Retinal Disease Course in Usher Syndrome 1B Due to *MYO7A* Mutations

Samuel G. Jacobson,^{*,1} Artur V. Cideciyan,¹ Dan Gibbs,² Alexander Sumaroka,¹ Alejandro J. Roman,¹ Tomas S. Aleman,¹ Sharon B. Schwartz,¹ Melani B. Olivares,¹ Robert C. Russell,¹ Janet D. Steinberg,¹ Margaret A. Kenna,³ William J. Kimberling,^{4,5} Heidi L. Rehm,⁶ and David S. Williams^{*,7}

PURPOSE. To determine the disease course in Usher syndrome type 1B (USH1B) caused by myosin 7A (*MYO7A*) gene mutations.

METHODS. USH1B patients ($n = 33$, ages 2–61) representing 25 different families were studied by ocular examination, kinetic and chromatic static perimetry, dark adaptometry, and optical coherence tomography (OCT). Consequences of the mutant alleles were predicted.

RESULTS. All *MYO7A* patients had severely abnormal ERGs, but kinetic fields revealed regional patterns of visual loss that suggested a disease sequence. Rod-mediated vision could be lost to different degrees in the first decades of life. Cone vision followed a more predictable and slower decline. Central vision ranged from normal to reduced in the first four decades of life and thereafter was severely abnormal. Dark adaptation kinetics was normal. Photoreceptor layer thickness in a wide region of central retina could differ dramatically between patients of comparable ages; and there were examples of severe losses in childhood as well as relative preservation in patients in the third decade of life. Comparisons were made between the mutant alleles in mild versus more severe phenotypes.

CONCLUSIONS. A disease sequence in USH1B leads from generally full but impaired visual fields to residual small central islands. At most disease stages, there was preserved temporal

peripheral field, a potential target for early phase clinical trials of gene therapy. From data comparing patients' rod disease in this cohort, the authors speculate that null *MYO7A* alleles could be associated with milder dysfunction and fewer photoreceptor structural losses at ages when other genotypes show more severe phenotypes. (*Invest Ophthalmol Vis Sci.* 2011;52:7924–7936) DOI:10.1167/iov.11-8313

The understanding of mechanisms underlying Usher syndrome (USH) has increased in recent years with the identification of the molecular bases of the diseases (reviewed in Refs. 1–3). The original clinical subcategories are now known to be caused by many different genes, and most of the gene products are postulated to play roles in an Usher protein network located in the region of the connecting cilium of the photoreceptor.^{3–5} All forms of USH, by definition, lead to retinal degeneration, although some of the USH-causing genes can also cause nonsyndromic deafness.¹

USH1B, the most common form of USH1, is caused by mutations in *MYO7A* (myosin 7A). Like most of the other forms of USH, there is no murine model with a retinal degeneration phenotype.^{1,6,7} The onus is thus placed on noninvasive human studies in patients with clarified genotypes to help define the retinal degenerative disease component of the syndrome. Given the prospect of therapy for USH1B,⁸ we have initiated studies to characterize in detail the retinal phenotype of USH patients with known genotypes. We first inquired in USH1B and in other USH genotypes about the earliest detectable site of disease and concluded that it was the photoreceptor.⁹ Then, we explored the microstructure of the central retina of USH1B patients using high-resolution optical coherence tomography.¹⁰ An unexpected result was that many patients showed a wide central region of structurally (and functionally) normal retina. This observation led to suggestions about candidate sites for treatment as well as retinal sites that would be ill-advised to treat in early safety trials. The finding of normal central retina in syndromic recessive retinal degenerations was extended recently to include USH1C¹¹ and nonsyndromic retinitis pigmentosa.¹²

Patterns of visual function have been published for various USH clinical and molecular subtypes (for example, Refs. 13–19). In the only previous study of USH1B, cross-sectional and longitudinal data for functional vision scores were analyzed, and deterioration rates were compared with those from USH2A.¹⁷ To increase the knowledge base of the USH1B phenotype in anticipation of clinical trials, we studied visual acuities, kinetic and chromatic static perimetry, and retinal imaging in a molecularly clarified group of USH1 patients with *MYO7A* mutations to determine the patterns of central, peripheral, and rod- and cone-based visual disturbances. A recent report of visual cycle abnormalities in *Myo7a*-deficient mice²⁰

From the ¹Scheie Eye Institute, Department of Ophthalmology, University of Pennsylvania, Philadelphia, Pennsylvania; the ²Salk Institute for Biological Studies, La Jolla, California; the ³Department of Otolaryngology and Communication Enhancement, Children's Hospital Boston, Boston, Massachusetts; the ⁴Usher Syndrome Center, Boys Town National Research Hospital, Omaha, Nebraska; the ⁵Department of Ophthalmology, University of Iowa Carver School of Medicine, Iowa City, Iowa; the ⁶Department of Pathology, Harvard Medical School, Boston, Massachusetts; and the ⁷Jules Stein Eye Institute, Department of Ophthalmology, University of California, Los Angeles, California.

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*Each of the following is a corresponding author: Samuel G. Jacobson, Scheie Eye Institute, University of Pennsylvania, 51 N. 39th Street, Philadelphia, PA 19104; jacobson@mail.med.upenn.edu.

David S. Williams, Jules Stein Eye Institute, Department of Ophthalmology, University of California, Los Angeles, CA 90095; dswilliams@ucla.edu.

also prompted us to study the kinetics of dark adaptation in some USH1B patients with preserved rod function. Once it became clear that there were milder as well as more severe phenotypes, we inquired whether the genotypes of the different phenotypes could help to explain the variation in disease expression among patients.

METHODS

Human Subjects

Thirty-three patients (ages 2–61 years) who had USH1 with *MYO7A* mutations (Table 1) were included. The patients had a complete eye examination, including electroretinograms (ERGs), which were tested with a standard protocol.^{21,22} Informed consent was obtained; procedures complied with the Declaration of Helsinki and had institutional review board approval.

Visual Function and Retinal Structure

Perimetry. Kinetic perimetry was performed and analyzed as published.²¹ Static thresholds were determined with 1.7°-diameter, 200-ms-duration stimuli under dark-adapted (500- and 650-nm stimuli) and light-adapted (600 nm) conditions. A full-field test of 71 loci on a 12° grid and a horizontal profile across the fovea (extending 60°, 2° intervals) were used. Photoreceptor mediation was determined by the sensitivity difference between detection of 500- and 650-nm stimuli.^{23,24} Rod (500 nm, dark-adapted) and cone (600 nm, light adapted) sensitivity losses at each test locus were calculated by comparison with normal mean sensitivities at the location. Loci were considered to have no measurable rod sensitivity if loss was >30 dB.²⁴ Rod and cone static field extents were defined as the number of locations with measurable function from rod-mediated dark-adapted (500 nm) or light-adapted (600 nm) perimetry, respectively. These extents were expressed as the percentage of total number of loci tested (12° grid; $n = 70$ extrafoveal loci for rods; $n = 71$ for cones).^{19,23} Central sensitivity averages were derived from an abbreviated set of central loci ($\pm 8^\circ$) from the dark-adapted horizontal profile.²⁴ Mean rod central sensitivity was defined as the average of sensitivities from all loci with rod-mediated detection (500 nm, dark adapted); mean cone central sensitivity was the average of sensitivities for locations detecting the 600 nm stimulus.

Dark Adaptometry. In retinal regions with evidence of rod-mediated function, dark adaptation testing was performed in a subset of patients to determine the kinetics of the rod and cone visual cycle.^{25–32} A short-duration (2-ms), yellow (Xenon filtered through Wratten 8; Eastman Kodak, Rochester, NY), full-field adapting exposure of 7 log scot-td · s was delivered by a flash unit mounted at the top of a 150-mm-diameter sphere with a white inner coating and an opening for the subject's eye. In a dark-adapted normal eye, ~60% of the available rhodopsin molecules would be expected to absorb a primary quantum with this flash. The yellow adapting flash was delivered under near-infrared (NIR) light viewing of the subject's pupil, to avoid reduction in retinal exposure due to partially closed eyelids. During testing, NIR LEDs illuminated the pupil and an NIR-sensitive camera allowed continuous monitoring of pupil position. The stimuli used to estimate psychophysical sensitivities were either blue or red LEDs illuminating an opal diffuser (1.7° diameter); for testing normal eyes, a 3-log-unit neutral-density filter was intercalated between the blue LED and the diffuser to shift the whole dynamic range of the instrument to lower illuminances. Under software control, LEDs were driven with amplitude and pulse-width modulation to achieve a 5.8-log unit dynamic range. Thresholds to blue and red stimuli were determined using a staircase procedure before and at regular intervals after the adapting flash. Differences between the sensitivities to blue and red stimuli were used to determine the type of photoreceptor mediating vision.

Optical Coherence Tomography (OCT)

Retinal cross sections were obtained with OCT. Data were collected mainly with the use of a spectral-domain (SD) OCT system (RTVue-100; Optovue Inc., Fremont, CA); a minority of patients were studied with time-domain (TD) OCT instruments (OCT1 and OCT3; Carl Zeiss Meditec, Dublin, CA). TD-OCT data, which antedated SD-OCT, were available for understanding the longitudinal natural history of retinal structural changes. Postacquisition processing of OCT data was performed with custom programs (MatLab 6.5; The MathWorks, Natick, MA). Our recording and analysis techniques have been published.^{32–36} In brief, SD-OCTs were performed along the vertical meridian extending to 9 mm from the fovea into superior and inferior retinas. Longitudinal reflectivity profiles (LRPs) making up the OCT scans were aligned by straightening the major RPE reflection. The outer nuclear layer (ONL) thickness was first defined at the foveal region as the major intraretinal signal trough delimited by the signal slope maxima of the LRPs. Then, the ONL boundaries were extended peripherally, and the thickness of the inner and outer segments (IS+OS) from ONL to the RPE layer was added. Quantitation occurred as a function of eccentricity, and comparisons were made to the normal range (mean–2 SD; $n = 9$; ages 8–44). The extent (in millimeters) of retina in the superior and inferior directions where photoreceptor (ONL+IS+OS) thickness remained within normal limits was defined. For TD-OCT data, similar analyses were performed, but only ONL thickness was considered.

Molecular Modeling of MYO7A Mutations

Homology Modeling of MYO7A Motor Domain Mutations. The amino acid sequence of the MYO7A motor domain (1–768 aa) was fit to the crystal structure of MYO5A-ADP (1w7j)³⁷ using the template identification and alignment functions of the SWISS-MODEL server (http://swissmodel.expasy.org/workspace/index.php?func=tools_targetidentification1; Swiss Institute of Bioinformatics, Geneva, Switzerland). Structural refinement of the MYO7A motor domain model was performed in Deepview (<http://spdbv.vital-it.ch/>; Swiss Institute of Bioinformatics) using the GROMOS96 forcefield energy calculation and global energy minimization functions. The point mutations G163R, K164N, R212H, and G519D were introduced independently into the model by using Deepview. For each mutated residue, energetically favorable sidechain rotamers were identified, and the impact on neighboring residues was assessed with GROMOS96. Affected residues were corrected with an exhaustive side chain search, and the global structure was refined by another round of energy minimization.

Analysis of Splice Site Mutations. The effects of the mutations IVS6+1 G>T, 19-2 A>G, 2187+1 G>A, and 4442-2 A>C on transcript splicing were analyzed by using the NNSPLICE 0.9 human splice site prediction server (http://www.fruitfly.org/seq_tools/splice.html) and compared to the genomic sequence of the consensus MYO7A isoform 1 long transcript (Ensembl ID ENST00000358342; <http://www.ensembl.org> The Ensembl Genome Database³⁸).

RESULTS

Clinical Characteristics of the USH1B Patients

In this cohort of 33 USH1B patients, representing 25 families, there were 8 sibling pairs (Table 1). Two sibling pairs (P7,P9 and P11,P12) were not known to be from the same family, but both pairs were from the Dominican Republic and all were homozygous for the c.999T>C, p.Y333X allele. Origins of the remainder of the patients were mainly European (Table 1). Severe bilateral hearing impairments were reported to be present by early childhood in all the patients.

Visual acuities in the first two decades of life were no worse than 20/63. In later decades of life, visual acuity could also be preserved in at least one eye, and only the oldest patient in the study (P33, age 61) had worse than 20/200 acuity in both eyes. Refractive errors (spherical equivalent; both eyes averaged) of

TABLE 1. Clinical and Molecular Characteristics of the USH1B Patients

Patient/ Family	Age at First Visit (y)/Sex	MYO7A Mutations	Ethnic or Geographic Origin	Eye	Visual Acuity*	Refraction†	Kinetic Visual Field Extent (V-4e/I-4e)‡	ERG Amplitudes§	
								Rod b-Wave	Cone Flicker
P1/F1	2/F	E166fs/H1109fs	British/European	RE	NAP	+2.50 sph	NAP	NP	NP
P2/F2	2/F	G1942X/F1963fs	British/European	LE	NAP	+2.50 sph	NAP	NP	NP
P3/F3	3/M	G519fs/Q1798X	European	LE	NAP	+2.00 sph	NAP	NP	NP
P4/F2	3/F	G1942X/F1963fs	British/European	RE	20/32	+6.25 – 1.00 × 149	NAP	NP	NP
P5/F4	4/F	H1355fs/R2024X	Ashkenazi Jewish/European	LE	20/32	+7.00 – 0.75 × 035	NAP	NP	NP
P6/F5	4/F	4442–2A>C/K1737fs	European	RE	20/50	+2.00 – 0.75 × 009	NAP	NP	NP
P7/F6	4/M	Y333X/Y333X	Spanish (Dominican Republic)	LE	20/50	+1.75 – 1.50 × 179	NAP	NP	NP
P8/F7	5/M	H133fs/R1883Q	European	RE	20/32	–0.75 – 2.00 × 005	81/<1	NP	NP
P9/F6	6/M	Y333X/Y333X	Spanish (Dominican Republic)	LE	20/32	–0.25 – 2.50 × 180	82/<1	NP	NP
P10/F8	6/F	R212H/R212H	Peruvian	RE	20/40	–5.00 – 1.25 × 178	NAP	NP	NP
P11/F9	8/M	Y333X/Y333X	Spanish (Dominican Republic)	LE	20/40	–4.25 – 1.25 × 159	NAP	NP	NP
P12/F9	9/M	Y333X/Y333X	Spanish (Dominican Republic)	RE	20/30	–0.75 – 1.50 × 180	NP	ND	3%
P13/F10	9/M	G955S/2187 + 1G>A	Eastern European	LE	20/50	–0.25 – 1.75 × 180	90/ND	ND	2%
P14/F3	9/M	G519fs/Q1798X	European	RE	20/32	+5.00 sph	74/1	NP	NP
P15/F5	10/F	4442–2A>C/K1737fs	European	LE	20/32	–2.75 – 2.25 × 015	67/<1	NP	NP
P16/F11	10/F	E968D/Q1178fs	European	RE	20/32	–1.25 – 2.00 × 165	84/2	NP	NP
P17/F12	11/F	19–2A>G/A1770D	British/European	LE	20/32	–4.75 – 1.00 × 175	81/ND	ND	8%
P18/F13	17/M	G163R/592 + 1G>T	European	RE	20/25	–6.50 – 0.75 × 176	81/ND	ND	ND
P19/F14	17/M	K164N/E968D	European	LE	20/32	–2.25 – 2.75 × 015	59/1	ND	ND
P20/F15	17/M	C31X/E495X	British/European	RE	20/32	–0.50 – 2.00 × 160	54/1	NP	NP
P21/F16	18/F	K1255fs/G1942X	American Indian/British/European	LE	20/32	–5.50 – 2.00 × 180	96/<1	NP	NP
P22/F17	19/F	A1288P/R1743W	European	RE	20/32	+1.00 – 1.00 × 180	12/1	NP	NP
P23/F18	19/M	G519D/R669X	European	LE	20/25	+1.50 – 1.00 × 180	12/1	NP	NP
					20/25	+3.25 – 2.00 × 035	92/<1	NP	NP
					20/25	Plano – 1.75 × 016	93/<1	NP	NP
					20/25	+0.25 – 1.50 × 008	95/1	NP	NP
					20/25	+2.25 – 2.75 × 009	97/1	NP	NP
					20/25	+1.50 – 2.75 × 177	81/3	ND	1%
					20/25	+2.75 – 1.25 × 010	80/2	ND	10%
					20/25	+2.50 – 1.25 × 173	32/1	ND	ND
					20/25	–2.00 sph	88/2	ND	ND
					20/25	–1.25 sph	93/2	ND	ND
					20/25	+1.00 – 1.25 × 180	19/5	ND	5%
					20/25	+1.25 – 1.25 × 180	21/6	ND	5%
					20/20	plano – 0.25 × 090	41/2	ND	5%
					20/20	–0.50 – 0.50 × 090	45/3	ND	1%
					20/40	+1.50 – 0.75 × 177	18/<1	ND	1%
					20/32	+1.00 – 1.00 × 019	13/<1	ND	ND
					20/32	–0.50 – 1.25 × 180	85/1	ND	ND
					20/32	+1.00 – 1.00 × 180	106/1	ND	1%
					20/25	+0.50 – 0.50 × 135	16/1	ND	1%
					20/20	+0.50 – 0.50 × 060	15/1	ND	1%

(continues)

TABLE 1 (continued). Clinical and Molecular Characteristics of the USH1B Patients

Patient/ Family	Age at First Visit (y)/Sex	MYO7A Mutations	Ethnic or Geographic Origin	Eye	Visual Acuity*	Refraction†	Kinetic Visual Field Extent (V-4e/I-4e)‡	ERG Amplitudes§	
								Rod b-Wave	Cone Flicker
P24/F19	19/M	D2010N/Y2015H	African	RE	20/25	-0.75 - 0.75 × 170	24/7	ND	ND
P25/F20	19/F	R1240Q/R1240Q	British	LE	20/20	-1.25 - 0.50 × 180	24/8	ND	ND
P26/F21	19/F	R634X/G1982E	British/European	RE	20/20	+2.00 - 2.00 × 014	15/3	ND	ND
P27/F16	21/F	K1255fs/G1942X	American Indian/British/European	RE	20/20	+1.50 - 2.00 × 007	19/3	6%	6%
P28/F21	21/F	R634X/G1982E	British/European	LE	20/20	+1.00 - 1.50 × 006	35/13	ND	ND
P29/F22	28/M	R159fs/R669X	European	RE	20/20	+1.50 - 2.00 × 176	36/15	ND	8%
P30/F17	31/F	A1288P/R1743W	European	RE	20/20	-0.25 - 0.75 × 180	13/<1	ND	ND
P31/F23	37/M	E1170K#	Spanish	RE	20/40	-0.75 - 0.75 × 180	13/<1	ND	ND
P32/F24	39/F	E1716X/L1858P	British/European	RE	20/32	+0.50 - 1.25 × 005	31/18	ND	ND
P33/F25	61/F	Q1798X/E1917X	European	LE	20/400	+1.00 - 2.00 × 179	34/19	ND	ND
					20/32	Plano - 0.50 × 090	1/<1	ND	ND
					20/40	+0.25 - 0.50 × 090	1/<1	ND	ND
					20/50	+0.50 - 0.50 × 180	1/N	ND	ND
					20/40	+0.25 - 1.00 × 157	<1/<1	NP	NP
					20/40	Plano - 0.50 × 096	<1/<1	NP	NP
					20/50	Plano	9/<1	NP	NP
					20/320	Plano	10/<1	NP	NP
					20/500	-3.75 - 0.50 × 025	ND/ND	NP	NP
						-3.75 - 1.50 × 051	ND/ND	NP	NP

ND, not detectable; NP, not performed; NAP (not able to be performed, mainly because of age); LP, light perception.

* Best corrected visual acuity.

† Cycloplegic retinoscopy was performed in P1, P2.

‡ Expressed as a percentage of normal mean of V-4e or I-4e target; 2 SD below normal equals 90% (Jacobson et al.²¹); average both eyes; similar in the two eyes unless specified.

§ Expressed as a percentage of normal mean amplitude (rod = 292 μ V; cone flicker = 172 μ V); 2 SD below normal equals 67% for rod b-wave and 60% for cone flicker (Aleman et al.²²). Shown as an average of results of the two eyes (if performed); some patients had testing in only one eye.

|| Data from age 8 visit.

Only one allele identified.

the USH1B patients in the present study ranged from -6.6 to $+6.25$ (mean $\pm$ SD = -0.4 ± 2.8 ; $n = 33$ patients; Table 1).

Electroretinograms were performed in 20 of the 33 patients. Only one patient (P26, age 19) had detectable rod ERG b-waves, and these were $\sim 5\%$ of normal mean amplitude. Cone flicker ERGs were detectable in 11 of 20 patients, and these waveforms ranged from 1% to 10% of mean normal amplitude.

Patterns of Visual Loss by Kinetic Perimetry

Kinetic visual fields with V-4e, the large bright target, were measurable in 25 patients; most young patients were unable to perform the test, and the oldest patient had no detectable kinetic field (Table 1). Twenty-three of the patients were able to detect I-4e, the small bright target. A normal extent of kinetic field in response to the V-4e target (defined as $\geq 90\%$; Ref. 21) was present in only six patients. None of the patients had a normal extent of kinetic field with the I-4e target.

Different patterns of kinetic visual field abnormalities were evident with the V-4e target in cross-sectional data. A sequence leading through several patterns was suggested by the longitudinal data available in some patients (Fig. 1A). A full extent of field with V-4e and no detectable absolute scotomas, albeit with only a small central island using I-4e, was designated pattern I. P9, P11, and P12 at ages 6 to 11 exemplify this pattern. At the other end of the severity spectrum, there were fields with only a residual small central island of function with V-4e; these were considered pattern V. Pattern II shows nasal field loss; and in pattern III, there were complete or incomplete midperipheral absolute scotomas. Pattern IVa showed a nearly complete annular midperipheral scotoma separating a central island from a temporal peripheral island; further reduc-

tion of central and peripheral islands was designated pattern IVb. Longitudinal data through at least three of the patterns are shown for P12 (I-IVa), P11 (I-III), and P24 (II, IVa-IVb). Data through two patterns are shown for P9 (I and III), P25 (IVb-V), P19 and P20 (IVa-IVb), and P32 and P18 (IVb-V).

Kinetic field data for all the patients and all visits are shown organized by pattern of disease and age at the time the perimetry was performed (Fig. 1B). Patterns I to IVa were present mainly in the first two decades of life (pattern I mean = 10.9 years, range 6-19; II mean = 16.3 years, range 13-20; III mean = 16 years, range 15-17; and IVa mean = 18.6 years, range 11-24). The average age of patients in patterns IVb and V tended to be older (IVb mean = 26.5 years, range 16-41, and V mean = 37 years, range 28-49) than those in earlier patterns.

Light-Adapted Visual Function in USH1B

The kinetic visual field extent, quantified for the V-4e target and expressed as a percent of normal mean, is plotted against age (Fig. 2A); longitudinal data are indicated by lines connecting symbols. There is a range of visual field loss in the first two decades of life; after the third decade, only approximately 10% or less of the visual field remains. The decline with age could be described by a log-linear relationship with a decay rate of 14% per year (half-life, 4.6 years) with longitudinal data (representing the average of individual rates for 14 patients with follow-up intervals between 5 and 15 years). When the cross-sectional data and the first visit of each patient were used, the decay rate was 10% per year.

Static threshold perimetry in the light-adapted state (600-nm stimulus) was performed in 22 of the patients. Cone sensitivity

FIGURE 1. Patterns of visual function in USH1B by kinetic perimetry. (A) Kinetic visual field maps of responses to the V- and I-4e targets. Fields are classified into six patterns (I-III, IVa and IVb, and V), with each column representing a different pattern. Longitudinal data for patients with multiple visits are shown on the left of each sequence of visits by patient number and connected by lines near the base of the fields. Isopters for the I-4e target are interior to the V-4e isopters. Shaded areas are absolute scotomas. Ages are given in years. All fields are depicted as right eyes. (B) Ages of patients within each pattern.

FIGURE 2. Light-adapted visual function in USH1B patients. **(A)** Kinetic field extent (as a percentage of normal mean extent; V-4e target) versus age. Longitudinal data are connected by *lines*. *Gray dashed lines*: illustrate linear regression results on cross-sectional data (first visits for each patient) with 95% prediction intervals. **(B) Left**: maps of light-adapted static perimetric cone sensitivity loss (600-nm stimuli) in two representative patients: P9 (age 12) and P32 (age 39). The scale has 16 levels of gray representing 0- to 30-dB losses; the physiological blind spot is a *black square* at 12° in the temporal field. *Right*: cone visual field extent (calculated by the number of loci with detectable perception as a percentage of total loci tested) plotted as a function of age. Longitudinal data are connected by *lines*. *Gray symbols*: the patients in the maps. *Gray dashed lines*: linear regression results on cross-sectional data (first visit of each patient) with 95% prediction intervals. **(C)** Visual acuity in logMAR as a function of age. Longitudinal data are connected by *lines*. **(D) Left**: profiles of light-adapted sensitivity (600-nm stimulus) horizontally across the central 60° of visual field in three patients between the ages of 28 and 31. *Dashed lines*: normal limits (± 2 SD from mean sensitivity). *Right*: mean light-adapted sensitivity to the same stimulus (central 16°) in the full cohort of patients; longitudinal data are connected by *lines*. *Gray symbols*: the patients illustrated with profiles to the *left* of the graph. *Hatched area*: physiological blind spot. LA, light-adapted; N, nasal; T, temporal; S, superior; I, inferior visual field.

maps across the visual field are shown for two USH1B patients (ages 12 and 39) to illustrate extremes of visual dysfunction (Fig. 2B, left). Patient 9 at age 12 has normal central cone function; extracentral loci with detectable function showed 1 to 2 log units of loss. Patient 32 (age 39) had detectable cone sensitivity only at the central locus. Data from all patients are summarized as the extent of cone visual field (Fig. 2B, right), for comparison with data from kinetic perimetry. The extent of cone field was defined as the number of loci with detectable function expressed as a percentage of the total number of loci analyzed. Cone field extent showed a decline with age that could be described by a log-linear relationship with a decay of $\sim 11\%$ per year (half-life, 5.9 years), using longitudinal data (representing the average of individual rates for 11 patients with follow-up intervals between 2 and 15 years). When cross-sectional data and the first visit of each patient were used, the decay rate was 10.5% per year.

Central cone function was assessed with visual acuity and central static profiles using 600-nm stimuli, light adapted. Best corrected visual acuity for USH1B in the first two decades of life varied between 20/20 (logMAR 0.0) and 20/63 (logMAR 0.49); individuals studied longitudinally during that interval showed progressive reduction in acuity (Fig. 2C). Data were limited after the third decade of life but some individuals retained relatively high visual acuity levels whereas others declined.

Static perimetry results in the central retina are illustrated in three patients, ages 28 to 31 years, showing there could be differences in central function in patients at similar ages (Fig. 2D, left). The summary of cone sensitivities across the central retina ($\pm 8^\circ$) of all patients, like the visual acuity results, showed that some patients retained normal sensitivity over three decades of life, whereas others had early losses (Fig. 2D, right).

In summary, there was a definable relationship of cone function with age, measured by parameters that included the peripheral and central retina. Central visual function measured independently of peripheral function was more variable.

Differences in Rod Functional Deficits among USH1B Patients

Maps of dark-adapted sensitivity to a 500-nm target and photoreceptor mediation at each of the loci with detectable function (based on two-color, dark-adapted perimetry with 500- and 650-nm stimuli) are illustrated (Fig. 3A, left). Patient 26 (age 19) had a substantial extent of normal central rod function but there was midperipheral and peripheral rod sensitivity loss of ~ 2 to 2.8 log units. At a comparable age, patient 27 (age 21) had no detectable rod function and only cone-mediated function.

Summaries of rod-mediated sensitivities across the visual field of all patients are plotted as a function of age (Fig. 3A,

the superior field in P9 (open symbols) and in a representative normal subject (gray symbols). Cone adaptation kinetics recorded with red stimuli immediately after the flash are fast and do not show a difference in sensitivity from healthy cones. Rod adaptation kinetics recorded with blue stimuli after emerging from the cone plateau is similar to that in normal rods. (D) Data from P21 show sensitivities of both rod and cone systems to be abnormally reduced but kinetics of adaptation resembles those in the normal. Hatched area: physiological blind spot. N, nasal; T, temporal; S, superior; I, inferior visual field; DA, dark-adapted.

right). The extent of rod visual field was defined as the number of loci mediated by rods (with ≤ 3.0 log units of sensitivity loss) and expressed as a percentage of the total number of loci analyzed. There were considerable differences among patients in measurable rod visual field extent in the first three decades of life. Seven patients retained at least 33% of rod visual field extent, whereas nine patients had $< 3\%$. By the fourth decade, rod field extent had diminished to 10% or less in all patients. Limited longitudinal data in four patients (P9, P11, P20, and P24) suggested progression rates ranging from 11% to 24% per year of reduction in rod visual field extent.

Rod-mediated vision in USH1B was also studied within the central field, excluding the fovea (Fig. 3B). Rod sensitivity could be within normal limits in some individuals for almost the first three decades of life, whereas others showed very reduced or nondetectable rod sensitivity at comparable ages (Fig. 3B, left). For example, three USH1B patients, ages 17 to

21, have contiguous central regions of normal rod sensitivity (Fig. 3B, left). The pattern of markedly reduced rod sensitivity or only cone-mediated sensitivity is illustrated by P13 at age 19 and P27 at age 21. Cross-sectional and longitudinal rod data plotted as a function of age (Fig. 3B, right) suggest that within the first two to three decades of life, the central field, like rod visual field extent measured across the entire field (Fig. 3A), can show different severities of rod disease.

Evidence of abnormalities in the visual cycle in the shaker1 mouse model of USH1B²⁰ prompted a study of kinetics of dark adaptation in patients. Chromatic sensitivities were measured before and after a desensitizing light flash in P7, P9, and P21 (Fig. 3C, 3D). In all patients, a retinal locus at 4° in the superior field was tested. P9 at age 17 showed rod sensitivities that were within 0.5 log unit of normal. Within a minute after the end of the light-adapting exposure, visual function was detectable, and it was mediated by the cone system. Cone-mediated func-

FIGURE 3. Dark-adapted visual function in USH1B patients. (A) *Top left:* Dark-adapted sensitivity in response to a 500-nm target at 71 loci (12° grid) across the visual field in two representative patients of similar age (P26, age 19; P27, age 21) displayed as maps of rod sensitivity loss. Scale has 16 levels of gray representing 0- to 30-dB losses. *Lower left:* photoreceptor mediation for loci with measurable sensitivities shown in (A), obtained by two-color, dark-adapted perimetry. R, rod-mediated perception of 500- and 650-nm targets; M, 500 nm mediated by rods, 650 nm by cones; C, perception of both stimuli by cones. The physiological blind spot is shown as a black square at 12° in the temporal field. *Right:* extent of rod-mediated (≤ 3.0 log units of loss) static field (expressed as a percentage of the total number of loci analyzed) as a function of age. Gray symbols: the two patients shown in the maps. (B) Profiles of dark-adapted sensitivity (500-nm stimulus) horizontally across the central 60° of visual field. Representative patients (ages 17–21) with rod- and mixed-mediated function are shown (above) as are patients of comparable age with severe rod loss or only cone-mediated function (below). Solid gray lines: normal limits of rod sensitivity (± 2 SD from mean); normal limits at cone plateau are shown in the lower graph with dashed lines. *Right:* mean dark-adapted sensitivity in the central 16° loci across the horizontal meridian for rods (500 nm, dark adapted; fovea excluded) in all patients studied; longitudinal data connected by lines. Gray symbols: patients included in plots. (C, D) Dark adaptation kinetics in USH1B. (C) Sensitivity to blue and red stimuli before and after a 7-log scot-td $\cdot$ s yellow adapting flash (presented at time 0) recorded at 4° in

tion was normal and remained on the plateau for approximately 8 minutes, similar to the 7 to 9 minutes in normal eyes. Rod function then became detectable and the shape and timing of the rod recovery function was normal (Fig. 3C). P21 at age 18 had ~ 1.6 log units of sensitivity loss at the test locus. After the light-adapting exposure, cone function was detectable and reached a plateau with normal kinetics within 5 minutes, but there was ~ 0.7 log unit of cone sensitivity loss. Rod function became measurable at ~ 8.5 minutes and the time course of recovery to baseline appeared to have normal kinetics (Fig. 3D). P7, at age 9 years, showed ~ 2 log units of rod sensitivity loss. The relatively young age of the patient prohibited the recording of as complete a dark adaptation function as in the older patients, but normal cone sensitivity was present before 9 minutes after the light-adapting exposure, and baseline rod levels were present after 45 minutes (data not shown).

Retinal Structural Differences in USH1B

Structure of the central retina was recorded with SD-OCT along the vertical meridian crossing the anatomic fovea and quantified in terms of the thickness of the laminae representing photoreceptor nuclei and inner/outer segments (Fig. 4). Structurally normal retina and photoreceptors could be present across extensive regions of central retina (e.g., P2 and P4, Fig. 4A) or might be limited to a small region at and around the fovea (P15, Fig. 4A), implying a centripetal component to the progressive retinal degeneration. A superior-inferior asymmetry was also often observed; the region of normal structure tended to extend farther into the superior retina compared with the inferior retina (Fig. 4A), implying intraretinal anisotropy of constriction rates with disease progression.

Superior to the fovea, the extent of normal photoreceptors plotted against age shows large interindividual differences in our cross-sectional sample of USH1B patients (Fig. 4B). Differences are especially notable in the first three decades of life. Some patients (P1, P2, P3, P4, P7, P24, P26, and P28) had normal retinal structure extending well into the rod ring³⁹ where rod:cone ratios peak at >20 , thus implying normal or near-normal rod photoreceptor density. Other patients in contrast had normal photoreceptors limited only to the cone-dominated fovea and its immediate surrounds. Age was not a good predictor of disease extent, as demonstrated by SD-OCT scans from P7 at age 6 and P5 at age 5, showing large differences in the extent of normal photoreceptor structure that were already present in the first decade of life. Similarly, P28 and P13, both at age 21, showed severity differences at the start of the third decade of life (Fig. 4B, right). Inferior to the fovea, there was a tendency to have a smaller extent of normal structure than in the superior retina, and thus interindividual differences appeared smaller due to the "floor" effect (Fig. 4C). Contributing to the superior-inferior asymmetry could be intraretinal differences in rod:cone ratios which are significantly different at eccentricities of 3 to 4 mm from the fovea (Figs. 4B, 4C).

Next, we tried to understand through quantitation the natural history of retinal structural changes in USH1B. Ideally, longitudinal measurements over decades provide the best estimates of natural history of retinal disease, but SD-OCT technique has been commercially available only recently. Therefore, we took advantage of our ONL thickness measurements from TD-OCTs in a subset of eight patients recorded longitudinally over an average of 6.9 years (range, 3–11). We hypothesized that the extent of normal ONL constricted along a delayed exponential trajectory in all patients. The rate of the exponential would be constant, but the delay would vary between patients, as has been described for several retinal function parameters in human retinal degenerations^{19,40–43}

and retinal structure parameters in animal models of retinal degeneration.⁴⁴ A simple exponential with a rate constant of 14.3% per year fit the shifted ONL data from USH1B patients well (Fig. 4D). Such an exponential progression would predict the initiation of retinal degeneration at 6 mm eccentricity superior to the fovea at or near birth in P5, P7, and P9 and at the ages of 17, 15, 24, 15, and 20 years in P18, P20, P24, P29, and P32, respectively. Exponential functions with the 14.3% per year rate constant but progressively delayed by one-decade intervals are plotted on the cross-sectional SD-OCT data (Figs. 4B, 4C) to show the predicted differences in the delay of disease initiation that could explain the interindividual differences observed.

Relationships between Phenotype and Genotype

There were notable differences in rod vision among patients of the same age (in the first two to three decades of life; Fig. 3) and there were also differences in the extent of normal retina observed by cross-sectional imaging of the photoreceptor layer in the central retina (Fig. 4). Recognizing that USH1B is a progressive retinal degeneration, we assumed that these differences in data acquired by cross-sectional studies were related to different rates of photoreceptor degeneration. An answerable question is whether those with milder disease expression (presumed slower natural history) had a different genotype than those with more rapidly aggressive retinal degeneration. To try to answer the question, we chose pairs of patients with extreme differences at comparable ages: those with normal rod sensitivities centrally (Fig. 3B, right) and age-related patients with at least 2 log units of rod sensitivity loss. The higher-lower sensitivity pairings by rod vision included sibling pairs P26,P28 (ages 19, 21) versus sibling pair P21,P27 (ages 18, 21); P20 (age 17) versus P13 (age 16); and P9 (age 12) and P11 (age 10) versus P15 (age 10). Retinal structural data (extent of normal photoreceptor layer in the central retina) yielded the following comparisons: sibling pair P26,P28 (ages 19, 21) versus P13 (age 21). There were also differences in patients by OCT in the first decade of life (e.g., Fig. 4B, right), but the different extents fell within the proposed rapidly progressive phase of the disease, which needs better definition by longitudinal studies.

The predicted consequences of the mutant *MYO7A* alleles in all 33 patients are listed (Table 2) and the bases of the predictions are explained (Supplementary Text and Supplementary Figs. S1, S2, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8313/-DCSupplemental>). Among the five patients with "milder" disease expressions were P9 and P11, who would be predicted to have two null *MYO7A* mutations (Y333X homozygous). P20 was a compound heterozygote with two mutations that were presumed nulls (C31X and E495X). P26 and P28 of family 21 (R634X heterozygous) would have a single null mutation. The other point mutation in this family (G1982E) would be predicted to interfere with the structure of the second FERM domain in the *MYO7A* tail (Supplementary Fig. S1, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8313/-DCSupplemental>). This interference may not be as severe as most other mutations in this domain, however, since G1982 is present in a rather unstructured loop so that the mutation may not affect folding of the domain or interaction of the domain with the MyTH4 domain.⁴⁵

Considering the four patients with "severe" disease expression, P21 and P27 (family 16) would have two alleles leading to a lack of a complete second FERM domain (K1255fs, G1942X). P15 would be heterozygous for a mutation with similar predicted consequence (K1737fs) and heterozygous for a splice acceptor site mutation that would result in aberrant splicing of exon 34 encoding the FERM1 domain (4442-2A>C). Patient 13

FIGURE 4. Retinal structure in USH1B. **(A)** SD-OCT scans along the vertical meridian through the fovea in a representative normal subject and USH1B patients P2, P4, and P15. Photoreceptor (PR) layers are colorized for visibility: ONL (dark blue) and inner and outer segments (light blue). **(B) Left:** superior extent of normal PR layer thickness along the vertical meridian in all USH1B patients with SD-OCT results. *Lines:* invariant exponentials delayed along the time axis by one-decade intervals. *Gray symbols:* the patients shown to the right. *Gray axis (left):* the rod/cone ratio in normal human eyes as a function of eccentricity.³⁹ **Right:** SD-OCT scans from pairs of USH1B patients with similar ages but dissimilar extents of normal PR structure demonstrating interindividual variation of disease severity. **(C)** Inferior extent of normal PR layer thickness along the vertical meridian in all USH1B patients with SD-OCT results. *Lines, gray axes, and gray symbols* are as described in **(B)**. **(D)** Longitudinal measurements of ONL thickness along the vertical meridian superior to the fovea using TD-OCT in a subset of eight USH1B patients. Data from each patient are shifted along the time axis to partially overlap with one another. *Gray line:* exponential fit to the resulting data set. The same exponential is redrawn in **(B)** and **(C)**.

had a point mutation that would affect a region without homologous crystal structure. This mutation (G955S) would appear to interfere with the structure of the post alpha helical domain. The

other mutation (2187+1G>A) is predicted to destroy the exon 18 splice donor site and result in aberrant splicing or truncation of the MYO7A neck region before the first IQ motif (Table 2; Supplemen-

TABLE 2. Molecular Characteristics of the MYO7A Mutations in the Patients

Family	Patient(s)	Allele 1 Mutation	Predicted Consequence	Allele 2 Mutation	Predicted Consequence
F1	P1	E166fs	Null*	H1109fs	
F2	P2, P4	F1963fs	Lacks complete second FERM domain	G1942X	Lacks complete second FERM domain
F3	P3, P14	Q1798X	Lacks complete second FERM domain	G519fs	Null*
F4	P5	H1355fs	Lacks complete second FERM domain	R2024X	Lacks complete second FERM domain
F5	P6, P15	4442-2A>C	Loss of exon 34 splice acceptor site. Predicted mis-splicing of FERM1 domain†	K1737fs	Lacks complete second FERM domain
F6	P7, P9	Y333X	Null*	Y333X	Null*
F7	P8	H133fs	Null*	R1883Q	MyTH4.2 domain, disrupts MyTH4-FERM interface§
F8	P10	R212H	Predicted loss of salt bridge between switch I and switch II‡	R212H	Predicted loss of salt bridge between switch I and switch II‡
F9	P11, P12	Y333X	Null*	Y333X	Null*
F10	P13	G955S	Structural interference, post SAH domain	2187+1G>A	Loss of exon 18 splice donor site. Predicted mis-splicing of IQ 1†
F11	P16	E968D	Structural interference, post SAH domain	Q1178fs	Lacks complete second FERM domain
F12	P17	A1770D	Affects second MyTH4 domain§	19-2A>G	
F13	P18	G163R	Interference, P-loop motif of motor domain‡	592+1G>T	Null*
F14	P19	E968D	Structural interference, post SAH domain	K164N	Structural interference, P-loop motif of motor domain‡
F15	P20	E495X	Null*	C31X	Null*
F16	P21, P27	K1255fs	Lacks complete second FERM domain	G1942X	Lacks complete second FERM domain
F17	P22, P30	R1743W	Affects second MyTH4 domain§	A1288P	Disrupt folding of first FERM domain§
F18	P23	G519D	Interference, switch II and relay loop of motor domain‡	R669X	Null*
F19	P24	Y2015H	Second FERM domain, likely to disrupt protein interaction§	D2010N	Second FERM domain, likely to disrupt protein interaction§
F20	P25	R1240Q	Disrupt folding of first MyTH4 domain§	R1240Q	Disrupt folding of first MyTH4 domain§
F21	P26, P28	R634X	Null*	G1982E	Affects loop in second FERM domain§
F22	P29	R669X	Null*	R159fs	Null*
F23	P31	E1170K	Disrupt folding of first MyTH4 domain§		
F24	P32	L1858P	Precedes second FERM domain, likely to disrupt folding§	E1716X	Lacks complete second FERM domain
F25	P33	Q1798X	Lacks complete second FERM domain	E1917X	Lacks complete second FERM domain

* Based on the absence of a truncated product in shaker1 mice with stop mutations within the motor domain (Liu et al.⁵⁴).† NNSPLICE0.9 server splice site analysis (Reese et al.⁶⁰).‡ Homology modeling based on MYO5A motor domain (Courcux et al.³⁷).§ Based on crystal structure analysis of MYO7A tail domains (Wu et al.⁴⁵).|| SAH, single alpha helix domain (Yang et al.⁵⁷), previously regarded as a predicted coiled-coil domain.

tary Fig. S1, <http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8313/-DCSupplemental>).

DISCUSSION

Genetic heterogeneity is a fact in Usher syndrome and focus has now shifted from gene discovery to pursuit of pathologic mechanisms.^{2,3} Hypotheses are now being tested experimentally to understand how the Usher proteins interact with each other and with other molecules.^{4,5} The prospect of clinical trials of treatment for these retinal diseases⁸ requires that we also move forward in the clinic from the classic three clinical USH subtypes³ to some greater understanding of how the different USH diseases are expressed. An estimate of natural history through cross-sectional data, for example, could help define when treatment would be appropriate. If a focal therapy is being considered, we should determine the location in the retina that would be safe and advantageous to target the treatment.

The present study of a cohort of molecularly defined USH1B patients provides an opportunity to increase understanding of this common form of USH1. Kinetic visual field abnormalities in the USH1B patients were able to be ordered in such a way as to suggest a sequence of visual field loss due to progression of the retinal degeneration. The visual fields in our USH1B patients without major losses resemble those previously described as pattern III in different forms of retinitis pigmentosa⁴⁶; our patients, however, appeared to have a predilection for early loss of the nasal midperipheral field. The ordering of patterns in the present study suggested that peripheral temporal field islands are retained until late stages of the disease. Considering the well-preserved central retina with normal structure and function in many USH1B patients,¹⁰ we previously speculated that focal treatment (e.g., subretinal gene delivery) could occur in transition zones from normal to abnormal retina adjacent to the central retina.¹⁰ Further results from the present study suggest that it may be even more prudent to begin focal treatment trials in the peripheral retina, assuming subretinal delivery is used. Subretinal injections of vector into the nasal retina of *RPE65*-LCA patients in our gene therapy clinical trial have proven safe (clinicaltrials.gov, NCT00481546). It is also conceivable, but awaits evidence, that those with only central islands remaining by kinetic perimetry (pattern V; Fig. 1) could have detectable temporal peripheral islands, given different test paradigms that produce higher intensity stimuli than the standard kinetic perimeter.⁴⁷

Cone-based static perimetry and light-adapted kinetic perimetry showed similar results with age. On average, there was a slowly progressive loss of visual field extent of 10% to 14% per year. Our progression rate estimates in USH1B lie within the ranges of 8% to 14% per year reported in studies combining USH1 and USH2 patients¹⁵ or examining only USH2 patients.^{16,19,48} Rod-mediated vision was less predictable. Some patients retained better rod function for a longer period of their lives than others, suggesting different natural histories of disease within USH1B. The limited longitudinal data in these patients indicated that there was progressive loss of rod function. The observations may thus represent another example of different onset ages of visual loss, as has been demonstrated in other retinal degenerations.⁴¹

Further insight into rod differences in the USH1B patients was gained from cross-sectional images of the retina and measurements of photoreceptor laminae vertically across a wide expanse of central retina. We measured the vertical meridian to try to capture the superior retinal region with the rod "hot spot" of high density of rod photoreceptors.³⁹ Commonly in USH1B and other USH genotypes,^{9,10} there is greater superior

than inferior extent of ONL, which we attribute to the higher numbers of rod cells leading to an apparently slower rate of degeneration in this region. The longitudinal OCT data in the present cohort could be described by an exponential constriction of the central extent of normal photoreceptors corresponding to a rapidly progressive centripetal sweep of the disease boundary¹⁰ in the first few years of life from the midperiphery toward central regions. An invariant function of photoreceptor degeneration⁴⁴ the onset of which is delayed as an exponential function of eccentricity could be the basis of the constriction observed. The ~14% per year rate of photoreceptor structure constriction along a single dimension implies ~28% per year constriction in terms of retinal area. Rod photoreceptor nuclei are the dominant contributors to the extrafoveal ONL thickness, and thus rod (rather than cone) vision may be expected to correlate closely with retinal structure. Indeed, higher rates of progression were observed in terms of rod-mediated visual field extent compared with cone-mediated visual field extent, even though measurable rod data were available from a very limited number of USH1B patients. Previous studies of other USH genotypes have also shown higher rates of rod than cone progression.¹⁹

As the extent of normal photoreceptor laminae of the retina becomes reduced, the residual extent is in central regions that are normally relatively cone-rich (lower rod:cone ratio). From the second decade of life onward, most patients retained only a central island of normal photoreceptor layer thickness that continued to constrict at a slower pace. This disease stage is likely to be dominated mainly by cone loss. Not surprisingly, the patients who were outliers to the photoreceptor progression model also showed greater retention of rod function measured psychophysically. Perfect overlap in the two data sets was not possible because psychophysical data were not available in young children, whereas OCT was able to be performed at all ages.

More than a decade ago, it was suggested that there should be an attempt to relate visual measures in USH1B patients with specific mutations in the *MYO7A* gene.⁴⁹ We acknowledge the complexity of USH syndromes at a molecular level and how difficult it may be to understand phenotype based on genotype. For example, there is recent evidence that there are modifiers in some forms of USH,⁵⁰ and other ciliopathies,^{51,52} and there are proposed interactions between USH proteins.^{2,3} Accepting the complexity, we still attempted to answer the question of whether there were any obvious differences in phenotype in patients within this USH1B cohort and whether there was any relation of these different phenotypes to the known or predicted consequences of their *MYO7A* mutations. It is noteworthy that patients with stop mutations within the coding region of the *MYO7A* motor domain tended to have relatively milder rod disease. Two alleles of shaker1 mice, *Myo7a*^{4494SB} and *Myo7a*^{4626SB}, contain stop mutations within the motor domain⁵³ and have been found to be null mutations.⁵⁴ Although fragments of the *MYO7A* motor domain thus appear to be unstable, truncated products that include a complete motor domain (and are thus somewhat comparable to S1 or HMM myosin fragments) are likely to be more stable. In contrast to the *Myo7a*^{4494SB} and *Myo7a*^{4626SB} mice, *Myo7a*^{3336SB} mice, which have a C2182X mutation,⁵³ have significant levels of mutant *MYO7A* protein in their tissues.⁵⁵ *Polka* mice possess a splicing mutation in *Myo7a* that results in loss of much of the FERM2 domain and the addition of 33 nonsense residues to the C terminus. These mice express mutant *MYO7A* at the normal level of wild-type protein in their retinas and brains.⁵⁶ Cells in patients with mutations that affect only the *MYO7A* tail may therefore contain significant levels of mutant *MYO7A*, which, especially in the absence of any wild-type protein, may

contribute to the more severe disease observed in some of these patients. MYO7A protein with a highly perturbed or absent second FERM domain could be particularly deleterious. This domain has been shown to be responsible for autoregulation of MYO7A activity,⁵⁷ as well as cargo binding that in turn promotes dimerization, which is necessary for progression along actin filaments.⁵⁸ The presence of dysfunctional MYO7A may interfere with other actin-related processes.

Missense mutations in the region coding for the MYO7A motor, which in many cases perturb ATP or actin interaction, can also result in a stable dysfunctional protein. Indeed, mice that are heterozygous for the MYO7A point mutation, I178F, have been observed to have vestibular and cochlear defects,⁵⁹ indicating that dysfunctional MYO7A can have dominant-negative effects.

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