Farber disease (FD; Online Mendelian Inheritance in Man number 228000) is an autosomal recessive lysosomal storage disorder caused by mutations in the ASAH1 gene, resulting in deficient acid ceramidase (ACDase) activity. ACDase is a key lysosomal enzyme that hydrolyzes the bioactive lipid ceramide into sphingosine (Sph) and a free fatty acid. Currently, there is no cure for FD, and with only 152 cases recorded in the literature to date, obtaining tissues and samples to study this disorder has been challenging. The clinical manifestations of FD are broad; patients with the classic variant die early in childhood. The cardinal features of FD are the presence of s.c. lipogranulomatous nodules, joint contractures, and aphonia. Patients with severe forms of FD will also develop respiratory complications, hepatosplenomegaly, and neurologic decline. Impaired ACDase activity leads to systemic ceramide accumulation in FD patients. Ceramide and other sphingolipids are key components of membranes and play a role in a variety of cellular functions, including inflammation, cell proliferation, and apoptosis. The balance of ceramide and other sphingolipids is critical for normal cellular function.
its metabolites are tightly regulated, and dysregulation results in disease and potential visual system defects.7,8

The most frequent ophthalmic manifestation that has been described in patients with FD is a cherry red spot in the macula.3,9–12 Other reported ocular phenotypes in patients with FD include corneal opacities, xanthoma-like growths on the conjunctiva, nystagmus, and macular degeneration.13–15

Ocular manifestations are a common feature in lysosomal storage disorders in general, and corresponding rodent models of the disorders have been instrumental for characterizing their pathogenesis.16–19 We previously reported the first viable model for ACDase deficiency, wherein a known human ASAH1 mutation, proline (P) 362 to arginine (R), was knocked in to the corresponding locus in murine Asah1 (P361R).20 Mice homozygous for this mutation mirror many FD patient features, including heightened inflammation and pathology in the hematopoietic, respiratory, and neuroglial systems that leads to early mortality.20–22

In this study, we investigated the consequences of ACDase deficiency by completing a comprehensive investigation of ocular manifestations in the Asah1P361R/P361R mouse model of FD. Noninvasive ocular imaging was used to monitor disease progression and highlight the abnormal sphingolipids present in the retina. Furthermore, ACDase deficiency was found to reduce visual function that is, in part, due to progressive inflammation, neurologic involvement, and abnormal storage pathology in cells of the visual system.

Materials and Methods

Animal Use, Breeding, and Genotyping

To generate homozygous Asah1P361R/P361R mice, Asah1+/P361R heterozygotes were crossed, as previously reported.20 Genotypes were confirmed by PCR using genomic DNA isolated from ear notches. To detect the wild-type Asah1 allele, the following primers were used: 5'-CAGAAAGTATGCCGCATCGTCATAC-3' (forward) and 5'-AGGGCCTACAGAAACCTGTTCCTC-3' (reverse). These primers yielded a 379-bp product. For the Asah1 knock-in allele, the following primers were used: 5'-TCAAGCTTGACTTGGGGGAC-3' (forward) and 5'-GCTGGACGTAAACTCCTCTTCA GACC-3' (reverse). These primers amplify a 469-bp product from the neomycin resistance cassette. All animal procedures were approved and performed in strict adherence to the policies of the Medical College of Wisconsin Institutional Animal Care and Use Committee. Animals used for this study were maintained in controlled ambient illumination on a 12-hour light/dark cycle, with an illumination level of 2 to 3 lux. Exposure to bright light was kept to a minimum for all study animals for the duration of this study.

Slit-Lamp Analysis

Mice were anesthetized with inhaled isoflurane (3% induction, 1% to 2% maintenance) in 0.6 L/minute oxygen flow. The cornea and the lens were evaluated and imaged with the Topcon SL-D81 slit-lamp biomicroscope (Topcon Medical Systems Inc., Oakland, NJ) with a digital camera (Nikon D810 36.3MP DSLR Camera; Nikon Inc., Melville, NY). The eye was then dilated and cyclopedged with one eye drop each of 2.5% phenylephrine hydrochloride and 1% tropicamide (Akron, Inc., Lake Forest, IL). The lens was then reevaluated and imaged after dilation. All examinations were performed by a board-certified ophthalmologist (I.S.K.) with experience in animal models of ocular disease.

Fundus Imaging and Confocal Scanning Laser Ophthalmoscopy

Mice were anesthetized and prepared for imaging as described above. Fundus images were taken with the Phoenix Micron IV (Phoenix Research Labs, Pleasanton, CA). Near-infrared (810-nm) reflectance imaging and blue autofluorescence (excitation, 486 nm; emission filter, 525/50 nm) imaging were performed with a customized Heidelberg Spectralis (Heidelberg Engineering, Heidelberg, Germany) confocal scanning laser ophthalmoscope (cSLO). The automatic real-time composite mode in the Spectralis software version 6.6.2.0 (Heidelberg Engineering, Heidelberg, Germany) was used to average 40 and 100 frames of the near-infrared and blue autofluorescence images, respectively.

Optical Coherence Tomography

To perform optical coherence tomography (OCT) imaging, mice were anesthetized and prepared for imaging as described above. Imaging was performed with a Bioptigen Envisu R2200 spectral domain–OCT system (Leica Microsystems, Wetzlar, Germany) equipped with a Superlum Broadlighter T870 light source centered at 878.4 nm with a 186.3-nm bandwidth (Superlum, Cork, Ireland). InVivoVue control software version 2.4.33 (Leica Microsystems, Wetzlar, Germany) and the Bioptigen mouse objective were used for retinal imaging. A customized Bioptigen mouse stage was used to aim the imaging beam to the desired retinal location. GenTeal lubricant eye gel and Systane Ultra lubricating eye drops (Alcon, Fort Worth, TX) were used as needed to maintain corneal hydration. Dispersion, reference arm position, and light power of the sample arm were optimized iteratively for each animal at each time point. During acquisition, all scans were displayed and acquired in logarithmic intensity mode. Horizontal line scans (1 mm, 1000 A-scans/B-scan; 100 repeated scans) of the retina were acquired with the optic nerve head (ONH) centered for each scan. With our system and these scan parameters, the pixel size was calculated to be 1.00 × 1.61 μm (μx axes, respectively). B-scans (20 to 50) were registered to a manually selected template frame and averaged using custom software described previously.23 The registration was limited to a displacement of approximately 10 μm to exclude scans acquired at different retinal locations. The Duke OCT Retinal Analysis Program version 63.6 (Duke...
University, Durham, NC) was used to segment the inner limiting membrane and the retinal pigmented epithelium, which were clearly visible in all animals. Total retinal thickness was defined as the optical path length between these boundaries, assuming a group refractive index of 1.38. The order of the images was randomized, the boundaries were manually corrected, and thickness was analyzed by an observer (A.E.S.) masked to the genotype.

**ERG**

Before testing herein, mice were dark adapted overnight. Apparatus setup and animal preparations were conducted under dim red illumination. Mice were anesthetized and prepared as they were for imaging. Mice were placed on a heated platform (38°C). A silver-coated nylon contact lens with a custom-made active electrode was positioned on the eye. To maintain electrical conductivity, two subdermal platinum needle electrodes were positioned in the scruff (reference) and base of the leg (ground). Prepared animals were then positioned inside the Ganzfeld dome of the Espion E2 system (Diagnosys LLC, Cambridge, UK). All recordings were completed in a custom-made Faraday cage. Signals from the electroretinogram (ERG) were differentially amplified and digitized at a rate of 5 kHz (bandpass filtered 0 to 100 Hz). Recording sessions started with the dark-adapted flash ERG, which consisted of a six-log intensity series (−4 to 1 log cd·second/m²). Twenty responses were collected and averaged for the −4 and −3 log cd·second/m² stimulus with an interstimulus interval of 5 seconds, 10 responses were collected and averaged for the −2 and −1 log cd·second/m² stimuli with an interstimulus interval of 10 seconds, and five responses were collected and averaged for the 0 and 1 log cd·second/m² stimuli with an interstimulus interval of 20 seconds. For the light-adapted series, mice were first exposed to a 30 cd·second/m² white light background for 10 minutes for rod saturation, then progressed to a light-adapted flash ERG over a two-log intensity series (0 to 1 log cd·second/m²). Twenty responses were collected and averaged for each condition. Recordings concluded with two flicker ERG tests performed with continuous 5- and 15-Hz flicker (30 cd·second/m²). Thirty responses were collected and averaged for each flicker condition.

**Visual Cliff Behavioral Test**

To evaluate visual perception, a mouse open-field setup was modified to replicate the presence of a visual cliff. In brief, a gray circular structure with a diameter of 49.5 cm was placed on top of a clear 60 × 60-cm plexiglass surface, half of which was hanging off a table. To enhance the edge effect, the portion of the plexiglass surface on the support table was covered with a checkered pattern. Lamps directed at the floor, which also had a checkered pattern, were used to enhance depth and add illumination. Mice were placed near the middle of the visual cliff and monitored for 5 minutes with a camera linked to the Any-Maze behavior tracking software version 3.9.6 (Any-Maze, Stoelting, IL). For data analysis, three zones were created: ground, air, and cliff zones. The cliff zone measured 3 cm in depth from the edge of both the ground and air side. This 3-cm area was excluded from data collection because normal animals frequently stretched their body over the cliff edge to assess the area. For data collection, total distance traveled and total movement time were used to measure activity. To reduce variability, circadian rhythms were taken into account and all experiments were performed between 7 and 11 AM. All tests were performed on the same apparatus, in the same room, and by the same individual.

**Histopathology and Eye Measurements**

For these studies, mice were euthanized via carbon dioxide inhalation and immediately perfused with ice-cold phosphate-buffered saline via cardiac puncture with a 24-gauge needle. Globes were enucleated with the optic nerve intact and fixed in 10% phosphate-buffered formalin or Davidson’s fixative for 24 to 48 hours. Whole-eye globes and separated optic nerves were dehydrated and embedded in paraffin. Globes were sectioned sagittally at the midline through the optic nerve (ON) and stained for hematoxylin and eosin (H&E) and Luxol fast blue. Histology slides were scanned on the Aperio AT2 histology slide scanner (Leica Biosystems, Buffalo Grove, IL) or NanoZoomer 2.0-HT histology slide scanner (Hamamatsu Photonics, Ichinocho, Japan). All analyses and measurements were performed using Aperio ImageScope analysis software version 12.3.3 (Leica Biosystems, Buffalo Grove, IL). Morphometric analyses of the retinal layers were obtained approximately two- to three-disc diameters away from the optic nerve. Samples that contained retinal folding two- to three-disc diameters from the optic nerve were excluded from measurements. The anterior-to-posterior globe measurements were taken from the midline of the cornea to the base of the retinal epithelium (Bruch membrane). The remainder of the globe, lens, and corneal measurements were obtained at the anterior/posterior or dorsal/ventral midlines.

**Retinal Dysplasia Scoring**

H&E-stained retinal sections from $Asah1^{-/-}$ and $Asah1^{P361R/P361R}$ mice at 3 to 4 and 8 to 9 weeks of age were evaluated for retinal dysplasia severity. Our retinal dysplasia scoring system contained three categories: normal, intermediate, and severe. Samples categorized as normal contained no overt folding but may have had minor ridges within the retina. The height of each ridge was ≤20 µm, and no more than two retinal layers were affected. Samples in the intermediate category consisted of one to two folds/whorls. Each fold/whorl affected up to three retinal layers, and the peak height of each fold/whorl was between 20 and
100 μm. Last, for the severe category, samples displayed more than two folds/whorls. The folds/whorls there also affected more than three retinal layers, and the peak height of each fold/whorl was >100 μm. Some cases of severe folding also showed signs of retinal detachment.

**Immunohistochemistry and Immunoﬂuorescence**

Eyes were ﬁxed in 10% formalin overnight for retinal sectioning, as described above. For immunohistochemistry (IHC), the following primary antibodies, secondary antibodies, and staining reagents were used: rat anti-mouse Mac-2 at 1:8000 (galectin-3 clone M3/38; Cedarlane, Burlington, ON, Canada); goat anti-mouse cathepsin D at 1:3000 (Santa Cruz Biotechnology, Dallas, TX); biotinylated rabbit anti-rat IgG antibody at 1:5000 (Vector Laboratories, Burlingame, CA); biotinylated donkey anti-rabbit IgG antibody at 1:500 (Vector Laboratories); avidin-biotin/horseradish peroxidase (Vector Laboratories); 3,3′-diaminobenzidine kit (Vector Laboratories); and Vectastain ABC Elite Standard kit (Vector Laboratories). For immunofluorescence staining, the following primary antibodies were used: rabbit anti-ionized, calcium-binding adapter molecule 1 (Iba1) at 1:2000 (Wako Chemicals USA, Cambridge, MA) and chicken anti–glial ﬁbrillary acidic protein (GFAP) at 1:2000 (Aves Lab Inc., Tigard, OR). DAPI at 1:7000 (Sigma Aldrich, St. Louis, MO) was used for nuclear staining. The following secondary antibodies were used to detect the primary antibodies: goat anti-chicken ﬂuorescein isothiocyanate 1:500 (Aves Lab Inc.) and donkey anti-rabbit Cy3 1:500 (Jackson ImmunoResearch USA, West Grove, PA). Immunofluorescence microscopy was performed on the Carl Zeiss LSM510 confocal microscope (Carl Zeiss Microscopy, LLC, Thornwood, NY) using the Zeiss Aim software version 4.2 (Carl Zeiss Microscopy, LLC).

**Electron Microscopy**

After euthanasia, mice were perfused with 4% paraformaldehyde. Eye globes with intact ONs were removed and placed in 4% paraformaldehyde for 24 to 48 hours. ONs were from the posterior pole of the eye globe; approximately 2-mm transverse sections were cut for transmission electron microscope processing. For analysis of the posterior chamber, the cornea was gently cut to expose and remove the lens. The remaining posterior segment structures were used for transmission electron microscope processing. In brief, samples were post-ﬁxed in 3% glutaraldehyde, washed, and placed in 2% OsO4 in phosphate buffer overnight for contrast. After dehydration, samples were embedded in Durcupan Epon (Fluka, Hatﬁeld, PA) for polymerization. Ultrathin sections (60 nm thick) were cut from tissue blocks of ON and posterior eye samples and placed on copper grids. Ultrathin sections were further stained with uranyl acetate and lead citrate. Samples were analyzed with the JEOL 1400+ (JEOL, Tokyo, Japan) transmission electron microscope equipped with an Olympus Veleta charge-coupled device camera (Olympus Soft Imaging Solutions GMBH, Münster Germany) and Radius software version 1.3 (Olympus, Tokyo, Japan).

To assess myelin sheath thickness, the G-ratio (axon diameter/total outer myelin sheath diameter) was measured on electron micrographs of ON cross sections from 8- to 9-week-old mice. Images of the ON were obtained at a magniﬁcation of ×2500 (xy axes image sampling density, 27.9 nm). The G-ratio was determined from 300 to 400 randomly chosen ﬁbers per nerve cross section. Images were analyzed using ImageJ software version 1.51 (NIH, Bethesda, MD; https://imagej.nih.gov/ij/index.html), and G-ratio measurements were performed with the G-ratio plugin and online source code (http://gratio.efil.de, last accessed June 12, 2018).

**Sphingolipid Mass Spectrometry**

After mice were euthanized and perfused as described above, globes were enucleated. Retinas were then carefully separated from the globes under a dissection microscope. Retinal tissue was homogenized in 300 μL phosphate-buffered saline with the Omni Bead Raptor 24 tissue homogenizer (Omni International, Inc., Kennesaw, GA) using 2.8-mm ceramic beads. Lipids were extracted from 100 μL of retinal tissue lysate with 400 μL of methanol. The supernatant was reconstituted with 300 μL of water for mass spectrometry analyses, as previously described.26 The following internal standards were spiked in to each retina homogenate before extraction: 50 ng each of deuterated ceramide-1-phosphate [d18:1/16:0 or d18:1/24:0 (Matreya Inc., Pleasant Gap, PA) and d18:1/24:1 (Avanti Polar Lipids Inc., Alabaster, AL)]; 50 ng of deuterated ceramide (d18:1/22:0; Medical University of South Carolina Lipidomics Core, Charleston, SC); 50 ng of C17 analog of monohexosylceramide (d:18:1/17:0; Avanti Polar Lipids Inc.); 500 ng of C17 analog of sphingomyelin (d18:1/17:0; Avanti Polar Lipids Inc.); 100 ng of d7-Sph (Avanti Polar Lipids Inc.); and 100 ng of d7, sphingosine-1-phosphate (S1P; Avanti Polar Lipids Inc.).

The samples were analyzed on the Shimadzu 20AD high-performance liquid chromatography system using reverse-phase C18 high-performance liquid chromatography columns (Agilent Co, Santa Clara, CA) and a Leap PAL autosampler coupled to a triple quadrupole mass spectrometer (API-4000; Applied Biosystems, Carlsbad, CA) operated in multiple reaction mode at the Medical University of South Carolina Lipidomics Core. Positive-ion electrospray ionization mode was used to detect all sphingolipids. Retinal extraction samples were injected in duplicate for data averaging. The Analyst software version 1.5.1 was used for data analysis (Applied Biosystems). Sphingolipid measurements were normalized for individual protein concentrations obtained via a bicinchoninic acid assay (Thermo Scientific Pierce, Waltham, MA) and expressed as fold-change over results from Asah1+/− mice.
Lipidomic analyses were conducted separately on retinal tissue from the two different age groups. Ceramide-1-phosphate (C1P) was analyzed in retinal samples from 8- to 9-week-old mice but could not be analyzed in 3- to 4-week-old mice because of insufficient tissue lysate and lipid standards. S1P levels were lower than the limit of detection in retinal samples from both 3- to 4- and 8- to 9-week-old mice.

Statistical Analysis

Data are expressed as means ± SEM and analyzed with a t-test unless otherwise stated. Statistics were performed using GraphPad Prism software version 5.0 (GraphPad Software Inc., La Jolla, CA) and MATLAB (MathWorks, Inc., Natick, MA). P < 0.05 was considered statistically significant.

Results

Noninvasive Imaging Reveals Ocular Pathology

In comparison to control 8- to 9-week-old Asah1+/+ mice (Figure 1A), slit-lamp examination of the anterior chamber revealed signs of uveitis in 8- to 9-week-old Asah1P361R/P361R mouse eyes (Figure 1B). These included corneal endothelial granulomatous keratic precipitates, Busacca and Koeppe nodules on the iris, and occasional pigmented deposits along the anterior lens capsule (Figure 1B). No corneal epithelial or stromal defects were observed in the slit-lamp examination.

En face infrared and blue autofluorescence cSLO images revealed significant hyperreflectivity and autofluorescence surrounding the periphery of the ONH in 8- to 9-week-old Asah1P361R/P361R mice in comparison to control Asah1+/+ mice (Figure 1, C and D). Variable patches of hyperreflective and autofluorescent lesions were also observed in the fundus.
of \(Asah1^{P361R/P361R}\) mice (Figure 1D). Examination of 3- to 4-week-old mice using infrared and blue autofluorescence cSLO revealed no abnormal reflectivity in either \(Asah1^{+/+}\) or \(Asah1^{P361R/P361R}\) mice at that age (data not shown). Colored fundus images captured from 8- to 9-week-old mice appeared to correspond with cSLO images (Supplemental Figure S1). The 8- to 9-week-old \(Asah1^{P361R/P361R}\) mice had significant accumulation of white deposits near the ONH, with occasional spots along the periphery of the fundus (Supplemental Figure S1).

To further investigate retinal involvement in \(Asah1^{+/+}\) and \(Asah1^{P361R/P361R}\) mice, OCT imaging was performed at two time points. The first time point was at 3 to 4 weeks of age (Figure 2, A and C); the second time point was at 8 to 9 weeks of age (Figure 2, B and D). Most OCT images obtained from 3- to 4-week-old \(Asah1^{P361R/P361R}\) mice appeared phenotypically to have normal retinal development (Figure 2C). However, some mice displayed less contrast within the individual retinal layers and on occasion there were hyperreflective spots within the vitreous (Figure 2C). This was most notable in the regions proximal to the ONH. OCT at the later time point revealed more pronounced impairment in the retinal layers (Figure 2, D and G), in comparison with control \(Asah1^{+/+}\) mice (Figure 2, B and E). Increased hyperreflectivity was most pronounced along the nerve fiber layer/retinal ganglion cell (RGC) layer, and the presence of hyperreflective specks was observed within the vitreous body of eyes from 8- to 9-week-old \(Asah1^{P361R/P361R}\) mice (Figure 2G and Supplemental Video S1). After OCT imaging, H&E staining of the corresponding globe confirmed retinal dysmorphia in the 8- to 9-week-old \(Asah1^{P361R/P361R}\) mice (Figure 2H), in comparison with control \(Asah1^{+/+}\) mice (Figure 2F).

**Impaired Retinal and Visual Function**

To test retinal function, dark-adapted, light-adapted, and flicker ERGs were performed on cohorts of both 3- to 4- and 8- to 9-week-old mice. A-waves, which in the dark-adapted condition measure rod photoreceptor function, were unchanged at lower intensities, but decreased starting at the 0 log cd·second/m² flash intensity in 3- to 4-week-old \(Asah1^{P361R/P361R}\) mice (Figure 3, A and C). A-wave implicit time compared with age-matched controls (Supplemental Figure S1). That said, 3- to 4-week-old \(Asah1^{P361R/P361R}\) mice also displayed no changes to A-wave implicit time compared with age-matched controls (Supplemental Figure S1). Dark-adapted, 3- to 4-week-old \(Asah1^{P361R/P361R}\) mice also displayed no changes to A-wave implicit time at the highest flash intensity (Supplemental Figure S2, A and B). Dark-adapted ERGs were repeated on the same cohorts of mice at 8 to 9 weeks of age. There was a significant reduction in signal from the \(Asah1^{P361R/P361R}\) mice (Figure 3B). A reduction in A- and B-wave amplitudes was detected at the −3 log cd·second/m² flash intensity, as well as increased implicit times, and a negligible response was observed at the −4 log cd·second/m² flash intensity (Figure 3, B, E, and F, and Supplemental Figure S2, C and D).

![Figure 2](https://example.com/figure2.png) Optical coherence tomography (OCT) highlights retinal pathology in \(Asah1^{P361R/P361R}\) mice. A–D: Representative OCT scans from the same eyes at 3 to 4 weeks of age and then again at 8 to 9 weeks of age from control \(Asah1^{+/+}\) and \(Asah1^{P361R/P361R}\) mice. C and D: Longitudinal OCT scans of eyes from \(Asah1^{P361R/P361R}\) mice at 3 to 4 and 8 to 9 weeks of age show increasing disorganization of retinal morphology as the animals age. E–H: OCT scans with corresponding hematoxylin and eosin (H&E) micrographs of the same eyes from 8- to 9-week-old \(Asah1^{+/+}\) and \(Asah1^{P361R/P361R}\) mice. G and H: Inflammatory cells in the vitreal space (black arrowheads) and retinal folding (white arrows) are present in 8- to 9-week-old \(Asah1^{P361R/P361R}\) mice. Scale bar = 100 μm.
In the light-adapted ERG, which is an inference of cone function, a decrease in both A- and B-wave amplitudes as well as an increase in B-wave implicit time at the higher flash intensity in 3- to 4-week-old Asah1<sup>P361R/P361R</sup> mice (Figure 3, G, I, and J, and Supplemental Figure S2, E and F) was detected. Flicker ERG, another indicator of cone function, revealed a decreased signal in both 5- and 15-Hz rates of flicker in 3- to 4-week-old Asah1<sup>P361R/P361R</sup> mice.
(Figure 3, M, O, and P). Repeating light-adapted ERGs and flicker ERG on the same mice at 8 to 9 weeks of age demonstrated a lack of response from the Asah1P361R/P361R mice (Figure 3, H, K, L, N, Q, and R, and Supplemental Figures S2, G and H).

To further validate visual impairment in this model, a modified visual cliff test was performed on 8- to 9-week-old mice. Control Asah1+/+ mice spent most of their time within the ground side of the apparatus (Figure 4, A and C–F). They also traveled a greater distance. In contrast, Asah1P361R/P361R mice demonstrated no preference for ground or air zones, spending roughly equal time in both sectors. They also traveled an equal total distance within the ground and air side (Figure 4, B–F). Although other behavioral deficits have been previously demonstrated in Asah1P361R/P361R mice,21 the results from this modified visual cliff test and the ERG data together suggest significant visual impairment in these animals.

Increased Retinal Thickness

Asah1P361R/P361R mice are smaller than age-matched controls. By 4 to 5 weeks of age, they also start to progressively lose weight until they succumb to the disorder.20 During examination of the eyes, Asah1P361R/P361R mice had smaller interpalpebral fissures compared with age-matched controls (Supplemental Figure S3). To assess whether eye development was affected, gross measurements were performed on micrographs obtained from H&E-stained globe sections. Measurements revealed no significant differences in globe or lens diameter in 3- to 4- and 8- to 9-week-old Asah1+/+ and Asah1P361R/P361R mice, respectively (Supplemental Figure S4, A–D). Measurements of the anterior chamber also revealed no differences in the thickness of the corneal epithelium and stroma between Asah1+/+ and Asah1P361R/P361R mice at both 3 to 4 and 8 to 9 weeks of age (Supplemental Figure S4, E and F). This phenotype may be
the clinical equivalent of ptosis or blepharophimosis, which has not been described in FD.

Surprisingly, Asahi\(^{P361R/P361R}\) mice displayed increased retinal thickness as early as 3 to 4 weeks of age when compared with age-matched Asahi\(^{+/+}\) mice (Figure 5, A and B). Specifically, by 3 to 4 weeks of age, the outer nuclear layer was significantly thicker (Figure 5C). By 8 to 9 weeks of age, increased thickness was observed in both the inner nuclear layer and outer nuclear layer (Figure 5D). To validate our histology measurements, retinal thickness was analyzed from our previous OCT images with OCT retinal analysis software.\(^23\) Analyses of OCT images revealed that 3- to 4-week-old Asahi\(^{P361R/P361R}\) mice exhibited increased nasal and temporal retinal thickness in comparison to age-matched Asahi\(^{+/+}\) mice (Figure 5E). This increased thickness became more progressive by 8 to 9 weeks (Figure 5F and H). No changes in thickness were seen between 3- to 4- and 8- to 9-week-old Asahi\(^{+/+}\) mice (Figure 5G).

**Variable Penetrance of Retinal Dysplasia**

To examine the presence of retinal dysplasia, H&E-stained retinas were examined to evaluate their morphology. All of the retinas from 3- to 4-week-old control Asahi\(^{+/+}\) and 3- to 4-week-old Asahi\(^{P361R/P361R}\) mice showed no dysplasia (Figure 6, A and B). Examination of 8- to 9-week-old Asahi\(^{P361R/P361R}\) mice, however, revealed a range in retinal dysplasia (Figure 6, C–F). Approximately 25% of globes from Asahi\(^{P361R/P361R}\) mice were within normal limits, 45% developed an intermediate degree of dysplasia, and 30% had severe retinal folding. Retinal dysplasia was most pronounced in regions closest to the ONH. Mice that developed severe retinal disturbances often had involvement of multiple layers, with folds even reaching into the vitreous body (Figure 6F).

**Inflammation, Astrogliosis, and Storage Pathology in the Retina, ONH, and Unmyelinated Optic Nerve**

Inflammatory cells were observed in the eyes of 8- to 9-week-old Asahi\(^{P361R/P361R}\) mice (Figure 7, B, E, I, and J). Control Asahi\(^{+/+}\) mice did not display such cellular recruitment (Figure 7, A, E, and H). Infiltrating inflammatory cells were observed in both the anterior chamber and vitreous humor of 8- to 9-week-old Asahi\(^{P361R/P361R}\) mice (Figure 7B). An increased abundance of inflammatory cells was most notable in the vitreal region above the ONH, presumably where the central retinal vein and artery are located (Figure 7, F and G).

Next, IHC staining was performed for Mac-2 (a macrophage marker) on globes from 8- to 9-week-old mice.
Many of these inflammatory cells were observed in \textit{Asah1}^{P361R/P361R} mice (Figure 7, H–J). Although they were commonly localized around the ONH, macrophages were also observed within the vessels of the RGC layer and along the choroid/pigment epithelium layer in regions where retinal folding occurred (Figure 7, I and J). In the 8- to 9-week-old \textit{Asah1}^{P361R/P361R} mice that developed significant retinal folding, detachment of the retina often occurred, as well as subretinal hemorrhages (Figure 7G).

IHC staining for cathepsin D revealed an increase in lysosomal staining, particularly in RGC and inner nuclear retinal layers of 8- to 9-week-old \textit{Asah1}^{P361R/P361R} mice compared with controls (Figure 7, C and D). Examination of the nerve fiber/RGC layers of 8- to 9-week-old \textit{Asah1}^{P361R/P361R} mice revealed increased vacuolation and a more intense granular cathepsin D staining (Figure 7D). Of the 8- to 9-week-old \textit{Asah1}^{P361R/P361R} mice that had severe retinal dysplasia, the nerve fiber/RGC layer showed degeneration and sloughing (Figure 7G).

To further characterize neuroinflammation, retinal sections were assessed from 8- to 9-week-old mice by immunofluorescence staining for GFAP, a label for glial cells such as astrocytes and Müller cells. Staining was also performed for Iba1, a marker for activated microglia (Figure 7, K and L). The retina of \textit{Asah1}^{P361R/P361R} mice did not contain many cells that were positive for Iba1. On occasion, some microglia-like cells that had branching projections in 8- to 9-week-old \textit{Asah1}^{P361R/P361R} mice were
observed; however, such occurrences were infrequent, and those cells were not found to be localized to one particular region. Furthermore, IHC on serial retinal sections revealed areas where Mac-2 staining was positive and Iba1 was negative (Supplemental Figure S5). This suggests that these recruited cells are predominantly macrophages.

Increased GFAP staining was found on multiple retinal layers, but most intense in the nerve fiber/RGC layer of retinal sections from 8- to 9-week-old Asah1P361R/P361R mice (Figure 7L). GFAP staining was also positive along the inner plexiform layer, the inner nuclear layer, and the outer plexiform layer of retinal sections from mutant mice. This observation suggests astrocytosis and Müller cell activation in the retina of the ACDase-deficient mice. To assess whether activation of microglia was present in the visual system, retinal tissue was stained for Iba1. On occasion, we observed an increased number of Iba1-positive cells; however, phenotypically, these labeled cells appeared similar to those from wild-type animals (Figure 7L).

Ultrastructural analyses of the retina and unmyelinated portion of the ON in 8- to 9-week-old Asah1P361R/P361R mice revealed extensive storage pathology in a range of cell types that was absent in samples from 8- to 9-week-old control mice (Figure 8, A–C). Ganglion cells in 8- to 9-week-old Asah1P361R/P361R mice contained an excess of zebra-like storage bodies (Figure 8, D and E). Zebra bodies were also present in endothelial cells of the vessels of the inner plexiform layer (Figure 8F). The inner plexiform layer also contained abnormal storage vacuoles in the abundant astrocytic processes (Figure 8G) and abnormal macrophage-like cells with an excess of curved-linear tubular body-like profiles in the vicinity of capillaries (Figure 8H) and zebra body-like storage vacuoles in the astrocytic processes (Figure 8I). In addition, storage vacuoles were observed within endothelial cells on the central retinal vessels of the ONH (Figure 8F). Similar to the rest of the retina and central nervous system,21 the perivascular space of the ONH contained storage-laden, macrophage-like abnormal cells (Supplemental Figure S6). Curved-linear
tubular body—like storage vacuoles were also observed within endothelial cells of the choroid capillaries and inside melanosome-containing cells along the outer choroid (Supplemental Figure S7). Last, zebra bodies (indicating storage) were also present within scleral fibroblasts (Supplemental Figure S7).

Pathology of the Myelinated Optic Nerve

Representative light micrographs of H&E-stained ON tissue from 8- to 9-week—old Asah1+/+ and Asah1P361R/P361R mice are shown in Figure 9, A and B. Asah1P361R/P361R mice displayed a thickening of the ON pial sheath (Figure 9B). H&E staining of ON tissue from 8- to 9-week—old Asah1P361R/P361R mice also revealed the presence of mononuclear cellular infiltrates with abundant vacuolated cytoplasm and/or cytoplasm distended with granular material that preferentially accumulated in the vicinity of the optic vein (Figure 9B). When the corresponding ON tissue from 8- to 9-week—old mice was stained with Luxol fast blue, the staining on Asah1P361R/P361R mice ON tissue appeared less intense (Figure 9, C and D).

IHC staining for Mac-2 confirmed the presence of infiltrating macrophages along the ON track in samples from 8- to 9-week—old Asah1P361R/P361R mice but not in samples from 8- to 9-week—old Asah1+/+ mice (Figure 9, E and F). Sections of ON tissue from 8- to 9-week—old Asah1P361R/P361R mice displayed greater GFAP staining than controls (Figure 9, G and H). This suggests that astrocytosis is also affecting the ON tissue of Asah1P361R/P361R mice.

Figure 8 Retinal and unmyelinated on ultrastructure pathology in the Asah1P361R/P361R mice. A: Electron micrographs highlighting the ganglion cells (nuclei; black asterisks) in the Asah1+/+ mouse. B: Inner plexiform layer (IPL) in the Asah1+/+ mouse. C: Optic nerve disk/unmyelinated part of the ON (astrocytic processes; black arrows) in the Asah1+/+ mouse. D and E: Ganglion cells (nuclei hallmarked by black asterisks) in the Asah1+/+ mouse are with numerous zebra-like storage vacuoles in their cytoplasm (black arrows). F–I: White arrows highlight zebra body—like storage profiles in the endothelial cell (F); zebra body—like storage vacuole (white arrow) in the astrocytic process (G); macrophage-like cells (white arrows) laden with curved-linear tubular body—like storage bodies (H); and zebra body—like storage vacuoles (black arrows) in the astrocytic processes (I; image corresponds to the boxed area in H). Scale bar = 5 μm.
Ultrastructure analysis of ON sections from 8- to 9-week-old Asah1P361R/P361R mice revealed abnormal storage in macrophages accumulating in the intraneuronal perivascular spaces (Figure 10, A–C). These macrophage- and fibroblast-like cells contained curved-linear tubular body storage in vacuoles. ON from Asah1P361R/P361R mice also showed discernible storage vacuoles in astrocytes and zebra-like storage vacuoles within the cytoplasm of oligodendrocytes (Figure 10, D–I). Abnormal storage vacuoles were also present in the endothelial cells of vessels (both pial and intraneuronal) and in capillaries (Supplemental Figure S8).

Neuroaxonal Dystrophy and Reduced Axonal Density

Ultrastructure analyses of tissue (interior and periphery) from 8- to 9-week-old animals revealed that the ON of mutant mice had a qualitative reduction in axon density compared with controls (Figure 10, J–L). Signs of neuroaxonal dystrophy in some axons via the formation of axonal spheroids were also observed in the ON of Asah1P361R/P361R mice (Figure 10, K and L).

To assess myelination, the G-ratio (axon diameter/fiber diameter; where fiber diameter = axon diameter + myelin sheath thickness) of the ON from 8- to 9-week-old mice was measured. The analysis revealed a significant reduction in the G-ratio in samples from Asah1P361R/P361R mice in comparison to those from control Asah1+/+ mice (Figure 10M). However, no differences were detected in the ON axon diameter between 8- to 9-week-old Asah1+/+ and Asah1P361R/P361R mice (Figure 10N). Further evaluation revealed a reduction in the density of myelinated axons in Asah1P361R/P361R ONs; however, of the axons that were assessed, no changes in the percentage of myelinated axons were observed (Figure 10, O and P).

Altered Sphingolipid Profile in the Retina

Ceramide, sphingomyelin (SM), monohexosylceramide (MHC), Sph, and S1P were measured via liquid chromatography–mass spectrometry in retinal lipid extracts from both 3- to 4- and 8- to 9-week-old Asah1+/+ and Asah1P361R/P361R mice. C1P was measured via liquid chromatography–mass spectrometry in retinal lipid extracts from 8- to 9-week-old Asah1+/+ and Asah1P361R/P361R mice.

Analyses of the individual species of each sphingolipid class revealed an accumulation of many ceramide species by 3 to 4 weeks of age and all ceramide species studied in retinal samples from 8- to 9-week-old Asah1P361R/P361R mice (Figure 11, A and G). SM was unchanged in retinal samples from both 3- to 4- and 8- to 9-week-old Asah1P361R/P361R mice (Figure 11B). SM 18:0 was increased, and SM 24:0 was decreased, in retinal samples...
from 8- to 9-week-old Asah1P361R/P361R mice (Figure 11H). Total MHC was elevated in retinal samples from both 3- to 4- and 8- to 9-week-old Asah1P361R/P361R mice (Figure 11, C and I). The C18:0 MHC species displayed the highest fold-change in retinal samples from both the 3- to 4- and 8- to 9-week-old Asah1P361R/P361R mice but were significantly decreased in retinal samples from 8- to 9-week-old Asah1P361R/P361R mice (Supplemental Figure S9,
A and B). S1P was also measured in retinal samples from both age groups; however, readings were lower than the limit of detection (data not shown). Although C1P was not measured in retinal samples from the 3- to 4-week-old mice because of insufficient sample and lipid standards, no alterations in C1P levels were observed in retinal samples from 8- to 9-week-old Asah1P361R/P361R mice (Supplemental Figure S9C).

Alterations in the relative abundance of sphingolipids were also detected in retinal samples from both the 3- to 4- and 8- to 9-week-old Asah1+/+ and Asah1P361R/P361R mice. The relative abundance of C16:0 ceramide decreased, whereas the relative abundance of several other ceramides increased, in retinal samples from 3- to 4-week-old Asah1P361R/P361R mice (Figure 11D). The relative abundance of C16:0 ceramide also decreased in retinal samples from 8- to 9-week-old Asah1P361R/P361R mice, whereas the relative abundance of all of the other ceramides measured increased (Figure 11J). Although the relative abundance of SM was almost unchanged in retinal samples from 3- to 4-week-old Asah1+/+ mice and 3- to 4-week-old Asah1P361R/P361R mice (Figure 11E), an increase in the SM18:0 species and a slight decrease in many of the other SM species were observed in retinal samples from 8- to 9-week-old Asah1P361R/P361R mice (Figure 11K). The largest change in MHC relative abundance was an increase in the C18:0 species, which coincided with decreased C16:0, C20:0, C24:1, and C24:0 species in retinal samples from both the 3- to 4- and the 8- to 9-week-old Asah1P361R/P361R mice (Figure 11, F and L). Because the quantification of sphingolipids in retinal samples from the 3- to 4- and 8- to 9-week-old mice was performed independently, no comparisons were made between the two age groups.

![Figure 11](image-url) Altered sphingolipid species in the retina of Asah1P361R/P361R mice. Sphingolipids were quantitated in retinal tissue from Asah1+/+ and Asah1P361R/P361R mice by liquid chromatography–mass spectrometry. Quantification of ceramide (Cer; A), sphingomyelin (SM; B), and monohexosylceramide (MHC; C) species in retinal tissue from 3- to 4-week-old Asah1+/+ mice and Asah1P361R/P361R mice. The relative abundance of Cer (D) and SM (E) and MHC (F) species in retinal tissue from 3- to 4-week-old Asah1+/+ mice and Asah1P361R/P361R mice. Quantification of Cer (G) and SM (H) and MHC (I) species in retinal tissue from 8- to 9-week-old Asah1+/+ mice and Asah1P361R/P361R mice. The relative abundance of Cer (J) and SM (K) and MHC (L) species in retinal tissue from 8- to 9-week-old Asah1+/+ mice and Asah1P361R/P361R mice. Data are expressed as means ± SEM. n = 5 to 6 animals for each genotype and age group. *P < 0.05, **P < 0.01, and ***P < 0.001.
ACDase deficiency is an ultrarare orphan disease that can manifest along a wide broad clinical spectrum. The cardinal phenotypes include painful joints, formation of lipo-granulomatous nodules, and aphony. In classic and more severe variants, neurologic involvement is common and is often associated with functional and behavioral deficits. Ocular manifestations are commonly seen in patients who also display neurologic dysfunction, with the most common phenotype being a cherry red spot in the macula. Herein, we have provided an analysis of the ocular pathology present in the ACDase-deficient mouse. The results demonstrate that a deficiency in ACDase leads to a broad range of ophthalmic abnormalities. We highlight findings from noninvasive ocular imaging, characterize the affected cell types, and demonstrate visual impairment.

Although ocular disease is often present in FD, reports of an eye phenotype have thus far been restricted to superficial/observational descriptions. In the literature, there are only two studies on the visual aspects of FD that were located. The first was published in 1966 and applied light microscopy and histologic techniques to reveal the presence of lipid granules within the RGCs of an FD patient who died at 11 months of age. The second report, published in 1985, described the retinal ultrastructure of an FD patient who died before the age of 3 years. Five types of cytoplasmic inclusions were identified in the second patient, with the most abundant being described as flattened stacks of osmophilic lamellae. The RGCs, glia, and phagocytic cells all showed significant inclusions. The findings from these reports are recapitulated in our murine model. The H&E retinal tissue staining revealed a disorganized and vacuolated RGC layer in Asah1P361R/P361R mice. Ultrastructural analyses of the retina also revealed significant storage pathology in various cell types—much like those reported by Zarbin and colleagues in 1985. Similar retinal pathology has also been noted in RGCs in other lysosomal storage disorders, such as Niemann-Pick disease type A, Pompe disease, and Tay-Sachs disease. Herein, significant storage pathology was further observed in endothelial cells, astrocytes, oligodendrocytes, and macrophage-like cells in the retina and optic nerves from Asah1P361R/P361R mice.

A heightened state of inflammation is characteristic of FD. An increased prevalence of inflammatory cells has been previously reported in the lungs, brain, and hematopoietic organs in this mouse model of FD. Thus, finding that a deficiency in ACDase activity also leads to inflammation in the eye and optic nerve could be expected. Through the use of noninvasive ocular imaging, the formation of granulomas and nodules was noted in the anterior chambers. In addition, deposits close to the ONH of the fundus were seen with cSLO, and hyperreflectivity was noted along the ONH and vitreous body by OCT. These observations can be explained by the presence of inflammatory cells. IHC staining for Mac-2 and ultrastructure analyses further demonstrated a significant presence of abnormal macrophage morphologies primarily around the choroid and other vessels along the retina and optic nerve.

We previously reported increased vascular permeability in the Asah1P361R/P361R mice. Although that study was primarily focused on lung pathology, one experiment involved a tail vein injection of Evans Blue Dye. Evans Blue Dye accumulation was measured in various organs in Asah1P361R/P361R mice. Although not reported in that study, qualitatively, Asah1P361R/P361R mouse eyes were more intensely stained than controls. Inflammatory cytokines, such as monocyte chemotactants, were not measured in the retina, it is possible the combination of vascular leakage and altered cytokine production previously reported contributes to the macrophage and neutrophil recruitment described herein.

The microglial and astroglial pathology was further characterized via Iba1 and GFAP immunofluorescence staining of retinal and optic nerve tissue. Asah1P361R/P361R mice showed a progressive accumulation of GFAP in the retina and optic nerve, suggesting activation of astrocytes and Müller cells. Although significant macrophage infiltration was observed, Iba1 staining was largely unchanged, suggesting limited microglia involvement and that most infiltrated macrophages did not express Iba1.

Retinal dysplasia was also a prevalent feature in Asah1P361R/P361R mouse globes. Over 75% of globes analyzed had intermediate to severe retinal folding. Within the retinal folds, inflammatory cells were often discernible. Retinal folding was most prevalent in the regions near the ONH. This may be because of the proximity of the central optic vein and artery, the region where increased macrophage infiltration was also observed. The phenotype seen in the eyes of Asah1P361R/P361R mice shares some similarities with the rodent models of experimental autoimmune uveoretinitis. During the early phase after experimental autoimmune uveoretinitis induction, cellular infiltrates can be observed in the vitreous and optic nerve head of mice eyes. As mice transition into the acute phase of experimental autoimmune uveoretinitis, inflammation is more severe, and retinal folding and edema might occur. Although edema within the retina was directly assessed, it may be occurring in the visual system of Asah1P361R/P361R mice. Specifically, hyperreflective deposits were observed from SLO imaging, and an increased retinal thickness and inflammation was seen in Asah1P361R/P361R mice, which is associated with edema. In addition, the lung and brain of Asah1P361R/P361R mice show signs of edema. Of interest, within the brain, approximately 70% of Asah1P361R/P361R mice at 9 weeks of age develop hydrocephalus. Although retinal pathology was not assessed previously, there may be a correlation between the
development of hydrocephalus and retinal dysplasia because the retina is a part of the central nervous system.

The association between inflammation and retinal dysplasia has been reported in other models, such as cats infected with feline leukemia virus, dogs that were inoculated with canine herpes virus, and mice that were infected with Sindbis virus. 39–41 The range of retinal dysplasia seen in the Asah1<sup>P361R/P361R</sup> mice may be because of varying rates of inflammation between individuals.

Sphingolipid metabolites have been shown to regulate both the function and the trafficking of inflammatory cells. 42 This has been shown in the eye, where cases of acute uveitis can change both lipid and sphingolipid profiles. 43 Conversely, there is evidence demonstrating that sphingolipid metabolism can be altered because of inflammation. 44–46 Direct intravitreal injection of bioactive sphingolipids, such as C8:0 in mice, can induce a dose-dependent effect in recruiting inflammatory cells. 47 From these studies, targeting the sphingolipid pathway has been suggested as an approach to treat uveitis. 48 One of the most promising agents is FTY720 or fingolimod, an approved drug for the treatment of multiple sclerosis. FTY720 has shown significant anti-inflammatory effects in experimental autoimmune uveoretinitis rodent models. 36,49,50 FTY720 is an analog of Sph that is derived from myriocin, a potent inhibitor of the de novo ceramide synthesis pathway. 51 FTY720 is phosphorylated by sphingosine kinase-2 in a similar manner to the modification of Sph to S1P. 52 In addition to anti-inflammatory properties via S1P binding, FTY720 has also been shown to inhibit ceramide synthase, the class of enzyme that regulates ceramide acyl chain length during synthesis. 53 Because of its anti-inflammatory effects, and potential decrease in ceramides, it is possible that treatment with FTY720 in conjunction with other anti-inflammatory compounds may impede pathogenesis of FD.

We characterized the sphingolipid profiles of retinal lysates from 3- to 4- and 8- to 9-week-old Asah1<sup>P361R/P361R</sup> mice. Ceramide accumulation is progressive and present as early as 3 to 4 weeks of age in the retina of Asah1<sup>P361R/P361R</sup> mice. It is possible that this early accumulation of ceramide in the retinas of Asah1<sup>P361R/P361R</sup> mice contributes to the abnormal ERG activity recorded in these animals. Retinal ceramide levels were significantly higher in the 8- to 9-week-old Asah1<sup>P361R/P361R</sup> mice. These older Asah1<sup>P361R/P361R</sup> mice display progressive retinal inflammation, dysmorphic retinal pathology, and severe visual impairment. Thus, our data suggest that increasing retinal ceramide accumulation over time is associated with the retinal dysfunction and pathology reported herein.

The downstream metabolites of ceramide were measured, and it was found that only MHC was elevated in retinal lysate from Asah1<sup>P361R/P361R</sup> mice. SM was unchanged in both age groups of Asah1<sup>P361R/P361R</sup> mice. Sph was unchanged in 3- to 4-week-old Asah1<sup>P361R/P361R</sup> mice and decreased in 8- to 9-week-old Asah1<sup>P361R/P361R</sup> mice. These findings appear specific to the retina because previous studies characterizing other organ systems in the ACDase-deficient mouse have noted accumulation of both ceramide and downstream ceramide metabolites. 21,22,26 Within the liver and lungs of Asah1<sup>P361R/P361R</sup> mice, SM, C1P, and MHC were significantly elevated. 22,26 In the brain of Asah1<sup>P361R/P361R</sup> mice, Sph and MHC were significantly elevated. 21,26 Future in-depth studies that investigate upstream and downstream metabolites of ceramide at various time points in different cells and tissues from the ACDase-deficient mouse may provide further insight into the complex biochemistry of this disorder.

Maintaining sphingolipid equilibrium is essential for proper health and vision. Ocular manifestations are commonly reported in lysosomal storage disorders, and animal models have been instrumental in understanding disease progression. Studies on the acid sphingomyelinase-deficient mouse, the model for Niemann-Pick A/B disease, have revealed progressive retinal degeneration and inflammation. 17,18 In the context of Sandhoff disease, work on the β-hexosaminidase mutant mouse showed robust storage pathology preferentially in the RGC and impaired neurite outgrowth from cultured retinal explants. 16,53 Decreased ERG amplitude has also been documented in mice deficient in acid sphingomyelinase, hexosaminidase, and ceramide synthases 1, 2, and 4. 17,18,54 The similarities in disease manifestations seen in these reports and our current study suggest that insults to sphingolipid metabolism lead to comparable pathologies because homeostatic control of sphingolipids is tightly regulated.

Ceramide is a central signaling lipid in the sphingolipid pathway. In more common retinal diseases, such as retinitis pigmentosa, increases in ceramide can lead to downstream effects, such as photoreceptor cell death. 55 One study in the retinal degeneration 10 mouse model showed that ceramides are increased with progressive photoreceptor degeneration and that treatment with the inhibitor myriocin could protect photoreceptors from apoptosis. 56 Another study in a rat model of light-induced retinal degeneration showed a neuroprotective effect of FTY720 that acted independent of its immunosuppressive action. 57 Together, these reports demonstrate the deleterious effects of ceramide in the retina. In the case of chronic ceramide accumulation, such as in FD, alternative pathways may be switched on in response to sphingolipid buildup. Further work will be required to fully understand the role of ceramide signaling in FD and visual biology.

This study has provided the first in-depth analysis of the ocular manifestations and optic nerve pathology in the ACDase-deficient mouse. It has highlighted parallels between human cases and our model, particularly in terms of inflammation, storage pathology, and central nervous system decline. Given the lack of patients with FD and the dearth of available human tissue to study, this mouse model has aimed to fill the gap. Our results demonstrate a progressive decline in retinal function in Farber mice that coincides with severe retinal and optic nerve pathology and
sphingolipid accumulation. These findings, although exploratory, may provide insight into the possible ocular pathology present in classic and severe variants of human FD.

Currently, there is an ongoing clinical trial (ID NCT03233841) to understand the natural history of FD. We have demonstrated the feasibility of noninvasive ocular imaging to assess and follow ocular pathology over time with serial testing in a mouse model of FD. Use of noninvasive ocular imaging may not only aid in diagnosis but serve as a screening modality to assess the inflammatory and neurologic status of patients with FD. In addition, recombinant enzyme therapy is currently being developed for the treatment of FD, and our laboratory has an ongoing interest in pursuing gene therapy to ameliorate this disorder.20,58,59 Noninvasive monitoring of the ocular conditions may also be a modality to assess the efficacy of these and future therapies.

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**Supplemental Data**

Supplemental material for this article can be found at https://doi.org/10.1016/j.ajpath.2018.10.018.

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