### Title

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# Sorting out co-occurrence of rare monogenic retinopathies: Stargardt disease co-existing with congenital stationary night blindness

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## Abstract

**Background:** Inherited retinal diseases are uncommon, and the likelihood of having more than one hereditary disorder is rare. Here, we report a case of Stargardt disease and congenital stationary night blindness (CSNB) in the same patient, and the identification of two novel in-frame deletions in the *GRM6* gene.

**Materials and Methods:** The patient underwent an ophthalmic exam and visual function testing including color vision, Goldmann visual field, and electroretinography (ERG). Imaging of the retina included fundus photography, spectral-domain optical coherence tomography (OCT), and fundus autofluorescence. Genomic DNA was PCR-amplified for analysis of all coding exons and flanking splice sites of both the *ABCA4* and *GRM6* genes.

**Results:** A 46-year-old woman presented with recently reduced central vision and clinical findings of characteristic yellow flecks consistent with Stargardt disease. However, ERG testing revealed an ERG phenotype unusual for Stargardt disease but consistent with CSNB1. Genetic testing revealed two previously reported mutations in the *ABCA4* gene and two novel deletions in the *GRM6* gene.

**Conclusions:** Diagnosis of concurrent Stargardt disease and CSNB was made on the ophthalmic history, clinical examination, ERG, and genetic testing. This case highlights that clinical tests need to be taken in context, and that co-existing retinal dystrophies and degenerations should be considered when clinical impressions and objective data do not correlate.

### Keywords

congenital stationary night blindness; electronegative ERG; Stargardt disease; *ABCA4*; *GRM6*

## INTRODUCTION

As sophistication and feasibility of genetic testing evolves, confirmation of whether clinical diagnoses are supported by known genetic causes becomes possible. Correlation of disease

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phenotype with genotype is the crux for understanding the function of the affected gene(s). Since inherited retinal diseases are rare, the likelihood of identifying multiple genetic etiologies for a given ocular phenotype in a single patient is exceedingly rare. Careful genotype and phenotype analysis may lead to discoveries of co-segregation of more than one gene. In the case of digenic diseases, only double heterozygotes develop the disease, whereas for co-existing monogenic diseases, independent phenotypes are conferred by separate gene changes.[1]

Stargardt disease is characterized by central vision loss and characteristic yellow, "pisciform" flecks on fundus ophthalmoscopy. It is caused by mutations in the *ABCA4* gene on the short arm of chromosome 1,[2] which encodes an ATP-binding cassette (ABC) transporter protein localized in the outer segment disc membranes of photoreceptors.[3] Stargardt disease is one of the more common recessively inherited macular dystrophies, with an estimated prevalence of 1:8000–1:10,000.[4]

Congenital stationary night blindness (CSNB) is a heterogeneous group of minimally progressive retinal disorders characterized by nyctalopia, an abnormal ERG, and minimal fundus changes other than those related to high myopia. CSNB is further divided into two subtypes based on whether the scotopic rod electroretinogram (ERG) is absent (CSNB1) or present (CSNB2). There is also a greater degree of cone-mediated ERG loss in CSNB2. X-linked CSNB1 is caused by mutations in the *NYX* gene, while autosomal recessive CSNB1 is caused by mutations in *TRPM1*, *GRM6*, or *GPR179*.[5,6] All CSNB1 genes encode proteins in ON-bipolar cells onto which photoreceptors synapse. X-linked and autosomal recessive CSNB2 are caused by mutations in *CACNA1F* and *CABP4*, respectively,[5] both of which encode pre-synaptic proteins in the photoreceptor inner segments.

A survey of military recruits in Israel estimated the prevalence of CSNB to be 1 in 10,661, affecting males more than females (1 in 7,143 males compared to 1 in 23,225 females).[7] Autosomal-recessive CSNB is estimated to be responsible for approximately 7% of all cases of CSNB, making its prevalence less than 1 in 100,000.[5]

Clinical exam findings are very different for Stargardt disease and CSNB, with Stargardt disease having classic fundus findings and characterized by a progressive course that can lead to severe central vision loss as atrophy develops. CSNB, on the other hand, is a stationary disease associated with mild reduction in visual acuity but with minimal foveo-macular changes. Finding evidence of the occurrence of both Stargardt and CSNB would have a rare likelihood of the product of their individual occurrences – and yet we do sometimes observe this, as in the case we describe below.

## CASE REPORT

A 46-year-old woman presented to our clinic for evaluation of decreased central vision for at least one year and with difficulty of night vision since childhood. The patient was otherwise healthy and had no family history of ocular disorders. She does not take any medications and was up-to-date with age-appropriate cancer screenings.

On presentation her best-corrected visual acuity was 20/250 OD and 20/32 OS. She had worn spectacle correction for myopic astigmatism since she was a child. Her current refraction was −11.50 +5.00 × 085 OD and −11.00 +5.00 × 095 OS. No changes in color vision were detected, and there was no nystagmus or ocular misalignment. Intraocular pressure and pupillary examination were unremarkable. Fundus examination revealed atrophy OD and parafoveal yellow flecks OU (Figure 1A). No peripheral flecks were noted. Fundus autofluorescence demonstrated central hypoflouresence surrounded by hyperautofluorescent flecks (Figure 1B).

## MATERIALS AND METHODS

### Visual Field Testing & Electroretinography

Goldmann kinetic perimetry was performed using the V4e, III4e, and I4e isopters at a test distance of 33cm. The patient was dark adapted for 40 minutes prior to the start of ERG recording. International Society for Clinical Electrophysiology of Vision (ISCEV) standard full-field flash ERGs were recorded from bipolar Burian-Allen electrodes using a commercial electrophysiology system (LKC, Gaithersburg, MD).

### Genetic Testing

The patient provided blood and informed consent for genetic testing. Total genomic DNA was extracted using standard techniques. Exons 1–50 of the *ABCA4* gene and the flanking splice sites were amplified by polymerase chain reaction, followed by direct Sanger sequencing. Direct testing for mutations in *TRPM1* and *GRM6* genes was performed by DNA amplification and direct Sanger sequencing of all coding exons and intron-exon boundaries.

### Verification of trans configuration of novel deletions in *GRM6*

The patient's mother's blood was obtained and analyzed for changes seen in the patient. The patient's mother was heterozygous for the c.50_64delCGCTGGCGTGGCTGG deletion. The patient's father was deceased, thus genetic testing could not be done.

### Bioinformatics Analyses

Polymorphism Phenotyping v2 (PolyPhen-2) (http://genetics.bwh/harvard/edu/pph2/) and Mutation Taster (http://www.mutationtaster.org/) analysis tools were utilized to predict the possible impact of amino acid substitution on the structure and function of protein.

## RESULTS

In addition to reduced visual actuity OD> OS, Goldmann perimetry demonstrated bilateral central scotomas to the I4e and V4e stimuli, with preserved peripheral fields (Figure 2). This was consistent with the autofluorescence changes seen on fundus imaging. Full-field scotopic ERG testing revealed loss of the dim flash rod response and the presence of an electronegative ERG to bright flashes. Photopic single flash and 30-Hz ERG responses demonstrated a broadened negative trough followed by a late sharply-rising positive response, which suggested the loss of ON-bipolar cell signaling that contributes to the b-

wave (Figure 3).[8] The long flash ERG confirmed loss of the b-wave to light onset, with preservation of the d-wave to light offset, consistent with loss of ON-bipolar but preservation of OFF-bipolar cell signaling, respectively.[9]

The patient was found to be heterozygous for two reported missense mutations in *ABCA4*: R2077G (c.6229 C>G)[10] and L12 1R (c.3602 T>G).[2,11,12] The patient was also heterozygous for a novel missense variation V513A (c.1538 T>C) in *ABCA4*, which is predicted to be a benign polymorphism because it is a conservative amino acid substitution at a position of the protein that is not evolutionary conserved. The presence of the two previously reported *ABCA4* missense mutations is consistent with the clinical diagnosis of Stargardt disease. Because of the unusual ERG changes and lifelong night blindness, we proceeded with testing for mutations in two of the genes (*TRPM1* and *GRM6*) associated with autosomal recessive CSNB1. The patient was heterozygous for two novel in-frame deletions in the *GRM6* gene: c.50_64delCGCTGGCGTGGCTGG and c.1835_1837delACA. A novel missense variation was also identified (Q23L; c.68 A>T) but this variation was predicted to be benign using PolyPhen-2 and Mutation Taster.[13] *TRPM1* testing was negative.

The patient returned for follow-up two years after her initial visit, and her visual acuity was stable at 20/200 OD and 20/40 OS. ERG and Goldmann visual fields had not changed further, and ocular exam revealed mild progression of fundus changes, consistent with Stargardt disease.

## DISCUSSION

Early descriptions of Stargardt disease reported normal or minimally subnormal electrophysiologic findings.[14–16] However, more recent studies suggest that ERG abnormalities may be more prevalent than previously appreciated.[17,18] Stargardt disease has been classified into three groups based on distinct electrophysiologic phenotypes: those with macula-only dysfunction; those with macular and generalized cone dysfunction; and those with macular and generalized rod and cone abnormalities.[17] A worse prognostic value is associated with patients who have both generalized rod and cone dysfunction. [19] In addition to an electronegative ERG, our patient displayed rod abnormalities in the presence of preserved cone function – an ERG phenotype not typically seen in Stargardt disease.

CSNB is clinically and genetically heterogeneous, but typically is minimally progressive. Electrophysiology and family history can be helpful is distinguishing different forms of CSNB. In our patient, electrophysiology was consistent with CSNB1, and we subsequently identified two novel in-frame deletions in the *GRM6* gene, specifically, c.50_65delCGCTGGCGTGGCTGG and c.1835_1837delACA. The 15-base pair deletion is located in the ligand-binding domain of the metabotropic glutamate receptor 6 (mGluR6) and is predicted to abolish the signal peptide. The 3-base pair deletion is located in the transmembrane domain of the mGluR6 and deletes a highly-conserved asparagine. Although paternal data was not available, genetic analysis of the patient's mother revealed the 15-base pair deletion, but not the 3-base pair deletion. It was presumed that the 3-base pair deletion

was inherited paternally, consistent with autosomal recessive inheritance. Her mother did not have symptoms of night-blindness.

The *GRM6* gene is located at the q35 region of human chromosome 5.[20] Located on both rod-bipolar and cone ON-bipolar cells, mGluR6 is required for signal transmission from the photoreceptors to the ON-bipolar cells.[21] In the dark, photoreceptors tonically release glutamate, which activates an mGluR6-mediated signaling pathway in ON-bipolar cells that closes TRPM1 channels and hyperpolarizes these cells.[22] Deactivation of mGluR6, in response to the light-induced reduction in glutamate from photoreceptors, results in depolarization of ON-bipolar cells and thereby upstream neural signaling. Absence or dysfunction of the mGluR6 receptor impairs ON-bipolar cell signaling, preventing generation of the ERG b-wave and simulating a light-adapted state in the dark that results in symptoms of night blindness.[23]

Prior studies have reported on twelve mutations in the *GRM6* gene in eight families.[23–26] A more recent study by Sergouniotis *et al.* reported on nine mutations from seven families, including six novel mutations that were not previously reported.[27] The two novel deletions in our case add to the growing collection of sequence changes identified in the *GRM6* gene associated with CSNB1. Although the exact significance of these two novel deletions is unknown, the patient's phenotype is convincing for CSNB and the nature of the deletions indicates they are pathogenic.

The electronegative ERG in our patient with clinical Stargardt disease led us to suspect that another retinal disorder was also present. CSNB and X-linked retinoschisis are the more common causes of electronegative ERGs[28–30] and are typically diagnosed in childhood. In an adult patient such as ours, the differential for an electronegative ERG includes acquired causes such as MAR, CAR, Birdshot chorioretinopathy, central retinal vein occlusion, and drug toxicity (e.g. quinine- and methanol-induced retinopathy). With the exception of MAR, these other conditions have fundus changes that suggest the diagnosis. For this patient, careful analysis of family history, ocular and medical history as well as ocular phenotype, specifically, electrophysiologic testing, lead to a concurrent diagnosis of CSNB.

The genes for *ABCA4* and *GRM6* are located on chromosomes 1 and 5 respectively, indicating that the co-existing mutations in these genes in our patient are not related. The known localization of the protein products of these genes places them in distinct areas of the retina, consistent with non-interacting and co-existing phenotypes. This case report highlights that rare monogenic retinal diseases can co-exist, and that careful phenotyping is critical to correct genotyping.

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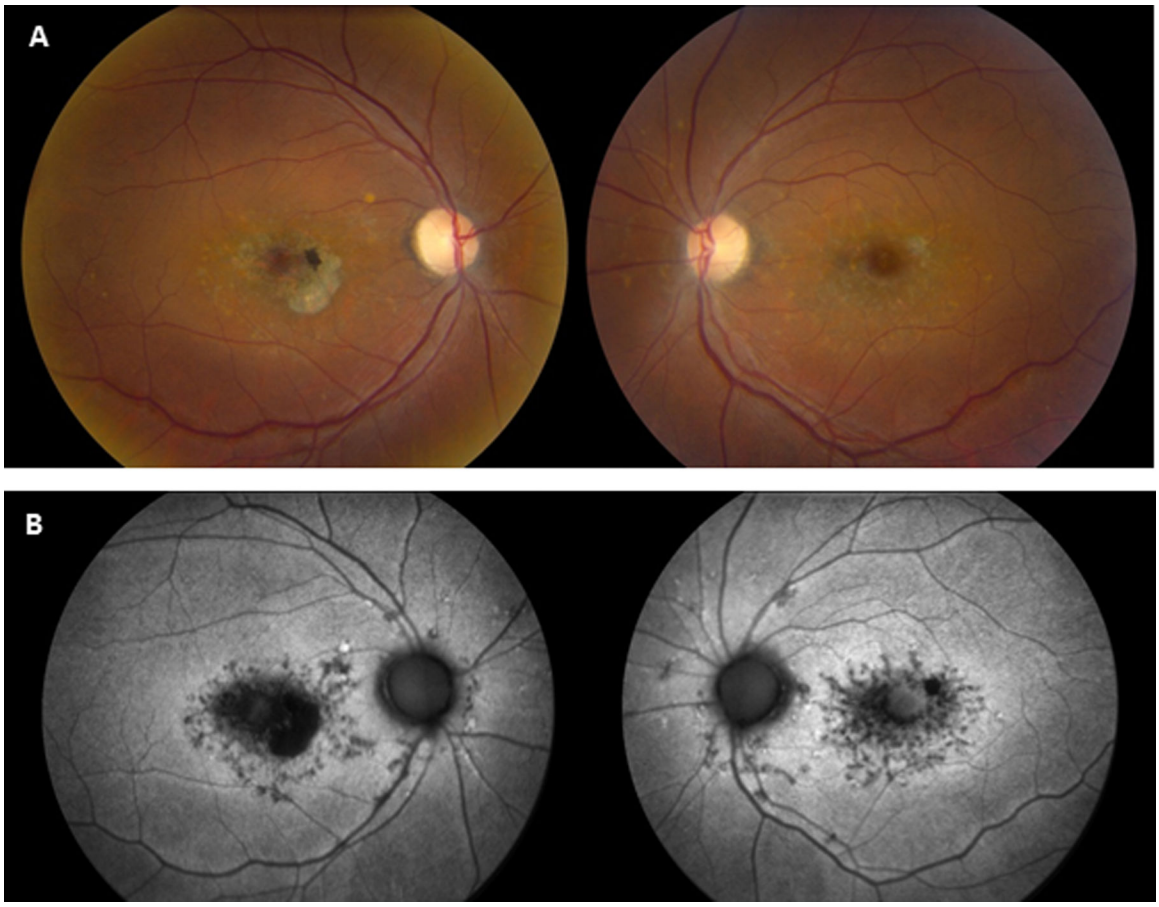


**FIGURE 1.**
(A) Fundus photographs demonstrating parafoveal flecks OU with central atrophy OD. (B) Fundus autofluorescence demonstrating central hypofluoresence OU.

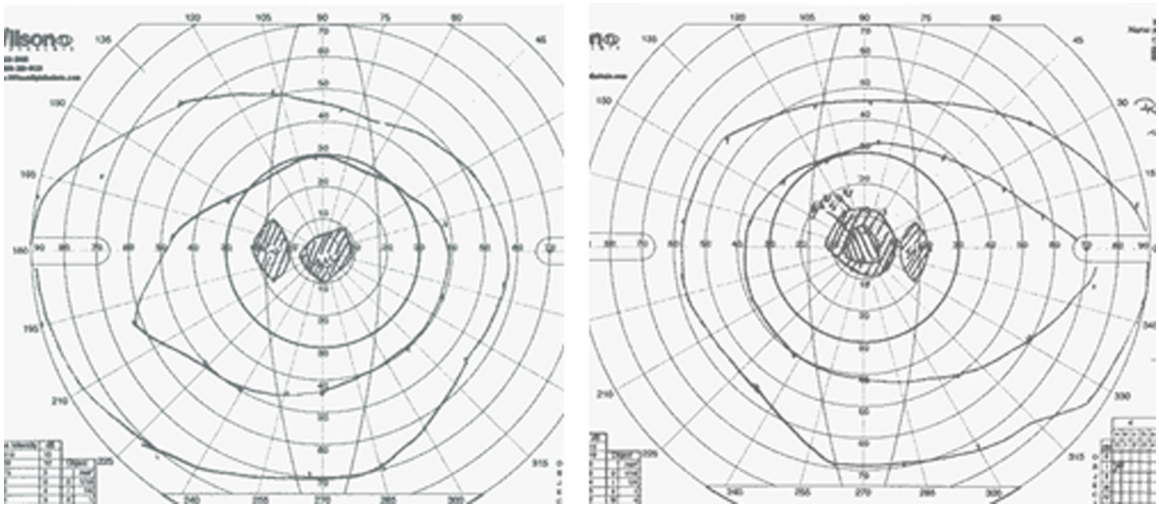

**FIGURE 2.**
Goldmann perimetry testing revealed central scotomas OU.

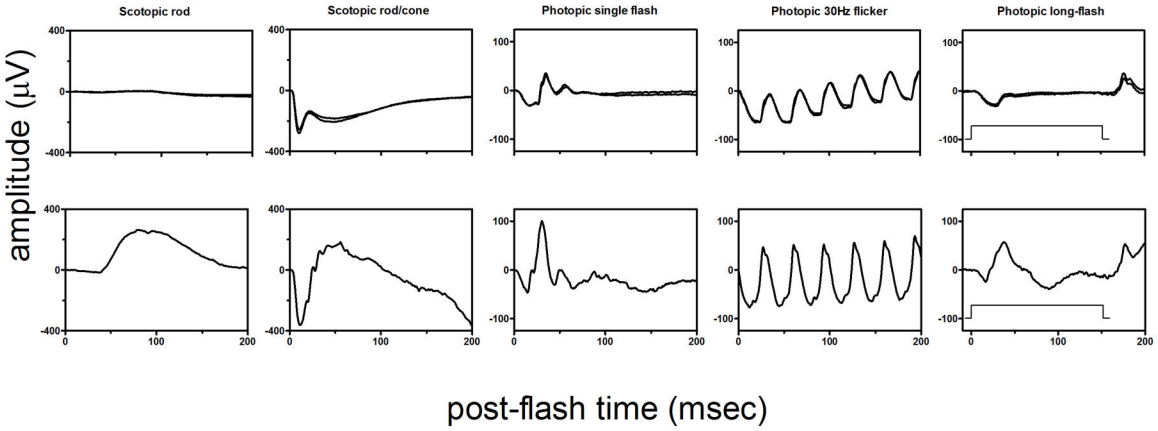


**FIGURE 3.**

Electrophysiologic testing from both eyes of the patient (top row) compared with an age matched control (bottom row). Responses were recorded to flash intensities (left to right) of 0.01, 30, 2.5, 2.5 and 185 photopic cd-s/m2. The square waves in the far right column indicate the time of light onset. Full-field scotopic revealed loss of the dim flash rod response and the presence of an electronegative ERG to bright flashes. Photopic single flash and 30-Hz ERGs demonstrated a broadened negative response followed by a late sharply-rising positive response. Long flash ERG confirms loss of the b-wave to light onset, with preservation of the d-wave to light offset.