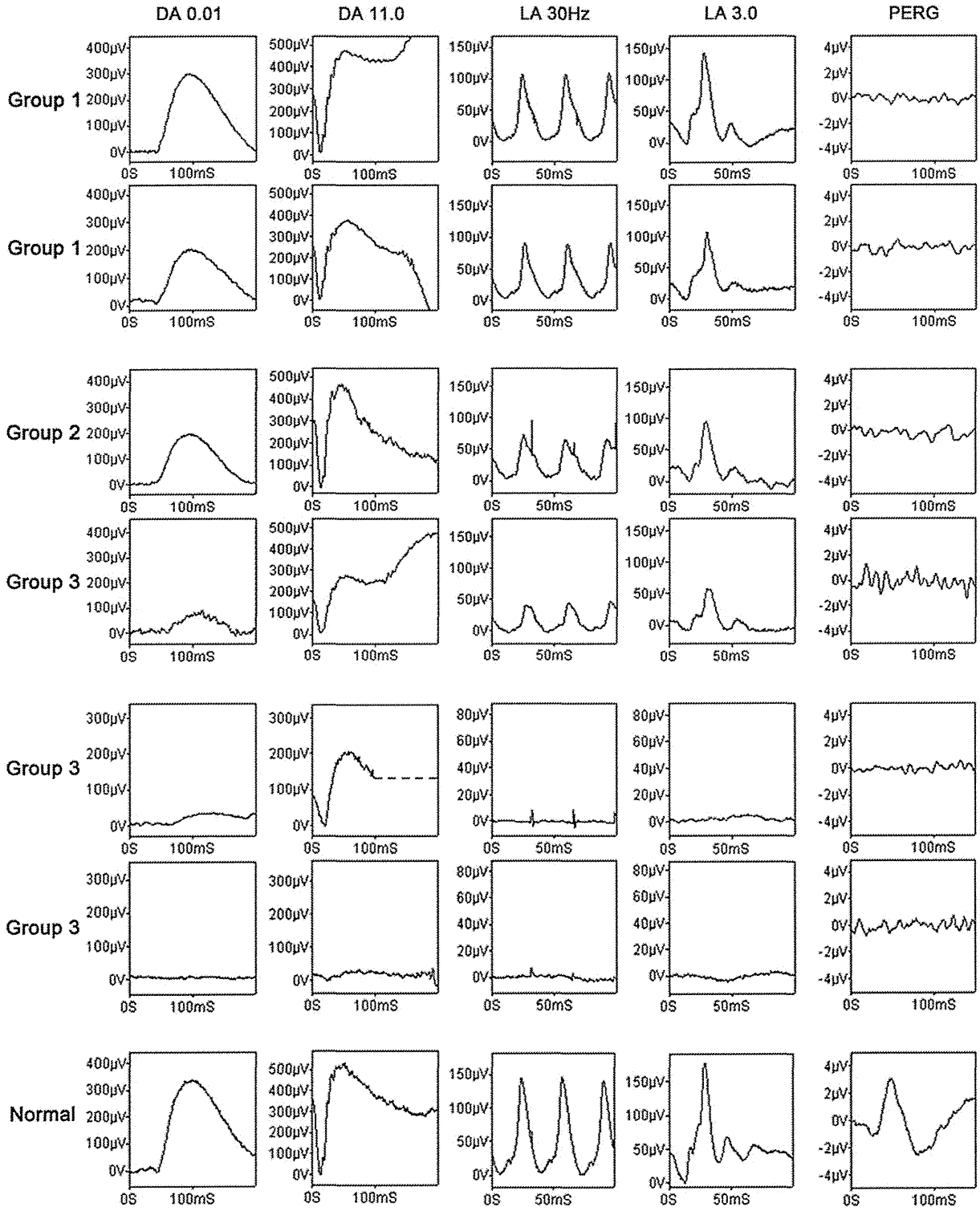

FIGURE 2. Full-field electroretinograms and pattern electroretinograms at baseline and at follow-up from the 3 representative cases of Stargardt disease illustrated in Figure 1 (Patients 17, 42, and 53). Patient 17 demonstrates undetectable pattern electroretinogram (PERG) and normal full-field electroretinograms (ERG) both at baseline (Top row) and at follow-up (Second row), consistent with ERG Group 1 both at baseline and at follow-up. Patient 42 has undetectable PERG and abnormal responses in light-adapted (LA) 3.0, while responses in dark-adapted (DA) 0.01, DA 11.0, and LA 30 Hz are normal at baseline (Third row). At follow-up, all the components of the ERGs are abnormal (Fourth row). Patient 42 demonstrates transition from ERG Group 2 to Group 3, with clinically significant electrophysiologic deterioration observed in rod-derived ERGs. Patient 53 at baseline shows undetectable responses for PERG, LA 30 Hz, and LA 3.0, with abnormal but detectable DA 0.01 and DA 11.0 responses (Fifth row), consistent with ERG Group 3. At follow-up there is only residual ERG activity in the DA 11.0 ERG, representing marked deterioration (Sixth row). (Bottom row) Normal traces are shown for comparison.

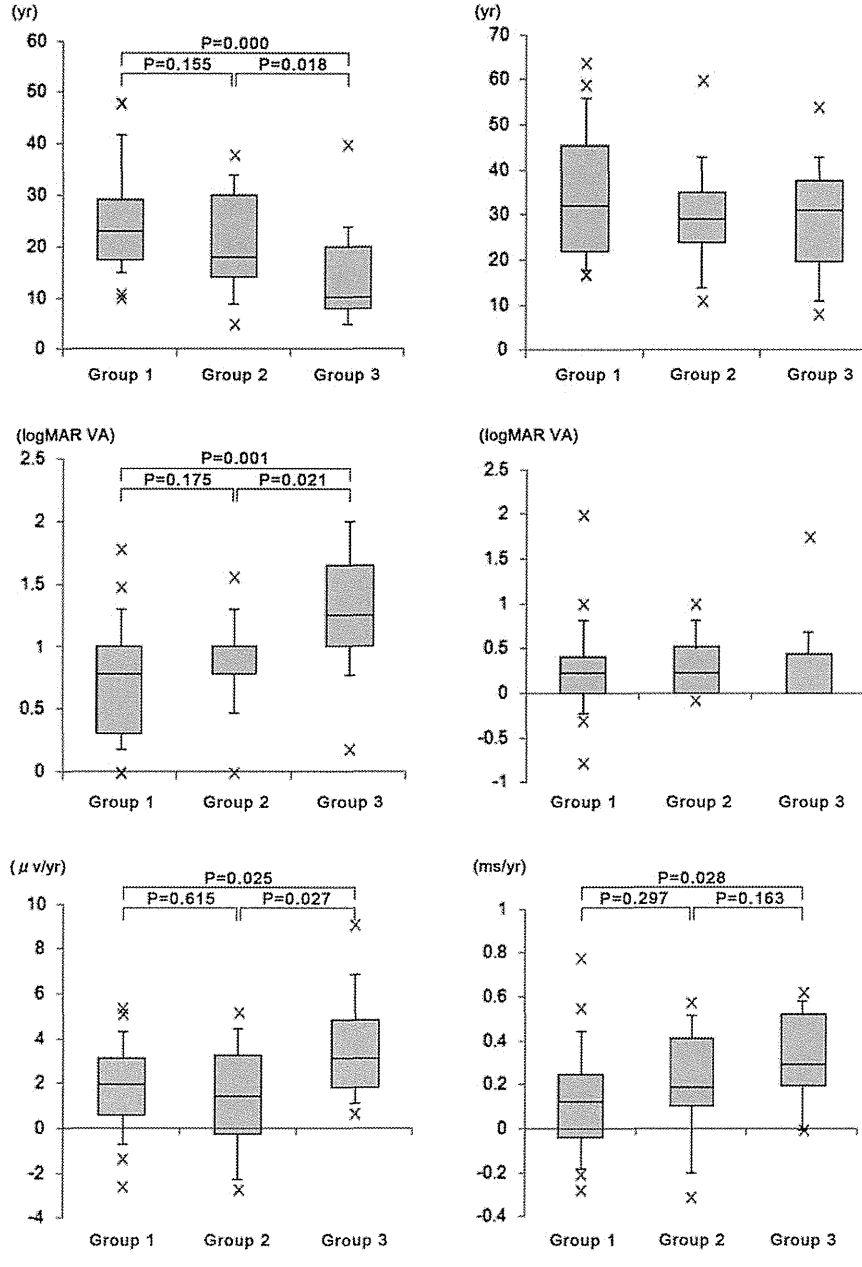

FIGURE 3. A comparison of selected clinical features and electrophysiologic findings associated with each electrophysiologic group at baseline in Stargardt disease, showing significant differences in age of onset, visual acuity at baseline, and electrophysiologic parameters between groups. Age of onset (Top left), age at baseline (Top right), logMAR visual acuity at baseline (Middle left), logMAR visual acuity reduction (Middle right), amplitude reduction per year in the a-wave of the dark-adapted (DA) 11.0 electroretinogram (ERG) (Bottom left), and peak time shift per year in the light-adapted 30 Hz flicker ERG (Bottom right) for the 3 electrophysiologic groups. The boxes show the median and 25% and 75% confidence intervals (lower and upper quartiles). The whiskers extend to what could be considered the 95% confidence interval. Crosses represent values outside the 95% confidence interval. *P* values obtained with the Mann-Whitney *U* test are shown for the parameters in which the Kruskal-Wallis test revealed significant differences. logMAR = logarithm of minimal angle of resolution.

11.0 a-wave and light-adapted 30 Hz of each genotype group are summarized in Tables 3 and 4. There was no statistically significant association identified between the severity of genotype and the extent of electrophysiologic dysfunction on the basis of baseline ERG grouping ($\gamma = -0.126$), although patients with 2 or more non-null variants (genotype B group) less frequently had rod ERG involvement (Table 5 and Supplemental Figure 1).

The distribution of patients with clinically significant electrophysiologic deterioration in each genotype group is

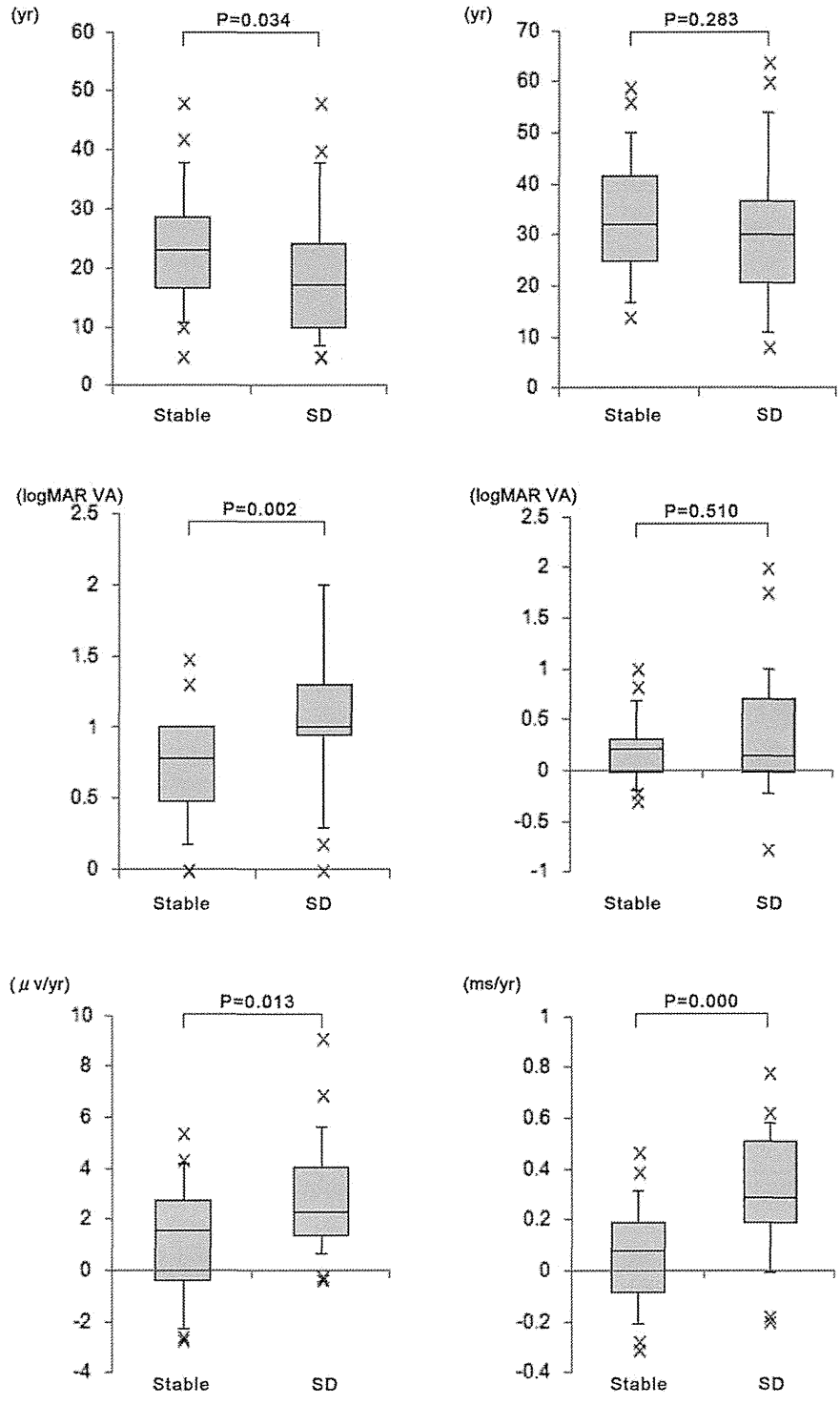

FIGURE 4. A comparison of the clinical findings and electrophysiologic data in Stargardt disease, between the subset of patients with evidence of electroretinogram progression and those without (stable electroretinogram), showing a significant difference in age of onset, visual acuity at baseline, and electrophysiologic parameters between subsets. Age of onset (Top left), age at baseline (Top right), logMAR visual acuity at baseline (Middle left), logMAR visual acuity reduction (Middle right), amplitude reduction per year in the a-wave of the dark-adapted 11.0 electroretinogram (ERG) (Bottom left), and peak time shift per year in light-adapted 30 Hz flicker ERG (Bottom right) for 2 subsets of Stargardt disease (those with and without clinically significant electrophysiologic deterioration). The subset with evidence of clinically significant ERG deterioration is labeled "SD" and the subset without deterioration is labeled "Stable." The boxes show the median and 25% and 75% confidence intervals (lower and upper quartiles). The whiskers extend to what could be considered the 95% confidence interval. Crosses represent values outside the 95% confidence interval. *P* values obtained with the Mann-Whitney *U* test are shown. logMAR = logarithm of minimal angle of resolution.

TABLE 4. Yearly Change[a] in Dark-Adapted Bright Flash Electrophysiologic Responses and Light-Adapted 30 Hz Flicker Responses With Respect to Electrophysiologic Group at Baseline, Electrophysiologic Deterioration, and Genotype Group, in 59 Subjects With Stargardt Disease

	Dark-Adapted 11.0 A-wave			Light-Adapted 30 Hz		
	Amplitude Reduction (μV/y)	Percentage Reduction (%/y)	Peak Time Shift (ms/y)	Amplitude Reduction (μV/y)	Percentage Reduction (%/y)	Peak Time Shift (ms/y)
Group 1 (n = 27)	5.5	1.7	0.10	2.7	2.2	0.14
Group 2 (n = 17)	4.5	1.5	0.09	1.1	1.7	0.19
Group 3 (n = 15)	4.9	3.6	0.18	1.5	3.1	0.32
Stable (n = 27)	3.9	1.2	0.04	2.2	1.9	0.07
Electrophysiologic Deterioration (n = 32)	6.0	2.9	0.18	1.7	2.7	0.31
Genotype A (n = 19)	6.5	3.0	0.14	2.3	3.0	0.23
Genotype B (n = 10)	2.3	0.5	-0.01	1.4	0.9	0.12
Genotype C (n = 18)	5.4	2.1	0.16	2.4	3.1	0.33
Genotype D (n = 12)	4.3	2.1	0.09	1.1	0.9	−0.04
Total (n = 59)	5.1	2.1	0.11	1.9	2.3	0.19

Dark-adapted 11.0 = dark-adapted bright flash electroretinogram (flash intensity 11.0 candela seconds (cd·s)/m^2); Light-adapted 30 Hz = light-adapted 30 Hz flicker electroretinogram (flash intensity 3.0 cd·s/m^2).

[a]A yearly amplitude reduction and a yearly percentage reduction were calculated by dividing the amplitude reduction or the percentage reduction by the follow-up time. A yearly peak time shift (difference between peak time at baseline and follow-up) was also calculated by dividing by the follow-up time.

TABLE 5. Distribution of the 4 Genotype Groups With Respect to Electrophysiologic Group at Baseline and Electrophysiologic Deterioration in Stargardt Disease

	Genotype A	Genotype B	Genotype C	Genotype D
Group 1 (n = 27)	8	5	9	5
Group 2 (n = 17)	4	4	4	5
Group 3 (n = 15)	7	1	5	2
Stable (n = 27)	6	9	7	5
Electrophysiologic deterioration (n = 32)[a]	13	1	11	7
Total (n = 59)	19	10	18	12

[a]The subset without evidence of significant deterioration is described as "Stable."

shown in Table 5 and Supplemental Figure 2 (available at AJO.com). Statistical analysis revealed a significant difference between genotype groups A and B and between genotype groups A and C in terms of age of onset. There was also a statistically significant difference between genotype groups A and B with respect to yearly amplitude reduction of dark-adapted 11.0 a-wave and light-adapted 30 Hz yearly peak time shift (Supplemental Table 5). No statistically significant difference was seen between genotype groups and the other ERG parameters (Supplemental Table 5).

Interestingly, 8 of the 9 patients harboring the variant c.5461-10 T>C (Patients 5, 25, 36, 39, 48, 50, 53-55) had clinically significant ERG progression. All 3 unrelated patients (1, 5, and 31) harboring p.Arg943Gln also had p.Gly863Ala, suggesting linkage disequilibrium of these 2 substitutions, with none of these subjects having clinically significant ERG deterioration.

DISCUSSION

THIS REPORT ADDRESSES LONGITUDINAL CHANGES IN CLINical and electrophysiologic features of Stargardt disease in a large, well-characterized cohort of patients, with 1 or both likely disease-causing *ABCA4* alleles identified in 80% of subjects (47/59). The findings confirm the prognostic value of ERG suggested by earlier cross-sectional data and are relevant to the design of future clinical trials.

Approximately one-fifth of Group 1 patients (dysfunction confined to the macula) progressed to either Group 2 or Group 3 (generalized retinal dysfunction) over a mean time period of 10.5 years, whereas 47% of subjects with Group 2 ERG at baseline changed to Group 3 over the same time period. Overall, there was clinically significant electrophysiologic deterioration in 54% of all patients (32/59), with progression in 22% (6/27) of Group 1 subjects, 65% (11/17) of Group 2, and 100% (15/15) of Group 3. These ERG changes far exceed estimates of normal age-related ERG decline.[39] Thus all patients with initial rod involvement (Group 3) demonstrated clinically significant electrophysiologic deterioration, but only 22% of the patients with normal ERGs (Group 1) at baseline showed clinically significant progression.

A transition in ERG group was seen in 14 patients, with all 14 also meeting the criteria for clinically significant

electrophysiologic deterioration. The 3 patients who progressed from Group 1 to Group 2 had abnormal light-adapted 30 Hz ERGs without any abnormalities in light-adapted 3.0 ERGs; the 30 Hz flicker ERG is known to be a more sensitive indicator of altered cone function than the single-flash photopic ERG. In contrast, both cone full-field ERGs were abnormal in the 3 patients who progressed from Group 1 to Group 3. All 6 patients had a >3 ms peak time shift over time; careful observation of the light-adapted 30 Hz ERGs is important in monitoring Stargardt disease patients with normal ERGs. All but 1 patient with abnormalities in dark-adapted 0.01 or dark-adapted 11.0 had abnormal cone responses, suggesting that generalized cone system dysfunction precedes generalized rod system dysfunction, as has previously been demonstrated.[31]

All 5 patients with undetectable cone responses at follow-up had a >50% amplitude reduction in dark-adapted 11.0 during follow-up. Four patients still had residual responses in dark-adapted 11.0 at follow-up and 1 patient had residual responses in dark-adapted 11.0 at baseline, which became undetectable at follow-up. These findings lend further support to the belief that generalized cone system function is abolished before generalized rod system loss, and that the amplitude of dark-adapted 11.0 responses may be helpful in assessing residual retinal function in cases with very severe retinal dysfunction.

The clinical characteristics of each ERG group showed a statistically significant difference between Groups 1 and 3 and Groups 2 and 3 in terms of age of onset, in keeping with the original cross-sectional data, with a younger age of onset associated with more generalized retinal dysfunction.[31] There was also a statistically significant difference in logMAR VA between Groups 1 and 3 and Groups 2 and 3, with worse VA associated with increasingly severe generalized retinal dysfunction, as has been previously proposed.[31] No statistically significant differences were observed between groups with respect to other parameters, including age at baseline, duration of disease, and interval of follow-up. In addition, the age of onset was earlier in subjects who had clinically significant ERG progression compared to those who did not meet criteria for clinically significant deterioration, further supporting the likelihood that age of onset in Stargardt disease is of prognostic value.[7] For ease of comparison between groups, a linear longitudinal relationship has been assumed and the rate of change expressed in terms of yearly amplitude reduction, yearly percentage reduction, and yearly peak time shift. This study has not examined the linearity of change between baseline and follow-up testing; a prospective study with additional, more frequent time point sampling will help address this pertinent question. It is likely that progression will be linear in some individuals and nonlinear in others, in keeping with the commonplace phenotypic heterogeneity of inherited retinal disorders.

ABCA4 mutations were originally reported in patients with autosomal recessive Stargardt disease but shortly thereafter were identified in association with cone dystrophy, cone-rod dystrophy, and "retinitis pigmentosa," with a genotype-phenotype relationship having been proposed.[10,13–15,21,24,40–43] In the present cohort, 82% of patients (22/27) in ERG Group 1 at baseline, 70% (12/17) in Group 2, and 87% (13/15) in Group 3 harbored at least 1 *ABCA4* variant.

A likely disease-causing *ABCA4* variant was identified in 47 out of 59 patients, with 6 putative novel mutations detected. There was no statistically significant association identified between the category of genotype and the extent of electrophysiologic dysfunction on the basis of ERG group, although patients with 2 or more non-null variants (genotype B group) less frequently had rod ERG involvement. A statistically significant greater percentage of patients with null variants (genotype A group) (68%, 13/19) had ERG deterioration, in comparison with patients harboring 2 or more non-null variants (10%, 1/10), with the majority therefore having a stable ERG (90%, 9/10). There was also a statistically significant difference between genotype groups A and B with respect to yearly amplitude reduction of dark-adapted 11.0 a-wave and light-adapted 30 Hz yearly peak time shift. There are several factors that may account for the relative lack of more clearly demonstrable genotype-phenotype correlations, including the relatively small sample size, the fact that only 1 disease-causing allele was identified in most cases, and the vast allelic heterogeneity of *ABCA4*. However, one particular variant (c.5461-10T>C) was found to be associated with electrophysiologic progression. This mutation has been previously reported to be associated with severe disease in both the homozygous and compound heterozygous states,[42,44] suggesting that it may be a marker for more severe disease, which is likely to show clinically significant progression.

Co-inheritance of p.Arg943Gln and p.Gly863Ala has been previously reported,[44,45] with p.Arg943Gln thought to be a benign polymorphism[29,45] and p.Gly863Ala believed to be associated with milder phenotypes,[42,45] although there has been a single report of a severe phenotype associated with p.Gly863Ala in the homozygous configuration.[44] Only 2 out of 8 patients harboring p.Gly863Ala in the present series had evidence of ERG progression, suggesting this variant is indeed likely to be associated with milder disease.

The longitudinal study described herein has identified that a patient's allocation to an individual ERG group, as proposed in the original cross-sectional study, may change over time—a conclusion that could not be made previously because of the inherent limitations of a cross-sectional survey. The rate of progression between groups and within groups has been determined, and age of onset and, to a lesser extent, visual acuity may predict the degree of eventual generalized retinal dysfunction and/or progression. It is important that only 20% of those patients with initially normal full-field ERGs showed evidence of progression

over a 10-year period, compared to 100% of those with an initial rod system ERG abnormality. These data assist the counseling of the patient in relation to visual prognosis and may inform the design, patient selection, and monitoring of current and future clinical trials for *ABCA4*-related retinopathy.

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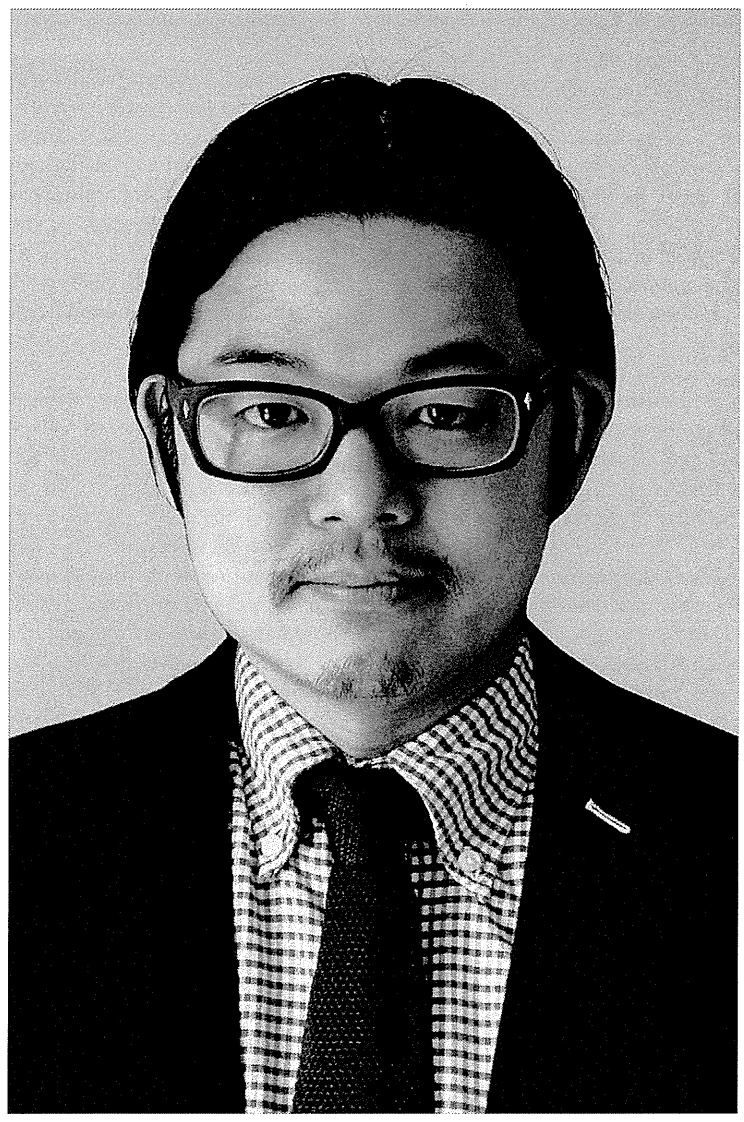
REFERENCES

1. Stargardt K. Uber familiare progressive degeneration in der makulagegend des auges. *Albrecht von Graefes Arch Klin Ophthalmol* 1909;71:534–550.
2. Franceschetti A, Francois J. Fundus flavimaculatus. *Arch Ophthalmol* 1965;25(6):505–530.
3. Michaelides M, Hunt DM, Moore AT. The genetics of inherited macular dystrophies. *J Med Genet* 2003;40(9): 641–650.
4. Lois N, Halfyard AS, Bird AC, Holder GE, Fitzke FW. Fundus autofluorescence in Stargardt macular dystrophy-fundus flavimaculatus. *Am J Ophthalmol* 2004;138(1):55–63.
5. McBain VA, Townend J, Lois N. Progression of retinal pigment epithelial atrophy in stargardt disease. *Am J Ophthalmol* 2012;154(1):146–154.
6. Jayasundera T, Rhoades W, Branham K, Niziol LM, Musch DC, Heckenlively JR. Peripapillary dark choroid ring as a helpful diagnostic sign in advanced stargardt disease. *Am J Ophthalmol* 2010;149(4):656–660.
7. Rotenstreich Y, Fishman GA, Anderson RJ. Visual acuity loss and clinical observations in a large series of patients with Stargardt disease. *Ophthalmology* 2003;110(6):1151–1158.
8. von Ruckmann A, Fitzke FW, Bird AC. In vivo fundus autofluorescence in macular dystrophies. *Arch Ophthalmol* 1997; 115(5):609–615.
9. Walia S, Fishman GA. Natural history of phenotypic changes in Stargardt macular dystrophy. *Ophthalmic Genet* 2009;30(2): 63–68.
10. 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(3):236–246.
11. Allikmets R, Shroyer NF, Singh N, et al. Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. *Science* 1997;277(5333):1805–1807.
12. Burke TR, Tsang SH. Allelic and phenotypic heterogeneity in ABCA4 mutations. *Ophthalmic Genet* 2011;32(3): 162–174.
13. 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(3):355–362.
14. Fishman GA, Stone EM, Eliason DA, Taylor CM, Lindeman M, Derlacki DJ. ABCA4 gene sequence variations in patients with autosomal recessive cone-rod dystrophy. *Arch Ophthalmol* 2003;121(6):851–855.
15. Klevering BJ, Deutman AF, Maugeri A, Cremers FP, Hoyng CB. The spectrum of retinal phenotypes caused by mutations in the ABCA4 gene. *Graefes Arch Clin Exp Ophthalmol* 2005;243(2):90–100.
16. Lois N, Holder GE, Fitzke FW, Plant C, Bird AC. Intrafamilial variation of phenotype in Stargardt macular dystrophy-Fundus flavimaculatus. *Invest Ophthalmol Vis Sci* 1999; 40(11):2668–2675.
17. Michaelides M, Chen LL, Brantley MA Jr, et al. ABCA4 mutations and discordant ABCA4 alleles in patients and siblings with bull's-eye maculopathy. *Br J Ophthalmol* 2007; 91(12):1650–1655.
18. van Driel MA, Maugeri A, Klevering BJ, Hoyng CB, Cremers FP. ABCR unites what ophthalmologists divide(s). *Ophthalmic Genet* 1998;19(3):117–122.
19. Fujinami K, Akahori M, Fukui M, Tsunoda K, Iwata T, Miyake Y. Stargardt disease with preserved central vision: identification of a putative novel mutation in ATP-binding cassette transporter gene. *Acta Ophthalmol* 2011;89(3):e297–e298.
20. Briggs CE, Rucinski D, Rosenfeld PJ, Hirose T, Berson EL, Dryja TP. Mutations in ABCR (ABCA4) in patients with Stargardt macular degeneration or cone-rod degeneration. *Invest Ophthalmol Vis Sci* 2001;42(10):2229–2236.
21. Fishman GA, Stone EM, Grover S, Derlacki DJ, Haines HL, Hockey RR. Variation of clinical expression in patients with Stargardt dystrophy and sequence variations in the ABCR gene. *Arch Ophthalmol* 1999;117(4):504–510.
22. Fumagalli A, Ferrari M, Soriani N, et al. Mutational scanning of the ABCR gene with double-gradient denaturing-gradient gel electrophoresis (DG-DGGE) in Italian Stargardt disease patients. *Hum Genet* 2001;109(3):326–338.

23. Jaakson K, Zernant J, Kulm M, et al. Genotyping microarray (gene chip) for the ABCR (ABCA4) gene. *Hum Mutat* 2003; 22(5):395–403.
24. Klevering BJ, Blankenagel A, Maugeri A, Cremers FP, Hoyng CB, Rohrschneider K. Phenotypic spectrum of autosomal recessive cone-rod dystrophies caused by mutations in the ABCA4 (ABCR) gene. *Invest Ophthalmol Vis Sci* 2002;43(6):1980–1985.
25. Lewis RA, Shroyer NF, Singh N, et al. Genotype/phenotype analysis of a photoreceptor-specific ATP-binding cassette transporter gene, ABCR, in Stargardt disease. *Am J Hum Genet* 1999;64(2):422–434.
26. Papaioannou M, Ocaka L, Bessant D, et al. An analysis of ABCR mutations in British patients with recessive retinal dystrophies. *Invest Ophthalmol Vis Sci* 2000;41(1):16–19.
27. Rivera A, White K, Stohr 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(4):800–813.
28. Rozet JM, Gerber S, Souied E, et al. Spectrum of ABCR gene mutations in autosomal recessive macular dystrophies. *Eur J Hum Genet* 1998;6(3):291–295.
29. Webster AR, Heon E, Lotery AJ, et al. An analysis of allelic variation in the ABCA4 gene. *Invest Ophthalmol Vis Sci* 2001; 42(6):1179–1189.
30. Zernant J, Schubert C, Im KM, et al. Analysis of the ABCA4 gene by next-generation sequencing. *Invest Ophthalmol Vis Sci* 2011;52(11):8479–8487.
31. Lois N, Holder GE, Bunce C, Fitzke FW, Bird AC. Phenotypic subtypes of Stargardt macular dystrophy-fundus flavimaculatus. *Arch Ophthalmol* 2001;119(3):359–369.
32. Holder GE, Brigell MG, Hawlina M, Meigen T. Vaegan, Bach M. ISCEV standard for clinical pattern electroretinography—2007 update. *Doc Ophthalmol* 2007;114(3):111–116.
33. Marmor MF, Fulton AB, Holder GE, Miyake Y, Brigell M, Bach M. ISCEV Standard for full-field clinical electroretinography (2008 update). *Doc Ophthalmol* 2009;118(1):69–77.
34. Robson AG, Webster AR, Michaelides MM, et al. "Cone dystrophy with supernormal rod electroretinogram": a comprehensive genotype/phenotype study including fundus autofluorescence and extensive electrophysiology. *Retina* 2010;30(1):51–62.
35. Lenassi E, Robson AG, Hawlina M, Holder GE. The value of two-field pattern electroretinogram in routine clinical electrophysiologic practice. *Retina* 2012;32(3):589–599.
36. Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc Natl Acad Sci U S A* 1989;86(8):2766–2770.
37. Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. *Nucleic Acids Res* 2003;31(13): 3812–3814.
38. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods* 2010;7(4):248–249.
39. Weleber RG. The effect of age on human cone and rod ganzfeld electroretinograms. *Invest Ophthalmol Vis Sci* 1981;20(3): 392–399.
40. Cella W, Greenstein VC, Zernant-Rajang J, et al. G1961E mutant allele in the Stargardt disease gene ABCA4 causes bull's eye maculopathy. *Exp Eye Res* 2009;89(1):16–24.
41. Martinez-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(1):11–12.
42. Schindler EI, Nylen EL, Ko AC, et al. Deducing the pathogenic contribution of recessive ABCA4 alleles in an outbred population. *Hum Mol Genet* 2010;19(19):3693–3701.
43. Simonelli F, Testa F, Zernant J, et al. Genotype-phenotype correlation in Italian families with Stargardt disease. *Ophthalmic Res* 2005;37(3):159–167.
44. Klevering BJ, Yzer S, Rohrschneider K, et al. Microarray-based mutation analysis of the ABCA4 (ABCR) gene in autosomal recessive cone-rod dystrophy and retinitis pigmentosa. *Eur J Hum Genet* 2004;12(12):1024–1032.
45. Maugeri A, van Driel MA, van de Pol DJ, et al. The 2588G–>C mutation in the ABCR gene is a mild frequent founder mutation in the Western European population and allows the classification of ABCR mutations in patients with Stargardt disease. *Am J Hum Genet* 1999;64(4): 1024–1035.



Biosketch

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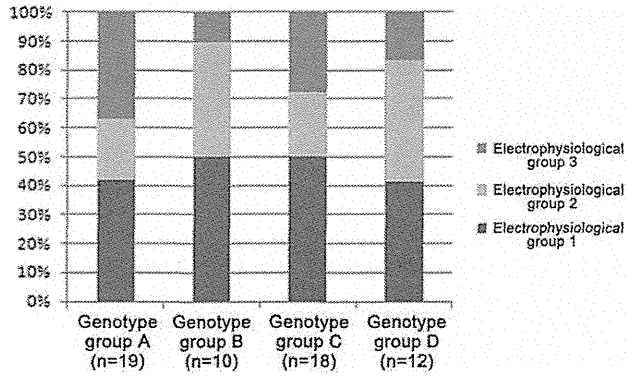
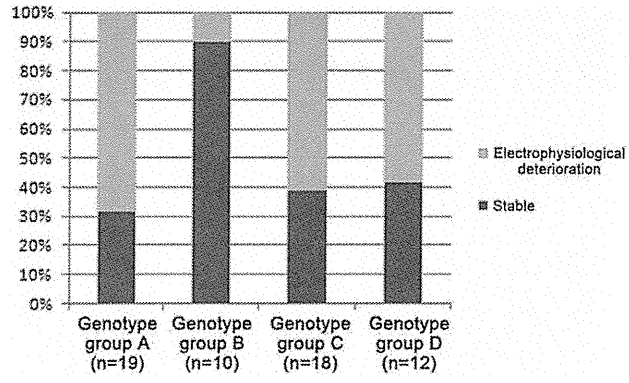


SUPPLEMENTAL FIGURE 1. The association between genotype group and electrophysiologic group at baseline in 59 patients with Stargardt disease, showing that patients with 2 or more null variants (genotype group A) more frequently had generalized rod involvement (electrophysiologic group 3).



SUPPLEMENTAL FIGURE 2. The association between genotype group and presence or absence of clinically significant electrophysiologic deterioration, showing that patients with Stargardt disease harboring 2 or more non-null variants (genotype group B) more frequently have stable electrophysiologic function over time compared with those with more severe mutations (genotype group A).

SUPPLEMENTAL TABLE 1. Normal Ranges for Each Component of International Standard Full-field Electroretinography in Young Adults

	Dark-Adapted 0.01		Dark-Adapted 11.0				Light-Adapted 30 Hz		Light-Adapted 3.0			
			A-wave		B-wave				A-wave		B-wave	
	Amplitude (μV)	Peak Time (ms)	Amplitude (μV)	Peak Time (ms)	Amplitude (μV)	Peak Time (ms)	Amplitude (μV)	Peak Time (ms)	Amplitude (μV)	Peak Time (ms)	Amplitude (μV)	Peak Time (ms)
Age group (<50 years old)	135-455	84-107	250-470	7-14	320-755	39-56	70-200	23-27	30-80	12-15	95-295	27-32

Dark-adapted 0.01 = dark-adapted dim flash electroretinogram with flash intensity 0.01 candela second $(cd \cdot s)/m^2$; Dark-adapted 11.0 = dark-adapted bright flash electroretinogram with flash intensity 11.0 $cd \cdot s/m^2$; Light-adapted 30 Hz = light-adapted 30 Hz flicker electroretinogram with flash intensity 3.0 $cd \cdot s/m^2$; Light-adapted 3.0 = light-adapted 2 Hz electroretinogram with flash intensity 3.0 $cd \cdot s/m^2$.

SUPPLEMENTAL TABLE 2. Normal Ranges for Full-field Electroretinography in Older Adults

	Dark-Adapted 0.01		Dark-Adapted 11.0				Light-Adapted 30 Hz		Light-Adapted 3.0			
			A-wave		B-wave				A-wave		B-wave	
	Amplitude	Peak Time	Amplitude	Peak Time	Amplitude	Peak Time	Amplitude	Peak Time	Amplitude	Peak Time	Amplitude	Peak Time
Age group (≥50 years old)	30-320	76-117	105-495	10-16	235-665	36-57	50-145	22-29	15-60	12-16	90-220	25-32

Dark-adapted 0.01 = dark-adapted dim flash electroretinogram with flash intensity 0.01 candela second (cd·s)/m^2; Dark-adapted 11.0 = dark-adapted bright flash electroretinogram with flash intensity 11.0 cd·s/m^2; Light-adapted 30 Hz = light-adapted 30 Hz flicker electroretinogram with flash intensity 3.0 cd·s/m^2; Light-adapted 3.0 = light-adapted 2 Hz electroretinogram with flash intensity 3.0 cd·s/m^2.

SUPPLEMENTAL TABLE 3. Primer Sequences and Annealing Temperatures for *ABCA4* Gene Screening

Primer	Sequence (5'–3')	Annealing Temperature (C)
Exon 2 forward	GTGTCTGCTCTGGTTACGTTTTC	61
Exon 2 reverse	CCTTTTGTCTAGAAAGATCTTGGG	
Exon 5 forward	TCCAATCGACTCTGGCTGTT	64
Exon 5 reverse	AGAGATCATGGGGCACAACC	
Exon 9 forward	CCAGCATGGAGTTGAATGAGAC	63
Exon 9 reverse	TAAGTGGACTCTTGCGTTTCCTC	
Exon 10 forward	TTAGATTCTGTCAGCCCAGGAAG	63
Exon 10 reverse	ACCAAGTGGGGTCACTGACTTT	
Exon 15 forward	AGAGAGCCCTTTAGGGCAGAAT	63
Exon 15 reverse	GTTTCCTTGGAAGGGTCCGTAG	
Exon 17 forward	AACTGCGGTAAGGTAGGATAGGG	63
Exon 17 reverse	GACCACCTTTCACAAGTTGCTG	
Exon 30 forward	GCCTAGGGATTTGTCAGCAACT	63
Exon 30 reverse	ACTAAACCAAACTCCCTGCACC	
Exon 38 forward	CCAGTTCACACACATCACCTCAG	63
Exon 38 reverse	ATGAGTGCCACTTTCTTCCTCC	
Exon 39 forward	GTGCTGTCCTGTGAGAGCATCTG	64
Exon 39 reverse	GAGGATTAGGGTGCCTCTGTTTC	
Exon 43 forward	CCCGTGTCAACTGGGACTTAG	63
Exon 43 reverse	ATAGTAGGGTGGCTCTGAGGCC	
Exon 44 forward	GCATTTCTGAAGCCAAATAGGAGA	63
Exon 44 reverse	GTGCATTCTCTTGGAGATGAGAAA	
Exon 46-47 forward	TCTTTACTCTTGGATCCACCTCCT	63
Exon 46-47 reverse	GTGTTCTCCATTGACACTTGGAAG	

SUPPLEMENTAL TABLE 4. Detailed Electrophysiologic Findings of 59 Patients With Stargardt Disease: Electrophysiologic Group, Electrophysiologic Deterioration, and Assessment of Each Component of Full-field Electroretinography

Pt	Selected Eye for Data Analysis	Electrophysiologic Group		Electrophysiologic Deterioration			Dark-Adapted 0.01 (R/L)		Dark-Adapted 11.0 (R/L)		Light-Adapted 30 Hz (R/L)		Light-Adapted 3.0 (R/L)	
		BL	FU	Yes/No	Amplitude Reduction	Peak Time Shift	BL	FU	BL	FU	BL	FU	BL	FU
1	R	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
2	L	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
3	L	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
4	R	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
5	L	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
6	R	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
7	L	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
8	L	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
9	R	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/NA
10	R	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
11	R	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
12	L	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
13	L	1	1	–	–	–	N/N	N/N	N/N	N/N	NA/N	NA/N	N/N	N/N
14	L	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
15	R	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
16	R	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
17	L	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
18	L	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	NA/N	NA/N
19	L	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
20	R	1	1	–	–	–	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
21	L	1	1	–	–	–	NA/NA	N/N	N/N	N/N	N/N	N/N	N/N	N/N
22	R	1	2	✓	–	✓	N/N	N/N	N/N	N/N	N/N	A/A	N/N	A/A
23	L	1	2	✓	–	✓	N/N	N/N	N/N	N/N	N/N	A/A	N/N	N/N
24	R	1	2	✓	–	✓	N/N	N/N	N/N	N/N	N/N	A/A	N/N	N/N
25	R	1	3	✓	✓	✓	N/N	N/A	N/N	N/A	N/N	A/A	N/N	A/A
26	L	1	3	✓	–	✓	N/N	N/N	N/N	A/A	N/N	A/A	N/N	A/A
27	L	1	3	✓	✓	✓	N/N	A/A	N/N	N/N	N/N	A/A	N/N	A/A
28	R	2	2	–	–	–	N/N	N/N	N/N	N/N	A/A	A/A	N/N	A/A
29	R	2	2	✓	✓	✓	N/N	N/N	N/N	N/N	N/N	A/A	A/A	A/A
30	L	2	2	–	–	–	N/N	N/N	N/N	N/N	A/A	A/A	N/N	A/A
31	L	2	2	–	–	–	N/N	N/N	N/N	N/N	A/A	A/A	A/A	A/A
32	R	2	2	–	–	–	NA/NA	N/N	N/N	N/N	A/A	A/A	A/A	A/A
33	L	2	2	✓	–	✓	N/N	N/N	N/N	N/N	A/A	A/A	NA/NA	A/A
34	R	2	2	–	–	–	N/N	N/N	N/N	N/N	A/A	A/A	A/A	A/A
35	R	2	2	–	–	–	N/N	N/N	N/N	N/N	A/A	A/A	A/A	A/A
36	L	2	2	✓	✓	–	N/N	N/N	N/N	N/N	A/A	A/A	A/A	A/A

Continued on next page

SUPPLEMENTAL TABLE 4. Detailed Electrophysiologic Findings of 59 Patients With Stargardt Disease: Electrophysiologic Group, Electrophysiologic Deterioration, and Assessment of Each Component of Full-field Electroretinography (*Continued*)

Pt	Selected Eye for Data Analysis	Electrophysiologic Group		Electrophysiologic Deterioration			Dark-Adapted 0.01 (R/L)		Dark-Adapted 11.0 (R/L)		Light-Adapted 30 Hz (R/L)		Light-Adapted 3.0 (R/L)	
		BL	FU	Yes/No	Amplitude Reduction	Peak Time Shift	BL	FU	BL	FU	BL	FU	BL	FU
37	L	2	3	✓	✓	✓	N/N	N/N	N/N	A/A	A/A	A/A	A/A	A/A
38	L	2	3	✓	✓	✓	N/N	A/A	N/N	A/A	A/A	A/A	A/A	A/A
39	R	2	3	✓	—	✓	N/N	NA/NA	N/N	A/A	A/A	A/A	A/A	A/A
40	L	2	3	✓	✓	✓	N/N	A/A	N/N	A/A	N/N	A/A	A/A	A/A
41	R	2	3	✓	—	✓	N/N	A/A	N/N	A/A	N/N	A/A	N/A	A/A
42	L	2	3	✓	✓	—	N/N	A/A	N/N	A/A	A/A	A/A	N/N	A/A
43	L	2	3	✓	✓	—	N/N	A/A	N/N	A/A	A/A	A/A	A/A	A/A
44	R	2	3	✓	✓	✓	N/N	A/A	N/N	A/A	A/A	A/A	A/A	A/A
45	R	3	3	✓	✓	✓	NA/NA	A/A	A/A	A/A	A/A	A/A	A/A	A/A
46	L	3	3	✓	—	✓	NA/NA	N/N	A/A	A/A	A/A	A/A	A/A	A/A
47	R	3	3	✓	—	✓	NA/NA	A/A	A/A	A/A	A/A	A/A	A/A	A/A
48	R	3	3	✓	✓	—	N/N	A/A	N/A	A/A	A/A	A/A	N/N	A/A
49	L	3	3	✓	✓	✓	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A
50	R	3	3	✓	✓	✓	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A
51	R	3	3	✓	—	✓	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A
52	L	3	3	✓	✓	✓	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/ND
53	L	3	3	✓	✓	✓	A/A	ND/ND	A/A	A/A	ND/ND	ND/ND	ND/ND	ND/ND
54	R	3	3	✓	✓	✓	A/A	ND/ND	A/A	A/A	ND/ND	ND/ND	ND/ND	ND/ND
55	L	3	3	✓	✓	—	A/A	ND/ND	A/A	A/A	A/A	ND/ND	A/A	ND/ND
56	R	3	3	✓	✓	—	A/A	ND/ND	A/A	ND/ND	ND/ND	ND/ND	ND/ND	ND/ND
57	L	3	3	✓	✓	✓	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A
58	L	3	3	✓	—	✓	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A
59	L	3	3	✓	✓	✓	A/A	A/A	N/A	A/A	N/A	A/A	N/A	A/A

✓ = yes; — = no; A = Abnormal; BL = baseline; Dark-adapted 0.01 = dark-adapted dim flash electroretinogram with flash intensity 0.01 candela second (cd·s)/m^2; Dark-adapted 11.0 = dark-adapted bright flash electroretinogram with flash intensity 11.0 cd·s/m^2; FU = follow-up; L = left; Light-adapted 30 Hz = light-adapted 30 Hz flicker electroretinogram with flash intensity 3.0 cd·s/m^2; Light-adapted 3.0 = light-adapted 2 Hz electroretinogram with flash intensity 3.0 cd·s/m^2; N = normal; NA = not available; ND = not-detectable; Pt = patient; R = right; VA = visual acuity.

SUPPLEMENTAL TABLE 6. Electrophysiologic Group Transition and *ABCA4* Variants[a] Identified in 59 Patients With Stargardt Disease

Pt	Electrophysiologic Group (BL / FU)	Genotype Group	Number of Variants	Exon	Nucleotide Substitution	Amino Acid Change	Screening Method (Yes/No) SSCP	APEX	DS
1	I / I	A	3	6	c.768 G>T	p.Val256Val/ Splice site	✓	✓	–
				17	c.2588 G>C	p.Gly863Ala	✓	✓	–
				19	c.2828 G>A	p.Arg943Gln	–	✓	–
2	I / I	C	1	29	c.4328 G>A	p.Arg1443His	–	✓	–
3	I / I	A	3	**10**	**c.1317 G > A**	**p.Trp439***	–	✓	✓
				17	c.2588 G>C	p.Gly863Ala	–	✓	✓
				43	c.5908 C>T	p.Leu1970Phe	–	✓	✓
4	I / I	C	1	44	c.6079 C>T	p.Leu2027Phe	–	✓	–
5	I / I	A	3	17	c.2588 G>C	p.Gly863Ala	–	✓	–
				19	c.2828 G>A	p.Arg943Gln	–	✓	–
				Int. 38	c.5461-10 T>C	Splice site	–	✓	–
6	I / I	C	1	28	c.4139 C>T	p.Pro1380Leu	–	✓	–
7	I / I	D	0				✓	–	–
8	I / I	B	2	10	c.1253 T>C	p.Phe418Ser	✓	–	✓
				44	c.6079 C>T	p.Leu2027Phe	✓	–	✓
9	I / I	A	2	Int. 28	c.4253+5 G>T	Splice site	✓	✓	–
				30	**c.4519 G > A**	**p.Gly1507Arg**	✓	–	✓
10	I / I	B	2	30	c.4469 G>A	p.Cys1490Tyr	–	✓	✓
				44	c.6089 G>A	p.Arg2030Gln	–	✓	✓
11	I / I	D	0				–	✓	–
12	I / I	C	1	3	c.286 A>C	p.Asn96His	✓	–	–
13	I / I	A	1	30	c.4537_4538insC	p.Gly1513Profs*1554	–	✓	–
14	I / I	D	0				✓	–	–
15	I / I	C	1	46	c.6320 G>A	p.Arg2107His	✓	–	–
16	I / I	D	0				–	✓	–
17	I / I	C	1	3	c.161 G>A	p.Cys54Tyr	✓	–	–
18	I / I	B	2	28	c.4139 C>T	p.Pro1380Leu	–	✓	–
				42	c.5882 G>A	p.Gly1961Glu	–	✓	–
19	I / I	C	1	22	c.3322 C>T	p.Arg1108Cys	✓	–	–
20	I / I	A	2	**10**	**c.1317 G > A**	**p.Trp439***	–	✓	✓
				17	c.2588 G>C	p.Gly863Ala	–	✓	✓
21	I / I	B	3	5	c. 466 A>G	p. Ile156 Val	✓	–	✓
				30	**c. 4363 C > T**	**p. Cys1455Arg**	✓	–	✓
				39	**c. 5516 T > C**	**p. Phe1839Ser**	✓	–	✓
22	I / II	C	1	46	c.6320 G>A	p.Arg2107His	–	✓	–
23	I / II	C	1	17	c.2588 G>C	p.Gly863Ala	–	✓	–
24	I / II	A	1	35	c.4956 T>G	p.Tyr1652*	–	✓	–
25	I / III	A	1	Int. 38	c.5461-10 T>C	Splice site	–	✓	–
26	I / III	D	0				✓	–	–
27	I / III	A	1	22	c.3211_3212insGT	p.Ser1071Cysfs*1084	–	✓	–
28	II / II	A	2	9	c.1222 C>T	p.Arg408*	✓	–	✓
				14	**c.2023 G > A**	**p.Val675Ile**	✓	–	✓
29	II / II	C	1	47	c.6449 G>A	p.Cys2150Tyr	–	–	✓
30	II / II	D	0				–	✓	–
31	II / II	B	3	17	c.2588G>C	p.Gly863Ala	✓	–	–
				22	c.3322 C>T	p.Arg1108Cys	✓	–	–
				19	c.2828 G>A	p.Arg943Gln	✓	–	–
32	II / II	B	2	14	c.1957 C>T	p.Arg653Cys	–	✓	–
				44	c.6089 G>A	p.Arg2030Gln	–	✓	–
33	II / II	D	0				✓	–	–
34	II / II	B	2	17	c.2588 G>C	p.Gly863Ala	✓	–	–
				22	c.3259 G>A	p.Glu1087Lys	✓	–	–

Continued on next page

SUPPLEMENTAL TABLE 6. Electrophysiologic Group Transition and ABCA4 Variantsa Identified in 59 Patients With Stargardt Disease (*Continued*)

Pt	Electrophysiologic Group (BL / FU)	Genotype Group	Number of Variants	Exon	Nucleotide Substitution	Amino Acid Change	Screening Method (Yes/No)		
							SSCP	APEX	DS
35	II / II	B	2	3	c.161 G>A	p.Cys54Tyr	✓	—	—
				17	c.2588 G>C	p.Gly863Ala	✓	—	—
36	II / II	A	2	19	c.2791 G>A	p.Val931Met	—	✓	—
				Int. 38	c.5461-10 T>C	Splice site	—	✓	—
37	II / III	C	1	28	c.4139 C>T	p.Pro1380Leu	—	✓	—
38	II / III	A	2	22	c.3211_3212insGT	p.Ser1071Cysfs*1084	—	✓	—
				28	c.4139 C>T	p. Pro1380Leu	—	✓	—
39	II / III	A	2	Int. 38	c.5461-10 T>C	Splice site	—	✓	—
				Int. 40	c.5714+5 G>A	Splice site	—	✓	—
40	II / III	D	0				✓	—	—
41	II / III	D	0				✓	—	—
42	II / III	C	1	3	c.161 G>A	p.Cys54Tyr	✓	—	—
43	II / III	D	0				✓	—	—
44	II / III	C	1	19	c.2894 A>G	p.Asn965Ser	✓	—	—
45	III / III	C	1	21	c.3056 C>T	p.Thr1019Met	✓	—	—
46	III / III	C	1	21	c.3056 C>T	p.Thr1019Met	✓	—	—
47	III / III	C	1	47	c.6449 G>A	p.Cys2150Tyr	✓	—	✓
48	III / III	A	2	Int. 38	c.5461-10 T>C	Splice site	—	✓	—
				44	c.6079 C>T	p.Leu2027Phe	—	✓	—
49	III / III	A	1	12	c.1721delAC	p.Asp574Aspfs*582	✓	—	—
50	III / III	A	1	Int. 38	c.5461-10 T>C	Splice site	—	✓	—
51	III / III	B	2	35	c.4918 C>T	p.Arg1640Trp	✓	—	—
				44	c.6079 C>T	p.Leu2027Phe	✓	—	—
52	III / III	C	1	22	c.3323 G>A	p.Arg1108His	✓	—	—
53	III / III	A	2	Int. 38	c.5461-10 T>C	Splice site	—	—	✓
				47	c.6449 G>A	p.Cys2150Tyr	✓	—	✓
54	III / III	A	2	Int. 38	c.5461-10 T>C	Splice site	—	—	✓
				47	c.6449 G>A	p.Cys2150Tyr	✓	—	✓
55	III / III	A	2	Int. 38	c.5461-10 T>C	Splice site	—	✓	✓
				47	c.6449 G>A	p.Cys2150Tyr	—	✓	✓
56	III / III	D	0				✓	—	—
57	III / III	A	1	**15**	**c.2239delC**	**p.Leu747Cysfs*787**	✓	—	✓
58	III / III	D	0				✓	—	—
59	III / III	C	1	5	c.466 A>G	p.Ile156 Val	✓	—	—

✓ = yes; — = no; APEX = arrayed primer extension microarray; BL = baseline; DS = Sanger direct sequencing; FU = follow-up; Int. = intron; SSCP = single-strand conformation polymorphism.

[a]Putative novel changes are in bold. All the variants are heterogeneous.

SUPPLEMENTAL TABLE 7. Investigation of the Pathogenicity of Identified *ABCA4* Variants

Exon	Nucleotide Substitution and Amino Acid Change	Number of Alleles	Previous Report	SIFT[a] Pred.	SIFT[a] Index (0-1)	PolyPhen 2[a] Pred.	PolyPhen 2[a] Hum Var Score (0-1)	HSF Matrix[a] Site Affected	HSF Matrix[a] Wt CV	HSF Matrix[a] Mt CV	HSF Matrix[a] CV % Variation	Allelic Frequency Observed by EVS[a]	Reference
3	c.161 G>A, p.Cys54Tyr	3	Lewis[25]	Tol.	0.11	PRD	0.994				No change	1/10 758	db SNP (rs150774447)
3	c.286 A>C, p.Asn96His	1	Papaioannou[26]	Tol.	0.14	PRD	0.994				No change	1/10 758	db SNP (rs61748529)
5	c. 466 A>G, p. Ile156 Val	2	Papaioannou[26]	Tol.	0.46	Benign	0.003				No change	11/10 758	db SNP (rs112467008)
6	c.768 G>T, p.Val256Val/Splice site	1	Klevering[24]	Tol.	0.56	NA		Don.	70.4	58	Site broken (−17.51)	ND	
9	c.1222 C>T, p.Arg408*	1	Webster[29]	NA		NA							
10	c.1253 T>C, p.Phe418Ser	1	Zernant[30]	Intol.	0	PRD	0.99				No change	ND	
10	c.1317 G>A, p.Trp439*	2	This study	NA		NA						ND	
12	c.1721delAC, p.Asp574Aspfs*582	1	Briggs[20]	NA		NA		Acc.	47.2	68.3	New site (44.5)	ND	
14	c.1957 C>T, p.Arg653Cys	1	Rivera[27]	Tol.	0.1	PRD	0.999				No change	1/10 758	db SNP (rs141823837)
14	c.2023 G>A, p.Val675Ile	1	This study	Tol.	0.07	PRD	0.989				NA	ND	
15	c.2239delC, p.Leu747Cysfs*787	1	This study	NA		NA		Don.	34.7	77	New site (+122)	ND	
17	c.2588 G>C, p.Gly863Ala	8	Allikmets[11]	Intol.	0.01	PRD	0.996				No change	53/10 758	db SNP (rs76157638)
19	c.2791 G>A, p.Val931Met	1	Allikmets[10]	Tol.	0.12	PRD	0.716				No change	18/10 758	db SNP (rs58331765)
19	c.2828 G>A, p.Arg943Gln	3	Webster[29]	Intol.	0.03	Benign	0.449	Acc.	52.2	81.1	New site (+55.48)	340/10 758	db SNP (rs1801581)
19	c.2894 A>G, p.Asn965Ser	1	Lewis[25]	Intol.	0	PRD	0.981	Acc.	53.4	82.3	New site (+54.26)	ND	
21	c.3056 C>T, p.Thr1019Met	2	Rozet[28]	Intol.	0	PRD	0.999				No change	ND	
22	c.3211_3212insGT, p.Ser1071Cysfs*1084	2	Allikmets[10]	NA		NA		Don.	69.3	28	Site broken (−59.55)	ND	
22	c.3259 G>A, p.Glu1087Lys	1	Lewis[25]	Intol.	0	PRD	0.997				No change	ND	
22	c.3322 C>T, p.Arg1108Cys	2	Rozet[28]	Intol.	0	PRD	0.986				No change	1/10 758	db SNP (rs61750120)
22	c.3323 G>A, p.Arg1108His	1	Webster[29]	Intol.	0	PRD	0.986				No change	ND	
28	c.4139 C>T, p.Pro1380Leu	4	Lewis[25]	Intol.	0.01	Benign	0.377				No change	2/10 758	db SNP (rs61750130)
Int. 28	c.4253+5 G>T, Splice site	1	Lewis[25]	NA		NA		Don.	87.9	75.6	Site broken (−14.02)	1/10 758	
29	c.4328 G>A, p.Arg1443His	1	Jaakson[23]	Tol.	0.19	PRD	0.996				No change	ND	
30	c. 4363 C>T, p. Cys1455Arg	1	This study	Tol.	0.34	PRD	0.994				NA	ND	
30	c.4469 G>A, p.Cys1490Tyr	1	Webster[29]	Intol.	0.03	PRD	0.994				No change	ND	
30	c.4519 G>A, p.Gly1507Arg	1	This study	Tol.	0.48	PRD	0.996	Acc.	78.9	78.9	New site (+58.11)	ND	
30	c.4537_4538insC, p.Gly1513Profs*1554	1	Briggs[20]	NA		NA		Acc.	91.7	33.3	Site broken (−63.76)	ND	
35	c.4918 C>T, p.Arg1640Trp	1	Rozet[28]	Intol.	0	PRD	1				No change	ND	
35	c.4956 T>G, p.Tyr1652*	1	Fumagalli[22]	NA		NA							
Int. 38	c.5461-10 T>C	9	Briggs[20]	NA		NA						3/10 758	db SNP (rs1800728)
39	c. 5516 T>C, p. Phe1839Ser	1	This study	Intol.	0	PRD	0.988				No change	ND	
Int. 40	c.5714+5 G>A, Splice site	1	Cremers[13]	NA		NA		Donor	85.5	73.3	Wild-type site broken (−14.23)	ND	
42	c.5882 G>A, p.Gly1961Glu	1	Allikmets[11]	Tol.	0.18	PRD	1				No change	29/10 758	db SNP (rs1800553)

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SUPPLEMENTAL TABLE 7. Investigation of the Pathogenicity of Identified ABCA4 Variants (*Continued*)

Exon	Nucleotide Substitution and Amino Acid Change	Number of Alleles	Previous Report	SIFT[a] Pred.	SIFT[a] Index (0-1)	PolyPhen 2[a] Pred.	PolyPhen 2[a] Hum Var Score (0-1)	HSF Matrix[a] Site Affected	HSF Matrix[a] Wt CV	HSF Matrix[a] Mt CV	HSF Matrix[a] CV % Variation	Allelic Frequency Observed by EVS[a]	Reference
43	c.5908 C>T, p.Leu1970Phe	1	Lewis[25]	Tol.	0.14	PRD	0.997				No change	ND	
44	c.6079 C>T, p.Leu2027Phe	4	Allikmets[11]	Intol.	0.02	PRD	0.999				No change	3/10 758	db SNP (rs61751408)
44	c.6089 G>A, p.Arg2030Gln	2	Lewis[25]	Tol.	0.1	PRD	0.999				No change	6/10 758	db SNP (rs61750641)
46	c.6320 G>A, p.Arg2107His	2	Fishman[21]	Intol.	0	PRD	0.996				NA	83/10 758	db SNP (rs62642564)
47	c.6449 G>A, p.Cys2150Tyr	5	Fishman[21]	Intol.	0	PRD	0.995	Don.	76.6	49.8	Site broken (−35.02)	1/10 758	db SNP (rs61751384)

Acc. = acceptor site; Don. = donor site; EVS = Exome Variant Server; HSF = Human Splicing Finder program; Hum Var = Human Var score; Int. = intron; Intol. = intolerant; Mt CV = mutant consensus value; NA = not applicable; ND = not detected; PRD = probably damaging; Pred. = prediction; SIFT = Sorting Intolerant from Tolerance program; Tol. = tolerant; Wt CV = wild-type consensus value.

[a]SIFT (version 4.0.4) results are reported to be tolerant if tolerance index ≥ 0.05 or intolerant if tolerance index < 0.05. PolyPhen-2 (version 2.1) appraises mutations qualitatively as Benign, Possibly Damaging, or Probably Damaging based on the model's false-positive rate. The cDNA is numbered according to Ensembl transcript ID ENST00000370225, in which +1 is the A of the translation start codon. Human Splicing Finder (HSF, version 2.4.1) reports the results from the HSF matrix: the higher the consensus value (CV), the stronger the predicted splice site. The values for the wild-type and mutant sequences are shown; the larger the difference between these values, the greater the chance that the variant can affect splicing. EVS denotes variants in the Exome Variant Server, NHLBI Exome Sequencing Project, Seattle, WA, USA (accessed January 12, 2012; http://snp.gs.washington.edu/EVS/).