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Electronegative electroretinogram in the modern multimodal imaging era

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Abstract

Background: The electronegative electroretinogram (ERG) reflecting inner retinal dysfunction can assist as a diagnostic tool to determine the anatomical location in eye disease. The aim of this study is to determine the frequency and aetiology of electronegative ERG in a tertiary ophthalmology centre and to develop a clinical algorithm to assist patient management.

Methods: Retrospective review of ERGs performed at the Save Sight Institute from January 2011 to December 2020. ERGs were performed according to ISCEV standard. The b:a ratio was analysed in dark adapted (DA) 3.0 or 12.0 recordings. Patients with ratio of ≤1.0 were included.

Results: A total of 4421 patients had ERGs performed during study period, of which 139 patients (3.1%) had electronegative ERG. The electronegative ERG patients' median age at referral time was 37 (0.7–90.6) years. The causative aetiologies were photoreceptor dystrophy (48, 34.5%), Congenital Stationary Night Blindness (CSNB) (33, 23.7%), retinal ischemia (18, 12.9%), retinoschisis (15, 10.8%), paraneoplastic autoimmune retinopathy (PAIR) and nonPAIR (14, 10.1%), batten disease (4, 2.9%), and inflammatory retinopathy (4, 2.9%). There were three patients with an unclassified diagnosis. Thirty-two patients (23%) had good vision and a normal fundus appearance. Eleven patients (7.9%) had good vision and normal results in all multimodal imaging.

Conclusions: The frequency of electronegative ERG in our referral centre was 3.1% with photoreceptor dystrophy as the main aetiology. A significant number of the cases had good vision with normal fundus or normal multimodal imaging. This further highlights the value of an ERG in this modern multimodal imaging era.

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KEYWORDS
electronegative electroretinogram, frequency, prevalence

1 | INTRODUCTION

In this modern multimodal imaging era, spectral domain optical coherence tomography (SD-OCT) and ultra wide-field fundus autofluorescence (UWF-FAF) have improved retinal assessment mainly enhancing structural evaluation.[1] Functional assessment of the visual system remains critical. The full-field electroretinogram (ffERG) provides an objective assessment of retinal function.[2] It is particularly important in patients who have poor visual function and a normal fundal appearance. The ISCEV standard ffERG provides a protocol to investigate the functional retinal signal processing for both the rod and cone systems.[3–7] The defined ISCEV standard ERG series includes six protocols. Two of these the Dark adapted (DA) 3.0 ERG and the DA 10.0 or DA 12.0 ERG consist of an initial negative-going a-wave reflecting phototransduction followed by a positive-going b-wave arising mainly from post-phototransduction processing in the inner retina. The conditions of testing determine which bipolar cells are involved (rod ON-bipolar cells in DA condition,[2,8] and cone ON- and OFF-bipolar cells under light adapted [LA] conditions[2]) (Figure 1A-C). The ISCEV extended protocol for the LA ON–OFF ERG was developed to further interrogate the post-phototransduction or post-receptoral pathway.[2] For the light-adapted vertebrate retina, the b- and d-waves reflect activity of the cone ON- and OFF-pathways, respectively (Figure 1D-E).[13]

An electronegative ffERG is defined as a selective reduction of the b-wave indicating post-phototransduction location for the pathology.[14,15] This is best seen in DA conditions with maximal stimulation but may be seen less commonly in LA testing.

The underlying aetiology of an electronegative ERG can be divided into inherited (e.g., retinoschisis, congenital stationary night blindness (CSNB)) or acquired (e.g., melanoma-associated retinopathy [MAR], autoimmune retinopathy, inner retinal ischemia).[8] In each of the conditions there is dysfunction in the inner retina either from disease, dystrophy or ischemia. Additionally, some electronegative conditions can be found with a normal fundal appearance, highlighting the importance of functional assessment with ffERG.[8,15,16]

The frequency and aetiology of electronegative ERG has only been reported on a few occasions.[15,17–20] The primary aim of this study is to determine the frequency and aetiology of an electronegative ffERG in a tertiary ophthalmology centre. The secondary aim is to apply these findings to develop a clinical algorithm to assist in managing patients who are suspected of having conditions that are associated with an electronegative ERG.

2 | METHODS

This study was approved by South-East Sydney Local Health district ethics committee and followed the tenets of Declaration of Helsinki.

2.1 | Patients

Retrospective review of ffERGs performed at the Save Sight Institute, the University of Sydney, from January 2011 to December 2020. Visual state was described using the World Health Organisation (WHO) classification. Good vision = 6/12 or better, mild visual impairment (VI) = worse than 6/12 to 6/18, moderate VI = worse than 6/18 to 6/60, severe VI = worse than 6/60 to 3/60, and blindness = worse than 3/60.[21] Refractive error state was defined using spherical equivalent (SE) and classified as emmetropia = > -0.5D but $\leq +0.5$D, myopia = ≤ -0.5D, and hyperopia = $> +0.5$D[22] in at least in one eye when the other eye is emmetropic. Antimetropia is defined as having myopia in one eye with hyperopia in the other eye and vice versa.[23]

2.2 | Technique

Full-field ERGs were performed according to International Society for Clinical Electrophysiology of Vision (ISCEV)[2,24,25] standard using a Diagnosys LLC Espion device (Lowell, MA, USA). The b:a wave ratio was calculated from either DA 3.0 or 12.0 ISCEV stimulus recording. By using both stimuli we optimised the chance of identifying an electronegative ERG. We recognise that b:a ratio with increasing flash strength reduces due to earlier saturation of b-wave amplitudes relative to a-wave amplitudes. We chose to assess both stimulus intensities to maximise the detection of an electronegative waveform. The study inclusion criteria was an electronegative ERG which was defined as a b:a ratio ≤ 1.0.[15–18]

An a-wave abnormality was defined as an a-wave amplitude 2-standard deviations below the normal range. The ISCEV extended protocol for the LA ON–OFF ERG was not available for all subjects and has not been included in the analysis. Ultra wide-field (UWF) fundus pseudocolour photographs and UWF-FAF were obtained using Optos

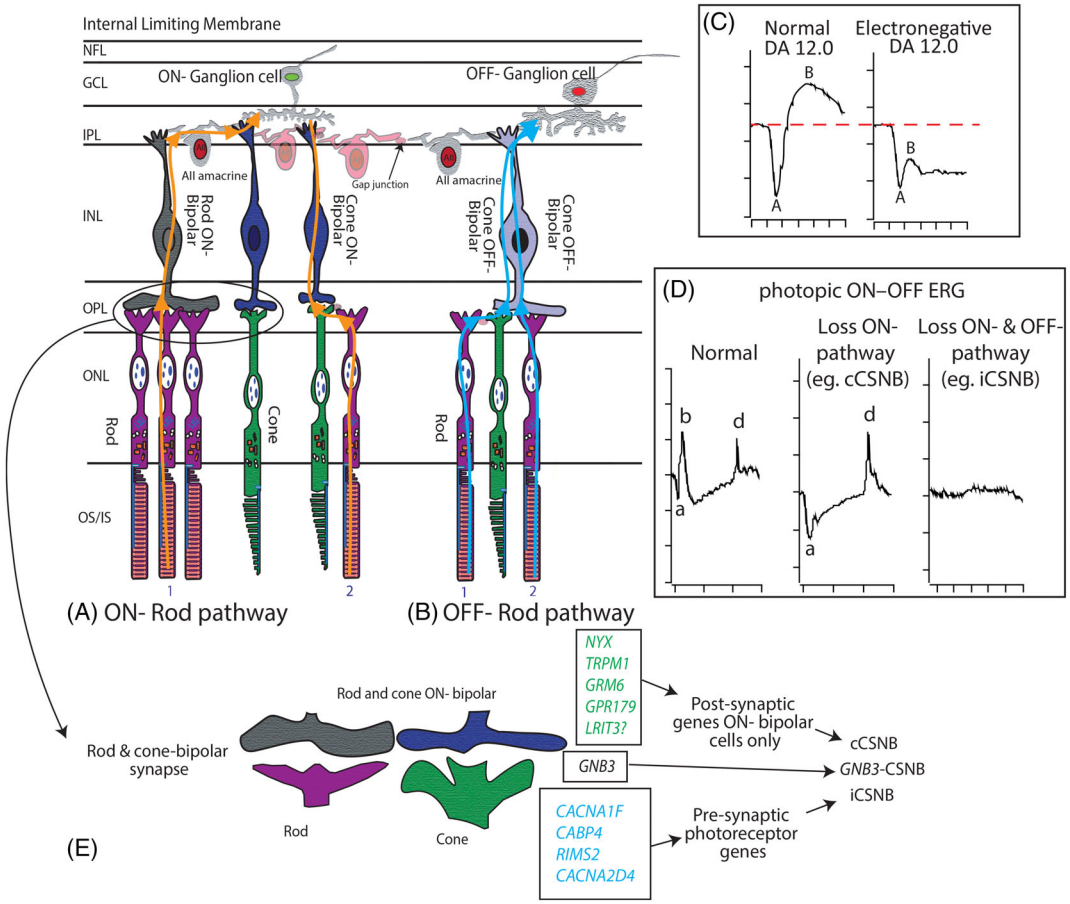

FIGURE 1 Retinal circuitry illustrating the principle of rod pathways in humans. Rod ON- pathways are displayed in orange arrows. Rod OFF- pathways are displayed in blue arrows. (A) Two main ON- rod pathways: (1) Rod→rod ON- bipolar cells→AII amacrine cell→cone ON- bipolar→ON- RGC and (2) Rod→cone (via gap junction)→cone ON- bipolar→ON- RGC.(B) Two main OFF- rod pathways: (1) Rod→cone→cone OFF- bipolar→OFF- RGC (2) Rod→cone OFF- bipolar→OFF- RGC.(C) In the ffERG the a-wave amplitudes are measured from baseline to a-wave trough, and b-wave amplitudes from a-wave trough to b-wave peak. The ffERG is termed electronegative if the b:a ratio is less than or equal to 1. Normal and electronegative wave (b:a ratio ≤1). Red line represents the recording baseline. (D) The ISCEV extended protocol for the LA ON–OFF ERG enables further analysis of the post phototransduction pathway. The normal ON-response has a negative a-wave and a positive b-wave arising from the ON-bipolar pathway. The OFF- d-wave is a positive component that arises from the OFF-bipolar pathway. Loss of the ON-pathway is seen in cCSNB. In iCSNB both ON- and OFF- pathways are affected. (E) Pre-synaptic gene mutations causing iCSNB (in blue) and post-synaptic gene mutation causing cCSNB (in green). Pre-synaptic proteins CACNA1F, CABP4, RIMS2 and CACNA2D4 are located at the rod and cone photoreceptor. Mutations in these genes are impacting both ON- and OFF- bipolar signalling and associated with iCSNB or cone/cone-rod dystrophies. The post-synaptic molecules GRM6, GPR179, NYX, and TRPM1 are important for glutamate-induced signalling from the photoreceptors to ON-bipolar cells work through the metabotropic glutamate receptor. LRIT3 is expressed in rod photoreceptors but takes action transsynaptically to arrange postsynaptic glutamate signalling complex comprising TRPM1. Mutations in these genes are associated with cCSNB. In contrast OFF- bipolar cells and horizontal cells employ ionotropic glutamate receptors. The post-synaptic defect is related to the bipolar glutamate receptor. During darkness, photoreceptors continuously release glutamate that binds to GRM6, which leads to the closure of the non-selective ion channel TRPM1. After light stimulation, TRPM1 channel opens, leading to depolarization of the ON-bipolar cells, which are largely responsible for generating the ERG b-wave. The proteins GRM6 and NYX are critical for the correct localization of TRPM1 in the dendritic tips of ON-bipolar cells. Mutations in *GRM6* and *NYX* lead to mislocalisation of TRPM1 resulting in the blocking of the signal transmission via this receptor. GNB3 protein is expressed in cone photoreceptors and ON-bipolar cells creating and unique form of CSNB, namely *GNB3*-CSNB. DA ERG is similar to iCSNB and cCSNB with decreased LA ERG but not as decreased as iCSNB.[4–6,9–12] cCSNB, complete congenital stationary night blindness; DA, dark adapted; ERG, electroretinogram; iCSNB, incomplete congenital stationary night blindness; LA, light adapted; RGC, retinal ganglion cell

TABLE 1 Prevalence of electronegative electroretinogram (ERG) diagnoses and comparison to other studies

	This study		Rocha et al.[20]		Kim et al.[18]		Renner et al.[17]	Koh et al.[15]	
Study centre	Sydney, Australia		Sao Paolo, Brazil		Atlanta, US		Berlin, Germany	London, UK	
Period	January 2011–December 2020		March 2004–November 2013		January 1999–March 2008		1992–2004	November 1995–December 1998	
Prevalence	3.1% (139/4421)		2.49% (41/1645)		4% (73/1837)		2.9% (47/1644)	4.8% (128/2640)	
Diagnoses		Abnormal a-wave							
Photoreceptor dystrophy	47 (33.8%)	35/47 (74.5%)	24 (58.5%)		4 (5.4%)		11 (23.4%)	34 (26.5%)	
RCD	23 (16.5%)	20/23 (87%)	-			3 (4.1%)	6 (12.8%)	-	
CRD	20 (14.4%)	11/20 (55%)	-			1(1.4%)	5 (10.6%)	-	
Enhanced S-cone syndrome	4 (2.9%)	4/4 (100%)	-		-		-	-	
CSNB	33 (23.7%)	11/33 (33.3%)	1 (4.1%)		29 (39.7%)		6 (12.8%)	17 (13.3%)	
Ischemia (including diabetic retinopathy)	18 (12.9%)	8/18 (44.4%)	2 (8.3%)		2 (2.7%)		-	13 (10.2%)	
Diabetic Retinopathy	1 (0.7%)	0		2 (8.3%)	-		-	-	
Retinoschisis	15 (10.8%)	6/15 (40%)	3 (7.3%)		7 (9.9%)		17 (36.2%)	19 (14.8%)	
PAIR and nPAIR	15 (10.8%)	9/15 (60%)	-		1 (1.4%)		1 (2.1%)	5 (3.9%)	
nPAIR	7 (5%)	3/7 (42.9%)	-			1 (1.4%)	-		1 (0.8%)
CAR (1 renal cancer, 1 breast cancer, 1 pancreatic cancer, and 2 prostate cancer)	5 (3.6%)	5/5 (100%)	-			-	-		-
MAR	3 (2.2%)	1/3 (33.3%)	-			-	1 (2.1%)		4 (3.1%)
Batten	4 (2.9%)	4/4 (100%)	-		-		-	1 (0.8%)	
Inflammatory retinopathy (unspecified, HIV, and birdshot chorioretinopathy)	4 (2.9%)	1/4 (25%)	5 (12.2%)		-		-	10 (7.8%)	
Unspecified	2 (1.4%)	1/2 (50%)		5 (12.2%)	-		-		3 (2.3%)
Birdshot chorioretinopathy	1 (0.7%)	0		-	-		-		7 (5.4%)
HIV	1 (0.7%)	0		-	-		-		-
Quinine toxicity	-	-	-		-		-	3 (2.3%)	
Vigabatrin toxicity	-	-	-		-		1 (2.1%)	1 (0.8%)	
Methanol toxicity	-	-	-		1 (1.4%)		-	-	
Multiple system atrophy	-	-	-		1 (1.4%)		-	-	
Choroideremia	-	-	-		-		1 (2.1%)	-	
Müller cell sheen dystrophy	-	-	-		-		1 (2.1%)	-	
Unknown	3 (2.2%)	0	6 (14.6%)		28 (38.3%)		9 (19.1%)	25 (19.5%)	
Total	139	73/139 (52.5%)	41		73		47	128	

Abbreviations: CAR, carcinoma associated retinopathy; CSNB, congenital stationary night blindness; HIV, human immunodeficiency virus; MAR, melanoma associated retinopathy; PAIR and nPAIR, paraneoplastic autoimmune retinopathy (PAIR) and nonPAIR (nPAIR).

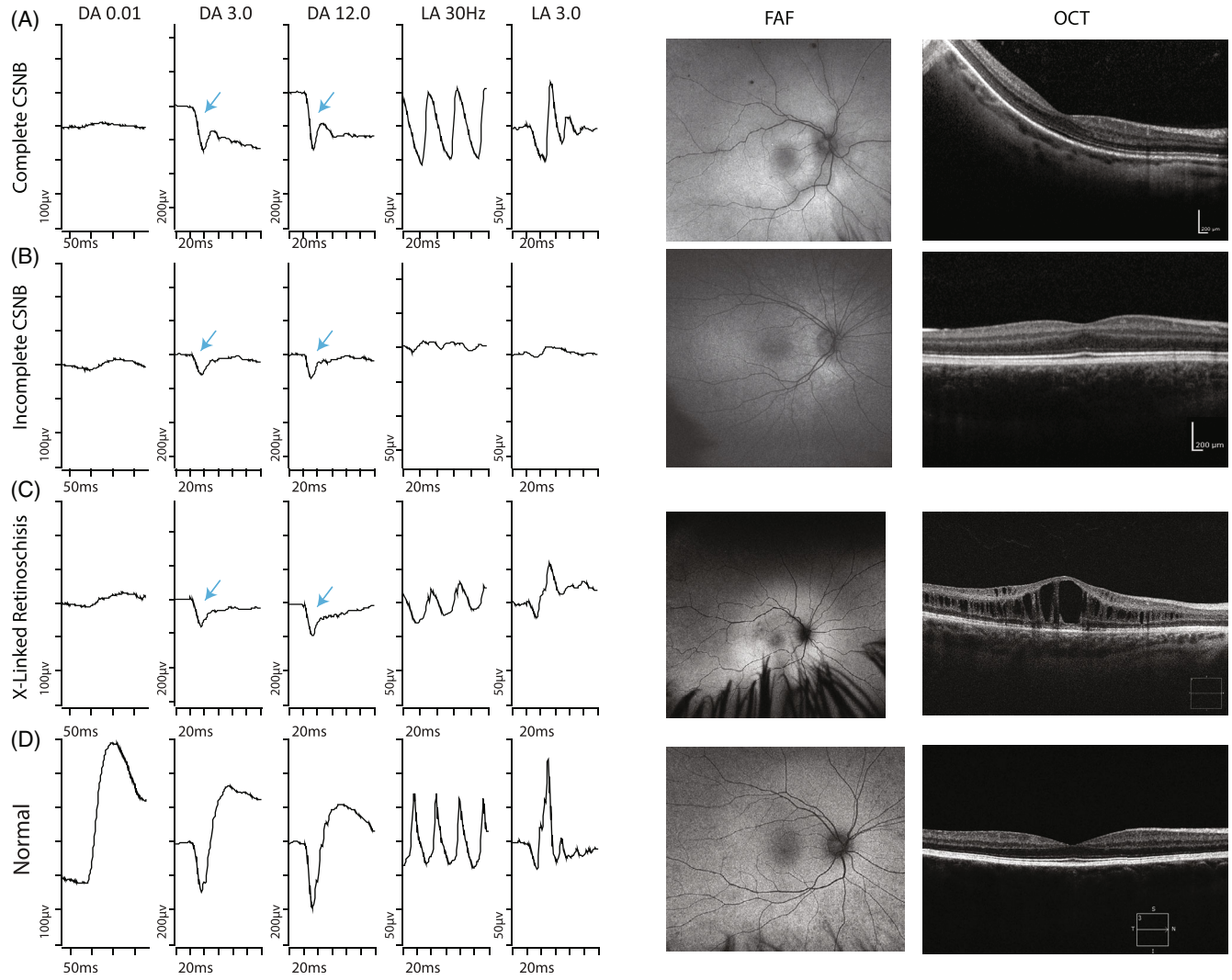

FIGURE 2 Examples of cCSNB, iCSNB, and retinoschisis in patients with an electronegative ERG. (A) cCSNB. The complete form of CSNB (cCSNB) is characterised by non-recordable or severely reduced rod ERG using dim light stimuli, an electronegative ERG pattern in response to bright white light stimuli in the DA state and a broadened a-wave in the single stimulus that is recorded in the LA ERG.[12] Normal UWF-FAF illustrating the foveal reduction in autofluorescence and normal macular SD-OCT with distinct lamination were identified. (B) iCSNB. A reduced, but measurable rod ERG an electronegative ERG pattern in response to bright white light stimuli in the dark-adapted state and a subnormal single flash light-adapted ERG of normal a-wave and b-wave pattern are found. Normal UWF-FAF illustrating the foveal reduction in autofluorescence and normal macular SD-OCT with distinct lamination were identified. (C) X-Linked Retinoschisis. An electronegative ERG can be observed in DA 3.0 and DA 12.0 ERG. UWF-FAF exhibited some parafoveal hypoAF dots which is a variation on the more commonly reported cartwheel appearance of hyperAF. Prominent schitic changes in the macular SD-OCT were found. Blue arrows indicate electronegative ERG waveform. (D) Normal ERG consists of five standard ISCEV recording. The weak flash (DA 0.01) ERG reflects the rod bipolar cells and the only one that selectively observes rod system activity. The standard flash (DA 3.0 and DA 10.0 or DA 12.0) ERG localise the dysfuction to either the rod photoreceptor (a- and b-wave reduction) or the inner retina (normal a-wave with a decreased b-wave). The DA 3.0 and DA 10.0/DA 12.0 ERGs reflect the response of rod and cone system. Nevertheless, the rod system dominates the response in a normal retina. The 30 Hz flash results in a post-receptoral response from the cone, making it a sensitive for the cone system. The single flash (LA 3.0) ERG comprises an a-wave, reflecting the cone and OFF- bipolar cell response, and a b-wave, reflecting both ON- and OFF-bipolar cells response. Thus, makes the LA 3.0 ERG useful in cone system localisation.[27] Normal UWF-FAF with foveal reduction of autofluorescence. The signal in the parafoveal area tends to be higher but still shows a relatively lower intensity compared with more peripheral retinal area.[28] Normal SD-OCT with normal thickness and distinct lamination. Four discrete hyper-reflective bands can be observed in the outer retina.[29] AF, autofluorescence; cCSNB, complete congenital stationary night blindness; DA, dark adapted; ERG, electroretinogram; iCSNB, incomplete congenital stationary night blindness; ISCEV, International Society for Clinical Electrophysiology of Vision; ffERG, full field electroretinogram; LA, light adapted; SD-OCT, spectral domain optical coherence tomography; UWF-FAF, ultra wide-field fundus autofluorescence

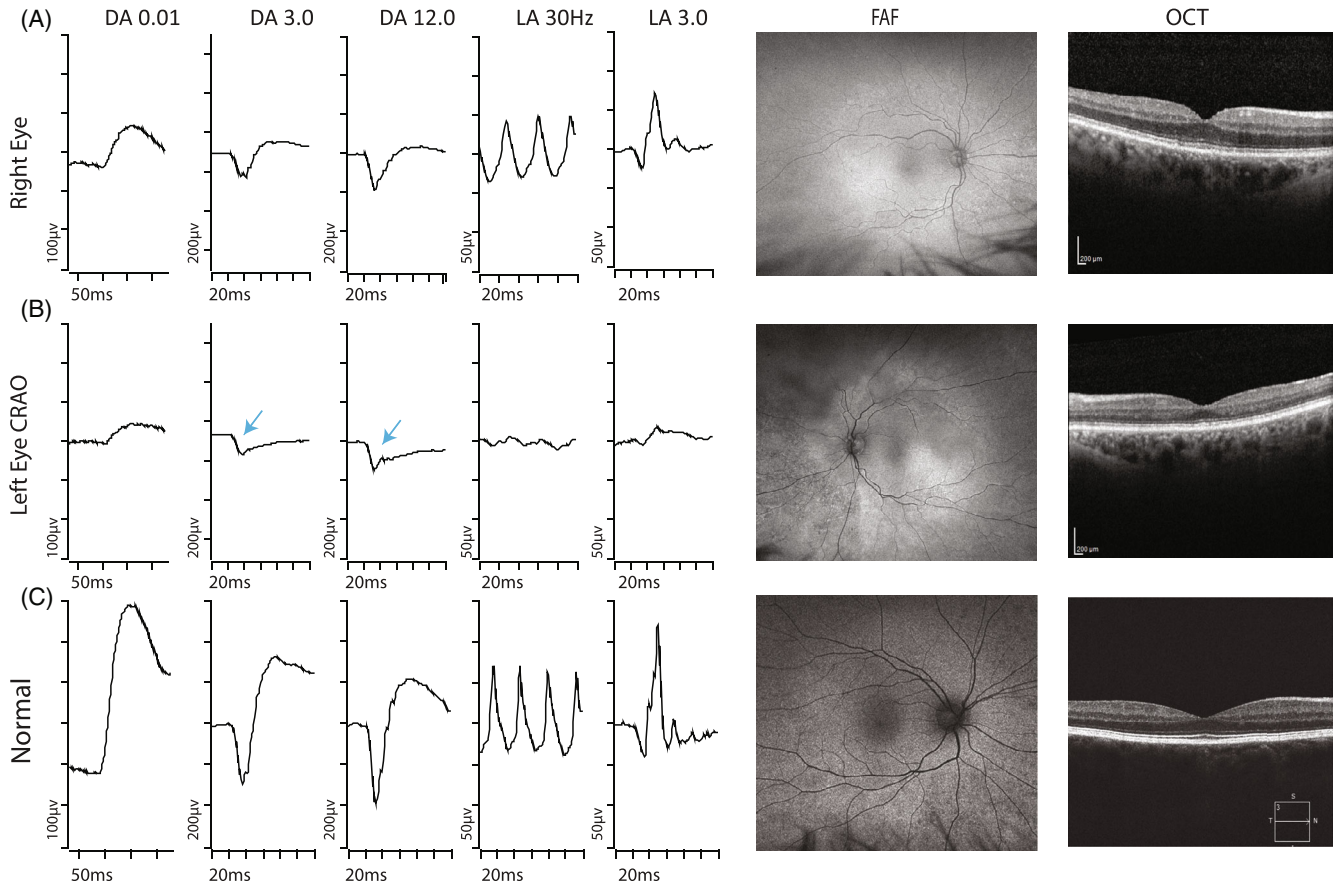

FIGURE 3 An electronegative ERG in a patient with a left CRAO. (A) Unaffected RE of the patient demonstrating the normal ffERG, normal UWF-FAF, and normal macular SD-OCT. (B) An electronegative ERG was identified in the DA 3.0 and DA 12.0 ffERG. Both DA and LA ffERG of LE showed reduced amplitudes. The LE UWF-FAF showed hypoAF fovea extending parafoveally to superior. HypoAF was also observed on peripheral retina outside vascular arcade. CMT was 297 µm on the right eye and 216 µm on the left eye. General thinning of retinal layer can be found on LE compared to RE. Blue arrows indicate electronegative ERG waveform. (C) Normal control with normal ffERG, normal UWF-FAF illustrating the foveal reduction in autofluorescence, and normal macular SD-OCT with distinct lamination and normal thickness. CMT, central macular thickness; CRAO, central retinal artery occlusion; DA, dark adapted; ffERG: full field electroretinogram; UWF-FAF, ultra wide-field fundus autofluorescence; LA, light adapted; LE, left eye; RE, right eye; SD-OCT, spectral domain optical coherence tomography

200TX and subsequently Optos California (Dunfermline, UK). SD-OCT were obtained using the Heidelberg Spectralis (Heidelberg Engineering, Heidelberg, Germany) and Zeiss Cirrus (Carl-Zeiss Meditec, Dublin, CA, USA) and classified as normal or abnormal. Routine genomic testing has evolved over the period of this study. In clinical practice there is a significant time interval to obtain all the genomic results, as these are incomplete they have been omitted. In understanding the paraneoplastic autoimmune retinopathy (PAIR), the type of the neoplasm was identified. However, the tumour staging details were not available.[26]

3 | RESULTS

A total of 4421 patients underwent a ffERG during this study period. A hundred and thirty-nine (3.1%) patients were identified to have an electronegative ERG. Eighty-nine were males and 50 females. The median age of the electronegative patients at referral was 37 (0.7–90.6) years.

The most prevalent initial complaint was reduced vision in 47 patients (33.8%) followed by nyctalopia in 29 (20.9%). Thirty-four patients had no recorded symptoms at time of testing (24.5%), either referred for screening due to a sibling's condition or due to suspected macular or photoreceptor dystrophy on examination. The patients' visual acuity (VA) was grouped according to the WHO criteria. Good vision was the majority with 58 patients (41.7%), mild VI in 28 patients (20.1%), moderate VI in 29 patients (20.9%), severe VI in 14 patients (10.1%), and 10 patients were blind (7.2%).

Myopia was the most prevalent refractive error with 46 patients (33.1%) followed by hypermetropia in

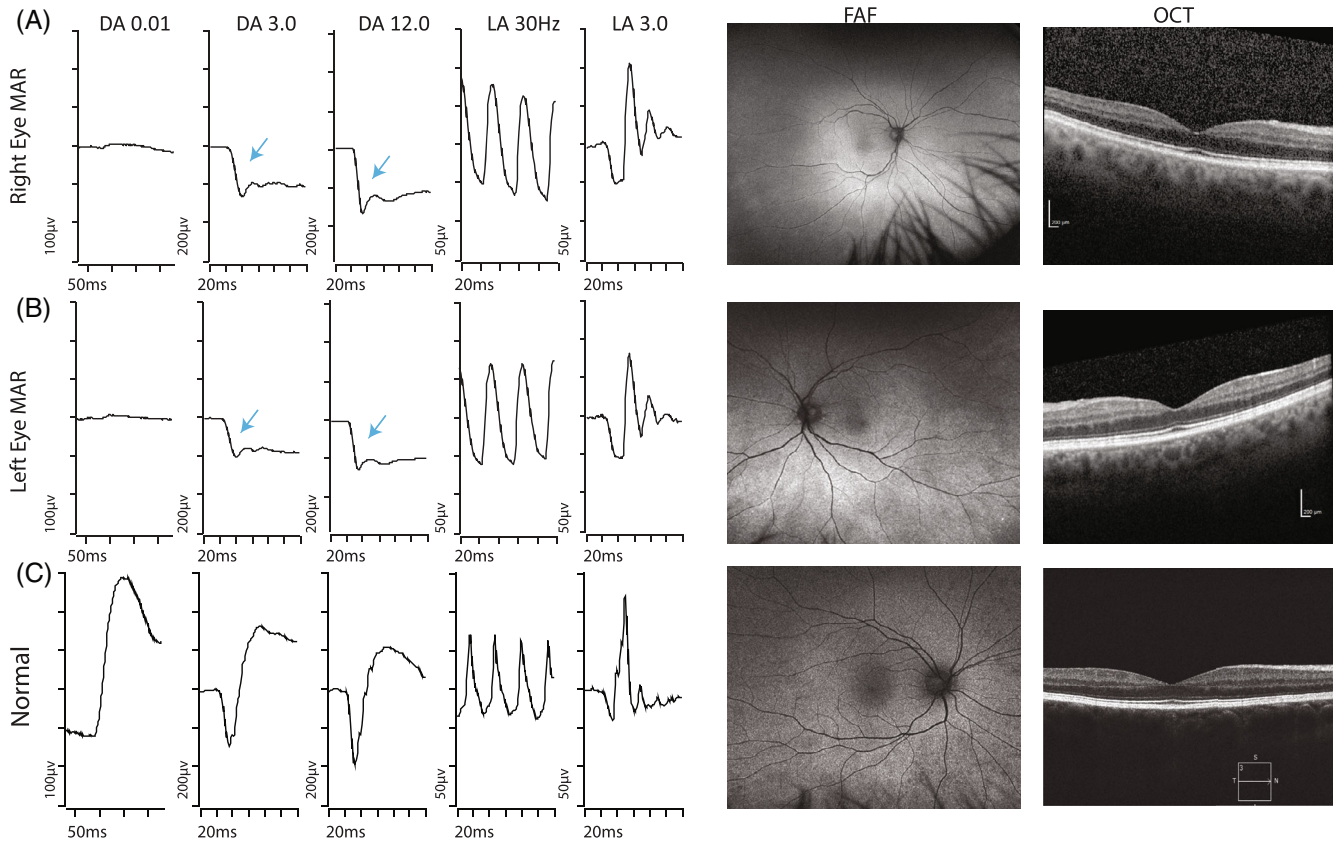

FIGURE 4 An example of a patient with MAR and a electronegative ERG. (A,B) RE and LE of MAR patient. Almost extinguished signal on DA 0.01. Electronegative waves were found in DA 3.0 and DA 12.0 with relatively normal LA ERG. Asymmetry is highlighted by the greater electronegative waveform in the RE compared with the LE. UWF-FAFs showed foveal reduction of autofluorescence. Macular SD-OCTs were normal in thickness and distinct lamination. Blue arrows indicate electronegative ERG waveform. (C) Normal control with normal ffERG, normal UWF-FAF illustrating the foveal reduction in autofluorescence, and normal macular SD-OCT with distinct lamination and normal thickness. ffERG, full field electroretinogram; hypoAF, hypo autofluorescence; LE, left eye; MAR, melanoma associated retinopathy; RE, right eye; SD-OCT, spectral domain optical coherence tomography; UWF-FAF, ultra wide-field fundus autofluorescence

44 patients (31.7%), emmetropia in nine patients (6.5%) and antimetropia in six patients (4.3%). Thirty-four patients (24.5%) had no refractive data documented.

Following electrophysiology testing and clinical assessment, a diagnosis was made in 136 patients (97.8%) while three patients had an unclassified diagnosis (2.2%) (Table 1). More than half or 73 patients had abnormalities in both a- and b-wave traces (52.5%). The largest group consisting of 48 patients (34.5%) were given a collective diagnosis of photoreceptor dystrophy. Congenital Stationary Night Blindness (CNSB) accounted for the next largest diagnostic grouping with 33 patients (23.7%) (Figure 2A,B). The remainder of the cohort of patients with electronegative ERGs had retinal ischemia in 18 patients (12.9%) (Figure 3), retinoschisis in 15 patients (10.8%) (Figure 2C), and paraneoplastic autoimmune retinopathy (PAIR) including MAR (Figure 4) and nonPAIR (nPAIR) in 14 patients (10.1%). A diagnoses of Batten disease (Figure 5) and inflammatory retinopathy (including birdshot chorioretinopathy) was made for 4 (2.9%) and 4 (2.9%) patients respectively (Figure 5).

Figures 2–5 compare representative cases with an electronegative ERG together with their multimodal imaging to normal subjects.Sixty-five patients (46.8%) had a normal or near normal fundus appearance, 64 patients (46%) had an abnormal fundal appearance and 10 patients did not have imaging available for analysis (7.2%). Thirty-two patients (55.2%) with normal fundus also had good vision. Among the 65 patients with normal fundus, 27 patients (19.4% of total patients) had abnormal UWF-FAF, or abnormal SD-OCT, or both abnormal UWF-FAF and SD-OCT. Furthermore, 11 patients (7.9%) had normal vision and normal multimodal imaging (wide-field imaging, macular SD-OCT, UWF-FAF). These patients included diagnosis of ischemia,[1] CSNB[13] and PAIR-nPAIR.[1] Thirty-nine patients (28.1%) had normal UWF-FAF with foveal reduction of autofluorescence. Seventy-seven patients (55.4%) had an abnormal

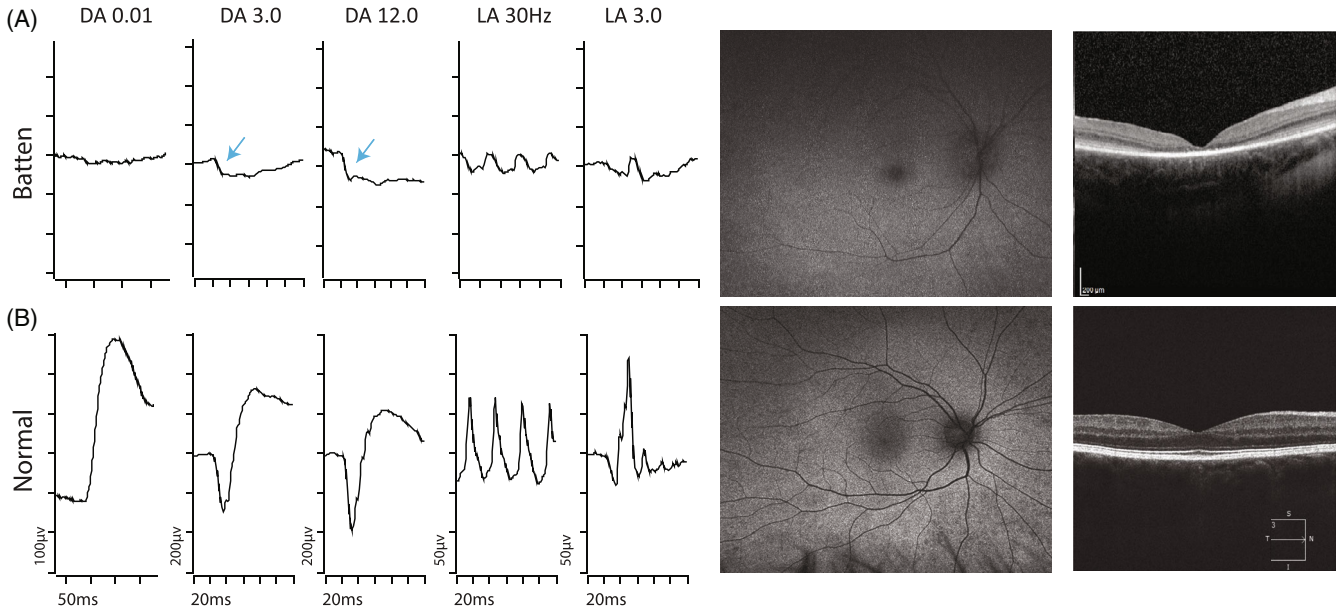

FIGURE 5 An example of a patient with Batten disease and an electronegative ERG. (A) Almost extinguished DA 0.01 traces, electronegative ERG on DA 3.0 and DA 12.0, reduced amplitude of LA ERG. The ffERG was performed with skin electrodes. BEM on UWF-FAF and foveal EZ loss on macular SD-OCT can be observed. Blue arrows indicate electronegative ERG waveform. (B) Normal eye as reference. Normal age matched skin electrode ffERG, normal UWF-FAF with foveal reduction of autofluorescence and normal SD-OCT with normal thickness and distinct lamination can be observed. BEM, bull's eye maculopathy; DA, dark adapted; EZ, ellipsoid zone; ffERG, full field electroretinogram; LA, light adapted; SD-OCT, spectral domain optical coherence tomography; UWF-FAF, ultra wide-field fundus autofluorescence

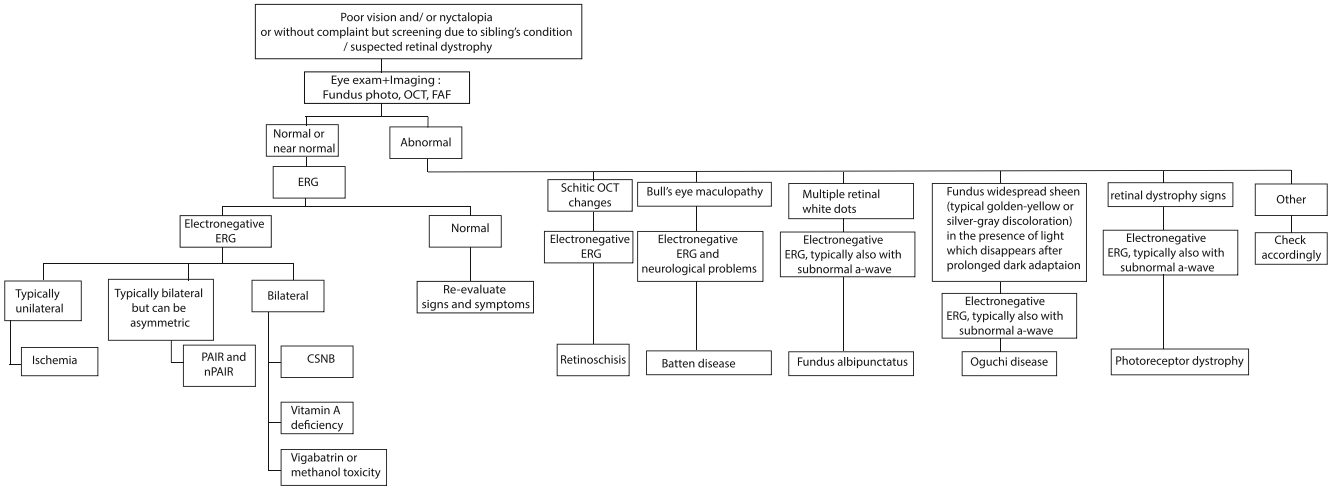

FIGURE 6 Diagnostic flow chart to assist in the clinical care of patients where an electronegative ERG is suspected. CSNB, congenital stationary night blindness; DA, dark adapted; ERG, electroretinogram; FAF, fundus autofluorescence; MAR, melanoma associated retinopathy; OCT, optical coherence tomography; PAIR and nPAIR, paraneoplastic autoimmune retinopathy and non-paraneoplastic autoimmune retinopathy

UWF-FAF appearance, and no UWF-FAF data was available for 23 patients (16.5%) respectively. Normal macular SD-OCT with symmetrical normal thickness and distinct lamination was found in 46 patients (33.1%) while abnormal macular SD-OCT consisted of below normal macular thickness, schitic changes, ellipsoid zone (EZ) loss and disturbed retinal pigment epithelium (RPE) with increased signal hypertransmission to choroid was found in 75 patients (54%). Eighteen patients (12.9%) had no macular SD-OCT on file

4 | DISCUSSION

The electronegative ERG is a specific clinical sign that usually indicates inner retinal dysfunction occurring post-phototransduction (e.g., at the level of the photoreceptor synapse or bipolar cell). When this functional data is combined with multimodal retinal imagining, a more precise clinical diagnosis can be made, even in normal or near normal fundal appearances. Recently an evaluation of a normal population found no electronegative ERGs in an adult cohort (211 subjects) using standard ISCEV DA 3.0 flash stimulus and borderline electronegative ERG in three patients using the stronger flash DA10.0 (67 cd/m^{2}s), endorsing the concept that a dark adapted electronegative ffERG is abnormal and requires further evaluation.[30]

Photoreceptor dystrophy, CSNB, ischemia, and retinoschisis were the most common aetiologies associated with an electronegative ERG in our cohort. This is comparable to previous studies using a similar classification.[15,17,18] Whilst Rocha et al. slightly differed with photoreceptor dystrophy, inflammatory retinopathy, retinoschisis, and diabetic retinopathy as their main diagnoses.[20] The complete diagnostic breakdown of aetiologies associated with an electronegative ERG for this study and previous published cohort studies[15,17,18,20] is shown in Table 1. We had less 'unknown' diagnoses reflecting the improved diagnostic rate with advances in multimodal imaging and functional investigations. Different referral criteria such as a large uveitis service referring for electrodiagnostic might contribute to the greater prevalence of PAIR and nPAIR compared to previous studies.

4.1 | Photoreceptor dystrophies

Thirty-six of 48 (75%) photoreceptor dystrophy patients in our study had both reduced a-wave and b-wave amplitudes a finding similar to other studies.[15,17,31,32] Photoreceptor dystrophies have their primary pathological defect in the photoreceptor contributing to the a-wave reduction. In the ISCEV standard DA 3.0 and DA 12.0, this stimulus parameters measure both dark adapted rod and cone responses. In 25% of this group the only abnormality was a reduced b-wave with a normal a-wave suggesting inner retinal dysfunction. A feature of cone system responses is that as flash strength increases, the a-wave amplitude increases while the b-wave increases then falls with further increases in flash strength. This is termed the 'photopic hill'.[8,16,33] This phenomenon is seen in rod dysfunction where there is preserved cone function. Therefore, electronegative finding with concurrent a-wave abnormality should be interpreted with cautions as the possible cause is either co-existing photoreceptor-post phototransduction defect or photoreceptor dystrophy with preserved cone function (photopic hill).

4.2 | CSNB

CNSB accounted for 23.7% of the electronegative cases in our series. This is a prevalence greater than all previous studies except the Kim et al. study.[15,17,18,20] The difference may reflect the improved awareness for CSNB diagnosis particularly in the milder forms leading to electrodiagnostic testing for suspected patients. CSNB is a non-progressive inherited retinal disorder caused by defective visual signal transmission from photoreceptor to the bipolar cells usually with a normal fundus appearance (Figure 2A,B). An ISCEV standard ffERG and the extended LA ON–OFF ERG assists in differentiating complete and incomplete forms (Figures 1D and 2A,B). The subclassification of CSNB was defined on ERG in 1986 and refined in 1987 by Miyake et al. studies.[34,35] Subsequently, advances in physiology and genomics has provided the explanation for the ERG pattern. Complete CSNB (cCSNB) is related to the post-synaptic ON-bipolar pathway while incomplete CSNB (iCSNB) is related to photoreceptor pre-synaptic defects which affects signalling of both the ON- and OFF-bipolar pathways (Figure 1E).[9,36]

4.3 | Retinoschisis

Retinoschisis represented 10.8% (Figure 2C) in our cohort similar to other studies except Renner et al. which found 36.2%.[17] Retinoschisis is a common X-linked juvenile macular degeneration.[8] This condition can often be difficult to diagnose due to subtle foveal schisis or the lesions might be replaced with non-specific macular atrophy with disease progression.[15,37] Six out of 15 (40%) retinoschisis patients in our study also had an abnormal a-wave ffERG highlighting the importance of correlating the multimodal imaging studies to the functional electrophysiology.

4.4 | Ischemia

Retinal ischemia was the underlying aetiology in 18 cases (12.9%) which was similar to the Koh et al. study.[15] Referral patterns to visual electrophysiology units will influence this diagnostic category. Retinal ischemia may arise from central retinal artery occlusion (CRAO) (Figure 3), diabetic retinopathy, or other causes. Identification of an electronegative ERG can greatly assist with diagnosis particularly when the presentation is distant from the acute

event and the retinal signs may have resolved or changed making diagnosis difficult.[8] Ocular coherence tomography-angiography (OCT-A) may provide a structural guide to corroborate the diagnosis.[38] This information is important for prognosis particularly for the other eye.

4.5 | PAIR and nPAIR

Autoimmune retinopathy (AIR) is a rare inflammatory condition that involves retinal antigens being aberrantly recognised as autoantigens, leading to retinal degeneration. It can be broadly grouped into PAIR which includes cancer associated retinopathy (CAR) and MAR, and nPAIR.[39,40] AIR is characterised by usually bilateral (asymmetric), relatively rapid, progressive, painless visual deterioration with little or no fundus findings.[41] This group represented 8.6% of the cases in our cohort which was more common than other studies.[15,17,18] MAR (Figure 4) is the most common PAIR associated with an electronegative ERG. In MAR, there are antibodies directed against the postsynaptic bipolar TRPM1 protein[42,43] while in nPAIR, antibodies have been reported against other retinal proteins.[40] However, the laboratory results are difficult to interpret as there is a significant overlap between the normal range and the affected individuals.[40,41,44,45]

4.6 | A guide to managing a patient with an electronegative ffERG

In 34 (24.5%) of our patients, the electronegative ERG findings preceded the symptoms which is larger than previous study of 0.8%.[15] The majority of our patients (58, 41.7%) had good vision. These patients may have had nyctalopia at presentation or had screening due to sibling's condition. Eleven patients (7.9%) had good vision along with normal fundus appearance, normal UWF-FAF, and normal macular SD-OCT, making it challenging to diagnose and emphasises the importance of ERG in such circumstances.

Using our cohort data and previous studies, we developed a flow chart to help guide further evaluation and analysis in patients with an electronegative ERG diagnosis (Figure 6). Patients with poor vision or nyctalopia as the presenting symptoms will require assessment of visual acuity, multimodal imaging of UWF fundus pseudocolour photograph, UWF-FAF, and SD-OCT. Normal or near normal results should be followed by an ISCEV standard ffERG. Identification of an electronegative ffERG will direct further assessments. Unilateral cases are usually caused by ischemia; bilateral cases are caused by CSNB, Vitamin A deficiency, or vigabatrin[46,47]/methanol toxicity; and typically bilateral but can be asymmetric cases are usually caused by PAIR-nPAIR.[16] Electronegative ERG with accompanying abnormal eye examination or imaging findings reflect other specific diagnoses. Schitic changes on the SD-OCT point to retinoschisis. A bull's eye maculopathy appearance in a child requires further systemic assessment including neurological review to identify Batten disease. Retinal dystrophies are a common cause of an electronegative ERG and the characteristic multimodal imaging will usually support the diagnosis as well as dark adapted a-wave reduction in amplitude.[16] While some diagnoses might have clinical variation, the flow chart (Figure 6) provides a guide for refining the diagnosis.

Limitations in this study are the retrospective nature which may have reduced data completeness. There were some cases where the diagnoses could not be established.

The prevalence of electronegative ERG in our referral centre was 3.1% with photoreceptor dystrophy as the main aetiology followed by CSNB, retinoschisis, retinal ischemia, and PAIR-nPAIR. An electronegative ERG is an important clinical sign that assists in localising the functional defect in the visual pathway. We present a flow-chart to assist in diagnosis and management of these patients. Numerous electronegative ERG diagnoses have normal or near normal fundus appearances and even preceding the symptoms, underlining the importance of a ffERG examination in the era of multimodal imaging and genomic therapy.

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CONFLICT OF INTEREST

Robyn V Jamieson and John R Grigg have been consultants for Novartis (Australia).

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