—Case Reports—

Novel *GUCY2D* Variant (E843Q) at Mutation Hotspot Associated with Macular Dystrophy in a Japanese Patient

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Background: The *GUCY2D* (guanylate cyclase 2D) gene encodes a photoreceptor guanylate cyclase (GC-E), that is predominantly expressed in the cone outer segments. Mutations in the *GUCY2D* lead to severe retinal disorders such as autosomal dominant cone-rod dystrophy (adCRD) and autosomal recessive Leber congenital amaurosis type 1. The purpose of this study was to identify the phenotype of a Japanese patient with a probably pathogenic *GUCY2D* variant.

Methods: Detailed ophthalmic examinations were performed, and whole exome sequencing was performed on DNA obtained from the patient. The variants identified by exome sequencing and targeted analysis were further confirmed by direct sequencing.

Results: A 47-year-old man had atrophic and pigmentary changes in the macula of both eyes. Amplitudes and implicit times on full-field electroretinograms (ERGs) were within normal limits; however, the densities of multifocal ERGs in the central area were reduced in both eyes. Whole exome sequencing identified heterozygous variant c.2527G>C, p.Glu843Gln in the *GUCY2D* gene within the mutation hot spot for adCRD. The allelic frequencies of this variant are extremely low and, according to American College of Medical Genetics and Genomics standards and guidelines, the variants are classified as likely pathogenic.

Conclusions: This is the first report of a heterozygous variant, c.2527G>C, p.Glu843Gln, in the *GUCY2D*, in a patient presenting with mild macular dystrophy without a general reduction in cone function. Our findings expand the spectrum of the clinical phenotypes of *GUCY2D*-adCRD and help clarify the morphological and functional changes caused by defects of dimerization of GC-E in the phototransduction cascade. (J Nippon Med Sch 2020; 87: 92–99)

Key words: *GUCY2D*, cone rod dystrophy, ERG, adCRD, hotspot

Introduction

The *GUCY2D* (guanylate cyclase 2D: OMIM 600179) gene encodes the photoreceptor guanylate cyclase (GC-E; also known as RETGC1), which is expressed predominantly in the cone outer segments[1,2]. GC-E is a key enzyme in phototransduction and has a role in restoring cytoplasmic cGMP to the dark state of the photoreceptors. Synthesis of cGMP by GC-E is regulated by guanylate cyclase-activating proteins (GCAPs), which in turn are activated by decreasing Ca2+ concentrations in the cell[3–5]. GC-E contains 5 functional domains: an extracellular domain, a hydrophobic transmembrane domain, a kinase-like domain, a dimerization domain, and a catalytic domain[6].

Mutations in the *GUCY2D* lead to inherited retinal disorders such as autosomal dominant cone-rod dystrophy

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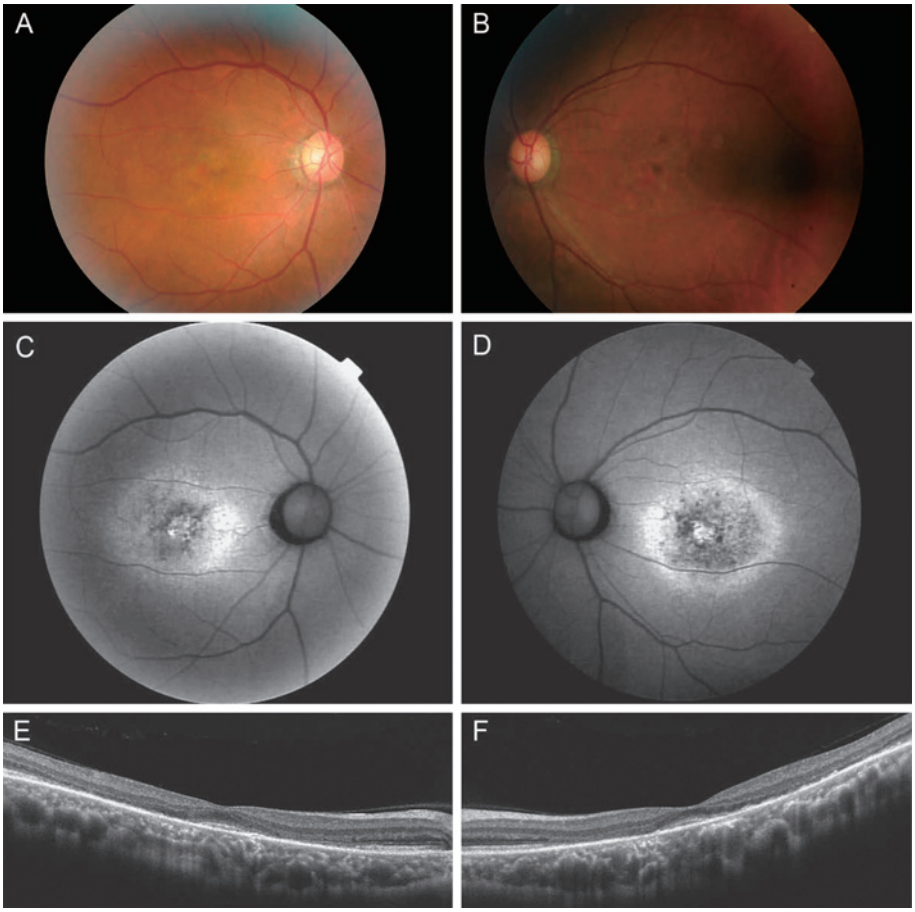

Fig. 1 Fundus photographs and autofluorescence and SD-OCT images of a patient with macular cone dystrophy caused by a variant in the *GUCY2D* gene.
Fundus photographs (A, B), fundus autofluorescence images (C, D), and SD-OCT images of the patient are shown.

(adCRD) and autosomal recessive Leber congenital amaurosis type 1 (arLCA1)[7,8]. *GUCY2D* mutations are the main cause of adCRD[9,10]. In eyes with cone-rod dystrophy (CRD), degeneration begins in the cones, which leads to loss of the central visual field. The LCA1 phenotype appears to be more severe and is associated with loss of photoreceptor function and blindness early in life[7]. Although more than 100 mutations in the *GUCY2D* have been described, most functional studies have focused on mutations in the dimerization domain (DD) of GC-E, which harbors a so-called "mutation hotspot"[3,11–13]. The purpose of this study was to determine the characteristics of a Japanese patient with a novel *GUCY2D* variant within this hotspot.

Materials and Methods

The protocol of this study conformed to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of Nippon Medical School. A signed written informed consent was obtained from the patient after the nature and possible complications of the study were explained.

The ophthalmological examinations included measurement of best-corrected visual acuity (BCVA) and refractive error (spherical equivalent), slit-lamp biomicroscopy, ophthalmoscopy, color fundus photography, fundus autofluorescence (FAF) imaging, spectral domain optical coherence tomography (SD-OCT; Zeiss cirrus HD-OCT), Goldmann kinetic perimetry, and recordings of full-field electroretinograms (ffERGs), and multifocal ERGs (mfERGs). Full-field ERGs were recorded by using an extended testing protocol conforming to the International Society for Clinical Electrophysiology of Vision standards.

Blood samples were collected from the patient, and genomic DNA was isolated from peripheral white blood cells using a blood DNA isolation kit (NucleoSpin Blood XL; Macherey Nagel, Germany). Whole exome sequencing (WES; Macrogen Japan) and targeted analyses were done in accordance with previously described methods[14].

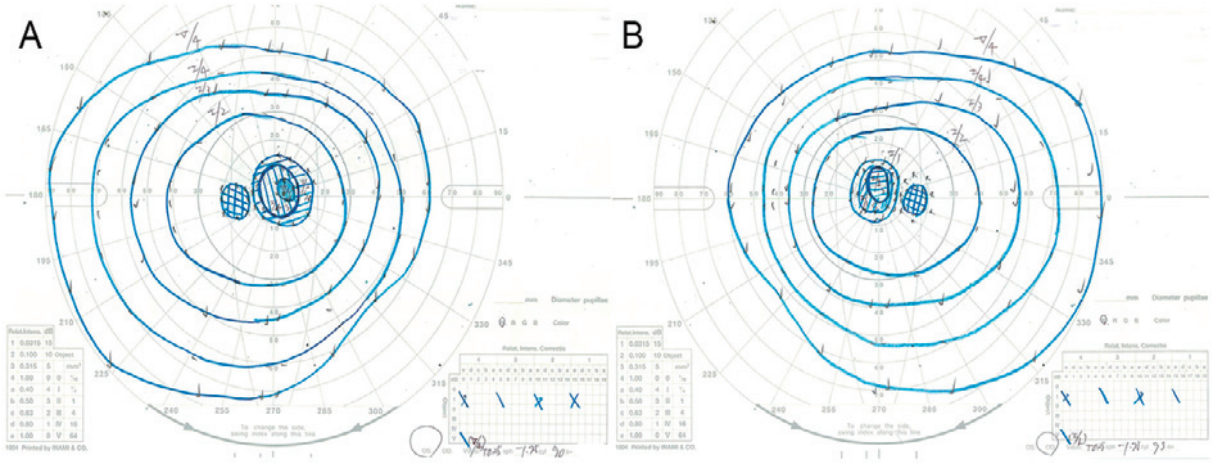

Fig. 2 Results of a Goldmann kinetic visual field test of a patient with a *GUCY2D* variant.
Results of Goldmann kinetic visual field testing of the left (A) and right (B) eyes of the patient are shown.

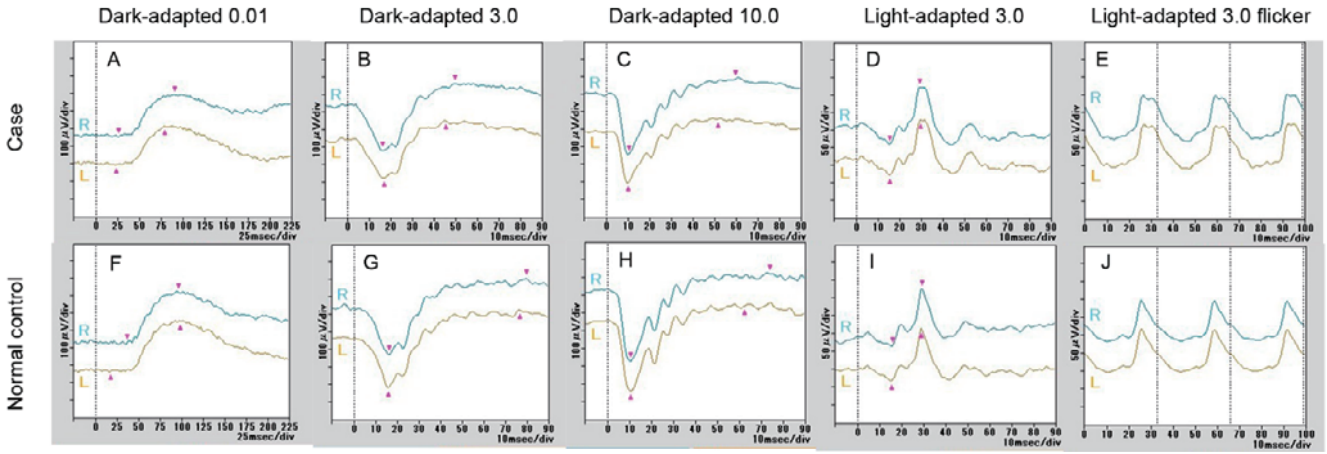

Fig. 3 Full-field electroretinograms (ERGs) of a patient with a *GUCY2D* variant.
Full-field ERGs recorded from the patient (A-E) and a normal control (F-J) are shown. Dark-adapted 0.01 (A, F), dark-adapted 3.0 (B, G), dark-adapted 10.0 (C, H), light-adapted 3.0 (D, I), and light-adapted 3.0 flicker ERGs (E, J) are shown.

The total number of read bases was 8.10 Gbp, and the percentage of >20× coverage was 97.4%. All called SNVs and INDELs of the 271 genes registered as retinal disease-causing genes on the RetNet database were selected for analysis (https://sph.uth.edu/retnet/home.htm). The identified variants were filtered with an allele frequency of less than 0.1% in the Human Genetic Variation Database (HGVD, http://www.genome.med.kyoto-u.ac.jp/SnpDB/), Integrative Japanese Genome Variation (iJGVD 3.5k, https://ijgvd.megabank.tohoku.ac.jp/download_3.5kjpn/) which are two allele frequency databases specific for the Japanese population, and gnomAD database (https://gnomad.broadinstitute.org/). A detected variant was analyzed with 3 different prediction programs: SIFT (https://www.shift.co.ul/), PROVEAN (http://provean.jcvi.org/index.php), and Polyphen 2 (http://genetics.bwh.harvard.edu/pph2/). Pathogenic classification of all detected variants was performed in accordance with the guidelines of the American College of Medical Genetics and Genomics (ACMG)[15].

GUCY2D variants identified by exome sequencing and targeted analysis were further confirmed by direct sequencing. The identified regions were amplified by polymerase chain reaction (PCR). PCR products were purified (ExoSAP-IT; USB Corp., USA) and used as the template for sequencing. Both strands were sequenced on an automated sequencer (Eurofins Genomics; JAPAN).

Results

Clinical Findings

A 47-year-old man reported a gradual decrease of vision in his left eye, which he first noticed at age 42 years. Age-related macular degeneration was diagnosed by a local doctor. Later, the patient developed macular dystro-

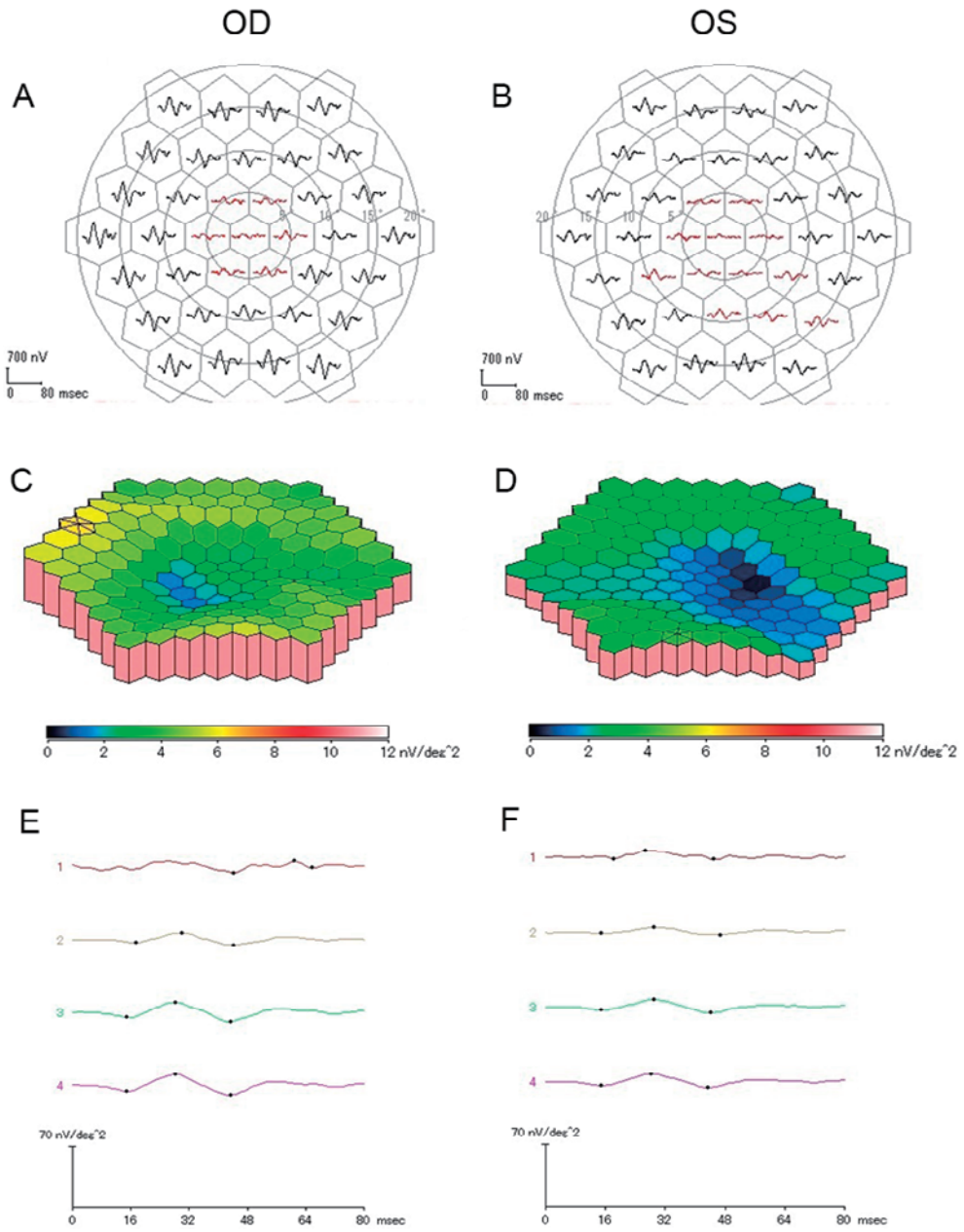

Fig. 4 Multifocal ERGs (mfERGs). The mfERGs (A, B), topographic map (C, D), and average densities of the rings of the mfERGs (E, F) of the patient are shown.

phy in the right eye and was referred to our hospital. Our examination showed a decimal BCVA of 1.0 in the right eye and 0.3 in the left eye, without obvious changes in the anterior segment or lens. His refractive error (spherical equivalent) was −2.0 diopters (D) in both eyes. Ophthalmoscopy showed atrophic and pigmentary changes in both macula (**Fig. 1A, 1B**). Fundus autofluorescence (FAF) showed symmetrical speckled Bull's eye-like hyperfluorescent macular lesions in both eyes but no remarkable findings outside the vascular arcade (**Fig. 1C, 1D**). SD-OCT images showed thinning of the outer nuclear layer (ONL) in the parafoveal area of both eyes. The ONL, ellipsoid zone, and interdigitation zone were more disrupted in the parafoveal area than in the foveal area and the peripheral retina (**Fig. 1E, 1F**). The visual fields were full on Goldman perimetry, and a relative reduction in central sensitivity was detected in both eyes (**Fig. 2**). The amplitudes and implicit times of the full-field ERGs of the patient were within normal limits (**Fig. 3**). The densities of the mfERGs in the central area were reduced in both eyes (**Fig. 4**).

Molecular Genetic Analyses

WES analyses of DNA samples from the patient showed a heterozygous variant, c.2527G>C, p.Glu843Gln, in the *GUCY2D*. No other variants of retinal disease-associated genes were detected. The allelic frequency of

Table 1 Results of analysis of in silico allele frequency, prediction, and pathogenicity of an identified GUCY2D variant

			Position	Allele frequency			Functional prediction				ACMG Classification			
No.	HGVS.c	HGVS.p	Chr	GRCh37	HGVD	iJGVD 3.5k	gnomAD East Asian Total	SIFT Prediction Score	PROVEAN Prediction Score	Polyphen2 HDIV_pred	Polyphen2 HDIV_score	Verdict	Identified classification rules	
V1	c.2527 G>C	p.Glu843Gln	17	7,918,033	0.000%	0.000%	0.0108%	0.00079%	Dam-aging	0.008	Deleteri-ous	−2.86	Damaging	1.0	Likely Patho-genic	PM1 PM2 PP3 PP4

NM_000180.3

this variant is extremely low in the iJGVD3.5k, HGVD, and gnomAD databases (**Table 1**). The variant was verified by Sanger sequencing (**Fig. 5C**). According to ACMG standards and guidelines, the variant is classified as PM1 (located in a mutational hotspot and/or critical and well-established functional domain without a benign variation), PM2 (absent from controls or at extremely low frequency), PP3 (multiple lines of computational evidence support a deleterious effect on the gene or gene product), and PP4 (patient's phenotype or family history is highly specific for a disease with a single genetic etiology), and the criterion will be likely pathogenic (**Table 1**). We asked the patient about his family history, and he reported that both his parents had no visual symptoms without glasses. He also reported that he had a younger brother and a son, both of whom had no visual symptoms. We asked him to bring his family to our hospital; however, he declined. From the results of the family history, we could not completely exclude the possibility that the variant found in this patient was a de novo variant.

Discussion

More than 150 *GUCY2D* mutations have been reported[3,16]. A genotype-phenotype correlation analysis of *GUCY2D* revealed that the vast majority of mutations caused arLCA1, while 13 mutations were reported to cause adCRD[3]. LCA-related mutations are usually recessive and null (mainly frameshift, non-sense, and splicing mutations), and they can affect all domains of the GC-E. CRD mutations are mainly dominant missense mutations clustered in a "hotspot region" in the DD at positions between 837 and 849 (**Fig. 5A, 5B**)[3,11,12]. The importance of the DD as a regulatory module or signaling helix has been demonstrated in different guanylate cyclases, and mutations in this region affect GC-E activity[17,18]. A very short stretch of 2 amino acids (positions 837 and 838) is particularly striking, and all these mutations caused a decrease in the basal activity and affected Ca2+-sensitive regulation by GCAPs.

Most individuals who are heterozygous for dominant mutations in *GUCY2D* are clinically diagnosed with cone dystrophy or CRD, while in a few cases the diagnosis was determined as macular degeneration[13]. However, the clinical characteristics of these 3 phenotypes overlap and they might just represent different stages of the same disorder.

Some clinical features were reported to be common in patients with heterozygous dominant *GUCY2D* mutations: disease onset is during childhood and vision dete-

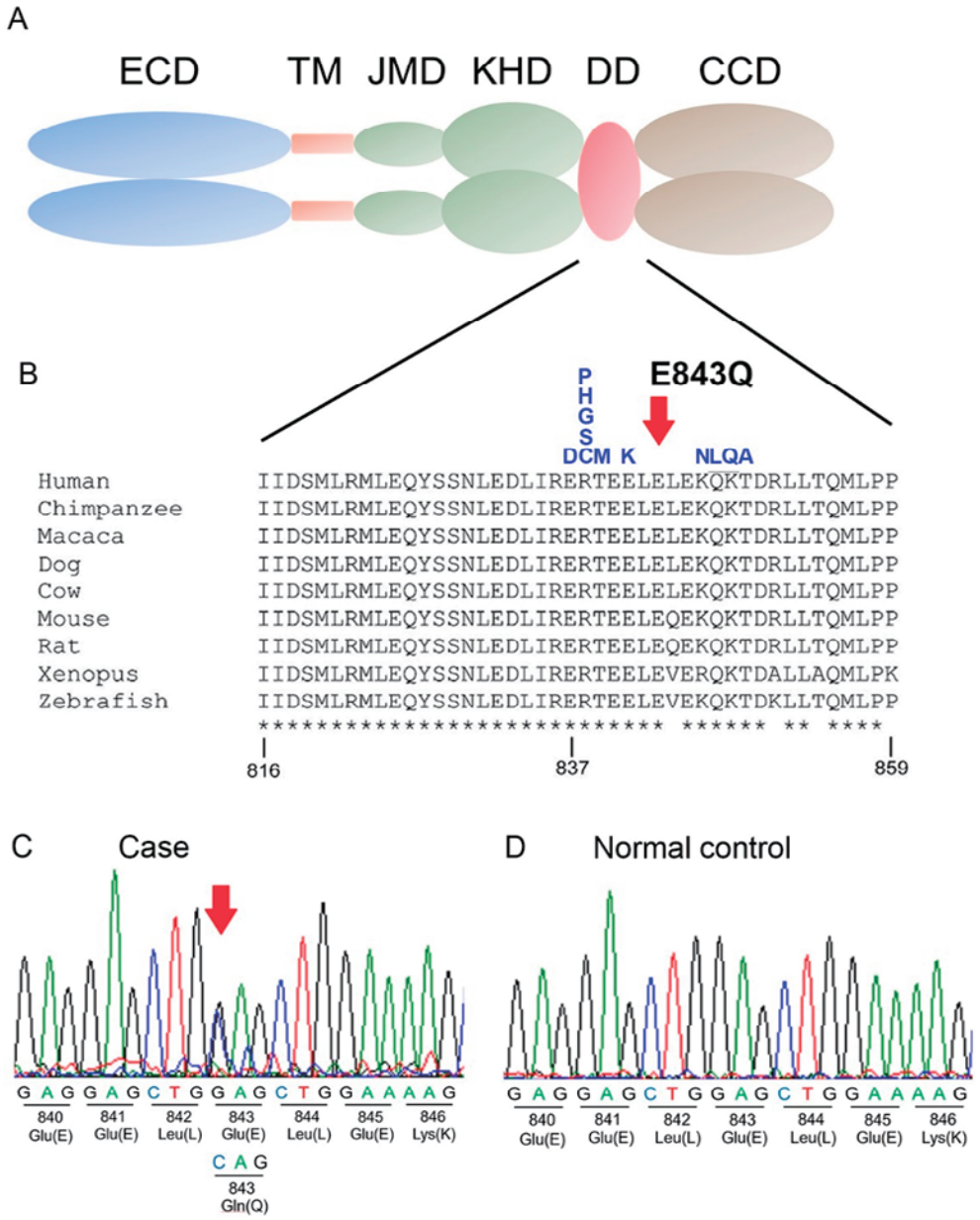

Fig. 5 The scheme of domain structure of GC-E and molecular genetic findings of the patient. The scheme of domain structure of GC-E is shown (A). (Abbreviations: ECD, extracellular domain; TM, transmembrane domain; JMD, hydrophobic transmembrane domain; KHD, kinase-like domain; DD, dimerization domain; and CCD, catalytic domain.) Amino acid (AA) sequence alignments of DD of GC-E (AA 816-859 in human) from 9 species are shown (B). AA residues of the DD domain are well conserved in these species. Previously reported AA changes associated with cone-rod dystrophy are shown in blue letters above the sequence. Red arrow indicated the AA residue mutated in the patient. Sequence chromatograms for Case 1 (top) and the normal control (bottom) are shown. Case 1 had c.3596 C>G variant in exon 4.

riorates to very low levels by the fifth decade of life; fundus appearance is initially normal in the periphery but the macula is usually abnormal, with large variations among the patients; central or paracentral scotoma with normal peripheral field of vision is present; and cone function is reduced, with normal or abnormal rod function[3,8,13].

The fundus appearance and visual fields in our patient were similar to those of patients with typical *GUCY2D*-adCRD. However, onset of symptoms was later in our patient than in typical *GUCY2D*-adCRD, and although the central sensitivities of the cone responses were reduced, no general cone dysfunction was observed.

The delayed age at onset and broad spectrum in the severity of clinical phenotypes could also be attributable to the following mechanisms[3]. Because GC-E is active in

dimer form (**Fig. 5A**), a few scenarios for homodimer versus heterodimer formation have been proposed. CRD mutations are missense and produce mutated proteins affecting the function of the wild-type (wt) proteins. In affected individuals with heterozygous adCRD mutations, both wt and mutant (mut) alleles produce 50% of the total protein. Upon dimerization, 25% wt-wt dimers, 50% wt-mut dimers, and 25% mut-mut dimers can be produced. Although wt-wt dimers are fully active, wt-mut dimers and mut-mut dimers have a different Ca2+ sensitivity profile. This combination of wt and mut dimers might result in the slow progressive nature of the disease over time. Because amino acid 838 is known to be at the most sensitive position and the effects of missense mutations in other parts of DD are less severe than that of 838. Thus, a variant at amino acid position 843 might have milder effects on mut-mut dimers, which could lead to later onset and a milder phenotype[19]. However, this hypothesis is speculative and needs to be confirmed by functional examinations.

This study has limitations. This is the first report of the characteristics of a patient with a heterozygous variant, p.Glu843Gln, in the *GUCY2D*. The patient presented with macular dystrophy without a general reduction in cone function. However, this is only one case, and we were unable to conduct segregation analysis in the family. Analysis of a larger number of patients, with different *GUCY2D* mutations within the mutation hotspot, would facilitate comprehensive characterization of the genotype-phenotype relationships of *GUCY2D*-adCRD.

In conclusion, our findings have expanded the spectrum of *GUCY2D*-adCRD clinical phenotypes and will help identify the morphological and functional changes caused by the defect of dimerization of GC-E in the phototransduction cascade.

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Conflict of Interest: Spouse of Dr. Kiyoko Gocho is Co-founder and CEO of Imagine eyes.

Other authors declare that they have no competing interests.

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