Published in final edited form as:

Retin Cases Brief Rep. 2021 July 01; 15(4): 473–478. doi:10.1097/ICB.0000000000000828.

Progression of scotopic single-flash electroretinography in the stages of *CAPN5* vitreoretinopathy

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Summary Statement:

Characterization of the degradation of scotopic electroretinography recordings from patients in various stages of ADNIV reveal photoreceptors may sustain early insult and reveals a unique time frame for potential therapeutic intervention.

Purpose: To characterize the changes found in the electroretinography (ERG) recordings of Autosomal Dominant Neovascular Inflammatory Vitreoretinopathy (ADNIV) patients and correlate with clinical stages of the disease.

Methods: Retrospective chart review. Bright and dim flash full-field scotopic, photopic, and 30 Hz flicker ERGs were obtained according to international standards. The scotopic ERGs were further processed to analyze the oscillatory potential (OP). The patient described in the case report underwent full ERG testing; five patients composed the archival case series data and included scotopic ERG recordings.

Results: Stage I ADNIV is characterized by a decrease in the *b*-wave amplitude on scotopic flash ERG and the disappearance of late OPs; however, the *a*-wave amplitude is normal. In stage II, attenuation of early OPs and the *c*-wave are observed in scotopic ERG recordings, but both *a*- and *b*-wave amplitudes are unchanged. For patients in stage III, there is a continued decline of both *a*-

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Conflicts of Interest: The authors declare no competing financial interests.

All work for this study was completed at Stanford University School of Medicine

and *b*-wave amplitudes in scotopic ERG recordings. There was a loss of recordable scotopic ERG response in patients with stage IV disease.

Conclusions: ERG may be valuable in determining optimal timing for therapeutic intervention and response prior to loss of recordable retinal function in *CAPN5* vitreoretinopathy.

Keywords

ADNIV; CAPN5; ERG; electroretinography; degeneration; retina; retinopathy

Introduction

Autosomal Dominant Neovascular Inflammatory Vitreoretinopathy (ADNIV, OMIM#193235)¹ is a rare and devastating disease that develops slowly over the lifetime of a patient to cause significant blindness. This disease has been extensively characterized in three families^{2–5}, and it is isolated to autosomal dominant mutations of the gene encoding for calcium-activated protease calpain-5 (CAPN5). Wert et al. have found that the resulting mutant protease displays hyperactivity from a lowered calcium threshold for activation;⁶ however, the specific downstream signaling cascades triggered by the CAPN5 protein are not well understood.

Various studies have shown that ADNIV patients are clinically asymptomatic for two decades, do not exhibit systemic manifestations,^{3, 7, 8} and display only ocular findings that are characterized into five distinct stages^{3, 4, 9} (Table). Stage I is clinically indistinguishable from a non-infectious uveitis. Progression to stage II exhibits photoreceptor degeneration similar to retinitis pigmentosa. Later stages include iris and retinal neovascularization, panuveitis, vitreoretinal fibrosis, tractional and rhegmatogenous retinal detachments, cystoid macular edema and vitreous hemorrhage. Ultimately, ADNIV patients degenerate into total blindness and phthisis bulbi.³

Current therapy using steroid implants for uveitis and anti-VEGF injections for neovascular hemorrhagic complications has had some success, but Tluczek et al. found that patients eventually lose sight from progressive retinal degeneration and fibrosis.¹⁰ Interestingly, a unique finding in ADNIV patients is the loss of the *b*-wave amplitude in electroretinography (ERG), a phenomenon that is not well understood. As new therapies are developed to target the primary gene and functional defects of mutant CAPN5, as described in Nelson et al.,¹¹ ERG may eventually become valuable in determining optimal timing for therapeutic intervention and response.

The purpose of the current study was to further characterize the changes found in the ERG recordings of ADNIV patients and to correlate these with the clinical stages of disease. Through our efforts, we hope to enhance our understanding of this devastating disease in its earlier stages for intervention.

Methods

The study was approved by the Institutional Review Board and adheres to the tenets set forth in the Declaration of Helsinki. A retrospective chart review of patients with ADNIV was

performed. Mahajan et al. previously described a clinical staging system was used for this study,³ and patients were categorized into four disease stages. Single bright-flash scotopic ERGs using Burian-Allen electrodes and Ganzfeld stimulation according to international standards were obtained from one patient described in the case report as well as five patients total in various stages of ADNIV in the case series portion. These recordings were further processed with a band-pass filter to isolate the oscillator potential (OP) for analysis. Photopic and flicker ERG recordings were obtained from a recent patient described in the case report section, although similar data was not available in archival records of patients in the case series section. Cases with a visually significant cataract or other media opacities were excluded from this study. Visual acuity was measured by Snellen.

Results

Case Report

A 39-year-old female with stage II ADNIV had previously undergone cataract surgery, trabeculectomy, and fluocinolone intravitreal implants (Retisert™) in both eyes. Despite control of inflammation, hemorrhages, and fibrosis, her visual acuity was 20/200 in the right eye and 20/160 in the left eye. Intraocular pressures measured by Goldmann applanation were 10 and 5 mmHg for the right and left eyes, respectively. The anterior chamber was deep and without cell or flare. A dilated fundus examination showed vitreous haze in both eyes without cells, bilateral fluocinolone intravitreal implants, rare peripheral pigmentary changes, and epiretinal membranes with moderate cystoid macular edema in both eyes (Figure 1A-C). Scotopic ERG recordings with both dim and bright stimuli showed a robust hyperpolarizing *a*-wave but a depressed depolarizing *b*-wave, indicating a normal photoreceptor response but a dysfunctional inner retina involving cells such as bipolar neurons and Müller glia (Figure 1D, E). Late OPs processed from scotopic recordings appeared attenuated in both dim and bright stimuli (Figure 1F). Photopic bright-flash ERG recordings showed a normal *a*-wave but a mildly depressed *b*-wave, reflecting findings from the scotopic recordings (Figure 1G). Furthermore, 30 Hz flicker ERG recordings showed a depressed response (Figure 1H).

Case Series

ADINV patients identified from a pedigree carrying the guanine to thymine nucleotide change at position 728 in exon 6 of the *CAPN5* gene previously described by Mahajan et al. had undergone full field single-flash scotopic ERG recordings.³ OCT imaging was not available for these patients at the time of data acquisition. Representative tracings from the various stages of disease are shown in Figure 2 for bright flash stimuli (left column) and OP (right column). Patients do not manifest the disease until the third decade of life, and the initial flash (Figure 2A, left) and OP (Figure 2A, right) recordings were indistinguishable from that of normal patients. The flash recording exhibited robust *a*- and *b*-waves. A hyperpolarizing *c*-wave was measured after peak *b*-wave response and is generated by the retinal pigment epithelium (RPE) as an indication of photoreceptor functional integrity. Finally, early and late OP, generated by amacrine cells, were appreciated (Figure 2A, right).

In stage I of ADNIV, patients typically display a mild and nonspecific anterior and/or posterior uveitis with rare inflammatory cells that can be documented on slit lamp biomicroscopy. The fundus exams otherwise appear normal. Our flash recording showed a reduced *b*-wave amplitude, while the *a*-wave amplitude was minimally affected (Figure 2B, left). The late OPs (OP3–4) were attenuated (Figure 2B, right). Together, these findings suggest that the initial functional detriment in ADNIV could be occurring at the interface between photoreceptors and the inner retina or within the inner retina itself.

Once ADNIV progressed to stage II, chronic ocular inflammation produces posterior subcapsular cataract, optic disc edema, cystoid macular edema, and peripheral pigmentary changes in the retina. Attenuation of the *c*-wave and early OPs were observed (Figure 2C). There did not appear to be significant changes in *a*- and *b*-wave amplitudes compared to Stage I patients. These findings suggest that photoreceptors are the site of progressive insult at this stage.

As ADNIV patients progress to stage III, there is a significant reduction of visual acuity between 20/50 and 20/200 as measured by Snellen. Clinical findings include worsening of cataract requiring surgery, development of fibrovascular membranes causing retinal traction, neovascularization of the retina, disc and iris, and development of epiretinal membranes. Electrophysiologically, patients exhibit reduced *a*- and *b*-wave amplitudes (Figure 2D). The OPs were found to have merged into one peak and were significantly attenuated. At this stage of the disease, we observed significant loss of both outer and inner retinal function.

Advanced ADNIV disease in patients is represented by stage IV, and visual acuity is frequently recorded to be count-fingers or light-perception only. Clinically, patients are found to have significant anatomical disease including neovascular glaucoma and tractional retinal detachment. In patients with clear media and no retinal detachment, there was extensive pigmentary retinal degeneration and loss of all detectable retinal electrophysiology (Figure 2E); therefore, we believe that *CAPN5* mutations are the underlying cause of the extinguished ERG instead of other anatomical explanations, such as vitreous hemorrhage or retinal detachment. Thus, despite control of inflammation, retinal neovascularization, and intraocular fibrosis, there was progressive retinal degeneration. Once patients progressed to the end-stage of disease, Stage V, their eyes become phthisical and their vision further declined to no-light perception.

Discussion

In the earliest stages of ADNIV, it is difficult to clinically distinguish the disease from other non-specific inflammatory conditions and retinal degeneration. The reduction in *b*-wave amplitude and loss of late OPs are the first confirmatory signs of this condition. Although genetic testing for *CAPN5* mutations has supplanted the use of ERG as a diagnostic tool, monitoring changes in scotopic bright-flash ERG recordings may be a useful method to follow disease progression and determine the timing and response to gene specific therapy. Indeed, much progress has been made in human gene therapy strategies as well as gene editing in models of retinal degeneration, which are further described by Nelson et al., Bassuk et al., Wu et al., DiCarlo et al., and Russell et al.^{11–15}.

Schaefer et al. recently showed that the CAPN5 protease is expressed in many cells throughout the retina,¹⁶ including retinal ganglion cells, outer nuclear layer neurons, and photoreceptors. Interestingly, CAPN5 expression is highly concentrated at photoreceptor synapses, suggesting that the initial insult in ADNIV may occur almost exclusively within photoreceptors to decrease signal transmission to the inner retina. The ERG recordings from stage II ADNIV lend further support where we observe a decrease in the *c*-wave amplitude with preservation of *a*- and *b*-wave amplitudes compared to stage I. The *c*-wave is generated by the RPE and is commonly used as a marker for photoreceptor health and its ability to interact with the RPE. Additional support comes from clinical observations of a degenerative pigmentary retinopathy with pigment clumping that occurs in stage II and has some similarity to that which is observed in retinitis pigmentosa. These results hopefully show that there is a window of opportunity for therapy before the health of photoreceptors decline.

Initiating long-term therapeutic interventions to control inflammation such as intravitreal implants of dexamethasone (OzurdexTM), fluocinolone (RetisertTM), or systemic immunomodulatory therapy may delay progression of the disease where further damage to the retina and surrounding structures are observed both clinically and functionally. Continued steroid therapy can have significant side effects such as cataract formation and glaucoma, thus minimizing steroid use has long-term benefits for patients. In our experience, however, corticosteroid or immunosuppressive therapy have not prevented the progression of retinal degeneration.

ADNIV provides a genetic model to elucidate the mechanisms by which neuroinflammation occurs within other uveitic conditions such as sarcoidosis, HLA-B27, and the White Dot Syndromes. CAPN5 is a calcium-activated protease expressed by photoreceptors and may be over activated in other retinal diseases where there is elevated intracellular calcium within photoreceptors. These conditions present characteristically with initial loss of the *b*-wave on ERG and inner retina dysfunction. Audo et al. have described initiating factors to span a wide range to include congenital (i.e. congenital stationary night blindness, Oguchi disease, Duchenne muscular dystrophy), acquired (i.e. central retinal artery and vein occlusions), inflammatory (i.e. birdshot chorioretinopathy, diffuse unilateral subacute neuroretinitis, melanoma-associated retinopathy), and retinotoxicity.¹⁷ Finally, Schaefer et al. describes other types of calpains expressed within the retina,¹⁶ and our ability to inhibit this family of proteases is hypothesized by Azuma and Shearer and Ozaki et al. to preserve functional target cells for subsequent rescue in different forms of retinal degenerative diseases, such as retinitis pigmentosa and cone-rod dystrophies.^{18, 19} A more precise understanding of the underlying mechanisms of CAPN5 in ADNIV will allow us to offer potential effective therapies for our patients and others with photoreceptor disease.

Acknowledgments

Funding Support:

VBM and AGB are supported by NIH grants [R01EY026682, R01EY024665, R01EY025225, R01EY024698 and R21AG050437], The Doris Duke Charitable Foundation Grant #2013103, and Research to Prevent Blindness (RPB), New York, NY. The Barbara & Donald Jonas Laboratory of Regenerative Medicine and Bernard & Shirlee Brown Glaucoma Laboratory are supported by the National Institute of Health [5P30EY019007, and R01EY018213], National Cancer Institute Core [5P30CA013696], the Research to Prevent Blindness (RPB)

Physician-Scientist Award, unrestricted funds from RPB, New York, NY, USA. SHT is a member of the RD-CURE Consortium and is supported by the Tistou and Charlotte Kerstan Foundation, the Schneeweiss Stem Cell Fund, New York State [C029572], the Foundation Fighting Blindness New York Regional Research Center Grant [C-NY05-0705-0312], the Joel Hoffman Fund, the Professor Gertrude Rothschild Stem Cell Foundation, and the Gebroe Family Foundation.

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Figure 1. Stage II ADNIV disease shows degradation in retinal structure and function. Fundus photography (right eye, **A**; left eye, **B**) and optical coherence tomography images (right eye, **C**; left eye, **D**) show formation of cystoid macular edema and epiretinal membranes. Scotopic dim (**D**) and bright (**E**) flash ERG recordings show normal *a*-wave amplitudes but decreased *b*-wave amplitudes. Scotopic OPs appear to be preserved (**F**). While photopic bright flash (**G**) ERG recordings show no significant changes, 30 Hz flicker recordings (**H**) show mild decreased cone function. OD – right eye; OS – left eye

Figure 2. Scotopic ERG recordings show decline in retinal function with progression of ADNIV disease stage.

Representative ERG tracings from scotopic bright flash stimulus (left column) are processed to evaluate for OP (right column). Recordings from a normal patient are shown for comparison (**A**). In stage I of ADNIV, *b*-wave amplitude is decreased, and late OPs (OP3–4) are attenuated (**B**). Progression to stage II shows loss of *c*-wave amplitude (**C**). In stage III, there is significant reduction of both *a*- and *b*-waves as well as reduction of OP (**D**). Once

patients progress to stage IV, the ERG recordings become nonrecordable (**E**). Scale bars represent 20 ms and 150 μ V for left column, and 20 ms and 30 μ V for right column.

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Table.

ADNIV Stages of disease and associated clinical findings

Stage	Anterior Segment	Posterior Segment	Electroretinogram
Stage I	Rare Cells	Rare Cells	Reduced b-wave
Stage II	Cells Mild posterior subcapsular cataract	Moderate Cells Optic Disc Edema Cystoid Macular Edema Peripheral pigmentation of the retina	Flat b-wave
Stage III	Anterior subcapsular cataract Moderate posterior subcapsular cataract Iris synechiae	Vitreous traction at posterior pole Epiretinal membrane Tractional retinal detachment Posterior vitreous bands (not fibrosis) Progressive pigmentary retinal degeneration Peripheral fibrosis Peripheral neovascularization Rare neovascularization of the disc Rare neovascularization elsewhere	Diminished a-wave Electronegative
Stage IV	Iris bombe Secluded pupil Peripheral anterior synechiae Angle closure glaucoma Neovascular Glaucoma	Progressive pigmentary retinal degeneration Vitreous hemorrhage Posterior tractional retinal detachment Anterior tractional retinal detachment	Non-recordable ERG
Stage V	Phthisis	Combined tractional and rhegmatogenous retinal detachment Phthisis	