MUTATION IN BRIEF

Mutational Analysis of the *NPHP4* Gene in 250 Patients with Nephronophthisis

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Nephronophthisis (NPH), a recessive cystic kidney disease, is the most frequent genetic cause for end-stage renal disease in the first two decades of life. Mutations in three genes (*NPHP1*, *2*, and *3*) were identified as causative. Extrarenal manifestations are known, such as retinitis pigmentosa (Senior-Løken syndrome, SLS) and ocular motor apraxia type Cogan. Recently, we identified a novel gene (*NPHP4*) as mutated in NPH. To date, a total of only 13 different *NPHP4* mutations have been described. To determine the frequency of *NPHP4* mutations, we performed mutational analysis by direct sequencing of all 30 *NPHP4* exons in 250 different patients with isolated NPH, SLS, or Cogan syndrome ascertained worldwide over 14 years. We identified 23 novel *NPHP4* sequence variants in 26/250 different patients (10%). Interestingly, we detected homozygous or compound heterozygous mutations of *NPHP4* in only 6/250 different patients (2.4%), but only one heterozygous *NPHP4* sequence variant in 20/250 different patients (8%). In the six patients with two *NPHP4* mutations, 5/8 mutations (63%) were likely loss-of-function mutations, whereas in the 20 patients with only one sequence variant, only 1/20 (5%) was a likely loss-of-function (i.e., truncating) mutation. We conclude that: i) two recessive mutations in *NPHP4* are a rare cause of nephronophthisis; ii) single heterozygous *NPHP4* sequence variants are three times more prevalent than two recessive mutations; iii) there is no genotype/phenotype correlation; iv) there must exist further genes causing nephronophthisis, since in 224/250 (90%) patients, no sequence variants in either of the four NPH genes were detected. © 2005 Wiley-Liss, Inc.

KEY WORDS: nephronophthisis; *NPHP4*; Senior-Løken syndrome; Cogan syndrome; mutational analysis

INTRODUCTION

Nephronophthisis (NPH) is an autosomal recessive cystic kidney disease that represents the most common genetic cause for end-stage renal disease in children and young adults (Hildebrandt et al., 2001). NPH leads to end-stage renal disease at a median age of 13 years. Characteristic renal histology shows a triad of renal interstitial fibrosis,

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interstitial cell infiltrates, and tubular atrophy with cyst development at the corticomedullary junction (Waldherr et al., 1982). NPH is a genetically heterogenous disorder. Four genes have been identified: Recessive mutations in *NPHP1* cause NPH type 1 (juvenile onset) (MIM# 256100) (Hildebrandt et al., 1997, Saunier et al., 1997). Its gene product nephrocystin (NPHP1) is a novel docking protein that plays a role in signaling at adherens junctions and focal adhesions of renal epithelial cells. Mutations in the *NPHP2* gene encoding *inversin* cause NPH type 2 (infantile onset) (MIM# 602088) (Haider et al., 1998, Otto et al., 2003). Mutations in *NPHP3* and its gene product nephrocystin-3 are responsible for NPH type 3 (adolescent form) (MIM# 604387, 608002) (Olbrich et al., 2003, Omran et al., 2000). Finally, we have identified mutations in *NPHP4* encoding nephrocystin-4/nephroretinin as causing NPH type 4 (juvenile onset) (MIM# 607215, 606966, 606996) (Mollet et al., 2002, Otto et al., 2002, Schuermann et al., 2002). With the exception of *inversin*, the NPH-causing genes represent novel genes. Recent evidence demonstrated expression of all 4 genes (*NPHP1-4*) in primary cilia of renal epithelial cells, a feature that has been recognized for virtually all proteins which, if defective give rise to renal cystic disease in mice or humans (Watnick et al., 2003). The gene products of the *NPHP2*, *3* and *4* genes bind to nephrocystin, the gene product of the *NPHP1* gene (Mollet et al., 2002). A number of extrarenal manifestations can be associated with NPH, such as retinitis pigmentosa (Senior-Løken syndrome, SLS) (Loken et al., 1961, Senior et al., 1961), ocular motor apraxia (Cogan syndrome) (Betz et al., 2000, Mollet et al., 2002), or liver fibrosis (Boichis et al., 1973). Retinitis pigmentosa is associated with NPH in about 10% of cases (Caridi et al., 1998). So far no genotype/phenotype correlation regarding the type of *NPHP* gene mutated or regarding distinct mutated alleles of these genes have been detected. The recently identified *NPHP4* gene extends over 130 kb, consists of 30 exons, and encodes 1426 amino acids. *NPHP4* is strongly conserved in evolution, with 23% amino acid identity in a protein of *C. elegans* (Mollet et al., 2002, Otto et al., 2002). So far only 13 different mutations in *NPHP4* have been reported in 17 different patients (Mollet et al., 2002, Otto et al., 2002).

To determine the frequency of *NPHP4* mutations in a large cohort of patients with NPH, we here performed mutational analysis by direct sequencing of all 30 exons of *NPHP4* in 250 unrelated NPH patients. We identified 23 novel *NPHP4* sequence variants in 26 unrelated patients with NPH. We detected both recessive mutations of *NPHP4* in only 6/250 different patients (2.4%), whereas we detected only one heterozygous *NPHP4* sequence variant in 20/250 different patients. From these data we conclude, i) mutations in *NPHP4* are a rare cause (2.4%) of nephronophthisis; ii) there is no genotype/phenotype relationship regarding extrarenal manifestations; iii) there must exist further genes causing nephronophthisis.

PATIENTS AND METHODS

Patients

For mutation screening in *NPHP4* we selected from a cohort of 375 patients with NPH on the basis of DNA availability 250 unrelated patients with at least one child affected by isolated NPH, SLS or Cogan syndrome. The diagnostic criteria were as follows: i) characteristic clinical phenotype of NPH, SLS or Cogan syndrome, as established by a pediatric nephrologist using a standard clinical questionnaire (www.renalgenes.org); ii) renal ultrasound or a renal biopsy result compatible with NPH; iii) exclusion of a homozygous deletion in *NPHP1*; iv) pedigree compatible with autosomal recessive inheritance. Renal biopsy result was considered characteristic for NPH if there was disruption of tubular basement membranes, tubulointerstitial round cell infiltration and fibrosis, and tubular atrophy with microcyst development (Waldherr et al., 1982). Renal ultrasound was considered characteristic for NPH if there was increased echogenicity with loss of corticomedullary differentiation or presence of corticomedullary cysts. Origin of patients was worldwide with an emphasis on European descent. We obtained blood samples, pedigrees, and clinical information following informed consent (www.renalgenes.org). To assess the genetic data about the frequency of *NPHP4* mutations, we evaluated only one sibling per family. This cohort of 250 unrelated patients consisted of 190 patients with isolated NPH (76%), 50 patients with SLS (20%), and 10 patients with Cogan syndrome (4%). Consanguinity was present in 9/190 NPH patients (5%), 6/50 SLS patients (12%), and in 1/10 patients with Cogan syndrome (10%). The diagnosis of NPH was confirmed by renal biopsy in 61/190 NPH patients (32%), 4/50 SLS patients (8%), and 1/10 patients with Cogan syndrome (10%). For all patients we had previously excluded a homozygous deletion of *NPHP1*, a combination of a heterozygous *NPHP1* deletion with a heterozygous *NPHP1* point mutation, and mutations in *NPHP3*. The diagnosis of infantile NPHP (NPHP2) was excluded by absence of the NPHP2-specific criterion of end-stage renal disease occurring within the first 5y of life.

Mutational analysis

Genomic DNA was extracted from blood samples using the QIAGEN Blood & Cell Culture DNA kit according to the manufacturer's instructions (Qiagen, Valencia, CA). For one individual from each family (250 patients) direct sequencing was performed for all 30 *NPHP4* exons on both strands using the dideoxy chain termination method on an ABI capillary sequencer. Resulting sequences were aligned and analyzed with the Sequencher™ software (Gene Codes Corporation, Ann Arbor, MI) and Mutation Surveyor™. Primers flanking the 30 exons of *NPHP4* were derived from genomic sequence, using the program PRIMER3 (http://zeon.well.ox.ac.uk/git-bin/primer3_www.cgi) (GenBank accession no. NT_015102, which corresponds to the published cDNA and amino acid sequences. Numbering is based on cDNA sequence. Position +1 corresponds to the A of the ATG translation initiation codon in the reference sequence. Mollet et al., 2002, Otto et al., 2002) . Primer sequences are available from the authors. For segregation analyses parental DNA, if available, was tested by direct sequencing. For mutational screening of healthy control individuals we employed allele-specific restriction digests, denaturing high-performance liquid chromatography (Transgenomic Wave™), or direct sequencing, as appropriate. For all patients with sequence variants in *NPHP4* direct sequencing was performed for all 17 exons of *NPHP2* on one strand using the dideoxy chain termination method on an ABI capillary sequencer.

RESULTS

In a cohort of 250 unrelated patients with NPH, SLS or Cogan syndrome, we performed mutational analysis by direct sequencing of both strands for all 30 exons of *NPHP4* (a total of 15,000 sequences). We detected altogether 23 different novel *NPHP4* sequence variants in a total of 26/250 unrelated patients (10%) (Table 1). However, in only 6/250 families (2.4%) we detected both mutated alleles, i.e. two heterozygous mutations or one homozygous mutation of *NPHP4* (Table 1). Most mutations were truncating mutations. Both missense mutations detected in F720 are conserved in *Nphp4* of mouse and *nph-4* of *C. elegans*. In contrast, in 20/250 families (8%) we found only a single heterozygous sequence variant of *NPHP4* (Table 1). All sequence variants were novel, i.e. they have not been reported previously (Mollet et al., 2002, Otto et al., 2002). All mutations were absent from at least 86 healthy control individuals and were not previously described as polymorphisms (Table 2). A total of 224/250 (90%) patients did not show any sequence variant in *NPHP4*. The total of 26 sequence variants consisted of 23 different novel sequence variants (Table 1). Of the patients, in whom only one sequence variant was found, 4 patients shared a mutation with at least one other patient (F270 with F1015, F94 with F726, F306 with F567, and F491 with F736). Of the patients in whom both *NPHP4* mutations were found, only 1 patient shared a mutation (F720 with F534) (Table 1). All shared sequence variants were missense. For none of the patients with *NPHP4* sequence variants parental consanguinity was reported. In the 3 families with 2 affected children the 2 recessive mutations and 1 single heterozygous sequence variant were present in each of the affected individuals. We also detected 6 additional new polymorphisms in *NPHP4*, that were at least detected once in > 86 healthy control individuals (Table 3). We thus identified 23 different novel *NPHP4* sequence variants in 26 unrelated families.

In the 6 families where both *NPHP4* mutations were detected (Table 1), 5 of the 8 different mutations detected (63%) were truncating mutations (nonsense or frame shift). In contrast, in the 20 families with only one heterozygous *NPHP4* sequence variant, only 1/20 mutations (5%) was a truncation mutation (c.1839_1840insGA), whereas the others were missense sequence variants (Table 1). Thus, the likelihood to detect a truncating mutation in patients with two mutated *NPHP4* alleles was 12.6 times as high (63% *vs*. 5%) as in patients with only one heterozygous sequence variant. Although the sequence variants identified as single heterozygous missense changes were absent from > 86 healthy controls, we cannot exclude that they might represent innocuous polymorphisms.

The presence or absence of extrarenal manifestation (retinitis pigmentosa, Cogan syndrome, hearing loss, or chronic bronchitis) did not correlate with either, i) the position of a mutation within the nephrocystin-4/nephroretinin sequence; ii) the type of mutation (truncating vs. non-truncating) or; iii) the fact whether both or only one *NPHP4* sequence variant was found (Table 1).

Table 1. Twenty-three Novel *NPHP4* Sequence Variants Detected in 26 NPH Families With and Without Extrarenal Manifestations

Family	Origin	Number of affecteds	Age at ESRD (yrs)	Nucleotide change[a]	Amino acid change	Evolutionary conservation	Exon, (segregation)	Renal cysts	Renal biopsy	Extrarenal manifestations
Families with both mutated *NPHP4* alleles detected										
F892	Netherlands	2	not yet	c.148delG c.1892_1895delAGAA	p.Val79fs p.Gln631fs	na na	3 (het, P) 15 (het, M)	+, nd	+, nd	Chronic bronchitis, nd
F720	Germany	1	not yet	c.1405C>T c.1961C>G	p.Arg469Trp p.Ala654Gly	mo, ce mo, ce	11 (het) 16 (het)	+	-	-
F88	Italy	1	14	c.1462C>T	p.Arg488X	na	12 (hom)	-	+	-
F617	USA	1	nd	c.2608_2617dupTGGAAGCTCA	p.Arg870fs	na	19 (hom)	-	+	Usher syndrome (hearing loss and RP)
F456	Italy	1	24	c.2836A>G	p.Thr946Ala	-	21 (hom)	-	+	RP
F704	Turkey	2	9, nd	c.3149_3150insC	p.Gln1050fs	na	22 (hom)	+	+	-
Families with only one single *NPHP4* sequence variant detected										
F270	Germany	1	nd	c.7G>T	p.Asp3Tyr	mo	2 (het)	nd	nd	RP
F1015	Germany	1	nd	c.7G>T	p.Asp3Tyr	mo	2 (het)	nd	nd	nd
F94	USA	2	nd	c.271T>C	p.Phe91Leu	mo	3 (het, M)	nd	nd	RP
F726	Germany	1	nd	c.271T>C	p.Phe91Leu	mo	3 (het)	nd	nd	nd
F719	Serbia	1	12	c.1024C>T	p.Arg342Cys	ce	9 (het)	nd	-	-
F848	Italy	1	40	c.1880C>T	p.Thr627Met	-	15 (het)	nd	+	RP
F116	Greece	1	nd	1839_1840insGA	p.Lys614fs	na	15 (het)	-	+	RP
F534	Germany	1	not yet	c.1961C>G	p.Ala654Gly	ce	16 (het)	nd	nd	nd
F586	Turkey	1	13	c.2213C>T	p.Arg735Trp	-	17 (het)	nd	+	-
F1142	Belgium	1	nd	c.2297A>G	p.Gln766Arg	mo	17 (het)	nd	+	Color blindness
F472	Germany	1	not yet	c.2327C>G	p.Pro776Arg	mo	18 (het)	-	+	-
F620	Germany	nd	nd	c.2346C>A	p.His782Gln	-	18 (het)	nd	nd	nd
F640	Germany	1	nd	c.2882G>A	p.Arg961His	-	21 (het)	+	+	-
F306	Russia	1	14	c.3292G>A	p.Ala1098Thr	mo, ce	23 (het)	nd	nd	nd
F567	Germany	1	nd	c.3292G>A	p.Ala1098Thr	mo, ce	23 (het, P)	nd	nd	Cogan syndrome
F491	Germany	1	nd	c.3574C>T	p.Arg1192Trp	mo	26 (het)	nd	nd	nd
F736	nd	1	nd	c.3574C>T	p.Arg1192Trp	mo	26 (het)	nd	nd	nd
FA2	Australia	1	6	c.3674C>T	p.Thr1225Met	mo, ce	27 (het, P)	+	+	RP, DD
F697	Germany	1	nd	c.3850C>T	p.Arg1284Cys	-	28 (het)	+	+	nd
F441	Switzerland	1	nd	c.3859C>G	p.Gln1287Glu	mo	28 (het)	+	-	Hearing loss

[a]All sequence variants were absent from at least 86 healthy control individuals; numbering based on cDNA, Position +1 corresponds to the A of the ATG translation initiation codon in the reference sequence NM_015102; ce, amino acid residue conserved in *C. elegans*; DD, developmental delay; ESRD, end-stage renal disease; M, maternal; mo, amino acid residue conserved in mouse; na, not applicable; nd, no data available; P, paternal; RP, retinitis pigmentosa (Senior-Løken syndrome).

Table 2. Spectrum of All 35 *NPHP4* Sequence Variants Known, Including 23 Novel Sequence Variants Detected in this Study

Type of sequence variant	Exon	Nucleotide change	Amino acid change	Segregation[a]	Extrarenal manifestations[b]	Origin	Reference
Nonsense	11	c.1334_1335TC>AA	p.Phe445X	hom	-	India	(Otto et al., 2002)
	12	c.1462C>T	p.Arg488X	hom	-	Italy	Present study
	16	c.1972C>T	p.Arg658X	hom	RP	France	(Mollet et al., 2002, Otto et al., 2002)
	16	c.2044C>T	p.Arg682X	het, sm	-	France	(Mollet et al., 2002, Otto et al., 2002)
	18	c.2335C>T	p.Glu779X	hom	RP	Turkey	(Otto et al., 2002)
	18	c.2368G>T	p.Glu790X	hom	-	Afghanistan	(Mollet et al., 2002, Otto et al., 2002)
	18	c.2377C>T	p.Gln793X	hom	-	Italy	(Mollet et al., 2002)
Missense	2	c.7G>T	p.Asp3Tyr	het, sm (x2)	RP (x1)	Germany	Present study (x2)
	3	c.271T>C	p.Phe91Leu	het, sm (x2)	RP (x1)	USA/Germany	Present study (x2)
	9	c.1024C>T	p.Arg342Cys	het, sm	-	Serbia	Present study
	11	c.1405C>T	p.Arg469Trp	het, sm	-	Germany	Present study
	15	c.1880C>T	p.Thr627Met	het, sm	RP	Italy	Present study
	16	c.1961C>G	p.Ala654Gly	het, sm (x2)	-	Germany	Present study (x2)
	17	c.2213C>T	p.Arg735Trp	het, sm	-	Turkey	Present study
	17	c.2260G>A	p.Gly754Arg	het	-	Germany	(Otto et al., 2002)
	17	c.2297A>G	p.Gln766Arg	het, sm	OA	Belgium	Present study
	18	c.2327C>G	p.Pro776Arg	het, sm	-	Germany	Present study
	18	c.2346C>A	p.His782Gln	het, sm	-	Germany	Present study
	21	c.2836A>G	p.Thr946Ala	hom	RP	Italy	Present study
	21	c.2882G>A	p.Arg961His	het, sm	-	Germany	Present study
	21	c.2972T>C	p.Phe991Ser	hom	-	North Africa	(Mollet et al., 2002)
	23	c.3292G>A	p.Ala1098Thr	het, sm (x2)	Cogan (x1)	Russia/Germany	Present study (x2)
	26	c.3574C>T	p.Arg1192Trp	het, sm (x2)	-	Germany	Present study (x2)
	27	c.3674C>T	p.Thr1225Met	het, sm	RP	Australia	Present study
	28	c.3850C>T	p.Arg1284Cys	het, sm	-	Germany	Present study
	28	c.3859C>G	p.Gln1287Glu	het, sm	Loss of Hearing	Switzerland	Present study
Deletions	3	c.148delG	p.Val79fs	het	Bronchitis	Netherlands	Present study
	15	c.1892-1895delAGAA	p.Gln631fs	het	Bronchitis	Netherlands	Present study
	23	c.3272delT	p.Val1091fs	hom, het, sm	OA, Cogan syndrome	Germany/France	(Mollet et al., 2002, Otto et al., 2002) (x2)
Insertions	15	c.1839_1840insGA	p.Lys614fs	het, sm	RP	Greece	Present study
	19	c.2608_2617dupTGGAAGCTCA	p.Arg870fs	hom	Usher syndrome	USA	Present study
	22	c.3149_3150insC	p.Gln1050fs	hom	-	Turkey	Present study
Splice site	IVS15	c.1955+1G>A	splice error	het	-	Finland	(Otto et al., 2002)
	IVS16	c.2144-1G>C	splice error	het	-	Germany	(Otto et al., 2002)
	IVS24	c.3472+1G>A	splice error	het	-	Finland	(Otto et al., 2002)

[a]het, heterozygous mutation; hom, homozygous mutation; sm, single mutation; numbering based on cDNA, Position +1 corresponds to the A of the ATG translation initiation codon in the reference sequence NM_015102; [b]OA, ophthalmologic abnormalities (unspecified); RP, retinitis pigmentosa (Senior-Løken syndrome).

Table 3. Spectrum of Polymorphisms

Type of sequence variant	Exon	Nucleotide change	Amino acid change	Reference
Polymorphisms[a]	2	c.86C>T	p.Thr29Met	Present study
	14	c.1631C>G	p.Ala544Gly	Present study
	15	c.1852G>A	p.Glu618Lys	Present study
	17	c.2219G>A	p.Arg740His	(Mollet et al., 2002)
	17	c.2293G>A	p.Val765Ile	Present study
	19	c.2542C>T	p.Arg848Trp	(Mollet et al., 2002)
	21	c.2818_2822delGCGCAG	p.940_941delAlaGln	(Mollet et al., 2002)

[a]The term polymorphism was used if the sequence variant was detected in at least 1 out of >172 chromosomes from healthy control individuals; numbering based on cDNA, Position +1 corresponds to the A of the ATG translation initiation codon in the reference sequence NM_015102.

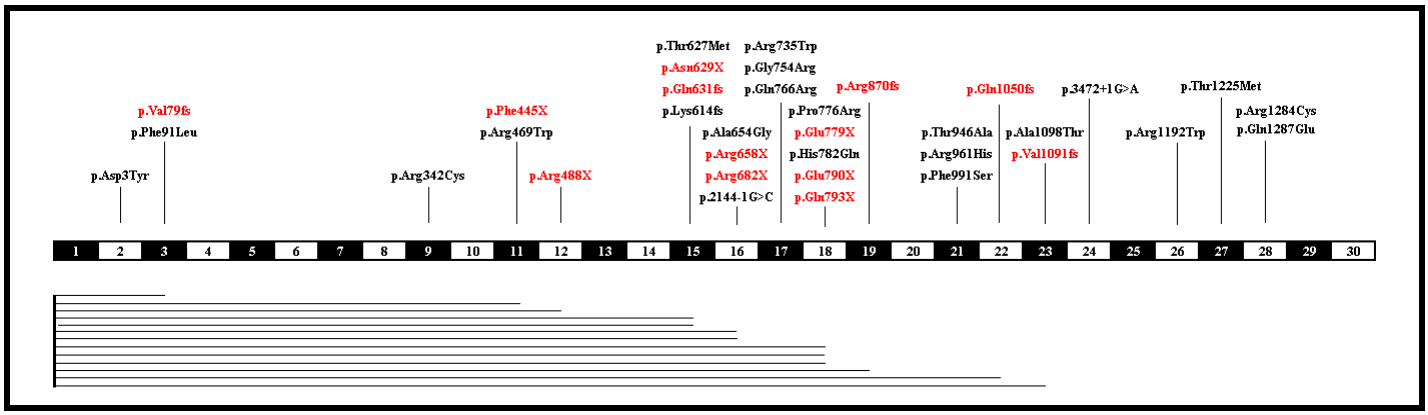

Figure 1. Linear representation of all sequence variants detected in the *NPHP4* gene in patients with NPH type 4. Numbering based on cDNA, Position +1 corresponds to the A of the ATG translation initiation codon in the reference sequence NM_015102. Black and white boxes represent the 30 exons encoding nephrocystin-4/nephroretinin. Positions of novel sequence variants detected in this study and of mutations described in the literature are indicated (see also Table 2). Truncating mutations are shown in red. The extent of the truncation is shown beneath the exon structure.

DISCUSSION

By positional cloning we and others have recently identified *NPHP4* as a novel gene which, if mutated, causes NPH (Mollet et al., 2002, Otto et al., 2002). In order to determine the frequency and the character of mutations in *NPHP4*, we here performed mutational analysis in 250 unrelated NPH patients with and without extrarenal manifestations. We detected 23 different novel *NPHP4* sequence variants in 26 NPH families. Interestingly, we detected homozygous or compound heterozygous mutations of *NPHP4* in only 6/250 families (2.4%), whereas we detected only one heterozygous *NPHP4* sequence variant in 20/250 families (8%). Our data indicate that: i) recessive mutations in *NPHP4* are a rare cause (2.4%) of nephronophthisis; ii) the presence of a single heterozygous *NPHP4* sequence variant is 3.3 times more prevalent than the presence of 2 recessive *NPHP4* mutations; iii) a genotype/phenotype correlation was not detected for the presence or absence of extrarenal manifestation; iv) and, there must exist further genes causing nephronophthisis, since in 224/250 (90%) patients no mutation in either of the four NPH genes was detected.

When examining a total of 515 unrelated NPH patients with and without extrarenal manifestations we had detected homozygous *NPHP1* deletions in 130 patients (25.2%) (Hoefele et al., unpublished). In addition, we found a combination of a heterozygous *NPHP1* deletion and a heterozygous *NPHP1* point mutation in 10/515 patients (2%) (Hoefele et al., unpublished). This indicates that in about 27% of all patients with NPH the disease is caused by mutations in *NPHP1* and thus represents nephronophthisis type 1. In this study we demonstrate that two

recessive mutations in *NPHP4* occur in only 2.4% of all NPH cases, thereby showing that NPH type 4 represents a rare cause of NPH with and without extrarenal manifestations. This finding supports the concept that NPH is a disorder that shows extensive genetic locus heterogeneity, and that most likely many additional genes are involved (Mollet et al., 2002). The finding that in 224/250 (90%) patients no *NPHP4* mutation was detected, further supports the notion there must exist additional genes causing NPH. All 23 *NPHP4* sequence variants identified in this study have not been described before (Mollet et al., 2002, Otto et al., 2002) and are therefore novel. We thus expanded the spectrum of distinct *NPHP4* sequence variants from 13 to a total of 35 (Table 2, Fig. 1). Although the sequence variants were distributed over all 30 exons of *NPHP4*, there was a propensity towards the C-terminal part of the protein, since 25/35 (71%) of all sequence variants identified in this study are located within exons 16-30 (Table 2).

We here observed the finding that the presence of a single heterozygous *NPHP4* sequence variant was 3.3 times more prevalent (20/250) than the presence of two *NPHP4* mutations (6/250). This is reminiscent of our recent finding in patients with NPH type 3, where 3/9 patients (33%) showed two mutations in *NPHP3*, whereas 6/9 patients (67%) had only a single *NPHP3* sequence variant (Olbrich et al., 2003). The finding that a single heterozygous *NPHP4* sequence variant was 3.3 times more prevalent than a two recessive *NPHP4* mutations may be interpreted in a variety of ways:

First, a second mutation may have been missed for technical reasons. We deem this possibility very unlikely, since we employed the optimal method for mutation detection: direct exon sequencing from both strands, yielding excellent sequence quality. In addition, we evaluated sequences by two independent computer programs (Sequencher™ and Mutation Surveyor™) and by two independent examiners, which in our experience leads to very sensitive mutation detection. Second, a mutation might be located in a non-exonic region. Although this possibility cannot be ruled out with certainty, this is a rare finding. It would therefore not explain the 3.3 fold higher prevalence of patients with a single sequence variant versus patients in whom both mutated alleles are detected. Third, another potential explanation for the high number of single sequence variants found in *NPHP3* and *NPHP4* would be the possibility of a dominant effect of these mutations. However, there is no evidence of any clinical signs or symptoms of NPH or extrarenal manifestations in the parents of the 515 NPH patients from our total cohort or in the literature. Fourth, it may well be possible that single heterozygous sequence variants may represent innocuous polymorphisms. Finally, it cannot be excluded altogether, that some of the single heterozygous sequence variants observed may be part of digenic or oligogenic mutations in other NPH-causing genes, as has been described for the related disease Bardet-Biedl syndrome (Badano et al., 2003).

It is known that in about 10% of all patients with NPH there is an association with retinitis pigmentosa (SLS) (Caridi et al., 1998). However, to date no correlation has been described for the presence or absence of extrarenal involvement in NPH with respect to the gene involved or with respect to specific allelic mutations. Similarly, in this study we did not detect any evidence for a genotype/phenotype relationship regarding extrarenal manifestations, since extrarenal manifestations (retinitis pigmentosa, Cogan syndrome, hearing loss, or chronic bronchitis) did not correlate with either, i) the position of a mutation within the nephrocystin-4/nephroretinin sequence, ii) the type of mutation (truncating vs. non-truncating) or, iii) the fact whether both or only one *NPHP4* sequence variant was found (Table 1). Nevertheless, further inter- and intrafamilial studies on a higher number of patients with *NPHP4* mutations might yield more information on genotype-phenotype correlations in NPH.

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