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Case Report

Phenotype–genotype correlations in patients with *GNB1* gene variants, including the first three reported Japanese patients to exhibit spastic diplegia, dyskinetic quadriplegia, and infantile spasms

Wakaba Endo [a,1], Satoru Ikemoto [b,1], Noriko Togashi [a,1], Takuya Miyabayashi [a], Erika Nakajima [b], Shin-ichiro Hamano [b], Moriei Shibuya [a], Ryo Sato [a], Yusuke Takezawa [a], Yukimune Okubo [a], Takehiko Inui [a], Mitsuhiro Kato [c], Toru Sengoku [d], Kazuhiro Ogata [d], Kohei Hamanaka [e], Takeshi Mizuguchi [e], Satoko Miyatake [e], Mitsuko Nakashima [e,f], Naomichi Matsumoto [e], Kazuhiro Haginoya [a,*]

[a] *Department of Pediatric Neurology, Miyagi Children's Hospital Hospital, Sendai 989-3126, Japan*
[b] *Division of Neurology, Saitama Children's Medical Center, Saitama 330-8777, Japan*
[c] *Department of Pediatrics, Showa University School of Medicine, Tokyo 152-8555, Japan*
[d] *Department of Biochemistry, Graduate School of Medicine, Yokohama City University, Yokohama, Japan*
[e] *Department of Human Genetics, Graduate School of Medicine, Yokohama City University, Yokohama 236-0004, Japan*
[f] *Department of Biochemistry, Hamamatsu University School of Medicine, Hamamatsu 431-3192, Japan*

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Abstract

We report the first three Japanese patients with missense variants in the *GNB1* gene. Patients exhibited severe dyskinetic quadriplegia with cortical blindness and epileptic spasms, West syndrome (but with good outcomes), and hypotonic quadriplegia that later developed into spastic diplegia. Whole-exome sequencing revealed two recurrent *GNB1* variants (p.Leu95Pro and p.Ile80Thr) and one novel variant (p.Ser74Leu). A recent investigation revealed large numbers of patients with *GNB1* variants. Functional studies of such variants and genotype–phenotype correlation are required to enable future precision medicine.

Keywords: *GNB1*; Hypotonic cerebral palsy; Epileptic and developmental encephalopathy; Hereditary spastic paraplegia; Infantile spasms

1. Introduction

Recently, *de novo* mutations in the *GNB1* gene have been identified as novel genetic causes of developmental delay [1]. *GNB1* encodes the guanine nucleotide-binding protein subunit beta-1 (Gβ1), a G-protein involved in signal transduction; the protein forms a heterotrimeric

* Corresponding author at: Department of Pediatric Neurology, Miyagi Children's Hospital, 4-3-17 Ochiai Aoba-ku, Sendai 989-3126, Japan.
E-mail address: khaginoya@kha.biglobe.ne.jp (K. Haginoya).
[1] These three authors contributed equally to this study.

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complex with the Gα and Gγ subunits [2]. Upon activation, heterotrimeric G proteins dissociate to form two functional molecules, the GTP-bound Gα monomer and Gβγ dimer, both of which bind to and activate downstream effector proteins [3]. Global developmental delay, cognitive delay, epilepsy, nystagmus, and severe infantile hypotonia evolving into hypertonia (such as spasticity or dystonia) have been recognized in patients with *GNB1* variants [1,4,5]. Here we report the first three Japanese patients with missense *GNB1* variants and their genotype–phenotype correlations; we compare these with data from previous reports.

2. Materials and methods

We collected clinical information and blood samples from three unrelated patients. Written informed consent was obtained from all parents to perform the diagnostic procedures and next-generation sequencing and for publication of this case report. The study was approved by the ethical review boards of Miyagi Children's Hospital (approval no. 287) and the Showa University School of Medicine (approval no. 219). The methods of whole-exome sequencing (WES) and Sanger sequencing were described in the Supplementary file. Structural consideration of G-protein and figure preparation was carried out with the program PyMOL (Schrödinger, LLC).

3. Results

3.1. Case presentations (Table 1)

3.1.1. Patient 1

A boy (3 years and 7 months of age) was born after 36 weeks of gestation by vaginal delivery without asphyxia to nonconsanguineous healthy Japanese parents. His birth weight, body length, and occipitofrontal circumference (OFC) were 2428 g (−1.4 SD), 44.5 cm (−2.1 SD), and 32.6 cm (−0.5 SD), respectively. He presented with congenital pes equinovarus of the right foot. He found feeding difficult and required nasogastric tube nutrition commencing a few days after birth. He also exhibited truncal hypotonia and paroxysmal tonic upgaze. At 4 months of age, he exhibited no visual pursuit, a poor auditory response associated with neck stiffness, an occasional opisthotonic posture, and twisting movement of the trunk, suggestive of dyskinetic quadriplegia. He suffered from repetitive epileptic spasms. An electroencephalogram (EEG) revealed frequent spike-and-wave episodes in the mid-central and bilateral occipital areas. He was diagnosed with infantile spasms. Soon after commencing adrenocorticotropic hormone (ACTH) therapy, his epileptic spasms disappeared but relapsed at 10 months of age. Magnetic resonance imaging (MRI) performed at 16 months revealed an enlarged subarachnoid space and lateral ventricles and a thin corpus callosum, but normal myelination. The visual evoked potentials were flat, but the auditory brainstem response was normal. Commencing at age 2 years, he exhibited occasional status dystonicus associated with an elevated blood creatine kinase level up to 60,000 U/L. The dystonic hypertonia was partially controlled by gabapentin, risperidone, dantrolene sodium hydrate, and phenobarbital. His seizures unexpectedly responded to sulthiam. Currently, his body weight, body length, and OFC are 12.2 kg (−1.2 SD), 88.0 cm (−2.0 SD), and 49.0 cm (−0.5 SD), respectively. He has not attained any developmental milestones (head control, head rolling, or purposeful hand use). His dysmorphic features include congenital pes equinovarus, low-set ears, carp-like lips and hypertelorism. He exhibits cortical blindness, continuous reverse ocular dipping, severe dyskinetic quadriplegia, and infantile spasms.

3.1.2. Patient 2

A boy (8 years and 6 months of age) was born at 38 weeks of gestation via cesarean section after an uncomplicated pregnancy without asphyxia. He was the first child of unrelated healthy Japanese parents. His birth weight, body length, and OFC were 2770 g (−0.6 SD), 46.4 cm (−1.2 SD), and 32.0 cm (−0.9 SD), respectively. He developed epileptic spasms at 5 months of age. An interictal EEG revealed hypsarrhythmia, which led to a diagnosis of West syndrome. Brain MRI performed at 7 months of age was normal. He exhibited normal tonus but dysmorphic features. His seizures disappeared after ACTH therapy. At the age of 10 months, he could sit independently but suffered a relapse of the epileptic spasms, which disappeared after repeat ACTH therapy at the age of 1 year and 6 months. Since then, he has experienced no seizures on zonisamide. EEG performed at 3 years of age revealed no paroxysmal discharge. Zonisamide was discontinued at 5 years and 4 months of age. His IQ at 5 years and 8 months was 46, as assessed by the Tanaka–Binet test. He is now attending a special school for intellectually handicapped children.

3.1.3. Patient 3

A girl (6 years of age) was the third girl born to nonconsanguineous Japanese parents and was born spontaneously after 41 weeks of gestation. There was no family history of any neuromuscular disorder. Her birth weight, body length, and OFC were 3,672 g (+1.7 SD), 50.7 cm (+1.1 SD), and 35.0 cm (+1.5 SD), respectively. She was referred to our hospital because of a developmental delay, presenting as inadequate head control, evident at age 6 months. She exhibited generalized hypotonia. Her developmental quotient (DQ) was 40 on the Enjoji Scale of Infant Analytical Development. EEG performed at age 3 years revealed no epileptic dis-

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Table 1
Summary of clinical, laboratory findings, and genetic studies of 3 Japanese patients and reported cases.

	Patient 1	Patient 2	Patient 3				total patients (n = 50)*
Mutation	c.284 T > C p. Leu95Pro	c.221C > T p. Ser74Leu	c.239 T > C p.Ile80Thr	c.284 T > C p. Leu95Pro (n = 4)*	Exon 5 mutations (n = 4)*	c.239 T > C p. Ile80Thr (n = 12)*	
Ethnicity	Japanese	Japanese	Japanese				
Gender, age	M, 3.6 years	M, 8.5 years	F, 6 years				
Feeding difficulties	+	–	–	4/4	0/2	4/12	9/22 (41%)
Global developmental delay	+	+	+	4/4	4/4	12/12	49/49 (100%)
infantile hypotonia	+	–	+	3/4	0/2	12/12	30/43 (70%)
hypotonia	+	–	+	4/4	1/4	12/12	37/48 (77%)
hypertonia (spasticity)	–	–	+	1/4	1/4	4/12	11/49 (22%)
hypertonia (dystonia)	+	–	–	3/4	0/4	3/12	8/49 (16%)
Epilepsy	+	+	–	2/4	1/4	9/12	25/50 (50%)
Well controlled seizures	–	+		1/2	1/1	2/5	
Abnormal EEG	+	–	–	3/4		6/12	26/50 (52%)
Abnormal MRI	+	–	–	1/4	1/4	8/12	19/49 (39%)
Nystagmus	+	–	–	4/4	0/2	6/12	15/49 (31%)
Cortical vision impairment	+	–	–	3/4	0/2	0/12	6/49 (12%)
Growth delay (body height and weight below −2SD)	–	–	–	1/4	0/4	0/12	12/49 (24%)
Microcephaly (below −2SD)	–	–	–	1/4	0/2	0/12	1/35 (3%)
Macrocephaly (below −2SD)	–	–	–	0/4	0/2	0/12	6/35 (17%)
Dysmorphic features	+	–	–	4/4	0/2	6/12	25/49 (51%)
Speach (Words)	–	+	–	0/4	2/2	2/9	17/25 (68%)
Non ambulatory (no independent walk)	+	–	+	4/4	0/2	10/12	12/23 (52%)

*Patients number including present cases. Other patients were adopted from previous reports (ref. 1, 4–5, 11–14).

charges. She was admitted for inpatient rehabilitation at 4 years of age, when she was diagnosed with spastic diplegia (she was unable to sit or crawl) and a severe intellectual disability (eye contact and social smiling were apparent, but she used no meaningful words). Brain MRI performed at age 4 years was unremarkable. She exhibited no dysmorphic features, seizures, or visual symptoms. Presently, she still does not speak meaningful words but responds to her surroundings via eye contact and changes in facial expression. She can eat and drink independently. She exhibits truncal hypotonia and spasticity (principally in the lower extremities). Hip subluxation is being treated using a hip abduction brace. Her body weight, body length, and OFC are currently 17.3 kg (−1.2 SD), 110.0 cm (−1.3 SD), and 50.8 cm (+0.1 SD), respectively.

3.2. Genetic testing

WES identified de novo missense variants in *GNB1* (c.284 T > C, p.Leu95Pro, in Patient 1; c.221C > T, p.Ser74Leu, in Patient 2; and c.239 T > C, p.Ile80Thr, in Patient 3), which were confirmed by Sanger sequencing (Table 1). The p.Leu95Pro and p.Ile80Thr variants have been reported previously [1,5], whereas the p.Ser74Leu variant is novel. The in silico phenotypic predictions of c.221C > T (p.Ser74Leu) was determined to be "deleterious" using SIFT and "probably damaging" using PolyPhen2. This variant is absent from the Exome Aggregation Consortium, which contains data on 60,706 individuals from general populations. Therefore c.221C > T (p.Ser74Leu) is "likely pathogenic", according to the guideline of the American College of Medical Genetics [6]. No other pathogenic variant was noted.

3.3. Three-dimensional structure consideration of G-protein

The three substitutions found in this study are all located near the interaction interface with Gα (Fig. 1a, d) [7] and two effector proteins, GRK2 (Fig. 1e) [8] and GIRK2 (Fig. 1f) [9]. Fig. 1b shows the location of Ser74 in the three-dimensional structure of Gβ1 (Gβ) bound to Gα and Gγ. The side chain OH group of Ser74 forms hydrogen bonds with the main chain atoms of Lys57 and Asp76, stabilizing the local conformation of the two loops that contain Lys57 or Asp76 (Fig. 1b). Substituting Ser74 with leucine not only eliminates these stabilizing interactions but also results in severe steric clashes between the leucine and Lys57 main chain atoms, as indicated by the modeling analysis (Fig. 1c). Thus, the Ser74Leu variant may cause a destabilization of the protein and/or a significant conformational change near Ser74, the latter of which could

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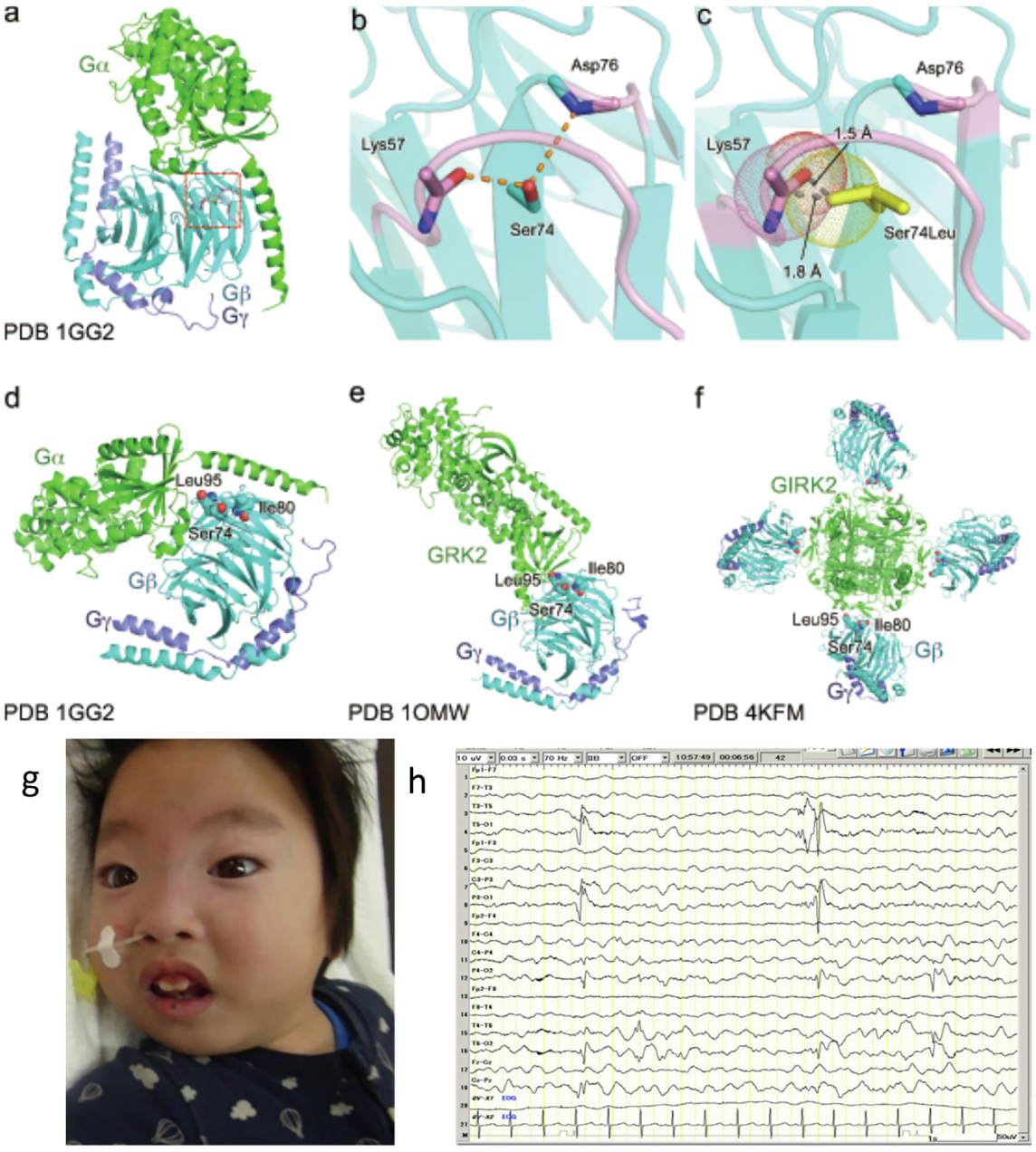

Fig. 1. Structures of Gβ1 (indicated as Gβ) in complex with Gα and Gγ, or Gγ and other factors (a-f), and photograph (g) and EEG (h) of patient 1. a) Structure of the Gαβγ trimer (PDB ID 1CG2). The Gα, Gβ, and Gγ subunits are colored green, cyan, and blue, respectively. The two loops containing Lys57 or Asp76 of Gβ are shown in pink. A dotted red box corresponds to the region shown in b. b) A close-up view of the Gβ Ser74 and nearby residues. c) Steric clash involving a modeled leucine at the position of Ser74 with nearby atoms. Steric clashes involving the Cδ1 atom of the substituted leucine are shown as dots with their interatomic distances indicated. d-f) Representative structures of complexes containing Gβγ. Ser74, Ile80, and Leu95 are shown as van der Waals spheres. d) The Gαβγ complex in a different view from a. e) The Gβγ-GRK2 complex with GRK2 colored green (PDB ID 1OMW). f) The tetrameric GIRK2 K+ channel (colored green) bound to one Gβγ heterodimer for each GIRK2 subunit (PDB ID 4KFM). g) Facial photograph at 2 years old showed carp-like lips and hypertelorism. The patient's parents gave permission to publish his image. h) EEG at 11 months exhibited independent irregular spikes in the bilateral occipital regions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

affect its interactions with partner proteins. As noted above, the Ile80 variant has been shown to affect the interactions between Gα and some effectors (such as GIRK), but not others (such as adenylyl cyclase 2) [10].

4. Discussion

To date, at least 24 pathogenic variants in *GNB1* have been described in the literature [1,4–5,11–14]. In

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this study, we added one novel pathogenic variant. Most germline mutations (40/50; 80%) are located in exons 6 and 7, encoding residues 76–118 involved in the Gα/Gβγ interaction in the G protein complex. The p.Ile80 substitution has been identified most frequently (14/50 patients; 28%), among which 12 patients have p.Ile80Thr (including one of ours) [1,5]. A previous study revealed that the p.Ile80 substitution significantly increases the inhibitory activity of a Gβγ-dependent calcium channel [10]. Of the 12 patients with the p.Ile80Thr mutation, 10 (83%) are unable to walk (Table 1). Interestingly, all 12 patients exhibited severe infantile axial hypotonia or hypotonic quadriplegia, and 4 of these patients later developed hypertonia or spasticity of the lower legs and 3 dystonic hypertonia, respectively. Although spastic paraplegia or diplegia is relatively rare among patients with *GNB1* variants, such variants may be considered as a causative gene for early-onset hereditary spastic paraplegia (HSP) [15]. On the other hand, hypotonic quadriplegia is a phenotype of cerebral palsy [16]. As 77% of patients with *GNB1* variants exhibit persistent hypotonic characteristics, such variants may be responsible for developmental and epileptic encephalopathy associated, in particular, with hypotonic cerebral palsy.

Four patients with the p.Leu95Pro variant are known, including one of our patients [1,5]. All exhibit dysmorphic features, axial hypotonia, and dystonic hypertonia of the extremities, perhaps attributed to dyskinetic quadriplegia. No patient can talk or walk independently, and epilepsy developed in two patients. Nystagmus was observed in all, but continuous reverse ocular dipping was exclusively found in one of our cases (Patient 1). To the best our knowledge, 6 of 49 patients (12%) with *GNB1* variants exhibit cortical vision impairments, whereas 3 of 4 with the p.Leu95Pro variant (75%) exhibit cortical blindness; these findings are remarkable.

Patient 2 exhibited relatively better outcomes. His seizures were controlled and the last EEG was normal; his intellectual and motor abilities were relatively well-preserved. The following missense variants in exon 5 have been reported in four patients including our Patient 2: p.Arg52Gly, p.Gly53Glu, p.Gly64Val, and p.Ser74Leu [4–5]. Three-dimensional structural consideration revealed that the Ser74Leu variant may cause a destabilization of the protein and/or a significant conformational change near Ser74. Arg52 is located near the sites of interaction with Gα and effector molecules, and that Gly64 is within an inner β-propeller structure [4], changes in either residue cause loss of function [4]. However, these four patients exhibited relatively mild phenotypes (Table 1). It remains unclear whether the clinically milder phenotype of Patient 2 is attributed to well-controlled epileptic seizures or the gene variant per se.

As shown in structural consideration, it is possible that various Gβ1 variants have different effects on the binding profiles of Gβ1 to Gα and multiple effector proteins, which may underlie the various symptoms exhibited by patients. Further structural determinations of unsolved Gβγ-effector complexes and functional analyses on these variants should help our understanding of the molecular bases of the symptoms caused by the *GNB1* variants.

In conclusion we report three first Japanese patients with *GNB1* variants who exhibited various clinical phenotypes. Acute leukemia has been recently reported in a patient with de novo variant of *GNB1*, which will require close attention in the follow-up of patients [11]. Functional and genotype–phenotype correlation studies are required to develop future precision medicine.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.braindev.2019.10.006.

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