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Case report

Phenotypic variance in Calpain-5 retinal degeneration

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ABSTRACT

Purpose: To characterize the phenotype of patients with mild calpain-5 Neovascular Inflammatory Vitreoretinopathy (ADNIV).
Observations: The *CAPN5* p.R243L mutation is typically associated with onset in the twenties and severe, progressive uveitis, retinal neovascularization, and intraocular fibrosis. Two subjects with this *CAPN5* variant only showed mild peripheral retinal pigmentary degeneration and loss of the ERG b-wave at age 45 and 69, respectively, without signs of uveitis or neovascularization.
Conclusions/Importance: The phenotypic penetrance of a specific variant in CAPN5-vitreoretinopathy may vary significantly in severity. Patients with pigmentary retinal dystrophy may consider *CAPN5* gene testing.

1. Introduction

Mutations to the gene encoding for calcium-activated cysteine protease-5 (*CAPN5*) lead to a rare and destructive condition termed Autosomal Dominant Neovascular Inflammatory Vitreoretinopathy (ADNIV, OMIM#193235).[1] While CAPN5 is expressed in a variety of tissues, its exact role is not well understood. There are currently six reported *CAPN5* human gene mutations, each resulting in varying levels of protease hyperactivity with a direct correlation to clinical severity. The most severe form of ADNIV results from a p.R289W mutation in the protease catalytic domain and presents with a syndrome of blindness before age ten along with hearing loss and developmental delay.[2] Most of the other mutations also occur in the catalytic domain, but appear to have less hyperactivity with visual symptoms occurring at a later age and without syndromic features. The p.G376S mutation is the only known mutation to occur outside of the catalytic domain within regulatory domain-III, but had a similar disease onset occurring at age nineteen.[3] To date, clinical reports show this dominant disease is highly penetrant and affected members within the same mutation have identical outcomes.

Of the different ADNIV gene mutations, the heterozygous p.R243L mutation is the most clinically studied, where disease onset typically begins around age twenty. The mutation lies within exon 6 and removes a charged residue adjacent to a nearby alpha helical domain within the catalytic domain.[4] Its disease phenotype was recreated in an animal study where mouse retinas virally transfected to express the human p.R243L *CAPN5* gene mutant displayed loss of electroretinography (ERG) function, photoreceptor degeneration, and an inflammatory response.[5]

Patients that carry the heterozygous p.R243L mutation exhibit disease onset at age 20 with progression through five distinct phases that each last approximately 10 years.[4–6] Stage I is clinically indistinguishable from a non-infectious posterior uveitis, and there is loss of the ERG b-wave. Once a patient progresses to stage II, they demonstrate a pigmentary photoreceptor degeneration that shows some similarities retinitis pigmentosa. Later stages include iris and retinal neovascularization, panuveitis, vitreoretinal fibrosis, tractional and rhegmatogenous retinal detachments, cystoid macular edema, and vitreous hemorrhage. The final phase of the disease results in total blindness and phthisis bulbi.[4] Previous work has shown that the different phases of ADNIV can be characterized by distinct electrophysiologic characteristics using scotopic single-flash ERG.[7]

Here we report a milder phenotype of p.R243L CAPN5-associated ADNIV in two subjects exhibiting later onset, no uveitis, and the clinical appearance of pigmentary retinal degeneration with functional vision. These findings further expand our understanding of this otherwise

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devastating disease and offer new insights into its pathophysiology.

2. 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 was performed and the Mahajan NIV clinical staging system was used.[4] Scotopic, photopic, and flicker ERGs were obtained using Diagnosys Espion 2 multi-focal ERG system equipped with DTL electrodes and Ganzfeld stimulation according to international Society for Clinical Electrophysiology (ISCEV) standards. These recordings were further processed with a band-pass filter to isolate the oscillator potential (OP) for analysis. Visual acuity was measured by Snellen.

3. Findings

3.1. Case 1

A 45-year-old male was referred for evaluation after receiving genetic testing at an outside facility that was positive for the *CAPN5* p.R243L c728G > T variant. His past ocular history was only significant for moderate myopia in both eyes (OU). Although clinical exam data was not available for his family, he reported a 70-year-old paternal uncle had the same *CAPN5* mutation with no visual complaints, while his brother and daughter are currently without any vision problems. Notably, his father began to lose visual acuity at age 40 that progressed to the point where he was no longer driving, but genetic testing was deferred. There were multiple other family members across more than three generations with poor vision (Fig. 1A), but more specific information was unavailable.

The patient himself denied any visual symptoms, and his best corrected visual acuity (BCVA) was 20/25 OU. Intraocular pressures measured by Goldmann applanation were 15 and 13 mmHg for right (OD) and left (OS) eyes, respectively. There were no findings of intraocular inflammation or other abnormalities on anterior segment examination OU. Evaluation of the fundus revealed pigmentary changes in the temporal periphery OU and vitreous syneresis OS; however, the remainder of the exam was unremarkable (Fig. 1B and C). Fundus autofluorescence demonstrated temporal peripheral hypoautofluorescence with a leading edge of hyperautofluoresence surrounding areas of pigmentary changes (Fig. 1D and E). Optical coherence tomography (OCT) imaging of the macula OU were unremarkable (Fig. 1F and G).

Compared to a normal control, dim-light scotopic ERG demonstrated an approximately 70–80% reduction of the rod photoreceptor driven response (Fig. 2A). Full-field scotopic 3.0 and 10.0 flash ERG demonstrated normal to mildly reduced *a*-wave amplitudes (approximately 5–20% of normal controls OU) but attenuation of the *b*-wave to 60–80% of normal in OU in the patient, similar to that seen commonly with the CAPN5 p.R243L mutation[5,7,8] (Fig. 2B and C). Maximal ERG had a low b:a ratio. Photopic single-flash ERG displayed an approximate 60% loss of cone driven response in the patient OU, also common for ADNIV patients carrying the p.R243L mutation with typical disease progression[5,7,8] (Fig. 2D). Additionally, 30 Hz flicker responses showed an approximate 70% reduction in peak amplitudes OU as expected for a loss of the cone driven response in the patient (Fig. 2E). At the 1 year follow up, his vision and repeat studies remained stable (data not shown).

3.2. Case 2

A 69-year-old female was referred for evaluation of persistent vitreomacular traction OU with cystoid macular edema in OD greater than OS; however, the patient felt symptomatically that OS was worse. Genetic testing at age 43 confirmed the patient had the *CAPN5* p.R243L c728G > T variant of ADNIV, although clinical exams showed she was asymptomatic and had no signs of ADNIV. Her past ocular history was also significant for pseudophakia OU and dense asteroid hyalosis OS. Multiple family members were diagnosed with ADNIV, including her paternal grandmother, father, and both her son and daughter (Fig. 3A). Furthermore, she had multiple extended family members with blindness; however, further information was unavailable. She had BCVA of 20/60 OU, and her intraocular pressures were 19 and 18 mmHg by applanation tonometry in OD and OS, respectively. Anterior segment examination was unremarkable and fundus examination of OD showed peripheral pigmentary changes (Fig. 3B and C). Fundus examination of OS was difficult due to the dense asteroid hyalosis. OCT imaging highlighted our findings OU (Fig. 3D and E). The patient remained stable at 1 year follow up.

4. Discussion

Our current understanding of the multiple phenotypes of ADNIV relates to the different genetic mutations that impart varying CAPN5 protease hyperactivity. For example, ADNIV patients with the p.R289W mutation display a clinically more severe phenotype, and this is thought to be correlated with a higher CAPN5 protease activity.[2]

Because of the phenotypic overlap with other ocular conditions, ADNIV patients may be misdiagnosed as other conditions, such as idiopathic uveitis, idiopathic vitreomacular traction, or ischemic neovascular retinopathy, especially if the physician is not aware of a patient's family history and genetic inheritance pattern. There are also significant distinguishing features from typical retinitis pigmentosa. The ADNIV pigmentary degeneration shows clumps of pigment rather than bone spicules. Compared to autosomal dominant retinitis pigmentosa (ADRP), where bone spicules are localized to the inferior and nasal parts of the retina, ADNIV pigment changes appear in the periphery such as the temporal area observed in case 1. Also, on fundus autofluorescence in ADRP, the leading edge of the hyper-fluorescent line is more straight and uniform, while in these ADNIV cases, the line much more irregular. On the ff-ERG in ADRP there is no sparing of the a-wave as in ADNIV. Moreover, the vitreomacular traction is more severe than retinitis pigmentosa.

In the two cases presented, an identical genetic mutation shows a milder disease phenotype compared to previously published reports.[5,7,8] Examination by electroretinography replicated the ADNIV p.R243L ERG phenotype from previously published reports, but at a later age in the patient. We have studied twins with ADNIV, and there was only minor asymmetry between patients.[9] In these cases where the disease is less severe, it appears that the electroretinographic findings precede the inflammatory uveitis signs. In case-1, we conduct annual follow-up examination to monitor for signs of uveitis that could be treated with immunosuppresses, such as inflammatory cells, cystoid macular edema, and for vitreomacular traction that might require surgical intervention. In case-2 where there is active vitreomacular traction, we recommend six-month exams with a cautionary approach to surgery, since vitrectomy can trigger an inflammatory response. If there is further vision deterioration and planned vitrectomy, we find that perioperative immunosuppression with oral and local steroid along with intravitreal methotrexate at the time of surgery can mitigate the postoperative inflammatory response.[10] We have also used off-label anti-interleukin-6 therapy for severe intraocular fibrosis with proliferative vitreoretinopathy causing tractional retinal detachment.[10]

The later disease development in these cases suggests that other factors may be in effect to modify the severity of disease phenotype. We have observed phenotypic variation in mice models of CAPN5 eye disease (unpublished observation), but it is not clear how this translates to humans. We have not identified, for example, gene modifiers or *in trans* sequence variants in *CAPN5* that modify its structural characteristics or enzymatic activity. Further laboratory investigation into the molecular targets and downstream signaling pathways of the CAPN5

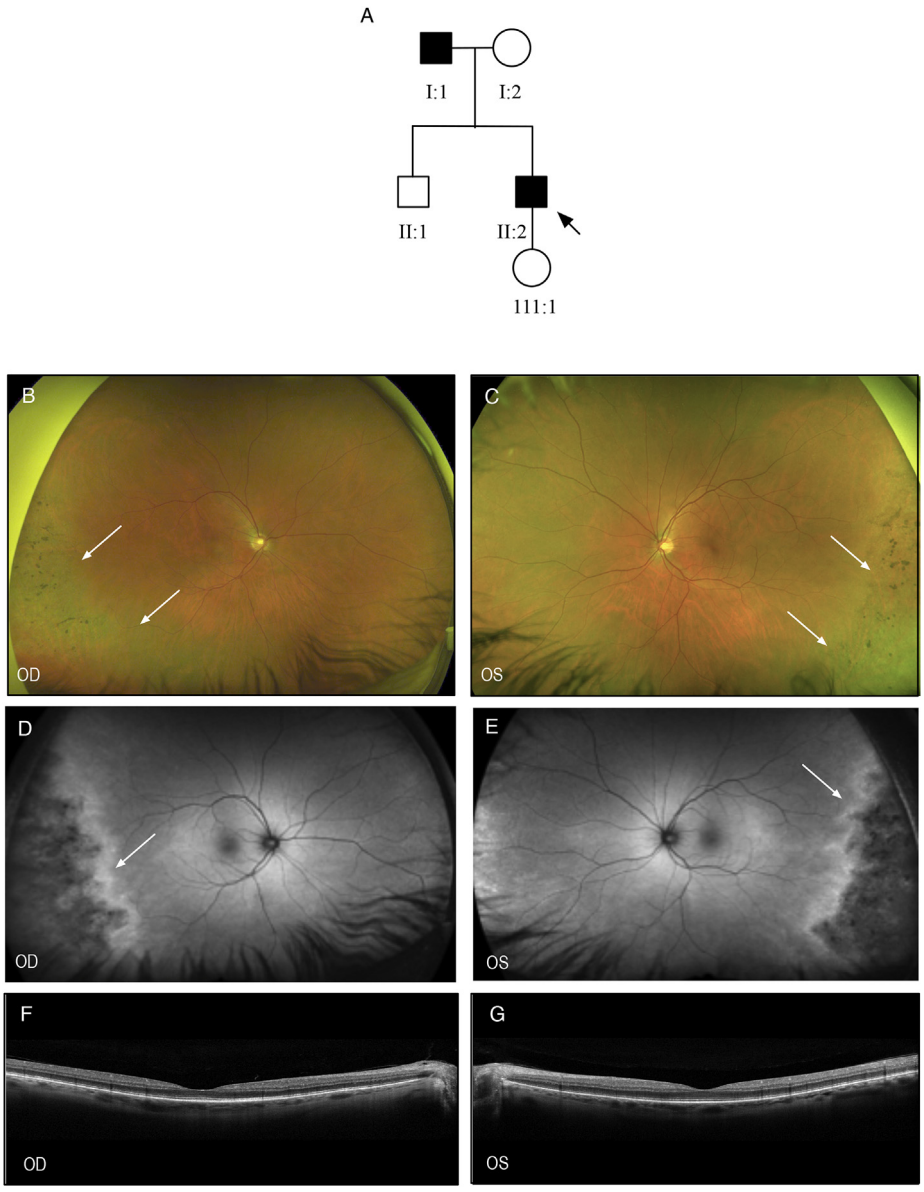

Fig. 1. Case 1. A. The family pedigree showed affected proband (arrow) and affected father. Black symbols represent clinically affected subjects. Open symbols represent unaffected subjects. Fundus photography (right eye, **B**; left eye, **C**) show normal nerve, macula, vessels. Arrows point to peripheral pigment degeneration. Autofluorescence (right eye, **D**; left eye, **E**) shows hyperfluorescent edge next to hypofluorescent spots. Optical coherence tomography (right eye, **F**; left eye, **G**) appears normal.

protease in the retina may reveal an important level of regulation that could eventually explain phenotypic variance.

5. Conclusions

Due to the clinically mild appearance and our previous understanding of the staging of ADNIV, these patients could have been clinically diagnosed with a retinal dystrophy. Therefore, it is possible that other patients with *CAPN5* variants who exhibit a clinic absence of uveitis and vitreoretinopathy features could be misdiagnosed. We propose that genetic evaluation of CAPN5 should be included in the evaluation panel of genetic testing for retinal dystrophy, especially in patients without a clear family history or classic ADNIV features of ocular inflammation and vitreoretinopathy.

Therapeutic interventions for ADNIV remain under investigation. Improving our understanding of the various phenotypic variance of this disease remains essential to find a therapeutic window within which treatment can be most effective.

Patient consent

Informed consents were obtained from all patients. This report does not contain any personal information that could lead to the identification of the patient.

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Authorship

All authors attest that they meet the current ICMJE criteria for Authorship.

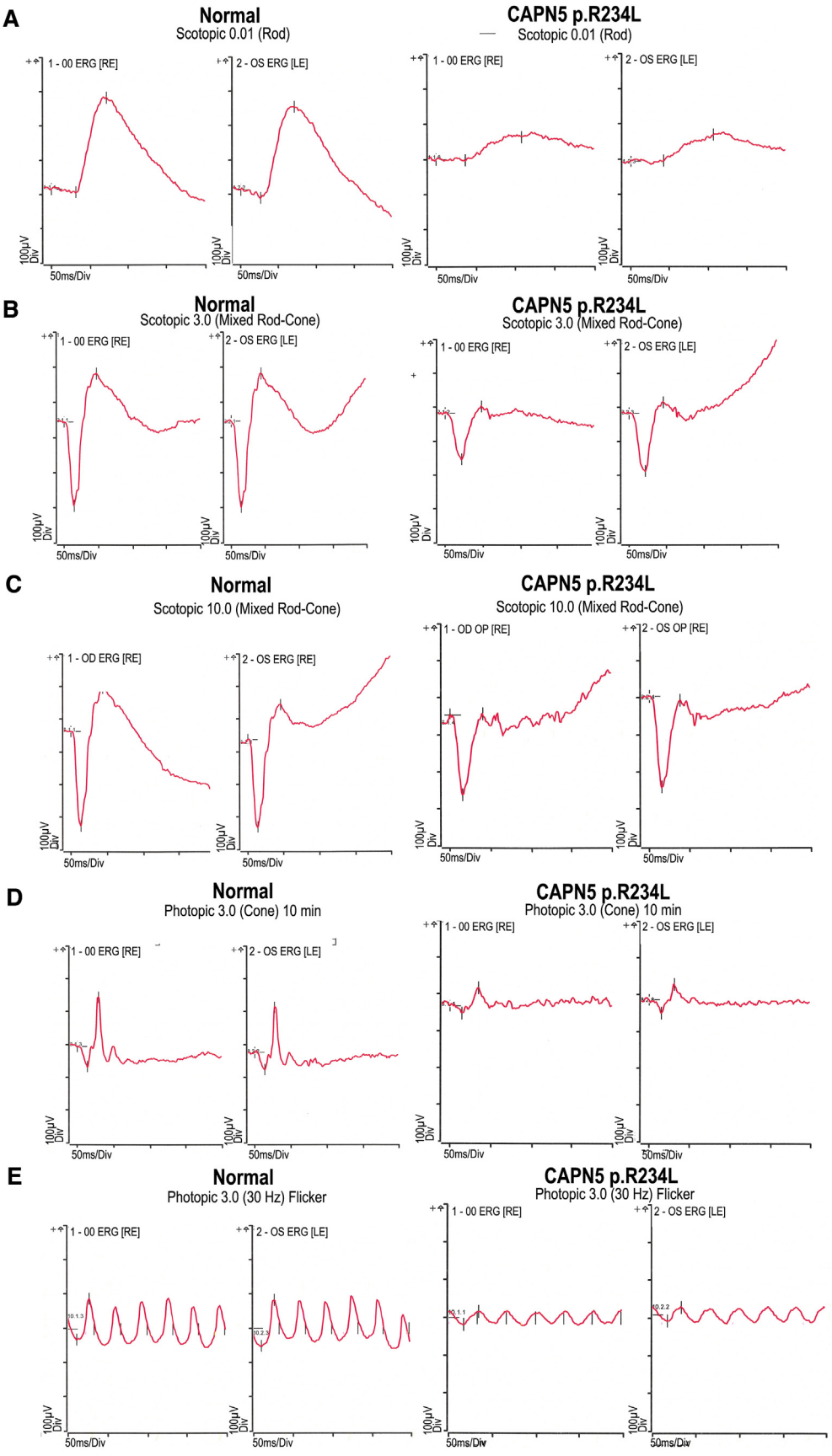

Fig. 2. Electroretinography (ERG). A. Scotopic 0.01 dim-light ERG recordings demonstrated an approximate 70–80% loss of the *b*-wave amplitude OU in the CAPN5 p.R243L patient (right) compared to a control (left). Full-field scotopic **B.** 3.0 and **C.** 10.0 flash ERG recordings demonstrated normal to mildly reduced *a*-wave amplitudes in the CAPN5 p.R243L patient (right) at approximately 5–20% of normal controls OU (left), as well as the attenuation of the *b*-wave to 60–80% of normal in OU in the patient. **D.** Photopic single-flash ERG displayed an approximate 60% loss of the cone driven response in the patient OU (right) compared to control (left). **E.** 30 Hz flicker responses showed delay and an approximate 70% reduction in peak amplitudes OU in the CAPN5 p.R243L patient (right) compared to normal control (left). OD/RE, right eye; OS/LE, left eye.

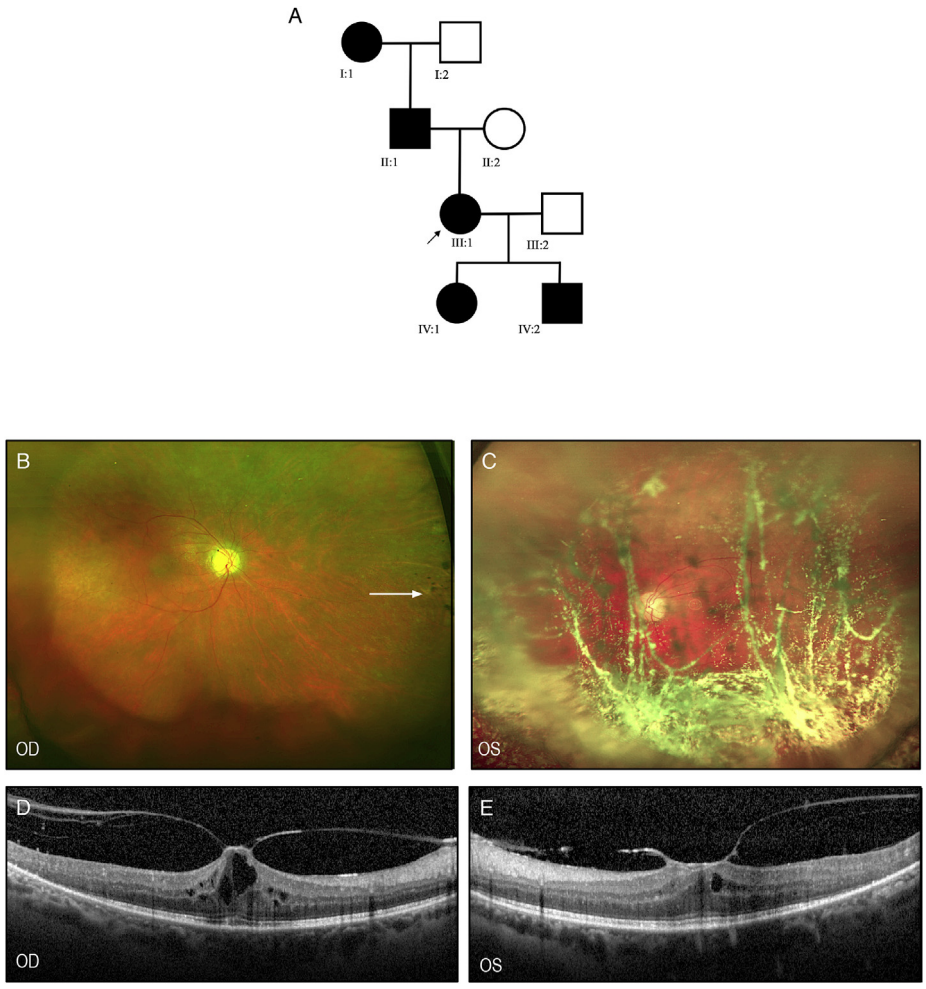

Fig. 3. Case 2. A. The family pedigree showed affected proband (arrow) and affected paternal grandmother, father, son, and daughter. Black symbols represent clinically affected subjects. Open symbols represent unaffected subjects. **B, C.** Right and left fundus photography, respectively, show normal nerve and vessels. Arrow points to peripheral pigment degeneration OD (left) and dense asteroid hyalosis OS (right). **D, E.** Right and left eye optical coherence tomography shows vitreomacular traction and cystoid macular edema.

Author contributions

Dr. Mahajan had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: PT, SHT, AGB, VBM. Acquisition of data: PT, TC, JF, VBM. Analysis and interpretation of data: PT, JF, SHT, AGB, VBM. Drafting of the manuscript: PT, TC, KW, MM, SHT, VBM. Critical revision of the manuscript for important intellectual content: SHT, AGB, JF, VBM. Obtained funding: VBM. Administrative, technical, and material support: TC. Study supervision: VBM.

Role of the sponsor

The funding organizations had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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