Variations in *NPHP5* in Patients With Nonsyndromic Leber Congenital Amaurosis and Senior-Loken Syndrome

Edwin M. Stone, MD, PhD, **Artur V. Cideciyan, PhD**, **Tomas S. Aleman, MD**, **Todd E. Scheetz, PhD**, **Alexander Sumaroka, PhD**, **Mary A. Ehlinger, BS**, **Sharon B. Schwartz, MS, CGC**, **Gerald A. Fishman, MD**, **Elias I. Traboulsi, MD**, **Byron L. Lam, MD**, **Anne B. Fulton, MD**, **Robert F. Mullins, PhD**, **Val C. Sheffield, MD, PhD**, and **Samuel G. Jacobson, MD, PhD**
Departments of Ophthalmology and Visual Sciences (Drs Stone, Scheetz, Mullins, and Sheffield and Ms Ehlinger) and Pediatrics (Dr Sheffield) and the Howard Hughes Medical Institute (Drs Stone and Sheffield and Ms Ehlinger), University of Iowa Carver College of Medicine, Iowa City; Scheie Eye Institute, Department of Ophthalmology, University of Pennsylvania, Philadelphia (Drs Cideciyan, Aleman, Sumaroka, and Jacobson and Ms Schwartz); Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago (Dr Fishman); Department of Pediatric Ophthalmology and Strabismus, Cole Eye Institute, Cleveland Clinic Foundation, Cleveland, Ohio (Dr Traboulsi); Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, Florida (Dr Lam); and Boston Children's Hospital, Boston, Massachusetts (Dr Fulton)

Abstract

Objective—To investigate whether mutations in *NPHP5* can cause Leber congenital amaurosis (LCA) without early-onset renal disease.

Methods—DNA samples from 276 individuals with non-syndromic LCA were screened for variations in the *NPHP5* gene. Each had been previously screened for mutations in 8 known LCA genes without identifying a disease-causing genotype.

Results—Nine of the 276 LCA probands (3.2%) harbored 2 plausible disease-causing mutations (7 different alleles) in *NPHP5*. Four of these have been previously reported in patients with Senior-Loken syndrome (F141del, R461X, H506del, and R489X) and 3 are novel (A111del, E346X, and R455X). All 9 patients had severe visual loss from early childhood but none had overt renal disease in the first decade of life. Two patients were diagnosed with nephronophthisis in the second decade. Retinal imaging studies showed retained photoreceptor nuclei and retinal pigment epithelium integrity mainly in the cone-rich central retina, a phenotype with strong similarities to that of *NPHP6* disease.

Conclusions—Mutations in *NPHP5* can cause LCA without early-onset renal disease. Abnormalities observed in the photoreceptor outer segments (a cilial structure) may explain the severe visual loss in *NPHP5*-associated LCA.

Clinical Relevance—The persistence of central photoreceptor nuclei despite severe visual loss in *NPHP5* disease is encouraging for future therapeutic interventions.

Many mendelian disorders have been recognized in which dysplasia, dysfunction, and/or degeneration of the retina are observed in combination with abnormalities of other organ systems. In the past decade, molecular geneticists have traced the cause of a number of these

syndromes to a phylogenetically ancient organelle, the sensory cilium. Cilia-associated proteins are essential for the normal development and function of a wide array of specialized tissues including the retina, inner ear, kidney, and brain. The many manifestations of Bardet-Biedl, Usher, Joubert, and Senior-Loken syndromes result from a loss or imperfect function of a component of the cilium-centrosome complex.[1,2]

Senior-Loken syndrome is an autosomal recessive oculo-renal condition.[3,4] The 2 major features of Senior-Loken syndrome are the cystic kidney disease known as nephronophthisis (NPHP) and an early childhood–onset retinal degeneration known as Leber congenital amaurosis (LCA).[5] To date, Senior-Loken syndrome has been associated with mutations in 5 of the 10 NPHP genes.[6–10] Certain genotypes of 1 of these genes (*NPHP6* [OMIM 610142]) cause a nonsyndromic retinal disease known as Leber congenital amaurosis (LCA).[11,12] *NPHP6* is thought to form a functional complex with *NPHP5*[13] (OMIM 609237) and knockdown of either of these genes in zebrafish embryos leads to a syndromic disease with ocular and systemic manifestations. These observations led us to investigate whether mutations in *NPHP5* could also cause LCA in the absence of early-onset renal disease.

With 1 exception, the retinal phenotypes associated with mutations in NPHP genes have not been described in detail. Only the nonsyndromic retinal disease caused by mutations in *NPHP6* has been studied extensively.[14] With the recent success in clinical trials of treatment of an inherited retinal degeneration,[15–18] we felt that it was important to investigate the retinal phenotype of patients with mutations in *NPHP5* for evidence that this retinal disease might be treatable.

METHODS

HUMAN SUBJECTS

The study was approved by the institutional review boards of the participating centers and adhered to the tenets set forth in the Declaration of Helsinki. Two hundred seventy-six individuals with the clinical diagnosis of nonsyndromic LCA and 276 ethnically similar control individuals were studied. The patients were chosen from a cohort of more than 1000 patients with LCA on the basis of the following criteria: (1) a clinical diagnosis of nonsyndromic LCA, (2) prior screening of the coding sequences of 8 genes known to cause the LCA phenotype (*AIPL1* [OMIM 604392], *CRB1* [OMIM 604210], *CRX* [OMIM 602225], *GUCY2D* [OMIM 600179], *RHD12* [OMIM 608830], *RPE65*[OMIM 180069], *RPGRIP1* [OMIM 605446], and *CEP290* [OMIM 610142]) without discovery of a plausible disease-causing genotype, (3) original medical records available for detailed review, and (4) sufficient DNA remaining to allow screening and molecular confirmation.

MUTATION ANALYSES

Fifty nanograms of genomic DNA from each patient with LCA were combined into a "patient DNA pool" and 50 ng of genomic DNA from each control individual were combined into a "control DNA pool." A series of oligonucleotide primers were designed to amplify the *NPHP5* coding sequence in fragments that were 200 to 300 base pairs in length. Each primer pair was used to amplify the corresponding candidate gene segment from both the patient DNA pool and the control DNA pool. Polymerase chain reaction (PCR) amplification was confirmed by agarose gel electrophoresis with ethidium bromide visualization and the products were quantified using densitometry of the agarose gels and comparison with standard DNA samples of known concentration. All PCR products amplified from the pooled DNA from patients with LCA were combined in an equimolar fashion into 1 sample and the PCR products amplified from the pooled control subjects' DNA were combined into a second sample. The amplified DNA segments from patients and

controls were separately ligated to emulsion PCR adapters (Roche, Branford, Connecticut) as directed by the manufacturer. The ligated fragments were hybridized to sequencing beads in an amplification mixture containing DNA polymerase, deoxynucleoside triphosphates, and buffer. The mixtures were mixed with oil, emulsified, and thermocycled for 50 cycles. The DNA-coated beads were recovered from the emulsions and subjected to pyrophosphate sequencing on a Roche 454 instrument using titanium chemistry. The beads corresponding to the samples from patients with LCA were analyzed on one-half of a picotiter plate and the beads corresponding to the control individuals were analyzed on the other half of the plate during a single run of the sequencing instrument. Roche GSAmplicon software was used to analyze the standard flowgram files and detect the presence of variations from the specified reference sequences of each amplimer. Variations that were significantly more common in patients than controls were confirmed using automated DNA sequencing with dye termination chemistry on an ABI 3730 sequencer (Applied Biosystems, Carlsbad, California). Six members of 1 family—the proband, both parents, and 3 unaffected siblings —were genotyped for 16 short tandem repeat polymorphisms, as previously described.[19] Five non–chromosome 3 markers (D6S1611, D1051174, D195902, D115956, and D55820) were used to confirm the biological relationships within the family while 11 chromosome 3 markers (D3S1568, D3S3664, D3S3513, D3S3620, D3S3720, D3S4497, D3S3709, D3S3576, D3S1269, D3S1267, and D3S3558) were used to investigate the possibility of uniparental isodisomy.

RETINAL CROSS-SECTIONAL IMAGING

Retinal cross-sections were obtained with a Fourier-domain OCT system (RTVue-100; Optovue Inc, Fremont, California) using recording and analysis techniques previously published.[20–23] For this work, the high-definition line protocol of the Fourier domain OCT system was used to obtain 4.5-mm-long scans composed of 4091 longitudinal reflectivity profiles (LRPs) acquired at 26 000 LRPs per second. Overlapping line scans were used to cover the vertical meridian up to 9-mm eccentricity from the fovea. In addition, topographic analysis was performed by obtaining dense overlapping raster scans sampling a rectangular region of the retina (18 mm × 12 mm) centered on the fovea.

Postacquisition processing of OCT data was performed with custom programs (MatLab 6.5; MathWorks, Natick, Massachusetts). The LRPs making up the OCT scans were aligned by straightening the major retinal pigment epithelium (RPE) reflection.[24] In some regions of patient retinas, the presumed RPE peak was sometimes the only signal peak deep in the retina; in other cases, it was apposed by other major peaks. In the latter case, the RPE peak was specified manually as previously described.[25] In normal subjects, the signal corresponding to the RPE was assumed to be the peak nearest the sclera within the multipeaked scattering signal complex.[25] Lateral sampling density of the Fourier-domain OCT scans was reduced by averaging 8 neighbors to increase the signal to noise ratio. Quantitative measurements of retinal laminae were performed after further reduction of lateral sampling density (sampling bins 0.07 mm). The outer photoreceptor nuclear layer (ONL) was defined in regions of scans showing stereotypical layering between the RPE and vitreoretinal interface. The boundaries of the ONL were determined by the minima and maxima of the LRP signal slopes and taking into account neighboring layers of higher reflectivity on LRPs; specifically, ONL boundaries were defined between the outer limiting membrane or RPE and the scleral boundary of the outer plexiform layer.

RETINAL *EN FACE* IMAGING

En face images of the retina were obtained with a confocal scanning laser ophthalmoscope (HRA2; Heidelberg Engineering GmbH, Heidelberg, Germany) using recording and analysis techniques previously published.[14,22,23,26–29] Imaging was performed in the "high-speed"

mode: $30° \times 30°$ of the retina was sampled onto a 768×768 pixel image at the rate of 8.8Hz. Natural melanin fluorophores in RPE cells[28] were imaged with autofluorescence (AF) using near infrared (NIR) excitation light at 790 nm and collecting emissions beyond 810 nm. This imaging mode provided an estimate of RPE health distribution since disease-related changes in RPE melanin content result in spatial variation of the NIR-AF signal intensity, appearance, or both. A sensitivity setting of 95% was invariantly used for all NIR-AF imaging. The automatic real-time averaging feature of the manufacturer's software was used whenever possible. When the automatic real-time feature failed, image stacks were exported from the manufacturer's software, spatially registered, and averaged. Automatic real-time–based or averaged images of the neighboring regions were digitally combined into a single mosaic image by manually specifying retinal landmark pairs.

RESULTS

DISEASE-CAUSING *NPHP5* VARIANTS IN PATIENTS WITH LCA

Plausible disease-causing genotypes were identified in *NPHP5* in 9 of the 276 patients with LCA (3.2%) (Table). Seven different disease alleles were observed, 4 of which have been previously observed in individuals with Senior-Loken syndrome (F141del, R461X, H506del, and R489X) and 3 of which have not been previously reported (A111del, E346X, and R455X). All of these variations would be expected to result in a truncated protein. Samples from 1 or both parents of 7 of the probands were available for assessing the phase of the observed variants. In 6 of these 7 probands, each parent contributed 1 disease allele to their affected child. In the remaining case, the affected individual was found to be homozygous for the H506del variant of *NPHP5* and his mother was found to be heterozygous for this change. The child's father was found to be homozygous for the consensus normal sequence of *NPHP5*. The proband, parents, and 3 unaffected siblings were then genotyped with a series of 5 short tandem repeat polymorphisms that lie on chromosomes other than chromosome 3, and these genotypes clearly confirmed the stated pedigree relationships (data not shown). Genotyping of an additional 11 short tandem repeat polymorphism markers from chromosome 3 revealed the affected child to be homozygous for a maternal allele at every locus tested, indicating maternal uniparental isodisomy (Figure 1).

In addition to the 7 disease-causing *NPHP5* variants, we also observed a missense variation at codon 435 (R435C) in 3 patients with LCA (1.1%) and 3 unaffected control individuals (1.1%). Although the frequency of this variant was identical in patients and controls, we noted that 2 of the 3 patients with LCA heterozygous for R435C were also heterozygous for the most common disease-causing variant in *NPHP6* (IVS 26 + 1655 A>G). Because only 12% of our LCA cohort harbored this *NPHP6* variant, the probability of 2 of the R435C heterozygotes harboring this variant by chance is less than 2%. However, investigation of the parents of these compound heterozygotes revealed that in 1 family, both alleles were inherited from the same unaffected parent. Thus, it is quite unlikely that these 2 alleles are acting together to cause LCA in these 2 families. Another potential modifying variant that we considered was A229T in *RPGRIP1L*, which was recently shown to accentuate the phenotype of *NPHP6*-associated disease.[30] None of the 9 patients with *NPHP5* mutations in this series was found to harbor this variant.

CLINICAL FINDINGS IN PATIENTS WITH *NPHP5* VARIATIONS: NONSYNDROMIC LCA IS THE EARLIEST DISEASE EXPRESSION

The clinical features of the 9 individuals with *NPHP5* variations are summarized in the Table. All patients had significantly reduced vision noted in the first few months of life and nystagmus. Only 1 of the 18 eyes of these 9 individuals had a visual acuity better than

20/200 and 5 of the patients had light perception or hand motions visual acuity in both eyes on their most recent visit. In both patients from whom multiple acuity measurements were available, there was progressive worsening of vision; 1 patient fell from 20/260 acuity at age 1 year to light perception at age 3 years. Three patients with visual acuity better than hand motions had visual field testing performed and all 3 exhibited only small (>20°) central islands of vision in each eye. Of the patients with recorded refractions, all had 4 to 9 diopters of hyperopia. Six of the patients were studied with electroretinography and there were no detectable responses under any stimulus condition. None of the patients exhibited signs or symptoms of renal disease in the first decade of life but 2 were diagnosed with NPHP at age 13 years. The oldest patient examined (age 23 years) had the best visual acuity (20/80 OD) but developed NPHP at age 13 years. In contrast, the second oldest patient examined (age 19 years) had the poorest vision (bare light perception) and no symptoms of renal disease. Specifically, she had no polydipsia, polyuria, or nocturia and had a normal blood urea nitrogen level of 11 mg/dL (to convert to millimoles per liter, multiply by 0.357) (reference range, 7–24 mg/dL). However, her serum creatinine level was slightly elevated (1.1 mg/dL [to convert to micromoles per liter, multiply by 88.4]; reference range, 0.6–1.0 mg/dL).

PHOTORECEPTOR AND RPE DISEASE IN *NPHP5* IS SIMILAR TO THAT IN *NPHP6*

We studied the retinal photoreceptor layer and the RPE of a subset of patients with *NPHP5*-associated retinal disease. Cross-sectional images using OCT of the central retina of a normal subject showed a foveal depression or pit and orderly retinal layers corresponding to nuclei, synapses, and axons (Figure2A, left panel). The ONL (highlighted in blue) was thickest at the fovea and thinned with increasing distance from it. In 3 patients (aged 4, 13, and 23 years) with *NPHP5* mutations, the images exhibited relative sparing of the ONL at the fovea but this was very diminished with increasing distance from the fovea (Figure 2A, right panels). A key observation was that the lamination attributed to the inner and outer segments and cilium of the photoreceptors (Figure 2A, bracketed with ONL in the normal subject and black arrows in patients) differed in the *NPHP5*-mutant retinas compared with normal. Whereas the laminae deep to the ONL were present in the normal retinal cross-section, a distinct laminar architecture was absent in the patients, suggesting disorganized inner and outer segment material.

The spatial distribution of the *NPHP5* retinopathy was shown in 2 of the patients (Figure 2B). Normal photoreceptor layer thickness has a central peak that declines with distance from the fovea. There is a shallower rate of decline in the superior direction that results in anisotropic contour lines extending superiorly. In the 13-year-old patient with *NPHP5* mutation, there was a central ellipsoid region approximately 6 mm wide and 4 mm high centered on the fovea with retained photoreceptors; a similar region with less extent was detectable in the 23-year-old patient with *NPHP5* mutation.

The topography of RPE integrity in eyes with *NPHP5* mutation was defined with NIR-AF imaging (Figure 2C); the signal is considered to originate from melanin and melanolipofuscin granules in the RPE and melanin in the choroid.[26,28,31] In normal human eyes, NIR-AF images show a circular central region of homogeneous appearance and higher brightness extending from the fovea. With increasing eccentricity, there is a decrease in brightness (Figure 2C). The NIR-AF topographies of eyes with *NPHP5* mutation showed a central ellipsoid of higher intensity surrounded by a more peripheral region of lower intensity. In the 23-year-old patient, there was evidence of 2 zones of RPE demelanization. Surrounding the central ellipsoid was a wide homogenous annulus of lower intensity likely corresponding to partially demelanized RPE cells. Further peripheral was a heterogenous annulus likely corresponding to loss of RPE cells and appearance of choroidal structures.

COMMENT

There are numerous examples in which clinical entities that were first described in the pregenomic era fail to map crisply to the genome. Some clinical phenotypes (eg, Senior-Loken syndrome[32] and LCA[11]) are caused by more than 1 gene while variations in some genes (eg, *NPHP5* and *NPHP6*) cause different clinical phenotypes in different patients. This situation would be challenging enough if all clinical findings were present at the same point and the clinician needed only to look for them during a patient's first examination. However, in many multisystem conditions, 1 or a few of the manifestations are prominent and occur early in the disease course while others are more subtle and manifest much later if at all.

The patients described by Senior et al[3] and Loken et al[4] in the early 1960s had profound renal disease that caused death from uremia as early as age 7 years. Knowledge of this literature might lead some clinicians to expect that if one of their patients with LCA were destined to have associated renal disease, it would be overtly manifest in the first decade of life. The patients in this report were all referred for molecular diagnosis before any sign of renal disease appeared, and 7 of them (1 now aged 19 years) are still free of overt renal disease. Similarly, Otto and colleagues[10] found that end-stage renal disease can occur relatively late in life in some patients with *NPHP5* mutations. These data illustrate that the frequencies of various clinical findings in patients with given genotypes will vary with the age of the patients and the degree to which they have been examined and tested. The clearest understanding of the phenotype of multisystem disorders will come from studies of large numbers of older patients. Unfortunately, for the past several decades, many children who were born blind were not carefully followed up by their ophthalmologists because of a prevailing sense that nothing could be done to help them. The advent of successful gene therapy for 1 form of LCA[15–18] is starting to reverse this attitude and kindle a greater interest in the natural history of all molecular forms of this disease (https://www.carverlab.org/project3000).

Examination of the Table reveals a wide variation in clinical findings among patients with seemingly similar disease alleles. That is, both *NPHP5* alleles of every patient in this series have frameshifts or nonsense mutations that might reasonably be expected to result in a complete loss of function of the protein (ie, through nonsense-mediated decay[33] or protein truncation). What then could account for the variations in phenotype? There are at least 3 nonexclusive possibilities that can be considered. First, some transcripts may escape nonsense-mediated decay and be transcribed into a partially functional truncated protein or "read through" to yield a small amount of normal protein. There is evidence that the most common LCA-causing mutation in human *NPHP6*, a cryptic splice variant in IVS 26,[12] results in some residual NPHP6 function. For example, most individuals who are homozygous for the IVS 26 variant have normal brain development as do individuals who are heterozygous for the IVS 26 change and either His406del2cAT[34] or Ile1372del2gaaAT.[12] In contrast, compound heterozygosity of His406del2cAT and Ile1372del2gaaAT has been reported to cause fatal maldevelopment of the brain (Meckel-Gruber syndrome).[34] If partial NPHP5 function is associated with some of the mutations observed in our series, some may result in more function than others. The patients with the best and worst visual acuity in our series each harbor alleles that have not been previously observed and may account in part for their phenotypic differences. A second potential explanation for variations in the phenotypes of these patients is the effect of some environmental factor. One could imagine that a partially dysfunctional kidney might be prone to injury by infections or dietary changes that would be completely harmless to a healthier organ. If such insults were nonuniform in the population, they could account for the kinds of variation seen in the Table. A third possibility is the effect of the genetic

background, that is, variations in other genes that by themselves do not alter an individual's phenotype but that do alter the pathogenicity of a disease-causing allele.

The most likely candidates for genes that could harbor such augmenting or mitigating variations would be genes already associated with congenital retinal dysfunction. All 276 patients with LCA enrolled in this study were extensively screened for variations in other LCA-causing genes and some of them were found to harbor a single disease-causing allele in a known LCA gene. For example, 21 of the 276 patients harbor the most common LCA-causing allele of *NPHP6* (IVS 26 + 1655 G>A) but none of these 21 individuals were among the 9 patients with disease-causing *NPHP5* variants. An extreme case of such "background" effects occurs when heterozygous alleles of 2 different genes—neither of which is capable of causing disease on its own—cause disease when present in the compound heterozygous state (ie, digenic inheritance). The R435C variant of *NPHP5* is present in approximately 1% of the general population while the IVS 26 1655G>A variant of *NPHP6* is present in fewer than 0.1% of the population.[11] Thus, it would seem extremely unlikely to observe 2 compound heterozygotes of these variations among only 276 patients in our LCA cohort. However, the frequency of the *NPHP6* variant in the LCA cohort was actually 7.6%, not the 0.1% of the general population; thus, we might have expected to see this combination once per 1300 patients with LCA, which is not statistically different from the 2 in 276 that we did observe. The most telling observation was that the normally sighted mother of 1 of the compound heterozygous patients with LCA was herself a compound heterozygote of these 2 variants, making the pathogenicity of this genotype extremely unlikely. The final modifying variant that we considered was A229T in *RPGRIP1L*, which was recently shown to accentuate the phenotype of *NPHP6*-associated disease.[30] None of the 9 patients with *NPHP5* mutations in this series was found to harbor this variant.

The foregoing analysis reveals the value of access to parental genotypes when evaluating their possible pathogenicity in affected individuals. Parental genotypes are also extremely valuable for detecting de novo mutations of various types. For example, nondisjunction during meiotic cell division results in gametes with abnormal numbers of chromosomes (nullisomy or disomy). When such a gamete is joined by a normal gamete to form a zygote, either trisomy or monosomy occurs. Loss of an extra chromosome in early mitotic cell division (trisomy rescue) or gain by duplication of a monosomic chromosome (monosomy rescue) results in an individual with 2 copies of a chromosome inherited from 1 parent and none from the other (uniparental isodisomy). If a recessive disease-causing allele lies on the disomic chromosome, it can result in the inheritance of a recessive disease from only 1 parent, as it did in patient 3 (Table) in our series.

Some chromosomes are more likely to become disomic than others. Of the cases of isodisomy reported in the literature, chromosomes 14 and 15 are the most likely to be involved.[35] Previous cases of isodisomic LCA observed involved a mutation in the *RPE65* gene on chromosome 1.[11,36] Isodisomy of chromosome 3 is very rare. We have found 2 instances of isodisomic LCA among a cohort of 260 individuals with both disease-causing alleles identified and with sufficient parental data to have revealed isodisomy if it existed. Thus, isodisomy seems to be involved in less than 1% of all cases of LCA. Despite its rarity, this inheritance pattern is important to recognize because the risk to subsequent siblings of an affected individual is near zero[35] in contrast to the recurrence risk of 25% when each parent carries a disease-causing allele.

The similarity of the retinal phenotypes of patients with *NPHP5*- and *NPHP6*-associated LCA has both mechanistic and therapeutic implications. The relative preservation of photoreceptor cells in the central 4 mm of the retina in patients with disease-causing mutations in either of these genes suggests some physiologic difference between these

photoreceptors and those elsewhere in the retina. The most obvious difference between the foveal and extrafoveal retina is the relative concentration of cone photoreceptors and the ratio of cone and rod photoreceptors. In vivo microscopy of the retina in our patients taken together with the normal anatomy[37] suggests that the majority of the remaining photoreceptors visible by OCT are cones. We suspect that cone photoreceptors in the central 4 mm of the retina have physiologic differences from cones elsewhere in the retina in addition to their obvious anatomical differences.[37] For example, the central cones could have much slower outer segment turnover than cones elsewhere and this difference could make them less dependent on the normal function of NPHP5, NPHP6, and other centrosomal proteins. Recent advances in noninvasive methods may allow testing of this hypothesis.[38]

Although there are certainly widespread retinal abnormalities in patients with *NPHP5*-associated LCA that would not be expected to respond to gene therapy, the persistence of a recognizable photoreceptor layer in the central retina of these patients is especially encouraging for future therapeutic interventions. For the subset of patients who develop end-stage renal disease, renal transplant is usually very successful and NPHP will not recur in a transplanted kidney.[39] Inquiring about polydipsia, polyuria, and nocturia in juvenile and adolescent patients with LCA can help the molecular diagnostic laboratory to focus their initial efforts on the NPHP gene family. Similarly, the more common *NPHP5* and *NPHP6* sequence variations should be screened in all patients with molecularly uncharacterized LCA because a subset of them may harbor mutations in these genes without any evidence of renal disease. These patients should be followed up periodically by nephrologists familiar with inherited renal disease so that if kidney disease does develop, the patients can be cared for in a timely fashion.

Acknowledgments

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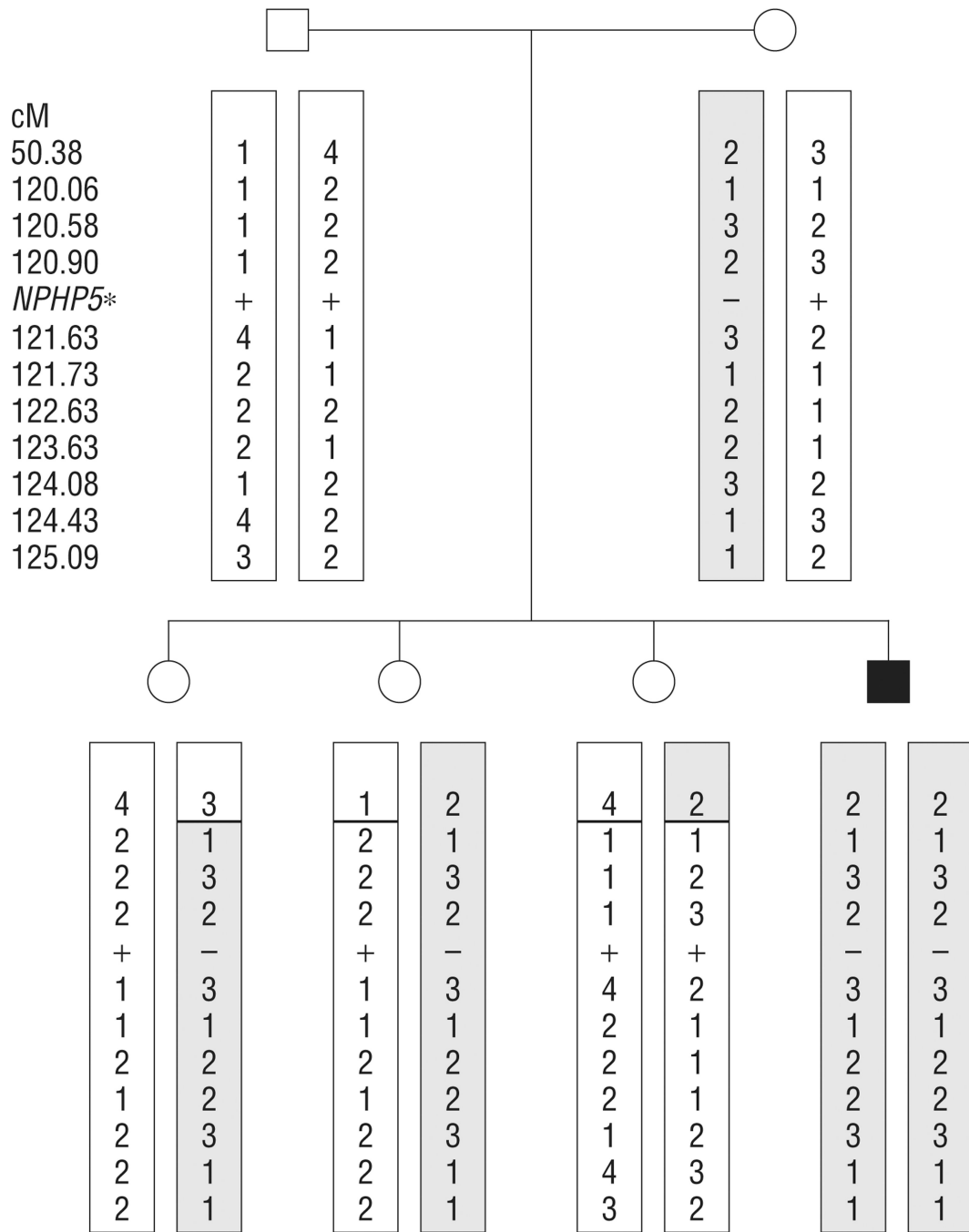
Figure 1.
Chromosome 3 genotyping of patient P3 and his family. These individuals were genotyped at 11 loci distributed across chromosome 3. The mutation (H506del) lies at 121.49 centimorgans (cM) (asterisk). For all 11 markers, the patient is homozygous for a maternal marker allele, consistent with maternal uniparental isodisomy.
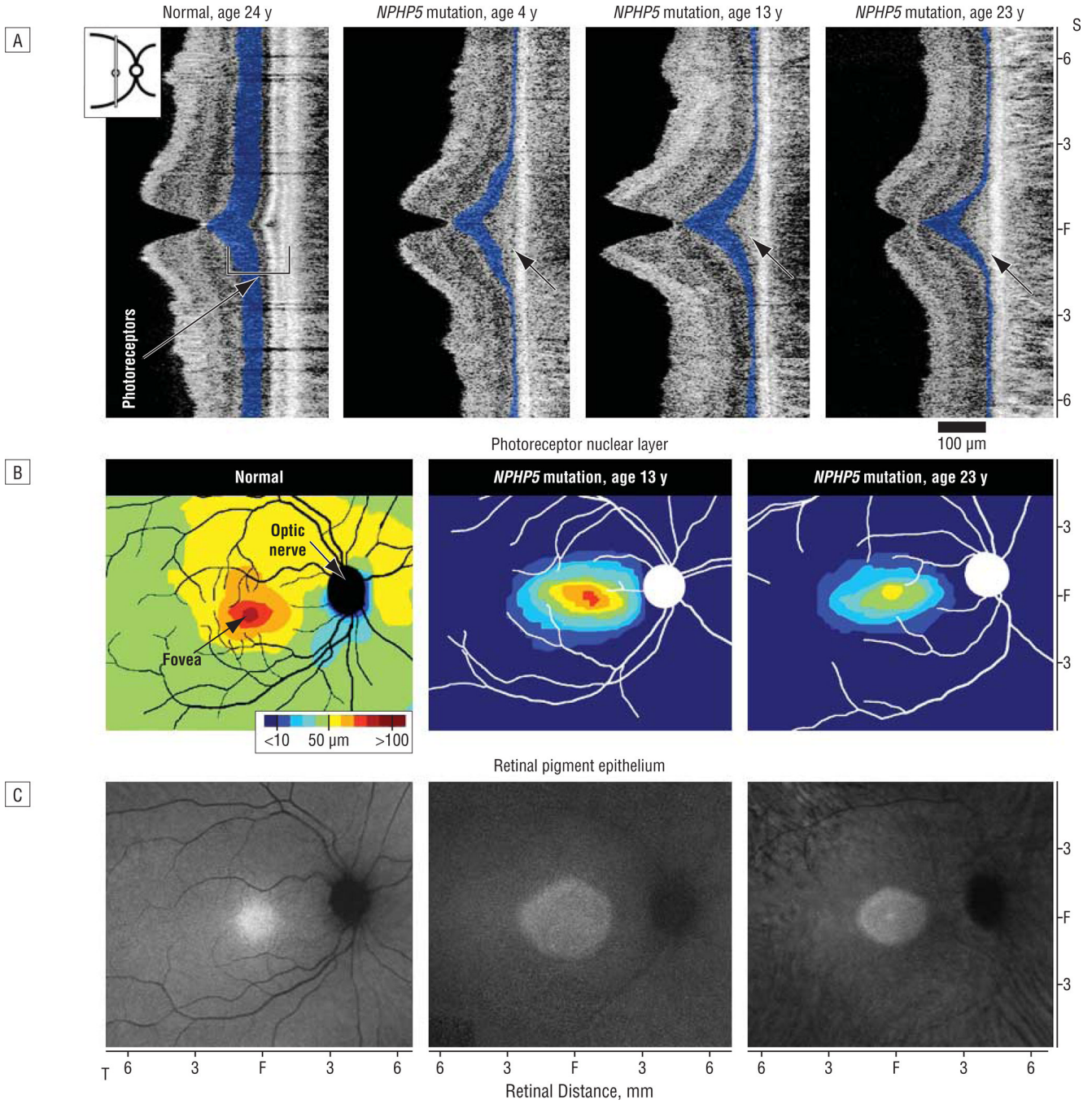
Figure 2.
NPHP5-mutant retinas analyzed for photoreceptor and retinal pigment epithelium disease. A, Cross-sectional images along the vertical meridian are shown in a normal subject (left panel) and 3 patients with *NPHP5* mutation of different ages (right 3 panels). The photoreceptor nuclear layer is highlighted (blue); inner and outer segment laminae and cilial structure are indicated (bracketed with the photoreceptor nuclear layer in the normal subject and unlabeled black arrows in patients). B, Topography of photoreceptor layer thickness in a normal retina (left) and 2 *NPHP5*-mutant retinas (right panels), displayed in pseudocolor. The location of the optic nerve head and major retinal blood vessels are overlaid (black or

white) for reference. The fovea and optic nerve head are labeled in the normal subject. C, Retinal pigment epithelium integrity as measured with near infrared autofluorescence in a representative normal subject (left panel) and in the patients with *NPHP5* mutation. Higher intensity represents greater melanin content. F indicates fovea; S, superior; T, temporal.

Table

Clinical and Molecular Characteristics of the 9 Patients With *NPHP5* Variations

Patient No./Sex	Age at Visit, y	Nucleotide Change Allele 1	Protein Change Allele 1	Nucleotide Change Allele 2	Protein Change Allele 2	Visual Acuity[a] OD	Visual Acuity[a] OS	Refraction[b]	ERG	Renal Disease
1/F	4	c.333delT	Ala111del1gcT	c.1381C>T	Arg461Stop	U	U	+6.75	ND	Severe (by age 13 y)
	23					20/80	20/200	NP		
2/M	5	c.1465C>T	Arg489Stop	c.1516-1517delCA	His506del2cagCA	LP	LP	+7.50	NP	None manifest[c,d]
3/M	7	c.1516-1517delCA	His506del2cagCA	c.1516-1517delCA	His506del2cagCA	20/400	20/800	+6.00	ND	None manifest[d,e]
4/M	13	c.1381C>T	Arg461Stop	c.1516-1517delCA	His506del2cagCA	20/200	20/200	+4.00	ND	None manifest[d]
5/M	14	c.1381C>T	Arg461Stop	c.1465C>T	Arg489Stop	HM	HM	NP	ND	Severe (by age 13 y)
6/F	8	c.1363C>T	Arg455Stop	c.421-422delTT	Phe141del2cacTT	20/200	20/200	+6.00	NP	None manifest[d]
7/F	3	c.1036G>T	Glu346Stop	c.1516-1517delCA	His506del2cagCA	20/1000	20/1000	+9.00	ND	None manifest[d]
	12					HM	HM	+5.00	NP	None manifest[d]
	19					LP	LP	NP	NP	Elevated creatinine level
8/F	1	c.1381C>T	Arg461Stop	c.1516-1517delCA	His506del2cagCA	20/260	20/290	+6.50	ND	None manifest
	3					LP	LP	NP	NP	
9/F	14	c.1381C>T	Arg461Stop	c.1381C>T	Arg461Stop	LP	LP	+5.25	NP	None manifest

Abbreviations: ERG, electroretinogram; HM, hand motions; LP, light perception; ND, not detectable; NP, not performed; U, unreliable.

[a] Best-corrected visual acuity.

[b] Spherical equivalent.

[c] Ureterocele, ureteral duplication.

[d] Normal blood urea nitrogen and creatinine levels.

[e] Hyperaldosteronism.