Novel GUCY2D variant (E843Q) at mutation hotspot
associated with macular dystrophy in Japanese patient

Running title: Novel GUCY2D mutated macular dystrophy

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Abstract

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 determine 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 the DNA obtained from the patient. The variants identified by exome sequencing and targeted analysis were further confirmed by direct sequencing.

Results: A 47-years-old man had atrophic and pigmentary changes in the macula of both eyes. The amplitudes and implicit times of the full-field electroretinograms (ERGs) were within normal limits, however the densities of the 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 was extremely low and according to ACMG standards and guidelines, the variants were classified as likely pathogenic.
Conclusions: This is the first report on the findings in a patient with a heterozygous variant, c.2527G>C, p.Glu843Gln in the GUCY2D, presenting with mild macular dystrophy without a general reduction of cone function. Our findings expand the spectrum of the clinical phenotypes of GUCY2D-adCRD and help identify the morphological and functional changes caused by defects of dimerization of GC-E in the phototransduction cascade.

Keywords: GUCY2D, cone rod dystrophy, ERG, adCRD, hotspot
The GUCY2D (guanylate cyclase 2D: OMIM 600179) gene encodes a photoreceptor guanylate cyclase (GC-E; also known as RETGC1) that is expressed predominantly in the cone outer segments\(^1,2\). GC-E represents a key enzyme in the phototransduction process and plays a role in the restoration of cytoplasmic cGMP to the dark state of the photoreceptors. The synthesis of cGMP by GC-E is regulated by guanylate cyclase-activating proteins (GCAPs) which in turn are activated by decreasing Ca\(^{2+}\) 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 (adCRD) and autosomal recessive Leber congenital amaurosis type 1 (arLCA1)\(^7,8\). GUCY2D mutations are the major cause of adCRD\(^9,10\). In eyes with CRD, the degeneration begins in the cones which leads to a loss of the central visual field. The LCA1 phenotype appears to be more severe with a loss of photoreceptor function and blindness developing very early in life\(^7\). While more than a hundred mutations in the GUCY2D have been described, most functional studies
have focused on mutations in the dimerization domain (DD) of the GC-E which harbors a so-called “mutation hot spot”\textsuperscript{3,11-13}.

The purpose of this study was to determine the characteristics of a Japanese patient with a novel \textit{GUCY2D} variant within the hot spot.

**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 the 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 measurements of the 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 the full-field electroretinograms (ffERGs), and multifocal ERGs (mfERGs). The full-field ERGs
were recorded 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 the 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 according to described methods\textsuperscript{14}. The total read bases were 8.10 Gbp, and the percentage of >20x 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 the analyses (https://sph.uth.edu/retnet/home.htm). The identified variants were filtered with allele frequency of less than 0.1% of 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_3.5kijpn/) which are two allele frequency databases specific for the Japanese population, and gnomAD database (https://gnomad.broadinstitute.org/). A detected variant was analyzed with three different prediction programs; SIFT (https://www.shift.co.uk/), PROVEAN (http://provean.jcvi.org/index.php), and Polyphen 2 (http://genetics.bwh.harvard.edu/pph2/). A pathogenicity classification of all detected
variants was performed based on the guidelines of the American College of Medical Genetics and Genomics (ACMG)\(^n\).

The \textit{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). The 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).

\textbf{Results}

\textbf{Clinical findings}

A 47-years-old man reported a gradual decrease of vision in his left eye that he first noticed at 42-years-of-age. He was diagnosed with age-related macular degeneration by a local doctor. Later, he developed macular dystrophy in the right eye and was referred to our hospital. Our examinations showed that his decimal BCVA was 1.0 in the right eye and 0.3 in the left eye without obvious changes in the anterior segment and 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 demonstrated a thinning of the ONL in the parafoveal area of both eyes. The ONL, ellipsoid zone (EZ), and interdigitation zone (IZ) were more disrupted in the parafoveal area than in the foveal area and the peripheral retina (Fig. 1E, 1F). The visual fields were full by Goldman perimetry and a relative reduction of the 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 on the DNA samples of the patient showed a heterozygous variant, c.2527G>C, p.Glu843Gln, in the GUCY2D. No other variants of retinal disease-associated genes were detected except GUCY2D. The allelic frequency of this variant was 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 guideline, the variant was classified as PM1 (located in a mutational hot spot 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, 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 for his family history and he answered that both his parents had no visual symptoms without glasses. He also reported that he had a younger brother and a son who also had no visual symptoms. We asked the patient to bring his family to our hospital, however he was not willing to bring them. From the results of the family history, we could not completely eliminate the possibility that the variant found in this patient was a de novo variant.

**Discussion**

More than 150 GUCY2D mutations have been reported. A genotype-phenotype correlation analysis of GUCY2D revealed that the vast majority of mutations caused arLCA1 while 13 mutations were reported to cause adCRD. The 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. The CRD mutations are mainly dominant missense clustered in a “hot-spot region” in the DD at positions between 837 and 849 (Figure 5A, 5B). 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 the GC-E activity. A very short stretch of two amino acids (positions 837 and 838) is particularly striking and all of these mutations caused a decrease in the basal activity and affected the 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. However, these three phenotypes overlap in their clinical appearance and might just represent different stages of the same disorder.

Some clinical features were reported to be common in patients with heterozygous dominant GUCY2D mutations; the disease onset is during childhood with the vision decreasing to very low levels by the 5th decade of life, their fundus appearance is initially normal in the periphery but the macular is usually abnormal with large variations among the patients, presence of a central or paracentral scotoma with normal peripheral field of vision, and reduced cone function with normal or abnormal rod function.
The features in our patient that were similar to those of patient with typical GUCY2D-adCRD were the fundus appearance and visual fields. However, the onset of the symptoms in our patient was delayed compared to typical GUCY2D-adCRD, and a general cone dysfunction was not observed although the central sensitivities of the cone responses were reduced.

The delayed age of onset and a broad spectrum of the severity of the clinical phenotypes could also be explained by the following mechanisms. Because GC-E is active in the dimer form (Fig. 5A), a few scenarios for homodimer versus heterodimer formation are proposed. CRD mutations are missense and produce mutated proteins affecting the function of the wild type (wt) proteins. In affected individuals carrying heterozygous adCRD mutations, both the 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 made. While 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 the missense mutations in the other part of DD are less severe than that of 838. Thus, a variant in the amino acid position 843 might have milder effect on mut-
mut dimers and that might lead to a more delayed onset and milder phenotype\textsuperscript{19}.

However, this suggestion is very speculative and needs to be confirmed by functional examinations.

There are limitations in this study. This is the first report of the characteristics of a patient with a heterozygous variant, p.Glu843Gln in the \textit{GUCY2D}. The patient presented with macular dystrophy without a general reduction of cone function. But this is only one case. Segregation analysis in a family could not be obtained. A larger number of patients with different \textit{GUCY2D} mutations within the mutation hotspot will allow us to determine more detailed genotype-phenotype relationships of \textit{GUCY2D}-adCRD.

In conclusion, our findings have expanded the spectrum of the clinical phenotypes of \textit{GUCY2D}-adCRD, and they will help identifying the morphological and functional changes caused by the defect of dimerization of GC-E in the phototransduction cascade.
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Disclosure 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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Figure legends

Figure 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.

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

Figure 3. Full-field electroretinograms (ERGs) of patient with a GUCY2D variant. Full-field ERGs recorded from the patient (A-E) and normal control (F-J) are shown. The 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.
Figure 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.

Figure 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 of domains are shown: ECD, an 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 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.
Fig. 1
Fig. 2
Fig. 3

- Dark-adapted 0.01
- Dark-adapted 3.0
- Dark-adapted 10.0
- Light-adapted 3.0
- Light-adapted 3.0 flicker
Fig. 4
Table 1. Results of in silico allele frequency, prediction and pathogenicity analysis of an identified GUCY2D variant

| No. | HGVS.c | HGVS.p | Position | Allele frequency | Functional prediction | ACMG Classification |
|-----|--------|--------|----------|------------------|-----------------------|---------------------|
|     |        |        | Chr GRCh37 | HGVD | JGVD 3.5k | gnomAD | SIFT | PROVEAN | Polyphen2 | Polyphen2 | HDIV_pred | HDIV_score | Verdict | Identified classification rules |
| V1  | c.2527G>C | p.Glu843Gln | 17 | 7,918,033 | 0.000% | 0.000% | 0.00108% | 0.00079% | Damaging | 0.008 | Deleterious | -2.86 | Damaging | 1.0 | Likely Pathogenic | PM1 | PM2 | PP3 | PP4 |

NM_000180.3