

Applicability of Electrophysiology Measurements in Congenital *PAX6*-Related Aniridia

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PURPOSE. Congenital *PAX6*-aniridia is associated with ocular developmental abnormalities, including photoreceptor specification. We focused on characterizing retinal function by electrophysiology (ERG) measurements and evaluated the usefulness of a handheld electrophysiology tool.

METHODS. Patients underwent a comprehensive ophthalmological examination including best corrected visual acuity (BCVA) and full-field ERG measurements using the RETeval system with sensor strip skin electrodes. In addition to International Society for Clinical Electrophysiology of Vision (ISCEV) standards, photopic negative responses (PhNRs) were recorded.

RESULTS. Forty-one eyes of 21 patients (10 and 11 male patients, age = 25.3 ± 16.9 years, range = 9–60 years) from 16 families were included. BCVA ranged from light perception to 0.3 logMAR (mean = 1.02 ± 0.54) and exhibited greater impairment in older patients. Despite considerable variability in ERG amplitudes, no statistically significant difference was observed in ERG amplitudes between patients and controls ($n = 18$). However, implicit times (ITs) were significantly delayed ($P < 0.001$). Although PhNR response amplitudes and ITs were not significantly different from controls, W-ratio was considerably lower in patients ($W = 0.89 \pm 0.1$) than in controls ($W = 1.09 \pm 0.05$), indicating reduced signal transmission through the different layers of the retina. We observed an effect of glaucoma status causing even lower W-ratios ($W = 0.94 \pm 0.13$ without confirmed glaucoma; and $W = 0.88 \pm 0.11$ with treated glaucoma). ERG recordings were well-tolerated.

CONCLUSIONS. This is the largest ERG study conducted in congenital aniridia. Results show that both photoreceptor-related functions and postreceptor signal transmission are affected in *PAX6*-related aniridia, suggesting retinal changes beyond the central macula. RETeval can serve as a useful instrument for the evaluation and surveillance of retinal function in aniridia, underscoring the potential risk of glaucoma.

Keywords: aniridia, retinal function, electrophysiology, photopic negative response (PhNR), glaucoma screening

Congenital aniridia (OMIM# 106210) represents a rare disorder affecting the entire eye, often grouped under anterior segment dysgenesis conditions, and arises during development. Aniridia is marked by the apparent absence or hypoplasia of the iris, of the fovea, and less frequently the optic nerve. As adults, individuals have an increased likelihood of developing progressive eye issues such as aniridia-associated keratopathy (AAK), cataracts, and glaucoma. Consequently, aniridia often leads to significant

visual impairment, is frequently accompanied by congenital nystagmus, and exhibits variable phenotypic expression both among and within families.^{1,2}

According to population studies from Norway, Sweden, Denmark, and the United States, the disease is estimated to have a prevalence ranging from approximately 1 in 64,000 to 1 in 96,000.^{3,4} Congenital aniridia is caused by loss-of-function variants in the *Paired box 6* gene (*PAX6*; OMIM 607108) or by chromosome rearrangements at 11p13. To

date, more than 700 genetic variants of the *PAX6* gene have been documented.^{5,6} Most *PAX6* mutations causing aniridia are heterozygous, sporadic, or familial with an autosomal dominant pattern, showing phenotypic diversity. About two-thirds of cases are familial, and one-third are sporadic.^{7,8} The majority of identified genetic variants result in the introduction of premature termination codons (PTCs) into the open reading frame of *PAX6*, consequently leading to haploinsufficiency of the *PAX6* transcription factor. This may happen because of mutations within the *PAX6* gene or its regulatory components, or, less commonly, via chromosomal deletions at 11p13.

The *PAX6* gene is commonly known as the “master regulator” of normal eye development due to its interactions with numerous other genes and proteins.^{1,7,8} It acts as a transcription factor that binds to DNA in a sequence-specific manner, influencing a wide range of developmental genes either positively or negatively. This accounts for the diverse phenotypes observed in *PAX6* mutations and the shared phenotypes arising from mutations in other developmental genes that either control or are controlled by *PAX6*, like *FOXC1* and *PITX2*. However, the majority of aniridia phenotypes arise from the loss of a single functional allele of the *PAX6* gene.^{1,7,8}

Mutations in *PAX6* may significantly influence retinal structure and function, as it is integral to the regulation of differentiation among various retinal nerve cell types. These include both early-born types such as retinal ganglion cells and cone photoreceptors, and later-born types like glycinergic amacrine cells and bipolar cells.⁹ Aniridia often presents with foveal hypoplasia, which significantly contributes to primary visual impairment. In addition, vision is further compromised by the emergence of corneal opacification, cataracts, and glaucoma.^{1,10,11} Retinal function in congenital aniridia has been investigated from several perspectives beyond central visual acuity. Disruption in foveal development affect L- and M-cone development, density, and their associated retinal circuitry, causing not only a reduction in visual acuity, but also leading to color discrimination problems along the red-green axis.¹² Dark adaptation threshold measurements have shown both rod- and cone-related impairments, suggesting that retinal anatomic and physiological changes extend beyond the macular area.¹³ Previous electroretinographic studies on small cohorts and case series have demonstrated varying degrees of retinal dysfunction, ranging from severely abnormal to almost normal full-field electroretinograms (ERGs).^{14–16} However, due to anterior segment disorders, increased sensitivity of the eyes, and nystagmus, ERG can be a challenging and undesirable examination, as the placement of corneal or conjunctival electrodes is not well-tolerated in this condition. Consequently, ERG measurements have not become part of the routine screening for patients with aniridia, even though they provide crucial insights into retinal function and the integrity of the visual pathways.

ERG research has made significant progress in recent years, improving diagnostic capabilities, standardization, and the objective evaluation of retinal function in everyday clinical practice. A commercially available portable ERG system has become a practical and economical option for both clinical and research purposes. With the use of sensor strip skin electrodes, more comfortable measurements have become available, broadening the usability of ERG recordings and minimizing patient anxiety and discomfort at the same time.^{17–21}

In this study, we focus on characterizing retinal function using ERG measurements and evaluating the validity and usefulness of a handheld electrophysiology tool in congenital aniridia. Our aim is to provide practical recommendations for the objective and detailed clinical evaluation and follow-up of the disease.

PATIENTS AND METHODS

Patient Recruitment, Consent, and Data Collection

The study was conducted in accordance with the ethical principles outlined in the World Medical Association's Declaration of Helsinki. Approval from the local ethics committee (Nr 80/2020) and informed consent from all patients or their legal guardians were obtained prior to clinical assessment. Patients with genetically confirmed pathogenic variants in the *PAX6* gene were recruited for comprehensive clinical examination.

Clinical Assessment

An extensive ophthalmic examination of the anterior and posterior segments was conducted using slit-lamp biomicroscopy. This included grading of AAK^{22,23} and iris hypoplasia, assessment of lens status, and evaluation of the optic disc and foveal hypoplasia. Where feasible both funduscopy and spectral-domain optical coherence tomography imaging (SD-OCT; Maestro2; Topcon Healthcare) were used to determine alterations of the posterior pole. Best corrected visual acuity (BCVA) was determined and full-field ERG measurements using the RETeval system with sensor strip skin electrodes (LKC Technologies Inc., Gaithersburg, MD, USA) were performed on patients and healthy controls. Despite the absence or hypoplasia of the iris, patients were administered tropicamide 0.5% eyedrops to ensure uniform conditions and consistent measurements in each case. Under light-adapted conditions, photopic negative responses (PhNRs) were recorded in both eyes, with eyes stimulated using red flashes (621 nm, 1.0 cds/m²) on a steady blue background (470 nm, 10 cd/m²). The stimulus frequency was 3.4 hertz (Hz), and 100 responses were averaged for each recording to enhance the signal-to-noise ratio. The ratio of the b-wave peak voltage to the PhNR trough voltage to b-wave amplitude was designated as the W-ratio. PhNR recordings were followed by the photopic ERG protocol on both eyes. Finally, after 20 minutes of dark adaptation, scotopic ERG measurements were performed. All ERG protocols adhered to the standards established by the Standardization Committee of the International Society for Clinical Electrophysiology of Vision (ISCEV).^{24,25} For comparison, age-matched healthy controls were examined with the same ERG protocols.

Genetic Evaluation

Prior to the current study, patients underwent genetic testing to confirm the causative role of *PAX6*. Evidence of pathogenicity was determined according to the guidelines provided by the American College of Medical Genetics and Genomics (ACMG).²⁶ To investigate whether genotype differences are reflected in the phenotype, we divided the patients into subgroups based on the underlying genotype: (1) genetic variants resulting in loss of function (the LOF group), such as PTCs or deletions, and (2) genetic variants associated with a predictably less severe phenotype, such as

missense mutations or mosaicism (reduced expression, the REX group).

Statistical Analysis

The statistical analysis of the data was conducted by using the JMP 16 statistical software (SAS Institute, Cary, NC, USA). Data normality was assessed by evaluating histogram plots. We also checked for interocular symmetry and calculated variances between the right and left eye in absolute values including coefficient of variation (CV). Correlation parameters were calculated using Pearson or Spearman analysis, as appropriate. To test for differences between groups, Holm-Bonferroni corrected pairwise *t*-tests or 1-way ANOVA were performed.

RESULTS

Forty-one eyes of 21 patients (10 female and 11 male patients, mean age [mean $\pm$ standard deviation] = 25.3 $\pm$ 16.9 years, range = 9–60 years) from 16 families were included. The symmetry between ocular parameters was assessed, revealing a CV of 0.7 or greater. Such values suggest the presence of interocular asymmetry; consequently, data from both eyes were incorporated into the analysis.

Glaucoma diagnoses in our patients had been established prior to this study in accordance with existing guidelines and recommendations²⁷ and as previously described by Fries et al. in studies on congenital aniridia subjects.^{28,29} Patients were categorized as “treated glaucoma” patients if they were receiving conservative glaucoma treatment or had undergone antiglaucoma surgery. In all other cases, glaucoma was not confirmed (IOP < 21 millimeters of mercury [mm Hg] and no documented increase in optic disc excavation in the patient history). Accordingly, we refer to the groups as “treated glaucoma” and “without confirmed glaucoma.” Healthy controls ($n = 18$, 36 eyes, 10 women and 8 men, 37.6 $\pm$ 12.1 years) were tested with the same electrophysiological procedure for comparison.

Clinical and genetic characteristics of our patients are presented in Table 1. All patients had nystagmus. BCVA ranged from light perception (3.7 logMAR) to 0.2 logMAR (mean = 1.1 $\pm$ 0.7) and showed a decline with age (Spearman's correlation $r_s = 0.5$). BCVA was better in the REX group (0.9 $\pm$ 0.5, $n = 4$, 8 eyes) than in the LOF group (1.15 $\pm$ 0.8, $n = 17$, 33 eyes), likely due to the difference in mean age between the two groups (14.2 $\pm$ 6 years in the REX group and 27.8 $\pm$ 17.6 years in the LOF group, respectively). In comparisons of patients with treated and without confirmed glaucoma, a significant difference in BCVA was observed: patients without confirmed glaucoma had more preserved BCVA (0.67 $\pm$ 0.28, $n = 10$, 19 eyes) compared with those with treated glaucoma (1.41 $\pm$ 0.88, $P = 0.002$, $n = 11$, 22 eyes), as expected.

Overall, ERG recordings performed with the RETeval system and sensor strip skin electrodes were well-tolerated by all participants, including pediatric subjects and those with significant anterior segment pathologies such as AAK. Wave morphology was relatively preserved in all recordings (Fig. 1). Although ERG amplitudes varied widely, no significant difference in dark-adapted ERG amplitudes was detected when comparing affected patients to the control group. However, implicit times were significantly delayed for dark adapted (DA) 0.01 cds/m² and DA 3.0 cds/m²

b-waves ($P < 0.001$). Light-adapted (LA) single flash a- and b-wave implicit times were significantly delayed ($P < 0.01$ and $P < 0.001$, respectively), and 30 Hz flicker implicit time was prolonged ($P < 0.001$), whereas response amplitudes showed no significant difference ($P = 0.17$; Fig. 2). In the subgroup analysis, scotopic and photopic response amplitudes were comparable between the LOF and REX groups and showed no significant difference compared with the controls. However, implicit times under both scotopic and photopic conditions were prolonged in the LOF group. This delay in responses was not observed in the REX group, where timing was close to normal. We acknowledge that the small sample size in the REX group, as well as their younger average age, limit the interpretation of phenotype differences between the groups. ERG parameters for each group and recording condition are shown in Table 2.

Furthermore, we observed a tendency for worsening with age in the dark and light adapted response amplitudes and implicit times (Fig. 3). The calculated age slope was -0.365 μ V/year for dark adapted rod function (DA 0.01 cds/m² b-wave amplitude) and -0.173 μ V/year for light adapted cone function (LA 3.0 cds/m² b-wave amplitude). These rates of decline were significantly steeper than the age correction factor determined in a normal population (-0.185 μ V/year and -0.091 μ V/year for rod and cone function, respectively), as provided by the manufacturer of the RETeval system. The reference population for their ERG tests included 309 individuals aged 4 to 85 years from 6 trial sites in the United States and Canada, all carefully examined to have normal vision.³⁰ On the other hand, implicit times of the dark-adapted weak flash response showed a slight increase over time, comparable to the reference values provided by the manufacturer. The DA 0.01 cds/m² b-wave implicit time slope in aniridia was calculated as 0.43 ms/year, compared to 0.45 ms/years in healthy subjects. Under light adapted conditions, the LA 3 cds/m² b-wave implicit time increment was calculated as 0.06 ms/year, compared to 0.04 ms/year in the controls.

Although PhNR amplitudes and implicit times did not differ significantly from controls, W-ratio was lower in patients ($W = 0.91 \pm 0.12$) compared with controls ($W = 1.09 \pm 0.05$), indicating reduced signal transmission through the different layers of the retina ($P < 0.0001$). No significant difference was observed between the LOF and REX groups (W-ratio was 0.89 ± 0.1 and 0.95 ± 0.17 , respectively), however, the REX group, with a predictably milder phenotype, appeared to have a more preserved retinal function. Considering the known glaucoma status in each case we also analyzed whether the W-ratio could serve as a helpful indicator of retinal nerve fiber layer loss in aniridia. In general, patients with aniridia showed significantly lower values than the controls. However, we observed an effect of glaucoma status, with lower W-ratios in patients with treated glaucoma ($W = 0.94 \pm 0.13$ without confirmed glaucoma and $W = 0.88 \pm 0.11$ with treated glaucoma; see Table 2). BCVA weakly correlated with the W-ratios ($r_s = -0.34$, $P = 0.04$), moderately correlated with implicit time delays ($r_s = 0.56$, $P = 0.0001$ for LA 3 cds/m² b-wave implicit time; $r_s = 0.47$, $P = 0.02$ for the LA 30 Hz flicker response implicit time), and amplitudes of the light-adapted cone response in the ISCEV standard ERG ($r_s = -0.38$, $P = 0.01$ for LA 3 cds/m² b-wave amplitudes; $r_s = -0.57$, $P = 0.0001$ for the LA 30 Hz flicker response amplitudes).

In a single case (patient 21 [P21]) with a mosaic PAX6 genetic variant and predictably milder phenotype, an ERG with reduced b/a ratios was observed (b/a ratio 1.09 in the

TABLE 1. Clinical and Genetic Characteristics of Patients With Congenital Aniridia

Patient	Gender	Age, Y	Eye	BCVA LogMAR	PAX6 Genetic Variant	ACMG Classification	Evidence of Pathogenicity	Genetic Group	AAK Grade	Iris Grade	Lens	Glaucoma, Yes or No	Optic Disc	Macula	W-Ratio
1	M	23	R	3.7	[c.130delC, p.Arg44Glu] 24] het	P*	PVS1, PM2, PP4	LOF	4	4	n.a.	Y	n.a.	n.a.	0.93
2	F	23	L	0.88					2	4	Pseudophakia	Y	n.a.	n.a.	0.97
			R	0.88	[c.399+1G>T] het	P*	PVS1, PS4, PM2, PP4	LOF	1	2	Cataract	N	n.a.	Hypoplasia	0.79
3	F	45	L	1.1					1	2	Cataract	N	n.a.	Hypoplasia	1.02
			R	1.1	[c.399+1G>T] het	P*	PVS1, PS4, PM2, PP4	LOF	3	2	Pseudophakia	Y	n.a.	n.a.	0.85
4	F	16	L	1.5					3	2	Pseudophakia	Y	n.a.	n.a.	1.00
			R	0.7	[c.664C>T, p.Arg222Trp] het	P	PS1, PS4, PP4	REX	1	1	Aphakia	Y	Hypoplasia	Hypoplasia	0.74
5	M	17	L	1.1					1	2	Aphakia	Y	Hypoplasia	Hypoplasia	0.82
			R	0.5	[c.184-5T>G] het	P	PS3, PS4, PM2, PP1, PP4	LOF	0	0	n.a.	N	n.a.	Hypoplasia	0.89
6	F	12	L	0.5					0	0	n.a.	N	n.a.	Hypoplasia	0.80
			R	0.5	[c.184-5T>G] het	P	PS3, PS4, PM2, PP1, PP4	LOF	2	2	Cataract	Y	Hypoplasia	Hypoplasia	1.0
7	F	14	L	0.6					2	2	Cataract	Y	Hypoplasia	Hypoplasia	1.11
			R	0.3	[c.184-5T>G] het	P	PS3, PS4, PM2, PP1, PP4	LOF	0	0	Cataract	N	n.a.	Hypoplasia	0.90
8	F	23	L	0.3					0	0	Cataract	N	n.a.	Hypoplasia	0.91
			R	1.6	[c.1075-1G>T] het	P*	PVS1, PS4, PM2, PP4	REX	4	4	Cataract	Y	n.a.	n.a.	0.98
9	M	35	L	1.7					4	4	Cataract	Y	n.a.	n.a.	1.05
			R	0.6	[c.121_127delGACATT, p.Asp41Profs 25] het	P*	PVS1, PM2, PP4	LOF	2	4	Cataract	N	Normal	Hypoplasia	0.98
10	F	11	L	enucleated					n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
			R	0.7	[c.850A>T, p.Lys284*] het	P*	PVS1, PM2, PP4	LOF	2	4	Cataract	Y	Hypoplasia	Hypoplasia	0.98
11	M	57	L	0.7					2	4	Cataract	Y	Hypoplasia	Hypoplasia	0.88
			R	2.3	[c.355A>T, p.Arg119*] het	P*	PVS1, PM2, PP4	LOF	4	1	n.a.	Y	n.a.	n.a.	0.69
12	M	26	L	2.3					4	1	n.a.	Y	n.a.	n.a.	0.71
			R	2	[c.355A>T, p.Arg119*] het	P*	PVS1, PM2, PP4	LOF	2	4	Cataract	Y	n.a.	n.a.	0.74
13	M	51	L	1.2					2	4	Cataract	Y	n.a.	n.a.	0.93
			R	0.8	11p13 (31783435–31803845) del	P*	PVS1, PM2, PP1, PP4	LOF	2	4	Cataract	N	n.a.	n.a.	0.94
14	F	9	L	1					2	4	Cataract	N	n.a.	n.a.	0.85
			R	0.8	[c.1225+1_1225+3delGTG] het	P	PVS1, PM2, PP1, PP4	REX	1	4	Cataract	N	n.a.	Hypoplasia	0.81
15	M	11	L	0.8					1	4	Cataract	N	n.a.	Hypoplasia	0.81
			R	1.2	[c.1130C>A, p.Ser377*] het	P	PVS1, PS4, PM2, PP4	LOF	1	2	Cataract	Y	Hypoplasia	Hypoplasia	0.88
16	M	10	L	1					1	2	Cataract	Y	Hypoplasia	Hypoplasia	0.83
			R	1	[c.715delC, p.Leu239TRPfs 19] het	P*	PVS1, PM2, PP4	LOF	2	4	Cataract	N	Hypoplasia	Hypoplasia	1.0
17	M	12	L	1.3					2	4	Cataract	Y	Normal	n.a.	0.98
			R	1.3	[c.349C>T, p.Arg117*] het	P	PVS1, PS4, PM2, PP4	LOF	2	4	Cataract	Y	Normal	n.a.	n.a.
18	M	50	L	3.7					2	4	Cataract	Y	n.a.	n.a.	0.4
			R	3.7	[c.349C>T, p.Arg117*] het	P	PVS1, PS4, PM2, PP4	LOF	4	4	n.a.	Y	n.a.	n.a.	0.4
19	F	60	L	3.7					4	4	n.a.	Y	n.a.	n.a.	0.78
			R	0.7	11p13(31810220–31819962) del	P	PVS1, PM2, PP4	LOF	3	1	Pseudophakia	N	n.a.	n.a.	0.84
20	F	17	L	0.7					4	1	Pseudophakia	N	n.a.	n.a.	0.82
			R	0.7	[c.817dupT, p.Ser273Phefs 2] het	P	PVS1, PS4, PM2, PP4	LOF	4	4	Cataract	N	n.a.	n.a.	0.88
21	M	9	L	0.8					4	4	Cataract	N	n.a.	n.a.	0.88
			R	0.2	[c.417_418delAG>T, p.Arg139Serfs 7] het mosaic	P	PVS1, PS4, PM2, PP4	REX	1	1	Clear lens	N	n.a.	n.a.	1.17
			L	0.3					1	1	Clear lens	N	n.a.	n.a.	1.23

L, left; LOF, loss of function; N, no; n.a., not available; R, right; REX, reduced expression; Y, yes.

Evidence of pathogenicity was determined according to the guidelines provided by the American College of Medical Genetics and Genomics (ACMG).²⁶

AAK Grade: Aniridia Associated Keratopathy Grade according to Lagali et al.^{22,23} Iris Grade: According to Lagali et al.^{22,23}

W-ratio = (b - pmin)/(b - a) where a, b, and pmin are the voltages relative to baseline defined as a: a-wave peak, b: b-wave peak, and pmin: the minimum of the PhNR (photopic negative response) wave.

*Genetic variant previously not reported.

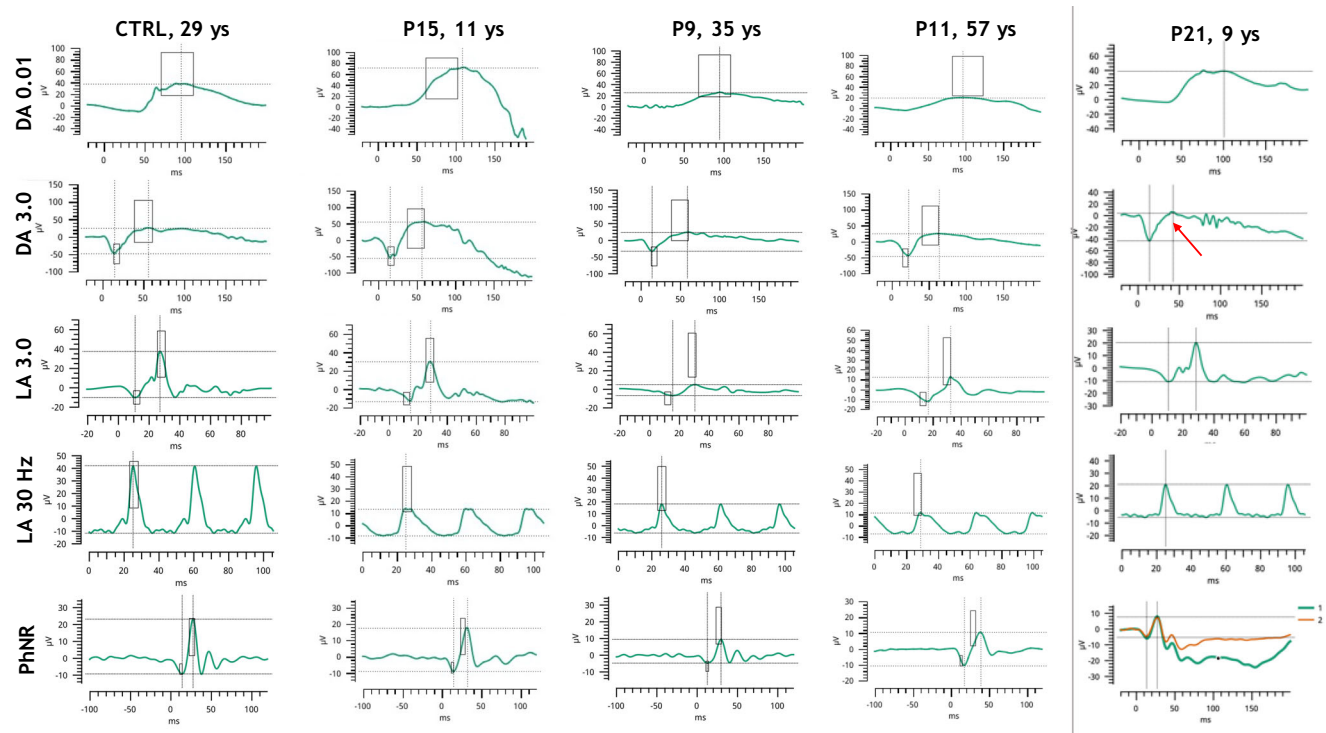


FIGURE 1. Representative ERG measurements from a control individual (ctrl) and four aniridia patients of different ages (9, 11, 35, and 57 years): In a case (patient 21 [P21]) with a mosaic *PAX6* genetic variant and a predictably milder phenotype, an ERG with reduced b/a ratios was observed (b/a ratio was 1.09 and 1.08 on the right and left eyes, respectively). The red arrow indicates the reduced b-wave in the dark adapted 3.0 ERG. DA 0.01: dark adapted rod response ERG, DA 3.0: dark adapted mixed cone-rod response ERG, LA 3.0: light adapted cone response ERG, LA 30 Hz: light adapted 30 Hz flicker ERG.

right eye and 1.08 in the left eye). The ISCEV standard ERG recordings otherwise showed implicit times within normal limits. The PhNR W-ratio in this case was also within the normal range (1.17 and 1.23 for the right and left eyes, respectively), and the patient was not diagnosed with glaucoma (see Fig. 1).

DISCUSSION

The issue of vision impairment associated with congenital aniridia is highly complex. Due to foveal hypoplasia and retinal developmental problems, vision impairment in congenital aniridia is present already at birth, as nystagmus is often observed. The partial or complete absence of iris tissue further impairs vision by increasing photophobia and optical aberrations caused by peripheral light entering the lens or by inducing excessive photopigment bleaching in the retina due to increased light exposure. Furthermore, visual acuity can be significantly compromised by opacification of the structures within the anterior segment, such as in cases of keratopathy and cataracts, typically manifesting later in life. Glaucoma, resulting from anterior segment dysgenesis, also contributes to visual impairment.^{1,2,11,31–33}

From a clinical perspective, the retina frequently exhibits indications of irregular cellular proliferation, including conditions such as foveal and optic nerve hypoplasia. It is plausible that there may be alterations in the distribution or quantity of photoreceptors. Supporting this, a reduction in cone density within the macula has been documented in aniridia using adaptive optics scanning light ophthalmoscopy.^{10,34} Moreover, investigations into color discrimi-

nation problems in aniridia suggest that the visual function deterioration is not solely confined to diminished visual acuity but is also likely attributable to modifications in retinal processing.¹²

Dark adaptation threshold measurements investigating the rod and cone system have also shown that both photoreceptor functions are affected, suggesting that retinal functional changes are not limited to the macular area, where hypoplasia is often observed.¹³ The impact on rod density remains uncertain; however, it is probable that it is influenced due to *PAX6*'s significant role in the initial stages of retinal development in embryos. *PAX6* is key in directing the development of various retinal cell types, such as cone and rod photoreceptors.^{9,13,35}

In young patients with aniridia, BCVA is likely correlated with the degree of retinal dysfunction, compounded by anterior segment dysplasia.^{10,14,33} Consequently, retinal dysfunction, as quantified by ERG, should be considered a useful additional feature of the aniridia phenotype. The visual impairment experienced by patients with aniridia may not solely result from anterior segment dysplasia.^{1,14,33} Previous smaller studies and case reports on ERG recordings indicated heterogeneity in retinal function among patients with aniridia, with observed ERG findings ranging from nearly normal to severely affected.^{14–16} Another study demonstrated that topography of the multifocal ERG (mfERG) responses in the macula was aberrant in patients with aniridia compared with healthy controls. Specifically, there was a slower drop-off of amplitude from center to periphery, and the amplitudes in the innermost central rings were lower than those in the controls.¹⁴

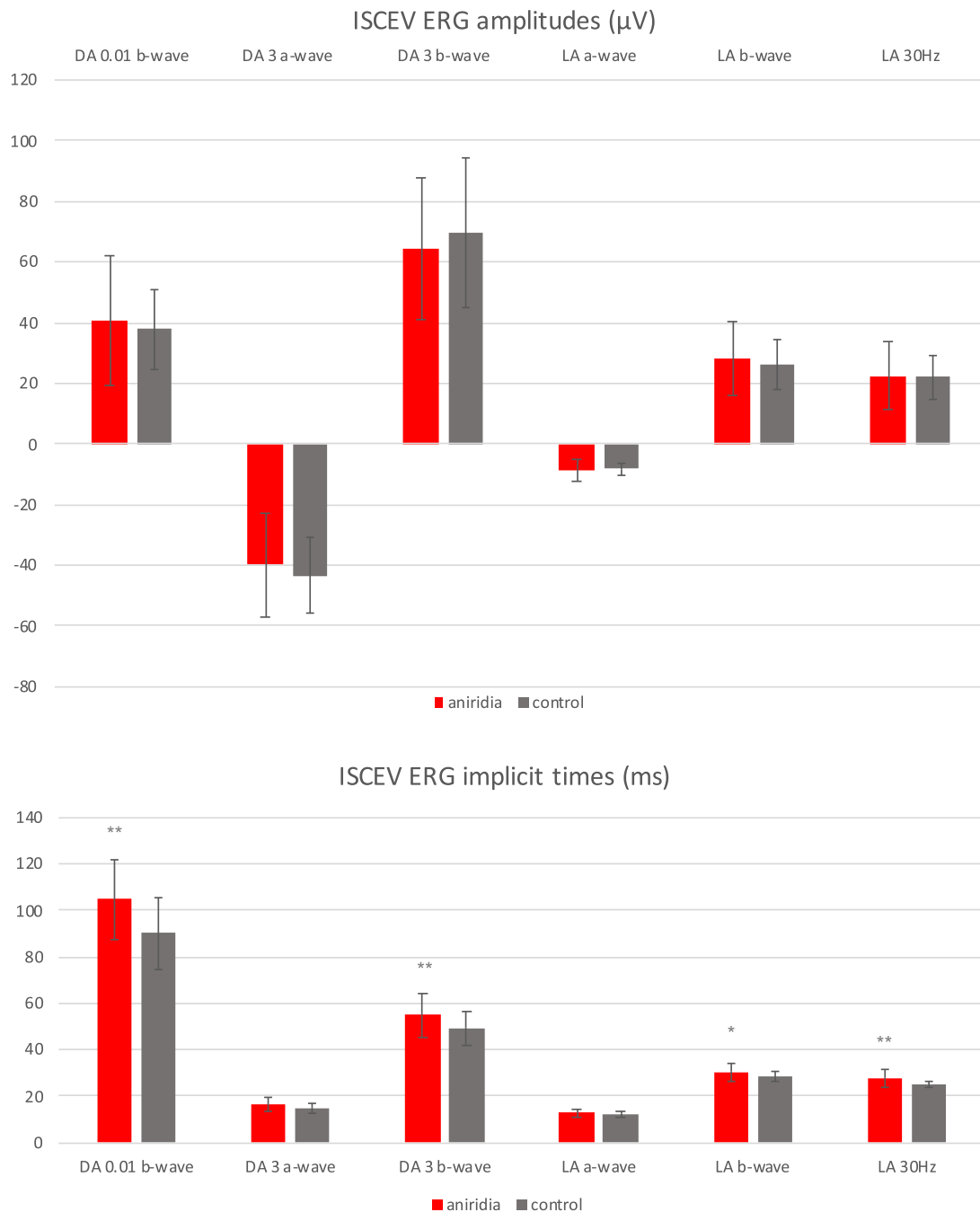


FIGURE 2. Amplitudes and implicit times of the ERG recordings in aniridia patients and in healthy controls. Although ERGs amplitudes varied, no significant difference was detected when comparing affected patients with the control (ctrl) group ($P = 0.17$). On the other hand, implicit times were significantly delayed under both scotopic and photopic conditions (* $P < 0.01$, ** $P < 0.001$).

Despite the ability of ERG to offer significant insights into retinal function and the condition of visual pathways, it is not commonly used in evaluating patients with aniridia. Patient discomfort often prevents the placement of electrodes on the cornea or conjunctiva, particularly in individuals with ocular surface problems or in children. Furthermore, reduced BCVA and nystagmus can affect the accuracy of mfERG recordings. The challenge of maintaining good fixation throughout a 5 to 10 minute recording session poses a significant hurdle, even for adults, not to mention children.³⁶ With the introduction of RETeval, a portable electrophysiology device, and the

sensor strip skin electrodes, most of these objective measurements have become more accessible for routine clinical practice.

Our current study reports on the largest cohort of patients with aniridia measured with ERG so far. Full-field ERG alterations were observed in the majority of patients, ranging from almost normal to severely affected, suggesting heterogeneity in retinal function, as described in previous studies.^{11,37} Although complications in the anterior segment and varying degrees of iris hypoplasia and opacification of ocular media may diminish the amount of light reaching the retina,

TABLE 2. ERG Response Amplitude and Implicit Time Values for Each Group Under Different Recording Conditions

	Age, Y	BCVA	DA 0.01		DA 3.0		DA 3.0		DA 3.0		LA 3.0		LA 3.0		LA 30 Hz		W-Ratio
			Amp, μ V	IT, ms	b-Wave	a-Wave	Amp, μ V	IT, ms	b-Wave	a-Wave	Amp, μ V	IT, ms	b-Wave	a-Wave	Amp, μ V	IT, ms	
Patients ($n = 21$, 41 eyes)	25.3 $\pm$ 16.9	1.1 $\pm$ 0.7	40.6 $\pm$ 21.3	104.9 $\pm$ 17.4**	16.4 $\pm$ 2.9*	-39.6 $\pm$ 17.1	16.4 $\pm$ 2.9*	64.3 $\pm$ 23.5	54.9 $\pm$ 9.6**	-8.6 $\pm$ 3.6	12.8 $\pm$ 1.6	28 $\pm$ 12.3	30.4 $\pm$ 3.4*	22.5 $\pm$ 11.4	27.7 $\pm$ 3.5**	0.91 $\pm$ 0.12**	
LOF group ($n = 17$, 33 eyes)	27.8 $\pm$ 17.6	1.2 $\pm$ 0.8	38.4 $\pm$ 17.8	104.8 $\pm$ 17.2**	16.6 $\pm$ 3*	-38.1 $\pm$ 17.1	16.6 $\pm$ 3*	62.2 $\pm$ 21.5	54.8 $\pm$ 7.8**	-8.6 $\pm$ 3.8	13 $\pm$ 1.7*	27.4 $\pm$ 3.7	30.7 $\pm$ 3.7*	22.1 $\pm$ 11.6	28 $\pm$ 11.6**	0.89 $\pm$ 0.1**	
REX group ($n = 4$, 8 eyes)	14.2 $\pm$ 6	0.9 $\pm$ 0.5	49.1 $\pm$ 31.7	105.2 $\pm$ 19.5**	15.3 $\pm$ 2.3	-45.8 $\pm$ 16.9	15.3 $\pm$ 2.3	72.7 $\pm$ 30.3	55 $\pm$ 15.7**	-8.9 $\pm$ 3.1	11.7 $\pm$ 1	30.7 $\pm$ 12.9	28.9 $\pm$ 0.8	24.4 $\pm$ 10.9	26.4 $\pm$ 1.7**	0.95 $\pm$ 0.17**	
Without confirmed glaucoma ($n = 10$, 19 eyes)	23.9 $\pm$ 18	0.7 $\pm$ 0.3	46 $\pm$ 24.7	97 $\pm$ 13.7*	15.4 $\pm$ 2.2	-40.2 $\pm$ 12.6	15.4 $\pm$ 2.2	67.6 $\pm$ 21.4	53 $\pm$ 8.1*	-10.3 $\pm$ 3.2	12.8 $\pm$ 1.5	32.8 $\pm$ 10.4	29.5 $\pm$ 1.3	25.3 $\pm$ 8.3	26.8 $\pm$ 2.8**	0.94 $\pm$ 0.13**	
Treated glaucoma ($n = 11$, 22 eyes)	26 $\pm$ 16.5	1.4 $\pm$ 0.9	35.9 $\pm$ 17.2	111.7 $\pm$ 17.7**	17.2 $\pm$ 3.2**	-39.1 $\pm$ 20.4	17.2 $\pm$ 3.2**	61.4 $\pm$ 25.4	56.5 $\pm$ 10.7**	-7.2 $\pm$ 3.3	12.7 $\pm$ 1.7	23.9 $\pm$ 12.5	31.1 $\pm$ 4.3*	19.9 $\pm$ 13.3	28.4 $\pm$ 4**	0.88 $\pm$ 0.11**	
Controls ($n = 18$, 36 eyes)	37.6 $\pm$ 12.1	0.0 $\pm$ 0.02	38 $\pm$ 13.2	90 $\pm$ 15.6	14.8 $\pm$ 2.2	-43.2 $\pm$ 12.2	14.8 $\pm$ 2.2	69.7 $\pm$ 24.6	49.3 $\pm$ 7	-8.3 $\pm$ 1.9	12.2 $\pm$ 1.5	26.3 $\pm$ 8	28.5 $\pm$ 1.8	25 $\pm$ 1.1	22 $\pm$ 7.3	1.09 $\pm$ 0.05	
Normal values	n.a.	n.a.	36.0 (34.1 to 37.6)	76.3 (74.1 to 78.0)	14.3 (14.0 to 14.7)	-36.8 (-38.8 to -34.8)	14.3 (14.0 to 14.7)	63.6 (57.5 to 71.3)	45.0 (43.6 to 46.6)	-8.3 (-8.9 to -7.7)	11.6 (11.3 to 11.9)	39.5 (37.3 to 41.8)	27.3 (27.0 to 27.5)	29.0 (26.9 to 30.6)	25.2 (24.8 to 25.6)	1.2 (1.1 to 1.2)	

Amp, amplitude; BCVA, best-corrected visual acuity; DA, dark adapted; IT, implicit time; LA, light adapted; LOF, loss of function; REX, reduced expression. Significant differences are marked accordingly (* $P < 0.01$, ** $P < 0.001$). Normal values, as specified by the manufacturer, are presented at the bottom for reference. A statistical comparison was conducted between the outcomes of patients and controls as measured in this study.

potentially leading to reduced ERG response amplitudes, no significant difference in ERG amplitudes was detected when comparing affected patients with the control group. This observation could be attributed to the relatively young age of the study population (mean age = 25.3 $\pm$ 16.9 years, range = 9–60 years), where retinal function is better preserved. On the other hand, implicit times were significantly delayed ($P < 0.001$), serving as an indicator of retinal dysfunction that is independent of the intensity of the stimulating light. Photopic ERG responses demonstrated a moderate correlation with the visual function (BCVA, $r_s = 0.5$). Furthermore, we observed a tendency of worsening with age in dark and light adapted response amplitudes, with a calculated age slope significantly steeper than the age correction factor determined in a healthy population. Conversely, implicit time delays increased at a similar rate over time for both rod and cone functions. The reduction in amplitudes without further worsening of the already delayed implicit times – compared with controls – supports the theory, that in young patients, visual function is likely correlated with the degree of retinal dysfunction. This dysfunction is subsequently worsened by the emergence of corneal opacification, cataracts, and glaucoma, which contribute to a further decrease in ERG amplitudes.^{10,14,33}

The changes noted in retinal function may stem from disruptions in synaptic integration and signal processing pathways, or from a decrease in the population of healthy retinal cells. Therefore, retinal dysfunction, as measured by ERG, should be regarded as a key characteristic of aniridia.¹¹

To examine the potential correlation between *PAX6* gene genotype variations and phenotypic expression, patients were categorized into subgroups according to their respective genotypes. Currently, more than 700 unique mutations have been discovered that impact *PAX6* and its regulatory regions, with most leading to *PAX6* haploinsufficiency, thus resulting in various ocular and systemic problems. The *PAX6* database indicates that the following 3 types represent 72% of all mutations linked to disease: nonsense mutations, splice mutations, and frame-shifting insertions or deletions, which usually add a PTC into the open reading frame. The resulting mRNAs containing PTCs are degraded before producing large quantities of truncated proteins through a nonsense-mediated decay (NMD) process, leading to loss of function.^{8,38–40}

In the present study, most of the patients harbored genetic variants ($n = 17$) leading to loss of function of the encoded protein. Clinical observations within the LOF group revealed a marked delay in the ERG response timing, with amplitudes exhibiting considerable variability yet maintaining averages comparable to those of the control group. On the contrary, patients who carry a missense mutation or display mosaicism are predicted to exhibit a less severe phenotype due to reduced expression of the *PAX6* protein. This was further corroborated by the ERG results, which indicated a better preservation of both delay time and amplitude in the ERG response in the REX group.

Interestingly, in a younger patient with a mosaic *PAX6* genetic variant, reduced b/a ratios of the ERG were observed. This waveform resembles a so-called electronegative or near-negative ERG, which occurs when the b-wave is selectively reduced, causing the ERG to fail to rise above the baseline following the a-wave. In the context of a normally sized a-wave, this indicates the retinal dysfunction site downstream of phototransduction, commonly at the photoreceptor to bipolar cell synapse.⁴¹ Mutations in the *PAX6* gene are linked to noticeable thinning of both the inner

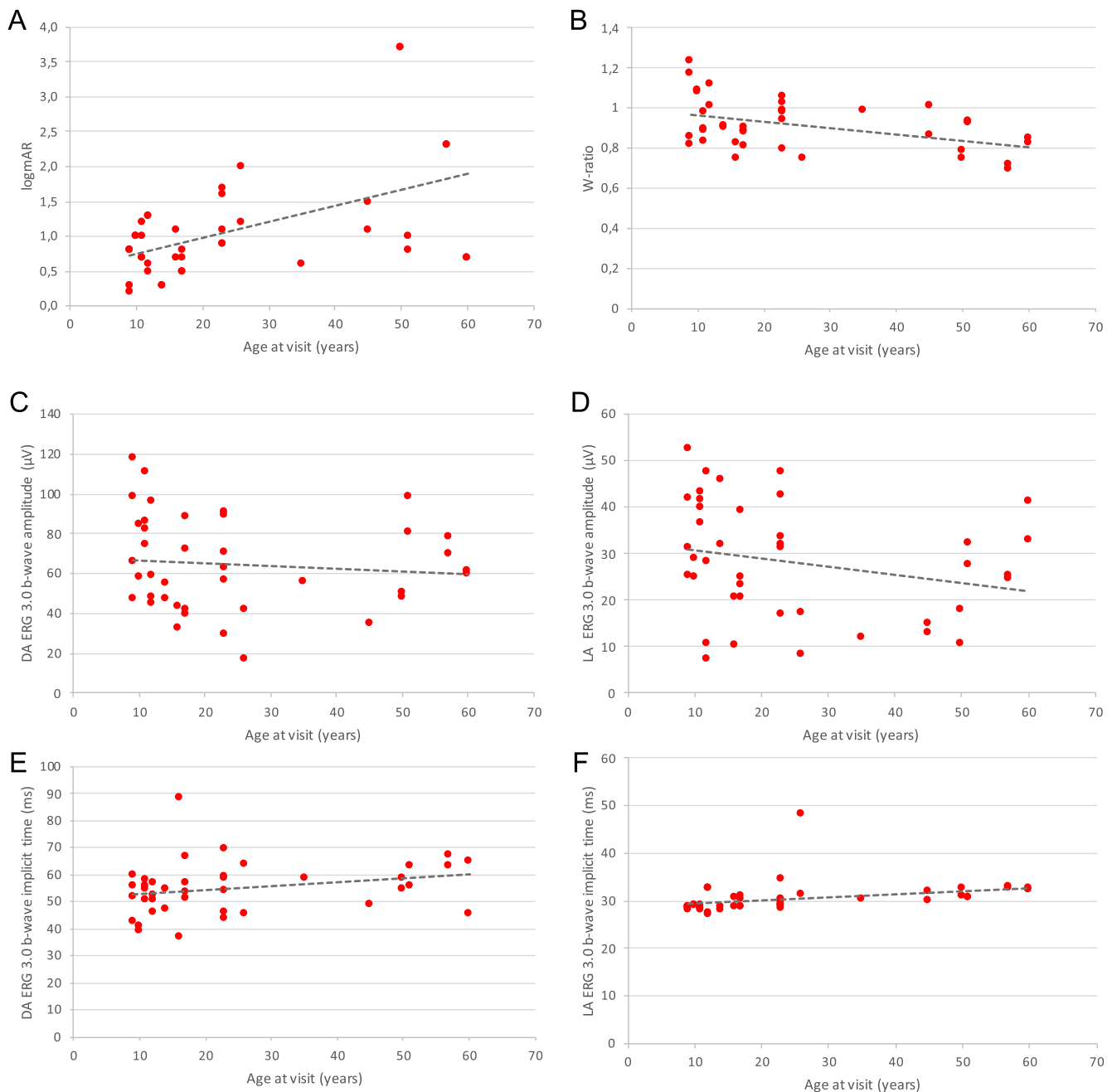


FIGURE 3. Functional results in correlation with age. BCVA deteriorated over time (A) and showed a strong correlation with age (Spearman's correlation $r_s = 0.51$). The W-ratio exhibited a decline over time (B), and, additionally, the amplitudes of rod and cone responses demonstrated a tendency to deteriorate with advancing age (C, D). In contrast, the implicit time delays exhibited slopes comparable to those observed in healthy controls (E, F).

and outer layers of the retina, aligning with improper retinal development that causes a decrease in the number of retinal neurons.^{34,35} It is possible that alterations in *PAX6* levels differentially affect the proliferation of various retinal progenitor cell subpopulations, leading to changes in the proportions of cell types within the retinal structure. Remez et al. investigated the functions of *Pax6* in late retinal progenitor cells by creating mosaic loss-of-function mutations in *Pax6* within neonatal mouse retinas and tracking the developmental paths of cells lacking *Pax6*.⁹ The authors discovered that deactivating *Pax6* resulted in alter-

ations to the destinies of inner nuclear layer retinal cell types produced later and showed that *Pax6* is crucial for the formation of late-born interneurons, while it suppresses the differentiation of photoreceptors. The research demonstrated distinct functions of *Pax6* unique to certain lineages in retinal cell types that develop later. Considering these findings, the mosaic *PAX6* genetic variant present in our patient may exert differential effects on retinal developmental characteristics, leading to modified impact on the inner and outer retinal layers. This, in turn, may result in normal photoreceptor function but a proportionally smaller postsy-

naptic response. Further, ERG recordings from various genotypes, including those with mosaic variants, would be valuable and could potentially reinforce our hypothesis in the future.

Glaucoma represents a significant concern for individuals with aniridia, with estimates suggesting that more than 50% of these patients will develop the condition over their lifetime, and the risk increases concomitantly with age.^{1,31} Similar to other forms of glaucoma, its diagnosis relies on the assessment of IOP, evaluation of the anterior chamber angle, visual field testing, and examination of the optic nerve. However, the diagnostic process in this patient population is complicated by ocular abnormalities associated with aniridia, such as keratopathy, cataracts, and nystagmus. These conditions pose challenges to examining both the anterior segment and the optic nerve, thereby impacting the accuracy of perimetry test results.^{1,31,42} Consequently, it is important that these patients are closely monitored by routine comprehensive examinations, including photographs of the optic nerve, and, where possible, other structural and functional tests to detect any changes. Nonetheless, glaucoma remains, along with limbal insufficiency, one of the major causes of blindness in congenital aniridia.

PhNR is reduced in disorders affecting the inner retina or involving retinal ganglion cell axon or glial cells. Thus, it has been used to aid in the diagnosis of retinal nerve fiber pathologies, such as glaucoma, retinal vascular diseases, and optic nerve atrophy.^{17,20,43,44} In our study, PhNR recordings followed the ISCEV recommendations for the standard protocols of the extended full-field ERG, with red flashes on a blue background used to stimulate the eye. For evaluation, we used the ratio of the b-wave peak voltage to the PhNR trough voltage to b-wave amplitude, designated as the W-ratio (b-wave voltage – P_{min} voltage)/(b-wave voltage – a-wave voltage).³⁰ This parameter has the advantage of demonstrating ganglion cell dysfunction with minimal influence from factors such as the absolute value of response amplitudes, the intensity of the stimulating light, pupil diameter, or gender. Furthermore, studies have shown that the sensitivity and specificity of the W-ratio for diagnosing optic nerve disorder are 75% and 83%, respectively, values comparable to those obtained using circumpapillary retinal nerve fiber layer thickness measurements.⁴³ PhNR is considered as useful as monitoring structural changes with OCT for diagnosing optic nerve diseases.^{17,45} Consequently, ERG recordings provide a valuable tool for improving the accuracy of diagnosing optic nerve disorders and monitoring the progression of glaucoma, even in cases of congenital aniridia. The PhNR test offers significant insights without the need for refractive correction, strict fixation monitoring, or clear ocular media. This test is particularly beneficial when visual field tests and/or OCT measurements prove to be inconclusive, unreliable, or challenging to conduct. Our study showed that optic nerve changes, such as optic nerve hypoplasia in aniridia, were associated with significantly lower W-ratio values compared with healthy controls. Furthermore, we observed a difference in the W-ratio between patients with treated and without confirmed glaucoma, indicating a further reduction of the W-ratio ($W = 0.94 \pm 0.13$ in those without confirmed glaucoma and $W = 0.88 \pm 0.11$ in patients with treated glaucoma).

Our study also had some limitations that could influence our evaluations, particularly the assessment of glau-

coma status and the presence of optic nerve hypoplasia within our study population. Both functional diagnostics and morphological examinations are often limited in this patient cohort. In our analysis, optic nerve hypoplasia was documented only in cases where AAK and additional anterior segment issues permitted a thorough visualization of the fundus. This was not achievable in all cases, resulting in missing data. Similar challenges were encountered in the diagnosis of glaucoma. The reliability of functional tests and fundus imaging is compromised in many cases, and even IOP measurements can be problematic when the cornea is significantly thickened. Furthermore, many patients had been previously assessed by other institutions, with glaucoma treatments already initiated. As a result, initial presentation at our clinic often occurred at an older age and at a more advanced stage of disease. These challenges are well-documented issues in the context of rare diseases, particularly in cases of high complexity, such as aniridia. Despite these limitations, longitudinal data remain crucial for validating the diagnostic accuracy of ERG—especially PhNR measurements—for the screening and monitoring of glaucoma in congenital aniridia. A more accurate characterization of glaucoma and optic nerve hypoplasia would require a prospective study initiated at an early age—ideally at the time of diagnosis. Such an approach should include ERG measurements to better evaluate and differentiate between retinal and optic nerve changes.

This study represents the most comprehensive ERG analysis of congenital aniridia conducted to date. The findings reveal that *PAX6*-related aniridia impacts both functions associated with photoreceptors and the transmission of signals beyond them, indicating that retinal alterations are not limited to the central macula region. In young patients, BCVA is likely correlated with the degree of retinal dysfunction, compounded by anterior segment dysplasia. Thus, ERG-measured retinal dysfunction ought to be considered a core feature of the aniridia phenotype. In later stages of life, additional pathologies, including aniridia-associated keratopathy, cataract or glaucoma complicate the disorder, with onset typically occurring most frequently after the second decade. Glaucoma is considered one of the major causes of blindness in congenital aniridia.³¹ ERG recordings performed with a portable device and sensor strip electrodes can be valuable for assessing retinal function and monitoring disease progression in aniridia, particularly considering the increased risk of glaucoma.

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