Novel homozygous in-frame deletion of GNAT1 gene causes golden appearance of fundus and reduced scotopic ERGs similar to that in Oguchi disease in Japanese family

Daiki Kubota**, Noriko Oishi**, Kiyoko Gocho*, Sachiko Kikuchi*, Kunihiko Yamaki*, Tsutomu Igarashi**, Hiroshi Takahashi*, Nobuo Ishida*, Takeshi Iwata*, Atsushi Mizota*, and Shuhei Kameya**

*Department of Ophthalmology, Nippon Medical School Chiba Hokusoh Hospital, Inzai, Chiba, Japan; **Department of Ophthalmology, Nippon Medical School, Tokyo, Japan; *Ishida Eye Clinic, Niigata, Japan; Division of Molecular and Cellular Biology, National Institute of Sensory Organs, Tokyo, Japan; **Department of Ophthalmology, Teikyo University School of Medicine, Tokyo, Japan

ABSTRACT
Background: The GNAT1 gene encodes the alpha-subunit of transducin in rod photoreceptors and is an important part of the phototransduction cascade. Defects in GNAT1 are very rare but have been identified in autosomal dominant and recessive congenital stationary night blindness (CSNB) and autosomal recessive rod-cone dystrophy. The purpose of this study was to determine the phenotype-genotype relationship in a non-consanguineous Japanese family with a GNAT1 mutation.

Methods: Detailed ophthalmic examinations were performed on the patients and their family members. Whole exome sequencing (WES) was applied to the DNA obtained from the family members. Sanger sequencing and co-segregation analyses were performed to identify the most likely pathogenic variant.

Results: Two female (13- and 11-years) and one male (15-years) patients from a family had night blindness from their childhood. The fundus had a mild golden appearance regardless of the state of light- or dark-adaptation. Electroretinographic (ERG) analyses showed that the scotopic a-wave was extinguished, and the mixed rod-cone responses were severely reduced with an electronegative form in patients. The shapes of the dark-adapted ERGs were similar to those recorded from patients with Oguchi disease. We identified a homozygous in-frame deletion c.818_820delAGA, p.Lys273del in the GNAT1 gene. Variants were verified by Sanger sequencing and co-segregated with the disease in five members of the family.

Conclusions: Our findings indicate that a recessive GNAT1 mutation found in this family could be the cause of the golden appearance of the fundus and negative ERGs with reduced a-waves, and nearly absent b-waves in the mixed rod-cone ERGs.

Introduction
The GNAT1 gene (OMIM *139330) encodes a subunit of rod transducin, a protein naturally expressed in vertebrate. Transducin is an important part of the phototransduction cascade (1,2) and is a trimeric G protein that consists of three subunits (3). The alpha-subunit binds to guanosine triphosphate (GTP) and activates cyclic guanosine monophosphate-phosphodiesterase (cGMP-PDE). The beta and gamma subunits form a complex that makes it possible for transducin to interact with rhodopsin (4). On exposure of the photoreceptor cells to light, rhodopsin is photoisomerized to its active form. This form activates transducin which then stimulates cGMP-PDE. The degradation of cGMP cause cGMP-gated ion channels to close resulting in hyperpolarization of the photoreceptors causing the electrical light response (5). The alpha-subunit is encoded by GNAT1 and is expressed in the rod cells, whereas the alpha-subunit that is encoded by GNAT2 is expressed in the cone cells (6).

The a- and b-waves of the electroretinograms (ERGs) of Gnat1 null mice are reduced under scotopic conditions. Morphologically, there is a shortening of the rod outer segments and a progressive loss of the photoreceptor nuclei (7). Institute for Cancer Research (ICR)-derived retinal dysfunction 1 (IRD1) and IRD2 mice strains are models of spontaneous rod-cone and rod dysfunction, respectively (8). Both strains carry a nonsense mutation in Gnat1, resulting in the absence or reduction of the alpha subunit of rod transducin (8).

Mutations in GNAT1 have been associated with autosomal dominant (AD) and autosomal recessive (AR) congenital stationary night blindness (CSNB) (9–14). Recently, a homozygous truncating GNAT1 mutation was identified in a patient with late-onset rod-cone dystrophy (RCD) (15).
has also been reported that GNAT1 variants can cause progressive AR-RCD (16).

Oguchi disease is a rare form of autosomal recessive congenital stationary night blindness in which all of the visual functions including the visual acuity, visual field, and color vision, are generally normal (17,18). A typical feature of the disease is a golden appearance of the fundus that disappears in the fully dark-adapted state and reappears shortly after the onset of light exposure (Mizuo-Nakamura phenomenon) (19). The course of dark-adaptation of the rod photoreceptors is severely retarded, whereas that of cones appears to progress normally. Oguchi disease-1 (CSNB1; MIM #258100) is caused by homozygous or compound heterozygous mutations in the arrestin gene (SAG gene; OMIM 181031) (18). Oguchi disease-2 (CSNB2; OMIM #613411) is caused by mutations in the rhodopsin kinase gene (GRK1 gene; OMIM 180381) (20). Dominant-acting mutations of SAG have been identified in autosomal dominant retinitis pigmentosa (adRP), and this mutation accounts for 3% of the 300 families in an adRP cohort and 36% of another cohort of Hispanic families (21).

The purpose of this study was to determine the genotype-phenotype relationship of a non-consanguineous Japanese family with a golden appearance of the fundus and reduced dark-adapted ERGs similar to that of Oguchi disease in Japanese patients.

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 patients and their parents after the nature and possible consequences of the study were explained.

The ophthalmological examinations included measurements of the best-corrected visual acuity (BCVA) and refraction error, slit-lamp biomicroscopy, ophthalmoscopy, fundus photography, fundus autofluorescence (FAF) imaging, spectral domain optical coherence tomography (SD-OCT), and full-field electroretinography (ERG). The ERGs were recorded using an extended testing protocol conforming to the International Society for Clinical Electrophysiology of Vision standards. The ERGs were elicited and recorded with a LED built-in electrode (LE4000, TOMEY, JAPAN). The FAF images were acquired with the TRC-NW8Plus retinal camera (TOPCON, Tokyo, Japan), and the SD-OCT images were acquired with a Cirrus HD-OCT (Carl Zeiss Meditec). For the fundus examinations, fundus photographs were recorded in both the dark-adapted and light-adapted state. For the dark-adaptation, the patient’s eye was patched, and the patient was placed in a dark room for 3 hours. Fundus photographs were recorded immediately after the patient was taken out of the dark room.

Blood samples were collected from the patients and their parents, and genomic DNA was isolated from the peripheral white blood cells using a blood DNA isolation kit (NucleoSpin Blood XL; Macherey Nagel, Germany). Exome sequencing (Macrogen Japan) and targeted sequence analysis were done according to the published protocol of NISO, a customized analysis protocol for the Japanese population (22,23). Paired-end sequence library construction and exome capturing were performed by the Agilent Bravo automated liquid-handling platform with SureSelect V5-post kit (Agilent Technologies, Santa Clara, CA). Enriched libraries were sequenced with the Illumina HiSeq2000 sequencer (San Diego, CA).

Reads were aligned to the UCSC human genome 19 reference sequence with Burrows-Wheeler Aligner software (24). Duplicated reads were removed by Picard MarkDuplicates module, and mapped reads around insertion-deletion polymorphisms (INDELS) were realigned by the Genome Analysis Toolkit (GATK) (25). Base-quality scoring was recalibrated by GATK. Mutation calling was performed by the GATK Unified Genotyper module.

Called single-nucleotide variants (SNVs) and INDELS were annotated by the snpEff software (snpEff score; “HIGH,” “MODERATE,” or “LOW”) (26). All called SNVs and INDELS of the 271 genes registered as retinal disease-causing genes on the RetNet database were selected for further analysis (https://sph.uth.edu/retnet/home.htm). The identified variants were filtered with allele frequency (less than 1%) of the Human Genetic Variation Database (HGVD; http://www.genome.med.kyoto-u.ac.jp/SnpDB/about.htm) which is specific for the Japanese population. Depth and coverage for the targeted areas were made visible and confirmed with the integrative Genomics Viewer (http://www.broadinstitute.org/igv/). All identified variants were analyzed using three software prediction programs; SnpEff, Sorting Intolerant from Tolerant (SIFT; http://sift.jcvi.org/) (27), and PolyPhen2 (http://genetics.bwh.harvard.edu/pph/index.html) (28). The allelic frequency of all of the variants was estimated in reference to three databases; HGVD, iJGVD, and gnomAD Browser. Pathogenicity classification of all detected variants was performed based on the guidelines of the American College of Medical Genetics and Genomics (ACMG) (29). Together with the clinical findings of the affected subjects, the mode of inheritance in the pedigree, as well as co-segregation, disease-causing variants were determined from the called variants in the retinal disease-associated genes.

The GNAT1 variants identified by exome sequencing and targeted analysis were further confirmed by direct sequencing of all family members. The identified regions were amplified by polymerase chain reaction (PCR) using primers synthesized by Greiner Bio-One (Tokyo, JAPAN). The PCR products were purified (ExoSAP-IT; USB Corp., USA) and were used as the template for sequencing. Both strands were sequenced on an automated sequencer (Bio Matrix Research; Chiba, JAPAN).

Results

Clinical findings

A male (15-years-old; II-1) and two female (13- and 11-years-old; II-2 and II-3) patients reported night blindness from their childhood. Their decimal BCVA was 1.0 in both eyes, and there was no nystagmus or strabismus. Fundus examination under light-adapted conditions showed a slight golden appearance (Figure 1c,e.g). Three expert doctors (KG, AM,
SK) who have experience in examining the fundus of Oguchi disease with the Mizuo-Nakamura phenomenon, found that the fundus appearance of these cases were similar to the light adapted state of Oguchi disease. Because we considered that they were possibly Oguchi disease, we tried fundus examination after dark-adaptation. However, the Mizuo-Nakamura phenomenon was not observed after three hours of dark-adaptation in all patients. The fundus autofluorescence images were normal (Figure 1d,f,h). The SD-OCT findings of all the patients were unremarkable with the ellipsoid and interdigitation zones intact (Figure 2b–d).

ERG analyses revealed that the scotopic a-waves were extinguished. The a-waves of the mixed rod-cone responses were markedly reduced, and the b-waves were present but

---

**Figure 1. Fundus photographs of family members.**

Dark-adapted (a, c, e and g) and light-adapted fundus photographs (b, d, f and h) are shown. Images from I-2 (a, b), II-1 (c, d), II-2 (e, f) and II-3 (g, h) are shown.
severely reduced. These changes resulted in the negative-type ERGs. The oscillatory potentials of the mixed rod-cone ERGs were relatively well-preserved in two patients (II-1 and II-3). These characteristics of the dark-adapted ERGs resembled those of patients with Oguchi disease (30). The amplitudes of the photopic b-waves were mildly reduced in two patients (II-1 and II-3) and severely reduced in one patient (II-2). The implicit times of the cone ERGs were delayed in all patients.

Analysis of their parents showed normal fundus, FAF, and OCT findings (Figures 1a,b for I-2, Data for I-1 not shown). The amplitudes and implicit times of the full-field ERGs of I-2 were within normal limits (Figure 3a).

**Molecular genetic analyses**

We identified a homozygous 1 amino-acid in-frame deletion c.818_820delAGA, p.Lys273del in the GNAT1 by WES in the DNA samples of the family (Figure 3). The GNAT1 variant was the only gene with two heterozygous or a homozygous variant with HIGH score of snpEff and less than 1.0% of allelic frequency in the RetNet genes. This variant was verified by Sanger sequencing, and it co-segregated with the disease in five members of the family (Figure 4). The parents were not consanguineous, however both parents had the same heterozygous mutation (Figure 4). Although, the mother (I-2) of the patients stated that her marriage was not consanguineous, the distance between the birthplaces of the parents (I-1 and I-2) was only 15 km. Therefore, the existence of common ancestors for the parents could not be excluded. The allelic frequencies of these variants were extremely low in the Japanese specific (HGVD, iJGVD) and gnomAD databases (Table 1). The results of three prediction programs indicated that the variant was deleterious by PROVEAN and was not applicable by SIFT and Polyphen2 because these programs could not predict truncated variants (Table 1). According to ACMG standards and guideline, the variant were categorized into PM4 [protein length changes as a result of in-frame deletions/insertions in a non-repeat region or stop-loss variants], PM2 [absent from controls (or at extremely low frequency if recessive) in the Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium], PM3 [for recessive disorders, detected in trans with a pathogenic variant], PP1 [co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease], and PP4 [patient’s phenotype or family history is highly specific for a disease with a single genetic etiology]. Therefore, the variant was classified as likely pathogenic. Pathogenic SAG and GRK1 variants were not found.

**Discussion**

A novel homozygous GNAT1 mutation was found in three Japanese patients from the same family. The characteristics of their retinal disorder resembled those of Oguchi disease. The mutation was found by WES in DNA samples. There are 6 families from 8 earlier reports describing the clinical phenotypes of patents with the GNAT1 mutations (Table 2) (9–16). The mode of inheritance and the phenotype correlations differed among these patients; AD-CSNB (3 families), AR-CSNB (1 family), and AR-RCD (2 families). Nevertheless, all of the patients have been reported to have night blindness with extinguished or severely reduced rod ERGs. Our cases also had night blindness with extinguished rod responses, and the alterations could be caused by the absence or functional suppression of the alpha subunit of the rod transducin.

The clinical features of our patients resembled those of patients with Oguchi disease. These were the golden appearance of the light-adapted fundus and severely reduced mixed rod-cone ERGs. Consistent with the findings of the mixed rod-cone ERGs in patients with Oguchi disease were; negative ERGs with reduced a-waves, nearly absent b-waves, relatively well-preserved OPs, and essentially normal cone-mediated ERGs (30). The ERGs of our patients were different from that of complete and incomplete CSNB (Schubert-Bornshein type) because patients with CSNBs have relatively normal amplitude a-waves in the mixed rod-cone ERGs (31,32). The ERGs of our patients were also different from that of patients with the Rigg’s type of ERGs caused by GNAT1, RHO, PDE6B, and SLC24A1 mutations because patients with the Rigg’s type of ERGs have small...
a- and b-waves in the mixed rod-cone ERGs without having a negative form (12,33–35). The features of the ERGs of our patients were similar to those of Oguchi disease, although there was a mildly reduced amplitude and delayed implicit time of the cone ERGs. Mutations in the SAG or GRKI gene cause the recessive form of Oguchi disease. Products of GNAT1 and SAG and/or GRKI interact closely in the phototransduction cascade (36), and we suggest that these relationships might contribute to the similarities of the ERG abnormalities of our cases and Oguchi disease. The dominant-acting mutations identified in SAG have been reported in autosomal dominant retinitis pigmentosa (21). A homozygous truncating GNAT1 mutation was identified in a patient with AR-RCD (15,16). These observations raise the possibility that our cases may be the early stages of rod-cone dystrophy rather than CSNB.

The golden appearance of the fundus without the Mizuo-Nakamura phenomenon was found in our patients. The underlying functional mechanism that causes the discoloration of the fundus observed in patients with Oguchi disease has not been definitively determined. Godara et al. examined the retina of patients with Oguchi disease by adaptive optics (AO) and OCT and reported that the rods, but not the cones, change their intensity after dark-adaptation. They suggested that the fundus changes in Oguchi disease were due to changes within the rods as opposed to changes at different retinal loci (37). Sandberg et al. hypothesized that the underlying functional mechanism of patients with GNAT1

Figure 3. Full-field electroretinograms (ERGs).

Full-field ERGs recorded from I-2 (a), II-1 (b), II-2 (c), and II-3 (d) are shown. The dark-adapted 0.01, dark-adapted 3.0, light-adapted 3.0, and light-adapted 3.0 flicker ERGs are shown. Extinguished scotopic a-waves are seen in II-1, II-2 and II-3. The a-waves of the mixed rod-cone ERGs are markedly reduced, and the b-waves are present but severely reduced. The ERGs have an electronegative form. The oscillatory potentials are relatively well-preserved in all patients. The photopic cone ERGs are mildly reduced in two patients (II-1 and II-3) and severely reduced in one of the two female patients (II-2). The implicit time of the cone ERGs are delayed in all the patients.
mutations was that the rod transducin encoded by the mutant GNAT1 gene was constitutively active, and the night blindness results from a partial desensitization of the rods caused by the...

**Figure 4.** Molecular genetic findings and a pedigree chart with segregation scheme. Sequence chromatograms of the I-2 (a), II-1 (b) and normal control (c) of GNAT1 variants are shown. Pedigree charts for the segregation analysis are shown (d). Segregation analysis of the variant of the GNAT1 gene in the family showed cosegregation of a putative disease-causing variant and phenotype. Affected patient is shown by a solid symbol and unaffected with open symbols.

| Allele frequency | gnomAD | PROVEAN | ACMG Classification | Functional prediction | Score | Verdict |
|------------------|--------|---------|---------------------|-----------------------|-------|---------|
|                  |        |         |                     |                       |       |         |
|                  |        |         |                     |                       |       |         |
|                  |        |         |                     |                       |       |         |
|                  |        |         |                     |                       |       |         |
|                  |        |         |                     |                       |       |         |
|                  |        |         |                     |                       |       |         |
| **c.818_820delAGA** | **p.Lys273del** | **Exon 7 of 9** | **ND** | **0.000%** | **0.000%** | **0.061%** | **0.000%** | **Deleterious** | **−14.04** | **Likely pathogenic** | **PM2** | **PM3** | **PM4** |

**Table 1.** Results of in silico molecular genetic analysis of the detected GNAT1 variant.
The constitutive activity (10). The underlying functional mechanism for the golden appearance of the fundus in our patients might be due to a similar mechanism, i.e., a partial desensitization of rods caused by the constitutive activity. Changes within the partially desensitized rods caused by GNAT1 mutation interacting with SAG may be implicated in this mechanism. However, these are very speculative.

There are limitations in this report. Although these are the first cases to show patients with the GNAT1 mutation resembling phenotypes of Oguchi disease, only three cases with this GNAT1 mutations were studied. A larger number of patients with different GNAT1 mutations will allow us to determine more detailed relationships of GNAT1 mutations and defects of the phototransduction cascade.

In conclusions, our findings indicate that the recessive GNAT1 mutation found in this family could be the cause of the golden appearance of the fundus and negative ERGs with reduced a-waves, and nearly absent b-waves in the mixed rod-cone ERGs. The findings in these three patients indicate genetic analyses need to be performed on individuals with night blindness and ERG responses resembling those of Oguchi disease.

Acknowledgments
We thank Professor Emeritus Duco Hamasaki of the Bascom Palmer Eye Institute, University of Miami School of Medicine, Miami, FL for discussions and editing our manuscript.

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.

Funding
No funding was received for this research.

Ethical approval
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent
Informed consent was obtained from all individual participants included in the study.

References
1. Fong SL. Characterization of the human rod transducin alpha-subunit gene. Nucleic Acids Res. 1992;20:2865–70. doi:10.1093/nar/20.11.2865.
2. Pugh EN Jr., Lamb TD. Amplification and kinetics of the activation steps in phototransduction. Biochim Biophys Acta. 1993;1141:111–49. doi:10.1016/0005-2728(93)90038-H.
3. Lerea CL, Somers DE, Hurley JB, Klock IB, Bunt-Milam A. Identification of specific transducin alpha subunits in retinal rod and cone photoreceptors. Science. 1986;234:77–80. doi:10.1126/science.3529395.
Erratum in: Nat Genet.

10.1371/journal.pone.0168271. eCollection 2016.

21. Sullivan LS, Bowne SJ, Koboldt DC, Cadena EL, Heckenlively JR, Bigham KE, Wigonaton DH, Johnson JD, Ruiz RS, Pennesi ME. A novel dominant mutation in SAG, the arrestin-1 gene, is a common cause of retinitis pigmentosa in Hispanic families in the Southwestern United States. Invest Ophthalmol Vis Sci. 2017;58:2774–84. doi:10.1167/iovs.16-21341.

22. Katagiri S, Yoshitake K, Akahori M, Hayashi T, Furuno M, Nishino J, Ikeo K, Tsunekoa H, Iwata T. Whole-exome sequencing identifies a novel ALMS1 mutation (p.Q2051X) in two Japanese brothers with Alström syndrome. Mol Vis. 2013;19:2393–406. eCollection 2013.

23. Katagiri S, Akahori M, Sergey Y, Yoshitake K, Ikeo K, Furuno M, Hayashi T, Kondo M, Ueno S, Tsunoda K. Whole exome analysis identifies frequent CNGA1 mutations in Japanese population with autosomal recessive retinitis pigmentosa. PLoS One. 2014;9:e108721. doi:10.1371/journal.pone.0108721. eCollection 2014.

24. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 2010;26:589–95. doi:10.1093/bioinformatics/btp698.

25. McKenna A, Hanna M, Banks E, Sikivu A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20:1297–303. doi:10.1101/gr.107524.110.

26. Cingolani P, Platts A, Wang L, Coon M, Nguyen T, Wang L, Land S, Lu X, Ruden D. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnvEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3, Fly (Austin). 2012;6:80–92. doi:10.1611/fly.19695.

27. Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res. 2003;31:3812–14. doi:10.1093/nar/gkg509.

28. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. Nat Methods. 2010;7:248–9. doi:10.1038/nmeth0410-248.

29. Richards S, Aziz N, Bale S, Bick D, Das S, Gaster-Foster J, Grody WW, Hegde M, Lyon E, Spector E, ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–24. doi:10.1038/gim.2015.30.

30. Miyake Y. Electrodiagnosis of retinal disease. Tokyo: Springer-Verlag; 2006.

31. Schubert G, Bornschein H. Analysis of the human electroretinogram. Ophthalmologica. 1952;123(6):396–413. doi:10.1159/000301211.

32. Zeitz C, Robson AG, Audo I. Congenital stationary night blindness: an analysis and update of genotype-phenotype correlations and pathogenic mechanisms. Prog Retin Eye Res. 2015;45:58–110. doi:10.1016/j.preteyeres.2014.09.001.

33. Deych D, Zinn W, Oprian DD. Heterozygous missense mutation in the rhodopsin gene as a cause of congenital stationary night blindness. Invest Ophthalmol Vis Sci. 1994;35:175–80. doi:10.1167/iovs.94.6.175.

34. Sanna Sandberg P, Miyake Y. Rod and cone function in the Nougaret form of congenital stationary night blindness. Acta Ophthalmol. 1998;76:867–9.

35. Naeem MA, Chavali VR, Ali S, Iqbal M, Riazuddin S, Khan SN, Zeitz C, Robson AG, Audo I. Congenital stationary night blindness: ERG findings, a new GNAT1 mutation and a systemic association. Doc Ophthalmol. 2018;137:57–62. doi:10.1007/s10633-018-9651-0.

36. Lamb TD. Evolution of phototransduction, vertebrate photoreceptors and retina. Prog Retin Eye Res. 2013;36:52–119. doi:10.1016/j.preteyeres.2013.06.001.

37. Godara P, Cooper RF, Sergouniotis PI, Diederichs MA, Streb MR, Genead MA, McAnany JF, Webster AR, Moore AT. Assessing retinal structural changes in complete congenital stationary night blindness and Oguchi disease. Am J Ophthalmol. 2012;154:987–1001.e1. doi:10.1016/j.ajo.2012.06.003.