Clinicopathological Case Series of Four Patients with Inherited Macular Disease

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Purpose. To correlate the phenotype of four patients with inherited macular disease with the immunohistopathology of retinal tissue collected at the time of retinal pigment epithelium (RPE)-choroidal transplantation.

Methods. A clinicopathologic case series describing the phenotype of four patients, including confocal immunohistochemistry and electron microscopy (EM), and the results of genetic testing.

Results. In Case 1, electrophysiology showed only macular dysfunction. Confocal microscopy revealed abnormal mitochondria. In case 2 (R172W mutation in RDS), electrophysiology demonstrated generalized cone system dysfunction with severe macular involvement. Peripherin labeling of outer segments was nonuniform, and EM showed discs arranged in whorl-like structures. Case 3 showed severe central macular dysfunction on multifocal electroretinogram (ERG). Peripherin staining was irregular and disorganized. EM revealed abnormal inner segment morphology, particularly in rods, and disorganized outer segments. Case 4 had localized central macular dysfunction on multifocal ERG. Confocal microscopy was grossly normal, with evidence of early redistribution of cone opsins to the inner segment. EM showed variable rod morphology and normal cones.

Conclusions. RPE transplantation provides a unique opportunity to gain insight into retinal disorders by enabling phenotypic correlation with the immunohistopathology of retinal tissue collected during surgery. (Invest Ophthalmol Vis Sci. 2009;50:3553–3561) DOI:10.1167/iovs.08-2715

The inherited macular dystrophies are characterized by bilateral central visual loss and atrophy of the macula and underlying retinal pigment epithelium (RPE). They exhibit considerable clinical and genetic heterogeneity and are incompletely characterized. Generalized photoreceptor dystrophies may also have macular involvement. To date, histopathologic study of human macular disease has been confined to post mortem specimens due to the difficulty in obtaining retinal samples.

Macular translocation is a recognized therapeutic option for age-related macular degeneration and has been described in the treatment of adult-onset foveomacular vitelliform dystrophy. RPE-choroidal transplantation has also shown potential as a surgical treatment for macular disease by transplanting healthier RPE and choroid from a more peripheral donor site to the macula. During this procedure, a small portion of peripheral neural retina is separated from the donor RPE-choroid patch and then removed from the eye, creating an opportunity for analysis of rapidly fixed retina in these patients.

A clinicopathologic case series is presented of four patients with inherited disease involving the macula who underwent RPE transplantation. A correlation between the phenotype and the immunohistopathology of retinal tissue collected during surgery has been performed in an attempt to aid in our understanding of these disorders.

Materials and Methods

This study received approval from the local Ethics Committee (ref. 05/Q0504/29) and adhered to the tenets of the Declaration of Helsinki.

Case Reports

Patients were ascertained from the medical retina clinics at Moorfields Eye Hospital. Four were considered suitable for RPE-choroid graft, and informed consent was obtained after a full explanation of the nature and possible consequences of the study.

Case 1. A 52-year-old man, reported a 4-year history of bilateral visual loss. He had high myopia (~8 D spherical equivalent) with no relevant family history. One year before transplantation, left-eye visual acuity (VA) declined over a 6-month period from 6/9 to 6/36 (logMAR 0.78). Over the following 6 months, right-eye visual acuity (VA) deteriorated from 6/9 to 6/36 (logMAR 0.76) with loss of the ability to read. He denied photoaversion or nystagmus. Fundoscopy revealed irregular but sharply defined areas of atrophy of the RPE and inner choroid extending superiorly and inferiorly from the perifoveal region to the arcades with foveal pigmentary hyperplasia (Fig. 1A-D). The nasal retina had small yellow flecks at the level of the RPE but no pigmentary change or vascular sheathing. Both optic nerve heads were of normal appearance surrounded by a well-defined rim of peripapillary atrophy. Fixation was foveal and stable on the left but eccentric and unstable on the right.

Case 2. A 45-year-old man had presented at age 36 years with increased photosensitivity. His mother and maternal uncle were affected by rod-cone dystrophy. Electrophysiology at initial presentation was reported as normal. VA declined over 2 years from 6/18 to 6/60 (logMAR 1.0), with eccentric fixation. Subsequently, over a 3-month period, VA deteriorated from 6/12 to 6/18 (logMAR 0.44).
Ocular examination revealed well-defined areas of RPE and inner choroid atrophy at both maculae (Figs. 1E–H). In the left eye, three bands of intact RPE interrupted the area of atrophy meeting at the fovea. Prominent peripapillary atrophy was present. Peripheral retina, retinal vessels, and optic nerve heads were unremarkable.

**Case 3.** A 45-year-old man had presented at the age of 41 with progressive visual loss since his late 30s. He denied photoaversion or nyctalopia. He had no significant medical or ocular history. Direct questioning revealed a paternal great aunt who was blind by the age of 12. After presentation, his VAL improved from 2/60 to 6/36 over a period of 3 to 4 years as he adopted eccentric fixation. However, during the subsequent 12 months, VAR declined gradually from 6/9 to 6/36 (logMAR 0.82) with inability to read.

Examination of his anterior segments, lens, and vitreous were normal. Fundoscopy revealed bilateral bull’s eye maculopathy with a small central island of RPE remaining on the right (Figs. 1I–L). The peripheral retina appeared healthy with no pigmentary or vascular abnormalities. Optic nerve heads were symmetrically cupped at 0.7 with no focal neuroretinal rim or nerve fiber layer loss.

**Case 4.** A 66-year-old woman presented with a 2-year history of bilateral sequential central visual loss. She denied photoaversion or nyctalopia. There were yellow deposits at the level of the RPE in both maculae associated with pigmentary hyperplasia (Figs. 1M–P). Peripheral retina, retinal vessels, and optic discs were normal.

A summary of the clinical features, genetic diagnosis and electrophysiology is given in Table 1.

**Genetic Analysis**

Blood samples were taken from the four patients and sent for genetic analysis at the Regional Molecular Genetics Laboratory, Manchester. Screening for Sorsby fundus dystrophy (SFD) was performed by bidirectional sequencing of the exon 5 and intron 4/exon 5 splice-acceptor site of the tissue inhibitor of metalloproteinases-3 (TIMP-3) gene. RDS/peripherin abnormalities were sought by bidirectional sequencing of the entire coding sequence of rhodopsin, including the intron/exon boundaries. Further samples were also sent to Asper Biotech (Tartu, Estonia) to look for variations in ABCA4 using an arrayed primer extension genotyping array chip.

**Preoperative Assessment**

The patients underwent a full ophthalmic examination. Color fundus photography was captured as 8-bit digital images, and fundus autofluorescence images were obtained with a scanning laser ophthalmoscope (HRA 2; Heidelberg Engineering GmbH, Dossenheim, Germany) by using five to nine averaged images over a 30° field, which were then
Table 1. Summary of Findings for Each Case

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
<td>48</td>
<td>36</td>
<td>Late 30’s</td>
</tr>
<tr>
<td>Age at biopsy</td>
<td>52</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Family History</td>
<td>Sporadic</td>
<td>Dominant</td>
<td>Sporadic</td>
</tr>
<tr>
<td>VA operated eye</td>
<td>0.76</td>
<td>0.44</td>
<td>0.82</td>
</tr>
<tr>
<td>Gene Identified</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrophysiology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cone ERG response</td>
<td>Normal</td>
<td>Subnormal</td>
<td>Normal</td>
</tr>
<tr>
<td>Rod ERG response</td>
<td>Absent</td>
<td>Normal</td>
<td>Mildly subnormal</td>
</tr>
<tr>
<td>Pattern ERG response</td>
<td>Localized to rod OS. Reduction in OS length and density.</td>
<td>Localized to rod OS. Reduction in OS length and density.</td>
<td>Localized to rod OS. Variable OS length and density.</td>
</tr>
<tr>
<td>Rod Opsin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M-cone opsin</td>
<td>Localized to cone OS. Reduction in OS length and density.</td>
<td>Localized to cone OS. Reduction in OS length and density.</td>
<td>Localized to cone OS. Variations in OS length.</td>
</tr>
<tr>
<td>Peripherin</td>
<td>Uniform staining of disc rims in OS.</td>
<td>Nonuniform staining. Clumps of peripherin within OS.</td>
<td>Staining irregular and disorganized</td>
</tr>
<tr>
<td>Cytochrome oxidase</td>
<td>Good staining of rod and cone IS</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Calbindin-D</td>
<td>Good staining of horizontal cells, amacrine cells and labeling of cones.</td>
<td>Good staining of horizontal cells, amacrine cells and labeling of cones.</td>
<td>Marked decreased labeling of cones. Normal horizontal and amacrine cells.</td>
</tr>
<tr>
<td>PNA</td>
<td>Normal interphotoreceptor matrix.</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>Normal cone synapse morphology.</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>7-G6</td>
<td>Normal cone labeling.</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>PKC</td>
<td>Normal rod bipolar cell morphology.</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>GFAP</td>
<td>Appeared increased; staining present within Müller cells from GCL to ONL.</td>
<td>Increased</td>
<td>Increased</td>
</tr>
</tbody>
</table>

Electrophysiology was performed on all patients to incorporate and exceed international standards and recommendations. Pattern ERGs were recorded to an alternating checkerboard and the P50 component was used as a measure of macular function. Multifocal ERG responses to a scaled array of 61 stimulus hexagons covering a visual field of 57° were recorded, allowing assessment of localized cone system function over foveal and discrete paracentral areas. Full-field ERGs were used to assess generalized rod and cone system function under conditions of dark and light adaptation, respectively. Scotopic responses included the dim flash rod ERG and bright-flash maximum ERG; the latter a flash strength 0.6 log units greater than the standard flash (3.0 cd·s·m⁻²) to better demonstrate the ERG a-wave. Photopic cone system function was assessed with the 50-Hz flicker ERG and transient photopic ERG. The macula contributes minimally to full-field ERGs.

Collection of Retinal Specimens

Peripheral RPE-choroid transplantation was performed on all four patients by one surgeon (LdC) using a technique previously described. In brief, a three-port pars plana vitrectomy was performed, a suitable site for RPE donation in the superior equatorial retina was identified, and diathermy was applied to a 2- to 3-mm² area. The neurosensory retina was then gently peeled off the surface of the RPE in two segments, removed from the eye, and immediately fixed. One segment was fixed in 4% Karnovsky solution for electron microscopy and the other in 4% paraformaldehyde solution for confocal microscopy.

Immunohistochemistry

After a rinse in PBS, the specimens that had been fixed in paraformaldehyde were embedded in 5% agarose (Sigma-Aldrich, St. Louis, MO) in sodium phosphate buffer (PBS). Seventy-five-micrometer-thick sections were cut with a vibratome (Technical Products International, Poly-}

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Table 2. Antibodies

<table>
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<tr>
<th>Antibody</th>
<th>Manufacturer</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>α-Calbindin-D</td>
<td>Sigma (St Louis, MO)</td>
<td>1:1000</td>
</tr>
<tr>
<td>α-GFAP</td>
<td>Dako (Carpinteria, CA)</td>
<td>1:400</td>
</tr>
<tr>
<td>α-Synaptophysin</td>
<td>Dako</td>
<td>1:100</td>
</tr>
<tr>
<td>α-M-cone opsin</td>
<td>Chemicon International (Temecula, CA)</td>
<td>1:500</td>
</tr>
<tr>
<td>aPKC</td>
<td>Biomol Research Labs, Inc. (Plymouth Meeting, PA)</td>
<td>1:100</td>
</tr>
<tr>
<td>α-Rhodopsin</td>
<td>Santa Cruz Biotechnology, Inc. (Santa Cruz, CA)</td>
<td>1:100</td>
</tr>
<tr>
<td>PNA-biotin</td>
<td>Vector Laboratories (Burlingame, CA)</td>
<td>1:1000</td>
</tr>
<tr>
<td>α-Cytochrome oxidase</td>
<td>Molecular Probes (Eugene, OR)</td>
<td>1:100</td>
</tr>
<tr>
<td>α-Peripherin 3B6</td>
<td>Robert Molday, University of British Columbia (Vancouver, BC, Canada)</td>
<td>1:10</td>
</tr>
<tr>
<td>α-7G6</td>
<td>Peter MacLeish, Morehouse School of Medicine (Atlanta, GA); Wolfgang Baehr, University of Utah (Salt Lake City, UT)</td>
<td>1:100</td>
</tr>
</tbody>
</table>

Electrophysiology

R172W mutation in the any of the genes tested. Genetic analysis of case 2 revealed an

Genetic Analysis

RESULTS

Transmission Electron Microscopy

The specimens that had been fixed in Karnovsky solution were rinsed in buffer, dehydrated in a graded ethanol series, placed in 100% resin oven.

for 6 hours with rotation, and embedded and cured overnight in a 60°C

Semithin (1 μm) sections for light microscopy and ultrathin (70 nm) sections for transmission electron microscopy (TEM) were cut with a microtome (Ultracut S; Leica, Deerfield, IL) fitted with a diamond knife. Semithin sections were stained with alcoholic toluidine blue. Ultrathin sections were contrasted by sequential staining with saturated uranyl acetate in 50% ethanol followed by lead citrate and viewed on a laser scanning confocal microscope (Fluoview 500; Olympus, Tokyo, Japan).

Confocal Microscopy

&middot; Normal cone morphology and interphotoreceptor matrix was shown by labeling with anti-7G6, which labels synaptic vesicle proteins in synaptic terminals (Fig. 4C, green), and anti-7G6, which labels cone arrestin in the entire cone (Fig. 4C, red). Labeling with anti-7G6 also showed rounded-off cone OS (Fig. 4C, arrow). Anti-calbindin D labels horizontal andamacrine cells in the INL, and the labeling was grossly normal (Fig. 4B, green). Similarly the morphology of the rod bipolar cells, demonstrated with anti-protein kinase C (PKC), was unremarkable (Fig. 4B, red). Glial fibrillary acidic protein (GFAP) expression was present throughout the ganglion cell layer to the outer nuclear layer within Müller cells (Fig. 4D, green) and appeared increased compared with that in normal retinas.

滿化al surgery. At presentation, residual pattern ERGs were present, but immediately before RPE transplantation, these were undetectable and there was severe multifocal ERG reduction consistent with severe central macular dysfunction (Fig. 3). Full-field ERGs had some mildly abnormal features suggestive of dysfunction occurring after phototransduction, although the a-wave in the bright-flash ERG was normal. There was no evidence of generalized rod photoreceptor or cone system dysfunction.

Case 4. Full-field, pattern, and multifocal ERGs were performed at 1 day (Figs. 2, 3) and 3 months before transplantation. VA was 6/60 in each eye on both occasions. Pattern ERGs immediately before surgery were normal in the surgical eye but multifocal ERGs confirmed the presence of localized central macular dysfunction (Fig. 3). There was no multifocal ERG evidence of peripheral macular cone system involvement and full-field ERGs were within age-matched normal limits bilaterally.

Electrophysiology

Case 1. Full-field and pattern ERGs were performed at 1 month (Fig. 2), 1 year, and 2 years before transplantation. Multifocal ERG was performed at 1 month before transplantation. Visual acuity was 6/9 in each eye at the time of the initial two recordings and 6/36 VAR and 3/36 VAL at the latest recording. Undetectable pattern ERGs (Fig. 2) and central multifocal ERG (Fig. 3) abnormalities, consistent with central macular dysfunction, were observed in both eyes. Full-field ERGs showed no electrophysiological evidence of generalized retinal involvement.

Case 2. Full-field, pattern, and multifocal ERGs were performed on two occasions: 2 weeks (Fig. 2) and 2 years before transplantation. VAR was 6/18 and VAL was 6/12 on that occasion. The full-field ERGs were consistent with a loss of generalized cone system function but rod-mediated ERGs were normal. This result was confirmed in a second ERG. Pattern and multifocal ERGs were consistent with severe macular involvement (Figs. 2, 3).

Case 3. Full-field and pattern ERGs were performed at 2 weeks (Fig. 2) and 4 years before transplantation. Multifocal ERGs were performed 2 weeks before transplantation (Fig. 3). VAR was 6/9 and VAL was CF at the time of the initial recording but had deteriorated to 6/60 and 6/56, respectively, before
Figure 2. Pattern and full-field ERGs recorded before surgery in cases 1 to 4. A normal example is shown for comparison (bottom row). Case 1: full-field ERGs were normal in both eyes. Case 2: pattern ERG was undetectable in both eyes; scotopic ERGs were normal but cone-mediated photopic ERGs were of subnormal amplitude in both eyes. Case 3: pattern ERG was undetectable in both eyes. The scotopic ERG was mildly subnormal in both eyes. The maximum ERG a-wave was normal, but the b wave was mildly subnormal bilaterally. Cone-mediated ERGs were normal in both eyes. Case 4: pattern ERG and all full-field ERGs were normal in both eyes.
peripherin observed within the OS (Fig. 5D, green, arrows). There was generalized increased distribution in labeling of Müller cells with GFAP (Fig. 4H, green). Findings are summarized in Table 2.

Case 3. The staining of rod OS with peripherin was irregular and disorganized (Fig. 5G, green; Table 2). Labeling of cones with anti-calbindin D was markedly reduced or absent; however, staining of horizontal and amacrine cells appeared normal (Fig. 4j; anti-calbindin D, green). Distribution of anti-GFAP labeling in Müller cells was generally increased (Fig. 4L, green).

Case 4. Cone OS structure was variable, with some being of normal length and others being shortened (Fig. 4M, red). In some areas there was evidence of early redistribution of cone opsin to the IS (Fig. 4M, arrow). Labeling with peripherin and rod opsin was grossly normal (Fig. 4J, peripherin green, rod opsin red). Staining with GFAP was increased and occasionally Müller cell end feet were seen to breach the outer limiting membrane (OLM; Fig. 4P, arrow).

Electron Microscopy

Case 1. TEM revealed abnormal cone IS with swollen mitochondria and apoptotic cell bodies (Fig. 5C, arrow). These abnormalities appeared to affect cones preferentially, with adjacent rod IS being relatively spared. Most OS were detached from their IS, although this may be an artifact of the detachment and sectioning procedures. Many appeared to be relatively normal with well-organized discs, but in others the discs were stacked in an inconsistent fashion (Fig. 5B).

Case 2. There were generalized abnormalities of both rod and cone structure, although rods appeared to be more severely affected, with mitochondria being grossly swollen (Fig. 5F). OS were dysplastic with distortion of discs into whorl structures (Fig. 5E, arrow).

Case 3. The cone IS morphology appeared to be abnormal; however, abnormalities in morphology were more marked in the rod population (Fig. 5I). In the rod IS the mitochondria were swollen and reduced in number. The OS were of variable size and shape with disorganized disc structure (Fig. 5H).

Case 4. Cone morphology was normal (Fig. 5L), but rod morphology was variable, with some appearing grossly normal and others appearing atrophic or with abnormal, swollen mitochondria (arrow). The OS were also grossly normal with well-organized disc structure (Fig. 5K).

A summary of the findings are presented in Table 2.

DISCUSSION

To our knowledge, this represents the first opportunity to study relatively well-preserved retinas of patients with inherited retinal disease involving the macula. The tissue was not from postmortem eyes but was obtained during surgery and placed immediately in fixative. Immunohistochemical and structural abnormalities in the peripheral retina of these four patients are discussed in relation to the electrophysiological findings.

In case 1, electrophysiology indicated a disease primarily of macular cone function, with no evidence of generalized retinal involvement. Marked abnormalities of cone IS mitochondria with relative sparing of the rods was observed on TEM. Confocal microscopy, however, showed little alteration in retinal structure apart from a slight reduction in OS length and an increased distribution of GFAP expression. Despite the presence of grossly abnormal cone mitochondria, labeling with cytochrome oxidase appeared to be normal by confocal microscopy. It is therefore possible that the findings demonstrated by TEM indicate subclinical disease with vulnerable, diseased cells, in this case cones, being more susceptible to oxidative stress during fixation. Although mild GFAP staining can be found in normal retinas, strong GFAP labeling was observed in all four of the retinal samples in this study, this is a nonspecific indicator of stress and may occur in response to several retinal diseases, including age-related macular degeneration.10,12–15 In one report, cone pedicles were also enlarged, but this was not found in our study (data not shown).16
Patient 2 was found to have an R172W mutation in the RDS gene, a condition in which the clinical and ERG phenotype is variable. In this case, the electrophysiology was consistent with generalized loss of retinal function confined to the cone system with severe macular involvement. Similar ERG findings have been previously reported in association with the R172W mutation. Peripherin/rds and rom1 are transmembrane proteins localized to rod OS disc rims and are involved in disc morphogenesis and maintenance of OS structure. In case 2, abnormalities of peripherin staining in rod photoreceptor OS were observed on confocal microscopy. Kedzierski et al. described whorl-like structures in rod OS of transgenic mice with RDS mutations, similar to those described in this study on TEM, and proposed that whorls result from the insufficiency of peripherin/rds which causes disc enlargement. Others have hypothesized that it may reflect disruption of other interactions required for maintenance of rod OS. Rod neurite sprouting has also been reported in cases of RDS mutation, but was not observed in this study.

In case 3 the rod specific ERG, arising in the ON-bipolar cells was subnormal. However, a normal a-wave in the bright flash ERG confirmed that the process of phototransduction was not compromised. Further examination of the ERGs showed a subnormal rod ERG and a maximum response with a low b- to a-wave ratio, suggestive of dysfunction occurring after phototransduction. Irregular peripherin staining of the rod OS was consistent with the disorganized disc structure observed on TEM. Reduced labeling with calbindin D, a calcium binding protein found in cones, but not rods, of many animal species, was observed in this case. The importance of this finding is difficult to determine, as calbindin expression in the human retina varies, being reduced or absent in the fovea or parafovea, but prevalent in the perifovea and peripheral retina. Variations in anti-calbindin labeling similar to that seen in this...
study (i.e., reduced labeling of cones and normal labeling of horizontal and amacrine cells) have also been noted in patients with inherited macular disease. Patients 1 and 4 showed uniform staining of disc rims suggesting normal morphology. Patients 2 and 3 demonstrated nonuniform clumps of peripherin (arrow) within the OS. Rod opsins (red) of rod OS appeared normal in all patients. TEM of OS showed grossly normal morphology in patients 1 and 4. An area of disorganized disc stacking with abnormalities in structure. In some areas the OS appeared to be relatively normal with well organized discs. TEM showed dysplastic OS with distortion of discs into whorl-like structures (arrow). Scale bar, 1 μm. Patient 2 demonstrates dysplastic OS with distortion of discs into whorl-like structures (arrow). Scale bar, 5 μm. Patient 3 shows OS of variable size and shape with disorganized disc structure. Scale bar, 2 μm. TEM of rods and cones. Scale bar, 5 μm. Swollen mitochondria were present in all patients, but the distribution varied among patients. Patient 1 showed swollen mitochondria in cone IS (arrowhead) with adjacent rods being relatively spared. Apoptotic cell bodies were also seen in this patient (arrow). Patients 2 and 3 appeared to have more marked abnormalities in the rod population. In patient 4, cone morphology was grossly normal, with variable involvement of the rod population.

In case 4, electrophysiological abnormalities were confined to the central macula. TEM of the equatorial retinal sample was grossly normal, consistent with full-field ERGs that were within age-matched normal limits. On fundoscopy, this patient was noted to have multiple yellow deposits at the level of the RPE in both maculae. Shortening of photoreceptor OS and isolated areas of cone opsin redistribution were observed on confocal microscopy and similar changes have also been described in photoreceptors overlying drusen in patients with age-related macular degeneration as well as retinal detachment.

Phenotypic variation of the ABCA4 gene is profound and is involved in several retinal dystrophies including Stargardt fundus flavimaculatus, retinitis pigmentosa, cone rod dystrophy and AMD. It is therefore conceivable that patients in this study may have had a mutation in ABCA4. Analysis of the ABCA4 gene was performed with a microarray gene chip designed to screen for known mutations in the gene; no patient studied had mutations detected. Although this chip is regularly updated and at the time of analysis was able to test ≥98% of the known genetic variations, screening of the ABCA4 gene is complicated by its large size and allelic heterogeneity with many polymorphisms, making it difficult to be certain that no disease-causing mutation exists.

The patients in this study presented with macular disease, but the samples obtained at the time of surgery were from the equatorial retina. Gene expression at the fovea may differ from that in the peripheral retina, possibly reflecting structural differences such as the high density of ganglion cells at the macula, and it therefore cannot be excluded that other abnormalities of photoreceptors and neural retina may exist in this region. Further, this study was confined to the analysis of the neural retina and offers no insight into the structure of the RPE or its interaction with photoreceptors. All the retinal samples were collected at the time of transplantation surgery, and although the neural retina was separated carefully from the underlying RPE, the sampling process may have caused cellular
damage. It would be expected that such artifacts would occur uniformly in rods and cones and is therefore unlikely to explain the differences observed in this study.

In this study, multifocal ERGs detected localized areas of cone system dysfunction, and it is possible that wider field multifocal ERG would better evaluate the function of potential graft sites before surgery. The need to perform full-field ERG is clearly demonstrated, as patients with a fundus appearance thought to reflect a macular dystrophy may show electrophysiological evidence of generalized retinal dysfunction.

In conclusion, samples obtained during transplantation surgery in these four patients with predominantly macular disease have enabled a correlation to be determined between histology and function in living eyes. There was a range of retinal disease in the equatorial retina, reflecting the underlying diseases. Retinal transplantation provides a unique opportunity to assess retinal tissue, allowing further characterization of retinal disease and dysfunction.

Acknowledgments

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References