



Identification of Pathogenic Variants in the *CHM* Gene in Two Korean Patients With Choroideremia

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Choroideremia is a rare X-linked disorder causing progressive chorioretinal atrophy. Affected patients develop night blindness with progressive peripheral vision loss and eventual blindness. Herein, we report two Korean families with choroideremia. Multimodal imaging studies showed that the probands had progressive loss of visual field with characteristic chorioretinal atrophy, while electroretinography demonstrated nearly extinguished cone and rod responses compatible with choroideremia. Sanger sequencing of all coding exons and flanking intronic regions of the *CHM* gene revealed a novel small deletion at a splice site (c.184_189+3delTACCAGGTA) in one patient and a deletion of the entire exon 9 in the other. This is the first report on a molecular genetic diagnosis of choroideremia in Korean individuals. Molecular diagnosis of choroideremia should be widely adopted for proper diagnosis and the development of new treatment modalities including gene therapy.

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INTRODUCTION

Choroideremia is a rare X-linked disorder causing progressive degeneration of the retina, retinal pigment epithelium (RPE), and choroid [1-3]. Affected male patients develop night blindness with progressive peripheral vision loss and central vision loss, usually observed later in their lives [4]. Female carriers may be

asymptomatic but can demonstrate patchy chorioretinal atrophy [5]. *CHM* was identified in the 1990s as a gene responsible for choroideremia. *CHM* encodes Rab Escort Protein-1 (REP-1), which facilitates posttranslational modification of Rab proteins regulating intracellular trafficking [6-8]. Various types of mutations in *CHM* have been identified including small deletions, non-sense mutations, missense mutations, frameshift mutations, splice

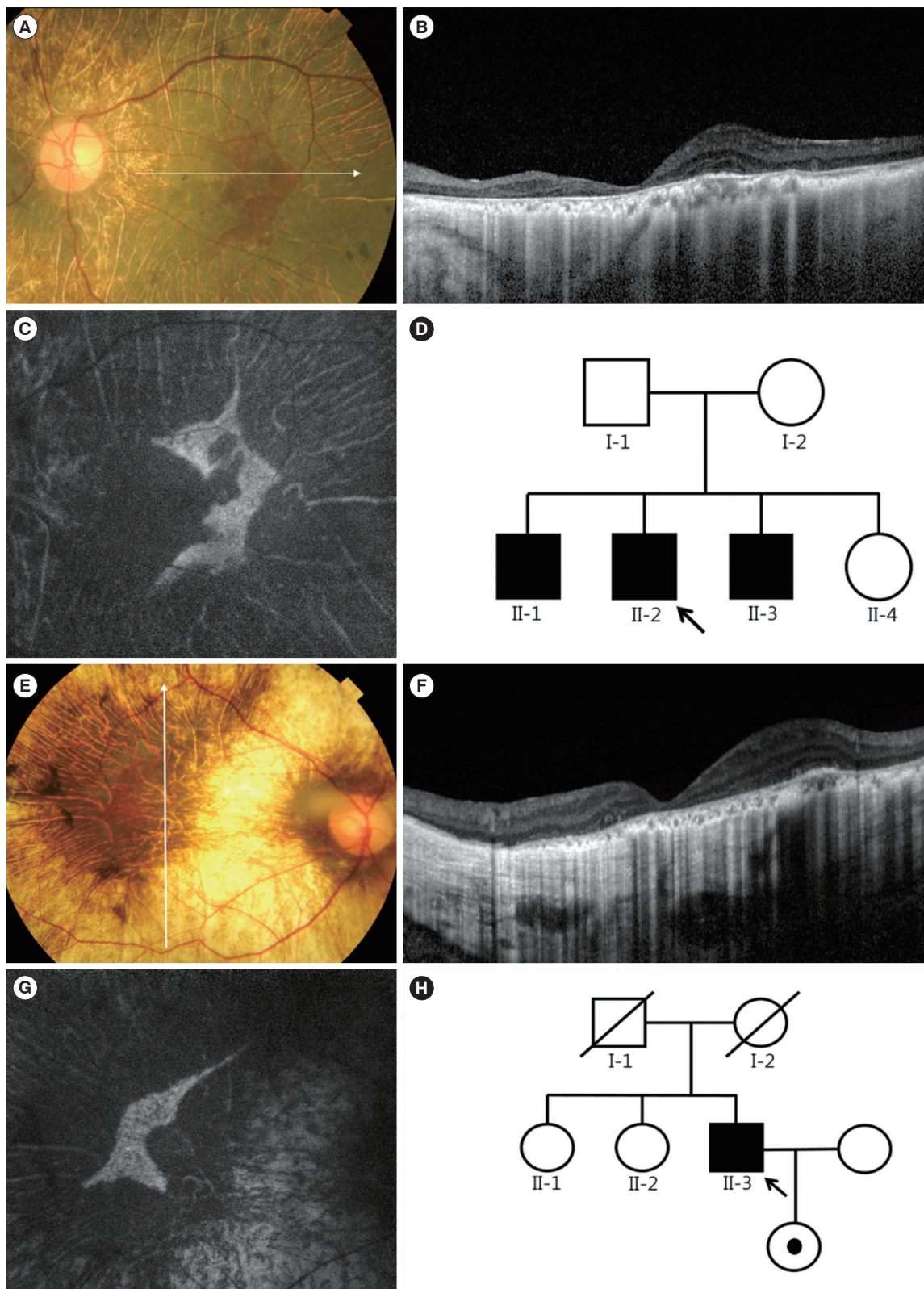


Fig. 1. Ocular phenotypes exhibited by the probands (indicated by arrows) and the pedigrees of family A (A-D) and family B (E-H). (A, E) Fundus photograph. (B, F) Spectral-domain optical coherence tomography. (C, G) Fundus autofluorescence photograph. (D, H) Family pedigree.

site defects, and deletion of the entire gene [9]. These mutations cause truncation, loss of functional domain, or absence of REP-1 [10]. In Korea, there has been no report on genetic diagnosis of choroideremia, although a few cases of clinical diagnosis of choroideremia have been reported [11, 12]. The purpose of this study is to report the first molecular diagnosis of choroideremia in two Korean families, one of which had a novel *CHM* mutation.

CASE REPORT

In family A, the proband was a 45-yr-old man complaining of night blindness and visual field defect with decreased visual acuity. His uncorrected visual acuity was 20/30 in the right eye and hand motion in the left eye. The proband had been taking immunosuppressant medication subsequent to undergoing kidney transplantation because of chronic glomerulonephritis. In addition, he underwent cataract surgery for posterior subcapsular opacity in both eyes eight years ago. The fundus exam showed bilateral chorioretinal atrophy and areas of RPE disruption with sparing of the central macula (Fig. 1A-D). The residual RPE tissue appeared as a well-demarcated hyperfluorescent area in fundus autofluorescence (FAF) photographs. Standard electroretinograms showed almost extinguished cone and rod responses. An automated visual field test showed a severely constricted visual field in both eyes. Spectral domain optical coherence tomography (SD-OCT) scans showed retinal thinning, choriocapillary atrophy, and abrupt transition to atrophic areas. Increased loss of outer nuclear layer and collapse of outer retina were observed compared to SD-OCT images taken five years ago. The proband's elder brother showed similar symptoms with severe vision loss, which was considered as legal blindness; the ocular phenotype was highly suggestive of choroideremia. In family B, the proband was a 41-yr-old man who was referred to the retina clinic with night blindness and visual field defect in both eyes. His best-corrected visual acuity was 20/40 in the right eye and 20/30 in the left eye. Prior to receiving refractive surgery, his eyes were highly myopic (-10 diopters in both eyes). The findings of the fundus exam, FAF, and SD-OCT scans were analogous to those of obtained for proband A (Fig. 1E-H).

To confirm choroideremia in the probands of family A and family B, genetic analysis of *CHM* was performed after obtaining informed consent. Genomic DNA was extracted from peripheral blood leukocytes by using the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Fifteen coding exons and their flanking introns were PCR amplified, and the resulting amplicons were sequenced

Table 1. Primers used for sequencing analysis of the *CHM* gene

Primer name	Primer sequence 5'-3'	Amplicon size (bp)
CHM_e01F	agcctggaaaatgagtcgag	414
CHM_e01R	ggagttggcagttacaggga	
CHM_e02F	agcaaggatgggtctctttg	334
CHM_e02R	gttagaagaagatcggagttgtt	
CHM_e03F	ccacttatgtgagcctcca	339
CHM_e03R	gcttcacctgtaacacagatt	
CHM_e04F	ttcttgggtactctgaggtga	396
CHM_e04R	cgtaatatgctggtttgcc	
CHM_e05F	tgagtcacataagcaaacgtaca	578
CHM_e05R	tgagatgcagaacattgttttg	
CHM_e06F	tcaattctgagcctgtaatagattgt	400
CHM_e06R	taaattccagtcctccgtgg	
CHM_e07F	actgatggacggtgatgtga	396
CHM_e07R	tctgcactatcaataggttagcca	
CHM_e08F	cctttgtgaggtctgtgaaca	499
CHM_e08R	acctacctatctaccacctaagtga	
CHM_e09F	tgccctctgagagattttaatactatg	399
CHM_e09R	acacacacacatcccacaaaca	
CHM_e10F	gaaaacatggaattgtaggcaag	395
CHM_e10R	ggctctggttttagggaagcc	
CHM_e11F	tttcatgagccaaggaaaga	378
CHM_e11R	tttttggtgagaaacacttaaga	
CHM_e12F	tgtttcaaatctgttccaaaa	431
CHM_e12R	tcatttcacaccatcccctt	
CHM_e13F	aacaaatgttgaaccaccatga	391
CHM_e13R	tgtctgcctaaacatgtggg	
CHM_e14F	acatacgaagctctgatttct	400
CHM_e14R	gcattctctcagtagtaccattctg	
CHM_e15F	acggaagtcatgtattctgattaag	491
CHM_e15R	tcacaaagggtatttcctt	

by using an ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with primers described in Table 1. Targeted sequencing of candidate retinal degeneration genes (including 98 known genes associated with inherited retinal degeneration) was also performed for proband B by using an Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA) with 101-bp paired-end reads.

A 9-bp deletion in exon 3 and adjacent intron sequences (c.184_189+3delTACCAGGTA; Fig. 2A) was identified in the proband of family A. The exon 9 PCR product was not detected in the proband of family B, indicating exon 9 deletion (Fig. 2B).

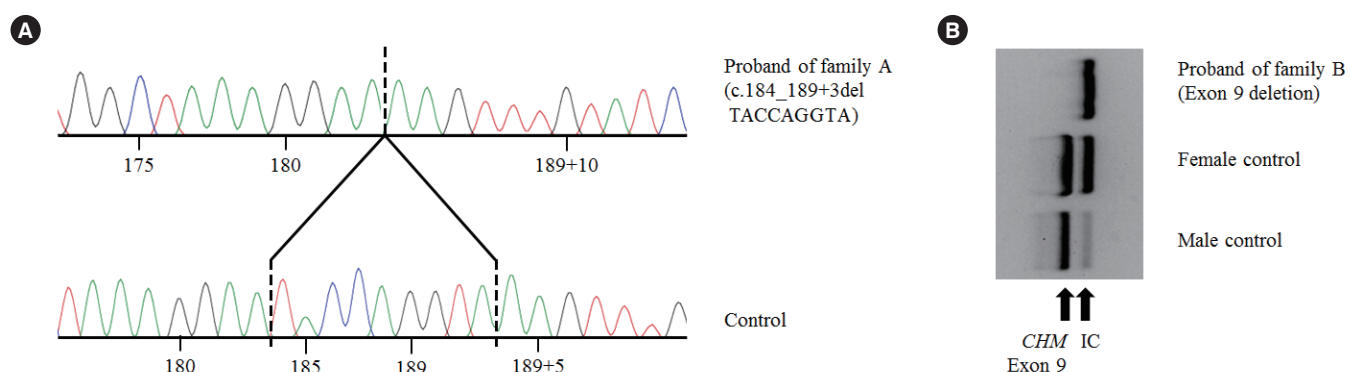


Fig. 2. *CHM* variants identified in the probands of family A and family B. (A) Chromatogram of c.184_189+3delTACCAGGTA (p.Tyr62_Gln-63del) detected in the proband of family A. (B) PCR products of exon 9. Exon 9 deletion was detected in the proband of family B.

Sequencing analysis of other amplified products did not uncover any pathogenic variants; targeted sequencing of 98 candidate retinal degeneration genes in proband B did not reveal any suspicious variations. However, exon 9 of *CHM* was not captured at all, indicating exon 9 deletion (see Supplemental data Figure S1). No genetic study of proband B's daughter has been performed; however, she is an obligate female carrier of the *CHM* exon 9 deletion, which is inherited in an X-linked recessive manner.

DISCUSSION

We present two Korean families with choroideremia diagnosed by sequencing *CHM*. One patient showed a novel small deletion at an exon/intron boundary, and the other revealed a full deletion of exon 9, a known mutation in choroideremia. The novel small deletion variant involves canonical ± 1 or 2 splice sites, which have been predicted to lead to a null effect and hypothesized to cause disease in choroideremia because the known *CHM* disease mechanism is loss-of-function. This variant is not present in the data from 622 Korean control exomes or approximately 8,600 East Asian alleles from the Exome Aggregation Consortium (ExAC) [13, 14]. According to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) 2015 guidelines [15], this variant is classified as a likely pathogenic variant based on one very strong piece of evidence (PVS1) and one moderate piece of evidence (PM2). However, an additional family study verifying cosegregation or functional analysis by RNA is needed to confirm the pathogenicity of this variant. Exon 9 deletion has been reported in several choroideremia families and patients [9, 16]. Deletion of exon 9 encompassing nucleotides 1,167 to 1,244

has been predicted to cause a frameshift, leading to a null variant. This deletion is not present in the data from 622 Korean control exomes or approximately 8,600 East Asian alleles from ExAC. Therefore, exon 9 deletion is classified as a pathogenic variant according to the ACMG-AMP 2015 guidelines.

Patients with choroideremia usually show characteristic retinal and choroidal features. However, at times it is difficult to clinically differentiate choroideremia from other inherited retinal degenerations including retinitis pigmentosa and cone-rod dystrophy [17, 18]. Therefore, a number of researchers have advocated next generation sequencing (NGS)-based approaches and have reported cases of successful diagnosis of choroideremia [17]. However, because choroideremia is often caused by large deletions in *CHM*, NGS alone may not enable a proper molecular diagnosis for a considerable number of patients with choroideremia. Therefore, when there is a clinical suspicion of choroideremia, a combined molecular genetics approach including direct *CHM* sequencing, multiplex ligation-dependent probe amplification, and RNA (cDNA) sequencing, as well as NGS-based methods should be considered [19].

In summary, we performed molecular diagnosis of choroideremia in two unrelated Korean families. To the best of our knowledge, this is the first report on the molecular genetic diagnosis of choroideremia in Korean individuals. This study will expand the mutational spectrum of *CHM* and may help in the development of new treatment modalities such as gene therapy.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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