

Review



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The gene for Stargardt disease, *ABCA4*, is a major retinal gene: a mini-review

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Abstract The gene *ABCA4* encodes the rod and cone photoreceptor Rim protein, which is a transmembrane transporter of vitamin A intermediates. *ABCA4* mutations are responsible for a large variety of retinal degenerations including all cases of Stargardt macular dystrophy and fundus flavimaculatus, some forms of cone-rod degeneration, and retinitis pigmentosa, and likely increase the risk of developing age-related macular degeneration (AMD). The purpose of this mini-review is to highlight the advances in our understanding of Stargardt disease and the *ABCA4* gene from the first description of the disease by Karl Stargardt in 1909 to gene discovery by Allikmets and colleagues in 1997. The knockout mouse model by Mata and co-workers has provided crucial pathophysiological information that has led to new ideas regarding treatment possibilities. These hypotheses were tested by Radu and colleagues in the mouse and shown to be efficacious.

Key words Stargardt disease; *ABCA4* gene; review; retinal dystrophy; macular degeneration

Introduction The gene for Stargardt disease, *ABCA4*, was discovered in 1997,¹ 88 years after the disease was first described by Karl Stargardt. In the past five years, this gene discovery has led to new insights into normal and abnormal retinal functioning in a host of macular and retinal degenerations while providing the first clues to future therapies.

***ABCA4* mutations are responsible for a large variety of retinal degenerations including Stargardt disease, fundus flavimaculatus, some forms of cone-rod degeneration, retinitis pigmentosa, and AMD** Mutations in *ABCA4* are responsible for the majority, if not all, of the juvenile macular dystrophies, Stargardt macular dystrophy (STGD), and fundus flavimaculatus (FF).¹ *ABCA4* mutations have also been found in recessive cone-rod degeneration (CRD)² and recessive retinitis pigmentosa (RP).² Heterozygous *ABCA4* mutations have been shown to raise the

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risk of age-related macular degeneration (AMD).³ The purpose of this review is to highlight the recent advances in the understanding of the pathophysiology of retinal degenerations associated with *ABCA4* and their potential treatments.

***ABCA4* encodes the rod and cone photoreceptor Rim protein, which is a transmembrane transporter of vitamin A intermediates** *ABCA4* encodes a protein called Rim protein, which is a member of the adenosine triphosphate (ATP)-binding cassette transporter family. It is expressed in the rims of both rod⁴- and cone⁵-photoreceptor disc membranes and is involved in the transport of vitamin A derivatives to the retinal pigment epithelium (RPE). To understand the role of the Rim protein in Stargardt and other retinal degenerations, it is necessary to recount previous histological and clinical findings and correlate them with recent genetic and biochemical discoveries.

The pathophysiology of Stargardt disease involves the accumulation of lipofuscin and intermediates of the vitamin A cycle Histopathologically, eyes with Stargardt disease reveal abnormal accumulations of lipofuscin in the RPE.⁶ Lipofuscin is made up of several compounds, one of which is A2E (N-retinylidene-N-retinyl-ethanolamine).⁷ Clinically, in-vivo measurements have shown abnormal amounts of lipofuscin (up to 5 times normal) in patients with Stargardt disease,⁸ while lipofuscin deposits have also been found in AMD patients⁹ and some cases of RP.¹⁰ The questions now are: does lipofuscin cause damage to the photoreceptors and why does it accumulate? Also, how is this accumulation related to Rim protein and can lipofuscin accumulation be prevented in the form of treatment for Stargardt, RP, and AMD?

In a normal eye, a photon of light is captured by rhodopsin and bound to 11-cis retinal to start the phototransduction cascade. This capture leads to the production of all-trans retinal, which leaves the disc membrane and enters the disc space, where it binds to a phospholipid called phosphatidylethanolamine (PE) and forms a complex called N-retinylidene-PE (N-RPE). Normal Rim protein is currently thought to transport N-RPE out of the disc space into the cytoplasm of rods and cones, where it cleaves the bond between all-trans retinal and PE.¹¹ All-trans retinal is then reduced to all-trans retinol and transported to the RPE, where it is recycled back to 11-cis retinal.

In mutant Rim protein, N-RPE is predicted to accumulate in the rod and cone disc spaces as no shuttling of N-RPE takes place from the intradiscal space to the cytoplasm. In the intradiscal space, another molecule of all-trans retinal combines with N-RPE to form A2PE, which is then hydrolyzed to A2E, the major component of lipofuscin.⁷ A2E is speculated to be toxic to RPE cells since it may dissolve cell membranes, destroy RPE cells, and cause secondary photoreceptor cell death.⁷ These predictions correlate well with the histopathology and clinical data of Stargardt disease. The *ABCA4*^{-/-} knockout mouse also confirms several of these predictions as mice lacking the Rim protein show delayed dark adaptation, increased levels of all-trans retinal after

light exposure, accumulation of N-RPE, and striking deposition of the major lipofuscin fluorophore A2E.¹²

Do *ABCA4* mutations play a role in AMD? Now that new discoveries are being made about the pathophysiology of Stargardt disease, a larger question has arisen: do *ABCA4* defects predispose to AMD and play a role in the pathophysiology? Several lines of evidence appear to suggest that heterozygous *ABCA4* mutations increase the risk of AMD development.^{3,13-15} In an international 15-center study, Allikmets and colleagues found that carriers of the heterozygous G1961E *ABCA4* mutation had a fivefold increased risk and the D2177N mutation a threefold risk of developing AMD.³ Earlier epidemiological studies, however, were unable to establish the increased risks, possibly because of differences in population or methodology.^{16,17} Nevertheless, further confirmation of increased risk comes from two biochemical studies of *ABCA4* mutations related to AMD. Mata and co-workers showed that A2E was fourfold more abundant in eight-month-old heterozygous *ABCA4*^{+/-} mice than in wild-type mice and that heterozygous mice exhibited a delayed recovery of rod sensitivity by ERG.¹³ In addition, Shroyer and colleagues showed that protein expression and ATP-binding capacities were significantly reduced in in-vitro biochemical assays of *ABCA4* mutations associated with AMD.¹⁴ Finally, Yatsenko et al.¹⁵ demonstrated that late-onset Stargardt disease (onset >35 years) is associated with missense mutations that map outside the known functional domains of *ABCA4* and seem to function as milder mutant alleles.

***ABCA4* and other retinal degenerations** In addition to increasing the risk of AMD, *ABCA4* mutations play a role in several other important retinal degenerations. It was recently found that *ABCA4* mutations are the major cause of autosomal recessive CRD, with 13/20 (65%) CRD patients harboring significant mutations.¹⁸ In a separate study of 12 CRD patients, it was shown that the phenotype resulting from the *ABCA4* genotype is heterogeneous and complex.¹⁹ In all patients, visual acuity was below 20/200, onset was at or before age 12, a bull's eye maculopathy was found, and night blindness was present in the final stages of degeneration (in two of the patients, night blindness was present early). A peripheral retinal degeneration developed late in the disease process in all patients, with an equal decline in the rod- and cone-mediated ERGs in most and an earlier cone- than rod-mediated decline in some. Several patients diagnosed as CRD with *ABCA4* mutations were unusual: one presented early with a diffuse chorioretinal degeneration, while another had features of Stargardt disease with subretinal flecks and developed features of retinitis pigmentosa (RP). The authors concluded that clinically 'separate' disorders may be genetically similar and that clinical categorization may be difficult and not always helpful. These studies also clearly point out that the gene for Stargardt disease (*ABCA4*) is implicated in a wide variety of retinal degenerations.

Different *ABCA4* mutations can cause distinct disease phenotypes even within the same family. Cremers et al.,² for example, discovered

that a consanguineous family with RP in one branch and CRD in another cosegregated with a variety of recessive *ABCA4* mutations. They found that the mutations in the RP patients were different from those in the CRD patients. The RP patients carried two homozygous splice-site mutations (IVS 30 +1 g to t/IVS 30 +1 g to t), while the CRD patients carried two different splice-site mutations (IVS 30 +1 g to t/IVS 40 +5 g to a). According to the authors, the IVS 30 +1 g to t mutation from the RP patients probably represents a null allele.

A classification system of retinal degenerations associated with *ABCA4* mutations based on residual function of the protein

Of interest is that no null alleles have yet been found in patients with Stargardt disease or fundus flavimaculatus. This fact, in addition to the findings above, have led several groups like that of Van Driel et al.²⁰ and Lewis et al.²¹ to the hypothesis that there is an inverse relationship between the residual function of the *ABCA4* allele and the severity of the retinal disorder. In other words, the more severe the retinal disorder, the less residual function of the Rim protein. This has led to the predictions that, depending on the type of mutation (mild, moderate, severe) and the remaining activity of the Rim protein, the phenotype associated with *ABCA4* can range from AMD at the mild end of the spectrum to RP at the severe end with fundus flavimaculatus, Stargardt disease, and CRD at intermediate positions. According to this model, a patient with one wild-type and one mutant allele has an elevated risk of developing AMD, while patients with two moderate or one severe and one mild allele probably have residual Rim protein activity and have either Stargardt disease or fundus flavimaculatus. Patients with one severe and one moderate allele and little Rim protein activity have CRD, while patients with two severe alleles and no Rim protein activity have RP. In-vitro studies can test these predictions and, if accurate, this model may be very helpful in new attempts to classify retinal degenerations based on molecular etiology instead of clinical appearance. In conclusion, the retinal phenotypes associated with *ABCA4* depend on the severity of the mutation and the remaining activity of the Rim protein.

Treatment for animal models with retinal degenerations associated with *ABCA4* mutations

Genetic, molecular, and biochemical studies have led to new therapeutic insights. Mata and co-workers, for example, recently discovered that *ABCA4*^{-/-} mice with no Rim protein product accumulated A2E at a rapid rate in light conditions and that this A2E accumulation was markedly reduced when the mice were raised in the dark.¹³ They also found that knockout mice raised in the light and then transferred to the dark did not show a reduction in the level of A2E.¹³ Darkness, therefore, appears to be a possible treatment for Stargardt disease patients and warrants further investigation in a clinical trial. In a separate attempt to utilize existing pharmaceutical agents in patients with Stargardt disease, Radu and associates studied the effects of isotretinoin (Accutane) on A2E accumulation in *ABCA4*^{-/-} mice as the compound is known to inhibit 11-cis retinol dehydrogenase, a key RPE enzyme in the regeneration of 11-

cis retinal.²² Surprisingly, complete inhibition of A2E accumulation was found in the treated *ABCA4*^{-/-} mice compared to the controls.²² These early results point to a possible avenue of further investigation: namely, by reducing the level of the vitamin A cycle and its intermediary products, lipofuscin levels may be lowered, with beneficial consequences for Stargardt, RP, CRD, and some AMD patients.

In the almost 100 years since the first description of Stargardt disease by Karl Stargardt in 1909 to the discovery of the causative gene *ABCA4* by Allikmets and colleagues in 1997, we have acquired an increased understanding of the pathophysiology of the disease through the development of the *ABCA* knockout mouse. Using this knowledge, we are now attempting to successfully treat the disease.

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