Abstract  Photoreceptors have high intrinsic metabolic demand and are exquisitely sensitive to metabolic perturbation. In addition, they shed a large portion of their outer segment lipid membranes in a circadian manner, increasing the metabolic burden on the outer retina associated with the resynthesis of cell membranes and disposal of the cellular cargo. Here, we demonstrate that deletion of both ABCA1 and ABCG1 in rod photoreceptors leads to age-related accumulation of cholesterol metabolites in the outer retina, photoreceptor dysfunction, degeneration of rod outer segments, and ultimately blindness. A high-fat diet significantly accelerates rod neurodegeneration and vision loss, further highlighting the role of lipid homeostasis in regulating photoreceptor neurodegeneration and vision.—Ban, N., T. J. Lee, A. Sene, Z. Dong, A. Santeford, J. B. Lin, D. S. Ory, and R. S. Apte. Disrupted cholesterol metabolism promotes age-related photoreceptor neurodegeneration. J. Lipid Res. 2018. 59: 1414–1423.

Supplementary key words  ATP binding cassette transporter G1 • cholesterol/dietary • cholesterol/efflux • eye retina • neurons • aging

Metabolic neurodegeneration is a central feature of diverse age-related pathologies such as Alzheimer’s disease (1) and retinal degeneration (2). The neurosensory retina, an extension of the CNS, is a complex neurovascular tissue whose primary function is to process light and color perceived by photoreceptor neurons and transmit this information to the visual cortex for further processing in order to generate a precise visual image. In the neurosensory retina, photoreceptor degeneration causes blindness in diseases such as retinitis pigmentosa (3) and age-related macular degeneration (AMD) (4).

The retina is also the most metabolically active tissue in the body (5, 6) and is exquisitely sensitive to metabolic flux (7). A significant portion of this metabolic demand is related to the circadian shedding of about ten percent of photoreceptor lipid bilayer membranes (7, 8). Photoreceptor outer segments once shed have to be regenerated, placing a very high biochemical burden on these cells. In addition, the lipid-rich shed outer segments have to be processed by the retinal pigment epithelium (RPE) underneath the neurosensory retina (9).

These cholesterol-containing lipids are then packaged onto carrier lipoproteins for transport from the eye to the bloodstream using a well-characterized process called reverse cholesterol transport or cholesterol efflux (7, 10). ABCA1 and ABCG1 play a central role in coordinating reverse cholesterol transport by packaging cholesterol and other lipids onto apolipoprotein carriers, such as ApoA1 and HDL, for transport through the bloodstream to the liver (10, 11). For these reasons, we hypothesized that maintaining physiologic cholesterol homeostasis by ABCA1 and ABCG1 would play a key role in photoreceptor function and survival. In the retina, there are two types of photoreceptors: rods responsible for dim vision and cones responsible for color and daytime vision. Although ABCA1 and ABCG1 have been reported to be expressed in the
retina, including in photoreceptors (12, 13), their function in photoreceptor physiology is largely unknown. In order to assess the functions of ABCA1 and ABCG1 in photoreceptors, we generated mice lacking both Abca1 and Abcg1 specifically from either rods (14, 15) or cones (15, 16). Retinas were structurally evaluated by biomicroscopy, histology, and electron microscopy at 3–18 months of age. In addition, retinal function and vision were evaluated by electrophysiology.

**MATERIALS AND METHODS**

**Animals**

All animal experiments were conducted in accordance with Washington University in St. Louis School of Medicine Animal Care and Use guidelines after approval by the Animal Studies Committee. All mice were housed in a temperature-controlled room under a 12 h light/dark cycle, with free access to food and water. All mouse species used in the experiments are summarized in Table 1. Abca1/g1F/F mice were previously characterized (17, 18) and were purchased commercially (Jackson Laboratory, Bar Harbor, ME). To generate mice lacking both Abca1 and Abcg1 specifically from rod photoreceptors, we crossed Abca1/g1F/F mice with mice carrying one copy of the rhodopsin-Cre transgene, which were provided by Dr. Ching-Kang Jason Chen and have been previously characterized (14, 15). To generate mice lacking both Abca1 and Abcg1 specifically from cone photoreceptors, we crossed Abca1/g1F/F mice with mice carrying one copy of the human red/green pigment-Cre (HRGP-Cre) transgene, which were provided by Dr. Yun Le and have been previously characterized (15, 16, 19). We confirmed that these mice did not carry the Cdh1 gene rd8 mutation (20) (data not shown). For high-fat diet (HFD) feeding experiments, only these mice did not carry the rd8 mutation (20) (data not shown). For high-fat diet (HFD) feeding experiments, only these mice did not carry the rd8 mutation (20) (data not shown). For high-fat diet (HFD) feeding experiments, only these mice did not carry the rd8 mutation (20) (data not shown).

**Isolation of rod or cone photoreceptors from the retina**

To isolate rod or cone photoreceptors from the retina, we used the Papain Dissociation System (Worthington Biochemical Corporation, Lakewood, NJ) following a previously described modified protocol (22), followed by EasySep Mouse PE or FITC positive selection kit (Stem Cell Technologies, Vancouver, Canada), respectively, following the manufacturer’s protocol. We used 1 mg/mL of PE-conjugated anti-CD75 antibodies (Miltenyi Biotec, Bergisch Gladbach, Germany) (15) for rod photoreceptors and 5 μg/mL of fluorescein peanut agglutinin (Vector Laboratories, Burlingame, CA) (23) for cone photoreceptors, respectively.

**TABLE 1. Mouse abbreviations**

| Abbreviations | Genotype |
|---------------|----------|
| Abca1/g1F/F    | Abca1flox/flox and Abcg1flox/flox-Cre-negative |
| Abca1/g1−/−/−   | Abca1flox/flox and Abcg1flox/flox-Rhod-iCre positive |
| Abca1/g1−/−/−   | Abca1flox/flox and Abcg1flox/flox-HRGP-Cre positive |
| Abca1/g1−/−/−   | Abca1flox/flox and Abcg1flox/flox-Rhod-iCre positive |
| Abca1/g1−/−/−   | Abca1flox/flox and Abcg1flox/flox-Rhod-iCre positive |

**RNA isolation and quantitative PCR**

Total RNA was extracted using an RNeasy Micro Plus kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. To synthesize cDNA, total RNA was added to the high-capacity cDNA reverse transcription kits (Thermo Fisher Scientific, Waltham, MA) and reverse-transcribed according to manufacturer’s instructions. Quantitative (q)PCR was performed in duplicate using the StepOnePlus real-time PCR system (Thermo Fisher Scientific) using TaqMan real-time PCR assays (Thermo Fisher Scientific), and the mRNA was quantified using the ΔΔCT method with Gapdh or Actb as the internal control.

**Electroretinography**

Electroretinography (ERG) was performed as previously described (21). A UTAS BigShot system (LKC Technologies, Inc., Gaithersburg, MD) was used. Mice were dark adapted overnight. Under red light illumination, mice were anesthetized with an intraperitoneal injection of 86.9 mg/kg ketamine and 13.4 mg/kg xyazine. Pupils were dilated with 1% atropine sulfate eye drops (Bausch and Lomb, Rochester, NY). Body temperature was maintained at 37°C with a heating pad. Contact lens electrodes were placed bilaterally with appropriate reference and ground electrodes. The stimuli consisted of a full-field white light flash (10 μs) in darkness or in the presence of dim [30.0 candela (cd)/m²] background illumination after 10 min adaptation time. Raw data were processed using MATLAB software (MathWorks, Natick, MA). The amplitude of the a-wave was measured from the average pretrial baseline to the most negative point of the average trace, and the b-wave amplitude was measured from that point to the highest positive point.

**Retinal imaging**

We performed digital color fundus photography and optical coherence tomography, as previously described (15, 24), using the Micron III animal fundus camera equipped with 830 nm optical coherence tomography (Phoenix Research Labs, Pleasanton, CA). Prior to fundus imaging and optical coherence tomography, we anesthetized mice with an intraperitoneal injection of 86.9 mg/kg ketamine and 13.4 mg/kg xyazine and administered 1.0% tropicamide eye drops (Bausch and Lomb) to dilate the pupils.

**Transmission electron microscopy**

We performed transmission electron microscopy analysis as previously described (15). For ultrastructural analyses, samples were fixed in 2% paraformaldehyde/2.5% glutaraldehyde (Polysciences Inc., Warrington, PA) in 100 mM sodium cacodylate buffer, and were postfixed in 1% osmium tetroxide (Polysciences Inc., Warrington, PA) in 100 mM sodium cacodylate buffer. Samples were dehydrated in a graded series of ethanol and embedded with Eponate 12 resin (Ted Pella Inc.). Ultrastructural analyses, samples were examined in a Leica EM 912 electron microscope (Leica Microsystems, GmbH, Wetzlar, Germany) at an acceleration voltage of 80 kV. Images were selected and adjusted for contrast and brightness using Adobe Photoshop (Adobe Systems Inc., San Jose, CA). Images were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD).

**Analysis of total cholesterol in the neurosensory retina**

We used Amplex Red cholesterol assay kits (Thermo Fisher Scientific) to measure total cholesterol in the neurosensory retina.
following the manufacturer’s protocol. Each neurosensory retina was homogenized with RIPA buffer (Thermo Fisher Scientific). Cholesterol concentrations were normalized to wet weights of the neurosensory retina (milligrams).

**Analyses of HDL, LDL/VLDL, and ApoA-1 in serum**

We collected blood samples from facial veins after overnight fasting. Blood samples were centrifuged at 800 g for 15 min, and then serum samples were collected. HDL and LDL/VLDL in serum were measured using HDL and LDL/VLDL quantitation kits (Sigma, St. Louis, MO) following the manufacturer’s protocol. ApoA-1 in serum was measured using an ApoA1 ELISA kit (LifeSpan BioSciences, Seattle, WA) following the manufacturer’s protocol.

**Analyses of oxysterols, free cholesterol, and cholesteryl esters in RPE complex**

To prepare RPE/choroid/sclera samples, we dissected the eyeballs to remove the anterior parts, including cornea, lens, and iris. After removing neurosensory retina, RPE/choroid/sclera samples were flash-frozen with liquid nitrogen and then stored at −80°C until the analyses. This RPE/choroid/sclera sample was named the RPE complex.

Each RPE complex was homogenized with 50-fold excess volume of PBS buffer using Omni Bead Ruptor 24 (Omni International Inc., Kennesaw, GA). All of the analyses listed above in 50 μl of the mouse retina homogenate were extracted with 200 μl of methanol. Prior to the extraction, triol-d7 (2 ng), 7-ketocholesterol-d7 (2 ng), 24-hydroxycholesterol (HC)-d7 (2 ng), 27-HC-d5 (2 ng), 4β-HC-d7 (2 ng), cholesterol esters (CEs) (18:2)-d7 (200 ng), and cholesterol-d7 (1 μg) were added as the internal standards to the homogenate samples. All oxysterols and cholesterol, as well as their deuterated standards, were derivatized with N,N-dimethylglycine to increase the MS sensitivity. Cholesteryl esters were not derivatized for the MS analyses.

The analysis of oxysterols and free cholesterol was performed with a Shimadzu 20AD HPLC system and a Shimadzu SIL 20AC autosampler coupled to a triple quadrupole mass spectrometer (API-6500 QTrap; Thermo Fisher Scientific) operated in MRM mode. The positive ion ESI mode was used for detection of the N,N-dimethylglycine-derivatized oxysterols and free cholesterol. An Agilent Erics XDB-C18 HPLC column (3.0 × 100 mm, 3.5 μm) was used for separation of all oxysterols. Mobile phase (a) 1% formic acid in water and mobile phase (b) 1% formic acid in 1:1 methanol/acetonitrile were programmed from 60% (b) to 99% in 4 min and held for 2 min at a flow rate of 1.2 ml/min. All samples were injected in triplicate for data averaging. Data processing was conducted with Analyst 1.6.3 (Thermo Fisher Scientific).

The analysis of two cholesteryl esters [CE(16:0) and CE(18:0)] was performed with a Shimadzu 20AD HPLC system and a LeapPAL autosampler coupled to a triple quadrupole mass spectrometer (API 4000; Thermo Fisher Scientific) operated with positive ion MRM mode. An Agilent Erics XDB-C8 HPLC column (4.6 × 100 mm, 3.5 μm) was used for these cholesteryl esters. The mobile phases, (a) 10 mmol ammonium acetate in 3:7 acetonitrile/water and (b) 10 mmol ammonium acetate in 1:1 methanol/isopropanol, were programmed from 80% (b) to 99% in 4 min and held for 2 min at a flow rate of 1.2 ml/min. The data processing was conducted with Analyst 1.5.1 (Thermo Fisher Scientific).

The analysis of the rest of the cholesteryl esters [CE(16:2), CE(18:1), CE(18:2), and CE(20:4)] was performed with a Shimadzu 10AD HPLC system and a Shimadzu SIL 20AC autosampler coupled to a triple quadrupole mass spectrometer (TSQ Quantum Ultra; Thermo Fisher Scientific) operated in MRM mode. A Thermo-Keystone betasil C18 HPLC column (2.0 × 100 mm, 5 μm) was used for the cholesteryl esters. The identical mobile phases [(a) and (b) listed above] were also used with an isocratic condition at 97% (b) for 12 min at a flow rate of 0.4 ml/min. The data processing was conducted with XCalibur (Thermo Fisher Scientific).

The relative quantification data is reported as peak area ratios of analytes to their internal standards normalized to wet weights (milligrams) of RPE complex.

**Statistics**

We performed statistical testing with GraphPad Prism (Version 6.0), using the appropriate test for each dataset. We defined statistical significance as a $P < 0.05$. Values are mean ± SE.

**RESULTS**

**Deletion of Abca1 and Abcg1 in rod photoreceptors increases retinal cholesterol, but does not affect retinal function in young mice**

The deletion of both Abca1 and Abcg1 in Abca1/g1–rod–rod mouse photoreceptors was confirmed by quantitative (q)PCR (supplemental Fig. S1A). Total cholesterol levels were higher in the neurosensory retinas of Abca1/g1–rod–rod mice compared with littermate controls (Abca1/g1/+) (supplemental Fig. S1B). However, Abca1/g1–rod–rod retinas were functionally and histopathologically normal at 3 months of age (Fig. 1A–C; supplemental Fig. S1C, D). Serum levels of HDL, LDL/VLDL, and ApoA-1 also did not show any differences (supplemental Fig. S1E–G).

**Aged Abca1/g1–rod–rod mice demonstrate lipid accumulation underneath the retina with associated rod neurodegeneration and vision loss**

Although young Abca1/g1–rod–rod mice did not demonstrate a functional phenotype at 3 months of age, upon analysis at 12 months of age, both scotopic a- and b-waves were significantly attenuated in Abca1/g1–rod–rod mouse retinas compared with littermate controls (Fig. 1D, E) and other controls (supplemental Fig. S2A, B), confirming rod photoreceptor dysfunction and vision loss. In contrast, photopic responses, a measure of cone function, were normal in Abca1/g1–rod–rod mouse retinas (Fig. 1F, supplemental Fig. S2C). Although fundus biomicroscopy at 12 months showed no gross abnormalities (Fig. 1G), electron microscopy demonstrated attenuation of normal vesicular structures at the rod inner and outer segment junction (25) in Abca1/g1–rod–rod mouse retinas (Fig. 1H). In addition, photoreceptor degeneration characterized by outer segment dysmorphic changes was identified in Abca1/g1–rod–rod mouse retinas, while littermate controls were normal (Fig. 1I). These structural changes were also associated with accumulation of large lipid droplets within the RPE (Fig. 1J). Further analysis by LC-MS/MS confirmed an increase in the levels of free cholesterol and several cholesterol metabolites underneath the neurosensory retina in the RPE complex (Fig. 2A) with no increase in any of the CEs analyzed (Fig. 2B).
Disrupted cholesterol metabolism promotes neurodegeneration

Retinal neurodegeneration increases in severity and affects cone photoreceptors with increasing age

We kept Abca1/g1⁻⁻/⁻⁻ rod mice up to 18 months to check to determine whether further aging might affect the neurodegenerative phenotype. At 18 months of age, vision loss was more severe with further degradation of scotopic rod responses on electrophysiology in Abca1/g1⁻⁻/⁻⁻ mice compared with littermate controls (Fig. 3A, B) and other
controls (supplemental Fig. S3A, B, D, E). A secondary decline in photopic cone responses was also observed (Fig. 3C; supplemental Fig. S3C, F). These findings recapitulate human diseases where rods are required for cone survival and where cone loss occurs after significant rod degeneration (26). At 18 months, photoreceptor neurodegeneration was identifiable on biomicroscopy, as seen by the atrophic areas within the retina (Fig. 3D, E). These atrophic areas corresponded to hyper-reflective outer retinal foci at and above the level of the RPE on optical coherence tomography imaging (Fig. 3F). Electron microscopy demonstrated degenerative vacuolization at the junction of the RPE and the retina, confirming the biomicroscopy and optical coherence tomography findings (Fig. 3G). On histology, degenerative vacuolization of the outer retina was clearly identifiable (Fig. 3H, I). The RPE also showed structural disruption including accumulation of lipid globules, undigested photoreceptor disk fragments, and vacuoles (Fig. 3J).

**Deletion of Abca1 and Abcg1 in cone photoreceptors does not cause neurodegeneration**

In addition to Abca1/g1<sup>−/−</sup>−rod mice, we also generated Abca1/g1<sup>−/−</sup>−cone mice to reveal the function of ABCA1 and ABCG1 in cone photoreceptors and analyzed them up to 18 months. The deletion of both Abca1 and Abcg1 in Abca1/g1<sup>−/−</sup>−cone mouse cone photoreceptors was confirmed by qPCR (supplemental Fig. S4). Similar to the phenotype seen in young Abca1/g1<sup>−/−</sup>−rod mouse retinas, young Abca1/g1<sup>−/−</sup>−cone mouse retinas did not show any neurodegenerative phenotype at 3 months old of age (Fig. 4A–C). However, in distinct contrast to old Abca1/g1<sup>−/−</sup>−rod mouse retinas, Abca1/g1<sup>−/−</sup>−cone mouse retinas were functionally (Fig. 4D–) and histopathologically (Fig. 4J–L) normal even at 18 months of age.

**ABCA1 and ABCG1 play vital roles in maintaining retinal function during metabolic stress**

In order to test whether this neurodegenerative phenotype was directly related to cholesterol uptake from the blood stream and associated metabolic stress, we placed Abca1/g1<sup>−/−</sup>−rod mice on a HFD. Abca1/g1<sup>−/−</sup>−rod mice placed on a HFD showed significant neurodegeneration and vision loss at 3 months compared with littermate controls on a HFD (Fig. 5A–C). Electron microscopy demonstrated rod outer segment degeneration (Fig. 5D), accumulation of lipid globules, and undigested photoreceptor disk fragments in the RPE (Fig. 5E) in Abca1/g1<sup>−/−</sup>−rod

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**Fig. 2.** Lipid analysis of Abca1/g1<sup>−/−</sup>−rod mouse eyes at 12 months of age demonstrates accumulation of cholesterol metabolites. A: Analyses of free cholesterol, 24-HC, 25-HC, 27-HC, and 7-ketocholesterol underneath the neurosensory retina in the RPE/choroid complex at 12 months of age (normalized peak area ratio per milligram wet tissue weight); n = 10 for each group. *P < 0.05 and **P < 0.01 by two-tailed unpaired t-test. B: Analyses of CEs underneath the neurosensory retina in the RPE/choroid complex at 12 months of age (normalized peak area ratio per milligram wet tissue weight); n = 10 for each group. No significant difference was detected by two-tailed unpaired t-test. Values are mean ± SE.
**Fig. 3.** *Abca1/g1^−/−^−* mouse retinas (aged 18 months) show decreased scotopic and photopic responses and atrophic retinal neurodegeneration. A–C: ERG of 18-month-old *Abca1/g1^+/+^* and *Abca1/g1^−/−^−* mice. *Abca1/g1^+/+^* (blue circles), n = 4; *Abca1/g1^−/−^−* (red squares), n = 4. A: Scotopic a-wave amplitude. B: Scotopic b-wave amplitude. C: Photopic b-wave amplitude. *P < 0.05, **P < 0.01, and ***P < 0.001 by two-way ANOVA with post hoc Bonferroni’s multiple comparison test. D: Representative fundus images of 18-month-old *Abca1/g1^+/+^* and *Abca1/g1^−/−^−* mouse retinas. Note the atrophic appearance of *Abca1/g1^−/−^−* mouse retinas (white arrowhead). E: Quantification of retinal atrophic foci per field. *Abca1/g1^+/+^* (blue circles), n = 8; *Abca1/g1^−/−^−* (red squares), n = 7. *P < 0.05 by two-tailed unpaired t-test. F: Representative optical coherence tomography images of 18-month-old *Abca1/g1^−/−^−* mouse retinas. Note the subretinal hyper-reflective foci in *Abca1/g1^−/−^−* mouse retinas (white arrowhead) corresponding to fundus atrophic appearance. G: Representative electron microscopy images of retinal atrophic foci in 18-month-old *Abca1/g1^−/−^−* mouse retinas. Note the vacuoles (asterisk) and photoreceptor disk fragments (yellow arrowheads). Scale bar: 2 μm. H: Representative H&E staining images of 18-month-old *Abca1/g1^+/+^* and *Abca1/g1^−/−^−* mouse retinas.
mice on a HFD, which is similar to the findings seen at 12–18 months of age in Abca1/g1<sup>−/−</sup> mice placed on a normal diet.

**DISCUSSION**

In this study, we hypothesized that maintaining physiologic cholesterol homeostasis in photoreceptors by ABCA1 and ABCG1 would play a key role in retinal function. In support of this hypothesis, we demonstrated that deletion of both Abca1 and Abcg1 in rod photoreceptors leads to age-related accumulation of cholesterol metabolites in the outer retina and retinal neurodegeneration. In addition, metabolic stress induced by a HFD significantly accelerated this neurodegenerative phenotype.

We generated Abca1/g1<sup>−/−</sup> mice to reveal the function of ABCA1 and ABCG1 in rod photoreceptors. As expected, total cholesterol levels were significantly increased in Abca1/g1<sup>−/−</sup> mouse retinas, whereas systemic lipoprotein levels were not changed. However, young Abca1/g1<sup>−/−</sup> retinas were functionally and histopathologically normal, suggesting that increased cholesterol levels in rod photoreceptors did not have acute effects on retinal structure and function. We were interested in examining the effects of aging on the retinal phenotype, as a number of neurodegenerative disease phenotypes, such as in AMD, occur with aging. As such, we aged Abca1/g1<sup>−/−</sup> mice and their littermate controls to 18 months of age and were able to demonstrate rod and subsequent cone photoreceptor degeneration and vision loss. In addition, we demonstrated that lipid droplets accumulated in the RPE of eyes from Abca1/g1<sup>−/−</sup> mice, but not in neurosensory retina, a finding consistent with the fact that one of the major functions of the RPE is phagocytosis of lipid-rich photoreceptor outer segments shed in a circadian manner. In addition, we examined the RPE complex for free cholesterol and cholesterol metabolites by LC-MS. The results confirmed increased levels of free cholesterol and several cholesterol metabolites in the RPE complex, without an increase in any of the CEs analyzed. These findings are in contrast to retinas from mice deficient in cytochrome P450 (CYP) enzymes, which showed an increase mainly in esterified cholesterols (27).

The majority of photoreceptors in murine retinas are rods, with only about 3% being cones. The ratio of rod and cone photoreceptors in human retinas is also very similar, but the distribution is different. Although aged Abca1/g1<sup>−/−</sup> mice showed significant retinal neurodegeneration, Abca1/g1<sup>−/−</sup> cone/−cone mouse retinas did not show any neurodegenerative phenotype, even at 18 months. These data suggest that either deletion of Abca1 and Abcg1 in a small percentage of photoreceptors does not impact visual function or that ABCA1 and ABCG1 are not important for normal cone function.

Finally, we tested to determine whether increased cholesterol uptake from the blood stream could affect the neurodegenerative phenotype of Abca1/g1<sup>−/−</sup> mouse retinas. In fact, cellular and tissue cholesterol level is regulated by a complex interplay between uptake and de novo biosynthesis of cholesterol, both responsible for cholesterol input and enzymatic elimination and efflux of cholesterol, which are responsible for cholesterol output. Remarkably, although short-term HFD feeding usually does not cause any retinal dysfunction in mice (21), even short-term HFD feeding to young Abca1/g1<sup>−/−</sup> mice accelerated photoreceptor neurodegeneration and vision loss, recapitulating the phenotype of aged Abca1/g1<sup>−/−</sup> mice on a normal diet. These results indicate that ABCA1 and ABCG1 play vital roles in maintaining cholesterol homeostasis in rod photoreceptors and retinal function during metabolic stress. Even in human studies, it is still controversial whether high serum cholesterol causes retinal neurodegeneration or cholesterol-lowering drugs prevent disease progression. For example, although the hallmark feature of AMD is accumulation of lipid-rich deposits underneath the RPE (called drusen), most previous studies failed to demonstrate the efficacy of cholesterol-lowering drugs, such as statins, to prevent disease progression (28, 29). Our study clearly revealed that, in the limited condition of disrupted cholesterol metabolism in rod photoreceptors, HFD caused retinal neurodegeneration. This fact might give us a new insight into the relationship between HFD or serum cholesterol and retinal neurodegenerative diseases such as AMD.

In summary, this study identifies cholesterol homeostasis as a critical regulator of age-related rod photoreceptor neurodegeneration and provides a unifying metabolic mechanism of vision loss in diverse retinal degenerative diseases. Accumulation of cholesterol and its metabolites in the outer retina may be a danger signal that leads to chronic lipotoxicity and neurodegeneration. These findings have potential translational relevance, as multiple genome-wide association studies have identified polymorphisms in genes involved in cholesterol metabolism, in general, and ABC transporters, in particular, in diseases such as AMD (30, 31) and Stargardt disease (32, 33) that are characterized by lipoprotein deposition and retinal neurodegeneration. In addition, these findings have potential implications beyond the eye, as recent studies also demonstrate a role for cholesterol clearance in regulating myelination of the CNS (34). Targeted metabolic interventions that regulate ABCA1 and ABCG1 in the retina may thus provide potential therapeutic avenues and prevent vision loss.

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**Figure legends:**

- **Figure 1:** Representative electron microscopy images of 18-month-old Abca1/g1<sup>−/−</sup> and Abca1/g1<sup>−/−</sup> mouse retinas. I. Vacuoles in outer segment of Abca1/g1<sup>−/−</sup> mouse retinas (asterisk). Scale bar: 1 µm. J. Accumulation of lipid globules (red arrowheads), undigested photoreceptor disk fragments (yellow arrowhead), and vacuoles (asterisk) within the RPE. Scale bar: 2 µm. Values are mean ± SE.
Fig. 4. Deletion of Abca1 and Abcg1 in cone photoreceptors does not cause detectable neurodegeneration. A–C: ERG of 3-month-old Abca1/g1^{+/+} and Abca1/g1^{−/−} cone mice. Abca1/g1^{+/+} (blue circles), n = 5; Abca1/g1^{−/−} (red squares), n = 6. A: Scotopic a-wave amplitude. B: Scotopic b-wave amplitude. C: Photopic b-wave amplitude. D–F: ERG of 12-month-old Abca1/g1^{+/+} and Abca1/g1^{−/−} cone mice. Abca1/g1^{+/+} (blue circles), n = 7; Abca1/g1^{−/−} (red squares), n = 7. D: Scotopic a-wave amplitude. E: Scotopic b-wave amplitude. F: Photopic b-wave amplitude. No significant difference was detected by two-way ANOVA. G–I: ERG of 18-month-old Abca1/g1^{+/+} and Abca1/g1^{−/−} cone mice. Abca1/g1^{+/+} (blue circles), n = 4; Abca1/g1^{−/−} (red squares), n = 6. G: Scotopic a-wave amplitude. H: Scotopic b-wave amplitude. I: Photopic b-wave amplitude. No significant difference was detected by two-way ANOVA. J: Representative fundus images of 18-month-old Abca1/g1^{+/+} and Abca1/g1^{−/−} cone mouse retinas. Scale bars: 20 μm. K: Representative H&E staining images of 18-month-old Abca1/g1^{+/+} and Abca1/g1^{−/−} cone mouse retinas. Scale bars: 2 μm. Values are mean ± SE.
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