Disease Boundaries in the Retina of Patients with Usher Syndrome Caused by MYO7A Gene Mutations

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PURPOSE. To study retinal microstructure in Usher Syndrome type 1B (USH1B) caused by MYO7A mutations as a prelude to treatment initiatives.

METHODS. Patients with MYO7A-USH1B (n = 17; ages 5–61) were studied with optical coherence tomography. Retinal laminae across horizontal and vertical meridians were measured. Colocalized visual sensitivity was measured with automated perimetry to enable comparisons of function and structure in the transition zones.

RESULTS. Laminar architecture of the central retina in MYO7A-USH1B ranged from normal to severely abnormal. Within the transition zone between normal and abnormal retina, the first detectable abnormality was an increase in prominence of the OLM (outer limiting membrane). Declining ONL thickness was accompanied by increased thickness of the OPL and normal or increased INL. Undetectable ONL and OPL and hyperthick INL were features of severe laminopathy at further eccentricities into the transition zone. Visual sensitivity in the transition zone declined with the decrease in ONL thickness.

CONCLUSIONS. Patients with MYO7A-USH1B can have regions of structurally and functionally normal retina with definable transitions to severe laminopathy and visual loss. The earliest detectable structural markers of disease may represent Müller glial cell response to photoreceptor stress and apoptosis. Visual losses were predictably related to a decline in ONL thickness. The prospect of focal treatment of MYO7A-USH1B, such as subretinal gene therapy, prompts the need to identify retinal locations that warrant consideration for treatment in early phase trials. The transition zones are candidate sites for treatment, and laminar architecture and visual sensitivity are possible outcomes to assess safety and efficacy. (Invest Ophthalmol Vis Sci. 2009;50:1886–1894) DOI:10.1167/iovs.08-3122

The Usher syndromes (USH) are a molecularly heterogeneous group of autosomal recessive diseases with dual sensory deficits of hearing impairment and retinal degeneration.3 The USH genes encode proteins of different classes. A unifying hypothesis is that USH gene products are part of a protein network in the region of the cilium of the photoreceptor. Dysfunction of this network is thought to lead to the retinal degeneration.2,5

USH1B is the most common form of USH1 and is caused by mutations in MYO7A, a gene encoding an unconventional myosin.4–6 Recent promise of somatic retinal gene therapy in MYO7A-USH1B7 prompts questions about the feasibility of treatment in humans. Transplantation to clinical trials is complicated by the fact that there is currently no Myo7a-deficient animal model with retinal degeneration approximating the human condition.8 Dual localization of the gene product in retinal pigment epithelium (RPE) and photoreceptors adds further complexity to any strategy for gene transfer.1,4,8 The onus falls on studies of molecularly defined patients to clarify the detailed retinal phenotype of MYO7A-USH1B and seek clues about disease mechanism that will enable translation.

Recently, we embarked on studies of human MYO7A-USH1B to quantify abnormalities in the photoreceptor cell layer and RPE. The pattern of results in MYO7A-USH1B was compared with those in other USH genotypes, for which there was experimental evidence of localization of gene products only to photoreceptors. We concluded that MYO7A-USH1B was behaving phenotypically like these other photoreceptor diseases.9 The present study seeks further details about the retinopathy resulting from human MYO7A mutations. Topographical maps of photoreceptor and inner retinal laminae indicate that there are not only photoreceptor layer losses but also inner retinal abnormalities. The transition zone between abnormal and normal-appearing retina, an area worth considering for focal treatment in a phase 1 trial, was investigated with high-resolution, cross-sectional imaging and visual thresholds.

METHODS

Human Subjects

There were 17 patients with USH1B caused by MYO7A mutations (Table 1). Patients underwent a complete eye examination. Normal subjects for optical coherence tomography (OCT; n = 33; ages 5–58 years) and for psychophysical testing (n = 9; ages 19–48 years) were also included. Informed consent was obtained for all subjects; procedures adhered to the Declaration of Helsinki and were approved by the institutional review board.

Optical Coherence Tomography

Retinal cross-sections were obtained with OCT. Data were acquired in 15 of the patients with Fourier-domain (FD) OCT imaging (RTVue-100; Optovue Inc., Fremont, CA). Two patients had imaging with either OCT3 (F7, P1) or OCT1 (F9, P1) (Carl Zeiss Meditec, Inc., Dublin, CA). The principles of the method and our recording and analysis technology are detailed elsewhere.10
Better definition of retinal laminae.10,14 Overall retinal thickness was aligned and averaged to increase the signal-to-noise ratio and allow disrupted local lateral isotropy of signals. Repeated scans were laterally when crossing structures (for example intraretinal pigment) inter­rupting signal originating from layers vitread and scleral to it. The presence of photoreceptor inner/outer segment signal and outer limiting membrane signals vitread to the RPE peak were also assessed.9

For topographic analysis, the precise location and orientation of each scan relative to retinal features (blood vessels, intraretinal pigment, and optic nerve head) were determined on the video images of the fundus. LRPCs were allotted to regularly spaced bins in a rectangular coordinate system centered at the fovea; the waveforms in each bin were aligned and averaged. For two-dimensional maps, 0.3-mm² bins were used for sampling, whereas 0.15-mm² bins were used for analysis along the horizontal and vertical meridians. Overall retinal, ONL, and inner retinal thicknesses were measured. Missing data were interpolated bilinearly, thicknesses were mapped to a pseudocolor scale, and the locations of blood vessels and optic nerve head were overlaid for reference.12,14,10

Visual Psychophysics

Static threshold perimetry was performed and analyzed as published.17 Static thresholds were determined with 1.7°-diameter, 200-ms duration white stimuli under light-adapted conditions. Thresholds were measured along the horizontal and vertical meridians (2° intervals) crossing fixation and corresponding to the same retinal regions examined with OCT. Sensitivity (1/threshold) for each location was compared with the mean normal data to determine locus-specific sensitivity loss.

RESULTS

Topographical Maps of Retinal Thickness in MYO7A-Mutant Retina

Maps of thickness topography across a wide expanse of central retina are illustrated (Fig. 1) for the full cross-section of retina (left column), the ONL (middle column) and the inner retina (right column) in a normal subject (Fig. 1A) and three patients with MYO7A-USH1B of different ages and disease stages (Figs. 1B–1D). Normal retina has a foveal depression surrounded by parafoveal thickening and then a decline in thickness with increasing eccentricity. Thickening at superior and inferior poles of the optic nerve represent converging axons (Fig. 1A, left). F5,P2, a 21-year-old patient with MYO7A-USH1B, showed a normal pattern of retinal thickness in a large region of central retina but thickness was reduced at greater eccentricities (Fig. 1B). F2,P1, a 6-year-old patient with MYO7A-USH1B, showed a pattern of retinal thickness that was also similar to normal in the more central retina, but there was loss of thickness inside and beyond the vascular arcades (Fig. 1C). F10,P1, age 35, has a normal pattern of retinal thickness in a large region of central retina (Fig. 1D).

Mapping ONL and inner retinal thickness across this region dissected the morphologic disease effects on photoreceptors from the effects on the postreceptoral retina. ONL topography of the normal retina peaked centrally and declined with distance from the fovea; parafoveal thinning occurred more gradually in the superior retina (Fig. 1A, middle). F5,P2 retained a large central island of ONL that extended more superiority than

### Table 1. MYO7A Mutations in Patients with USH1B

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* Families in which some genotype and/or phenotype information has been reported in Reference 9.
inferiorly (Fig. 1B). The inset comparing the map of F5,P2 to the lower limit of normal (mean \( \pm 2 \) SD) shows that most of this central island had normal ONL thickness (Fig. 1B, inset). There was a decrement of ONL with eccentricity and it became undetectable at greater eccentricities. F2,P1 had a relatively smaller island of preserved ONL compared with that of F5,P2. Again, there was greater superior than inferior extension (Fig. 1C). Most of the remaining ONL was within normal limits (white, defined as mean ± 2 SD), compared with normal. Optic nerve outlines were schematized in both the topographic and thickness difference maps. T, temporal; N, nasal; S, superior; I, inferior, F, fovea.

**Figure 1.** Retinal thickness topography of MYO7A-Marin1B. Topographical maps of total retinal (left), ONL (middle), and inner retinal (right) thicknesses in a normal 24-year-old subject (A) and three patients with MYO7A-Marin1B of different ages and disease stages (B–D). Traces of major blood vessels and location of optic nerve head are overlaid on each map (depicted as right eyes). Pseudocolor scales are shown beneath the normal maps. Insets: thickness difference maps showing regions that were abnormally thin (blue), within normal limits (white, defined as mean ± 2 SD), or thick (pink), compared with normal. Optic nerve outlines were schematized in both the topographic and thickness difference maps. T, temporal; N, nasal; S, superior; I, inferior, F, fovea.

Normal inner retinal thickness topography had a foveal depression surrounded by an annulus of increased thickness and a crescent-shaped thickening extending toward the optic nerve from superior and inferior retina (Fig. 1A, right). Inner retinal topography in two patients with MYO7A-Marin1B, F5,P2 and F2,P1, was normal in the central retina but there was increased thickness at greater eccentricities (Figs. 1B, 1C, insets). F10,P1, who had far more advanced disease, showed abnormally increased thickness of the inner retina across the entire region sampled except for a small central region (Fig. 1D, inset). These observations led to a locus-by-locus quantitation of the thickness parameters along horizontal and vertical meridians in our entire cohort of patients with MYO7A-Marin1B (Fig. 2).

**Inner and Outer Retinal Architecture in MYO7A-Mutant Retinas**

Cross-sectional images along the horizontal and vertical meridians in a normal subject and in F3,P1, an 11-year-old patient with MYO7A-Marin1B, are shown (Fig. 2A). In the normal retinal cross section, there was a foveal depression and the surrounding retina was laminated with prominent low-reflectivity cellular layers (ONL and INL) and intervening hyperreflective synaptic laminae. F3,P1 also showed a foveal depression with normal-appearing foveal ONL thickness. The ONL, however, diminished in thickness with eccentricity from the fovea and became abnormally thinned or undetectable at greater eccentricities. The inner retina appeared thickened at the eccentricities with reduced ONL.
Overall retinal, ONL, and inner retinal thicknesses across the horizontal and vertical meridians were quantified for the 17 patients with MYO7A-USH1B (Figs. 2B–2D). Patients are identified by individual symbols; colors distinguish younger (yellow, 4–30 years) from older (red, 34–61 years) ages. Also plotted are normal limits (gray, \( \pm 2\) SD from mean). Retinal thickness shows a wide spectrum of results from normal to abnormally reduced (Fig. 2B). ONL thickness in all the younger patients was normal at the fovea while many of the older patients had abnormally reduced foveal ONL (Fig. 2C). A minority of the younger patients had normal ONL extending to \( >6 \) mm from the fovea. Most patients with MYO7A-USH1B, however, had abnormally thinned ONL by \( 2 \) to \( 4 \) mm eccentric to the fovea, and ONL was not detectable at greater eccentricities. Inner retinal thickness is normally at its minimum in the fovea. Parafoveal thickening, a feature of the normal retina, is also present in MYO7A-USH1B. At eccentricities beyond approximately \( 3 \) mm eccentric to the fovea, some younger pa-
Details of Laminar Architecture in the Transition Zone from Normal to Abnormal Retina

Cross-sectional images from the fovea along the temporal retinal meridian in a normal subject and a patient with MYO7A-USH1B are shown (Fig. 3A). There was an obvious transition zone in F5,P2 from more normal-appearing retina to markedly abnormal retina. What were some of the features of the transition zone? There appeared to be greater prominence of the hyperreflective layer considered to be the outer limiting membrane (OLM) beginning at approximately 3 mm temporal from the fovea and continuing until this layer was no longer detectable at approximately 7 mm eccentricity. The ONL thinned with eccentricity gradually from ~5 to 6 mm eccentric to the fovea until it was not discernible at distances of >8 mm. The OPL, the hyperreflectivity immediately vitread to the ONL, appeared to increase in thickness beginning at ~6 mm eccentricity; after >7.5 mm it thinned again and became indistinct. The INL appeared nearly normal from the fovea through most of the transition zone but after ~8 mm, lamination became indistinct; there may be some thickening at this eccentricity. Quantititation of thickness of the ONL, OPL, and INL in F5,P2 compared with normal subjects over this temporal retinal region is shown (Fig. 3B). OLM prominence (not quantitated) is also indicated. The ONL thickness was within normal limits until approximately 6 mm eccentricity, but OLM prominence was evident at lesser eccentricities. As the ONL thinned to below the normal limits, the OPL thickened; with increased eccentricity, OPL thickness returned to normal and then was not discernible. In the region of decreasing ONL and increasing OPL, the INL was slightly thicker than normal.

Subdividing the transition zone allowed us to ask if the results in F5,P2 were generalizable to other patients with MYO7A-USH1B. The transition zone was divided into four contiguous subzones. Subzones a to d are marked on the scans of F5,P2 and the quantitative analyses of thickness (Figs. 3A, 3B). Subzone a represented retina with abnormal prominence of the OLM and normal or nearly normal thickness of the ONL, OPL and INL. In subzone b, the ONL decreased in thickness to below the normal limits; the OPL in this region increased in thickness; and the INL was slightly increased or within normal limits. The OLM varied from visible to not detectable. Subzone c still showed definable retinal lamination but the ONL was reduced to barely detectable; the OPL decreased and the INL increased in thickness. There was no visible OLM. Subzone d is what we previously termed “bilaminar” retina—no measurable ONL or OPL and a thickened hyperreflective layer that may well represent intermingled INL and residual ONL nuclei.

Representative scans with demarcation of the subzones are shown in another region of F5,P2 and in three other patients (Fig. 4A). Quantitation of ONL, OPL and INL thickness in examples of each of the subzones are also shown (Fig. 4B). Not all subzones were detectable in each patient, but the order of laminar architectural changes from most normal (a) to most abnormal (d) was never contravened.

Visual Function in the Transition Zone of MYO7A-USH1B

Full-field electrotoretinography (ERG), the traditional measure of retinal-wide function, was performed (or results were available through medical records) in 13 of 17 (76%) patients. Among the detectable ERGs (8/13; 62%), b-wave amplitudes to a maximum dark-adapted white flash ranged from 10 to 73 μV (mean, 30 μV; SD, 20 μV; normal mean, 497 μV; SD, 111 μV). Localized visual function, specifically in the transition zone, was measured with light-adapted static perimetric profiles, centered at the fovea and along the horizontal (Fig. 5A) and vertical (Fig. 5B) meridians. The results, shown for seven representative patients with MYO7A-USH1B, indicate that sensitivity could be normal in a wide extent of central field, as in F5,P1 and F8,P1. A decline of visual sensitivity was noted at 6 to 7 mm eccentricity in these two patients along the temporal and superior retina; inferior retinal abnormalities begin closer to fixation (Figs. 5A, 5B). Vertically asymmetric sensitivity profiles were also present in two other patients (F4,P1 and P9,P1), with sensitivity extending more into superior than into inferior retina. There were also patients (F11,P1; F12,P2; and F12,P1) with reduced sensitivity throughout the profiles, but more function centrally than at greater eccentricities.

Do the transitions from normal to abnormal function relate to transitions from normal to abnormal retinal structure noted in the OCT data analyses? Cross-sectional OCT images through the horizontal meridian from the fovea into temporal retina in a patient with MYO7A-USH1B (F4,P1) illustrate the structural changes within the transition zone and the accompanying changes in visual sensitivity (Fig. 5C). There is normal lamination, normal ONL thickness, and normal sensitivity that extend from the fovea to ~2 mm into temporal retina. From 2 to 4 mm the retina retained lamination, but there was gradual ONL thinning and loss of IS/OS and OLM signals. OPL and INL thickened and sensitivity declined below normal limits (dashed line, Fig. 5C). Between 4 and 5 mm temporally, the ONL is barely discernible and there is further loss (>2 log units) of visual sensitivity. Beyond 5 mm, there is a change in retinal structure with loss of measurable ONL and of normal lamination. This structural change was accompanied by >3 log units of visual sensitivity loss.

A relationship between ONL thickness and visual function has been established in normal human retinas and in retinal degenerations. We used this general function-structure relationship to study colocalized visual sensitivity and ONL thickness along the horizontal and vertical meridians of patients with MYO7A-USH1B (Fig. 5D). ONL thickness, expressed as a fraction of mean normal thickness (in log units) for any given location, was related to the colocalized loss of visual sensitivity (slope, 0.3; correlation, r = 0.76). Thus, in MYO7A-USH1B, transition zone visual sensitivity behaved as if photoreceptor loss is the dominant contributor to the visual dysfunction.

Discussion

USH1 has been considered to be the most severe form, based mainly on the profound hearing loss and vestibular involvement. The associated retinal disease is often described as a “prepubertal onset of retinitis pigmentosa.” Molecular discovery has identified many different causative genes within the clinical category of USH1, but detailed information on phenotype has not usually accompanied the reports of new genotypes. Proof of concept that subretinal gene delivery may be useful to treat USH1B caused by MYO7A mutations is a key reason to refine our understanding of this retinal phenotype. The lack of a retinal degeneration component in the myo7a-mutant mouse and no available human postmortem donor retinas from patients with MYO7A-USH1B makes noninvasive study of the human retinal disease essential for developing a focal intervention strategy.

OCT imaging studies of retinal laminar architecture with colocalized visual sensitivity measurements in patients with
MYO7A-USH1B led to our finding that large regions of retina can be normal in structure and function. Such findings were not anticipated because the traditional notion is that retinal degenerations are retina-wide diseases with various levels of severity, a percept that derives from full-field ERGs, the gold standard for diagnosis and monitoring of RP. Indeed, full-field ERGs in patients with MYO7A-USH1B are severely reduced in amplitude, but plans to deliver a focal treatment demand understanding of the disease details in relatively small retinal regions.

**FIGURE 3.** Details of the retinal laminar structure in MYO7A-USH1B examined by FD-OCT. (A) Cross-sectional OCT along the horizontal meridian from the fovea extending into the temporal retina in a normal subject and a patient (F5,P2) with MYO7A-USH1B. Arrows: INL, OPL, ONL, and OLM layers in both scans. Inset: scan location on a schematic of the left retina. Calibration bar at right. (B) INL, OPL, and ONL thickness measurements along the horizontal meridian into temporal retina in the patient (circles) compared with normal limits (shaded gray areas: mean ± 2 SD; INL and OPL, n = 9, ages 8–48 years; ONL, n = 25, ages 5–58 years). The change of symbol in INL measurements at 8 mm temporal to the fovea (from circles to diamonds) represents change to a hyporeflective layer that is continuous with the INL (but not known to be composed only of INL cells). Bar beneath the ONL thickness graph defines OLM visibility (white) and abnormal prominence (hatched). Brackets above patient scan in (A) and graphs in (B) define locations of four contiguous subzones (a–d) of the transition between normal and severely abnormal retinal architecture.
Adjacent to normal retina in many patients was a readily identifiable transition zone to abnormal retina, and there were stereotypical anatomic features within this zone. The assumption is that these features are markers of a continuum of change that leads from normal retinal architecture with normal function to remodeled retina with little or no function. Among the more subtle OCT signs of abnormality was increased prominence of the OLM. The OLM represents the most distal processes of Müller glia, which interconnect with neighboring Müller cells and photoreceptors by specialized junctions. Increased OLM visibility on OCT is likely a marker of altered Müller cell properties, such as swelling of microvilli or hyperreactivity, in response to local photoreceptor cell stress or death. Of interest, endothelin receptor B immunoreactivity, a Müller cell response to photoreceptor injury or disease, was reported to be especially prominent in the OLM after light exposure to the rodent retina. Further, it has been postulated that the OLM is the site of interconnection between the Usher protein network and the proteins forming the Crumbs complex. How such interactions lead to this optical-structural abnormality is not known. The increase in OLM visibility is in contrast to morphologic evidence of OLM disruption in more advanced stages of retinal degeneration.

The defining histopathologic feature of inherited retinal degenerations, whether in postmortem human donor tissue or in animal models, is loss of photoreceptors. Traditionally, quantitation has occurred by ONL cell counting or ONL thickness measurements. In vivo micron-level measurements of ONL thickness in human retinal degenerations were not possible until the advent of OCT. Quantitative comparisons of frozen sections of normal and abnormal animal retina to OCT images of the same retinas indicated that OCT lamination was plausible to relate to retinal histopathology. OCT has now become nearly routine as a method to understand retinal degenerative diseases and disease mechanisms, and more recently, OCT has been used to identify appropriate candidates for therapeutic initiatives. Higher resolution imaging in the present study led to the observation that there is a constellation of laminar changes that accompany reduction in ONL thickness in the MYO7A-USH1B retina. There was abnormal thickening of the OPL and INL in what we termed subzone b. The OPL is the site of photoreceptor synapses and these are enveloped by Müller cell processes; the INL is a layer with many retinal neurons and Müller cell nuclei. A parsimonious explanation is that both OPL and INL thickening are phenomena related to Müller cell hypertrophy or hyperre-
activity in response to the photoreceptor apoptosis that accounts for ONL thinning. In subzone b, the OLM varies from visible to not detectable. ONL thinning has been noted histopathologically to be associated with a disappearance of the OLM.28

Subzone c shows further ONL thinning, a return of OPL to normal thickness, and greater increases of INL thickness. The underlying histopathology would most likely be major photoreceptor loss with possible rod neurite outgrowth,28 disturbance of the integrity of the OPL, and eventual intermingling of the two nuclear layers.13,19 Subzone d, a late-stage OCT appearance that we have documented in many other retinal degenerations with lesser resolution OCT instruments,13,15,16,32–35 is characterized by a loss of normal laminar architecture. Underlying histopathology is speculated to be nearly complete photoreceptor loss, a residual cell layer populated by hypertrophic Müller glial cell nuclei and residual INL cells almost approximate to RPE cells, and a thick vitread layer of IPL processes, ganglion cells, nerve fibers, and epiretinal membrane from Müller glial response. These laminar abnormalities are likely to represent more longstanding disease and extend into the periphery where there may be another transition zone to less advanced retinopathy.28,57

What is the value of defining and then subdividing the transition from normal to abnormal retina? In focal treatment strategies, such as subretinal gene replacement, there is a need to know where exactly in the retina it is most sensible and safe to perform the injection of vector-gene. Gliotic and remodeled retina representing late stages of retinal degeneration (subzone d) could be valuable as sites for the retinotomy, but not sites for the entire injection volume. Subfoveal injections may lead to greater risk than potential benefit.38,39 in those patients who retain normal central function and structure. That leaves subzones b and c as candidate regions for treatment. Assuming that photoreceptor apoptosis is the provocative event leading to OLM, OPL, and INL changes in these two subzones, an alteration in the pattern of laminar architecture may be a structural parameter suggesting efficacy. For example, there would be no expectation of ONL increase but a decrease in OPL and INL thickness could be an indirect sign of less photoreceptor stress or loss. From an optimistic viewpoint, the region could also show a difference in relationship between structure and function; outer segment material could increase as photoreceptor ciliary function improves, and sensitivity gains would follow. Our finding of similar subzone patterns in other retinal meridians in the same individual should be followed up by formal interocular comparisons. A potentially expeditious method of evaluating a focal therapy may be to compare the properties of structure and function in the region of the injection to noninjected sites that show similar properties on baseline examinations.

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References


