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ORIGINAL RESEARCH ARTICLE

Coexistence of *GNAT1* and *ABCA4* variants associated with Nougaret-type congenital stationary night blindness and childhood-onset cone-rod dystrophy

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Abstract

Purpose A single variant (p.G38D) in the *GNAT1* gene, encoding the rod-specific transducin α-subunit in phototransduction, has been reported only in one French family with Nougaret-type autosomal dominant congenital stationary night blindness (CSNB). We identified a Japanese family with Nougaret-type CSNB and cone-rod dystrophy (CORD).

Methods Five patients with CSNB and two patients with childhood-onset CORD were recruited. We performed a comprehensive ophthalmic examination including electroretinography (ERG). Disease-causing variants were identified by whole exome sequencing, with candidates confirmed by Sanger sequencing in nine family members.

Results The *GNAT1* variant (p.G38D) was identified in all four CSNB patients, whereas the two CORD patients carried biallelic truncated known *ABCA4* variants as well as the *GNAT1* variant. Clinically, no remarkable findings were observed in fuduscopy, fundus autofluorescence, or optical coherence tomography images from the CSNB patients. No response was detectable by rod ERG. The a-waves of standard and bright flash ERG were delayed and broadened rather than biphasic, and b/a-wave amplitude ratio was negative. Cone and 30-Hz flicker responses were normal, and overall, the ERG findings were compatible with previous descriptions of Nougaret-type CSNB. ERG of the CORD patients with macular atrophy showed non-recordable rod response and severely decreased standard flash, cone and 30-Hz flicker responses.

Conclusions This is the second report of a Nougaret-type CSNB family with the *GNAT1* variant. Our novel findings suggest that coexistence of the *GNAT1* and biallelic *ABCA4* variants is associated with an overlapping phenotype with both Nougaret-type CSNB and CORD.

Keywords Congenital stationary night blindness · *GNAT1* · Nougaret-type · *ABCA4* · Cone-rod dystrophy · Japanese

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Introduction

Congenital stationary night blindness (CSNB) is a group of clinically and genetically heterogeneous non-progressive retinal diseases. Nougaret-type CSNB with autosomal dominant inheritance is well described in a single large French family in which all affected patients descended from the first reported patient, Jean Nougaret [1, 2]. A heterozygous variant (p.G38D) in the *GNAT1* gene, encoding a rod-specific transducin α-subunit, has been identified in patients with Nougaret-type CSNB [2]. At present, the family is the only known Nougaret-type CSNB family with *GNAT1* variant (p.G38D). Other variants in three genes, rhodopsin (*RHO*) [3], rod-specific cGMP phosphodiesterase type 6 β-subunit (*PDE6B*) [4, 5], and *GNAT1* [6–8], have been associated with "Riggs-type" autosomal dominant CSNB. Functional defects of these three genes affect the rod phototransduction cascade.

A large number of variants in the *ABCA4* gene, encoding an ATP-binding cassette superfamily transmembrane protein, have been reported in patients with autosomal recessive Stargardt macular dystrophy (STGD1, MIM #248200) since its first description in 1997 [9]. Biallelic variants in the *ABCA4* gene are responsible for a spectrum of retinal dystrophies (*ABCA4*-associated retinopathies) including STGD1, cone (COD), and cone-rod dystrophies (CORD) [10–12].

More than 270 other genes causing inherited retinal diseases (IRDs) have been cataloged in the Retinal Information Network (RetNet, https://sph.uth.edu/retnet/home.htm), and this database has grown in recent years with the application of whole exome sequencing (WES), a popular next-generation sequencing technology. This has allowed ophthalmologists to identify disease-causing gene variants not only in IRDs but also in undiagnosed retinal diseases.

To date, autosomal dominant CSNB with any known disease-causing gene variant has never been reported in the Japanese population. Recently, we encountered five patients with autosomal dominant CSNB and two patients with childhood-onset CORD in a Japanese family. The purpose of this study was to characterize clinical and genetic features of those patients. Genetic analysis using WES revealed that the CSNB patients carried the known heterozygous *GNAT1* variant (p.G38D), while the CORD patients carried biallelic *ABCA4* variants as well as the *GNAT1* variant.

Methods

Ethical approval

The Institutional Review Boards of the Jikei University School of Medicine (approval no. 24-232 6997) and Hamamatsu University School of Medicine (approval no. 14-040) approved the protocol for this study. The protocol adhered to the tenets of the Declaration of Helsinki, with informed consent obtained from each participant or their legal guardians.

Clinical examinations

A total of nine family members from three different generations of a Japanese family were recruited in this study (Fig. 1A). Four (II-2, II-3, III-1, and III-2) of the nine were ophthalmologically assessed at the Jikei University Hospital. We performed a comprehensive ophthalmic examination, including decimal best corrected visual acuity (BCVA), slit-lamp examination, funduscopy, fundus autofluorescence imaging (FAF) using a Spectralis HRA (Heidelberg Engineering, Heidelberg, Germany), and an Optos 200Tx, Ultra-Wide Field Retinal Imaging System (Optos, Dunfermline, UK), and optical coherence tomography (OCT; Carl Zeiss Meditec AG, Dublin, CA, USA). Horizontal B-scan images through the fovea were obtained. Full-field electroretinography (FF-ERG) using a light-emitting diode built-in electrode (LE-4000, Tomey, Nagoya, Japan) was recorded in accordance with the protocols of the International Society for Clinical Electrophysiology of Vision [13]. Details on the procedure and experimental conditions have been previously reported [14]. ERG recording of the proband (II-2, JU#1542) was dark-adapted for 30 min or 24 h in either eye. On–off ERG with 100-ms stimulus duration was also recorded for the proband.

Whole exome sequencing

Genomic DNA was extracted from peripheral blood leukocytes using a Gentra Puregene Blood Kit (Qiagen, Hilden, Germany). WES was performed on four

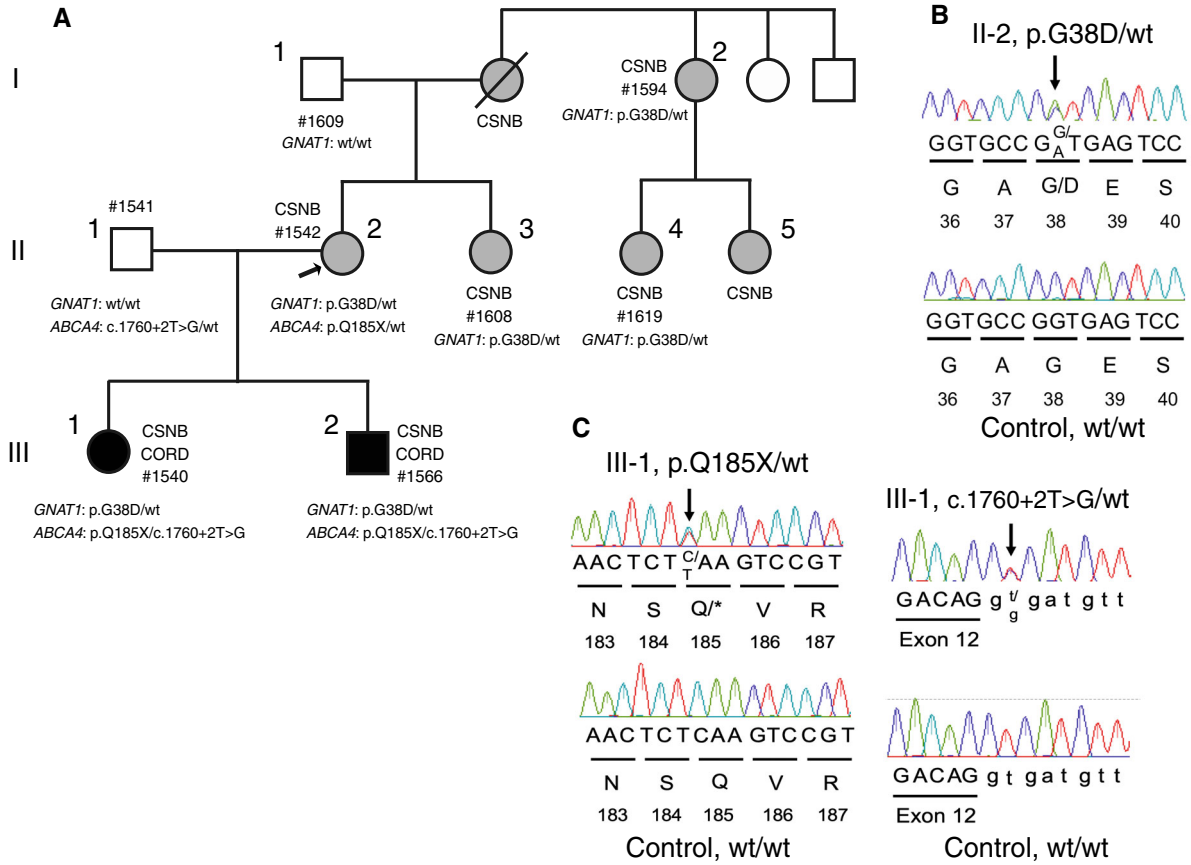

Fig. 1 Pedigree of the Japanese family and nucleotide sequences of exon 2 of the *GNAT1* gene. **A** Unaffected members are shown as unfilled circles (female) or squares (males), whereas patients with congenital stationary night blindness (CSNB) or cone-rod dystrophy (CORD) are shown as gray or black symbols, respectively. The female proband (II-2) is indicated by an arrow. The "wt" denotes wild-type genotype. A heterozygous *GNAT1* variant (p.G38D) is found in all examined CSNB patients including the proband, whose nucleotide sequencing is shown in **B**, while the CORD patients (III-1) carry compound heterozygous *ABCA4* variants (p.Q185X and c.1760 + 2 > G), whose nucleotide sequencing is shown in **C**, as well as the *GNAT1* variant

family members (II-1, II-2, III-1, and III-2; Fig. 1A). Our detailed WES methodology has been described elsewhere [14, 15]. To find out disease-causing variants, we focused on nonsynonymous variants and splice site variants, which are within 10 bp of exon–intron boundaries, and excluded synonymous and noncoding exonic variants from the analysis. We treated common genetic variants (allele frequency > 0.01 for recessive variants or > 0.001 for dominant variants) in any of the ethnic subgroups found in the following single-nucleotide polymorphism databases and synonymous variants as putative nonpathogenic sequence alterations: 1000 Genomes database (http://www.1000genomes.org/), Exome Aggregation Consortium database (http://exac.broadinstitute.org/), Genome Aggregation Database (https://gnomad.broadinstitute.org/), Human Genetic Variation Database (http://www.genome.med.kyoto-u.ac.jp/SnpDB/), and Tohoku Medical Megabank Organization database (https://ijgvd.megabank.tohoku.ac.jp/).

Sanger sequencing for validation and co-segregation analysis

Sanger direct sequencing was performed to confirm the detected variants and to conduct co-segregation analysis on eight family members (I-1, I-2, II-1, II-2, II-3, II-4, III-1, and III-2). Considering clinical features of patients, mode of inheritance in the family,

and co-segregation analysis, disease-causing variants were finally confirmed from the detected variants in the IRD genes. For variants of the *GNAT1* and *ABCA4* genes, the following primer sets were used. *GNAT1* exon 2: forward 5′-GGTCCACCCTGCCAACTC-3′, reverse 5′-ATAAACTCGAGGCACTCTTCCAG-3′; *ABCA4* exon 5: forward 5′-TTACAAGTGTTTC-CAATCGACTCT-3′, reverse 5′-GTTGAAAATGAT-CACGATCTCAAG-3′; *ABCA4* exon 12: forward 5′-CTGGACACGTTGAAAAATTAACAC-3′, reverse 5′-ATTCAAGGACTCTTTGTTGAGCTT-3′. NCBI Reference Sequences of *GNAT1* (NM_144499.2) and *ABCA4* (NM_000350.2) were used.

Results

Congenital stationary night blindness (CSNB) phenotypes

Patient II-2

A 34-year-old female proband (II-2, JU#1542) was referred to The Jikei University Hospital for assessment of her congenital night blindness. She had never complained of visual symptoms except for night blindness, nor had systemic medical history. The condition of night blindness was stationary, and therefore, she was diagnosed with CSNB. Her decimal BCVA was 1.2 (− 1.75 diopter sphere) in the right and 1.5 (− 1.75 diopter sphere) in the left eye. Slit-lamp examination showed no abnormal findings in the anterior segment and media. No remarkable findings were observed in funduscopy, fundus autofluorescence (FAF), or cross-sectional macular OCT images (Fig. 2). FF-ERG (Fig. 3) showed non-recordable rod response to a weak flash (dark-adapted 0.01 cd s m^{-2}; DA 0.01) in rod ERG but at least half of a- and b-wave responses in DA 3.0, DA 10.0, and DA 200 ERG after 30 min of DA in the right eye, as well as after 24 h of DA in the left eye. Notably, the DA 3.0 responses showed distinguishing delayed and broadened a-waves and negative-type waveforms (b/a ratio less than 1.0). The photopic cone (light-adapted 3.0 cd s m^{-2}; LA 3.0) and 30-Hz flicker (LA 3.0 flicker) responses were completely normal. Clear on and off responses were observed.

Patient II-3

A 37-year-old female patient (II-3, JU#1608, elder sister of the proband) was ophthalmologically evaluated and diagnosed with CSNB. Her decimal BCVA was 1.5 (− 1.00 diopter sphere) in the right and 1.5 (− 0.50 diopter sphere) in the left eye. No remarkable findings were observed in slit-lamp examination, fuduscopy, fundus autofluorescence (FAF), or cross-sectional macular OCT images (Fig. 2). The FF-ERG findings (Fig. 3) were essentially very similar to those of the proband.

Patients I-2, II-4, and II-5

Patients I-2 (JU#1594, a 74-year-old female, maternal aunt of the proband), II-4 (JU#1619, a 49-year-old female, maternal cousin of the proband), and II-5 (45-year-old female, maternal cousin of the proband), who had good visual acuities, had never complained of visual symptoms except for congenital night blindness. The diagnosis of these three patients was also compatible with CSNB. Incidence of the "CSNB" disease in this family was consistent with autosomal dominant inheritance (Fig. 1A).

Cone-rod dystrophy (CORD) phenotypes

Patient III-1

A 7-year-old female patient (III-1, JU#1540, daughter of the proband), who complained of congenital night blindness, was evaluated because of progressive deterioration of visual acuity. Her decimal BCVA was 0.35 (+ 2.50 diopter sphere) in the right and 0.2 (+ 3.25 diopter sphere) in the left eye, although her BCVA was nearly 1.0 at 5 years of age. Slit-lamp examination showed no remarkable findings. Retinal examination revealed gray discoloration within the vascular arcade in the fundus photographs, hypoautofluorescent macular area with a hyperautofluorescent ring in FAF, and disrupted ellipsoid zone (EZ) with foveal thinning in OCT (Fig. 4). Dark-adapted FF-ERG of the right eye (Fig. 3) showed non-recordable rod response to a weak flash (DA 0.01), severely decreased a- and b-wave responses to a strong flash (DA 3.0), but notably about one-third of normal response to a stronger flash (DA 200).

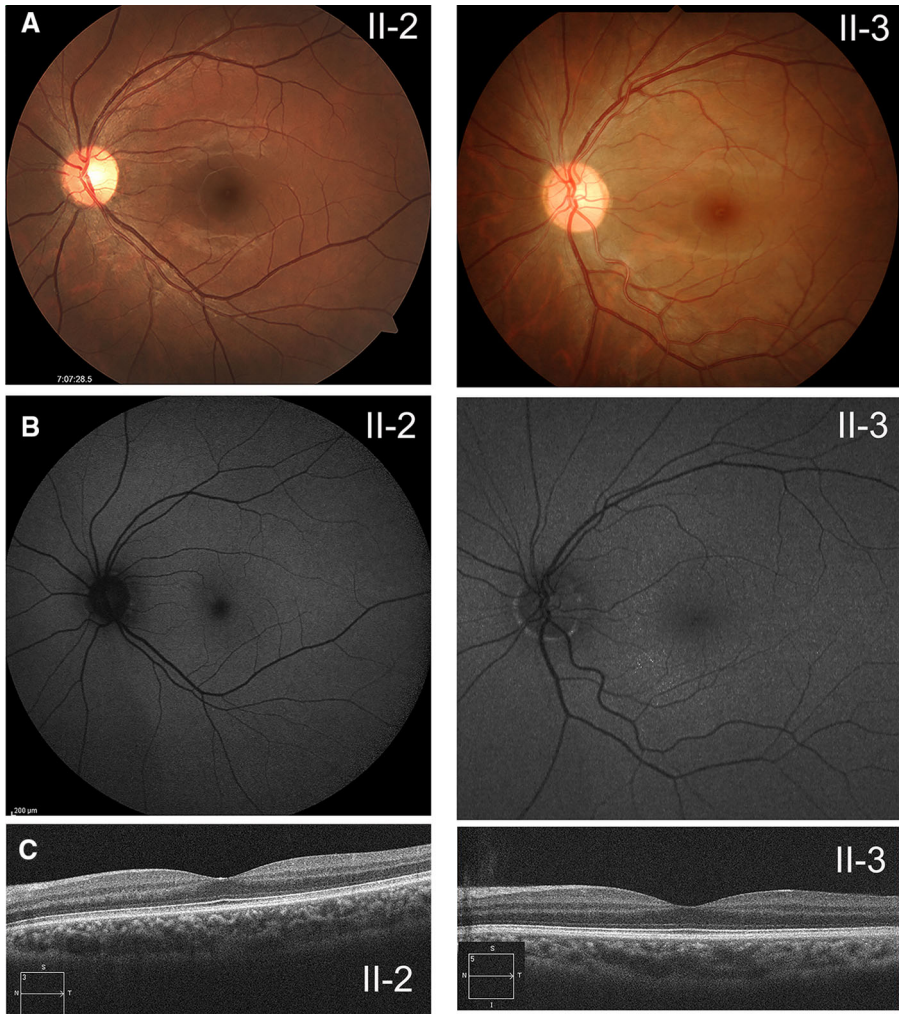

Fig. 2 Fundus photograph, fundus autofluorescence (FAF), and B-scan optical coherence tomography (OCT). No remarkable findings were present in fundus photographs (**A**), FAF (**B**), or OCT (**C**) of patients (II-2, II-3) with congenital stationary night blindness

Patient III-2

A 5-year-old male patient (III-2, #JU1566, son of the proband), who complained of congenital night blindness, was evaluated. At 5 years of age, his BCVA was 0.7 (+ 0.25 diopter sphere) in the right and 0.7 (+ 0.25 diopter sphere) in the left eye. Slit-lamp and funduscopy examination showed no remarkable findings (Fig. 4). FAF showed a hyperautofluorescent ring around the macula (Fig. 4). OCT showed a thickened external limiting membrane (ELM) as previously reported [16]. Later, when he was 6 years, 8 months of age, his BCVA decreased to 0.1 in the right and 0.15 in the left eye. Surprisingly, OCT revealed disappearance of the thickened ELM and thinner foveal thickness compared to that at 5 years of age (Fig. 4), demonstrating progressive loss of visual acuity. Dark-adapted FF-ERG findings (Fig. 3) were essentially very similar to those of patient III-1 (JU#1540). The photopic cone (LA 3.0) and 30-Hz flicker (LA 3.0 flicker) responses were severely decreased (Fig. 3), consistent with known features of CORD.

Identification of pathogenic variants of the *GNAT1* and *ABCA4* genes

We examined all known IRD genes to screen for variants from WES data, which we then analyzed based on the autosomal dominant, autosomal recessive, and de novo models. After synonymous variants

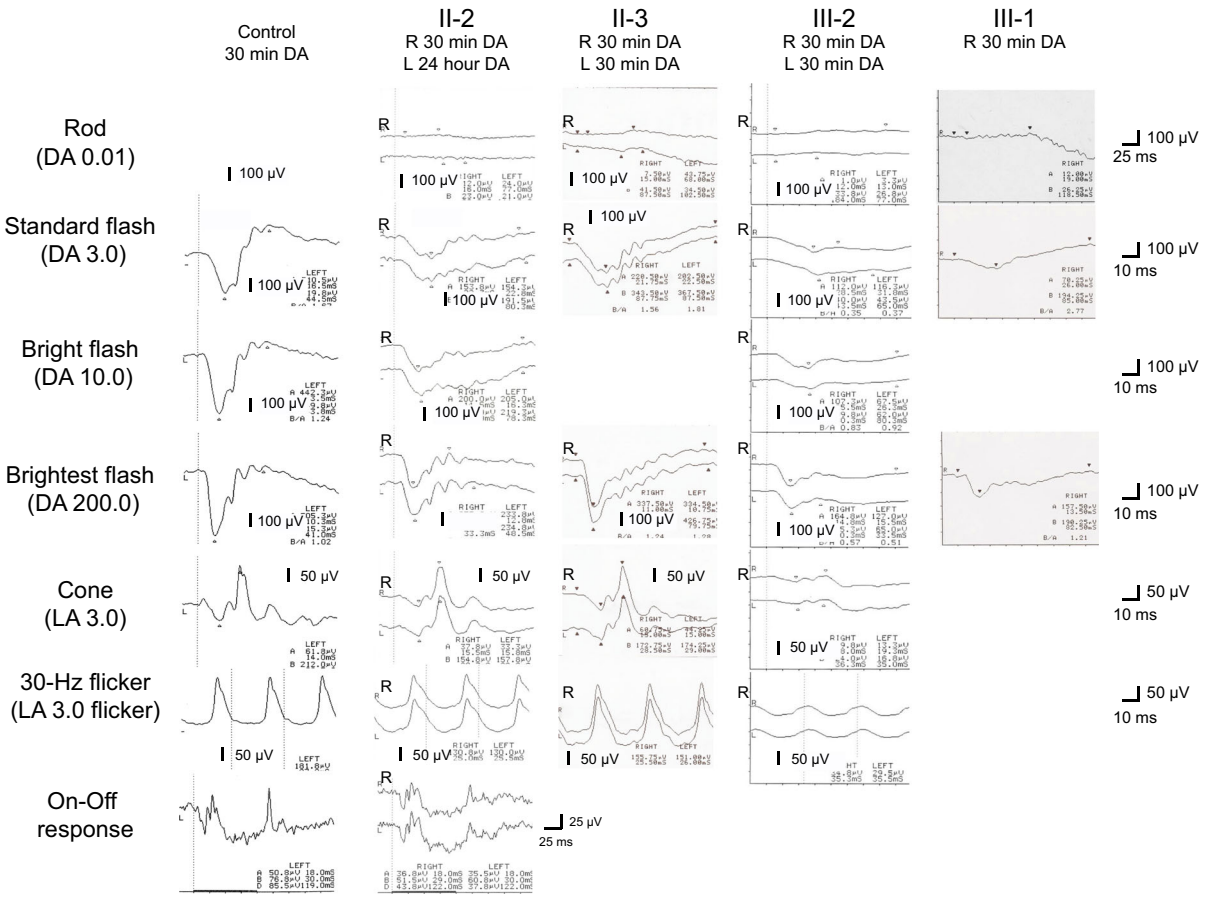

Fig. 3 Full-field electroretinography (ERG). ERG findings of a control, patients (II-2 and II-3) with congenital stationary night blindness (CSNB), and patients (III-1 and III-2) with cone-rod dystrophy (CORD). ERG of CSNB patient II-2 shows non-recordable rod response (dark-adapted 0.01 cd s m^{-2}; DA 0.01) but at least 50% greater than normal a- and b-wave responses in DA 3.0, DA 10.0, and DA 200 ERG after 30 min of DA in the right eye, as well as after 24 h of DA in the left eye. The cone (light-adapted 3.0 cd s m^{-2}; LA 3.0) and 30-Hz flicker (LA 3.0 flicker) responses are completely normal. Clear on and off responses are observed. The ERG findings of CSNB patient II-3 are compatible with those of patient II-2. In CORD patients III-2 and III-1, DA ERG shows non-recordable rod response (DA 0.01), and severely decreased a- and b-wave responses to a strong flash (DA 3.0), but notably about one-third of normal response to a stronger flash (DA 200). The LA 3.0 and LA 3.0 flicker responses are severely decreased in III-2, consistent with features of CORD

were excluded, a total of nine rare variants remained (data not shown). We then selected remaining variants that matched the patients' phenotypes and the disease phenotypes known to be caused by the IRD genes listed in the RetNet database. As a result of WES analysis, a heterozygous variant (c.113G > A, p.G38D) in the *GNAT1* gene, in agreement with autosomal dominant inheritance, was identified in the proband (II-2) (Fig. 1B). The variant was also found in the CORD patients (III-1 and III-2), but not in the unaffected member (II-1). In addition, compound heterozygous *ABCA4* variants, c.553C > T, p.Q185*, and c.1760 + 2T > G, were also identified in the CORD patients (Fig. 1A, C), whose parents (II-1 and II-2) had either of the variants (Fig. 1A), compatible with autosomal recessive inheritance. Sanger sequencing also confirmed these *GNAT1* and *ABCA4* variants. Co-segregation analysis revealed that the *GNAT1* variant was found in all CSNB patients except for patient II-5 who did not undergo genetic testing, but not in the unaffected members (I-1, II-1) (Fig. 1A). The *GNAT1* variant (p.G38D) has been reported only in Nougaret-type CSNB [2]. On the other hand, each of the nonsense (p.Q185*) and splice site (c.1760 + 2T > G) variants in *ABCA4* has been reported in *ABCA4*-associated retinopathies [17, 18].

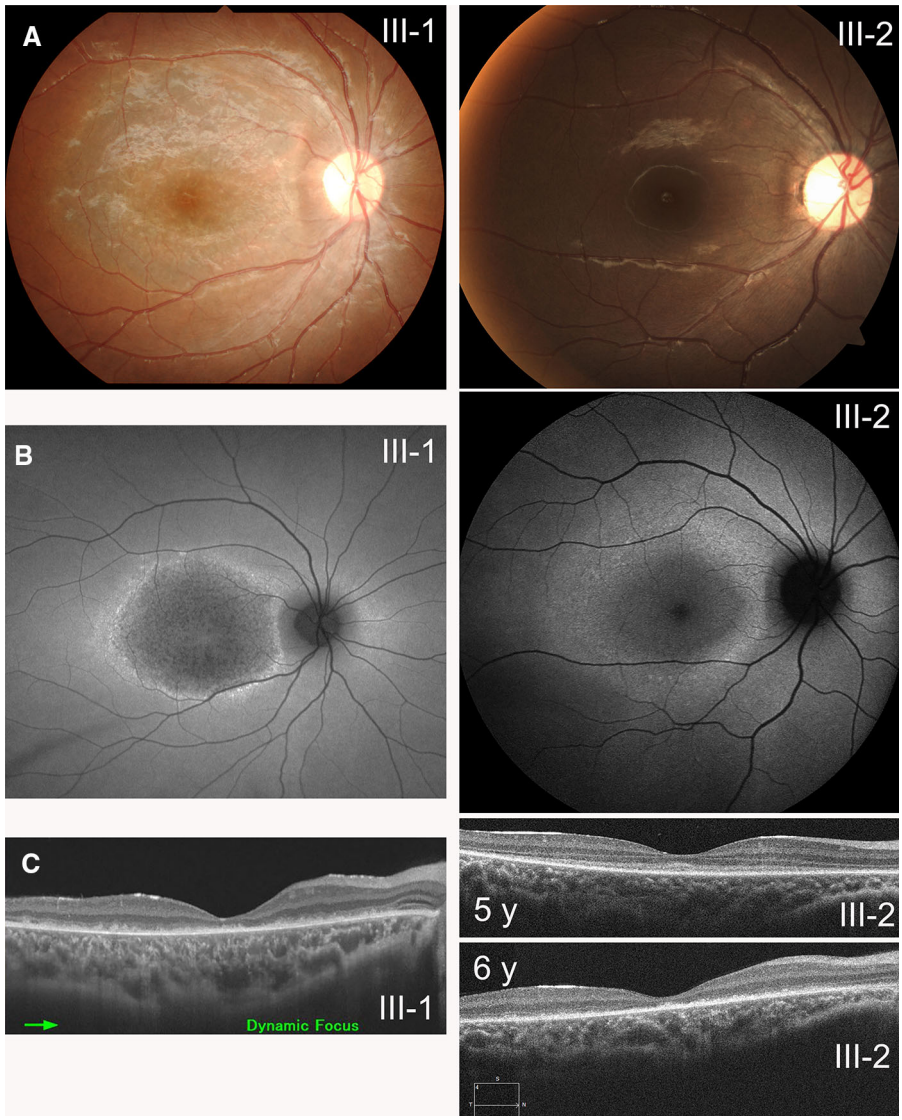

Fig. 4 Fundus photograph, fundus autofluorescence (FAF), and B-scan optical coherence tomography (OCT). **A** Fundus photographs show gray discoloration within the vascular arcade in III-1 and no remarkable finding in III-2. **B** FAF shows hypoautofluorescent macular area with hyperautofluorescent ring in III-1 and hyperautofluorescent ring around the macula in III-2. **C** OCT shows disrupted ellipsoid zone (EZ) with foveal thinning in III-1. In III-2, OCT reveals a prominent hyperreflective line, corresponding to thickened external limiting membrane, underneath the outer nuclear layer at 5 years of age (5 years), but later disappearance of the line and thinner foveal thickness at 6 years, 8 months of age (6 years)

Discussion

In this study, we identified a previously documented heterozygous *GNAT1* variant (p.G38D) in CSNB patients of a Japanese family. Also, we found coexistence of the *GNAT1* and biallelic *ABCA4* variants in two patients with both CSNB and childhood-onset CORD from this family.

To date, the *GNAT1* variant has been found only in a large French family with Nougaret-type CSNB [2, 19]. Details of characteristic ERG findings were described in a father and a son with Nougaret-type CSNB, including undetectable rod response and decreased biphasic a-waves revealed by bright flash ERG, with a positive b/a-wave amplitude ratio and at least 50% greater than normal amplitudes [19]. Their

LA 30-Hz flicker responses were normal. Notably, a characteristic feature was larger amplitudes of bright flash ERG than could be generated by cone responses alone. In addition, it was demonstrated from detailed ERG data that Nougaret-type CSNB is characterized by 100-fold reduction in rod sensitivity [19]. Our ERG findings (Fig. 3) of patients II-2 and II-3 were similar to those of Nougaret-type CSNB, but with minor differences. The a-waves from DA 3.0 ERG were delayed and broadened rather than biphasic, and b/a-wave amplitude ratio was negative. However, clear on and off responses were observed, which is inconsistent with Schubert–Bornschein-type CSNB or complete-type CSNB [20, 21]. Further, our pathognomonic ERG findings are different from Riggs-type CSNB, which is characterized by DA 3.0 and/or DA 10.0 ERG with minimal a-waves as well as b-waves derived only from cone responses. In other words, Riggs-type CSNB exhibits essentially no rod signal. Our comprehensive ERG results were therefore most compatible with those documented in Nougaret-type CSNB. The slight differences in the ERG findings might contribute to the difference in the flash intensity used between the previous [19] and current studies.

Previously, heterozygous missense *GNAT1* variants have been reported in both Nougaret-type (p.G38D) and Riggs-type CSNB (p.I52N and p.Q200E) with autosomal dominant inheritance (Table 1) [2, 6–8, 19], while homozygous missense (p.D129G) and truncated *GNAT1* variants (p.Q302* and p.C321*) are known to cause Riggs-type CSNB and progressive retinal degeneration with autosomal recessive inheritance, respectively (Table 1) [22–24]. So far, the *GNAT1* variant (p.G38D) is the only documented cause of Nougaret-type CSNB. Experimental studies using transgenic mice with p.G38D have revealed two crucial deficiencies: poor ability to activate PDE6 (rod cGMP phosphodiesterase) and partially decreased GTPase activity in rod phototransduction [25, 26]. On the other hand, in Riggs-type CSNB, constitutive activation of rod phototransduction by impaired GTPase activity is suggested as the pathogenic mechanism leading to the absence of rod function [6]. In Nougaret-type CSNB, although it is unclear why the rod sensitivity and rod ERG responses are extremely reduced, the mutant (p.G38D) α-transducin in heterozygotes can be activated only by strong light stimuli, resulting in at least 50% greater than normal hyperpolarizing broadened a-waves in DA 3.0, DA 10.0, and DA 200 ERG (Fig. 3). These findings suggest that transducing activity in heterozygotes with the p.G38D variant might be suppressed incompletely.

Biallelic variations in the *ABCA4* gene are responsible for *ABCA4*-associated retinopathies including STGD1, COD, and CORD [9–12]. Previously, phenotypic subtypes have been classified into three groups based on ERG abnormalities: normal full-field ERG in Group 1, normal rod ERG but reduced cone ERG in Group 2 (COD phenotype), and decreased both rod and cone ERG in Group 3 (CORD phenotype) [27]. In genotype–phenotype correlations of *ABCA4*-associated retinopathies, it has been reported that missense variants are associated with milder and later onset phenotypes, whereas null/truncated alleles are associated with more severe and earlier onset phenotypes [10]. Fujinami et al. [28] report that a higher proportion of *ABCA4*-associated retinopathy patients with childhood-onset phenotype were in Group 3 compared with adult-onset phenotype. Our childhood-onset CORD patients (III-1 and III-2) had biallelic truncated *ABCA4* variants as well as the *GNAT1* variant (p.G38D). In patient III-2, the cone and 30-Hz responses, which cannot be affected by the *GNAT1* variant, were severely decreased (Fig. 3), indicating that the phenotype of *ABCA4*-associated retinopathy is classifiable into either Group 2 or Group 3. Although it is uncertain whether non-recordable rod responses in patients III-1 and III-2 were derived from either the *GNAT1* or *ABCA4* variants, the DA 200 ERG responses were much more decreased than those of patients (II-2 and II-3) with Nougaret-type CSNB alone (Fig. 3), suggesting that the biallelic *ABCA4* variants themselves may contribute much more to rod dysfunction than the *GNAT1* variant itself. Our ERG findings revealed that the biallelic *ABCA4* variants alone are sufficient to develop the CORD phenotype seen in patients III-1 and III-2. This confirmed that patients III-1 and III-2 belonged to Group 3 [27]. Further, our FAF and OCT findings (Fig. 4) were consistent with *ABCA4*-associated retinopathy. Notably, the hyperreflective line corresponding to thickened ELM in OCT we observed in patient III-2 is one of the features previously observed to characterize childhood-onset *ABCA4*-associated retinopathy [16].

Patients suffering from simultaneous occurrence of STGD1 and other IRDs such as CSNB and X-linked ocular albinism (OA) have been reported previously. Huynh et al. [29] report that a female patient

Table 1 Clinical and genetic characterization of patients with reported *GNAT1* variants

Number (nationality)	Variants	Variant types	Diagnosis	Progressive	Fundus appearance	ERG findings	References
Autosomal dominant inheritance							
#1 (French)	p.G38D	Heterozygous	Nougaret-type CSNB	No	Normal	Rod: non-recordable, Combined: half of normal with biphasic a-wave, Cone: normal	Dryja et al. [2] and Sandberg et al. [19]
#2 (Japanese)	p.G38D	Heterozygous	Nougaret-type CSNB	No	Normal	Rod: non-recordable, Combined: more than half of normal with broadened a-wave, Cone: normal	This study
#3 (Chinese)	p.I52N	Heterozygous	Riggs-type CSNB	No	Mild myopic change	Rod: non-recordable, Combined: cone-like response, Cone: normal	Zeitx et al. [7] and Marmor Zeitz [8]
#4 (Danish)	p.Q200E	Heterozygous	Riggs-type CSNB	No	Normal	Rod: non-recordable, Combined: cone-like response, Cone: normal	Szabo et al. [6]
Autosomal recessive inheritance							
#5 (Pakistani)	p.D129G	Homozygous	Similar to Riggs-type CSNB	No	Normal	Rod: non-recordable, Combined: nearly non-recordable, Cone: slightly decreased	Naeem et al. [22]
#6 (Irish)	p.Q302*	Homozygous	CSNB with late-onset retinal degeneration	Yes	Retinal degeneration	Rod: non-recordable, Combined: decreased, Cone: decreased	Carrigan et al. [23]
#7 (French)	p.C321*	Homozygous	Rod-cone dystrophy	Yes	Retinal degeneration	Rod: non-recordable, Combined: non-recordable, Cone: non-recordable	Mejecase et al. [24]

CSNB congenital stationary night blindness

exhibiting a complex phenotype with both STGD1 and complete-type CSNB carried biallelic *ABCA4* variants (p.L1201R and p.R2077G) and biallelic *GRM6* gene variants (c.50_64del and c.1835_1837del). Lee et al. [30] report two female patients with STGD1 and carrier status of OA, who exhibited bull's eye macular lesions, elevated FAF signals, and retinal mosaic patterns seen in OA, and carried biallelic *ABCA4* variants (p.L541P and p.G1961E) and a heterozygous *GPR143* gene variant (p.Y257C). Apart from IRDs, a large cohort study has recently reported that nearly 5% (101/2076) of patients with genetic disorders, who underwent WES, have two or more disease loci/disease-causing variants [31], indicating the importance of identifying concurrent phenotypes in a single pedigree. Our ERG findings (Fig. 3) of patients III-1 and III-2 with two monogenic disorders, Nougaret-type CSNB and childhood-onset CORD, were associated with coexistence of the *GNAT1* and biallelic *ABCA4* variants. This clearly reflects the principal that developmental mechanisms of complex phenotypes can be resolved only when multiple disease-causing gene variants are identified. On the other hand, we cannot exclude the possibility that the childhood-onset CORD phenotype could occur by interactions of the respective mutated proteins of *ABCA4* and *GNAT1*, because both are co-localized to rod photoreceptors. This suggests that, where there are two different IRD phenotypes in a family, coexistence of two gene variants should be considered.

In conclusion, this is the second family of Nougaret-type CSNB with the *GNAT1* variant (p.G38D). Also, our novel findings suggest that coexistence of the *GNAT1* and biallelic *ABCA4* variants is likely to be associated with an overlapping phenotype with both Nougaret-type CSNB and childhood-onset CORD.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest regarding this paper.

Statements of human rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Statement on the welfare of animals This article does not contain any studies with animals performed by any of the authors.

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

References

1. François J, Verriest G, de Rouck A, Dejean C (1956) Les fonctions visuelles dans l'héméralopie essentielle nougarienne. Ophthalmologica 132:244–257
2. Dryja TP, Hahn LB, Reboul T, Arnaud B (1996) Missense mutation in the gene encoding the alpha subunit of rod transducin in the Nougaret form of congenital stationary night blindness. Nat Genet 13:358–360. https://doi.org/10.1038/ng0796-358
3. Dryja TP, Berson EL, Rao VR, Oprian DD (1993) Heterozygous missense mutation in the rhodopsin gene as a cause of congenital stationary night blindness. Nat Genet 4:280–283. https://doi.org/10.1038/ng0793-280
4. Gal A, Orth U, Baehr W, Schwinger E, Rosenberg T (1994) Heterozygous missense mutation in the rod cGMP phosphodiesterase beta-subunit gene in autosomal dominant stationary night blindness. Nat Genet 7:64–68. https://doi.org/10.1038/ng0594-64
5. Manes G, Cheguru P, Majumder A, Bocquet B, Senechal A, Artemyev NO, Hamel CP, Brabet P (2014) A truncated form of rod photoreceptor PDE6 beta-subunit causes autosomal dominant congenital stationary night blindness by interfering with the inhibitory activity of the gamma-subunit. PLoS ONE 9:e95768. https://doi.org/10.1371/journal.pone.0095768
6. Szabo V, Kreienkamp HJ, Rosenberg T, Gal A (2007) p.Gln200Glu, a putative constitutively active mutant of rod alpha-transducin (*GNAT1*) in autosomal dominant congenital stationary night blindness. Hum Mutat 28:741–742. https://doi.org/10.1002/humu.9499
7. Zeitz C, Mejecase C, Stevenard M, Michiels C, Audo I, Marmor MF (2018) A novel heterozygous missense mutation in *GNAT1* leads to autosomal dominant Riggs type of congenital stationary night blindness. Biomed Res Int 2018:7694801. https://doi.org/10.1155/2018/7694801
8. Marmor MF, Zeitz C (2018) Riggs-type dominant congenital stationary night blindness: ERG findings, a new *GNAT1* mutation and a systemic association. Doc Ophthalmol 137:57–62. https://doi.org/10.1007/s10633-018-9651-0
9. Allikmets R, Singh N, Sun H, Shroyer NF, Hutchinson A, Chidambaram A, Gerrard B, Baird L, Stauffer D, Peiffer A, Rattner A, Smallwood P, Li Y, Anderson KL, Lewis RA, Nathans J, Leppert M, Dean M, Lupski JR (1997) A photoreceptor cell-specific ATP-binding transporter gene (*ABCR*) is mutated in recessive Stargardt macular dystrophy. Nat Genet 15:236–246. https://doi.org/10.1038/ng0397-236
10. Tanna P, Strauss RW, Fujinami K, Michaelides M (2017) Stargardt disease: clinical features, molecular genetics, animal models and therapeutic options. Br J Ophthalmol 101:25–30. https://doi.org/10.1136/bjophthalmol-2016-308823
11. Tanaka K, Lee W, Zernant J, Schuerch K, Ciccone L, Tsang SH, Sparrow JR, Allikmets R (2018) The rapid-onset chorioretinopathy phenotype of *ABCA4* disease. Ophthalmology 125:89–99. https://doi.org/10.1016/j.ophtha.2017.07.019
12. Gill JS, Georgiou M, Kalitzeos A, Moore AT, Michaelides M (2019) Progressive cone and cone-rod dystrophies: clinical features, molecular genetics and prospects for therapy. Br J Ophthalmol. https://doi.org/10.1136/bjophthalmol-2018-313278
13. McCulloch DL, Marmor MF, Brigell MG, Hamilton R, Holder GE, Tzekov R, Bach M (2015) ISCEV Standard for full-field clinical electroretinography (2015 update). Doc Ophthalmol 130:1–12. https://doi.org/10.1007/s10633-014-9473-7
14. Katagiri S, Hosono K, Hayashi T, Kurata K, Mizobuchi K, Matsuura T, Yoshitake K, Iwata T, Nakano T, Hotta Y (2018) Early onset flecked retinal dystrophy associated with new compound heterozygous *RPE65* variants. Mol Vis 24:286–296
15. Kurata K, Hosono K, Hikoya A, Kato A, Saitsu H, Minoshima S, Ogata T, Hotta Y (2018) Clinical characteristics of a Japanese patient with Bardet-Biedl syndrome caused by *BBS10* mutations. Jpn J Ophthalmol 62:458–466. https://doi.org/10.1007/s10384-018-0591-8
16. Khan KN, Kasilian M, Mahroo OAR, Tanna P, Kalitzeos A, Robson AG, Tsunoda K, Iwata T, Moore AT, Fujinami K, Michaelides M (2018) Early patterns of macular degeneration in *ABCA4*-associated retinopathy. Ophthalmology 125:735–746. https://doi.org/10.1016/j.ophtha.2017.11.020
17. Jiang F, Pan Z, Xu K, Tian L, Xie Y, Zhang X, Chen J, Dong B, Li Y (2016) Screening of *ABCA4* gene in a Chinese

cohort with Stargardt disease or cone-rod dystrophy with a report on 85 novel mutations. Invest Ophthalmol Vis Sci 57:145–152. https://doi.org/10.1167/iovs.15-18190
18. Fukui T, Yamamoto S, Nakano K, Tsujikawa M, Morimura H, Nishida K, Ohguro N, Fujikado T, Irifune M, Kuniyoshi K, Okada AA, Hirakata A, Miyake Y, Tano Y (2002) *ABCA4* gene mutations in Japanese patients with Stargardt disease and retinitis pigmentosa. Invest Ophthalmol Vis Sci 43:2819–2824
19. Sandberg MA, Pawlyk BS, Dan J, Arnaud B, Dryja TP, Berson EL (1998) Rod and cone function in the Nougaret form of stationary night blindness. Arch Ophthalmol 116:867–872
20. Schubert G, Bornschein H (1952) Analysis of the human electroretinogram. Ophthalmologica 123:396–413. https://doi.org/10.1159/000301211
21. Miyake Y, Yagasaki K, Horiguchi M, Kawase Y, Kanda T (1986) Congenital stationary night blindness with negative electroretinogram. A new classification. Arch Ophthalmol 104:1013–1020
22. Naeem MA, Chavali VR, Ali S, Iqbal M, Riazuddin S, Khan SN, Husnain T, Sieving PA, Ayyagari R, Riazuddin S, Hejtmancik JF, Riazuddin SA (2012) *GNAT1* associated with autosomal recessive congenital stationary night blindness. Invest Ophthalmol Vis Sci 53:1353–1361. https://doi.org/10.1167/iovs.11-8026
23. Carrigan M, Duignan E, Humphries P, Palfi A, Kenna PF, Farrar GJ (2016) A novel homozygous truncating *GNAT1* mutation implicated in retinal degeneration. Br J Ophthalmol 100:495–500. https://doi.org/10.1136/bjophthalmol-2015-306939
24. Mejecase C, Laurent-Coriat C, Mayer C, Poch O, Mohand-Said S, Prevot C, Antonio A, Boyard F, Condroyer C, Michiels C, Blanchard S, Letexier M, Saraiva JP, Sahel JA, Audo I, Zeitz C (2016) Identification of a novel homozygous nonsense mutation confirms the implication of *GNAT1* in rod-cone dystrophy. PLoS ONE 11:e0168271. https://doi.org/10.1371/journal.pone.0168271
25. Muradov KG, Artemyev NO (2000) Loss of the effector function in a transducin-alpha mutant associated with Nougaret night blindness. J Biol Chem 275:6969–6974. https://doi.org/10.1074/jbc.275.10.6969
26. Moussaif M, Rubin WW, Kerov V, Reh R, Chen D, Lem J, Chen CK, Hurley JB, Burns ME, Artemyev NO (2006) Phototransduction in a transgenic mouse model of Nougaret night blindness. J Neurosci 26:6863–6872. https://doi.org/10.1523/JNEUROSCI.1322-06.2006
27. Lois N, Holder GE, Bunce C, Fitzke FW, Bird AC (2001) Phenotypic subtypes of Stargardt macular dystrophy-fundus flavimaculatus. Arch Ophthalmol 119:359–369
28. Fujinami K, Zernant J, Chana RK, Wright GA, Tsunoda K, Ozawa Y, Tsubota K, Robson AG, Holder GE, Allikmets R, Michaelides M, Moore AT (2015) Clinical and molecular characteristics of childhood-onset Stargardt disease. Ophthalmology 122:326–334. https://doi.org/10.1016/j.ophtha.2014.08.012
29. Huynh N, Jeffrey BG, Turriff A, Sieving PA, Cukras CA (2014) Sorting out co-occurrence of rare monogenic retinopathies: Stargardt disease co-existing with congenital stationary night blindness. Ophthalmic Genet 35:51–56. https://doi.org/10.3109/13816810.2013.865762
30. Lee W, Schuerch K, Xie Y, Zernant J, Tsang SH, Sparrow JR, Allikmets R (2016) Simultaneous expression of *ABCA4* and *GPR143* mutations: a complex phenotypic manifestation. Invest Ophthalmol Vis Sci 57:3409–3415. https://doi.org/10.1167/iovs.16-19621
31. Posey JE, Harel T, Liu P, Rosenfeld JA, James RA, Coban Akdemir ZH, Walkiewicz M, Bi W, Xiao R, Ding Y, Xia F, Beaudet AL, Muzny DM, Gibbs RA, Boerwinkle E, Eng CM, Sutton VR, Shaw CA, Plon SE, Yang Y, Lupski JR (2017) Resolution of disease phenotypes resulting from multilocus genomic variation. N Engl J Med 376:21–31. https://doi.org/10.1056/NEJMoa1516767