

# Stargardt Disease Due to an Intronic Mutation in the *ABCA4*: A Case Report

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**Purpose:** To report on a patient with Stargardt disease (STGD1) and with an intronic mutation in the *ABCA4* gene.

**Patients and Methods:** A 69-year-old female patient presented to the clinic complaining of progressive vision loss. The ophthalmic evaluation was remarkable for a best corrected visual acuity of counting fingers at 5' in the right eye and 3' in the left eye. Imaging revealed deep extensive atrophy of the central macula, epithelial pigment hyperplasia, and other areas of multifocal atrophy in the right eye. Furthermore, fundus autofluorescence imaging of the macula showed central hypoautofluorescence with bilateral expansion to the periphery in both eyes. A full-field electroretinogram showed a normal rod response, with decreased cone response, bilaterally. Genetic testing was positive for a homozygous intronic mutation in the *ABCA4* gene of the variant c.5714+5G>A.

**Conclusions and Importance:** Patients with STGD1 due to presumed mild or moderate mutations in the *ABCA4* gene may have a more severe presentation and progression of the disease. Based on this, the first report of a genotype–phenotype correlation in a Puerto Rican patient with STGD1 disease, genotyping all Puerto Rican patients is warranted.

**Keywords:** Stargardt disease, intronic mutation, *ABCA4* gene, macular dystrophy

## Introduction

Stargardt disease (STGD1) is known to be one of the most common causes of inherited macular dystrophy in both adults and children. Stargardt disease has a typical onset during the early part of the second decade of life and presents initially with central vision loss, yellow flecks around the macula and retinal mid-periphery, and progressive atrophy of the retinal pigment epithelium (RPE).<sup>1–4</sup> The progression of the loss of retinal function is usually slow; however, there is great variability in the progression rate among patients.<sup>3</sup>

Mutations in the *ABCA4* gene have been associated with STGD1, cone-rod dystrophy, and retinitis pigmentosa.<sup>1,3,5</sup> *ABCA4* is an ATP-binding cassette transporter found at the rim of the outer segments of the rods and cones.<sup>1,2,6</sup> Defects in this protein lead to the accumulation of N-retinylidene-phosphatidylethanolamine in the photoreceptor disc membrane. This, in turn, condenses to phosphatidyl-pyridinium bisretinoid and accumulates in the RPE, forming a major component of lipofuscin, which is toxic to this tissue.<sup>7</sup>

STGD1 is inherited in an autosomal recessive manner and is caused by mutations in the *ABCA4* gene.<sup>1–7</sup> Made up of 50 exons, *ABCA4* is a highly polymorphic gene. Hundreds of disease-associated variants have been reported.<sup>1–7</sup> This gene's highly polymorphic nature leads to great phenotypic variation, making STGD1 a clinical and diagnostic challenge. Most patients are compound heterozygous for pathogenic variants of the gene.<sup>4,8</sup> Studies have suggested that missense variants are associated with a milder and later onset of disease and that those with null alleles are associated with more severe disease.<sup>3</sup> Intronic mutations in the *ABCA4* gene have been described. Studies in Newfoundland and Greece found the intronic allele c.5714+5G>A to be the most common in their cohorts.<sup>4,8</sup> This allele was associated with mild-to-moderate disease. Both studies found some degree of founder effect in patients affected with this mutation.

Up to this day, there is no study describing the genotype–phenotype correlation of a Puerto Rican patient with STGD1. Herein, we report on a Puerto Rican patient with STGD1 who had a homozygous intronic mutation in the *ABCA4* gene.

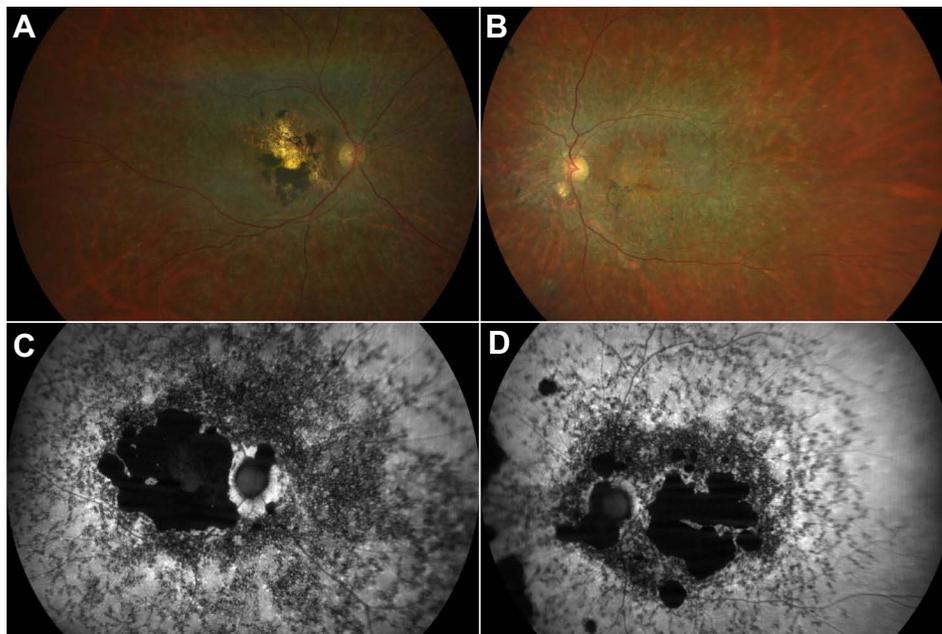
## Case Report

A 69-year-old female patient complained of progressive vision loss. Her parents were first cousins. She had been diagnosed with retinitis pigmentosa 27 years previously. The patient had been a smoker for 15 years. She had a past medical history of hyperlipidemia and hypothyroidism.

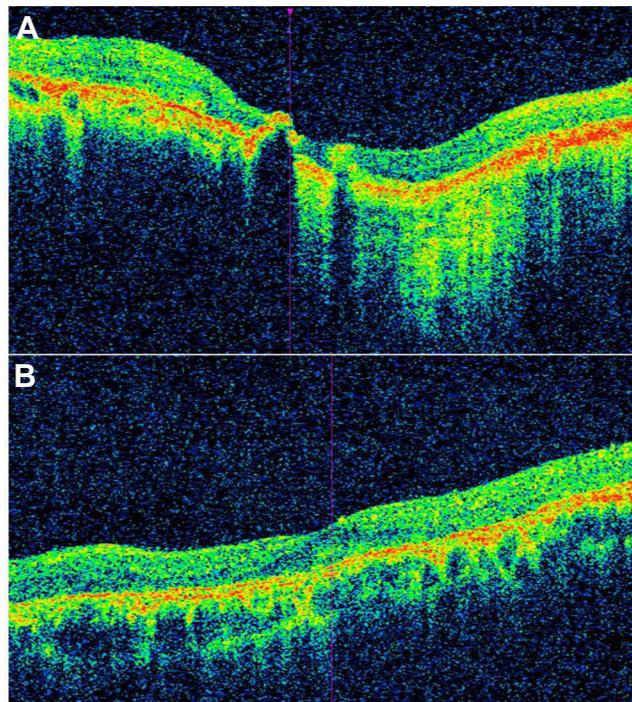
Upon a comprehensive ophthalmic evaluation, the patient was found to have a best corrected visual acuity (BCVA) of counting fingers at 5' and 3' in the right and left eyes, respectively. Retinoscopy showed a refraction of  $-1.00 + 0.50 \times 70$  and  $-2.00 + 1.00 \times 5$  in the right and left eyes, respectively. As depicted in [Figure 1A and B](#), the patient had pale optic discs with extensive deep atrophy of the central macula, epithelial pigment hyperplasia, and other areas of multifocal atrophy in the right eye. In addition, the patient had macular atrophy in the left eye. In the patient's fundus autofluorescence (FAF) imaging, there was evidence of central hypoautofluorescence of the macula, with diffuse outward centrifugal extension from its center to its periphery (depicted in [Figure 1C and D](#)).

Macular optical coherence tomography (OCT) determined that the average macular thickness was 191  $\mu\text{M}$  and 193  $\mu\text{M}$  in the right and left eyes, respectively. Macular volume was 6.9  $\text{mm}^3$  and 7.0  $\text{mm}^3$  in the right and left eyes, respectively. No macular edema, cysts, or subretinal fluid was found in either eye. [Figures 2A and B](#) depict Macular OCT imaging.

A visual field test revealed that the patient had mean deviations of  $-23.75$  dB and  $-24.56$  dB in the right and left eyes, respectively. Pattern standard deviations were  $+3.69$  dB and  $+4.69$  dB in the right and left eyes, respectively ([Supplementary Figures 1 and 2](#)). The results of a full-field electroretinogram (ERG) showed a normal rod response and decreased cone response, bilaterally ([Supplementary Figure 3](#)). For these reasons, a clinical diagnosis of STGD1 disease was made.



**Figure 1** Color fundus photography and autofluorescence showing the extent of macular dystrophy. **(A)** Right eye, demonstrating pale optic discs with extensive deep atrophy of the central macula, epithelial pigment hyperplasia, and other areas of multifocal atrophy. **(B)** Left eye, depicts macular atrophy. **(C)** Right eye, central area of hypoautofluorescence at the macula. Diffuse outward centrifugal extension from the center to the periphery of the macula and at the nasal side of the optic disc, with sparing of peripapillary area. Very few hyperfluorescent flecks near the outer border of the affected area. **(D)** Hypoautofluorescent areas at the central macula and nasal to the optic disc. Outward centrifugal extension from the center to the periphery of the macula and at the nasal side of the optic disc. Very few hyperfluorescent flecks near the outer border of the affected area.



**Figure 2** Macular optical coherence tomography demonstrating macular dystrophy with retinal pigment epithelium atrophy. (A) Right eye. (B) Left eye.

A single pathogenic homozygous intronic variant c.5714+5G>A of the *ABCA4* gene was found upon sequencing and deletion/duplication analysis using next-generation sequencing (Invitae Corporation, San Francisco, California). She had six additional variants of uncertain significance (VUS) at the *CACNA1F*, *EYS*, *KCNV2*, *KLHL7*, *MKKS*, and *USH2A* genes (Table 1, [Supplementary Figure 4](#)).

## Discussion

Previous studies have reported that patients with a mutation on the *ABCA4* gene have a broad spectrum of phenotypic presentations due to many variants. In general, patients with STGD1 have progressive bilateral central vision loss. Disease onset typically occurs in childhood, early adulthood, or, sometimes, after the fourth decade of life. Several studies have found that disease severity is linked to the commencement of disease presentation, with earlier onset being associated with more severe disease.<sup>3,9–11</sup> In early-onset STGD1, spectral-domain OCT usually shows a loss of central macular architecture and the relative preservation of the peripheral macula.<sup>3</sup> Later-onset STGD1 usually has a milder phenotype with a better prognosis and is associated with foveal sparing. Using OCT, a study in Hungarian patients with

**Table 1** Next-Generation Sequence Analysis of Our Patient

Gene	Variant	Zygosity	Variant Classification
<i>ABCA4</i>	c.5714+5G>A (Intronic)	Homozygous	Pathogenic
<i>CACNA1F</i>	c.3901A>G (p. Met1301Val)	Heterozygous	Uncertain significance
<i>EYS</i>	c.2027C>T (p. Thr676Met)	Heterozygous	Uncertain significance
<i>KCNV2</i>	c.731G>A (p. Arg244His)	Heterozygous	Uncertain significance
<i>KLHL7</i>	c.1372A>C (p. Thr458Pro)	Heterozygous	Uncertain significance
<i>MKKS</i>	c.1161+3A>G (Intronic)	Heterozygous	Uncertain significance
<i>USH2A</i>	c.14531C>T (p. Thr4844Met)	Heterozygous	Uncertain significance

STGD1 found decreased foveolar thinning and decreased macular thinning.<sup>12</sup> Investigators also found a linear correlation between BCVA and foveolar and macular thickness. Upon OCT, our patient had significant visual acuity loss associated with decreased macular thickness and volume. Our patient findings are comparable with those of the Hungarian cohort.

Additionally, Hargitai et al<sup>12</sup> found that all the patients with the c. 5714+5G>A allele had a retinal phenotype defined by extensive atrophy of the RPE. Our patient had retinal ophthalmoscopic and OCT findings compatible with macular atrophy in both eyes. Our findings were consistent with those of Hargitai's study.<sup>12</sup>

Fundus autofluorescence of patients with STGD1 initially demonstrates reduced central autofluorescence in the macula, which represents RPE atrophy, surrounded by an increased signal or a bull's eye maculopathy appearance.<sup>3</sup> Longitudinal studies on the course of STGD1 disease have observed RPE atrophy expansion to progress at a rate of 4.47 mm<sup>2</sup> per year in patients with FAF imaging showing multiple areas of low autofluorescence signal at the posterior pole with a heterogeneous background.<sup>13</sup> This rate was faster compared to those of patients with other morphologies visible on FAF imaging.<sup>13</sup> Our patient had extensive RPE atrophy, which is similar to the increased rate of atrophy found in the former group.

Similarly, a genotype–phenotype study by Lee et al in patients with STGD1 found that patients with an FAF-identified morphology of macula-wide atrophy, affected mid-periphery, and central scotoma with confluent fleck accumulation to be associated with the allele c.5714+5G>A.<sup>14</sup> In addition, this study found, contrary to prior studies, that this allele was associated with a clinically severe phenotype.<sup>14</sup> Our patient's findings are comparable to those of Lee and team, in terms of FAF findings of and the presence of the pathogenic allele in our patient.

Later-onset STGD1 patients usually have good visual acuity and isolated macular dysfunction that will be present on a normal full-field ERG.<sup>9,11</sup> Full-field electroretinography can be helpful in confirming the diagnosis and serves as a classification and prognosis predictor. Patients with ERGs that show a generalized loss of cone function but preserved rod function are classified as having moderately severe disease.<sup>3</sup> Our patient's ERG showed a loss of cones with preserved rod function. Our patient's history and clinical findings are compatible with those of later-onset-STGD1 studies.<sup>3,9</sup> Furthermore, her ERG showed functional cone loss. This finding is consistent with Green et al's study on patients with homozygous mutations on the c.5714+5G>A allele.<sup>8</sup>

Earlier studies on the c.5714+5G>A allele have described homozygous patients with this mutation as having milder disease with later onset and slower progression.<sup>4,8,15</sup> Green et al observed that, in their study population, this mutated allele was associated with a “definite” phenotype that leads to vision loss by middle age.<sup>8</sup> Interestingly, a genotype–phenotype analysis of patients with this variant showed that, in patients with the c.5714+5G>A allele, the vision loss was mild to moderate and that homozygotes had less severe disease that appeared later compared to compound heterozygotes. Patients in this cohort with the homozygous inheritance of this allele experienced disease onset at or after the fourth decade of life, mild-to-moderate loss of visual acuity, central scotoma, and normal rod response with normal to decreased cone response. Our patient had late-onset disease and bilateral central scotoma. Our findings are compatible with those of Green's report.

A study of patients with late-onset STGD1 (after the fourth decade of life) found that late-onset disease had clinical overlap with juvenile-onset disease except for the factors of later age at onset, slower disease progression, and greater visual acuity.<sup>9</sup> However, visual acuity, fundus imaging results, and disease progression varied greatly in their cohort. Our patient had late-onset disease and a significant loss of visual acuity. The severity of her disease could have been due to factors such as smoking and late diagnosis.

*ABCA4* intronic variants have been previously studied.<sup>16</sup> Intronic variants that are located at splice donor sites are mostly situated at +3 to +6 downstream of exons, while intronic noncanonical splice-site variants at acceptor sites are located at positions −14 to −3 upstream of exons. Sangermano and co-workers found that the intronic allele c.5714G>A, which corresponds to exon 40, resulted in approximately 40% normally spliced *ABCA4* mRNA, which leads to residual protein activity.<sup>16</sup> Therefore, the residual activity of *ABCA4* mutants may play a significant role in determining disease severity.<sup>16–18</sup> Our patient had an intronic mutation in the *ABCA4* gene, which could explain the severity of her disease. Moreover, she had additional variants of uncertain significance (VUS) in other retinal disease-associated genes. These results should be considered with caution due to the clinical unpredictability of VUS.<sup>19</sup> There is no consensus on the interpretation of VUS, and further studies should clarify their roles on phenotypic expression of inherited macular dystrophies and the possibility of molecular interactions.<sup>19–21</sup> Findings could potentially reveal a role in our patient's disease severity.

The consanguinity present in relatively isolated populations may be responsible for the founder effect that is commonly found in patients of this disease. Our patient's parents were first cousins.<sup>4,9,12</sup> Finding this degree of consanguinity in the parents of an individual with STGD1 is not unusual, which is supported by previous reports.

Much of the immigration from Canada to Puerto Rico took place during the War of 1812. This gene may have reached the island during that period of time. Additionally, this gene is relatively common in European populations,<sup>22</sup> which is an interesting finding that reflects social and migratory genetics.

## Conclusion

This is the first report of a genotype–phenotype correlation in a Puerto Rican patient with STGD1. Patients with STGD1 due to presumed mild or moderate mutations in the ABCA4 gene may have a more severe presentation and progression of the disease. Genotyping all Puerto Rican patients with STGD1 is warranted.

## Ethics Approval and Informed Consent

Institutional Review board (IRB) approval for this study was not required. The patient provided informed written consent for the case and images to be published.

## Disclosure

Dr Natalio J Izquierdo-Encarnacion reports personal fees from Rhythm Corporation, outside the submitted work. The authors report no other conflicts of interest in this work.

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