Published in final edited form as:

Retina. 2019 December ; 39(12): 2311–2325. doi:10.1097/IAE.0000000000002316.

CLINICAL CHARACTERIZATION OF STARGARDT DISEASE PATIENTS WITH THE p.N1868I ABCA4 MUTATION

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Abstract

Purpose: To investigate the Stargardt disease phenotype associated with an unusually common and “extremely hypomorphic” *ABCA4* variant, p.N1868I.

Methods: The charts of 27 patients with p.N1868I on one allele and a severe/deleterious mutation on the other allele were reviewed. Subjective age of onset, best-corrected visual acuity, and stage of disease were recorded for all 27 patients, 18 of whom had multiple visits. When available, fundus photography, spectral domain optical coherence tomography, fundus autofluorescence, full-field electroretinograms, Goldmann visual fields, and fluorescein angiography were included. Five families with multiple affected members were analyzed.

Results: The median age at symptom onset was 41.5 years, and 3 p.N1868I patients had not developed visual symptoms as of the most recent eye examination. Median best-corrected visual acuity in the better-seeing eye at baseline was 20/25⁻², and the median duration from symptom onset to legal blindness was 25 years. The five families described in this study demonstrated clinically significant intrafamilial variability, and affected family members who did not share the p.N1868I variant had relatively more severe phenotypes.

Conclusion: This study demonstrates the consistency of foveal sparing, the variation in age at onset, the intrafamilial variability, and the prognosis with regard to visual acuity in p.N1868I-associated Stargardt disease.

Keywords

ABCA4; foveal sparing; p.N1868I; Stargardt disease

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None of the authors has any financial/conflicting interests to disclose.

ABCA4-associated autosomal recessive Stargardt disease¹ is the most common monogenic macular dystrophy.² Onset of Stargardt disease symptoms is most common in childhood or the teenage years.^{3,4} However, a proportion of patients can have onset in early adulthood (adult-onset) or late in adulthood (late-onset).^{5,6} Late-onset *ABCA4*-associated retinal dystrophy tends to be associated with parafoveal atrophy and relative foveal sparing.^{7–10}

Over 1,000 different disease-associated mutations have been found on the *ABCA4* gene.¹¹ On the more severe end of the *ABCA4*-associated inherited retinal degeneration spectrum are early-onset retinitis pigmentosa and cone-rod dystrophy phenotypes,^{12–14} which are typically associated with null or otherwise severe mutations on both *ABCA4* alleles. Early-onset Stargardt disease is often associated with severe and/or moderate *ABCA4* mutations.¹⁵

Adult- and late-onset Stargardt disease phenotypes are often associated with milder missense mutations,^{8,15} which may retain partial *ABCA4* protein function. Some especially mild missense mutations are highly associated with less severe forms of Stargardt disease.^{8,16} However, many late-onset Stargardt disease cases have been found to have one mutant allele, and in many of those cases, the second allele was not found.^{8,9} A recent report by Zernant et al¹¹ explains a large proportion of these adult- and late-onset Stargardt disease cases by demonstrating that the second allele in many of these cases harbored a single *ABCA4* variant, p.N1868I (c.5603A>T). This variant was previously considered not to be disease-associated.^{17–19} The frequency of the p.AsnN1868Ile allele, ~7%, is unusually high in the general population for a disease-associated allele (gnomAD database; <http://gnomad.broadinstitute.org/>). Because of the high frequency of p.N1868I in the general population, some investigators found similar frequencies of the p.N1868I variant in patients with Stargardt disease and unaffected control subjects (Stone, 1998, Rivera, 2000).^{18,20} Other studies found the p.N1868I variant to occur more frequently in patients with Stargardt disease than in control populations.^{19,21–24} Both biochemical¹⁷ and computational analyses²² have shown that the p.N1868I mutation may have a small negative effect on *ABCA4* protein function. The combination of these factors led some authors to describe the allele as a “risk factor” for Stargardt disease.^{22–24} Recently, we demonstrated that the p.N1868I variant does cause adult-onset and late-onset Stargardt disease when the opposing *ABCA4* allele carries a deleterious mutation.¹¹ These could be stop mutations, frame-shift mutations, severe splice-altering mutations, or deleterious missense mutations.

Clinical descriptions of patients with the genotype investigated in this study, with p.N1868I on one allele and a deleterious mutation on the other *ABCA4* allele, are sparse in the literature. Thus, we present in this study clinical data on 27 such patients with Stargardt disease, demonstrating a number of clinical findings in this group of patients, including the consistency of foveal sparing, and an adult- or late-onset phenotype among these patients. We further demonstrate the variability of presentations among family members who share the p.N1868I mutation, and we also demonstrate the relatively milder phenotype p.N1868I patients can have when compared with family members who have *ABCA4*-associated retinal degenerations but do not share the p.N1868I variant.

Methods

We retrospectively reviewed the charts of 27 patients with Stargardt disease, all of whom were compound heterozygous for *ABCA4* mutations, with one allele harboring the p.N1868I variant and the other allele carrying a deleterious mutation. In this article, patients with only the p.N1868I variant on one *ABCA4* allele and a deleterious mutation on the other allele are referred to as “p.N1868I patients.” This name would therefore exclude patients who have complex p.N1868I alleles, which have other mutations on that same allele and can therefore be more severe than the p.N1868I allele alone.¹¹ The patients were examined and diagnosed by one of the authors (G.A.F.), and all had findings consistent with Stargardt disease, including yellow-white retinal flecks with atrophic lesions in the macula. Exceptions include one case in which flecks were not evident until a follow-up visit, another who did not have overt flecks as of the most recent visit, and a third who exhibited flecks without ophthalmoscopically apparent atrophy in the macula (a “fundus flavimaculatus” phenotype). Genetic testing was performed at The Laboratory of Molecular Genetics at Columbia University. The entire *ABCA4* gene locus was sequenced in 17 patients; the *ABCA4* gene, including all exons and intron/exon boundaries were sequenced in 4 patients. In the remaining 6 cases representing family members, only targeted testing was performed. *ABCA4* variants and subjective age at onset for 19 of these patients were reported in a previous article.¹¹

Several findings were recorded from the patient records: subjective age at onset, first subjective complaint(s), age at each examination date, best-corrected visual acuity (BCVA) at each examination, and retinal findings, including macular findings, peripheral findings, and stage. Visual acuities were tested with either a Snellen acuity chart or a Feinbloom Low Vision chart, and these acuities were converted to logarithm of the minimum angle of resolution (logMAR) format for analyses. The stage of Stargardt disease was defined as follows: Stage 1 was characterized by flecks confined to the macula; Stage 2 was characterized by extension of flecks beyond the macula; Stage 3 was characterized by predominantly resorbed flecks; and Stage 4 was characterized by extensive atrophy of the retinal pigment epithelium (RPE) and choroid involving both the macula and peripheral retina.²⁵ Longitudinal analyses were performed on BCVA and stage because those findings were consistently documented at all visits.

Ancillary imaging and functional tests, where available, were also included in the analysis. Color fundus photographs of the macula and mid-periphery were available in 25 cases. These color photographs, in addition to chart sketches and descriptions, were used to determine the presence of peripheral retinal lesions, such as peripheral reticular degeneration. Spectral domain optical coherence tomography (SD-OCT), near-infrared autofluorescence (NIR-AF), and short-wavelength autofluorescence (Heidelberg Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany) of the central 30° were available in 18, 17, and 7 cases, respectively, and aided in the assessment of macular changes (such as foveal sparing) and stage of disease. Goldmann (kinetic) visual field results were available in 15 cases and were analyzed qualitatively. Clinical full-field electroretinography (ERG) had been performed on eight patients using a variety of systems, including a Nicolet Viking IV system (Nicolet Biomedical, Inc, Madison, WI) or a

Diagnosys ColorDome (Diagnosys LLC, Lowell, MA), and one was performed by an outside clinic; thus, different normative ranges were used depending on the instrument. The dark-adapted dim flash (rod-isolated) as well as the light-adapted single-flash and 32-Hz flicker responses (both cone-isolated) were analyzed according to the Lois criteria.²⁶ Fluorescein angiography, which was available in the charts of four patients (all but one of these performed by an outside clinic), was analyzed for the “dark choroid” sign.

Four subjects with either early-onset Stargardt disease or cone-rod dystrophy were included in the study as a contrast to the p.N1868I patients. These 4 unrelated subjects were first-degree relatives of at least one of the 27 p.N1868I patients, but none of these family members harbored the p.N1868I allele. One of these four family members was genotyped by The John and Marcia Carver Nonprofit Genetic Testing Laboratory and another was genotyped by the Casey Eye Institute Molecular Diagnostic Laboratory.

Statistical analyses were performed with Microsoft Excel (Redmond, WA) and vassarstats.net (Richard Lowry, Poughkeepsie, NY).

This study was approved by the Western IRB and the IRB of Columbia University and conformed to the principles of the Declaration of Helsinki. Informed consent was obtained from all patients.

Results

Demographics

Twenty-seven p.N1868I patients (18 females and 9 males, all non-Hispanic white) from 21 families were seen for the first time by one author (G.A.F.) at a mean age of 46.6 years (range 17.3–73.8, median 49.4). Demographics and *ABCA4* mutations and pathogenicity classes for *ABCA4* variants^{17,27,28} are listed in Table 1. Four affected family members who did not share the p.N1868I allele are not listed in the tables.

p.N1868I *ABCA4* Case Families

Four of the p.N1868I probands in this cohort had family members who were diagnosed as having either Stargardt disease or cone-rod dystrophy due to mutations other than p.N1868I. In all four of these families, the patients with the p.N1868I allele had a considerably milder form of Stargardt disease by comparison with the affected family members without the p.N1868I allele. In two of these families, multiple members had the p.N1868I mutation and showed some variability in the onset of visual symptoms even within these families and among patients who shared both *ABCA4* alleles. In a fifth family, two sisters with the p.N1868I allele were affected with very similar forms of late-onset Stargardt disease. Table 1 lists the mutations and family numbers of the p.N1868I patients discussed below, and Figure 1 shows pedigrees for each of the five families.

Family #1.—The proband of Family #1 (Patient #1, II:1 in pedigree Figure 1A) and his paternal female cousin (Patient #2, II:2) both carried the complex allele p.[L541P; A1038V] and p.N1868I. The proband developed blurred vision at age 30 years, while his cousin developed a paracentral black spot at the age of 25 years. Both cousins developed foveal

atrophy in the left eye. Patient #2 also developed optic atrophy from an episode of optic neuritis in the left eye. However, both cousins maintained some relative foveolar sparing in their right eyes, with BCVAs of 20/4022 at age 40 years in Patient #1 and 10/2522 (Feinbloom chart) at age 33 years in Patient #2.

The proband's paternal aunt (mother of the Patient #2, I:4) was diagnosed with Stargardt disease at age 15 years, although her symptom of poor vision was known in childhood. She was found to have the complex allele p.[L541P; A1038V] and p.G1961E. At her first full examination in our clinic at age 31 years, she was found to have Stage 1 Stargardt disease with foveal atrophy and BCVA of 20/200 in the right eye and 20/300 in the left eye. And, at the most recent follow-up visit at age 61 years, she was found to have large atrophic macular lesions and fundus flecks extending out to the mid-periphery with some resorption (Stage 2–3) and BCVA of 5/180 in the right eye and 5/350 in the left eye with the Feinbloom Low Vision chart.

Family #2.—Representative NIR-AF images of four p.N1868I patients from Family #2 are shown in Figure 2, and the family pedigree is shown in Figure 1B. The proband of this family (Patient #4) was found to have the p.[L541P; A1038V] complex allele and p.N1868I. He reported metamorphopsia in the right eye at the age of 48 years. At his initial visit at age 52 years, he was found to show bull's eye-appearing macular lesions without evident fundus flecks. His full-field ERG at the time showed normal rod-mediated amplitudes, as well as a quite normal cone-mediated single-flash response, although his 32-Hz flicker amplitude was slightly reduced compared with normal. By the time of the most recent visit at age 61 years, he had developed modest flecks at the outer margins of his bull's eye macular lesions. He had maintained foveal sparing in both eyes, with acuities of 20/25+2 in the right eye and 20/25+1 in the left eye.

The proband's sister (Patient #3) had identical *ABCA4* alleles, and she developed symptoms of difficulty with night driving around age 52 years. She did not notice problems with central vision until age 58 years. By the time of her first examination in our clinic (age 62 years), she was found to have Stage 1 flecks, with moderately large macular lesions, showing both RPE and choroidal atrophy in both eyes. A small island of foveolar sparing was noted in the left eye. Her BCVA at that visit was 20/200 (10/80–1 Feinbloom) in the right eye and 20/8021 in the left eye. Although the ages at symptom onset were similar between the two siblings, these macular findings are in contrast with those of her brother, whose macular lesions were considerably smaller and spared more of the fovea at a similar age (Figure 2A vs. Figure 2B).

The proband's son was found to have the complex allele p.[L541P; A1038V] and p.P1380L. He failed a school eye screening in kindergarten and was eventually diagnosed with cone-rod dystrophy at age 7 years based on a full-field ERG (done by an outside laboratory). The ERG reportedly showed essentially nondetectable cone-mediated responses and subnormal scotopic responses. At his first visit to our clinic at age 8 years, his macula showed mottling in both eyes and his BCVA was 20/200 in each eye. At his most recent follow-up visit at age 27 years, his BCVA was 10/225 in the right eye and 10/400 in the left eye.

A daughter (Patient #6) of the proband had the p.N1868I and p.P1380L mutations and developed symptoms of mild central vision reduction and photoaversion around age 17 years. She was a high myope, with spherical equivalents of -14.00 D in the right eye and -13.00 D in the left eye at her most recent visit at age 30 years, when her BCVA was still 20/40–1 in the right eye and 20/70 in the left eye. Her retina showed round atrophic macular lesions with relative foveolar sparing, more evident in the right eye, but no definite fundus flecks.

A second daughter (Patient #5) of the proband was asymptomatic as of her only examination at age 27 years, with BCVA of 20/15 in the right eye and 20/15–2 in the left eye and no ophthalmoscopically evident changes consistent with Stargardt disease. Of interest, she was found to possess the same mutations as her sister, p.N1868I and p.P1380L, despite the difference in phenotypes between the two sisters. Near-infrared autofluorescence showed subtle parafoveal hyperautofluorescence in both eyes. Spectral domain OCT demonstrated enhancement of the parafoveal external limiting membrane (ELM) and hyperreflective foci in the outer nuclear layer, but otherwise normal retinal anatomy (Figure 3). Spectral domain OCT line profiles of the outer retina demonstrate that her parafoveal ELM showed a distinctly enhanced reflectance relative to control subjects, whereas her foveal ELM was only slightly enhanced.

Family #3.—Patient #7 of family #3 had p.N1868I and p.P1380L mutations, and she was asymptomatic with only punctate drusenoid changes in the macula and no definite changes on either NIR-AF or SD-OCT. Her SD-OCT, along with line profiles showing a normal ELM, is shown in Figure 3. Of note, she had been treated with 400 mg per day hydroxychloroquine for 2 years for lupus before the visit in which the images in Figure 3 were obtained. Her son (the proband of Family #3, pedigree in Figure 1C) had early-onset Stargardt disease, and genetic testing found that he received the p.P1380L mutation from his mother and a complex *ABCA4* allele containing p.N1868I and IVS38:c.5461–10T>C from his unaffected father. He first noticed problems with reading at age 10 years, at which time his BCVA was 20/200 in the right eye and 20/50–2 in the left eye. His retinas showed oval foveal lesions with Stage 2 flecks.

Family #4.—The proband of Family #4 (Patient #8, pedigree shown in Figure 1D) was found to have p.N1868I and p.R653C *ABCA4* mutations. Her first symptoms were difficulty with night vision starting at age 45 years. Her BCVA at age 50 years was 20/20 in each eye, and her fundus examination showed Stage 2 fundus flecks and bull's eye-appearing atrophy in the macula. The proband's daughter shares the p. R653C with her mother but has the p.G1961E mutation on the other allele. The daughter had symptoms that started with difficulty in focusing around age 19 years, and her BCVA at the first visit (age 25 years) was 20/200–1 in the right eye and 20/80–1 in the left eye.

Family #5.—Two sisters who shared the p.N1868I and IVS36:c.5196+1G>A mutations had very similar clinical appearances, with Stage 2 fundus flecks, round atrophic lesions in the macula sparing the fovea in their 7th decade. The proband (Patient #20, pedigree shown in Figure 1E) first noted localized paracentral blur in the right eye around age 62 years. Nonetheless, she has maintained foveal sparing with BCVA of 20/20–3 and 20/20–2 up to

her most recent visit at age 70 years. The affected sister (Patient #19) had been previously diagnosed with pattern dystrophy and tested negative for the RDS gene by an outside clinic and laboratory. She reported photoaversion starting at age 57 years and glare during night driving starting at age 62 years. These symptoms may be at least partially attributed to modest nuclear sclerotic and cortical cataracts in both eyes. Her BCVA at age 67 years was 20/20–2 in each eye.

Symptoms and Onset

Subjective age at symptom onset and initial symptoms for p.N1868I patients only are listed in Table 2. Three of the 27 p.N1868I patients had no symptoms (Patients 5, 7, and 13, at ages 27, 48, and 60 years, respectively). The younger two of those three patients had no fundus flecks, and the older two described mild sensitivity to bright lights that could be considered normal variation. Another 2 patients who reported no symptoms at the baseline examination both developed symptoms within 2 years after that examination (Patients 9 and 16, at ages 29 and 50 years, respectively). The 24 symptomatic patients had onset of symptoms attributable to Stargardt disease at a mean age of 39.7 years (range 17–71, median 41.5). The majority of the initial complaints involved central or paracentral visual changes. Several patients also complained of significant photoaversion, glare, slow dark adaptation, or nyctalopia. Two noted mild color discrimination difficulties along with other symptoms. Note that these were the initial symptoms only, and many patients eventually developed a mix of visual symptoms.

Visual Acuity

Visual acuity data at the first and last clinic visits of the 27 p.N1868I patients are listed in Table 3, and BCVA in the better-seeing eye at all visits is shown in a spaghetti plot in Figure 4. The mean BCVA in the better-seeing eye of the 27 p.N1868I patients was 20/25 Snellen (0.10 logMAR), with 25 patients having BCVA of 20/40 Snellen or better, 1 having BCVA of 20/50, and 1 having 20/80 in the better-seeing eye. Thus, none of the 27 patients were legally blind at the first visit, although two of the lesser-seeing eyes were around 20/200.

Fourteen patients were examined at least one time between 1 and 3.5 years after the baseline visit, and none of these 14 patients had BCVA worse than 20/50 in the better-seeing eye in that time frame. As can be seen in Figure 4, visual acuities between 20/60 and 20/100 were uncommon, with loss of acuity tending to jump from 20/50 or better to worse than 20/100. Once foveal sparing was lost, patients tended to stabilize between 20/125 and 20/200 (0.80–1.00 logMAR), but 3 eyes reached the 20/250 to 20/400 range (1.10–1.30 logMAR), and one eye was 20/800 (1.60 logMAR) at the final visit. The first detected loss of visual acuity to worse than 20/100 in the better-seeing eye (legal blindness) was at 6 years after symptom onset (in two patients) or after 4 years of follow-up (in three patients). Using a Kaplan–Meier analysis, we found the median interval from subjective onset of symptoms (in the 24 symptomatic patients) to loss of vision in both eyes worse than 20/100 was 25 years (95% confidence interval 10–34 years). Considering right eyes only, the median time was 17 years (95% CI 10–34 years). When measured with the first visit as the baseline (in 18 patients with more than one visit), the median interval to loss of BCVA worse than 20/100 in the better-seeing eye was 9 years (95% CI 8–24 years). For right eyes only, the median time was

also 9 years (95% CI 8–17 years). Inclusion of three asymptomatic patients did not significantly affect these calculations because those three patients were only seen once.

Stage

Stargardt disease stages at the first and last examinations are listed in Table 3. Most patients below age 40 years had a Stage 1 appearance, whereas the patients older than 40 years were primarily Stage 2. The two patients who had neither flecks nor macular atrophy (#5 and #7) were not staged. Two other patients had parafoveal atrophic changes without definite flecks at the first visit and were arbitrarily included as Stage 1. Of the 23 patients with evident fundus flecks, 7 were Stage 1, 1 was between Stages 1 and 2, 14 were Stage 2, and 1 was Stage 3. Among the 18 patients with at least one follow-up visit, one patient without definite flecks developed overt flecks (Stage 1). Another Stage 1 patient progressed slightly to between Stages 1 and 2. Three of the Stage 1 or Stage 1 to 2 patients progressed to Stage 2, while two Stage 2 patients progressed to between Stages 2 and 3. In some cases (Figure 5B), fundus flecks appeared to be smaller in number, larger in size, and more spread out than usually seen in Stargardt disease, as described by Zernant et al.¹¹

Macula

Representative macular photographs are shown in Figure 5. There was at least partial foveal sparing in all 27 p.N1868I patients at their first visits, ranging from ophthalmoscopically normal in 2 patients (#5 and #7) to a very small island of foveolar sparing in only one eye of Patient #3. One patient (#13) had a fundus flavimaculatus phenotype, with evident Stage 2 flecks, but no definite atrophic lesion in the macula. Macular atrophic changes in the remaining 23 patients ranged from single small atrophic parafoveal changes to partial or complete “bull’s eye” atrophy with foveal sparing.

Peripheral Retina

Peripheral retina findings are listed in Table 4 and example images of peripheral findings are shown in Figure 6. Some patients showed a net-like pattern to their flecks, and some had pigmented flecks; an example patient with both is shown in Figure 6A. Of 25 patients who had available retinal photography that included at least the retinal mid-periphery, 6 were determined to have deep pigment consistent with peripheral reticular degeneration. Two of these patients were younger than 50 years, one was 53 years old, and the remaining 3 were older than 60 years. One of these six patients with peripheral reticular degeneration also had a large hypertrophic pigmented and fibrotic lesion temporal to the macula (Figure 6B).

Goldmann Visual Field, Full-Field Electroretinography, and Fluorescein Angiography

Goldmann visual field, full-field ERG, and fluorescein angiography findings are summarized in Table 4.

Two representative Goldmann visual fields are shown in Figure 7. Centrally, one patient had no detected sensitivity losses to the II2e target, several had paracentral and/or central relative scotomas (Figure 7A), and one had large central scotomas to the V4e target (Figure 7B). Among 15 p.N1868I patients who had at least one Goldmann visual field, all had normal peripheral boundaries to the size II4e target. Three demonstrated mild or moderate

constriction when tested with the II2e (the smallest, dimmest target regularly used in this clinic), and one subject had gross loss to the II2e target (Figure 7B). An additional three patients eventually developed mild or moderate constriction to the II2e target only.

Eight patients who had full-field ERGs were found to have both light-adapted and dark-adapted responses that were within normal limits. The light-adapted single-flash b-wave was slightly below the lower limit of normal in Patient 11, but his 32-Hz flicker amplitudes were well within normal limits. Patient #4 had the opposite findings, with a normal single-flash b-wave amplitude and a slightly subnormal 32-Hz amplitude. Patient #6, who was highly myopic, had reduced full-field ERG amplitudes; however, because of a noisy signal, the test result was felt to be unreliable.

Fluorescein angiography images were available in four p.N1868I patients. The “dark choroid” sign was evident in three patients and uncertain in one patient.

Discussion

Our findings demonstrate the Stargardt disease phenotypes associated with an “extremely hypomorphic” *ABCA4* mutation (p.N1868I). This variant is found in approximately 6.6% of the general population of European descent and 20% of *ABCA4*-associated retinal dystrophy cases.^{11,19,22} Because the p.N1868I mutation is so highly prevalent (and therefore presumably ancient), it is also found on the same allele (“in *cis*”) with several other *ABCA4* mutations of varying severity.¹¹ In fact, of the 31 patients discussed in this article, 4 had p.N1868I complex alleles. The recent elucidation of the role of the p.N1868I mutation, which was previously primarily considered a benign variant, provided the second allele in 57% of previously unsolved *ABCA4* cases in whom only one disease-associated mutation had been found.¹¹ More specifically, around 80% of previously monoallelic late-onset Stargardt disease cases were solved.¹¹ The focus of this study is on the phenotype of these newly solved cases in which the p.N1868I mutation accounts for one allele and the other *ABCA4* allele contains a relatively severe mutation. We find consistency in the phenotype of these patients, including later onset of symptoms and relative foveal sparing.

Five (18.5%) of the subjects in this cohort of p.N1868I patients had no symptoms (or minimal symptoms such as very mild sensitivity to light) at the baseline examination (mean age 43 years, range 27–61). Two of those were followed long enough that they eventually developed symptoms attributable to Stargardt disease, at the ages of 29 and 50 years. The finding of asymptomatic patients among a cohort of foveal-sparing Stargardt disease is not uncommon^{8,10} due to incidental detection of retinal changes by referring doctors, or referral of siblings for examination once one sibling is found to have Stargardt disease. One p.N1868I patient was first symptomatic in her late teens, and the rest developed symptoms after age 20 years. The age cutoff for adult- versus late-onset Stargardt disease has varied among studies between 35 years and 45 years of age.^{7,15,29} With a median onset of symptoms at age 41.5 years in this cohort, roughly half of the symptomatic patients were “adult-onset” and the other half were “late-onset.”

The median logMAR BCVA was 0.14 (20/25–2 Snellen) at a mean age of 46.6 years at the first visit among the p.N1868I patients in this study. This initial-visit BCVA is quite similar to that found in other studies of late-onset and/or foveal-sparing Stargardt disease, where patients were selected based on phenotype rather than by specific *ABCA4* mutations (Fujinami et al, 2013; Lambertus et al, 2016a).^{8,10} On the other hand, one study of p.G1961E patients found a median logMAR BCVA of 0.87 (~20/150 Snellen) in the better eye at a mean age of 27.6 years at first visit.¹⁶ Thus, the phenotype of the p.N1868I mutation is demonstrably milder than even the “mild” p. G1961E mutation, with later onset of symptoms¹¹ and characteristically better visual acuity at a later age.

Excluding the patient who was 20/80–1 in the better eye at her only visit, our longitudinal analysis found no patients who lost visual acuity to worse than 20/50 in the first few years after the baseline visit. We found the median interval from subjective onset of symptoms to loss of vision worse than 20/100 in both eyes to be at least a decade. Our estimate overlaps that found in a similar type of analysis by Lambertus et al,¹⁰ who reported a median interval between disease onset and visual acuity decline to 20/200 of 11.4 years in late-onset patients with Stargardt disease. Confidence intervals in this study are broad because of the relatively small number of subjects with extended follow-up. However, we may comment from these longitudinal BCVA data that the time from symptom onset to legal blindness in this group is at least 5 years for the most rapidly progressing patients, and it is one or more decades for the average p.N1868I patient.

Although relative foveal sparing and good initial visual acuity appear to be defining features of the p.N1868I phenotype, several patients in this cohort did eventually show progressive visual acuity loss. Therefore, therapeutic intervention in these patients may still be considered, keeping in mind that progression is slow, and efficacy may be difficult to prove due to the slow disease progression. Other biomarkers, such as area of decreased fundus autofluorescence (and change in that area), may be needed to shorten the duration of treatment trials.^{10,30}

Peripheral reticular degeneration, which was found in 6 (22%) of the p.N1868I patients in this study, has not been previously associated with Stargardt disease to our knowledge. This phenotype, which has been referred to by various other names, such as senile reticular degeneration,³¹ is a benign, deep pigmentary change that forms polygonal patterns in the peripheral retina. It starts at the equator in the nasal (or superior-nasal) retina, with more advanced cases spreading anteriorly and posteriorly, as well as circumferentially.^{32,33} Peripheral reticular degeneration is highly associated with advancing age,³² as well as age-related macular degeneration.^{31,32,34} In one report of 104 patients with peripheral reticular degeneration, only one was younger than 50 years, and 82% were older than 60 years.³⁴ In postmortem histology, none of the 129 subjects younger than 50 years were found to have peripheral reticular degeneration.³² In this context, our finding of 2 patients younger than 50 years out of a total of 6 patients with peripheral reticular degeneration suggests that the finding of deep peripheral pigment in our cohort could, at least in part, be associated with Stargardt disease, and not an incidental finding due to the relatively advanced age of the cohort.

We found that ERG amplitudes were preserved in all seven patients with reliable testing. This compares with 67% in ERG group 1 in a study of 33 patients with foveal sparing.⁸ Similarly, peripheral boundaries on Goldmann perimetry generally did not show restriction. Only subsets of the patients in this study had available full-field ERGs and perimetry. However, a combination of findings in this study, such as the full-field ERG and Goldmann visual fields, as well as very few patients reaching Stage 3, suggests that diffuse retinal degeneration in this group is rare. Notably, none of our patients exhibited extensive atrophy beyond the macula, such as the “fundus pattern 4” described in a subset of foveal-sparing Stargardt disease patients by Fujinami et al.⁸

The families described in this study demonstrate variability among patients with p.N1868I mutations, even among members of the same family with identical *ABCA4* genotypes. The comparison to non-p.N1868I *ABCA4* disease patients within four families highlights the hypomorphic nature of the p.N1868I phenotype. These families were also instrumental in determining the severity of the non-p.N1868I mutations. The p.P1380L mutation has been found to be more common in nonfoveal-sparing Stargardt disease than patients with foveal sparing.⁸ In two cases of early-onset *ABCA4*-associated retinal degeneration (Family 3 II:1 and Family 4 II:1, from Figure 1, C and D), the p.P1380L mutation was found in *trans* with known deleterious variants. These data suggest that the p.P1380L variant is a notably severe, likely deleterious, missense mutation. With three p.N1868I patients (Patients #5, #6, and #7) in this study having the p.P1380L mutation in *trans* with p.N1868I, we can deduce the severity of the p.P1380L variant from a different perspective, using the “litmus test” of the p.N1868I allele.¹¹ The p.P1380L variant appears to be just deleterious enough to cause Stargardt disease when found on the opposing allele to the p.N1868I mutation. The variability of expression among these three patients could conceivably relate to other factors such as unaccounted-for modifier genes, environmental factors, or other ocular factors, such as the mechanical stresses of high myopia.

The consensus among investigators about the innermost hyperreflective outer retinal band is that it represents Müller cell footplates in the form of the ELM.^{35–37} We found an enhanced ELM and hyperreflective foci in the ONL on the SD-OCTs of several of the patients in this study,³⁸ one example of which is shown in Figure 3. In that case (Patient #5), the ELM was demonstrably enhanced in the parafoveal area, but not in the fovea. This finding contrasts with the greatly enhanced “convex” foveal ELM found in some patients with early-onset Stargardt disease.³⁸ One interpretation of this finding is that the lack of foveal ELM enhancement in our patient (relative to the parafovea) suggests that the foveal Müller cells are under relatively lower stress than the parafoveal Müller cells in our case (and other cases of adult-onset, foveal-sparing Stargardt disease). This interpretation would suggest that thickening of ELM precedes loss of photoreceptors and RPE, rather than being protective.³⁸

Conclusion

As others have suggested,⁸ the dichotomy of foveal involvement in most Stargardt disease and foveal sparing in a large subset might indicate that multiple mechanisms are at play in *ABCA4*-associated retinal degeneration. The finding of a mutation (p.N1868I) that is consistently associated with foveal sparing could lead to identification of those mechanisms.

³⁹ Many, but not all the patients in this cohort maintained foveal sparing (and the attendant good visual acuity) up to their latest follow-up visit. Any preventative treatment of this group would need to balance the risk of treating some of these patients who might not lose significant acuity for several years untreated, with the potential benefits to those who eventually do lose their foveal sparing. With the p.N1868I allele accounting for a significant number of patients with Stargardt disease, the relative homogeneity of this group of patients should lend itself to longitudinal studies of visual acuity loss as well as more sensitive biomarkers, such as progression of macular atrophy,^{10,30,40} conceivably leading to treatment trials requiring smaller numbers of patients than might otherwise be required to demonstrate efficacy. Delineating the prognosis of these patients may also suggest that one particular avenue of treatment, such as gene therapy, stem-cell transplantation, or pharmacological approaches,⁴¹ may be more appropriate than others for this foveal-sparing form of Stargardt disease.

Acknowledgments

Supported in part by grants from the Pangere Family Foundation, National Eye Institute/NIH P30EY001792 (core grant), EY021163, EY019861, and EY019007 (Core Support for Vision Research); and unrestricted funds from Research to Prevent Blindness to both the Department of Ophthalmology, Columbia University, and the Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago.

References

1. Allikmets R, Singh N, Sun H, et al. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nat Genet* 1997;15: 236–246. [PubMed: 9054934]
2. Stone EM, Andorf JL, Whitmore SS, et al. Clinically focused molecular investigation of 1,000 consecutive families with inherited retinal disease. *Ophthalmology* 2017;124:1314–1331. [PubMed: 28559085]
3. Fishman GA, Farber M, Patel BS, Derlacki DJ. Visual acuity loss in patients with Stargardt's macular dystrophy. *Ophthalmology* 1987;94:809–814. [PubMed: 3658351]
4. Aaberg TM. Stargardt's disease and fundus flavimaculatus: evaluation of morphologic progression and intrafamilial coexistence. *Trans Am Ophthalmol Soc* 1986;84:453–487. [PubMed: 3590477]
5. Noble KG, Carr RE. Stargardt's disease and fundus flavimaculatus. *Arch Ophthalmol* 1979;97:1281–1285. [PubMed: 454263]
6. Oh KT, Weleber RG, Oh DM, et al. Clinical phenotype as a prognostic factor in Stargardt disease. *Retina* 2004;24:254–262. [PubMed: 15097887]
7. Westeneng-van Haaften SC, Boon CJ, Cremers FP, et al. Clinical and genetic characteristics of late-onset Stargardt's disease. *Ophthalmology* 2012;119:1199–1210. [PubMed: 22449572]
8. Fujinami K, Sergouniotis PI, Davidson AE, et al. Clinical and molecular analysis of Stargardt disease with preserved foveal structure and function. *Am J Ophthalmol* 2013;156:487–501.e1. [PubMed: 23953153]
9. van Huet RA, Bax NM, Westeneng-Van Haaften SC, et al. Foveal sparing in Stargardt disease. *Invest Ophthalmol Vis Sci* 2014;55:7467–7478. [PubMed: 25324290]
10. Lambertus S, Lindner M, Bax NM, et al. Progression of late-onset stargardt disease. *Invest Ophthalmol Vis Sci* 2016;57: 5186–5191. [PubMed: 27699414]
11. Zernant J, Lee W, Collison FT, et al. Frequent hypomorphic alleles account for a significant fraction of ABCA4 disease and distinguish it from age-related macular degeneration. *J Med Genet* 2017;54:404–412. [PubMed: 28446513]
12. Cremers FP, van de Pol DJ, van Driel M, et al. Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene ABCR. *Hum Mol Genet* 1998;7:355–362. [PubMed: 9466990]

13. Martínez-Mir A, Paloma E, Allikmets R, et al. Retinitis pigmentosa caused by a homozygous mutation in the Stargardt disease gene ABCR. *Nat Genet* 1998;18:11–12. [PubMed: 9425888]
14. Maugeri A, Klevering BJ, Rohrschneider K, et al. Mutations in the ABCA4 (ABCR) gene are the major cause of autosomal recessive cone-rod dystrophy. *Am J Hum Genet* 2000;67:960–966. [PubMed: 10958761]
15. Yatsenko AN, Shroyer NF, Lewis RA, Lupski JR. Late-onset Stargardt disease is associated with missense mutations that map outside known functional regions of ABCR (ABCA4). *Hum Genet* 2001;108:346–355. [PubMed: 11379881]
16. Genead MA, Fishman GA, Stone EM, Allikmets R. The natural history of Stargardt disease with specific sequence mutation in the ABCA4 gene. *Invest Ophthalmol Vis Sci* 2009;50: 5867–5871. [PubMed: 19578016]
17. Sun H, Smallwood PM, Nathans J. Biochemical defects in ABCR protein variants associated with human retinopathies. *Nat Genet* 2000;26:242–246. [PubMed: 11017087]
18. Rivera A, White K, Stöhr H, et al. A comprehensive survey of sequence variation in the ABCA4 (ABCR) gene in Stargardt disease and age-related macular degeneration. *Am J Hum Genet* 2000;67:800–813. [PubMed: 10958763]
19. Maugeri A, Flothmann K, Hemmrich N, et al. The ABCA4 2588G>C Stargardt mutation: single origin and increasing frequency from South-West to North-East Europe. *Eur J Hum Genet* 2002;10:197–203. [PubMed: 11973624]
20. Stone EM, Webster AR, Vandenburgh K, et al. Allelic variation in ABCR associated with Stargardt disease but not age-related macular degeneration. *Nat Genet* 1998;20:328–329. [PubMed: 9843201]
21. Webster AR, Héon E, Lotery AJ, et al. An analysis of allelic variation in the ABCA4 gene. *Invest Ophthalmol Vis Sci* 2001; 42:1179–1189. [PubMed: 11328725]
22. Aguirre-Lamban J, González-Aguilera JJ, Riveiro-Alvarez R, et al. Further associations between mutations and polymorphisms in the ABCA4 gene: clinical implication of allelic variants and their role as protector/risk factors. *Invest Ophthalmol Vis Sci* 2011;52:6206–6212. [PubMed: 21330655]
23. Fritsche LG, Fleckenstein M, Fiebig BS, et al. A subgroup of age-related macular degeneration is associated with monoallelic sequence variants in the ABCA4 gene. *Invest Ophthalmol Vis Sci* 2012;53:2112–2118. [PubMed: 22427542]
24. Schulz HL, Grassmann F, Kellner U, et al. Mutation spectrum of the ABCA4 gene in 335 Stargardt disease patients from a multicenter German cohort-impact of selected deep intronic variants and common SNPs. *Invest Ophthalmol Vis Sci* 2017;58:394–403. [PubMed: 28118664]
25. Fishman GA. Fundus flavimaculatus. A clinical classification. *Arch Ophthalmol* 1976;94:2061–2067. [PubMed: 999551]
26. Lois N, Holder GE, Bunce C, et al. Phenotypic subtypes of Stargardt macular dystrophy-fundus flavimaculatus. *Arch Ophthalmol* 2001;119:359–369. [PubMed: 11231769]
27. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the american college of medical genetics and genomics and the association for molecular pathology. *Genet Med* 2015;17:405–424. [PubMed: 25741868]
28. Zhang N, Tsybovsky Y, Kolesnikov AV, et al. Protein misfolding and the pathogenesis of ABCA4-associated retinal degenerations. *Hum Mol Genet* 2015;24:3220–3237. [PubMed: 25712131]
29. Collison FT, Fishman GA. Visual acuity in patients with Stargardt disease after age 40. *Retina* 2017. doi: 10.1097/IAE.0000000000001903.
30. Strauss RW, Muñoz B, Ho A, et al. Progression of Stargardt disease as determined by fundus autofluorescence in the retrospective progression of Stargardt disease study (ProgStar report No. 9). *JAMA Ophthalmol* 2017;135:1232–1241. [PubMed: 29049437]
31. Writing Committee for the OPTOS PEripheral RetinA (OPERA) study (Ancillary Study of Age-Related Eye Disease Study 2), Domalpally A, Clemons TE, Danis RP, et al. Peripheral retinal changes associated with age-related macular degeneration in the age-related eye disease study 2: age-related eye disease study 2 report number 12 by the age-related eye disease study 2 optos PEripheral RetinA (OPERA) study Research group. *Ophthalmology* 2017;124:479–487. [PubMed: 28089680]

32. Lewis H, Straatsma BR, Foos RY, Lightfoot DO. Reticular degeneration of the pigment epithelium. *Ophthalmology* 1985;92:1485–1495. [PubMed: 4080322]
33. Bae K, Cho K, Kang SW, et al. Peripheral reticular pigmentary degeneration and choroidal vascular insufficiency, studied by ultra wide-field fluorescein angiography. *PLoS One* 2017;12: e0170526. [PubMed: 28114409]
34. Humphrey WT, Carlson RE, Valone JA. Senile reticular pigmentary degeneration. *Am J Ophthalmol* 1984;98:717–722. [PubMed: 6507543]
35. Jonnal RS, Kocaoglu OP, Zawadzki RJ, et al. The cellular origins of the outer retinal bands in optical coherence tomography images. *Invest Ophthalmol Vis Sci* 2014;55:7904–7918. [PubMed: 25324288]
36. Cuenca N, Ortuño-Lizarán I, Pinilla I. Cellular characterization of optical coherence tomography and outer retinal bands using specific immunohistochemistry markers and clinical implications. *Ophthalmology* 2018;125:407–422. [PubMed: 29037595]
37. Staurenghi G, Sadda S, Chakravarthy U, Spaide RF. International Nomenclature for Optical Coherence Tomography (IN • OCT) Panel. Proposed lexicon for anatomic landmarks in normal posterior segment spectral-domain optical coherence tomography: the IN • OCT consensus. *Ophthalmology* 2014; 121:1572–1578. [PubMed: 24755005]
38. Lee W, Nõupuu K, Oll M, et al. The external limiting membrane in early-onset Stargardt disease. *Invest Ophthalmol Vis Sci* 2014;55:6139–6149. [PubMed: 25139735]
39. Nõupuu K, Lee W, Zernant J, et al. Recessive Stargardt disease phenocopying hydroxychloroquine retinopathy. *Graefes Arch Clin Exp Ophthalmol* 2016;254:865–872. [PubMed: 26311262]
40. Lambertus S, Bax NM, Fakin A, et al. Highly sensitive measurements of disease progression in rare disorders: developing and validating a multimodal model of retinal degeneration in Stargardt disease. *PLoS One* 2017;12:e0174020. [PubMed: 28355279]
41. Tanna P, Strauss RW, Fujinami K, Michaelides M. Stargardt disease: clinical features, molecular genetics, animal models and therapeutic options. *Br J Ophthalmol* 2017;101:25–30. [PubMed: 27491360]

Fig. 1.

Pedigrees illustrating the segregation of multiple *ABCA4* variants and discordant Stargardt disease phenotypes in five families. Affected individuals are represented by filled shapes (black = classic Stargardt disease or cone-rod dystrophy; gray = hypomorphic Stargardt disease; square = male; circle = female). The index patient or affected proband of each family is indicated by a white arrow. Affected individuals were observed across successive generations in three families (Families 1, 2, and 4; **A**, **B**, and **D**), with the patients possessing the p.N1868I variant having consistently later subjective onset than affected family members without that variant. Some phenotypic discordance was found among p.N1868I patients, even between patients with identical *ABCA4* genotypes; this was especially notable in Family 2 (see main text and Figure 2). Family 3 (**C**) segregated c.5603A>T (p.N1868I) in both a single allele and a complex allele (in *cis* with c.5461-10T>C). The proband in Family 3 was diagnosed with Stargardt disease with onset in childhood; his mother (I:2, Patient #7) was biallelic for *ABCA4*, with p.N1868I and p.P1380L, and is indicated by a question mark to denote uncertainty whether she will develop overt Stargardt disease. Two affected sisters

(Family 5, **E**; Patients #20 and #19) with the genotype p.N1868I/c.5196+1G>A were both found to have late-onset Stargardt disease with similar phenotypes.

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Fig. 2.

Near-infrared autofluorescence, measuring the presence of melanin, in four different patients from Family 2. Panel A (proband, Patient #4, age 61 years) shows a bull's eye-like ring of hypoautofluorescence representing RPE atrophy, with inhomogeneity (dark spots) primarily at the temporal border representing flecks, and a small island of foveolar sparing. Panel B (sister of proband, Patient #3, age 62 years) shows a large macular lesion with visibility of choroidal vessels representing at least RPE and choriocapillaris atrophy, with hyperautofluorescent and hypoautofluorescent flecks at the borders of the lesion and a very small island of foveolar sparing. Panel C (daughter of proband, Patient #6, age 30 years) shows hypoautofluorescence centrally with a ring of homogeneous hyperautofluorescence and no definite foveal sparing (although BCVA in this eye was 20/70 at this visit, suggesting some foveal function). Panel D (also daughter of proband, Patient #5, age 27 years) shows an incomplete ring of subtle hyperautofluorescence.

Fig. 3.

Macular SD-OCT line profiles, derived from SD-OCT b-scans. Reflectivity (arbitrary units; normalized to the RPE) is plotted as a function of retinal depth. The gray regions represent the range of normal and the two profiles (red and blue) are from two p.N1868I patients. The top-left panel shows measurements performed within a 1° region centered at the fovea (filled arrows), whereas the topright panel shows measurements from the nasal and temporal parafovea (0.5 – 5.5° from the fovea, broad arrow outlines). Below the graphs are the horizontal SD-OCT b-scans of the right macula in both p.N1868I patients, neither of whom had definite flecks or atrophy on ophthalmoscopy, with the measured regions of the fovea and parafovea demarcated by vertical lines. Note that Patient #7 had essentially normal line profiles, whereas the ELM of Patient #5 was hyperreflective relative to the control subjects in the parafovea. The ellipsoid zone (EZ) was similar to controls in both patients. Near-infrared autofluorescence in the right eye from Patient #7 is displayed in the lower right

corner, showing no definite abnormalities; this is in contrast to the subtle NIR-AF changes found in Patient #5 (Figure 2D).

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Fig. 4.

Spaghetti plot showing BCVA in the better eye of all 27 p. N1868I patients, with the initial visit at time zero. Patients with only one visit are represented as dots. Note that in the first few years, none of the patients were worse than 20/50, but in later years, a number of patients (N = 6) did go on to lose foveal vision and were worse than 20/100 in the better eye.

Fig. 5.

Fundus photographs of macular lesions and fundus fleck distributions found in *ABCA4* p.N1868I patients. Panel A (Patient #23) shows Stage 1 fundus flecks with subtle bull's eye hypopigmentation. Panel B (Patient #11) shows a bull's eye-appearing lesion with Stage 2 flecks with a relatively sparse distribution. Panel C (Patient #25) shows an incomplete bull's eye with several round areas of parafoveal hypopigmentation becoming confluent. Panel C also shows both nonpigmented and pigmented Stage 2 flecks. Panel D (Patient #14) shows Stage 3 (primarily resorbed flecks) and macular atrophy with a relatively spared fovea.

Fig. 6. Peripheral lesions in two patients with *ABCA4* p.N1868I Stargardt disease. In the near-periphery of the left eye shown in Panel A (Patient #25), there are nonpigmented flecks in the macula and near-periphery, as well as reticular degeneration-like deep pigment in the mid-periphery. Panel B (Patient #10, right eye) shows a hypertrophic, highly pigmented lesion temporally.

Fig. 7.

Goldmann visual fields of the right eyes in two patients with *ABCA4* p.N1868I Stargardt disease. In both examples, the peripheral boundaries are normal to the II4e target. Panel A (Patient #26) shows a central relative scotoma to the II2e (filled in red), but only paracentral loss to II4e (filled in blue); the peripheral boundaries to the II4e and II2e targets are normal. Panel B (Patient #14) shows the most advanced visual field loss among the 27 p. N1868I patients, yet the peripheral sensitivity is still largely spared. There is a large central scotoma to all targets, including V4e (the largest and brightest available target). Peripherally, the II4e and larger targets are essentially normal; only the II2e target is considerably constricted.

Table 1.*ABCA4* Mutations in Patients With p.N1868I *ABCA4* Stargardt Disease

Patient	Family	Sex	<i>ABCA4</i> Mutation in <i>trans</i>	Pathogenicity Class
1	F1-II:1 *	M	c.[1622T>C; 3113C>T] (p.[L541P;A1038V])	PS3
2	F1-II:2	F	c.[1622T>C; 3113C>T] (p.[L541P;A1038V])	PS3
3	F2-I:1	F	c.[1622T>C; 3113C>T] (p.[L541P;A1038V])	PS3
4	F2-I:2 *	M	c.[1622T>C; 3113C>T] (p.[L541P;A1038V])	PS3
5	F2-II:2	F	c.4139C>T (p.P1380L)	PS3
6	F2-II:3	F	c.4139C>T (p.P1380L)	PS3
7	F3-I:2 *	F	c.4139C>T (p.P1380L)	PS3
8	F4-I:2 *	F	c.1957C>T (p.R653C)	PS4
9		F	c.6229C>G (p.R2077W)	PS3
10		M	c.[4469G>A; 5603A>T] (p.[C1490Y;N1868I])	PS3
11		M	c.3259G>A (p.E1087K)	PS3
12		M	c.2894A>G (p.N965S)	PS3
13		F	c.52C>T (p.R18W)	PS4
14		M	c.5923G>C (p.G1975R)	PM2
15		F	c.3261A>C (p.E1087D)	PM2
16		F	c.161-1G>A (p.?)	PVS1
17		F	c.[5461-10T>C; 5603A>T] (p.[?; N1868I])	PVS1
18		M	c.[5461-10T>C; 5603A>T] (p.[?; N1868I])	PVS1
19	F5-I:1	F	c.5196+1G>A (p.?)	PVS1
20	F5-I:2 *	F	c.5196+1G>A (p.?)	PVS1
21		F	c.1906C>T (p.Q636 *)	PVS1
22		M	c.6166A>T (p.K2056 *)	PVS1
23		F	c.45G>A (p.W15 *)	PVS1
24		F	c.[1027_1028del; 5603A>T] (p.[N343 *; N1868I])	PVS1
25		M	c.4234C>T (p.Q1412 *)	PVS1
26		F	c.3846delA (p.G1283fs)	PVS1

Patient	Family	Sex	ABCA4 Mutation <i>in trans</i>	Pathogenicity Class
27		F	c.1903C>T (p.Q365*)	PVS1

All 27 patients listed in this table were white and carried the p.N1868I variant on the opposite allele.

Nucleotide positions and protein translation correspond to CCDS747.1 and NP_000341.2, respectively. Nucleotide numbering uses the A of the ATG translation initiation start site as nucleotide 1. Pathogenicity class is determined according to Richards et al.²⁷

Functional consequences for missense variants were reported in Zhang et al.²⁸ for the c.[1622T>C; 3113C>T] (p.[Leu541Pro; Ala1038Val]) variant and by Sun et al.¹⁷ for the c.4139C>T (p.Pro1380Leu), c.6229C>G (p.R2077W), c.4469G>A (p.C1490Y), c.2894A>G (p.N965S), and c.3259G>A (p.E1087K) variants.

* Proband.

PM, moderate evidence of pathogenicity; PS, strong evidence of pathogenicity; PVS, very strong evidence of pathogenicity.

Table 2.Subjective Onset and Symptoms in Patients With p.N1868I *ABCA4* Stargardt Disease

Patient Number	Subjective Onset Age	First Symptom
1	30	Blur
2	25	Paracentral black spot OS only
3	52	Night vision and glare
4	48	Metamorphopsia
5		No symptoms
6	17	Central and photoaversion
7		No symptoms (mild light sensitivity)
8	45	Night vision
9	29	Reading and missing text (also slow dark adaptation, photoaversion)
10	35	Reading
11	40	Reading and slow dark adaptation
12	45	Uncertain (photoaversion, nyctalopia, and central)
13		No symptoms (mild light sensitivity)
14	48	Central, mild color
15	67	Paracentral (missed spots while cleaning)
16	50	Night vision
17	32	Reading (misreads words and photoaversion)
18	35	Photoaversion
19	57	Glare and photoaversion
20	62	Paracentral blurry spot OD
21	21	Reading (also photoaversion and color)
22	28	Night driving (age 29 years central blur)
23	32	Micropsia OD
24	43	Reading
25	43	Metamorphopsia
26	52	Reading
27	71	Central (OD) and night vision

Patients 4, 12, and 21 were asymptomatic and were each only seen one time.

Table 3.

Visual Acuity and Stage at Baseline and Most Recent Follow-up in Patients With p.N1868I *ABCA4* Stargardt Disease

Patient Number	Age at First Visit	First Visit					Last Visit					
		Snellen VA OD	Snellen VA OS	LogMAR VA OD	LogMAR VA OS	Stage	Age at Last Visit (Duration)	Snellen VA OD	Snellen VA OS	LogMAR VA OD (Change)	LogMAR VA OS (Change)	Stage
1	33.9	20/25-3	20/70-2	0.16	0.58	1	40.7 (6.8)	20/40-2	10/80-1	0.34 (0.18)	0.92 (0.34)	2
2	26.7	20/40-2	20/40-2	0.34	0.34	1	33.6 (6.9)	10/25-2	10/80-3	0.44 (0.10)	0.95 (0.61)	1
3	62.6	10/80-1	20/80-1	0.92	0.62	2						
4	52.4	20/30-2	20/25-2	0.22	0.14	1	61.3 (8.9)	20/25+2	20/25+1	0.06 (-0.16)	0.08 (-0.06)	1
5	27.1	20/15	20/15-2	-0.12	-0.08							
6	17.3	20/30+2	20/30+1	0.14	0.16	1	30.2 (12.9)	20/40-1	20/70	0.32 (0.18)	0.54 (0.38)	1
7	48.7	20/15	20/15	-0.12	-0.12							
8	49.4	20/20	20/20	0.00	0.00	2						
9	27.1	20/20-1	20/20-1	0.02	0.02	1	33.4 (6.3)	20/15-3	20/20	-0.06 (-0.08)	0.00 (-0.02)	1
10	37.0	20/30	20/100+2	0.18	0.66	2	46.4 (9.4)	10/140	10/100	1.15 (0.97)	1.00 (0.34)	2-3
11	41.2	20/30+1	20/30	0.16	0.18	1-2	50.0 (8.8)	20/200	20/200	1.00 (0.84)	1.00 (0.82)	2
12	67.3	20/30-1	20/25-2	0.20	0.14	2	75.0 (7.7)	10/	10/400	1.10 (0.90)	1.60 (1.46)	2-3
13	60.6	20/20-3	20/20-1	0.05	0.02	2		120-1				
14	51.1	20/30+2	20/40+1	0.14	0.28	3	56.0 (4.9)	20/30-2	20/50-1	0.22 (0.08)	0.42 (0.14)	3
15	71.7	20/50+2	20/60-2	0.36	0.52	2						
16	49.4	20/15-1	20/15	-0.10	-0.12	2	51.0 (1.6)	20/15-1	20/15	-0.10 (0.00)	-0.12 (0.00)	2
17	33.5	20/25-2	20/25-2	0.14	0.14	1	34.8 (1.3)	20/25-2	20/25-2	0.14 (0.00)	0.14 (0.00)	1
21	22.4	20/25+2	20/2022	0.06	0.04	1	34.0 (11.6)	10/80	10/6023	0.90 (0.84)	0.84 (0.80)	1-2
18	52.6	20/200	20/30+1	1.00	0.16	1	60.2 (7.6)	20/200	20/200	1.00 (0.00)	1.00 (0.84)	2
19	67.7	20/20-2	20/20-2	0.04	0.04	2						
20	63.1	20/20+1	20/15-1	-0.02	-0.10	2	70.7 (7.6)	20/20-3	20/20-2	0.05 (0.07)	0.04 (0.14)	2
21	22.4	20/25+2	20/20-2	0.06	0.04	1	34.0 (11.6)	10/80	10/60-3	0.90 (0.84)	0.84 (0.80)	1-2
22	29.9	20/20-3	20/25-3	0.06	0.16	2	36.4 (6.5)	10/80-1	10/100+1	0.92 (0.86)	0.98 (0.82)	2
23	33.8	20/30	20/20	0.18	0.00	1	35.0 (1.2)	20/50	20/25	0.40 (0.22)	0.10 (0.10)	1
24	51.6	20/20	20/20	0.00	0.00	2						
25	53.0	20/25+3	20/20	0.04	0.00	2	56.3 (3.3)	20/25-2	20/20-1	0.14 (0.10)	0.02 (0.02)	2

Patient Number	Age at First Visit	First Visit					Last Visit				
		Snellen VA OD	Snellen VA OS	LogMAR VA OD	LogMAR VA OS	Stage	Snellen VA OD	Snellen VA OS	LogMAR VA OD (Change)	LogMAR VA OS (Change)	Stage
26	54.0	20/30+1	20/50+1	0.16	0.38	2	20/40-1	20/50+1	0.32 (0.16)	0.38 (0.00)	2
27	72.9	20/40+2	20/30	0.26	0.18	2					

Duration, time from first to last visit (years); VA, best-corrected visual acuity.

Table 4.

Clinical Findings in Patients With p.N1868I *ABCA4*/Stargardt Disease

Patient Number	Peripheral Retinal Findings	Dark Choroid?	ERG LoIs Classification	Goldmann Visual Field Findings	Other Findings
1				Central scotomas II4e; mild-moderate constriction II2e	
2				Central scotoma II2e and II4e OU	Optic atrophy OS and multiple sclerosis
3					
4			1		
5					Subtly thickened ELM on OCT
6	White without pressure		3? Unreliable ERG	Paracentral scotomas II2e and II4e	~13 diopter myope
7					Hydroxychloroquine use × 2 years
8	RDPE				
9			1	Partial paracentral ring scotoma II4e	
10	RDPE	Yes		Paracentral ring scotomas II4e and central scotomas II2e OU	Hypertrophic scar OD
11	Net-like flecks		1	Central scotomas II4e OU	Amblyopia OS
12	RDPE			Central scotomas II4e OU; mild-moderate constriction II2e	Type 2 diabetes mellitus × 31 years
13					Pigmented flecks
14				Large central scotoma V4e; extensive peripheral loss to II2e	
15				Central scotomas III4e; mild constriction II2e	
16				Paracentral scotomas II2e and central scotoma II2e OS	
17		Yes	1 (outside)		
18		Yes	1	Small central scotomas II4e OD and II2e OS	
19	RDPE				
20	RDPE			Normal to II2e OD and OS	
21		Uncertain	1	Paracentral ring scotomas II4e and central scotomas II2e	~6 Diopter myope
22	Net-like flecks		1	Small central scotomas II4e OD and II2e OS	Pigmented flecks
23					
24					
25	RDPE				Pigmented and net-like flecks
26				Paracentral scotomas II4e and central scotomas II2e OU	
27	Net-like flecks				

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Goldmann visual field periphery was normal to II4e and II2e, unless otherwise noted. For the “Dark Choroid?”, “ERG Lois classification”, and “Goldmann visual field findings” columns, a blank space indicates that no data were available for that test.

RDPE, reticular degeneration of the pigment epithelium (peripheral reticular degeneration).