Novel Insights Into the Phenotypical Spectrum of KIF11-Associated Retinopathy, Including a New Form of Retinal Ciliopathy

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PURPOSE. This study sought to characterize the ophthalmic and extraocular phenotype in patients with known and novel KIF11 mutations.

METHODS. Four patients (3, 5, 36, and 38 years of age, on father-daughter constellation) from three unrelated families were characterized by retinal examination including multimodal retinal imaging, investigation for syndromic disease manifestations, and targeted next-generation sequencing. The subcellular localization of Kif11 in the retina was analyzed by light and electron microscopy.

RESULTS. There was considerable interindividual and intrafamilial phenotypic heterogeneity of KIF11-related retinopathy. Two patients presented with a progressive retinal dystrophy, one with chorioretinal dysplasia and one with familial exudative vitreoretinopathy (FEVR) in one eye and thinning of the photoreceptor layer in the fellow eye. Obvious syndromic disease manifestations were present only in the youngest patient, but minor signs (e.g. reduced head circumference) were present in the three other individuals. Immunohistochemistry results demonstrated Kif11 localization in the inner segment and ciliary compartments of photoreceptor cells and in the retinal pigment epithelium.

CONCLUSIONS. Progressive retinal degeneration in KIF11-related retinopathy indicates a role for KIF11 not only in ocular development but also in maintaining retinal morphology and function. The remarkable variability of the ocular phenotype suggests four different types of retinopathy which may overlap. KIF11 should be considered in the screening of patients with retinal dystrophies because other syndromic manifestations may be subtle. Evaluation of head circumference may be considered as a potential shortcut to the genetic diagnosis. The localization of Kif11 in photoreceptor cells indicates a retinal ciliopathy.

Keywords: KIF11, next-generation sequencing, retinal dystrophy, retinopathy, ciliopathy

Monogenic retinal diseases are a frequent cause of severe visual impairment in children and young adults. The disease entities may differ in their mode of inheritance, progression of retinal lesions and functional alterations, and their manifestation as isolated retinopathy or syndrome. Thus, a correct diagnostic classification may guide appropriate counseling and may impact the patient’s perceived burden of disease, personal plans for the future, and family planning.

Mutations in kinesin family member 11 (KIF11) encoding a homotetramer spindle motor protein (EG5) of the kinesin family have recently been found to be causative for a rare syndromic form of chorioretinal alteration with autosomal dominant inheritance (Online Mendelian Inheritance in Man [OMIM] #152950).1,2 Characteristic extraocular manifestations include microcephaly, developmental delay or mental retardation and, less consistently, congenital lymphedema of the dorsa of the feet. A characteristic facial phenotype with upward-slanting palpebral fissures, broad nose with rounded tip, long philtrum with thin upper lip, prominent chin, and prominent ears has been described.3–7 The syndromic features show incomplete penetrance, with some heterozygous carriers of KIF11 mutations lacking some or all features of the syndrome.

The chorioretinal lesions have so far been considered to represent a stationary dysplasia, although reports of long-term observations are scarce.8 Moreover, alterations resembling a retinal dystrophy with retina-wide dysfunction have been
reported, but again no progression of these changes over time has been reported. Additional but inconsistently observed ocular changes include retinal detachment, angle-closure glaucoma, nyctagmus, optic atrophy, retinal folds, persistent hyperplastic primary vitreous, cataract, pseudocoloboma, persistent hyaloid artery, hyperopia, myopia, and astigmatism.3–5,9–11

Recently, mutations in KIF11 have also been reported in 5.6% to 8.3% of patients with familial exudative vitreoretinopathy (FEVR).12 A rare inherited developmental disease of the retina.13 Although patients were not systematically investigated for syndromic features, most had microcephaly and/or mental retardation.

Here, we present a detailed characterization of the retinal phenotype and extraocular signs in four patients from three families with KIF11 mutations, two of them novel. In addition, we provide first evidence for progression of KIF11-related retinal degeneration and demonstrate expression of Kif11 in the adult retina, indicating a new form of retinal ciliopathy. Finally, our observed intrafamilial phenotypic variability in conjunction with the heterogeneity of previously reported cases allowed us to propose distinct types of KIF11-related retinopathy.

METHODS

Patients, Retinal Imaging, and Functional Testing

Patients were identified in a referral center for rare retinal diseases at the Department of Ophthalmology, University of Bonn, Germany. In this retrospective single-center study, three index patients harboring KIF11 mutations and their families were included. One patient has been described previously but without a detailed characterization of the ocular phenotype.3 The study adhered to Declaration of Helsinki tenets. Institutional review board approval (Ethics Committee, Medical Faculty, University of Bonn) and patients' informed consent were obtained. For all patients, a full medical and family history was obtained, and syndromic disease manifestations were recorded.

Clinical assessment included standardized anterior segment and dilated fundus examination, best corrected visual acuity (BCVA) and electroretinography (ERG). Retinal imaging consisted of spectral-domain optical coherence tomography (OCT), fundus autofluorescence (FAF) imaging (Spectralis HRA+OCT, Heidelberg Engineering, Heidelberg, Germany), fundus photography (Visucam, Zeiss, Oberkochen, Germany), and wide-field fundus imaging (Optos PLC, Dunfermline, United Kingdom).

Genetic Counseling and Mutation Analysis

Genetic counseling included a detailed documentation of possible syndromic manifestations and family history assessment performed by a medical geneticist. Asymptomatic relatives of index patients received genetic counseling prior to mutation testing.

In one instance (patient 4), the disease-causing mutation had already been reported.3 Genetic testing for the other two index patients was conducted through targeted next-generation sequencing (NGS) using a HiSeq 1500 platform (Illumina, San Diego, CA, USA). The gene panel analyzed includes a large number of genes known to cause developmental and progressive inherited retinal diseases (including syndromic forms). Bioinformatic software used for evaluation of variants' pathogenicity initially focused on 44 genes known to be associated with retinal degeneration preferentially affecting central regions of the retina such as macular dystrophy, cone dystrophy, and cone-rod dystrophy (status as of August 2013), as described previously.14,15 If no mutation was identified in this subset, the remaining retinal disease genes, including KIF11, were analyzed for potentially pathogenic variants. Verification of identified variants was carried out by polymerase chain reaction amplification of the corresponding exon, followed by Sanger sequencing. The National Center for Biotechnology Information reference sequence for KIF11 is NM_004523.3.

Analysis of Retinal Kif11 Expression and Localization

To investigate Kif11 expression in the adult mouse retina and testis, Western blot analysis was performed using polyclonal antibodies against Kif11. Immunohistochemistry with antibodies against Kif11 was used to study the localization of Kif11 in the adult murine retina. Additional colabeling was performed relative to centrin-3 as a marker for the centriole, basal body, and connecting cilium of the photoreceptor cells, relative to the cilary marker acetylated tubulin (acTub), and to Ribeye as a marker for the synaptic ribbon of presynaptic terminals in the inner plexiform layer. Nuclear DNA was labeled using 4,6-diamidino-2-phenylindole (DAPI). Immunoelectron microscopy was performed to identify high-resolution localization of Kif11. A more detailed description of the Kif11 expression analysis can be found in Supplementary Methods.

RESULTS

Age, sex, BCVA, refraction, and genetic characteristics of the reported patients are given in Table 1. The mutations identified are either likely truncating (e.g., nonsense mutation and splice-site mutation in patient 3 and patient 4) or have eliminated a highly conserved amino acid, indicating likely loss-of-function alleles. Allele frequencies have not been documented in the general population. All mutations arose as a de novo event in the 3 families, further supporting their pathogenicity. Additional genetic findings are reported in Supplementary Results.

Patients 1 and 2 (Father and Daughter)

Index patient 1 presented at 5 years of age with decreasing vision. Ophthalmic examination revealed myopia, astigmatism, bilateral outer retinal atrophy at the posterior pole with relative sparing of the fovea, and an abnormal vitreoretinal interface in the (mid-)periphery (Fig. 1; Supplementary Fig. S1). Fundus autofluorescence imaging revealed a decreased signal within atrophic areas which was surrounded by a border of increased FAF (Fig. 1). Optical coherence tomography imaging showed severe outer retinal thinning within the area of retinal atrophy and milder but widespread thinning of the photoreceptor layer throughout the scanned area (Fig. 2). Peripapillary retinal nerve fiber thickness was within normal limits. Although no comprehensive electrophysiology examination was achievable, reduced photopic and scotopic responses were detected.

The patient's parents reported she had poor concentration at school, difficulties in mathematics, and physical hyperactivity. However, she had not had the explicit diagnosis of attention deficit or hyperactivity. The patient's head circumference (at the 42nd percentile), height, and weight were within normal ranges. At clinical examination, mild upward-slanting palpebral fissures, a slightly broadened nose tip, and two light stork bites, one in the neck and one in the lumbar region, were noted (Table 2; Fig. 3). Overall, extraocular...
### TABLE 1. Patient Demographics, Visual Acuity, Refraction, and KIF11 Mutations

<table>
<thead>
<tr>
<th>Patient/ Sex</th>
<th>Age/ Follow-up</th>
<th>BCVA, RE; LE</th>
<th>Refraction, RE; LE</th>
<th>KIF11 Mutation</th>
<th>Exon</th>
<th>Protein Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F</td>
<td>5 y/56 mo</td>
<td>20/40; 20/40</td>
<td>−3.0/−1.0 × 45°; −4.0/−1.75 × 157°</td>
<td>c.1844_1846delCAG</td>
<td>14</td>
<td>p.Ala615del</td>
</tr>
<tr>
<td>2/M†</td>
<td>36 y/24 mo</td>
<td>20/800; 20/20</td>
<td>+2.0/−2.0 × 86°; +1.5/−0.25 × 6°</td>
<td>de novo in P2</td>
<td>14</td>
<td>p.Ala615del</td>
</tr>
<tr>
<td>3/M</td>
<td>38 y/10 mo</td>
<td>20/2000; 20/500</td>
<td>−5.75/−5.75 × 5°; −5.0/−1.25 × 17°</td>
<td>c.808G&gt;T de novo</td>
<td>8</td>
<td>p.Glu270*</td>
</tr>
<tr>
<td>4/F</td>
<td>3 y/40 mo</td>
<td>20/63; 20/125</td>
<td>+4.0/−0.5 × 60°; +5.5/−0.25 × 179°</td>
<td>c.2267+1G&gt;A de novo</td>
<td>17i</td>
<td>Donor splice site</td>
</tr>
</tbody>
</table>

BCVA, best corrected visual activity; LE, left eye; RE, right eye.
† Patient 2 is the father of patient 1.

**FIGURE 1.** Variable phenotype of KIF11-associated retinopathy in patients 1-3. Wide-field fundus (first column), fundus color (second column), wide-field fundus autofluorescence (third column), and 55-degree fundus autofluorescence images (fourth column). Findings are suggestive for a predominantly central retinal dystrophy. Patient 2 presented with a retinal fold in his right eye, whereas the left eye was unremarkable on these imaging modalities. LE, left eye; RE, right eye.
manifestations were subtle and had not led to detailed medical investigations such as cranial magnetic resonance imaging (MRI).

The father of patient 1 (patient 2) presented to our department at 36 years of age. At 1 year of age, he had received a diagnosis of unilateral radial retinal fold and reduced visual acuity in the right eye. Strabismus surgery was performed for esotropia at the age of 9 years. His medical records since then indicated stable vision and funduscopic findings, with a right eye fibrotic mass at the peripheral end of the retinal fold, a pale, dysmorphic optic nerve head with V-shaped vascular formation, and a hypopigmented nasal fundus with reduced vascularity (Fig. 1). Fluorescein angiography to assess potential peripheral retinal avascularity was not performed. There was no chorioretinopathy except for a small chorioretinal atrophic patch in the nasal periphery of the fundus in the left eye. The patient reported normal vision with his left eye, and there were no functional deficits on visual acuity and static 30-degree visual field testing. However, retinal thickness mapping of the left eye using OCT imaging revealed a borderline low retinal thickness, which appeared to be due mainly to thinning of the photoreceptor layer (Fig. 2; Supplementary Fig. S2). Electroretinography examination revealed retina-wide photoreceptor dysfunction (right > left), which was more pronounced in the scotopic than in the photopic recordings. Ocular hypertension in both eyes was well controlled with topical beta-blocker therapy. Peripapillary retinal nerve fiber thickness, as measured by OCT, appeared severely reduced in the right eye (most likely due to the abnormalities associated with retinal fold, which also made the assessment unreliable) but was within normal limits in the left eye.

The patient reported difficulties with mathematics at school but an otherwise normal development. At 31 years of age,

FIGURE 2. Structural retinal changes of KIF11-associated retinopathy in comparison to those in a healthy control on OCT images. The photoreceptor layer is highlighted in yellow. Images in the right column are magnifications of the foveal region. The right eye of the control and patients 1, 3, and 4 and the left eye of patient 2 are shown.

TABLE 2. Clinical Features of Patients With KIF11 Mutations

<table>
<thead>
<tr>
<th>Patient / Age at Genetic Assessment</th>
<th>Neuroimaging</th>
<th>Behavioral</th>
<th>Gastroesophageal Reflux</th>
<th>Heart Defect</th>
<th>Neurological</th>
<th>Hearing Loss</th>
<th>Gastroesophageal Reflux</th>
<th>Hypospadias</th>
<th>Behavioral Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1, 10 y</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Patient 2, 38 y</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Patient 3, 38 y</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Patient 4, 10 mo</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

Patient reports: +, present; ( ), questionable; –, not present; EU, European; ID, intellectual disability; NA, not assessed.

- Possible variant of genotype-phenotype correlation.
- Some features indicating attention deficit/hyperactivity (poor concentration, easily distracted at school, always physically in motion).
- Assessable only in males.
- Others features indicating attention deficit/hyperactivity (poor concentration, easily distracted at school, always physically in motion).

- Assessment only in males.
epilepsy with sleep-bound grand mal seizures and focally complex seizures was noted and medically treated. An MRI scan revealed a cystic lesion in the left precentral gyrus of unknown origin. Height and head circumference were in the lower normal range (13th and 5th percentiles, respectively). At clinical examination, a sloping forehead, hypotelorism, upward-slanting palpebral fissures, a broad nose tip, and a thin upper lip were noted (Table 2; Fig. 3).

**Patient 3**

The patient was observed to have reduced visual acuity and an increased sensitivity to glare at 2 years of age. Based on previous medical records, retinal dystrophy was diagnosed in childhood but was not further specified. At 19 years of age, visual field testing revealed severe concentric constriction.

When the patient presented to our department at 38 years of age, funduscopic findings were consistent with a retinal dystrophy of the cone-rod type, including diffuse retinal pigment epithelial degeneration, pigment clumping mainly at the posterior pole, pale optic discs, and attenuated vessels. Fundus autofluorescence imaging revealed an irregular area of decreased FAF in the central fundus, with a surrounding area of increased FAF (Fig. 1). There was severe outer retinal atrophy on OCT imaging (Fig. 2). Electroretinography testing revealed undetectable dark-adapted rod-driven responses and minor light-adapted cone-driven responses.

The patient’s parents reported dyscalculia that became apparent in puberty but an otherwise unremarkable development apart from visual impairment. Head circumference was at the 2nd percentile, and at clinical examination, a sloping forehead, upward-slanting palpebral fissures, a broad nose, and a thin upper lip were noted (Table 2; Fig. 3).

**Patient 4**

The patient and her molecular diagnosis had been described before but without a detailed characterization of the ocular phenotype. Briefly, the patient, born full term, initially presented at 4 months of age with microcephaly, bilateral congenital pedal lymphedema, dysmorphic features, and developmental delay. Delayed myelination was found on MRI neuroimaging, and relatively wide outer cerebral fluid inter-spaces due to microcephaly. She had possible seizure activity during infancy, but on ERG, no pathology was detected.

Ophthalmic examination at 3 years of age revealed a convergent strabismus in the left eye and bilateral hyperopia and astigmatism. Funduscopy revealed pale optic discs and areas of sharply demarcated chorioretinal atrophy involving mainly the macula and the inferior mid-periphery (Fig. 4). Optical coherence tomography imaging showed severe outer retinal thinning and atrophy (Fig. 2). Electroretinography, retinal wide-field and AF imaging were not performed due to reduced cooperation.

**Progression of Retinal Disease**

Visual acuity of all four patients remained stable within the observational period at our department (Table 1). The two older patients (patients 2 and 3) and their relatives were interviewed about perceived long-term changes in visual function over the decades. Patient 2 had noted no change over time, which was consistent with his medical records. However, patient 3 reported progressive night blindness in childhood, and he also reported worsening of overall visual function noticeable in daylight. According to previous external medical records, BCVA was 20/100 in both eyes at 10 years of age, and a slow but steady decline was documented over the decades to 20/2000 and 20/500 in the right and left eyes, respectively (Table 1).

Disease progression was also observed by tracking morphologic changes over time. In patient 1, OCT imaging revealed slight foveal thinning, and FAF imaging showed an increasing area of reduced AF, whereas the surrounding border of increased AF shifted accordingly (Fig. 4A). Of note, this mild progression of retinal atrophy was not apparent on fundus color images.

No significant morphologic changes could be detected in patient 2 with FEVR in one eye and a very mild retinal phenotype in the fellow eye, and over a relatively short follow-up time in patient 3 with advanced atrophic changes of the central retina. In patient 4, similar high-quality longitudinal imaging (SD-OCT and FAF) was not possible due to reduced cooperation. Thus, a possible enlargement of the chorioretinal...
atrophy (Fig. 4B) on fundus color images remained uncon-

**Expression and Localization of Kif11 in the Mammalian Retina**

Western blot analysis for expression of Kif11 in the retina revealed distinct bands at 28, 40, and 60 kDa, respectively (Fig. 5A), which differ from the calculated molecular weight of 118 kDa and most probably represent splice variants of the frequently spliced *Kif11* gene (https://www.ncbi.nlm.nih.gov/IEB/Research/Assembly/av.cgi?db=mouse&q=Kif11) or degradation products were also detected in testis lysate used as reference tissue. In testis, in addition to the two polypeptides of 40 kDa and 60 kDa, the high-molecular full-length KIF11 protein is expressed, as documented for lysates of HEK293T cells overexpressing KIF11 in the company’s data sheet (https://atlasantibodies.com/#/products/KIF11-antibody-HPA006916). Immunostaining of Kif11 in the murine retina showed Kif11 localization in the retinal pigment epithelium, in the inner segment and the ciliary region of the photoreceptor layer, and in the inner and outer plexiform layers where the synaptic contacts of retinal neurons are present (Fig. 5B). Double-staining of Kif11 and centrin-3, a molecular marker for centrioles, basal bodies, and the connecting cilia of the photoreceptor cells, revealed colocalization of both of the proteins in the ciliary region of photoreceptor cells (Fig. 5C). In contrast, costaining of Kif11 and Ribeye, a molecule localized in the synaptic ribbon of presynaptic terminals, did not show colocalization indicating the Kif11 is not part of the presynaptic ribbon (data not shown).

Localization of Kif11 in the ciliary region of photoreceptor cells prompted us to pinpoint the specific localization of Kif11 in photoreceptor cilia applying high-resolution analysis of immunofluorescence double labeling (Figs. 5D, 5E) and immunoelectron microscopy (Fig. 5F). This correlative analysis by both techniques consistently demonstrated Kif11 localization at the adjacent daughter centriole, the basal body, and in the connecting cilium but not in the axoneme of the photoreceptor cilium (Fig. 5G).

**DISCUSSION**

Here, we report a comprehensive ophthalmologic characterization of four patients with retinal disease carrying three different mutations in *KIF11*. The molecular diagnosis was achieved by targeted NGS of a retinal disease gene panel in two of them. Intrafamilial variability over two generations is illustrated, and for the first time, progression of retinal degeneration is being documented.

**Phenotype of KIF11-Related Retinal Disease**

Analysis of the patients’ phenotype described herein and in previous reports indicates four distinct types of *KIF11*-related retinal changes which we use as a framework for classifying types 1 to 4 (Table 3). An overlap of the four different subtypes appears likely with phenotypic characteristics of different subtypes occurring in the same eye, although evidence from systematic investigations using multimodal imaging is not yet available. It also seems possible that types 1 to 3 represent a spectrum of a retinopathy with common pathophysiology, where phenotypic expression is mildest in type 1 and most severe in type 3 disease. Furthermore, intrafamilial cooccurrence of the four subtypes, with subtle or isolated symptoms to syndromic manifestation, was observed herein and elsewhere. Reduced outer retinal thickness on OCT images, indicating thinning of the photoreceptor layer, was a consistent
**FIGURE 5.** Expression and subcellular localization of Kif11 in the retina. (A) Western blot analyses revealed expression of Kif11 protein in the neuronal retina and testis (control tissue) of mice. Anti-Kif11 detected three protein bands in retinal lysates distinct from the high-molecular weight bands of the putative full-length Kif11 present in testis (arrowhead). (B) Longitudinal cryosections through a mouse retina stained for Kif11 (green) and counterstained for the ciliary marker centrin-3 (Cen3 [red]) and DAPI for nuclear DNA (blue). Differential interference contrast image overlaid with the blue DAPI nuclear staining of the outer nuclear layer (ONL) further demonstrated retina layering. Kif11 localized to the ciliary region (CR) and the inner segments (IS) but not to the outer segments (OS) of the photoreceptor layer. In addition, Kif11 is found at synapses of the outer plexiform layer (OPL) and to a small amount in the retinal pigment epithelium (RPE). (C) Increased magnification of the photoreceptor cells shows co-localization of Kif11 and Cen3 in the photoreceptor CR. (D, E) High magnification of the ciliary region of photoreceptor cell layer double stained for Kif11 (green) with Cen3 (red) and the ciliary marker acetylated tubulin (acTub [red]), respectively, reveal Kif11 localization at the adjacent centriole (Ce), basal body (BB), and connecting cilium (CC) but not in the axoneme (Ax) of the photoreceptor cilium. (F) Anti-Kif11 immunoelectron microscopy analysis of the ciliary region of a murine rod photoreceptor cell confirmed subciliary localization of Kif11 in the Ce, BB, and CC. (G) Schematic representation of Kif11 localization in a rod photoreceptor cilium. Scale bars B, C: 5 μm; D, E: 1 μm; F: 200 nm.

**TABLE 3.** Four Types of KIF11-Associated Retinopathy

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
<th>Type 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenotype</strong></td>
<td>Subclinical retinal changes. Findings may include retinal thinning or decreased full-field ERG responses.</td>
<td>Retinal dystrophy with characteristic findings on OCT- and FAF imaging.</td>
<td>Chorioretinal atrophy. Sharply demarcated atrophy mainly but not invariably located outside the vascular arcades.</td>
<td>FEVR-like phenotype with retinal folds.</td>
</tr>
<tr>
<td><strong>Examples in this study and references</strong></td>
<td>Patient 2, left eye</td>
<td>Patients 1 and 3*</td>
<td>Patient 45, 5, 5*</td>
<td>Patient 2, right eye12, 13</td>
</tr>
</tbody>
</table>

*Progression of degeneration was not reported in these previous studies. FEVR, familial exudative vitreoretinopathy.
Novel Insights in KIF11-Related Retinopathy

finding in KIF11-related retinal disease across types 1 to 4. This includes subclinical retinal changes in the left eye of patient 2. Classifying KIF11-associated retinopathy as cone-rod or rod-cone dystrophy/retinitis pigmentosa is not straightforward. Although functional testing indicates higher vulnerability of rods (ERG recordings, symptoms, and visual field testing in patients 2 and 3), the most pronounced retinal degeneration in type 2 disease is observed at the posterior pole. Such mismatch between early rod dysfunction but predominantly central retinal degeneration might characterize this form of retinal dystrophy, similar to few other molecularly defined diseases such as CERKL or CDHR1-associated retinopathies.18,19

Progression of Retinal Changes

As yet, no disease progression has been shown for any of the four KIF11-associated retinal phenotypes. We show that atrophic fundus changes in type 2 disease (patients 1 and 3) may progress over time and are not stable, as has been assumed to date. In patient 1, progression of retinal atrophy was shown in a relatively early disease stage on serial SD-OCT and FAF imaging over an approximately 4-year interval. In patient 3, functional deterioration was demonstrated through a detailed history and analysis of medical records over more than 3 decades. His existing substantial atrophic alterations and the relatively short follow-up interval may have precluded detection of further structural decline.

Balikova et al.8 described long-term observation of three patients with a similar KIF11-associated retinal phenotype over 2, 6, and 9 years without progression of fundus examination or FAF imaging findings. Possible reasons for the inconsistency with our observations include our detailed long-term patient history and our in-depth analysis of SD-OCT and FAF images. These observations indicate the need for high-quality imaging and detailed long-term functional analysis to detect disease progression, as serial visual acuity testing and fundus color images over limited observation periods may not be adequate to monitor the slow structural and functional decline in type 2 KIF11-associated retinopathy.

Possibly, type 3 KIF11-associated retinopathy may also progress as suggested based on the serial fundus color photographs of patient 4. These were possible despite reduced cooperation due to the apparently rather uncommon central localization of the atrophic changes. However, additional imaging modalities were not available for confirmation, and alternative explanations for the apparent lesion growth include ocular growth and depigmentation of the retinal pigment epithelium bordering the atrophy. Additional longitudinal high-quality imaging studies are needed to investigate if areas of chorioretinal atrophy (type 3 KIF11-associated retinopathy) indeed enlarge or if they are developmental without further changes in later life.

Extraocular Phenotypic Variability

A pleiotropic spectrum of anomalies in the lymphatic, central nervous system, and diverse clinical phenotypes has been reported in patients with KIF11 mutations and syndromic manifestations.3–5 In addition to the known features, we identified a mild microcephaly with a sloping forehead and some weaknesses in mathematics as an indicator for a mild mental affection as possible KIF11 characteristics.

The phenotype in the two patients with type 2 KIF11-associated retinopathy reported herein was initially classified as nonsyndromic retinopathy. This supports the hypotheses by Jones et al.4 that KIF11 mutations might be disease-causing in a small proportion of patients with apparently isolated retinal degeneration. In a cohort of about 250 patients with predominantly central retinal atrophy we identified two index patients with type 2 KIF11-related retinal dystrophy (frequency of ~0.8%). Better estimates on the frequency may derive from future large-scale targeted NGS (including KIF11) or whole-exome sequencing in patients with retinal dystrophies. Jones et al.4 reported microcephaly (head circumference ≤3 SD) in 86% of patients with KIF11-related syndrome. Notably, they also screened otherwise healthy family members and found microcephaly in 75% of the mutation carriers.3 Our observations demonstrate that in some patients with KIF11 mutations, syndromic manifestations are too subtle to be noted in routine opthalmologic workup. For instance, a head circumference in the low-normal or mild microcephalic range, which can be considered a strong indicator of a KIF11 mutation, may remain unnoticed. Consideration of KIF11 in the molecular genetic analysis may then be helpful to identify underlying mutations.

Pathophysiological Considerations

Many genes involved in the pathophysiology of retinal dystrophies are specifically or predominantly expressed in the retina. However, mutations in ubiquitously expressed genes such as PRPF31 and other splicing factors have been shown to cause ‘only’ retinitis pigmentosa. Due to the high metabolic activity of the photoreceptors, and thus high splicing activity in photoreceptor cells, slightly reduced PRPF31 activity levels may lead to retinal disease, whereas other tissues remain unaffected.20 Mutations in REP1 which are the cause for choroideremia represent another example for a retina-specific disease manifestation of a ubiquitously expressed gene product that plays a role in fundamental cellular processes throughout the body.21 However, REP1 can functionally be compensated for by REP2 only in cells outside the eye, due to the inability of REP2 to support Rab27a, which plays a key role in the retina.22

The progressive retinal alterations may be the leading finding in patients with KIF11 mutations indicating that KIF11 may play an important role in the maintenance of retinal cells. For instance, Kif11 has been shown to be important during polypeptide synthesis which, if dysfunctional, may primarily cause disease in cells (e.g., photoreceptors) with high metabolic activity.

Many genes whose mutations cause primary or syndromic microcephaly have been identified.23 The respective proteins fulfill related functions which, for example, are important for the establishment and maintenance of structures of the mitotic spindle, the centrosome, and primary cilia.24 Besides Kif11, three other proteins have been implicated in, albeit recessively inherited, microcephaly with (chorio-)retinopathy: PLK4,25 its phosphorlylation substrate TUBGCP6,26,27 and TUBGCP4.27 In case of plk4, immunostaining indicated localization at the connecting cilia and basal bodies of photoreceptor cells, and a reduction of the number of photoreceptor cells was observed in plk4-zebrafish morphants. Moreover, plk4-depleted cells had less primary cilia.25

Kif11 has been identified as regulator of ciliogenesis among 2174 mouse genes in the primary screen of a recent siRNA-based functional genomics screen, but this potential role had not been further validated.28 Here, we show for the first time that Kif11 is associated with the ciliary apparatus of retinal photoreceptor cells. Kif11 is also known as Eg5, a microtubule-dependent, minus-end directed motor protein of the kinesin-5 family.29 In contrast to conventional kinesins, Kif11 motor molecules are homotetramers with two motor domains arranged at each pole, and thus can crosslink two adjacent microtubules.30 Although Kif11 is known as a spindle motor, it regulates a number of additional important microtubulic-
dependent functions in neuronal and non-neuronal cells, growth cone turning, and microtubule organization in axons and dendrites. The specific role of Kif11 in microtubule organization and transport function in primary cilia has to be determined in future studies. Nevertheless, in view of the localization of Kif11 in the modified primary cilium of retinal photoreceptor cells, KIF11-mediated disease could represent a syndromic ciliopathy.

A large proportion of the so far identified KIF11 mutations are truncating, indicating loss-of-function and haplinsufficiency as likely disease mechanism. Retinal disease due to heterozygous KIF11 mutations may thus result from less efficient microtubule organization and/or transport of ciliary cargo from the inner to the outer segment of photoreceptor cells. Reduced trafficking of proteins to the outer segment could explain the progressive course of retinal disease and why the phenotype usually is not congenitally severe (as in Leber’s congenital amaurosis).

CONCLUSIONS

Based on the findings reported herein and previously, we propose different types of KIF11-associated retinopathy that may occur within the same family or individual. We demonstrate that compromised vision may be the leading symptom of KIF11-related disease, and that extraocular manifestations are also highly variable. The observed progression of retinal atrophy suggests that KIF11 should be included in genetic testing for both isolated and syndromic retinal dystrophies. The expression and localization of Kif11 protein in specific compartments of photoreceptor cilia suggest that KIF11-associated disease represents a ciliopathy.

Note: Around the time of the acceptance of this manuscript, we became aware of a case report published ahead of print, showing progression of KIF11-associated retinopathy, thus confirming our findings.

Acknowledgments

The authors thank Rainer Guthoff for providing history information for patient P3. The authors also thank Ulrike Maas, Elisabeth Sehn, and Gabriele B. Stern-Schneider for skillful technical assistance.

Supported by ProRetina, Aachen, Germany, and by the Oxford NIHR Biomedical Research Centre. The funding organization had no role in the design or conduct of this research.

Disclosure: J. Birtel, None; M. Glien, None; E. Mangold, None; L. Tebbe, None; I. Spier, None; P.L. Müller, None; F.G. Holz, None; C. Neuhaus, None; U. Wolfrum, None; H.J. Bolz, None; P. Charbel Issa, None.

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