



Clinical and imaging findings of choroideremia in a pediatric patient due to a novel frameshift mutation

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ABSTRACT

Purpose: To describe the clinical characteristics, imaging findings and genetic testing results of a young simplex male with choroideremia.

Observations: A 6-year-old Hispanic-Chinese male was referred to the retina clinic for peripheral retinal pigmentary changes observed in both eyes on routine exam. The patient has an unremarkable family history and developmental history. Best corrected visual acuity was 20/25 in both eyes. Optical coherence tomography demonstrated attenuation of the ellipsoid and interdigitation zones. Widefield fundus autofluorescence demonstrated nummular hypo-autofluorescence peripherally in both eyes. Genetic testing revealed a variant originally described as a variant of uncertain significance (VUS) a c. 1775_1814del (p.Glu592Valfs*44) identified in the *CHM* gene, which was reclassified as pathogenic following segregation analysis. The patient was diagnosed with choroideremia due to a *CHM* pathogenic variant.

Conclusions: The multimodal imaging findings demonstrated here illustrate important clues to the diagnosis of Choroideremia in a simplex male.

1. Introduction

Choroideremia (CHM, OMIM #303100) is an X-linked hereditary chorioretinal dystrophy, with a prevalence of 1 in 50,000–100,000 people.¹ Caused by pathogenic variants in the *CHM* gene, Choroideremia classically manifests as progressive atrophy of the retinal pigment epithelium (RPE), photoreceptors, and choroid.^{2,3} Though various types of genetic variants including translocations, point mutations, small deletions, and insertions have been documented, nonsense and frameshift variants account for approximately 70% of all disease causing variants.^{4,5} *CHM* encodes Rab escort protein 1 (REP1) which plays a critical role in vesicle trafficking.⁶ However, the pathogenesis of the disease remains poorly understood. Herein, we demonstrate benefit of multimodal imaging, particularly fundus autofluorescence, in diagnosing choroideremia at an early stage in a simplex pediatric patient with a novel frameshift mutation.

2. Case report

A 6-year-old Hispanic-Chinese male was referred to the retina clinic for peripheral retinal pigmentary changes observed during a routine eye exam. The patient had an unremarkable birth history, family history, and developmental history. The patient and mother denied he had any visual difficulty at school, but upon targeted questioning, reported two episodes of relative difficulty navigating in a dark environment compared to his sister.

Examination showed corrected visual acuity of 20/25 OD and 20/25 OS. Measured spherical equivalent refraction was -1.25 OD, and -2.00 OS. Extraocular movements were intact. Anterior segment examination was unremarkable. Fundus examination and widefield fundus photography (Optos, Marlborough, MA, USA) revealed subtle peripheral pigment mottling and peripheral sheen in both eyes (Fig. 1). Widefield fundus autofluorescence (Optos, Marlborough, MA, USA) revealed nummular hypo-autofluorescence in the mid periphery and adjacent to the optic nerves. Optical coherence tomography (OCT) (Spectralis, Heidelberg, Germany) showed foveal retinal thickness of 178 and 180

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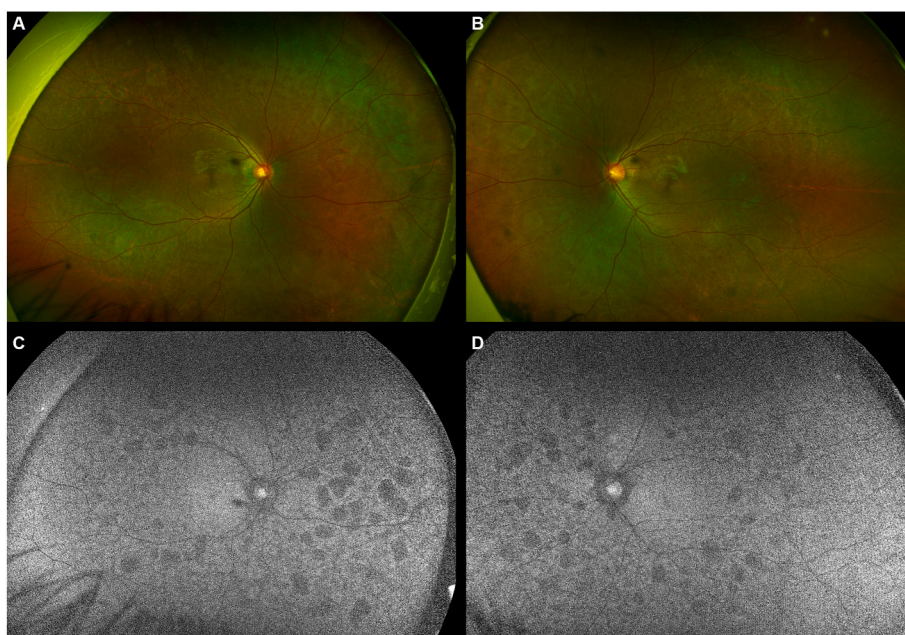


Fig. 1. Ultra-widefield pseudocolor fundus photos (A,B) show peripheral sheen and pigment mottling, peripapillary atrophy, good foveal reflex and normal vessels. Ultra-widefield fundus Autofluorescence (C,D) demonstrates numerous nummular patches of hypo-fluorescence peripherally and mild peripapillary atrophy OU.

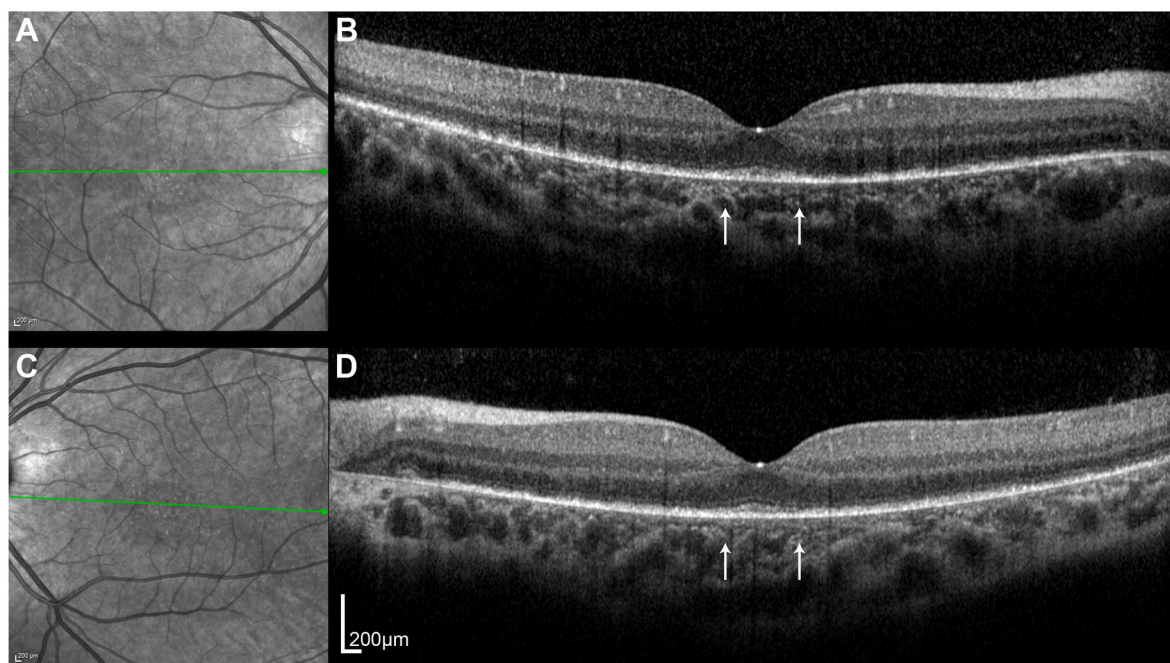


Fig. 2. Scanning laser ophthalmoscope and OCT image from right (A,B) and left (C,D) eyes respectively. SLO images (A,C) show punctate hyper and hypo-reflective clusters across the macula. OCT (B,D) shows preserved ellipsoid zone (between arrows) only at the fovea, with diffuse disruption elsewhere.

µm respectively in right and left eyes and foveal choroidal thickness of 374 µm in both right and left eyes. There was intact ellipsoid zone at the foveal center only, while the interdigitation zone band was poorly resolved across the entire macular scan (Fig. 2). The RPE band was mottled, but intact, across the entire scan except in the peripapillary regions in both eyes. There was grossly intact outer nuclear layer thickness throughout.

Full field electroretinography (ERG) was recorded conforming to the International Society for Clinical Electrophysiology of Vision protocol and revealed a rod-cone pattern of dysfunction, with rod function at approximately 20% of normal OU and cone function at approximately

85% of normal OD and borderline OS (Fig. 3). Scotopic testing showed a 0.01 ERG response that was 20% normal while 3.0 ERG combined rod-cone response demonstrated an a-wave that was 30% normal and a b-wave that was 65% normal. The photopic 3.0 ERG cone response was 85% normal OD, and within normal limits OS for amplitude. The 3.0 31 Hz flicker response was 80% normal OD with borderline OS amplitude. Delayed peak implicit time was noted on both 3.0 ERG cone response and 31 Hz flicker response. Goldmann visual field testing showed good cooperation given his age with mildly enlarged physiologic blind spots and slight constriction of the I4e isopter in each eye.

The patient was diagnosed on basis of his clinical phenotype,

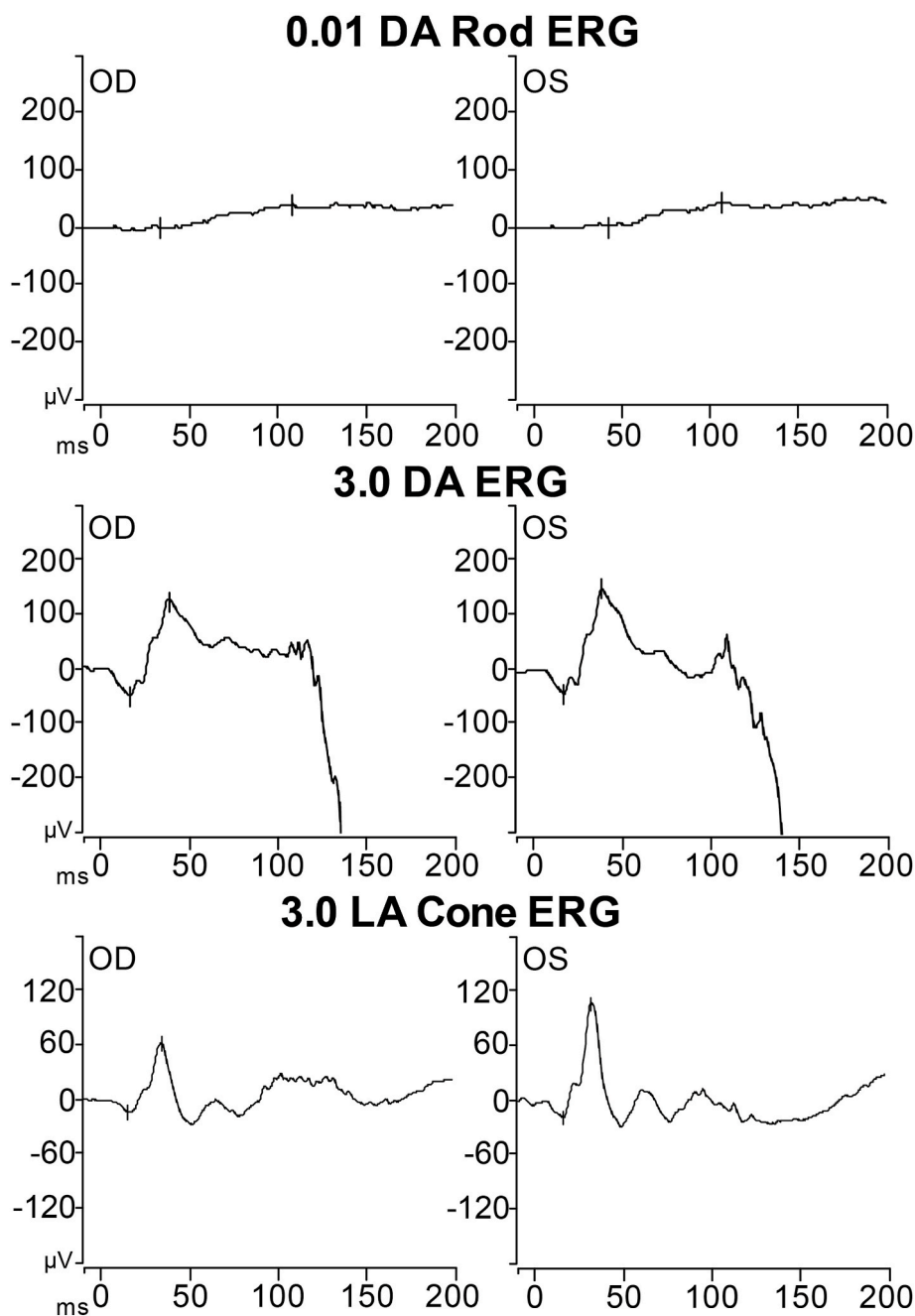


Fig. 3. Electroretinogram results showed nearly flat 0.01 ERG with B-wave amplitudes of 42.3/41.95 in the right/left eyes respectively. The 3.0 scotopic ERG amplitudes were also significantly reduced at 48.61/42.93 and 178.5/193.7 in the right/left eyes respectively. The 3.0 photopic ERG amplitudes were near normal in the right eye at 12.56/75.5 and within normal limits in the left eye at 13.79/126.2.

imaging and testing with a rod-cone dystrophy. His nummular peripheral fundus autofluorescence, however, was suggestive of choroideremia.^{6–8} A commercial gene panel (Invitae Inherited Retinal Disorders Panel, San Francisco, CA)⁹ with sequence analysis and deletion/duplication testing of 293 genes was performed on the patient. Variant analysis was performed by Invitae clinical testing lab based on a modified American College of Medical Genetics/Association for Molecular Pathology (ACMG-AMG).¹⁰ The patient was found to have a frameshift variant originally classified as a variant of unknown significance in the *CHM* gene (c. 1775_1814del (p.Glu592Valfs*44)). This variant has not previously been reported in the literature and is not present in large genomic databases. The variant has subsequently been reported to Clinvar by Invitae (Accession RCV001959165.3). Following segregation analysis of the variant showing that the mother did not carry

the p. Glu592Valfs*44 variant, it was presumed to be *de novo*. As the variant was a frameshift creating a premature stop signal, not present in population databases, and presumed to be *de novo*, the variant was upgraded to pathogenic. Given the fundus and autofluorescence findings, this *CHM* pathogenic variant was presumed to be the cause of disease in this patient.

3. Discussion

In evaluating a simplex male for rod-cone dystrophy, a comprehensive clinical examination including refraction, multi-modal imaging and genetic testing with segregation analysis is often necessary to reach the correct diagnosis. Choroideremia specifically is not only rare but shares a noticeable overlap in clinical features with other

retinal dystrophies, hence, why it is often misdiagnosed, or diagnosed after symptoms have progressed to advanced stages.¹¹ Here, the wide-field FAF provided the most influential contribution to the phenotype of suspected CHM, as previously demonstrated by Mucciolo et al.⁷ Other notable phenotypic features include excellent vision despite advanced central photoreceptor attenuation on OCT as well as preserved central autofluorescence. The diagnosis was confirmed with genetic analysis revealing a presumed *de novo* CHM late frameshift mutation expected to cause a truncated REP1 protein, similar to previously reported late frameshift variants which have been demonstrated to be pathogenic.^{5,12} Specifically, frameshift variants in exon 15 been identified as the likely cause of disease in other patients affected with choroideremia.¹³ Due to the 5' location of the frameshift, the variant was initially classified as a VUS, but segregation testing in this case promoted it to a pathogenic classification, demonstrating the importance of segregation testing in establishing pathogenicity. In this case, the *de novo* pathogenic variant was the reason for the simplex pedigree, but X-linked disease should be on the differential for any simplex male, who may have inherited disease from a long line of female carriers. This is best demonstrated in retinitis pigmentosa, in which 15% of simplex males have X-linked disease.¹⁴

There is limited published data available on CHM in children less than 10 years old, especially simplex cases, given that diagnosis is often delayed due to slow progression of central vision loss and nyctalopia. Khan *et al.* identified twenty-nine patients with a mean age at referral of 9 years. They reported that peripapillary FAF loss was one of the earliest disease manifestations, while central OCT is sometimes normal. They recommended longitudinal measurement of central retinal thickness, and sub foveal choroidal thickness for evaluation of disease progression.¹⁵ Others have reported that the earliest anatomical alteration in CHM was photoreceptor shortening (loss of the interdigitation zone, similar to what was seen in our patient) on macular OCT, despite normal foveal thickness in patients <15 years old.^{4,8} Widefield FAF was not available in these reports, and thus the relative timing of interdigitation loss compared to appearance peripheral and peripapillary FAF alterations is not yet known.

There is yet to be a consensus regarding the pathogenesis of Choroideremia, as controversy regarding the sequence of retinal degeneration remains. Some studies suggest that abnormalities at the photoreceptor level precede RPE abnormalities,^{8,16} while other studies suggest that the disease initially afflicts the RPE and is followed by an independent photoreceptor degeneration and ultimately choroidal atrophy.^{17,18} Histopathology from a donated eye affected by choroideremia demonstrates widespread irregularities in RPE pigment and thickness even in areas of normal photoreceptors.¹⁹ Studies in RPE models of choroideremia demonstrate abnormalities in phagocytosis.^{20,21} The mouse model suggests independent primary pathology in both photoreceptors and RPE.²² Our young patient demonstrated diffuse photoreceptor anatomical changes out or proportion to RPE loss centrally, although this does not preclude primary RPE dysfunction as a pathologic mechanism.

4. Conclusion

In conclusion, our case demonstrates the utility of FAF in evaluating a pediatric patient with a novel CHM pathogenic variant. Early clinical and genetic diagnosis of choroideremia is increasingly clinically relevant and timely given current gene therapy trials.

Patient consent

Verbal informed consent for publication of this report was obtained from the mother of the patient. Written consent to publish this case has not been obtained. This report does not contain any personal identifying information.

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Authorship

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

Declaration of competing interest

The following 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: MM, DS. ATF owns stock in Ionis Pharmaceuticals and is a consultant for Janssen Pharmaceuticals. KB is a consultant for Biogen, ProQR Therapeutics, and Janssen. AC is a consultant for Novartis, consultant for Synergetics, founder and equity owner of FocusROP and Retinal Solutions.

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