™ software, we mapped an average of 177,000 (92 %) reads per patient to a human reference. Alignment resulted in mean exon coverage of 185×, with 70 % of the targeted coding regions covered at least fivefold and 66 % being covered tenfold. Insufficient coverage was found in 20 % of targeted exons randomly distributed across all genes investigated (Suppl. Table 1).

Variant filtering, validation, and parameter setting

Variant calling resulted in altogether 52,063 single nucleotide variant calls and 7,181 indels. We found a total of 26,534 known dbSNP135 (exonic and intronic) variants across the dataset derived from all 1,056 patients analyzed. As in our pilot project, we set a threshold of 20 % minimal allele frequency as a filter parameter and considered variants below this threshold as most likely “false positives” (Halbritter et al. 2012). Due to the low coverage, compared to our pilot project, we evaluated all truncating and obligatory splice-site variants with a count of at least 2. In contrast, missense variants were evaluated applying a cutoff parameter of ≥10 counts. Only missense variants with PolyPhen2 scores above 0.9 were further analyzed. In summary, filtering and ranking led to the selection of 315 potentially truncating mutations (including nonsense, frameshift, and obligatory splice-site mutations) and 80 missense variants/mutations for validation by standard Sanger sequencing. We were able to confirm 194 of the truncating mutations and 20 of the selected missense variants. In total, 214 out of 395 variants (specificity: 54 %) have been confirmed by Sanger sequencing.

Mutation detection in positive control samples

In order to calculate the sensitivity, we included 27 DNA samples with 44 known mutations as positive controls. Overall, only 27 out of these 44 mutations have been re-detected in the present study ("mutation" sensitivity 61 %). The low total coverage resulted in the detection of only one heterozygous mutation in some of these patients who knowingly carried a compound heterozygous mutation. When taking these patients into account, we were able to identify 20 out of 27 patients ("patient" sensitivity 74 %). Identified control samples are indicated as underlined in Table 2.

Identification of mutations in a cohort of 1,056 individuals

Combination of high-throughput multiplex-PCR and bar-coded subsequent NGS in a worldwide cohort of 1,056 independent patients revealed the molecular diagnosis in 90 patients. Furthermore, one single heterozygous truncating mutation was found in 68 additional patients. In order to screen for a potential second mutated allele, standard amplification of all coding exons of the respective gene and barcoded consecutive NGS on an Illumina MiSeq Personal Sequencer System was conducted. Using this approach, a second mutated allele could be identified in 36 of those 68 patients. Due to low DNA quality, sequencing on the MiSeq failed for seven samples. However, after Sanger sequencing, one additional patient with a second heterozygous mutation was detected.

In summary, high-throughput mutation analysis led to the molecular diagnosis in 127 (90 + 36 + 1) out of 1,056 (12.0 %) independent NPHP-RC patients. Segregation analysis in multiplex families resulted in the identification of causative mutations in an additional 15 affected siblings. A molecular genetic diagnosis has been obtained in 142 patients derived from 127 families who carried mutations on both alleles. Recessive mutations have been identified in the following genes: *NPHP1* (26 patients/23 families), *INVS/NPHP2* (2 patients/2 families), *NPHP3* (20 patients/17 families), *NPHP4* (24 patients/22 families), *IQCB1/NPHP5* (18 patients/16 families), *CEP290/NPHP6* (22 patients/20 families), *GLIS2/NPHP7* (1 patient/1 family), *SDCCAG8/NPHP10* (3 patients/3 families), *TMEM67/NPHP11* (15 patients/14 families), *TTC21B/NPHP12* (6 patients/5 families), and *WDR19/NPHP13* (5 patients/4 families) (Table 2). No causative mutation was identified in the gene *NEK8/NPHP9* for which only four mutations have been reported to date (Otto et al. 2008b; Frank et al. 2013). Overall, we identified 51 independent individuals with homozygous mutations, 4 individuals with hemizygous mutations (all in *NPHP1*), and 72 individuals with compound heterozygous mutations. In 93 patients, truncating mutations (nonsense, frameshift or obligatory splice-site mutations) were found on both alleles, whereas 18 patients carried one truncating mutation in combination with a non-synonymous missense mutation. The remaining 16 patients exhibited missense mutations only.

After evaluation of all coding regions and intron/exon boundaries in the respective genes, 31 patients remained with only one heterozygous truncating mutation (Table 3).

In total, we discovered 99 novel pathogenic mutations in the genes *NPHP1* (14), *INVS*/*NPHP2* (6), *NPHP3* (16), *NPHP4* (26), *IQCB1*/*NPHP5* (2), *CEP290*/*NPHP6* (12), *GLIS2*/*NPHP7* (1), *RPGRIP1L*/*NPHP8* (1), *SDCCAG8*/*NPHP10* (1), *TMEM67*/*NPHP11* (6),

TTC21B/*NPHP12* (6), and *WDR19*/*NPHP13* (8). These mutations add an additional 20 % to the previously reported 492 mutations in the genes *NPHP1–NPHP13*, according to the HGMD®-Professional mutation database “Biobase” (September 28th 2012 release) (Table 4).

Discussion

High-throughput mutation analysis of 13 *NPHP* genes in a large worldwide cohort of 1,056 patients using the Fluidigm/NGS system led to the identification of the causative mutations in 127 different families with 142 affected individuals with NPHP-RC. In addition, we detected single heterozygous truncating mutations, which do not fully explain the phenotype in a recessive disease in 31 patients. Individuals with mutations in *NPHP1* (23), *NPHP4* (22), *CEP290*/*NPHP6* (20), *NPHP3* (17) and *IQCB1*/*NPHP5* (16) were the most frequent findings. Combined with previous studies and the results of the homozygous *NPHP1* deletion analysis, which has been applied to every affected individual in our cohort of 1,540 families, we hereby obtain a representative frequency distribution of genes implicated in NPHP-RC with 63.8 % of cases remaining still unsolved (Fig. 1). By identifying 99 novel mutations, our study generated the largest number of previously unreported mutations in patients with a NPHP-RC phenotype, adding an additional 20 % to publicly available databases.

In contrast to previously reported phenotypical findings, it is noteworthy that two patients with truncating mutations in *INVS/NPHP2* did not present as infants and did not exhibit extrarenal manifestations. Another striking observation is that *NPHP3* represents the most common gene implicated in infantile NPHP in this study. Remarkably, two patients with homozygous *WDR19* mutations additionally displayed Caroli disease, a rare inherited disorder characterized by dilatation of the intrahepatic bile ducts.

In *GLIS2/NPHP7*, only one homozygous splice-site mutation (c.755+1G>T) has been published to date (Attanasio et al. 2007). We hereby report the second mutation, an evolutionary highly conserved (*Drosophila melanogaster*) homozygous missense mutation, located at the first nucleotide of exon 4, potentially affecting the splicing of the respective exon (c.523T>C, p.C175R). Similarly, in *WDR19/NPHP13* we added an additional eight mutations to the five currently known (Table 4). Interestingly, in this project we have not found any indication for the presence of oligogenicity in NPHP unlike described earlier (Hoefele et al. 2007). Except for one (F1369, Table 3), none of the patients showed truncating mutations in more than one NPHP gene. Still, we cannot exclude oligogenicity for those patients with only one truncating mutation detected. To address the oligogenicity hypothesis, one might have to analyze even more genes in parallel, take missense alleles into account, and compare with the results derived from an ethnically matched cohort of healthy individuals.

Regarding the 31 patients with only one heterozygous truncating mutation, one has to consider the possibility that some of these truncating mutations, although rare, might have been found by chance in concordance with the frequency seen in the general population. Using the data derived from about 6,500 individuals deposited in the Exome Variant Server

database (EVS, http://evs.gs. washington.edu/EVS/), we previously calculated that 10 heterozygous deleterious truncating mutations within an *NPHP* gene is expected to be present by chance in a cohort of 1,000 individuals (Halbritter et al. 2012). Interestingly, four out of the above mentioned 31 patients indeed do carry rare truncating variants listed in the EVS server.

There are multiple reasons why we did not detect mutations in about 900 patients. First, in comparison with our pilot project, the mutation detection sensitivity was substantially lower (Halbritter et al. 2012). In the current study, NGS was performed on a GAIIx instead of a HiSeq2000, resulting in fewer reads per lane, significantly lower mean exon-coverage, and thus lower sensitivity. We estimate that we therefore might have overlooked about 10 % of patients with exonic mutations. Second, many disease causing mutations are not exonic and therefore not detectable with our exon-resequencing method. Third, some patients in our cohort might have been misdiagnosed with NPHP but suffer from other cystic kidney diseases like autosomal recessive polycystic kidney disease (ARPKD). Fourth, some of the cases might be explained by disease causing mutations implicated in JBTS or MKS that were not part of the present study such as *AHI1*, *ARL13B*, *CC2D2A*, *INPP5E*, *TCTN1-3*, *MKS1*. Nevertheless, the high number of still “unsolved” cases indicates that additional extensive heterogeneity in NPHP-RC is likely.

To improve the method, in subsequent projects we have begun testing bidirectional sequencing of 150 bp reads on a HiSeq2000. As a consequence, we are able to increase the sequence output from 25.5 million reads up to 200–300 million reads per lane.

Identification of the remaining unknown genes in genetically heterogeneous diseases like nephronophthisis and other ciliopathies still represents a major challenge. Discovery of these genes can be achieved by applying high-throughput methods like whole exome/genome sequencing (WES/WGS). The Fluidigm/NGS approach is an affordable method to screen large cohorts for a predefined set of genes and should be considered before applying WES/WGS.

In summary, we successfully introduced the use of a high-throughput mutation analysis in a large NPHP-RC cohort and were able to detect the largest number of novel mutations in a single experiment. Further method optimizations will lead to a higher sensitivity and specificity and will enable rapid screening of large cohorts in an efficient and streamlined way.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

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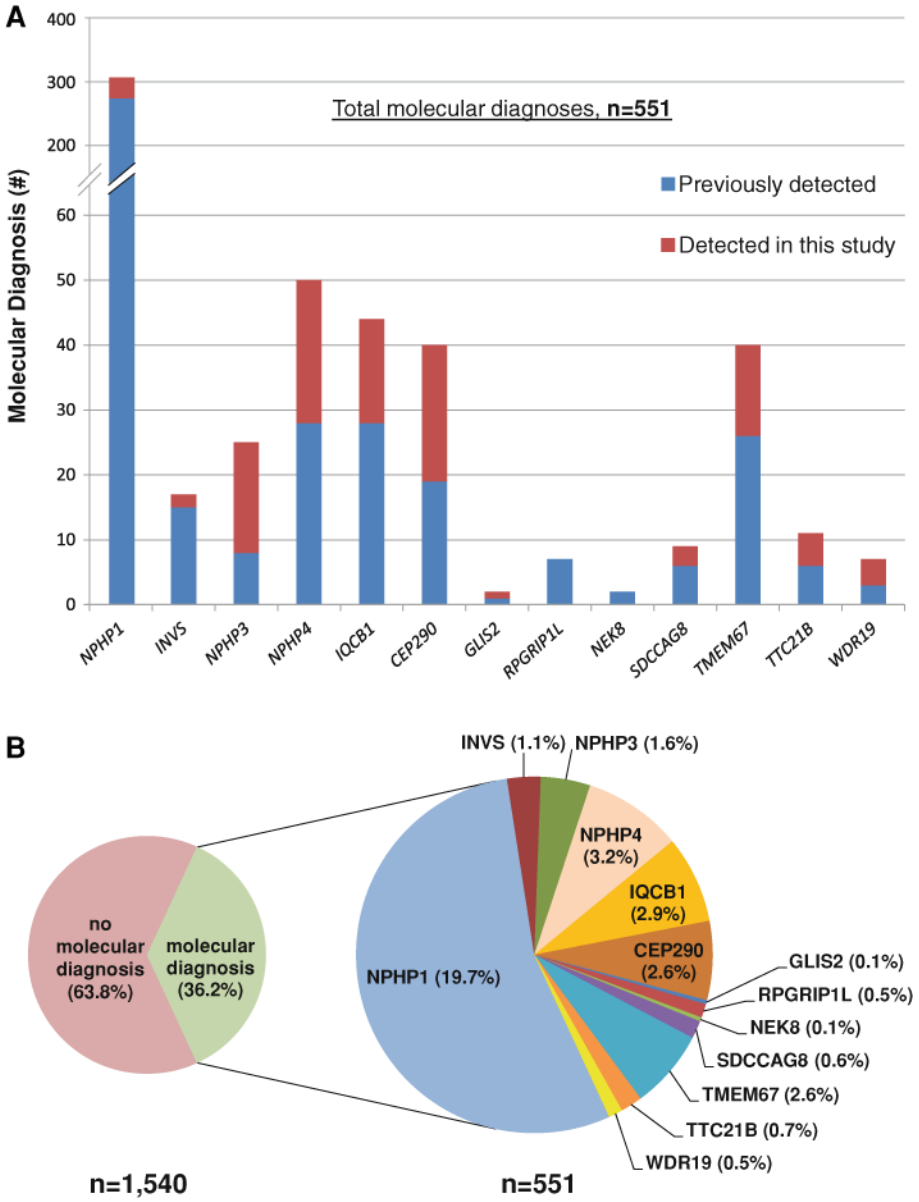

Fig. 1.

a Distribution of established molecular NPHP-diagnoses for the genes *NPHP1–NPHP13* detected previously in our total cohort of 1,540 individuals (*in blue*) and in the subset of patients identified within the present study (*in red*). All affected individuals were screened for the presence of a homozygous *NPHP1* deletion prior to being entered into the present study. **b** Percentage of patients with a molecular diagnosis versus patients without a molecular diagnosis in our total cohort of 1,540 NPHP-RC patients (*left*). Distribution of molecular diagnoses across the genes *NPHP1–NPHP13* (*right*) (color figure online)

Table 1

13 *NPHP* genes investigated using Fluidigm 48.48 Access Array™ amplification and consecutive next-generation resequencing (NGS)

Gene	Locus/protein	Chromosome	Accession #	Exon count	Coding exon count	Open reading frame size (bp)
NPHP1	*NPHP1*/nephrocystin 1	2	NM_000272.2	20	20	2,202
INVS	*NPHP2*/inversin	9	NM_014425.2	17	16	3,198
NPHP3	*NPHP3*/nephrocystin 3	3	NM_153240.3	27	27	3,993
NPHP4	*NPHP4*/nephroretinin	1	NM_015102.2	30	29	4,281
IQCB1	*NPHP5*/IQ motif containing B1	3	NM_001023570.1	15	13	1,797
CEP290	*NPHP6*/centrosomal protein 290 kDa	12	NM_025114.3	54	53	7,440
GLIS2	*NPHP7*/GLIS family zinc finger 2	16	NM_032575.2	6	6	1,575
RPGRIP1L	*NPHP8*/RPGRIP1-like	16	NM_015272.2	27	26	3,948
NEK8	*NPHP9*/NIMA-related kinase 8	17	NM_178170.2	15	15	2,079
SDCCAG8	*NPHP10*/serologically defined colon cancer antigen 8	1	NM_006642.3	18	18	2,139
TMEM67	*NPHP11*/meckelin	8	NM_153704.5	28	28	2,988
TTC21B	*NPHP12/tetratricopeptide repeat domain 21B*	2	NM_024753.4	29	29	3,951
WDR19	*NPHP13/WD repeat domain 19*	4	NM_025132.3	37	36	4,029
				323	316	43,620

Table 2

Genotypes and phenotypes of 127 families (142 patients) with mutations in *NPHP1*, *INVS*, *NPHP3*, *NPHP4*, *IQCB1*, *CEP290*, *GLIS2*, *SDCCAG8*, *TMEM67*, *TTC21B*, and *WDR19*

Patient[c]	Kidney ESRD age (years)	Extrarenal manifestations	Origin	Gene	Nucleotide change[a] (zygosity)	Amino acid change (segregation)	Mutation count/ coverage	PolyPhen2 score[b]	Reference
A867-21	11	OMA	Germany	*NPHP1*	c.84_87delTTCT (Horn)	p.S29Rfs*4	10/10	NA	Novel
A2527-21/22	19	No	UK	*NPHP1*	c.112G>T (hem)	p.E38*	7/7	NA	Novel
A2229-25	6	No	Arab	*NPHP1*	c.143G>A (Horn)	p.R48K (m)	31/31	0.99	Novel
A3244-21	17	No	Turkey	*NPHP1*	c.400G>T (Horn)	p.E134*	10/10	NA	Novel
A13-21	15	No	USA	*NPHP1*	c.555dupA (het)	p.P186Tfs*2 (p)	446/1,113[d]	NA	Caridi et al. (2006)
					c.1438-1G>A (het)	Splice site (m)	15/25	NA	Novel
A1754-21	>10	No	The Netherlands	*NPHP1*	c.1027G>A (Horn)	p.G343R	805/809	1.0	Caridi et al. (2006)
A2169-21	10	No	USA	*NPHP1*	c.1027G>A (Horn)	p.G343R	523/528	1.0	Caridi et al. (2006)
A3171-21	12	No	Germany	*NPHP1*	c.1027G>A (Horn)	p.G343R	575/579	1.0	Caridi et al. (2006)
A4618-21	>13	JBTS, Nystagmus, T1 DM	Germany	*NPHP1*	c.1027G>A (Horn)	p.G343R	816/820	1.0	Caridi et al. (2006)
A4840-21	12	No	Czech Republic	*NPHP1*	c.1057C>T (Horn)	p.Q353*	114/114	NA	Novel
A3484-21	9	No	Turkey	*NPHP1*	c.1252-1G>T (Horn)	Splice site	707/708	NA	Novel
A2369-21	12	No	Philippines	*NPHP1*	c.1298delA (Horn)	p.K433Sfs*55	998/1,010	NA	Novel
A661-21	17	No	Germany	*NPHP1*	c.1520+1delG (Horn)	Splice site	28/28	NA	Hildebrandt et al. (1997)
F430-22	25	No	Germany	*NPHP1*	c.1520+1delG (Horn)	Splice site (p)	25/25	NA	Hildebrandt et al. (1997)
F845-21	8	Ataxia	Germany	*NPHP1*	c.1520+1delG (Horn)	Splice site (m)	14/14	NA	Hildebrandt et al. (1997)
F1213-21	ND	ND	Germany	*NPHP1*	c.1520+1delG (Horn)	Splice site	27/27	NA	Hildebrandt et al. (1997)
A157-21	8	OMA	USA	*NPHP1*	c.1719delT (Horn)	p.I573Mfs*10	292/294	NA	Novel
A749-21	14	No	Turkey	*NPHP1*	c.1786_1787delGA (hem)	p.D596Qfs*8	265/274	NA	Novel
A232-21	22	OMA	USA	*NPHP1*	c.1884+1G>T (hem)	Splice site	973/977	NA	Otto et al. (2008a)
A2548-21	6	No	Turkey	*NPHP1*	c.1884+1G>A (Horn)	Splice site	919/923	NA	Otto et al. (2008a)
A3630-21	7	RD, deafness, CAKUT, microcephaly, JBTS, heart anomalies	India	*NPHP1*	c.1884+2T>C (Horn)	Splice site	743/749	NA	Novel
F1422-21	10	No	Turkey	*NPHP1*	c.2006delG (Horn)	p.R669Pfs*60	7/7	NA	Novel
A4432-21/22/23	20/12/16	No	Turkey	*NPHP1*	c.2153C>A (hem)	p.S718*	10/10	NA	Novel
A3483-21	13	No	Turkey	*INVS*	c.1417delG (het)	p.A473Qfs*37	321/646[d]	NA	Novel
					c.3125delA (het)	p.N1042Tfs*64	75/128	NA	Novel
F1433-21	10	No	Germany	*INVS*	c.2695C>T (het)	p.R899*	1,020/2,502[d]	NA	Otto et al. (2003)

Patient[c]	Kidney ESRD age (years)	Extrarenal manifestations	Origin	Gene	Nucleotide change[a] (zygosity)	Amino acid change (segregation)	Mutation count/ coverage	PolyPhen2 score[b]	Reference
					c.2782C>T (het)	p.R928*	41/58	NA	Novel
A4301-21	<1	LF	USA	*NPHP3*	c.406delA (het)	p.T136Rfs*13	819/1,546[d]	NA	Novel
					c.2570+lG>T (het)	Splice site	47/136	NA	Halbritter et al. (2012)
A3865-21	11	LF	Germany	*NPHP3*	c.518A>G (het)	p.K173R	658/1,278[d]	1.0	Novel
					c.2694-2_2694-ldelAG (het)	Splice site	5/14	NA	Bergmann et al. (2008)
A173-21	<1	HF, BDP	USA	*NPHP3*	c.537_542delAGAAAA (het)	p.K179_E180del (de novo)	3/5	NA	Halbritter et al. (2012)
					c.2570+lG>T (het)	Splice site (m)	154/349	NA	Halbritter et al. (2012)
A4040-22	<1	Cholangitis	Egypt	*NPHP3*	c.671-3C>G (Horn)	Splice site	51/51	NA	Novel
A1122-21	9	No	Austria	*NPHP3*	c.682C>T (het)	p.Q228*	71/145	NA	Novel
					c.3329+lG>A (het)	Splice site	29/83	NA	Novel
A633-21/22	<1	PFO	USA	*NPHP3*	c.1206delA (het)	p.V403Sfs*9 (m)	35/105	NA	Novel
					c.3003delT (het)	p.F1001Lfs*61 (p)	384/540	NA	Novel
F300-21	ND	ND	Germany	*NPHP3*	c.1304_1306delAAG (het)	p.E435del	276/563[d]	NA	Novel
					c.2104C>T (het)	p.R702*	587/1,197	NA	Simpson et al. (2009)
A4695-21	<1	Heart anomalies, HSM	USA	*NPHP3*	c.2369T>C (het)	p.L790P	75/138[d]	0.99	Novel
					c.2694-2_2694-ldelAG (het)	Splice site	17/28	NA	Bergmann et al. (2008)
A1444-21	<1	LF, heart anomalies	USA	*NPHP3*	c.2541delG (het)	p.K847Nfs*2	86/189	NA	Novel
					c.2570+lG>T (het)	Splice site	52/97	NA	Halbritter et al. (2012)
A2361-21	3	LF, hydrocephalus, recurrent subdural bleeding	Norway	*NPHP3*	c.2563C>T (het)	p.Q855* (p)	184/477	NA	Tory et al. (2009)
					c.3812+2dupT (het)	Splice site (m)	47/68	NA	Otto et al. (2008a)
F1215-21	>11	LF	Germany	*NPHP3*	c.2694-2_2694-ldelAG (het)	Splice site	24/45	NA	Bergmann et al. (2008)
					c.3020T>G (het)	p.L1007R	2,900/5,702[d]	1.0	Novel
A2425-21/22	<1	MKS	UK	*NPHP3*	c.2694-2_2694-ldelAG (het)	Splice site	21/45	NA	Bergmann et al. (2008)
					c.3619C>T (het)	p.R1207*	10/10	NA	Novel
A4405-21	16	No	USA	*NPHP3*	c.3133C>T (Horn)	p.Q1045*	193/194	NA	Novel
A3499-21	5	HSM	Turkey	*NPHP3*	c.3329+2T>G (Horn)	Splice site	87/87	NA	Novel
A3999-21/22	4/3	LF	USA	*NPHP3*	c.3466G>T (het)	p.E1156*	56/333	NA	Novel
					c.3570+5G>A (het)	Splice site	89/150	NA	Novel
A2225-21	<1	ID, LF	Turkey	*NPHP3*	c.3567_3568delAA (Horn)	p.K1189Nfs*5	107/192	NA	Halbritter et al. (2012)

Patient[c]	Kidney ESRD age (years)	Extrarenal manifestations	Origin	Gene	Nucleotide change[a] (zygosity)	Amino acid change (segregation)	Mutation count/ coverage	PolyPhen2 score[b]	Reference
A1451-21	2	No	Egypt	*NPHP3*	c.3812+lG>C (Horn)	Splice site (p,m)	47/47	NA	Halbritter et al. (2012)
A2393-21	25	RD	Italy	*NPHP4*	c.175C>T (Horn)	p.R59*	80/81	NA	Otto et al. (2008a)
A1539-21	18	Malformation of thoracic vertebrae	Canada	*NPHP4*	c.257_258delCG (het)	p.P86Lfs*6	23/57	NA	Novel
					c.3316-lG>C (het)	Splice site	150/277	NA	Novel
F1291-21	7	No	Germany	*NPHP4*	c.305delA (het)	p.N102Tfs*76	40/80	NA	Otto et al. (2011)
					c.1956-2A>G (het)	Splice site	39/83	NA	Novel
A137-21	9	No	USA	*NPHP4*	c.641delT (het)	p.I214Nfs*101	73/177	NA	Novel
					c.3920T>C (het)	p.L1307P	867/1,767[d]	1.0	Novel
A3285-21	8	No	Egypt	*NPHP4*	c.673G>A (Horn)	p.G225S	124/124	0.95	Novel
A3443-21	11	PD	Turkey	*NPHP4*	c.685C>T (Horn)	p.R229*	39/39	NA	Novel
A3165-21	15	RD	Germany	*NPHP4*	c.750dupC (het)	p.S251Lfs*6	92/177	NA	Novel
					c.3703C>G (het)	p.R1235G	946/1,909[d]	1.0	Novel
A4421-21	ND	No	Czech Republic	*NPHP4*	c.1082_1083dupAG (het)	p.Y362Sfs*45	485/1,193	NA	Novel
					c.3272delT (het)	p.V1091Gfs*31	1,945/4,662	NA	Mollet et al. (2002)
A4243-21/22	15/14	Dextrocardia	USA	*NPHP4*	c.1228C>T (het)	p.Q410*	124/319	NA	Novel
					c.3769_3772delACAG (het)	P.T1257*	433/872	NA	Novel
A647-21	17	No	USA	*NPHP4*	c.1271delA (het)	p.K424Rfs*7	44/69	NA	Novel
					c.3644+lG>T (het)	Splice site	1,137/1,752	NA	Novel
F10-21/22	14/8	No	Germany	*NPHP4*	c.1956-2A>G (het)	Splice site (p)	158/185	NA	Novel
					c.3773_3776delTGAG (het)	p.V1258Gfs*3 (m)	480/1,058	NA	Novel
A4021-21	12	No	Belgium	*NPHP4*	c.2001T>A (het)	P.Y667*	26/44	NA	Novel
					c.3196C>T (het)	p.Q1066*	35/78	NA	Novel
A3261-21	11	Gastroschisis	Australia	*NPHP4*	c.2011C>T (het)	p.Q671*	29/55	NA	Novel
					c.3272delT (het)	p.V1091Gfs*31	1,403/2,583	NA	Mollet et al. (2002)
A3411-35	7	No	Egypt	*NPHP4*	c.2044C>T (Horn)	p.R682*	85/85	NA	Mollet et al. (2002)
F824-21	16	No	Turkey	*NPHP4*	c.2145delG (Horn)	p.S716Lfs*6	83/83	NA	Novel
A3863-21	7	No	India	*NPHP4*	c.2265delC (Horn)	p.S756Pfs*12 (p,m)	272/277	NA	Novel
A3228-34	11	Ataxia	Egypt	*NPHP4*	c.2356dupG (Horn)	p.V786Gfs*5	21/24	NA	Novel
A2377-21	15	No	Italy	*NPHP4*	c.2511_2512delAG (het)	p.G838Lfs*2	177/828	NA	Novel
					c.3272delT (het)	p.V1091Gfs*31	1,447/3,746	NA	Mollet et al. (2002)

Patient[c]	Kidney ESRD age (years)	Extrarenal manifestations	Origin	Gene	Nucleotide change[a] (zygosity)	Amino acid change (segregation)	Mutation count/ coverage	PolyPhen2 score[b]	Reference
A1967-26	10	No	Egypt	*NPHP4*	c.2618dupA (Horn)	p.H873Qfs*14 (m)	194/195	NA	Chaki et al. (2011)
A1195-21	15	No	Sweden	*NPHP4*	c.3010dupA (het)	p.T1004Nfs*99	47/148	NA	Otto et al. (2008a)
					c.3866T>C (het)	p.L1289P (m)	1,047/2,03l[d]	1.0	Novel
A2265-21	11	No	Germany	*NPHP4*	c.3272delT (Horn)	p.V1091Gfs*31 (p,m)	3,047/3,107	NA	Mollet et al. (2002)
A3540-22	12	No	Egypt	*NPHP4*	c.3557delT (het)	p.V1186Gfs*ll	979/2,302	NA	Novel
					c.3773_3776delTGAG (het)	p.V1258Gfs*3	105/212	NA	Novel
A4031-21	20	RD	Germany	*IQCBl*	c.273dupT (het)	p.V92Cfs*15	9/18	NA	Novel
					c.1518_1519delCA (het)	p.H506Qfs*13	21/98	NA	Otto et al. (2005)
A3618-21	19	RD	UK	*IQCBl*	c.424_425delTT (Horn)	p.F142Pfs*5	16/21	NA	Otto et al. (2005)
A1973-22	13	RD	USA	*IQCBl*	c.424_425delTT (het)	p.F142Pfs*5	NA	NA	Otto et al. (2005)
					c.897_900dupCTTG (het)	p.I301Lfs*42 (m)	2/8	NA	Halbritter et al. (2012)
F62-21/22	>11/7	LCA, ID, LCA	Germany	*IQCBl*	c.424_425delTT (het)	p.F142Pfs*5 (m)	14/27	NA	Otto et al. (2005)
					c.1518_1519delCA (het)	p.H506Qfs*13 (p)	19/124	NA	Otto et al. (2005)
F849-21	51	RD	France	*IQCBl*	c.758delG (het)	p.C253Sfs*9	5/8	NA	Halbritter et al. (2012)
					c.1381C>T (het)	p.R461*	2/15	NA	Otto et al. (2005)
A1902-21	43	RD	Austria	*IQCBl*	c.825_828delACAG (het)	p.R275Sfs*6	NA	NA	Otto et al. (2005)
					c.1518_1519delCA (het)	p.H506Qfs*13	64/249	NA	Otto et al. (2005)
A4253-21	26	RD	Canada	*IQCBl*	c.897_900dupCTTG (Horn)	p.I301Lfs*42	34/37	NA	Halbritter et al. (2012)
F58-21/24	33/30	RD	The Netherlands	*IQCBl*	c.897_900dupCTTG (het)	p.I301Lfs*42	11/42	NA	Halbritter et al. (2012)
					c.1333C>T (het)	p.R445*	9/33	NA	Halbritter et al. (2012)
A3125-21	22	LCA, pituitary cysts	Canada	*IQCBl*	c.897_900dupCTTG (het)	p.I301Lfs*42	53/112	NA	Halbritter et al. (2012)
					c.1465C>T (het)	p.R489*	90/181	NA	Otto et al. (2008a)
A3084-21	13	RD	Germany	*IQCBl*	c.994C>T (het)	p.R332*	3/4	NA	Otto et al. (2005)
					c.1518_1519delCA (het)	p.H506Qfs*13	30/200	NA	Otto et al. (2005)
A3122-21	17	RD	USA	*IQCBl*	c.1465C>T (het)	p.R489*	84/113	NA	Otto et al. (2008a)
					c.1518_1519delCA (het)	p.H506Qfs*13	23/137	NA	Otto et al. (2005)
A3333-21	14	RD	Turkey	*IQCBl*	c.1504C>T (Horn)	p.R502*	72/73	NA	Estrada-Cuzcano et al. (2011)
A2227-21	15	RD, ASD	USA	*IQCBl*	c.1518_1519delCA (Horn)	p.H506Qfs*13	120/191	NA	Otto et al. (2005)
A4418-21	12	RD	Brazil	*IQCBl*	c.1518_1519delCA (Horn)	p.H506Qfs*13	32/70	NA	Otto et al. (2005)

Patient[c]	Kidney ESRD age (years)	Extrarenal manifestations	Origin	Gene	Nucleotide change[a] (zygosity)	Amino acid change (segregation)	Mutation count/ coverage	PolyPhen2 score[b]	Reference
A4606-21	>10	LCA	Germany	*IQCBl*	c.1518_1519delCA (Horn)	p.H506Qfs*13	63/142	NA	Otto et al. (2005)
F1150-21	7	RD	USA	*IQCBl*	c.1518_1519delCA (Horn)	p.H506Qfs*13	93/179	NA	Otto et al. (2005)
A3535-21	6	OMA, ID	Germany	*CEP290*	c.57_58delCC (het)	p.R20Sfs*7	NA	NA	Novel
					c.828delA (het)	p.E277Kfs*16	45/92	NA	Novel
A1664-21	13	RD	Canada	*CEP290*	c.95T>C (het)	p.L32S	12/32	0.617	Halbritter et al. (2012)
					c.5226+5_5226+8delGTAA (het)	Splice site	19/43	NA	Novel
A4638-21	8	RD	USA	CEP290	c.164_167delCTCA (het)	p.T55Sfs*3	36/91	NA	Helou et al. (2007)
					c.6072C>A (het)	P.Y2024*	9/16	NA	Brancati et al. (2007)
F641-21/22	ND<33	JBTS	Greece	*CEP290*	c.164_167delCTCA (het)	p.T55Sfs*3 (p)	82/217	NA	Helou et al. (2007)
					c.7320_7321delCT (het)	p.L2441Rfs*14 (m)	592/1,167[d]	NA	Novel
A2-21	6	RD, LCA	Australia	*CEP290*	c.270_274delAGTAA (het)	p.K90Nfs*6	90/186[d]	NA	Novel
					c.6277delG (het)	p.V2093Sfs*4 (m)	49/92	NA	Brancati et al. (2007)
A4460-21	9	Dandy-Walker malformation, RD	Egypt	*CEP290*	c.1606C>T (Horn)	p.Q536*	28/28	NA	Novel
A2818-21	1	LF, ID, nystagmus, strabismus	Canada	*CEP290*	c.1936C>T (het)	p.Q646*	27/55	NA	Perrault et al. (2007)
					c.4723A>T (het)	P.K1575*	NA	NA	Perrault et al. (2007)
F283-21	17	RD	Germany	*CEP290*	c.1984C>T het)	p.Q662*	55/163	NA	Baala et al. (2007)
					c.4723A>T (het)	P.K1575*	365/736[d]	NA	Perrault et al. (2007)
A3100-21	16	ID, RD	Slovenia	*CEP290*	c.1987A>T (het)	P.K663*	82/179	NA	Halbritter et al. (2012)
					c.4723A>T (het)	P.K1575*	183/336[d]	NA	Perrault et al. (2007)
A2422-21/22	<1	MKS	Germany/France	*CEP290*	c.2251C>T (het)	p.R751*	16/53	NA	Tory et al. (2009)
					c.4864_4865delinsT (het)	p.R1622Ffs*9	3/3	NA	Novel
A3493-21	1	ID, CVH	Turkey	*CEP290*	c.2457_2458delAA (het)	p.S820Ffs*4	46/99[d]	NA	Novel
					c.5722G>T (het)	P.E1908*	13/21	NA	Brancati et al. (2007)
F335-22	10	RD, LCA	Saudi-Arabia	*CEP290*	c.2915T>C (Horn)	p.L972P	38/38	1.0	Otto et al. (2011)
F91-21	10	RD, JBTS	Germany	*CEP290*	c.3175dupA (het)	p.I1059Nfs*ll	19/47	NA	Sayer et al. (2006)
					c.6331C>T (het)	p.Q2111*	10/16	NA	Sayer et al. (2006)
A4663-21	5	RD, ID	Egypt	*CEP290*	c.3572delA (het)	p.Q1191Rfs*22	26/37	NA	Novel
					c.4792_4795delAAAT (het)	p.K1598Sfs*8	149/288[d]	NA	Novel
A1210-21	22	RD	Germany	*CEP290*	c.3802C>T (het)	p.Q1268* (m)	54/106	NA	Halbritter et al. (2012)

Patient[c]	Kidney ESRD age (years)	Extrarenal manifestations	Origin	Gene	Nucleotide change[a] (zygosity)	Amino acid change (segregation)	Mutation count/ coverage	PolyPhen2 score[b]	Reference
					c.4723A>T (het)	P.K1575*	586/l,109[d]	NA	Perrault et al. (2007)
F891-21	5	Dandy-Walker malformation	Germany	*CEP290*	c.4144delT (het)	p.Y1382Mfs*37	7/20	NA	Novel
					c.6277delG (het)	p.V2093Sfs*4	40/85	NA	Brancati et al. (2007)
F118-21	>10	RD	Austria	*CEP290*	c.4452_4455delAGAA (het)	p.K1484Nfs*4	NA	NA	Halbritter et al. (2012)
					c.4723A>T (het)	P.K1575*	422/760[d]	NA	Perrault et al. (2007)
F351-21	ND	RD	Germany	*CEP290*	c.5182G>T (het)	P.E1728*	NA	NA	Otto et al. (2011)
					c.6277delG (het)	p.V2093Sfs*4	47/47	NA	Brancati et al. (2007)
A1048-21	ND	ND	Turkey	CEP290	c.5714delC (Horn)	p.A1905Vfs*5	15/15	NA	Novel
A1413-22	>3 mo	ID, JBTS, pituitary cysts,	Germany	*CEP290*	c.5714delC (Horn)	p.A1905Vfs*5 (p,m)	7/7	NA	Novel
A1924-21	15	No	Turkey	*GLIS2*	c.523T>C (Horn)	p.C175R	368/368	1.0	Novel
F99-21	17	RD	Germany	*SDCCAG8*	c.679A>T (het)	P.K227* (p)	38/55	NA	Otto et al. (2010)
					c.784G>T (het)	P.E262* (m)	41/58	NA	Novel
A3945-21	5	No	Turkey	*SDCCAG8*	c.696T>G (Horn)	P.Y232*	5/5	NA	Otto et al. (2010)
A4665-21	>15	RD, PCO, HM	USA	*SDCCAG8*	c.1444delA (Horn)	p.T482Lfs*12	16/16	NA	Otto et al. (2010)
A4313-21	6	JBTS	UK	*TMEM67*	c.407-2A>G (het)	Splice site	17/38	NA	Novel
					c.1918G>A (het)	p.D640N	56/163[d]	1.0	Novel
A2431-21/22	<1	MKS	UK	*TMEM67*	c.579_580delAG (het)	p.G195Ifs*13	3/3	NA	Brancati et al. (2009)
					c.622A>T (het)	p.R208*	9/18	NA	Khaddour et al. (2007)
A4485-21	4	JBTS, heart anomalies (VSD), VUR	Poland	*TMEM67*	c.579_580delAG (het)	p.G195Ifs*13	44/89[d]	NA	Brancati et al. (2009)
					c.1843T>C (het)	p.C615R	18/34	0.98	Tallila et al. (2009)
F1431-21	<1	JBTS	Germany	*TMEM67*	c.622A>T (het)	p.R208*	4/8	NA	Khaddour et al. (2007)
					c.1538A>G (het)	p.Y513C	22/42[d]	1.0	Novel
A382-21	ND	RD	Italy	*TMEM67*	c.622A>T (het)	p.R208*	4/7	NA	Khaddour et al. (2007)
					c.1289A>G (het)	p.D430G	16/24	0	Halbritter et al. (2012)
F912-21	20	LF, Morning glory papillary	Germany	*TMEM67*	c.622A>T (het)	p.R208*	2/3	NA	Khaddour et al. (2007)
					c.2498T>C (het)	P.I833T	121/279[d]	0.97	Brancati et al. (2009)
A4019-21	6	LF	Australia	*TMEM67*	c.726T>G (het)	P.N242K	1,526/3,016[d]	1.0	Novel
					c.1843T>C (het)	p.C615R	13/24	0.98	Tallila et al. (2009)
A3473-21	ND	JBTS, LF	UK	*TMEM67*	c.755T>C (het)	P.M252T (p)	24/65	0.38	Khaddour et al. (2007)

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					c.2498T>C (het)	P.I833T (m)	152/285[d]	0.97	Brancati et al. (2009)
F631-21	<21	JBTS, LF	Germany	*TMEM67*	c.1046T>C (het)	p.L349S	694/1,265	0.95	Khaddour et al. (2007)
					c.1843T>C (het)	p.C615R (p)	6/14	0.98	Tallila et al. (2009)
A3858-21	>7	LF, Nystagmus	Czech	*TMEM67*	c.1815_1831del17 (het)	p.Q605Hfs*17 (m)	NA	NA	Novel
			Republic		c.1843T>C (het)	p.C615R (p)	13/13	0.98	Tallila et al. (2009)
A3187-21	10	HSM	USA	*TMEM67*	c.1843T>C (Horn)	p.C615R	20/20	0.98	Tallila et al. (2009)
A4439-21	14	HSM	Czech Republic	*TMEM67*	c.1843T>C (Horn)	p.C615R	26/26	0.98	Tallila et al. (2009)
F529-21	<10	No	Germany	*TMEM67*	c.1843T>C (Horn)	p.C615R	26/26	0.98	Tallila et al. (2009)
A3669-21	9	RD	Poland	*TMEM67*	c.1843T>C (het)	p.C615R (m)	6/12	0.98	Tallila et al. (2009)
					c.2345A>G (het)	p.H782R (p)	3/5	0.96	Brancati et al. (2009)
A3260-21	3	SI, polysplenia, GIT malformation, PD	USA	*TTC21B*	c.264_267dupTAGA (Horn)	p.E90*	35/37	NA	Novel
A999-21	2	LF	Germany	*TTC21B*	c.626C>T (het)	p.P209L	2,898/5,811[d]	1.0	Davis et al. (2011)
					c.1240G>T (het)	P.E414*	3/3	NA	Novel
A4291-21	3	LF, cone-shaped epiphysis (hands/feet)	USA	*TTC21B*	c.626C>T (het)	p.P209L	2,608/5,486[d]	1.0	Davis et al. (2011)
					c.2868+lG>T (het)	Splice site	471/853	NA	Novel
A1065-21	10	SI, Hepatopathy	Germany	*TTC21B*	c.626C>T (het)	p.P209L	52/113	1.0	Davis et al. (2011)
					c.3923A>G (het)	p.D1308G	36/68	1.0	Novel
A3511-21/22	>8	Chondrodysplasia, Bell's palsy, hypertension	UK	*TTC21B*	c.1231C>T (het)	p.R411*	3/3	NA	Davis et al. (2011)
					c.1445dupA (het)	p.T483Dfs*25	30/93	NA	Novel
F1229-21	17	RD	Spain	*WDR19*	c.641dupT (het)	p.L214Ffs*5	64/200	NA	Novel
					c.1477G>C (het)	p.D493H	183/383[d]	0.99	Novel
A2556-21/22	5	Caroli disease	Egypt	*WDR19*	c.682C>T (het)	p.Q228*	71/108	NA	Novel
					c.3703G>A (het)	P.E1235K	1,125/2,262[d]	1.0	Novel
A4436-22	<1	PD, Caroli disease, RD	Oman	*WDR19*	c.3533G>A (Horn)	p.R1178Q	294/296	1.0	Novel
A3241-21	<1	Cortical blindness, pancreatic cysts, hepatic cysts	USA	*WDR19*	c.3533G>A (het)	p.R1178Q	2,701/5,319[d]	1.0	Novel
					c.3565+lG>A (het)	Splice site	22/48	NA	Novel

Mutation numbering is based on cDNA position according to reference sequences of *NPHP1* (NM_000272.3), *INVS* (NM_014425.2), *NPHP3* (NM_153240.4), *NPHP4* (NM_015102.3), IQCB1 (NM_001023570.2), *CEP290* (NM_025114.3), *GLIS2* (NM_032575.2), *SDCCAG8* (NM_006642.3), *TMEM67* (NM_153704.5), *TTC21B* (NM_024753.4) and *WDR19* (NM_025132.3) with +1 corresponding to the A of the ATG translation initiation codon

ASD atrial septal defect, *BDP* bilary ductal proliferation, *CAKUT* congenital anomalies of the kidney and urinary tract, CVH cerebellar vermis hypoplasia, *ESRD* end-stage renal disease, *GIT* gastrointestinal tract, (*het*) heterozygous mutation, (*Hom*) homozygous mutation, *HM* hepatomegaly, *HSM* hepato-splenomegaly, *ID* intellectual disability, JBTS Joubert syndrome, *LCA* Leber congenital amaurosis, *LF* liver fibrosis, (*m*) maternal heterozygous mutation, *MKS* Meckel–Gruber syndrome, *NA* not applicable, *ND* no data available, *OMA* ocular motor apraxia, (*p*) paternal heterozygous mutation, *PCO* polycystic ovaries, *PD* polydactyly, *PFO* patent foramen ovale, *RD* retinal dystrophy, *SI* situs inversus, *T1 DM* type 1 diabetes mellitus, *VSD* ventricular septal defect, *VUR* vesicoureteral reflux

[a] All mutations were absent from at least 192 healthy control subjects

[b] PolyPhen-2 scores above 0.9 are predicted to be disease causing

[c] Samples found in pilot run and included as positive controls are underlined

[d] Samples sequenced on Illumina MiSeq Personal Sequencer

Table 3

31 single heterozygous truncating or obligatory splice variants in 31 different patients in *NPHP1*, *INVS*, *NPHP3*, *NPHP4*, *IQCB1*, *CEP290*, *RPGRIP1L*, *SDCCAG8*, *TMEM67*, *TTC21B* and *WDR19*

Patient[c]	Kidney ESRD (yrs)	Extrarenal manifestations	Origin	Gene	Nucleotide change[a] (zygosity)	Amino acid change (segregation)	Mutation count/coverage	EVS (exome variant server)	Reference
F1369-21[#]	>16	No	Germany	*NPHP1*	c.1274dupT (het)	p.R426Qfs*7	573/701	NA	Novel
A903-21	16	Pulmonary stenosis, microcephaly, LF	Turkey	*INVS*	c.465G>A (het)	p.W155*	93/209	NA	Novel
A1936-22	12	Nystagmus	Candada	*INVS*	c.1078+1G>A (het)	Splice site	28/44	A= 1 G = 13,005	Novel
F964-21	ND	ND	Germany	*INVS*	c.2069-1G>T (het)	Splice site	58/84	NA	Novel
A10-21	ND	ND	France	*INVS*	c.2908delG (het)	p.E970Nfs*2	116/231	NA	Otto et al. (2003)
F1135-21	>14	OMA, JBTS	Germany	*NPHP3*	c.2104C>T (het)	p.R702*	585/1,230	NA	Simpson et al. (2009)
A918-21	<1	Neonatal hepatitis	Turkey	*NPHP3*	c.2570+lG>T (het)	Splice site	139/304	NA	Halbritter et al. (2012)
A3865-21	11	LF	Germany	*NPHP3*	c.2694-2_2694-ldelAG (het)	Splice site	5/14	NA	Bergmann et al. (2008)
A4419-12	2	ID	USA	*NPHP3*	c.2694-2_2694-ldelAG (het)	Splice site	8/15	NA	Bergmann et al. (2008)
A3843-21	>10	No	USA	*NPHP4*	c.133C>T (het)	p.Q45*	162/287	T= 1 C = 12,485	Novel
A165-21	11	Developmental delay	Canada	*NPHP4*	c.517C>T (het)	p.Q173*	669/1,497	NA	Novel
A385-21	ND	RD	Germany	*NPHP4*	c.1956-2A>G (het)	Splice site	21/48	NA	Novel
F1348-21	12	RD	Germany	*NPHP4*	c.1956-2A>G (het)	Splice site	90/138	NA	Novel
A821-21	>1	No	Germany	*IQCB1*	c.1632_1638dupTGTGGCA (het)	p.A547Cfs*31	19/22	NA	Novel
F1051-21	>5	No	Sweden	*CEP290*	c.1992delT (het)	p.P665Lfs*10	56/107	NA	Perrault et al. (2007)
F1386-23	14	Dental and skeletal malformations	Poland	*CEP290*	c.1992delT (het)	p.P665Lfs*10	114/190	NA	Perrault et al. (2007)

Patient[c]	Kidney ESRD (yrs)	Extrarenal manifestations	Origin	Gene	Nucleotide change[a] (zygosity)	Amino acid change (segregation)	Mutation count/coverage	EVS (exome variant server)	Reference
F122-22	7	JBTS	Germany	*CEP290*	c.2249T>G (het)	p.L750*	13/18	NA	Den Hollander et al. (2006)
F417-22	25	LF	Germany	*CEP290*	c.3175dupA (het)	p.I1059Nfs*ll	56/182	NA	Sayer et al. (2006)
A711-21	ND	LCA	Canada	*CEP290*	c.4966G>T (het)	P.E1656*	285/539	NA	Den Hollander et al. (2006)
A2615-21	>1	JBTS	Germany	*CEP290*	c.6277delG (het)	p.V2093Sfs*4	29/69	NA	Brancati et al. (2007)
A2156-21	ND	PD, microcephaly, VUR	USA	*RPGRIP1L*	c.1700-1G>A (het)	Splice site	Sanger	NA	Novel
A963-21	12	RD	Spain	*SDCCAG8*	c.1420delG (het)	p.E474Sfs*20	18/46	NA	Otto et al. (2010)
A1010-21	>10	HMSN type 1	Germany	*TMEM67*	c.622A>T (het)	p.R208*	Sanger	T= 1 A = 13,005	Khaddour et al. (2007)
F128-21	ND	JBTS	Germany	*TMEM67*	c.622A>T (het)	p.R208*	14/22	T= 1 A = 13,005	Khaddour et al. (2007)
F1392-21	>10	No	Germany	*TMEM67*	c.622A>T (het)	p.R208*	8/12	T= 1 A = 13,005	Khaddour et al. (2007)
F1307-21	>1	ND	Germany	*TMEM67*	c.1774-1G>A (het)	Splice site	21/54	NA	Novel
F1369-21[#]	16	No	Germany	*TTC21B*	c.93delG (het)	p.R32Gfs*17	56/164	NA	Novel
A4609-21	>19	No	Taiwan	*TTC21B*	c.264_267dupTAGA (het)	p.E90*	12/39	NA	Novel
F889-21	12	RD	Turkey	*WDR19*	c.407-2A>G (het)	Splice site	125/364	NA	Novel
F754-22	>7	No	USA	*WDR19*	c.781dupA (het)	p.T261Nfs*13	15/27	NA	Novel
A4395-21/22	5	JATD	USA	*WDR19*	c.781dupA (het)	p.T261Nfs*13	6/13	NA	Novel

Mutation numbering is based on cDNA position according to reference sequences of *NPHP1* (NM_000272.3), *INVS* (NM_014425.2), *NPHP3* (NM_153240.4), *NPHP4* (015102.3), *IQCB1* (NM_001023570.2), *CEP290* (NM_025114.3), *GLIS2* (NM_032575.2), *RPGRIP1L* (NM_015272.2), *SDCCAG8* (NM_006642.3), *TMEM67* (NM_153704.5), *TTC21B* (NM_024753.4) and *WDR19* (NM_025132.3) with +1 corresponding to the A of the ATG translation initiation codon

ESRD end-stage renal disease, (*het*) heterozygous mutation, *HMSN* hereditary motor and sensory neuropathy, *ID* intellectual disability, *JATD* Jeune asphyxiating thoracic dystrophy, *JBTS* Joubert syndrome, *LCA* Leber congenital amaurosis, *LF* liver fibrosis, *NA* not applicable, *ND* no data available, *OMA* ocular motor apraxia, *PD* polydactyly, *RD* retinal dystrophy, *VUR* vesicoureteral reflux

[a] All mutations were absent from at least 192 healthy control subjects

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[b] Polyphen 2 scores >0.9 are predicted to be disease causing

[c] Samples found in pilot run and included as positive controls are underlined

[#] Note that F1369-21 has one variant in two different genes

Table 4

Novel mutations found in this study compared with previously reported mutations (HGMD®-Professional "Biobase") in the genes *NPHP1–NPHP13*

Gene	Biobase (# mut)	Novel (# mut)	Percent added
NPHP1	27	14	52
INVS/NPHP2	20	6	30
NPHP3	31	16	52
NPHP4	59	26	44
IQCB1/NPHP5	21	2	10
CEP290/NPHP6	146	12	8
GLIS2/NPHP7	1	1	100
RPGRIP1L/NPHP8	31	1	3
NEK8/NPHP9	4	–	–
SDCCAG8/NPHP10	13	1	8
TMEM67/NPHP11	102	6	6
TTC21B/NPHP12	33	6	18
WDR19/NPHP13	5	8	160
Total	493	99	20