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

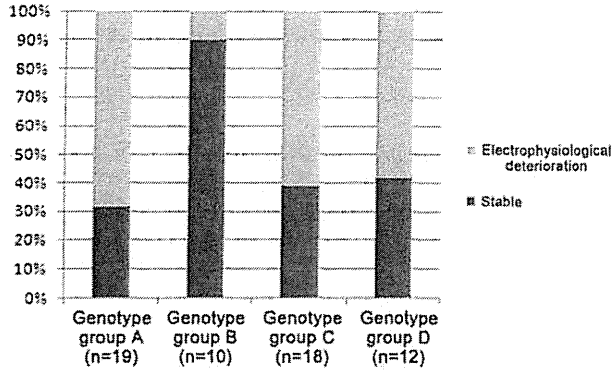
	Dark-Adapted 0.01		Dark-Adapted 11.0				Light-Adapted 30 Hz		Light-Adapted 3.0			
			A-wave		B-wave				A-wave		B-wave	
	Amplitude	Peak Time	Amplitude	Peak Time	Amplitude	Peak Time	Amplitude	Peak Time	Amplitude	Peak Time	Amplitude	Peak Time
Age group (≥50 years old)	30-320	76-117	105-495	10-16	235-665	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.

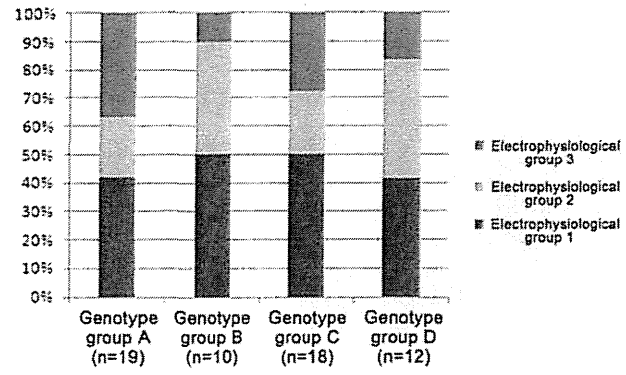
SUPPLEMENTAL TABLE 1. Normal Ranges for Each Component of International Standard Full-field Electroretinography in Young Adults

	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

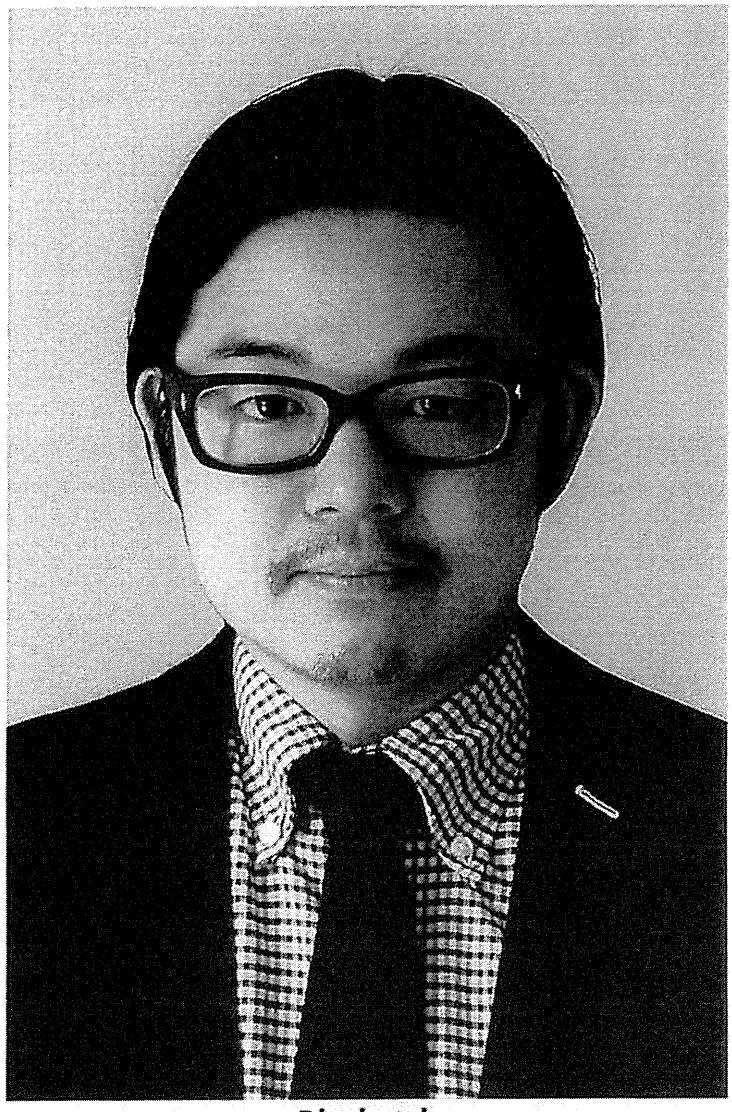
Dark-adapted 0.01 = dark-adapted dim flash electroretinogram with flash intensity 0.01 candela second (cd·s)/m^2; Dark-adapted 11.0 = dark-adapted bright flash electroretinogram with flash intensity 11.0 cd·s/m^2; Light-adapted 30 Hz = light-adapted 30 Hz flicker electroretinogram with flash intensity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



Biosketch

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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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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

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

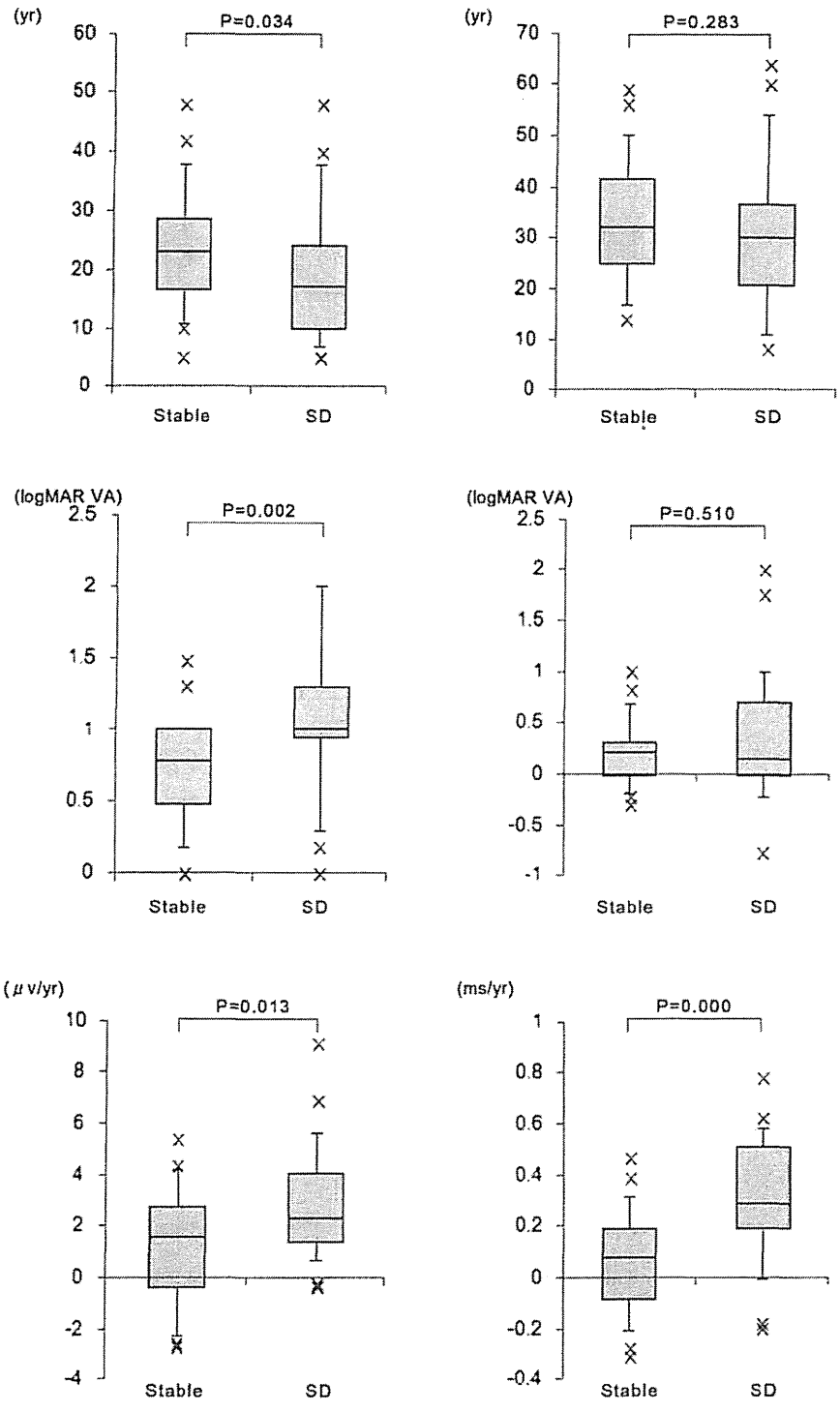

FIGURE 4. A comparison of the clinical findings and electrophysiologic data in Stargardt disease, between the subset of patients with evidence of electroretinogram progression and those without (stable electroretinogram), showing a significant difference in age of onset, visual acuity at baseline, and 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.

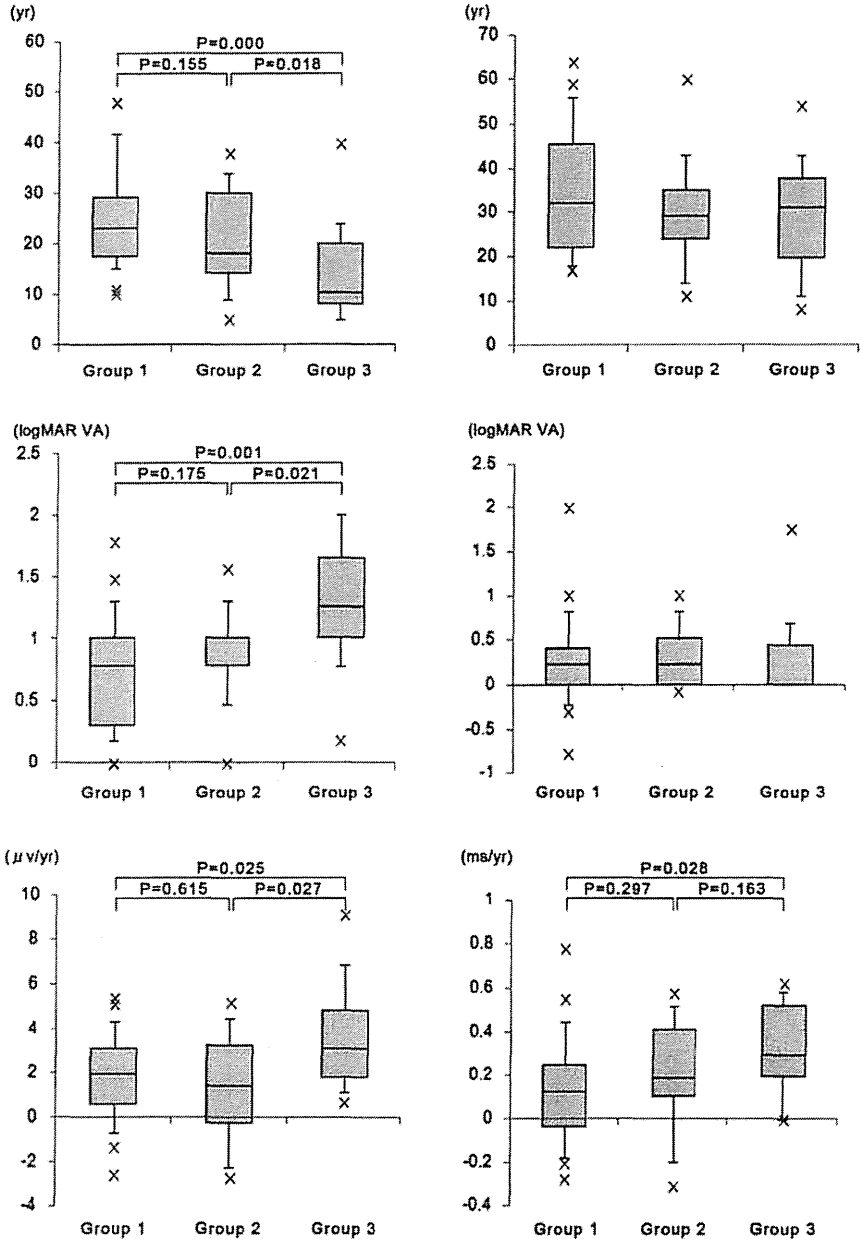

FIGURE 3. A comparison of selected clinical features and electrophysiologic findings associated with each electrophysiologic group at baseline in Stargardt disease, showing significant differences in age of onset, visual acuity at baseline, and electrophysiologic parameters between groups. Age of onset (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

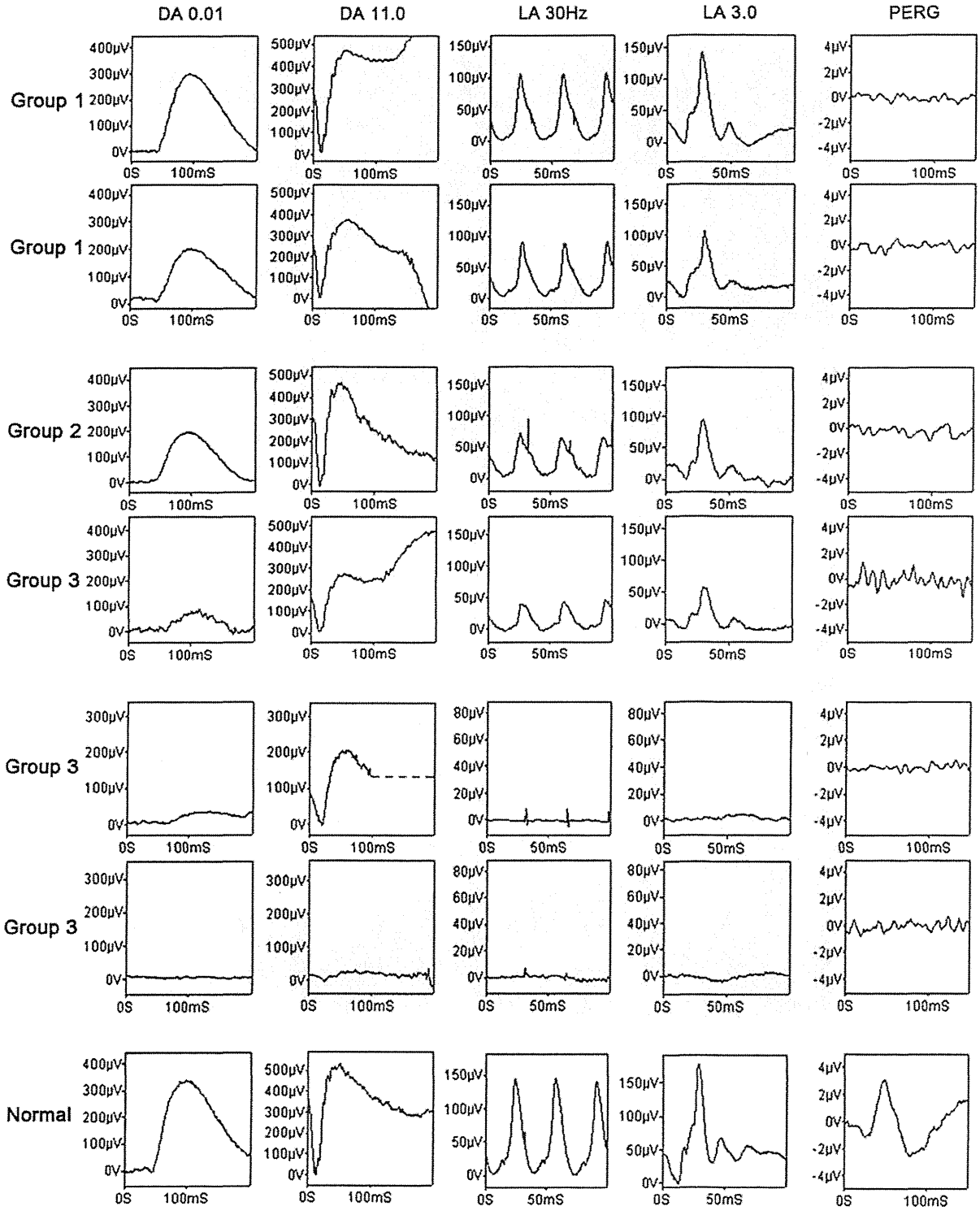

FIGURE 2. Full-field electroretinograms and pattern electroretinograms at baseline and at follow-up from the 3 representative cases of Stargardt disease illustrated in Figure 1 (Patients 17, 42, and 53). Patient 17 demonstrates undetectable pattern electroretinogram (PERG) and normal full-field electroretinograms (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.

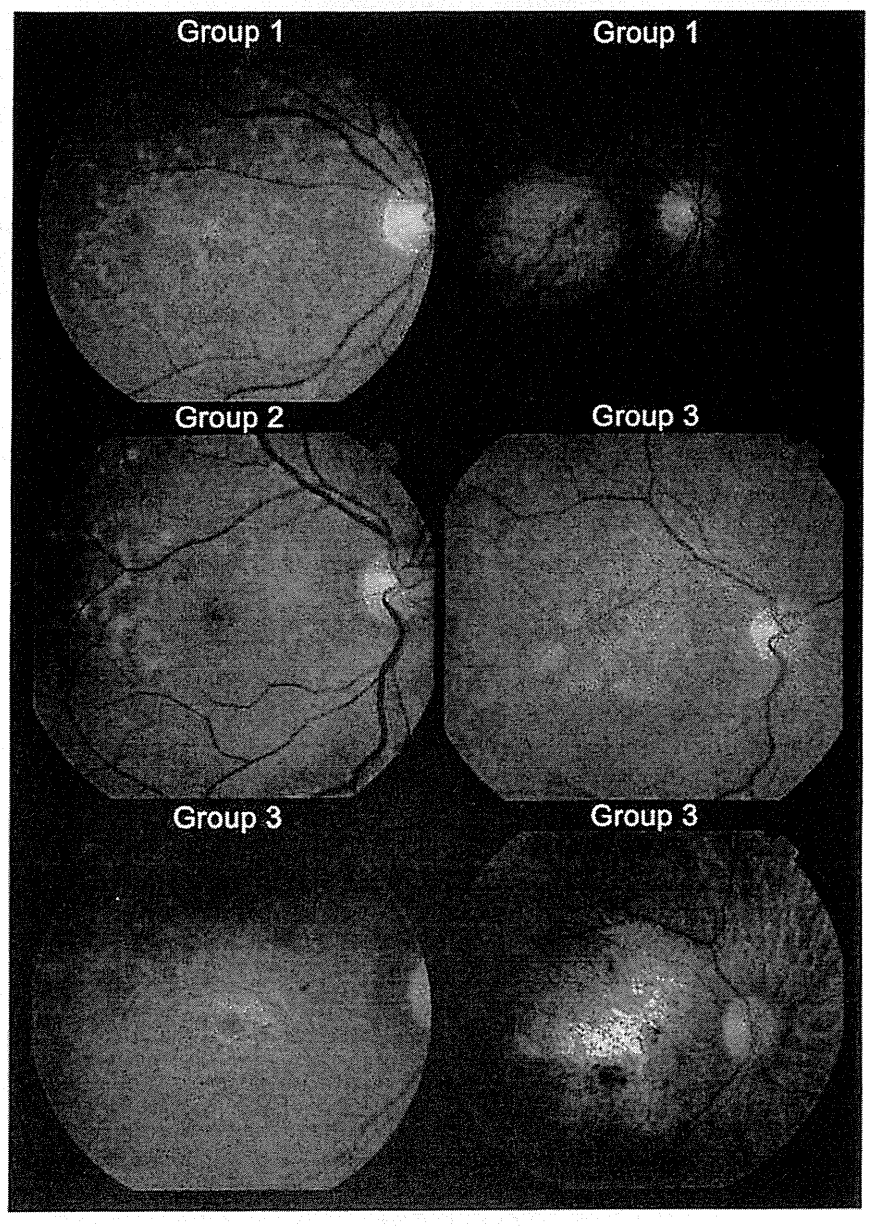

FIGURE 1. Fundus photographs of 3 representative cases of Stargardt disease (Patients 17, 42, and 53) at baseline and at follow-up depicting change over time, with the electrophysiologic group at each time point annotated. (Top) Color fundus photographs of Patient 17 showing macular atrophy surrounded by flecks at baseline (left) and severe well-defined macular atrophy surrounded by atrophic flecks at follow-up (right). Neither electrophysiologic group transition (Group 1 both at baseline and at follow-up) nor clinically significant electrophysiologic deterioration was observed in Patient 17. (Middle) Patient 42 had foveal mottling surrounded by confluent flecks at baseline (left) and multiple areas of macular atrophy at follow-up (right). Electrophysiologic transition from Group 2 to 3, with clinically significant electrophysiologic deterioration, was observed in Patient 42. (Bottom) Patient 53 had multiple areas of macular atrophy with mild pigmentation at baseline (left) and more marked macular atrophy and pigmentation at follow-up (right). Patient 53 was in Group 3 at baseline and experienced clinically significant electrophysiologic deterioration.

p.Cys1455Arg, (5) c.4519G>A, p.Gly1507Arg, and (6) c.5516T>C, p.Phe1839Ser (Supplemental Tables 6 and 7). At least 1 variant was identified in 22 patients (81%, 22/27) in ERG Group 1 at baseline, 12 (71%, 12/17) in Group 2, and 13 (87%, 13/15) in Group 3. At least 1 null variant was found in 8 patients (30%, 8/27) in ERG Group 1 at baseline, 4 (24%, 4/17) in Group 2, and 7 (47%, 7/15) in Group 3 (Supplemental Table 6 and Supplemental Figure 1, available at AJO.com).

• GENOTYPE-PHENOTYPE CORRELATIONS: Clinical features at baseline and electrophysiologic progression in dark-adapted

TABLE 3. Clinical Features Associated With Electrophysiologic Group at Baseline, Electrophysiologic Deterioration, and Genotype Group in 59 Patients With Stargardt Disease

		Median Age of Onset (y)	Median Age		Median logMAR Visual Acuity	
			BL	FL	BL	FL
Baseline electrophysiologic group	Group 1 (n = 27)	24.9	34.4	45.0	0.78	1.00
	Group 2 (n = 17)	20.4	29.6	39.4	1.00	1.00
	Group 3 (n = 15)	14.0	29.1	40.3	1.25	1.30
Evidence of clinically significant electrophysiologic deterioration[a]	Stable (n = 27)	23.4	33.5	43.8	0.78	1.00
	Significant deterioration (n = 32)	18.7	30.1	40.8	1.00	1.19
Genotype grouping[b]	Genotype A (n = 19)	17.6	32.6	42.1	1.08	1.39
	Genotype B (n = 10)	22.3	35.7	48.2	0.84	0.94
	Genotype C (n = 18)	20.0	27.8	38.4	0.90	1.20
	Genotype D (n = 12)	26.1	32.7	43.5	0.69	1.19
	Total (n = 59)	20.8	31.7	42.2	0.93	1.22

BL = baseline; FL = follow-up; logMAR = logarithm of minimal angle of resolution.

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

[b]Each patient was classified into 4 mutually exclusive genotype groups on the basis of the molecular analysis: (A) patients with at least 1 null variant, (B) subjects with 2 or more non-null variants, (C) individuals with 1 non-null variant, and (D) patients with no detectable variants.

a statistically significant difference in logMAR VA between Groups 1 and 3 and between Groups 2 and 3. No statistically significant difference was seen between groups with respect to age at baseline, duration of disease, and follow-up interval. Mean yearly electrophysiologic progression within each baseline ERG group with respect to dark-adapted 11.0 a-wave and light-adapted 30 Hz is summarized in Table 4 and Figure 3. Statistical analysis revealed a significant difference between Groups 1 and 3 and between Groups 2 and 3 in terms of yearly amplitude reduction of dark-adapted 11.0 a-wave (Supplemental Table 5). There was also a statistically significant difference in light-adapted 30 Hz yearly peak time shift between Groups 1 and 3. No significant difference was seen between groups with respect to amplitude reduction in light-adapted 30 Hz.

Thirty-two patients showed evidence of clinically significant electrophysiologic deterioration (Table 2 and Supplemental Table 4). Twenty-one subjects showed a greater than 50% amplitude reduction and 26 patients had more than a 3 ms peak time shift (Supplemental Table 4). The clinical findings were compared between the subset of patients with evidence of ERG progression and those without (stable ERG) (Table 3 and Figure 4). There was a statistically significant difference between the 2 subsets in terms of age of onset and logMAR VA at baseline (Supplemental Table 5 and Figure 4). There were no statistically significant differences between the 2 subsets with respect to age at baseline, duration of disease, interval of follow-up, and reduction in logMAR VA (Supplemental Table 5 and Figure 4).

There was clinically significant deterioration of ERG parameters in 22% (6/27) of patients in ERG Group 1, 65% (11/17) in Group 2, and 100% (15/15) in Group 3 (Table 2). Patients with a Group 1 ERG phenotype both at baseline and at follow-up did *not* show significant electrophysiologic deterioration (78%, 21/27), with the Group 1 subjects (22%, 6/27) who did show ERG progression all moving to either Group 2 or Group 3 in equal proportions. Mean yearly electrophysiologic progression was compared between patients with and without clinically significant ERG deterioration (Table 4 and Figure 4). Statistical analysis revealed a significant difference in terms of both amplitude reduction and peak time shift of dark-adapted 11.0 a-wave (Supplemental Table 5 and Figure 4). There was also a statistically significant difference in light-adapted 30 Hz peak time shift. No significant difference was seen with respect to rate of amplitude reduction in light-adapted 30 Hz (Supplemental Table 5).

• MOLECULAR GENETICS: Likely disease-causing variants in *ABCA4* were detected in 47 out of 59 patients, with 2 or more variants identified in 22 patients and 1 variant in 25 subjects (Table 1 and Supplemental Table 6, available at AJO.com). Nineteen patients had at least 1 null variant, 10 subjects had 2 or more non-null variants, 18 individuals were identified with 1 non-null variant, and 12 patients had no detectable variants. Detailed results, including *in silico* analysis to assist in the prediction of pathogenicity of the variants, are shown in Supplemental Table 7 (available at AJO.com).

Thirty-eight different variants were found in 47 patients: 11 null mutations with 3 predicted to affect splicing, and 27 non-null variants (Supplemental Tables 6 and 7). Eighteen patients harbored at least 1 null variant, with a single subject having 2 null mutations. Thirty-two of these 38 variants have been previously reported and 6 are putative novel mutations: (1) c.1317G>A, p.Trp439*, (2) c.2103G>A, p.Val675Ile, (3) c.2239delC, p.Leu747Cysfs*787, (4) c.4363C>T,

TABLE 1. Clinical Data and Molecular Genetic Status of 59 Patients With Stargardt Disease (*Continued*)

Pt	Onset (y)	Age (y)		logMAR VA		Variants Identified[a]
		BL	FU	BL	FU	
52	11	31	42	1.3/1.3	2.0/2.0	p.Arg1108His
53	5	32	43	2.0/2.0	2.0/2.0	c.5461-10 T>C / p.Cys2150Tyr
54	5	32	43	2.0/2.0	2.0/2.0	c.5461-10 T>C / p.Cys2150Tyr
55	7	36	47	1.3/1.3	3.0/1.3	c.5461-10 T>C / p.Cys2150Tyr
56	13	39	50	1.25/1.56	3.0/3.0	ND
57	23	42	52	1.56/1.0	1.0/1.0	p.Leu747Cysfs*787
58	40	43	54	0.18/0.18	0.78/0.78	ND
59	23	54	65	0.78/1.0	1.0/1.0	p.Ile156Val

BL = baseline; FU = follow-up; logMAR = logarithm of minimal angle of resolution; ND = not detected; Pt = patient; VA = visual acuity.
[a]Putative novel changes are shown in bold.

TABLE 2. Distribution of Electrophysiologic Groups at Baseline and at Follow-up in Stargardt Disease

Electrophysiologic Group[a] at Baseline[b]	Electrophysiologic Group[a] at Follow-up[b]		
	Group 1	Group 2	Group 3
Group 1 (n = 27, **6**)	21	3 (**3**)	3 (**3**)
Group 2 (n = 17, **11**)		9 (**3**)	8 (**8**)
Group 3 (n = 15, **15**)			15 (**15**)
Total (n = 59, **32**)	21	12 (**6**)	26 (**26**)

[a]Patients were classified into 3 groups based on electrophysiologic findings: Group 1 had dysfunction confined to the macula; Group 2 had macular and generalized cone system dysfunction; Group 3 had macular and both generalized cone and rod system dysfunction.
[b]Numbers in bold show the numbers of patients who demonstrated electrophysiologic evidence of deterioration. An amplitude reduction of over 50% in any electrophysiologic component and/or a peak time shift of over 3 ms for the light-adapted 30 Hz electroretinogram or dark-adapted 11.0 electroretinogram a-wave were considered evidence of significant electrophysiologic deterioration.

respective electrophysiologic traces appear in Figure 2. Patient 17 showed no ERG group transition (Group 1 at baseline and Group 1 at follow-up). ERG transition from Group 2 to Group 3, with clinically significant ERG deterioration, was demonstrated in Patient 42. Patient 53 was in ERG Group 3 at baseline and had evidence of clinically significant ERG deterioration.

• ELECTROPHYSIOLOGIC FINDINGS: The electrophysiologic findings are summarized in Supplemental Table 4 (available at AJO.com). PERG P50 components were undetectable (93%, 51/55) or moderately reduced (7%, 4/55; Patients 16, 24, 42, and 55) at baseline, in keeping with severe or moderately severe macular dysfunction; and were undetectable in 53 individuals (96%, 53/55) or moderately reduced in 2 patients (4%, 2/55; Patients 16 and 24) at follow-up. There were no available PERG data both at baseline and at follow-up in 2 subjects (Patients 7 and 23), and no available baseline PERGs in 2 further individuals (Patients 45 and 46), who had undetectable PERGs at follow-up.

Complete ERG data sets were available at baseline and follow-up, with few exceptions (Supplemental Table 4). The dark-adapted 0.01 and dark-adapted 11.0 ERGs were abnormal in 11 and 15 patients (20%, 11/54 and 25%, 15/59), respectively, at baseline, and in 22 and 24 subjects (36%, 22/59 and 41%, 24/59), respectively, at follow-up. All those with abnormal dark-adapted 0.01 ERGs had abnormal light-adapted 30 Hz and light-adapted 3.0 ERGs. Three out of 4 patients (Patients 53-56) with undetectable dark-adapted 0.01 responses at follow-up had undetectable light-adapted ERGs at baseline and at follow-up.

Light-adapted 30 Hz and light-adapted 3.0 ERGs were abnormal in 29 and 26 patients (49%, 29/59, and 45%, 26/58), respectively, at baseline; and in 38 and 36 subjects (64%, 38/59 and 61%, 36/59), respectively, at follow-up. An abnormal light-adapted 3.0 ERG was the only baseline ERG abnormality in 2 patients (Patients 29 and 41); isolated light-adapted 30 Hz ERG abnormality occurred in another 4 subjects (Patients 28, 30, 42, and 48). All 6 of these patients showed abnormal responses in both light-adapted tests at follow-up. Isolated light-adapted 30 Hz ERG abnormality occurred in another 2 patients at follow-up.

Four out of 5 sibships were concordant (the same ERG group) both at baseline and at follow-up (Patients 11 and 14; 40 and 42; 45 and 46; 53-55). Two siblings from 1 family had discordant ERG groups, with 1 sibling in Group 3 at baseline and follow-up and the other sibling in Group 2 at baseline and follow-up (Patients 47 and 29) (Supplemental Table 4).

The clinical features of each baseline group are summarized in Table 3 and Figure 3. There was a statistically significant difference between Groups 1 and 3 and between Groups 2 and 3 in terms of onset of disease (Supplemental Table 5, available at AJO.com). There was also

TABLE 1. Clinical Data and Molecular Genetic Status of 59 Patients With Stargardt Disease

Pt	Onset (y)	Age (y)		logMAR VA		Variants Identified[a]
		BL	FU	BL	FU	
1	16	17	26	0.0/1.0	0.0/0.48	c.768G>T / p.Gly863Ala / p.Arg943Gln
2	15	17	25	0.78/0.78	1.0/1.0	p. Arg1443His
3	11	18	27	0.78/1.0	1.0/1.0	**p.Trp439*** / p.Gly863Ala / p.Leu1970Phe
4	19	21	32	0.78/0.78	1.0/1.0	p.Leu2027Phe
5	10	22	30	0.48/0.48	1.0/0.78	p.Gly863Ala / p.Arg943Gln / c.5461-10T>C
6	18	26	37	0.78/1.0	1.0/1.0	p.Pro1380Phe
7	25	28	40	0.78/1.0	1.3/0.78	ND
8	24	29	38	1.0/0.78	1.0/1.0	p.Phe418Ser / p.Leu2027Phe
9	24	31	44	1.0/1.0	1.3/1.0	c.4253+5 G>T / **p.Gly1507Arg**
10	26	32	44	0.78/0.78	1.0/1.0	p.Cys1490Tyr / p.Arg2030Gln
11	31	34	46	0.18/0.3	0.6/0.7	ND
12	17	35	47	1.0/1.0	1.0/1.0	p.Asn96His
13	23	35	45	1.0/0.3	1.0/0.48	p.Gly1513Profs*1554
14	33	37	48	0.18/1.48	1.0/1.3	ND
15	38	40	51	0.18/0.78	1.0/1.0	p.Arg2107His
16	42	43	53	0.0/0.0	1.0/1.0	ND
17	22	48	59	1.0/1.0	1.0/1.0	p.Cys54Tyr
18	20	49	59	1.0/0.6	1.0/1.0	p.Pro1380Leu / p.Gly1961Glu
19	35	50	61	1.0/0.3	1.0/1.0	p.Arg1108Cys
20	25	56	67	1.3/0.18	1.0/1.0	**p.Trp439*** / p.Gly863Ala
21	48	59	71	1.0/0.78	1.0/1.0	p. Ile156 Val / **p. Cys1455Arg** / **p. Phe1839Ser**
22	21	22	31	0.3/1.0	1.0/1.0	p.Arg2107His
23	21	23	33	1.0/1.0	1.0/1.0	p.Gly863Ala
24	48	64	73	0.0/1.0	0.18/3.0	p.Tyr1652*
25	17	19	29	0.78/0.3	1.0/1.0	c.5461-10 T>C
26	17	21	33	1.0/0.78	1.0/1.0	ND
27	27	53	66	1.78/1.78	1.3/1.0	p.Ser1071Cysfs*1084
28	5	14	21	0.78/0.78	1.0/1.0	p.Arg408* / **p.Val675Ile**
29	9	15	27	1.08/1.08	1.0/1.0	p.Cys2150Tyr
30	14	24	32	1.0/0.78	1.0/1.0	ND
31	18	28	39	1.0/1.0	1.0/1.0	p.Gly863Ala / p.Arg1108Cys / p.Arg943Gln
32	14	29	37	1.0/1.0	1.0/1.0	p.Arg653Cys / p.Arg2030Gln
33	19	29	40	1.0/1.0	1.0/1.08	ND
34	34	40	49	0.3/0.48	1.0/1.0	p.Gly863Ala / p.Glu1087Lys
35	25	43	54	1.0/1.0	1.0/1.0	p.Cys54Tyr / p.Gly863Ala
36	38	60	69	1.0/1.0	1.3/1.08	p.Val931Met / c.5461-10 T>C
37	10	11	20	1.0/0.78	1.3/1.3	p.Pro1380Leu
38	10	15	23	1.0/1.0	1.3/1.3	p.Ser1071Cysfs*1084 / p.Pro1380Leu
39	24	25	38	1.56/0.3	2.0/2.0	c.5461-10 T>C / c.5714+5 G>A
40	18	26	36	1.3/1.3	2.0/1.3	ND
41	32	33	45	0.48/0.48	1.0/1.0	ND
42	32	35	46	1.3/0.0	3.0/1.0	p.Cys54Tyr
43	30	35	45	0.48/0.48	2.0/1.3	ND
44	15	41	49	1.3/1.3	2.0/1.3	p.Asn965Ser
45	8	8	20	0.78/0.78	1.0/1.0	p.Thr1019Met
46	10	11	23	1.0/1.0	1.0/1.0	p.Thr1019Met
47	8	12	24	2.0/1.56	1.78/1.48	p.Cys2150Tyr
48	17	18	26	1.0/0.78	1.3/1.0	c.5461-10 T>C / p.Leu2027Phe
49	8	21	33	1.3/1.3	2.0/2.0	p.Asp574Aspfs*582
50	8	27	39	2.0/1.56	1.78/1.48	c.5461-10 T>C
51	24	31	43	1.18/1.18	1.08/1.3	p.Arg1640Trp / p.Leu2027Phe

Continued on next page

classified into 2 subsets: those with clinically significant ERG deterioration and those without significant ERG deterioration (stable ERG).

• MUTATION SCREENING: Mutation analysis was performed using the single-stranded conformation polymorphism (SSCP) strategy of the whole coding region of *ABCA4* in 33 subjects[36] and the arrayed primer extension (APEX) microarray (ABCR400 chip; Asper Ophthalmics, Tartu, Estonia) for previously reported variants in 27 patients.[23] Direct Sanger sequencing was done in siblings of probands and parents, when available, to confirm segregation of alleles, as well as in 8 subjects either to confirm putative novel variants or where the variants found with SSCP and APEX differed (Supplemental Table 3, available at AJO.com).

Non-null variants were analyzed using 2 software prediction programs: SIFT (Sorting Intolerant from Tolerant; http://sift.jcvi.org/)[37] and PolyPhen2 (http://genetics.bwh.harvard.edu/pph/index.html).[38] All variants were also analyzed for their effect on splicing using the Human Splicing Finder program, version 2.4.1 (http://www.umd.be/HSF/). All variants were compared with variants in the Exome Variant Server, NHLBI Exome Sequencing Project, Seattle, Washington, USA (http://snp.gs.washington.edu/EVS/).

Each patient was classified into 4 mutually exclusive genotype groups on the basis of the molecular analysis: (A) patients with at least 1 null variant, (B) subjects with 2 or more non-null variants, (C) individuals with 1 non-null variant, and (D) patients with no detectable variants. Null variants were those that would be expected to affect splicing, or to introduce a premature truncating codon in the protein if translated. The term "variants" for the purpose of this study includes those sequence changes previously shown to be enriched in Stargardt patients from prior studies, or for very rare variants, those not found at an allele frequency greater than 0.1% on the exome variant database (Accessed March 1, 2012).

• STATISTICAL ANALYSIS: Statistical analysis has been undertaken using data from only 1 eye in each subject. For the 57 patients with the same ERG grouping in both eyes, the eye used for analysis was selected according to the Random Integer Generator (http://www.random.org/). For the 2 patients (Patients 26 and 48) with a different ERG group in each eye, the eye with the more severe ERG grouping (ie, more generalized retinal dysfunction) was selected for analysis.

The Mann-Whitney *U* test was used to explore whether differences observed between patients with clinically significant electrophysiologic deterioration and those without were statistically significant with regard to age of onset, duration of disease, age at baseline, the interval of observation, logMAR visual acuity at baseline, logMAR visual acuity reduction (defined as the difference between visual acuity at baseline and at follow-up), and yearly percentage amplitude reduction and yearly peak time shift in both the light-adapted 11.0 a-wave and light-adapted 30 Hz.

The Kruskal-Wallis test with Steel-Dwass multiple comparisons was performed to compare the 3 baseline ERG groups (ERG Group 1, 2, and 3) and the 3 genotype groups (genotype A, B, and C) for the 10 aforementioned parameters. Where evidence was found of a difference between these groups, all pairwise comparisons were made.

The association between genotype group classification and baseline ERG group classification was tested using the Goodman-Kruskal gamma, a measure of association for ordered categories ranging between −1 and +1 for perfect negative or positive association, respectively. *P* values less than .05 were considered to indicate statistical significance.

All analyses were conducted using MedCalc statistical software version 9.2.1.0 (MedCalc Software, Ostend, Belgium) and Excel Tokei 2010 (Social Survey Research Information Co Ltd, Tokyo, Japan).

RESULTS

• CLINICAL FINDINGS: Fifty-nine patients, 31 female (52%, 31/59) and 28 male (48%, 28/59), were included in the study. All complained of central visual loss with a median age of onset of 20.8 years (range, 5-48 years) and a median duration of disease of 10.9 years (range, 0-31 years). The median ages at baseline and at follow-up were 31.7 and 42.2 years (range, 8-64 and 20-73 years), respectively. The mean follow-up interval was 10.5 years (range, 7-13 years). Seven patients (12%, 7/59) presented before 16 years of age and 52 (88%, 52/59) presented after age 16 years. The median logMAR visual acuities (VA) at baseline and at follow-up were 0.93 (range, 0.0-2.0) and 1.22 (range, 0.0-3.0), respectively, with a median logMAR VA reduction during the follow-up interval of 0.29 (range, −0.78-2.0). The clinical findings are summarized in Table 1 and the eye selected for data analysis is shown in Supplemental Table 3 (available at AJO.com).

At baseline, there were 27 patients (46%, 27/59) in Group 1, 17 (29%, 17/59) in Group 2, and 15 (25%, 15/59) in Group 3, compared at follow-up to 21 patients (36%, 21/59) in Group 1, 12 (20%, 12/59) in Group 2, and 26 (44%, 26/59) in Group 3 (Table 2). The median age of onset for each baseline ERG group was 24.9 years in Group 1, 20.4 years in Group 2, and 14.0 years in Group 3. The median age (years) at examination/logMAR visual acuity at baseline and follow-up for each baseline ERG group was 34.4/0.78 and 45.0/1.00, respectively, in Group 1; 29.6/1.00 and 39.4/1.00, respectively, in Group 2; and 29.1/1.25 and 40.3/1.30, respectively, in Group 3 (Table 3).

Color fundus photographs of eyes in 3 representative cases (Patients 17, 42, and 53) are shown in Figure 1; their

The purpose of the present study was to determine whether longitudinal data from a cohort of Stargardt disease patients support the value of full-field ERG to visual prognosis previously suggested by cross-sectional data. We have assessed the progression of Stargardt disease by repeated clinical and electrophysiologic examinations over time and probed whether the initial phenotype predicts long-term prognosis.

PATIENTS AND METHODS

A COHORT OF 59 PATIENTS WITH A CLINICAL DIAGNOSIS OF Stargardt disease and a minimum of 7 years of follow-up were ascertained at Moorfields Eye Hospital. All patients were first diagnosed between 1997 and 2000, with the latest examinations performed between 2009 and 2011. The baseline clinical and electrophysiologic characteristics of 33 of these 59 patients have been previously reported.[31] The panel included 5 sibships (4 sibling pairs and 1 set of 3 siblings). Informed consent was obtained from all participants. Blood samples were taken from all individuals for DNA extraction. The protocol of the study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Moorfields Eye Hospital.

• CLINICAL ASSESSMENT: Fifty-nine patients were assessed on at least 2 occasions, with the first and most recent visits taken as the baseline and "follow-up" examinations, respectively, for the purposes of data analysis. A full medical history was obtained and a comprehensive ophthalmologic examination performed for all patients. The age of onset was defined as the age at which visual loss was first noted by the patient. The duration of the disease was calculated as the difference between age at onset and age at the baseline examination when an electrophysiologic assessment was obtained. The interval of observation was determined by the difference between the age at baseline and the age at the most recent electrophysiologic examination. Clinical assessment included best-corrected Snellen visual acuity (converted to equivalent logarithm of minimal angle of resolution [logMAR] visual acuity for the purpose of data analysis), dilated ophthalmoscopy, and color fundus photography.

• ELECTROPHYSIOLOGY: All patients underwent electrophysiologic assessment, to include full-field ERG and pattern electroretinography (PERG), incorporating the minimum standards of the International Society for Clinical Electrophysiology of Vision (ISCEV).[32,33] ERG examination was comprehensive and included: (1) dark-adapted dim flash 0.01 candela second (cd·s)/m^2 (dark-adapted 0.01); (2) dark-adapted bright flash 11.0 cd·s/m^2 (dark-adapted 11.0); (3) light-adapted 3.0 cd·s/m^2 30 Hz flicker ERG (light-adapted 30 Hz); and (4) light-adapted 3.0 cd·s/m^2 at 2 Hz (light-adapted 3.0). All recordings were performed with gold-foil recording electrodes with reference electrodes at the ipsilateral outer canthi.

The patient data were compared against those of 16 healthy subjects younger than 50 years and 19 subjects older than 50 years, to maintain consistency with the original cross-sectional study.[4,31,34] PERGs were compared against those from 28 normal subjects, with N95 peak time not being used for interpretation because of its accepted variability.[35] The limits of ERG normality were defined for all the components of the ERG and PERG as the mean value ±2 standard deviations (Supplemental Tables 1 and 2, available at AJO.com). The threshold values for the minimum amplitude/maximum peak time for subjects younger than 50 years were defined as 135 μV/107 ms (dark-adapted 0.01), 250 μV/13 ms and 320 μV/56 ms (dark-adapted 11.0 a- and b-wave, respectively), 70 μV/27 ms (light-adapted 30 Hz), and 30 μV/15 ms and 95 μV/32 ms (light-adapted 3.0 a- and b-wave, respectively); and for patients older than 50 years as 30 μV/117 ms (dark-adapted 0.01), 105 μV/16 ms and 235 μV/57 ms (dark-adapted 11.0 a- and b-wave, respectively), 50 μV/29 ms (light-adapted 30 Hz); and 15 μV/16 ms and 90 μV/32 ms (light-adapted 3.0 a- and b-wave, respectively). The threshold values for the PERG P50 minimum amplitude/maximum peak time were defined as 2.1 μV/58.5 ms.

All the components of the ERG and PERG from each eye were taken into account when classifying patients into 1 of the 3 ERG groups at baseline and follow-up. Group 1 was defined as PERG abnormality with normal ERGs. In Group 2, there was PERG abnormality and abnormal cone function (assessed with light-adapted 30 Hz and light-adapted 3.0) on ERG. In Group 3, there was additional rod ERG abnormality (assessed using dark-adapted 0.01 and dark-adapted 11.0). The overall classification was based on the more severe eye in the small number of patients with different ERG groups between eyes. The data obtained at follow-up were compared with those at baseline. Concordance for ERG group between siblings was defined as siblings having the same ERG group classification both at baseline and at follow-up.

Amplitude reduction was calculated as the difference between amplitude at baseline and at follow-up. The percentage reduction in amplitudes was obtained by dividing the amplitude reduction by baseline amplitude. 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 at follow-up) was also calculated by dividing by the follow-up time.

An amplitude reduction of over 50% in any ERG component and/or a peak time shift of over 3 ms for the light-adapted 30 Hz ERG or dark-adapted 11.0 ERG a-wave were considered evidence of clinically significant ERG deterioration/progression. Patients were thereby

A Longitudinal Study of Stargardt Disease: Clinical and Electrophysiologic Assessment, Progression, and Genotype Correlations

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• PURPOSE: To investigate the clinical and electrophysiologic natural history of Stargardt disease and correlate with the genotype.
• DESIGN: Cohort study of 59 patients.
• METHODS: Clinical history, examination, and electrophysiologic assessment were undertaken in a longitudinal survey. Patients were classified into 3 groups based on electrophysiologic findings, as previously published: Group 1 had dysfunction confined to the macula; Group 2 had macular and generalized cone system dysfunction; and Group 3 had macular and both generalized cone and rod system dysfunction. At baseline, there were 27 patients in Group 1, 17 in Group 2, and 15 in Group 3. Amplitude reduction of >50% in the relevant electroretinogram (ERG) component or a peak time shift of >3 ms for the 30 Hz flicker ERG or bright flash a-wave was considered clinically significant ERG deterioration. Molecular screening of *ABCA4* was undertaken.
• RESULTS: The mean age at baseline was 31.7 years, with the mean follow-up interval being 10.5 years. A total of 22% of patients from Group 1 showed ERG group transition during follow-up, with 11% progressing to Group 2 and 11% to Group 3. Forty-seven percent of patients in Group 2 progressed to Group 3. There was clinically significant ERG deterioration in 54% of all subjects: 22% of Group 1, 65% of Group 2, and 100% of Group 3. At least 1 disease-causing *ABCA4* variant was identified in 47 patients.
• CONCLUSIONS: All patients with initial rod ERG involvement demonstrated clinically significant electrophysiologic deterioration; only 20% of patients with normal full-field ERGs at baseline showed clinically significant progression. Such data assist counseling by providing more accurate prognostic information and are also highly relevant in the design, patient selection, and monitoring of potential therapeutic interventions. (Am J Ophthalmol 2013;155:1075–1088. © 2013 by Elsevier Inc. All rights reserved.)

STARGARDT DISEASE IS ONE OF THE MOST COMMON inherited retinal disorders, with a prevalence of 1 in 10 000. It is inherited as an autosomal recessive trait.[1–3] Most cases present with central visual loss and there is typically macular atrophy with yellow-white flecks at the posterior pole, which are at the level of the retinal pigment epithelium (RPE). Autofluorescence (AF) imaging and fluorescein angiography can be helpful in confirming the diagnosis.[4–8] The age of onset is usually in the early teens, but there is wide variation, with a later age of onset being associated with a better visual prognosis.[7,9]

Since the discovery of *ABCA4* variants underlying Stargardt disease, multiple studies have described the wide phenotypic variability in *ABCA4*-associated retinopathy.[9–19] There is also extensive allelic heterogeneity, with more than 600 sequence variations having been reported to date in the *ABCA4* gene.[10,13,20–30] These 2 features make comprehensive genotype/phenotype correlations challenging. A previous cross-sectional study of 63 patients with Stargardt disease classified subjects into 3 functional electroretinogram (ERG) phenotypes: Group 1: dysfunction confined to the macula; Group 2: macular and generalized cone ERG abnormalities; and Group 3: macular and both generalized cone and rod ERG abnormalities.[31] Differences in rod or cone function between groups could not be explained by differences in age of onset or duration of disease. It was thereby concluded that these 3 groups may represent distinct phenotypic subtypes of Stargardt disease and it was suggested, based on the cross-sectional data, that patients in Group 1 were likely to have a more favorable prognosis.

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