LETTER TO JMG

# Mutation analysis of *NPHP6/CEP290* in patients with Joubert syndrome and Senior–Løken syndrome

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*J Med Genet* 2007;**44**:657–663. doi: 10.1136/jmg.2007.052027

**Background:** Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease that constitutes the most common genetic cause of renal failure in the first three decades of life. Using positional cloning, six genes (*NPHP1-6*) have been identified as mutated in NPHP. In Joubert syndrome (JBTS), NPHP may be associated with cerebellar vermis aplasia/hypoplasia, retinal degeneration and mental retardation. In Senior–Løken syndrome (SLSN), NPHP is associated with retinal degeneration. Recently, mutations in *NPHP6/CEP290* were identified as a new cause of JBTS.

**Methods:** Mutational analysis was performed on a worldwide cohort of 75 families with SLSN, 99 families with JBTS and 21 families with isolated nephronophthisis.

**Results:** Six novel and six known truncating mutations, one known missense mutation and one novel 3 bp pair in-frame deletion were identified in a total of seven families with JBTS, two families with SLSN and one family with isolated NPHP.

Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease, which is the most common genetic cause of end-stage renal disease (ESRD) in the first three decades of life.[1] Using positional cloning, six genes (*NPHP 1–6*) have been identified to date,[2–8] causing disease variants NPHP types 1–6, respectively. NPHP can occur with isolated kidney involvement or in combination with diverse extrarenal manifestations. Specifically, it can be associated with cerebellar vermis dysplasia/hypoplasia, retinal degeneration and mental retardation in Joubert syndrome (JBTS).[8–10] NPHP may also be associated with retinal degeneration in Senior–Løken syndrome (SLSN) in about 10% of patients with mutations in *NPHP1*, *2*, *3* or *4*[4 5 11 12] and in >90% of patients with mutations in *NPHP5* or *6*.[7 8]

JBTS is an autosomal-recessive developmental disorder with multiple organ involvement characterised by a typical neuroradiological feature, the "molar tooth sign" (MTS), which describes a cerebellar and brainstem malformation. This entails dysplasia or hypoplasia with or without dysplasia of the cerebellar vermis, thick and misoriented superior cerebellar peduncles and an abnormally deep interpeduncular fossa.[10 13] Accompanying neurological symptoms of JBTS include neonatal hypotonia, transient abnormal breathing patterns in the neonatal period (apnoea and/or hyperpnoea), ataxia, nystagmus, mental/psychomotor retardation and/or oculomotor apraxia.[10 14 15] Seizures and behavioural problems within the autism spectrum have been described.[10] JBTS and JBTS-related disorders (JSRD), as defined by the presence of MTS with multiorgan involvement, are clinically heterogeneous.[13] Two forms of JBTS have been determined: types A and B, the latter being characterised by the co-occurrence of ocular and renal features, namely retinal dystrophy and nephronophthisis.[14] At least eight distinct syndromes that have MTS as a feature have been reported, showing wide phenotypic variability both within and between families.[13] One example is COACH syndrome[15] in which cerebellar vermis hypoplasia occurs along with oligophrenia, congenital ataxia, ocular coloboma and hepatic fibrosis. Additional extrarenal organ manifestations may include retinal coloboma, congenital amaurosis, hepatic fibrosis and postaxial polydactyly. MTS has also been described in association with complex midbrain–hindbrain malformations including Dandy–Walker malformation[15] and occipital encephalocoele.[10 13]

JBTS is genetically heterogeneous. Four genes have been identified and two additional loci mapped for JBTS,[16] including the *JBTS1* locus at chromosome 9q34.3 (OMIM 213300)[17] and the *JBTS2* or *CORS2* locus at chromosome 11p12-11q13.3 (OMIM 608091).[18] The gene defects identified in patients with JBTS include: (1) deletions in *NPHP1* (JBTS4; OMIM 607100);[10] (2) mutations in the *AHI1* (*Abelson helper integration site*) gene (JBTS3; OMIM 608894),[10 19–21] and (3) mutations in the *MKS3/TMEM67* (*Meckel syndrome type 3*) gene (JBTS6) which was initially found in patients with Meckel–Gruber syndrome (OMIM 607361).[22] In addition, using positional cloning, we recently identified mutations in a novel gene *NPHP6/CEP290* (OMIM 610142), as causing JBTS5,[8] describing eight different mutations in seven families with JBTS.[8] Mutations in the same gene were identified in five families with pleiotropic forms of JBTS by Valente *et al*[9] and as a major cause of JBTS with oculorenal involvement by Brancati *et al*.[23] Recently, a heterozygous truncating mutation in *NPHP6/CEP290* and a heterozygous missense mutation in *AHI1* have been found in combination with the homozygous *NPHP1* deletion in patients with NPHP and JBTS-related neurological symptoms.[24]

The most common extrarenal manifestation of nephronophthisis is retinal involvement. In SLSN there is the association of NPHP with retinitis pigmentosa, tapetoretinal degeneration or retinal dysplasia.[1] About 10% of patients with mutations in *NPHP1*[1] or *NPHP4*[6] present with SLSN. In one patient with SLSN, a mutation in *NPHP2* was identified.[12] In *NPHP3*, only single heterozygous mutations were found in four patients with SLSN.[4] Whether a mutation in an unknown modifier gene is causative for the retinal involvement is unknown.[1] In *NPHP5*, all patients described to date have had early-onset SLSN.[7] Recently we reported a homozygous 5 bp deletion in *NPHP6/CEP290* that altered an obligatory splice site in a patient with SLSN.[8]

Another condition that can occur concomitant with NPHP is Leber congenital amaurosis (LCA; OMIM 204000) a severe

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**Abbreviations:** ESRD, end-stage renal disease; JBTS, Joubert syndrome; JSRD, Joubert syndrome-related disorders; LCA, Leber congenital amaurosis; MTS, molartooth sign; NPHP, nephronophthisis; OMIM, Online Mendelian Inheritance in Man; SLSN, Senior–Løken syndrome retinal dystrophy causing blindness or severe visual impairment at birth or within the first 2 years of life. Mutations in nine genes (*AIPL1*, *CRB1*, *CRX*, *GUCY2D*, *IMPDH1*, *RDH12*, *RPE65*, *RPGRIP1* and *NPHP6/CEP290*) have been found to cause LCA.[25 26] A hypomorphic mutation of *NPHP6/CEP290* in humans represents the most common cause of LCA.[25 26] This intronic *NPHP6/CEP290* mutation (c.2991+1655A→G) accounts for 21% of LCA cases. It creates a strong abnormal splice-donor site leading to an insertion of a cryptic exon in intron 27 of the *CEP290* messenger RNA.[25] Similarly, an in-frame deletion in the orthologue of the gene *NPHP6/CEP290* identified in the mouse mutant *rd16* causes retinal degeneration without renal or cerebellar involvement.[27] The high frequency of *NPHP6/CEP290* mutations in patients with LCA was confirmed by Perrault *et al*.[26]

Thus, mutations in *NPHP6/CEP290* have been found in disorders with cerebello-renal, cerebello-oculo-renal, cerebello-retinal, retinal-renal and retinal phenotypes.[9 11 23 25 28] To date, mutations in *NPHP6/CEP290* had not been identified in patients with isolated nephronophthisis. In this study we performed mutation analysis in a worldwide cohort of 195 families with NPHP, SLSN or JBTS. We examined all translated exons and adjacent intronic sequence and intron 27 for mutations in *NPHP6/CEP290*. We found seven novel mutations (six of them being truncating mutations), in four families with JBTS, two families with SLSN and one family with isolated nephronophthisis.

## METHODS

### Patients

We performed mutational analysis in a worldwide cohort of 195 families with JBTS, SLSN or isolated NPHP. Our cohort comprised 99 families with JBTS (5 with diagnosis of LCA), 75 families with SLSN (6 with LCA and NPHP) and 21 families with isolated NPHP.

We collected blood samples, pedigrees, clinical information and informed consent (www.renalgenes.org). Approval for studies on human subjects was obtained from the University of Michigan institutional review board.

In all patients the diagnosis of NPHP was based on one or more of the following criteria: (1) clinical course with characteristic clinical signs of polyuria, polydipsia, anaemia and growth retardation; (2) presence of chronic renal failure; (3) renal ultrasound or renal biopsy compatible with the diagnosis of NPHP as judged by a (paediatric) nephrologist; and (4) pedigree compatible with autosomal recessive inheritance. Neurological criteria for JBTS were based on the following clinical hallmarks of this cerebello-oculo-renal syndrome: (1) MTS or (2) diagnosis of JBTS by a (paediatric) neurologist or geneticist. Associated JBTS symptoms were recorded: optic nerve or retinal coloboma, tapetoretinal degeneration, cerebellar vermis aplasia/hypoplasia, ataxia and periodic apnoea/tachypnoea. The diagnosis of SLSN was based on the presence of NPHP in association with tapetoretinal degeneration.

### Analysis of *NPHP1-5*, *AHI1* before analysis for *NPHP6/CEP290*

Genomic DNA from peripheral blood samples was extracted by standard methods. Before mutation analysis for *NPHP6/CEP290* described in this study, the homozygous *NPHP1* deletion and mutations in *NPHP5*, both known to cause SLSN and LCA, were excluded in patients with eye involvement. All patients with JBTS were tested for the homozygous *NPHP1* deletion and for mutations in *AHI1*. All patients with isolated NPHP were tested for the homozygous *NPHP1* deletion.[29] To exclude mutations in other known NPHP genes prior to this study, 40 patients with infantile NPHP were tested for mutations in *NPHP2*,[29] 50 patients were tested for mutations in *NPHP3*[4 29] and 95 patients of the cohort were screened for *NPHP4* mutations.[28 29]

### *NPHP6/CEP290* mutation analysis

In total, 195 samples underwent *NPHP6/CEP290* mutation analysis. Intron 27 and all 54 translated exons of *NPHP6/CEP290* were amplified by PCR using 51 exonic flanking primers. Initially, all amplicons were prescreened by heteroduplex formation and a subsequent CEL I endonuclease digest as described previously.[29] The CEL I enzyme recognises single-base mismatches present in heteroduplex DNA and cleaves both strands. Mutations can be detected with a sensitivity of 92%.[29 30] Samples showing aberrant bands in agarose-gel electrophoresis were purified and directly sequenced. For each mutation, 94 healthy control individuals were examined by restriction-enzyme digest or CEL I endonuclease assay.

## RESULTS

We analysed a cohort of 99 families with JBTS, 75 families with SLSN and 21 families with isolated NPHP for mutations in *NPHP6/CEP290*. Using heteroduplex analysis with CEL I endonuclease for all 54 coding exons of *NPHP6/CEP290*, we found 59 aberrant banding patterns. Direct sequencing of these mismatches revealed 15 different nucleotide changes in 7 families with JBTS, 2 families with SLSN and 1 family with isolated NPHP (tables 1 and 2, fig 1). These consisted of: (1) five nonsense mutations (C3811T, C4882T, G5941T, T2249G and C5932T) resulting in premature protein truncation (R1271X, Q1628X, E1981X, L750X and R1978X); (2) four deletions (5734delT, 5163delT, 1419-1423delAATAA and 164-167delCTCA) generating frameshifts and premature stop codons (W1912fsX1923, T1721fsX1723, K473fsX478 and T55fsX57); (3) one start codon mutation (A1G); and (4) two obligatory splice-site mutations (1066-1G→A, 3104-2A→G) (table 1). Seven of these sequence variations are novel, whereas C3811T (R1271X)[23] and 5734delT (W1912fsX1923)[23] in A372, C4882T (Q1628X)[23 26] and G5941T (E1981X)[23] in A989, 5163delT (T1721fsX1723)[23 26] in F101, T2249G (L750X)[25] in F122, and A1991G (D664G)[23] have already been described. We did not detect the intron 27 mutation[25] in our cohort.

In four families (A372, A989, F101 and F57) both mutations were found in *NPHP6/CEP290*, thereby indicating the phenotype to be autosomal recessive (table 1). In four patients (F122, A1332, F938 and F848), only single heterozygous loss-of-function mutations could be found (table 1). Individual F848 is known to harbour a heterozygous missense mutation in *NPHP4* (C1880T, T627M).[28] When parental DNA was available, segregation analysis was performed and confirmed that the sequence variants were transmitted as autosomal recessive alleles. All the detected sequence variants were absent from 188 control chromosomes of people of Central European, Middle Eastern, East Asian and American origin.

We also identified several sequence variants of unknown significance, present in the heterozygous state: one known missense mutation (A1991G)[23] and a 3 bp in-frame deletion (7311–7313delGAA) (fig 1, table 2). The first sequence change results in a non-conservative amino acid substitution (D664G) and the conserved residue of the protein (K2437 is conserved in *Ciona intestinalis*). However, as this family (A854) was consanguineous, the significance of this change is unknown.

### Eight compound heterozygous loss-of-function *NPHP6/CEP290* mutations in four families with JBTS or SLSN

The members of the three families with JBTS (A372, A989 and F101) and one family with SLSN (F57) in whom eight compound heterozygous loss-of-function mutations were found (table 1) exhibited the following disease phenotypes.

**Table 1** Twelve different truncating *NPHP6/CEP290* mutations found in 6 families with JBTS and 2 families with SLSN

| Family (individual) | Country of origin | Nucleotide alteration(s)* | Alteration(s) in coding sequence | Exon (segregation)** | Parental consanguinity | Age at ESRD‡ (years) | Phenotype | Ocular features (age of diagnosis, years) | Central nervous system features (other) |
|---|---|---|---|---|---|---|---|---|---|
| A372 (II-1, II-2) | Italy | C3811T**<br>5734delT** | R1271X**<br>W1912fsX1923** | 32 (het, P)<br>43 (het, M) | – | ND | JBTS | LCA, RC (ND) | MR |
| A989 (II-1) | Switzerland | C4882T**<br>G5941T** | Q1628X**<br>E1981X,** | 38 (het, ND)<br>44 (het, ND) | – | 5 | JBTS | LCA, NY | CVA, MR, spastic paresis, liver fibrosis |
| F101 (II-2) | USA | 1066-1G>A<br>5163delT** | splice site<br>T1721fsX1723** | 13 (het, P)<br>39 (het, $P_{wt}$, M ND) | – | 9 | JBTS<br>Empty sella | LCA (5 months) | MR, empty sella, hypoplasia of the optic chiasm, hypothalamic hypoplasia, (breathing, abnormality, pes planus) |
| F57 (II-1) | Germany | A1G, 1419-1423del AATAA | Start codon defect<br>K473fsX478 | 2 (start codon) (het, ND)<br>15 (het, ND) | – | 24 | SLSN | NY, early-onset TRD keratoconus, vision <1 (early childhood) | Complex focal seizures (hyperlipidaemia, scoliosis) |
| F122 (II-2) | Germany | T2249G,?** | L750X, ? | 23 (het, M) | – | >6§ | JBTS | LCA, NY | CVA, AT, MR |
| A1332 (II-1) | Syria | 164_167del CTCA, ? | T55fsX57, ? | 3 (het, ND) | + | None at 1.5 | JBTS | LCA, NY | CVA, MR |
| F938 (II-1) | Bosnia | 3104-2A>G,? | Splice site, ? | 29 (het, M) | – | 13 | JBTS | NY, esotropia, retinal dystrophy (14) | MR, hypotonia (scoliosis) |
| F848 (II-1)¶ | Italy | C5932T,? | R1978X, ? | 44 (het, ND) | – | 40 | SLSN | TRD (vision 1/10 at 44) | ND |

?, Second mutation not found; AT, ataxia; CA, congenital amaurosis (bilateral); CVA, cerebellar vermis aplasia/hypoplasia; ESRD, end-stage renal disease; het, heterozygous in affected individual; hom, homozygous in affected individual; LCA, Leber congenital amaurosis (blindness within the first 2 years of life); JBTS, diagnosed with Joubert syndrome; M, mutation identified in mother; MEC, occipital menigoencephalocele; MR, mental retardation/psychomotor retardation; $M_{wt}$ maternal sequence is wild type; ND, no data or DNA available; NY, nystagmus; P, mutation identified in father; $P_{wt}$ paternal sequence is wild type; SLSN, diagnosed with Senior–Løken syndrome; RC, retinal coloboma; TRD, tapetoretinal degeneration.

*All mutations were absent from at least 188 chromosomes of healthy controls.

‡All patients had renal ultrasonography results compatible with nephronophthisis (increased echogenicity and/or corticomedullary cysts).

§Renal function significantly reduced.

¶This patient is also known to have a heterozygous missense mutation in the *NPHP4* gene (C1880T, T627M).[28]

**Previously published.[23 25 26]

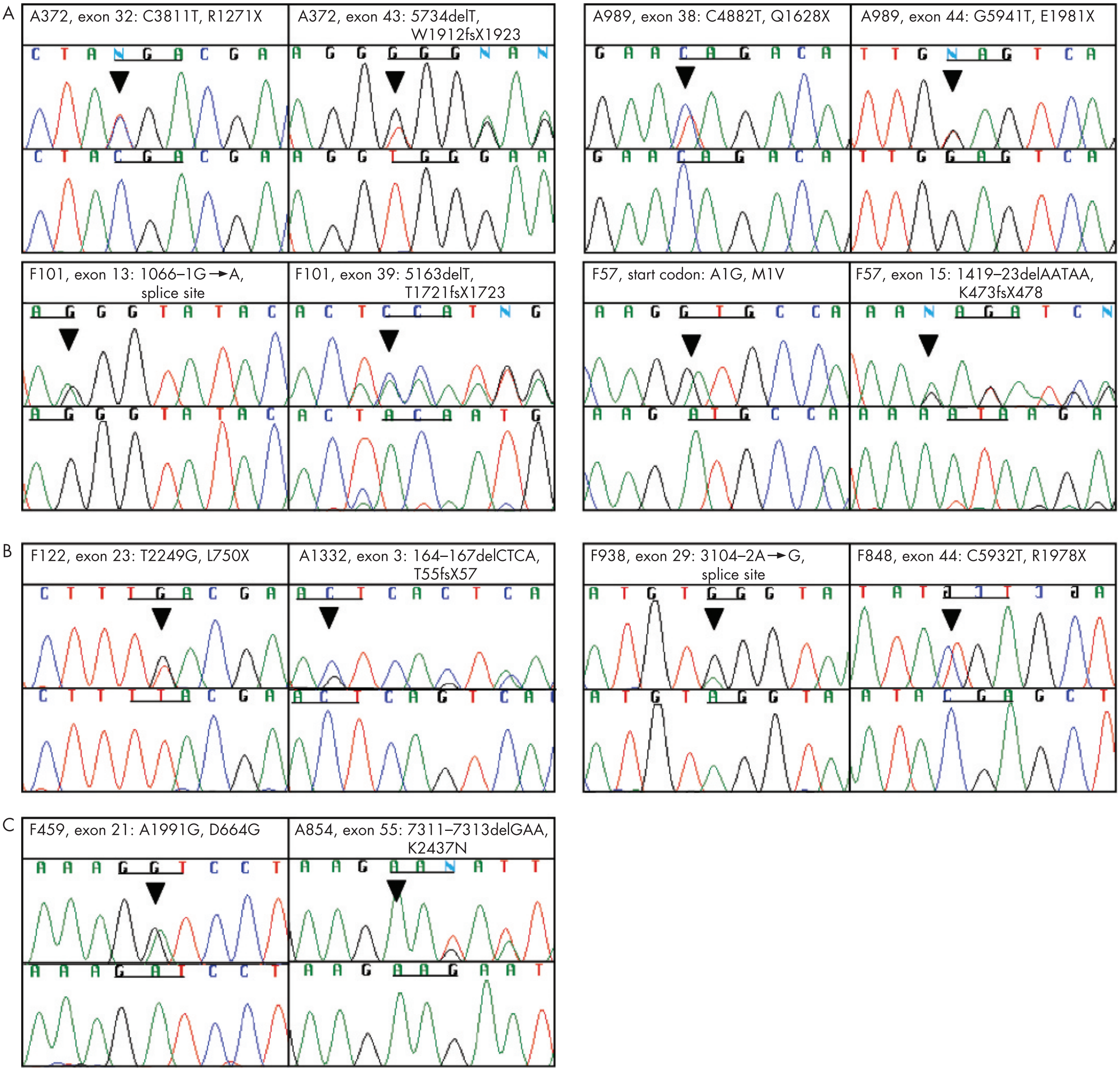

**Figure 1** Mutations and sequence variants found in *NPHP6/CEP290*. (A) Eight different compound heterozygous truncating mutations in *NPHP6/CEP290* were found in three families with JBTS and one family with SLSN; (B) four single heterozygous *NPHP6/CEP290* mutations were found in three families with JBTS and one family with SLSN; (C) two heterozygous sequence variants of unknown significance were found: one missense mutation in a patient with JBTS and a 3 bp in-frame deletion in a patient with isolated NPHP (see table 2). Family number, altered nucleotide and amino acid change are given above sequence traces and wild-type sequence below mutated sequence. One codon triplet in each chromatogram is underlined to indicate reading frame.

(1) Two siblings (II-1) and (II-2) from family A372 were diagnosed with JBTS, LCA and retinal colobomas.

(2) In patient II-1 from family A989, ESRD secondary to NPHP occurred at 5 years of age. This patient had retinal dystrophy type LCA, nystagmus, severe mental/psychomotor retardation, spastic quadraparesis and liver fibrosis. The MRI showed cerebellar vermis aplasia, supratentorial hypomyelinisation and hypoplastic brain stem.

(3) In patient II-2 from family F101, ESRD secondary to NPHP was diagnosed at 9 years of age, and he was diagnosed with LCA. Despite the fact that some of the symptoms presented by this patient are common to JBTS (severe hypotonia in the first year of life, mental and psychomotor retardation, sleep apnoea episodes), the CT scan lacked the typical MTS and showed empty sella, hypoplasia of the optic chiasm and hypothalamic hypoplasia. An MRI was not available for review.

(4) Patient II-1 from family F57 has been visually impaired since early childhood, lost vision progressively and was diagnosed with retinitis pigmentosa at the age of 34 years. He also had keratoconus. Owing to progressive renal failure secondary to NPHP, he underwent kidney transplantation at 24 years. He had complex focal seizures at 17 years.

**Table 2** *NPHP6/CEP290* sequence variants of unknown significance

| Family (individual) | Country of origin | Nucleotide alteration(s)* | Alteration(s) in coding sequence† | Exon (segregation) | PC | Age at ESRD‡ [years] | Pheno-type | Ocular features (age of diagnosis, years) | Central nervous system features (other) |
|---|---|---|---|---|---|---|---|---|---|
| F459 (II-2) | USA | A1991G, ?‡ | D664G, ? *(NC)* | 21 (het,$M_{wt}$) | – | ND | JBTS | ND | CVA, AT, MR, RC (hepatic fibrosis, hearing loss) |
| A854 (II-9) | Pakistan | 7311-7313delGAA, ? | K2437del (*C. i.*), ? | 55 (het, ND) | + |  | NPHP | None | None |

?, Second mutation not found; AT, ataxia; CVA, cerebellar vermis aplasia/hypoplasia; ESRD, end-stage renal disease; het, heterozygous in affected individual; JBTS, diagnosed with Joubert syndrome; M, mutation identified in mother; MR, mental retardation/psychomotor retardation; $M_{wt}$ maternal sequence is wild type; ND, no data or DNA available; P, mutation identified in father; PC, parental consan-guinity; SLSN, diagnosed with Senior–Løken syndrome; RC, retinal coloboma.
*All mutations were absent from at least 188 chromosomes of healthy controls.
†Evolutionary conservation: NC, non-conserved; *C. i.*, *Ciona intestinalis*.
‡All patients had renal ultrasonography results compatible with nephronophthisis (increased echogenicity and/or corticomedullary cysts).
‡Previously published.[23]

### Four single heterozygous *NPHP6/CEP290* loss-of-function mutations in four families with JBTS and SLSN

In three patients from families with JBTS (F122, A1332, F938) and one patient from a family with SLSN (F848), single heterozygous mutations were found, three of them truncating (in F122, A1332 and F848) and one splice-site mutation (in F938) (table 1).

Patient II-2 from family F122 had LCA and features of JBTS, such as cerebellar vermis aplasia, muscle hypotonia at birth and later mental/motor retardation. Symptoms and typical ultrasound findings of NPHP had been present since 6 years of age.

A1332 presented with dilated calyces bilaterally on ultrasound and normal renal function by blood testing at 18 months of age. He had congenital amaurosis (flat electroretinogram at 15 months of age), nystagmus since birth, cerebellar vermis aplasia and MTS, and mental and psychomotor retardation. As his parents are consanguineous but he carries a heterozygous mutation in *NPHP6/CEP290*, it is likely that there may be recessive mutations present in other NPHP genes.

In patient II-1 from family F938, ESRD secondary to NPHP occurred at 13 years of age. During birth, asphyxia occurred with hypotonia. In addition, horizontal nystagmus and esotropia were seen. In early childhood, this patient presented with severe scoliosis and psychomotor retardation. An ophthalmological examination revealed retinal dystrophy.

In patient II-1 from family F848, initial symptoms of NPHP occurred during infancy. However, ESRD occurred late, at the age of 40 years. The patient progressively lost vision and at 44 years of age, retinitis pigmentosa (SLSN) was diagnosed. He also had bilateral cataracts. This patient is known to harbour a heterozygous missense mutation in the *NPHP4* gene, which has been published previously.[28]

### Phenotypes of families with sequence variants of unknown significance

In patient II-1 from family F459, a nonconservative missense mutation, A1991G (D664G), was found. In a recessive disease, this sequence variant alone cannot be disease causing. The patient had cerebellar vermis hypoplasia, ataxia, developmental delay and progressive hepatic fibrosis; the latter has previously been described in COACH syndrome.[15] In addition, the patient presented with hearing loss, which has not been described previously in association with *CEP290/NPHP6* mutations (table 2).

As oligogenic mutations of the *NPHP1*, *AHI1* and *NPHP6* genes have been described in JBTS, we examined the patients who carried two mutations or one single heterozygous mutation in *NPHP6* for additional mutations in *NPHP1* and *AHI1*. However, we did not detect any additional mutations in either gene.

### *NPHP6/CEP290* mutations in isolated NPHP

Interestingly, in a patient with isolated NPHP (patient II-9 from family A854) a 3 bp in-frame deletion (7311–7313delGAA; K2437del) was found (table 2), which was absent in 188 healthy control chromosomes. It deletes an amino acid residue conserved in the *C intestinalis* orthologue of *NPHP6/CEP290*. Renal symptoms (polyuria, polydipsia) and increased echogenicity on ultrasonography occurred at 10 years of age. To test the possibility of *NPHP6/CEP290* mutations in isolated NPHP, we analysed 5 families that had been found to be homozygous at the *NPHP6* locus when we had performed a total genome search for homozygosity in 105 families, and found these 5 families to be homozygous for markers in the *NPHP6/CEP290* gene region. We did not find any sequence variants in these families.

## DISCUSSION

In this study, we performed mutational analysis in 195 families with JBTS, SLSN or isolated NPHP. In total, 15 different nucleotide changes were found, 7 of them novel. In four patients, both compound heterozygous truncating or splice site mutations were found. In another four patients, only single heterozygous mutations were found; three of these mutations were truncating and one was a splice defect (table 1). The single heterozygous mutations are not disease-causing in themselves in the recessive disease NPHP, but could be disease-causing in combination with mutations in other genes.[26 31]

We report here one family (F848) with a heterozygous nonsense mutation in *NPHP6/CEP290* and an additional known missense mutation in *NPHP4*, as published previously.[28] In one consanguineous family (A1332) only a single heterozygous truncating mutation could be found. Tory *et al* reported a patient with a homozygous deletion in *NPHP1* and a heterozygous truncating mutation in *NPHP6/CEP290*.[24] These mutations may potentially indicate a situation of oligogenic inheritance as described in patients with Bardet–Biedl syndrome.[31]

In the mouse model of retinal degeneration, *rd16*, a 300 amino acid in-frame deletion as a hypomorphic allele was found to be associated with early-onset retinal degeneration.[27] This is consistent with the finding that the hypomorphic allele of a partial splice defect in intron 27[25] in humans leads to an ocular phenotype only, and not to renal or cerebellar involvement. Interestingly, neither kidney nor gross brain pathological changes could be found in the *rd16* mice. Mutations in *NPHP6/CEP290* have been described as the most common genetic cause

## Key points

- Mutations in the gene *NPHP6/CEP290*, which encodes the centrosomal protein nephrocystin-6, cause nephronophthisis-associated ciliopathies.
- In this study, we examined a worldwide cohort of 195 families with Senior–Løken syndrome (SLSN), Joubert syndrome (JBTS) and isolated nephronophthisis for mutations in NPHP.
- We identified seven novel and seven known mutations in seven families with JBTS, two families with SLSN and one family with isolated NPHP, thus confirming the clinical heterogeneity of patients with *NPHP6/CEP290* mutations.

of LCA/early onset retinal degeneration.[25] In our cohort there were 11 patients with diagnosed LCA. Interestingly, mutations in *NPHP6/CEP290* were found in five of these patients. All of the patients with JBTS with either compound or single heterozygous *NPHP6/CEP290* mutations identified in this study presented with an ocular phenotype, confirming that this gene is a major cause of JBTS with oculorenal involvement as described previously.[23]

*NPHP6/CEP290* mutations can be found in patients with a spectrum of phenotypes, mainly JBTS or early-onset retinal degeneration with additional extrarenal manifestations. We also found *NPHP6/CEP290* mutations in a patient with empty-sella syndrome and in a patient with JBTS and an additional manifestation of liver fibrosis. In summary, we have found six novel loss-of-function mutations of *NPHP6/CEP290*.

## ACKNOWLEDGEMENTS

We express our sincere appreciation to the patients and their families for their participation in this study. We gratefully acknowledge the cooperation of the following colleagues: A Kampik and G Rudolph (Eye Hospital, LMU München, Germany); R Weleber (Casey Eye Institute, Oregon Health and Sciences University, USA); R Beetz (Children's Hospital, Johannes-Gutenberg Universität Mainz, Germany); L Schimmenti, (Pediatrics, UCLA, USA); Y Pirson (Universite? Catholic de Louvain, Brussels, Belgium); D Walb (Wiesbaden, Germany); T J Neuhaus and G Laube (Children's Hospital, Zurich, Switzerland); and S Signori (Department of Child Neurology and Psychiatry, IRCCS "C Mondino" Foundation, University of Pavia, Italy). H Omran is supported by grant no. SFB592; FH is a Frederick G L Huetwell professor and Doris Duke Distinguished Clinical Scientist, and is supported by grants from the NIH (DK068306, DK064614 and DK069274).

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Competing interests: None declared.

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Received 27 May 2007
Revised 19 June 2007
Accepted 20 June 2007
**Published Online First 6 July 2007**

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