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Novel *GUCA1A* mutation identified in a Chinese family with cone-rod dystrophy

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HIGHLIGHTS

- A novel *GUCA1A* mutation detected in one of 130 families with CORD.
- Associated phenotypes were presented.
- All reported *GUCA1A* mutations were summarized.

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ABSTRACT

Ten mutations in the guanylate cyclase activator 1A (*GUCA1A*) have been previously identified and reported in patients with retinal degeneration, including patients from 12 families with cone-rod dystrophy (CORD) and in an isolated patient with retinitis pigmentosa (RP). In this study, the coding exons and adjacent regions of *GUCA1A* were evaluated in 130 probands with CORD from 130 unrelated Chinese families using Sanger sequencing. A novel heterozygous c.464A>C (p.Glu155Ala) mutation was detected in a proband from a large family. The mutation presented in all nine patients examined in that family, but it was absent in six unaffected family members and 192 normal controls. All the nine patients in that family expressed typical CORD in eight cases and atypical CORD in one case. The results of this study suggested that the *GUCA1A* mutation only contributes to a small portion of CORD in people of Chinese descent.

1. Introduction

Cone-rod dystrophy (CORD) is a form of heterogeneous hereditary retinal degeneration with primary cone dysfunction and milder or subsequent rod involvement [6]. Mutations in at least 26 genes have been reported to be responsible for autosomal dominant (adCORD, 10 genes), autosomal recessive (arCORD, 14 genes), or X-linked (xlCORD, 2 genes) CORD, respectively (RetNet: https://sph.uth.edu/Retnet/sum-dis.htm) [3,5,8,13,19,24]. Analysis of multiple genes suggested that mutations in these genes may explain about 48% of the autosomal dominant CORD [13] and 40% of the autosomal recessive CORD [3]. Analysis of individual genes revealed that mutations in *GUCY2D* (23% adCORD) [13], *GUCA1A* (8% adCORD) [12,13], or *ABCA4* (65% arCORD) [3,14] are the relatively common causes of CORD.

GUCA1A encodes guanylyl cyclase activating protein 1 (GCAP1), which plays an important role in the recovery of retinal photoreceptors from photobleaching [17,18]. *GUCA1A* was localized at 6p21.1 [22], contained four coding exons, and encoded 201 residues. To date, 10 variants in *GUCA1A* have been reported [10–12,16,20,25]. Only nine mutations in GUCA1A have demonstrated to cause CORD so far. There is no solid evidence supporting another variant, THr114Ile, is a causative variant. However, analysis of *GUCA1A* mutations has not been reported in people of Chinese descent. In this study, the *GUCA1A* mutation was screened in 130 probands with CORD from unrelated Chinese families. A novel *GUCA1A* mutation was identified in a large Chinese family with autosomal dominant CORD.

2. Materials and methods

2.1. Patients

One-hundred-thirty unrelated probands with CORD were recruited for this study at the Pediatric and Genetic Eye Clinic, Zhongshan Ophthalmic Center, Guangzhou, China. The entire process was in compliance with the guidelines of the Declaration of

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Table 1
Sequences of primers used to amplify the coding regions of GUCA1A.

Primer name	Primer sequence (5′-3′)	Size of amplicons	Annealing temperature (°C)
GUCA1A-EX1-582-F	TCTGGCATCTGTGAGTTTG	582	56
GUCA1A-EX1-582-R	ACCCACCTATCAAATAAACA		
GUCA1A-EX2-582-F	GTGGATGCTGGCTTATTAGAT	582	57
GUCA1A-EX2-582-R	CAAGGAAGGGAAGGAACG		
GUCA1A-EX3-442-F	TGGCCTCAGTTTCCTCATCAATAC	442	64
GUCA1A-EX3-442-R	GGGCAGAGGCAGGTGGAGGAAGT		
GUCA1A-EX4-666-F	TGCCCCAGCCACAAAGTT	666	64
GUCA1A-EX4-666-R	GGGAAAAGCTGGGAGAGGAGTCA		

Helsinki and the Guidance of Sample Collection of Human Genetic Diseases (863-Plan) of the Ministry of Public Health of China. All participations or their guardians provided informed consent prior to the collection of clinical data and genomic samples. This study was approved by the Institutional Review Board of Zhongshan Ophthalmic Center.

2.2. Detection of GUCA1A mutation

Genomic DNA was extracted from venous blood leukocytes using the reported methods [23]. Four pairs of primers, used to amplify the four coding exons and the adjacent regions of *GUCA1A*, were designed (Table 1) (reference sequence from the NCBI: NC_000006.11 for gDNA, NM_000409.3 for mRNA, and NP_000400.2 for protein). Touch-down polymerase chain reaction (PCR) was used to amplify the sequences [26] (Table 1). The amplicons were purified and then analyzed with the ABI BigDye Terminator Cycle Sequencing Kit, v3.1, using an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). The sequences of the CORD patients were compared to the *GUCA1A* consensus sequences from the NCBI database, using the SeqManII assembly program (DNAstar, Madison, WI) to find the variants. Every variant was bidirectionally sequenced and verified in 192 normal controls. The description of the variants was in accordance with nomenclature for the description of sequence variants (HGVS: http://www.hgvs.org/mutnomen/) [2]. The effect of a missense mutation on the encoded protein was predicted using the PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/) online tools [1,15].

3. Results

3.1. Genetic analysis

Upon completion of the sequencing analysis for the four coding exons and the adjacent regions of *GUCA1A*, a novel c.464A>C (p.Glu155Ala) mutation in exon 4 was identified in one of the 130 probands, who came from a large family with four generations of CORD (Fig. 1). This mutation cosegregated with the disease in the family and was absent in 192 normal controls. Further analysis demonstrated that the residue with the mutation was evolutionarily highly conserved (Fig. 1). The mutation was predicted to be pathogenic using PolyPhen-2 and SIFT. Linkage analysis of the mutation yielded a positive LOD score of 2.27 at theta = 0, which is close to the theoretical maximum LOD score for this type of family.

3.2. Clinical information about the family

The disease was transmitted in the family as an autosomal dominant trait. In this family, patients with the c.464A>C

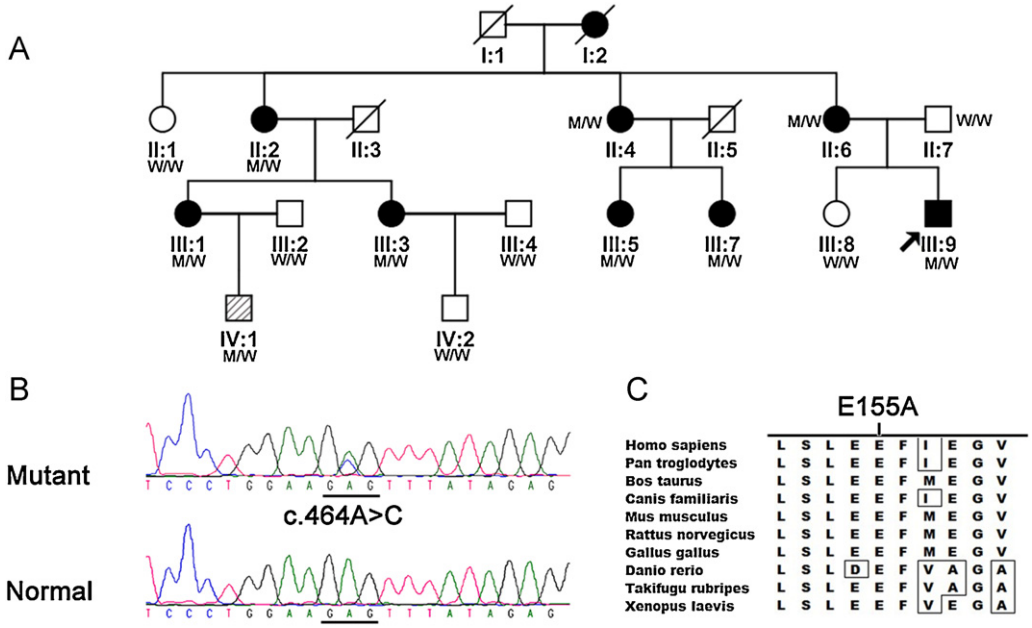

Fig. 1. Pedigree and *GUCA1A* mutation. (A) The blackened symbols indicate affected individuals and the open symbols indicate unaffected individuals. Individual IV:1 (17-years-old) showed mild reduced visual acuity, high myopia, attenuated retinal artery, and color vision defects, but without photophobia and typical macular atrophy; therefore, a different symbol was used. Under each individual, M represents the allele with the p.Glu155Ala mutation while W indicates a wild allele. (B) Sequence chromatography of *GUCA1A* fragments revealed a novel heterozygous c.464A>C (p.Glu155Ala or p.E155A) mutation in a patient when compared with a normal sequence. (C) Protein sequence alignment of 10 *GUCA1A* orthologs demonstrated that the residue at 155 is highly conserved.