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Exome Reports

A *de novo GNB2* variant associated with global developmental delay, intellectual disability, and dysmorphic features

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Abstract

Heterotrimeric G proteins are composed of α, β, and γ subunits and are involved in integrating signals between receptors and effector proteins. The 5 human Gβ proteins (encoded by *GNB1*, *GNB2*, *GNB3*, *GNB4*, and *GNB5*) are highly similar. Variants in *GNB1* were identified as a genetic cause of developmental delay. D*e novo* variant in *GNB2* has recently been reported as a cause of sinus node dysfunction and atrioventricular block but not as a cause of developmental delay. Trio-based whole-exome sequencing was performed on an individual with global developmental delay, muscle hypotonia, multiple congenital joint contractures and dysmorphism such as brachycephalus, thick eyebrows, thin upper lip, micrognathia, prominent chin, and bilateral tapered fingers. We identified a *de novo GNB2* variant c.229G>A, p.(Gly77Arg). Notably, pathogenic substitutions of the homologous Gly77 residue including an identical variant (p.Gly77Arg, p.Gly77Val, p.Gly77Ser, p.Gly77Ala) of GNB1, a paralog of GNB2, was reported in individuals with global developmental delay and hypotonia. Clinical features of our case overlap with those of *GNB1* variants. Our study suggests that a *GNB2* variant may be associated with syndromic global developmental delay.

Keywords: *GNB2*, global developmental delay, whole-exome sequencing

Introduction

Whole-exome sequencing (WES) has identified genetic causes of global developmental delay, which is genetically heterogeneous. Studies have uncovered disorders caused by dysfunction of heterotrimeric guanine nucleotide-binding proteins (G proteins), which regulate signaling pathways by transducing extracellular signals from G protein-coupled receptors (GPCRs) to effector proteins, thereby regulating cellular function (Syrovatkina et al., 2016). Heterotrimeric G proteins are comprised of guanine nucleotide-bound Gα subunits and Gβγ subunits, with each subunit is encoded by a member of corresponding gene families (Syrovatkina et al., 2016).

Recently, pathogenic variants were identified in 5 human Gβ proteins. For example, *de novo* variants in *GNB1*, which encodes G protein β subunit 1, were identified as a genetic cause of developmental delay (Petrovski et al., 2016; Hemati et al., 2018), and *GNB5* nonsense variants were reported as a cause of an autosomal-recessive multisystem syndrome with sinus bradycardia and cognitive disability (Lodder et al., 2016). It was recently shown that a heterozygous variant (p.Arg52Leu) in *GNB2* causes dysfunction of sinus node and atrioventricular conduction in a large 3 generation family (Stallmeyer et al., 2017). More reports showing association between *GNB2* variants and human phenotypes will establish essential roles of *GNB2* in humans and delineate clinical features related to *GNB2* variants.

Here, we report an individual with global developmental delay, intellectual disability, and dysmorphic findings with a *de novo* missense variant p.(Gly77Arg) in *GNB2*, which is very likely to impair function of *GNB2*.

Clinical report

The patient is a Japanese girl who is the 5th child of non-consanguineous healthy parents. She has an elder brother and three younger sisters with no neurodevelopmental abnormality. She had no prenatal alcohol exposure. She was born by Caesarean section at 37 gestational weeks and 5 days because of placental dysfunction. Her birth weight, body length, and head circumference were 2,558 g (−0.6 standard deviation [SD]), 46.5 cm (−0.7 SD), 33 cm (0.1 SD), respectively. She cried weakly and experienced hypoxia after delivery. Her multiple joints were congenitally contracted. At birth, she had internally rotated shoulders and flexion contractures in elbows, knees, hips, and ankle plantar. Her upper arms contacted the chest. Wrists were extended and palms and fingers were firmly gripped with thumbs being positioned in palms. Torticollis was not shown. She also displayed brachycephaly and micrognathia. Muscle hypotonia was seen since infancy. Her developmental milestones were delayed: crawling at 26 months, and walking independently at 5 years. She begun to speak meaningful words at 2 years and begun to combine two words at 5 years and 6 months. Developmental quotient (DQ) at 5 years was 49 by Kyoto Scale of Psychological Development 2001, suggesting moderate developmental delay

The biochemical analyses of blood and metabolic screenings including amino acids, lactic acid, and pyruvic acid were unremarkable. Electroencephalogram (EEG) showed no paroxysmal discharges. Nerve conduction studies showed normal median motor nerve conduction velocities and normal amplitude of median compound muscle action potentials (CMAPs) (56.5 m/s and 6.9 mv, respectively), and did not suggest demyelinating peripheral neuropathy or axonal peripheral neuropathy. Brain magnetic

resonance imaging (MRI) and MR angiography (MRA) at age 4 years 7 months revealed no brain hypoplasia and no delayed myelination (Fig. 1A-C). Spine MRI, auditory brain stem response and echocardiogram were all normal. Electrocardiogram showed all the important intervals, including PR interval within normal limits. G-banded analysis and array CGH analysis showed normal findings (46,XX).

Arthrogryposis has been gradually improving with physical therapy. The final physical examination at 7 years 6 months showed a body weight of 23.6 kg (-1.0 SD), height 116.8 cm (-0 SD), and head circumference 50 cm (10th percentile). She showed cranial and facial dysmorphism with brachycephalus, thick eyebrows, thin upper lip, micrognathia, prominent chin, and dental crowding (Fig. 1E, F). She had exotropia, tapered fingers, hyperextensibility of finger joints, and hypertrichosis in her back. She had muscle hypotonia, ankle planter flexion contracture and supine lordosis. Her deep tendon reflexes were normal. X-ray revealed small intermediate phalanx of bilateral fifth fingers (Fig. 1D). She never had epileptic seizures until her last visit.

Methods

This study was approved by the Institutional Review Board Committee at Hamamatsu University School of Medicine. After receiving the written informed consent, the genomic DNA were extracted from the patient and her parents. Data processing, variant calling, annotation, and filtering were performed essentially as previously described (Hiraide et al., 2018). Briefly, trio-based WES was performed using the SureSelectXT Human All Exon v6 (Agilent Technologies, Santa Clara, CA) with Illumina NextSeq 500 (Illumina, San Diego, CA). Reads were aligned to reference genome (GRCh37) using BWA (Version

0.7.12). Variants were identified by GATK Version 3.5. The final variants were annotated with Annovar (Kircher et al., 2014) for predictive value of functional impact of the coding variants. We focused on rare variants with minor allele frequencies below 1% in the following databases (in-house 218 exomes, EAS data in gnomAD [http://gnomad.broadinstitute.org/], Human Genetic Variation Database (HGVD) [http://www.hgvd.genome.med.kyoto-u.ac.jp/] and allele frequency data of 2,049 Japanese individuals (2KJPN) [https://ijgvd.megabank.tohoku.ac.jp/download_2kjpn/]). At least 96.8% of target RefSeq coding sequences were covered by 20 reads or more in trio samples.

Results

We identified one *de novo* variant, and no compound heterozygous or homozygous variants among rare nonsynonymous variants. A *de novo* variant in *GNB2* (NM_005273.3: c.229G>A, p.(Gly77Arg)), which was validated by Sanger sequencing (Fig. 1G). This variant was absent in our 218 in-house Japanese control exomes and from public databases including the gnomAD, HGVD, and 2KJPN. This substitution showed a Grantham score of 125 and was predicted to be deleterious by multiple *in silico* pathogenicity prediction tools: CADD PHRED score was 31 (http://cadd.gs.washington.edu/snv), PP2_HVAR score was 0.921 (http://genetics.bwh.harvard.edu/pph2/), and SIFT score was 0 (http://sift.jcvi.org/) (Supplemental Table S1). Notably, pathogenic substitutions of the homologous Gly77 residue, including the identical variant and pathogenic variants affecting adjacent residues of GNB1 had been reported in patients with global developmental delay and hypotonia

(Fig. 1H) (Brett et al., 2017; Hemati et al., 2018; Petrovski et al., 2016; Szczałuba et al., 2018). Both *GNB1* and *GNB2* encode for 340 amino acids, and GNB1 and GNB2 proteins exhibit 90% identity. Therefore, the p.Gly77Arg variant is very likely to affect function of *GNB2*. This c.229G>A variant of *GNB2* was submitted to the Leiden Open Variation Database (https://databases.lovd.nl/shared/genes/GNB2, variant number: #0000578276).

Discussion

There are five human Gβ proteins encoded by five different *GNB* genes. Three *GNB* genes (*GNB1*, *GNB2,* and *GNB4*) are paralogs and share 90% identities in amino acid sequences (Gautam et al., 1998). Among them, *GNB1* and *GNB4* heterozygous variants have been associated with global developmental delay and Charcot-Marie-Tooth disease (CMT), respectively, suggesting important roles of these paralog genes in the human nervous system (Petrovski et al., 2016; Soong et al., 2013). *GNB1* and *GNB2* showed moderately high Z score for missense variants in gnomAD (3.86 and 3.1, respectively) and high pLI score for loss-of-function variants (both 1.0), suggesting that *GNB2* may be intolerant to pathogenic variants in humans.

The G-protein β subunit forms a propeller-like structure containing 7 blades. Each blade is made by β-sheet of 4 antiparallel β strands, and this Gβ protein surface interacts with Gα subunits and downstream effectors in the G protein complexes (Gautam et al., 1998). Pathogenic *GNB1* variants are clustered in the Gα-Gβγ binding region (Petrovski et al., 2016), including the Gly77 residue (Supplemental Fig. S1). Therefore, the p.Gly77Arg variant in GNB2 is likely to affect inhibition or activation of the downstream effector proteins.

159 Recently, a heterozygous *GNB2* variant (p.Arg52Leu) was reported in a large 3
160 generation family with dysfunction of sinus node and atrioventricular conduction
161 (Stallmeyer et al., 2017). In this report, we identified a *de novo GNB2* variant in a sporadic
162 case of global developmental delay and intellectual disability. *GNB2* is widely expressed
163 in human tissues including brain and cardiac sub-compartments (Stallmeyer et al., 2017).
164 It was reported that Gβ2 protein had important roles for neuronal migration, acting as a
165 scaffold protein to organize the extracellular signal-regulated kinase 1/2 (ERK1/2)
166 signaling pathway (Guo et al., 2017). Thus, we suggest that *GNB2* could play a role in
167 the central nervous system, and *GNB2* dysfunction caused global developmental delay,
168 intellectual delay, and central hypotonia in our case.
169 Our case also showed arthrogryposis, which is defined as multiple congenital
170 contractures due to decreased fetal movement. Congenital contractures in our case were
171 recognized both in distal and proximal. As growing up, her congenital contractures has
172 been gradually improving, and muscle hypotonia became more distinct. Hypotonia may
173 be related to arthrogryposis. Several genes causing arthrogryposis were also reported to
174 cause hypotonia like CMT, and *GNB4* variants cause CMT diseases, suggesting that
175 arthrogryposis in our case might be associated with peripheral nerve impairment (Soong
176 et al., 2013; Hall and Kiefer, 2016; Yoshimura et al., 2019). However, nerve conduction
177 studies showed normal findings in our case, suggesting that peripheral nerve impairment
178 are less likely. Many neurodevelopmental syndromes are known to cause multiple
179 congenital contractures (Hall, 2014). Thus arthrogryposis in our case seems to be caused
180 by fetal akinesia which is related to central nervous system dysfunction.
181 Another possible mechanism for *GNB2* variant causing hypotonia is altering

182 neuromuscular synapses. Gβ1 and Gβ2 were reported to be localized to the post-synaptic
183 compartment in skeletal muscle, suggesting that Gβ-mediated signaling such as ERK1/2
184 pathway may be important for synapse formation and/or function (Lok et al., 2007).
185 Therefore it might be possible that the *GNB2* variant would also cause impairment of
186 neuromuscular function through altered neuromuscular synapses.

187 The clinical findings of our case with *GNB2* variant and the individuals having *GNB1*
188 variants are summarized in Table S2 (Brett et al., 2017; Hemati et al., 2018; Lohmann et
189 al., 2017; Petrovski et al., 2016; Steinrücke et al., 2016; Szczałuba et al., 2018). All of
190 them had global developmental delay. Intellectual disability, muscle hypotonia, and
191 dysmorphic findings are reported in more than 70% of patients with *GNB1* variants, and
192 these features were seen in our case. Facial dysmorphism was the most common, and
193 similar to our case, 24% of patients with *GNB1* variants showed dysmorphism of hands
194 and feet, among which the tapered fingers were observed in two individuals with *GNB1*
195 variants. Strabismus is recognized in 24% of cases in *GNB1* variants, and exotropia (a
196 kind of strabismus) was found in our case. These findings suggest that clinical features
197 related to *GNB2* variants might largely overlap with those of *GNB1* variants.

198 Our report suggests that *GNB2* is essential for normal brain function in addition to heart
199 function in humans. Consistent with this, a mutant mice (*Gnbem1*$^{(IMPC)J}$) showed
200 abnormal behavioral response to light, and increased heart rate and shortened RR, PQ,
201 and ST interval (http://www.mousephenotype.org/data/genes/MGI:95784). Although
202 node dysfunction was not observed in our case, electrocardiogram check should be kept
203 in mind.

204 In conclusion, we identified a case of a *de novo GNB2* variant with global

developmental delay, intellectual disability, and dysmorphic findings. Our case suggests that *GNB2* may be one of the candidate genes for syndromic global developmental delay. However, further accumulation of cases will be necessary for establishing the role of *GNB2* in global developmental delay.

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Conflict of interest

The authors declare no conflict of interest.

Figure Legends

Figure 1. Cranial and digital features and the *GNB2* variant in the patient

(A-C) Brain MRI taken at 4 years and 2 months of age. (A) Coronal image in fluid attenuated inversion recovery, (B) axial image in T1-weighted image, and (C) axial images in T2-weighted image reveal no delayed myelination or abnormality. (D) X ray taken at 7 years and 6 months shows small intermediate phalanx of bilateral fifth fingers. (E, F) Photos taken at 6 years and 4 months of age show thick eyebrows, thin upper lip, micrognathia, prominent chin and brachycephalus. (G) Sanger sequencing of the *GNB2* showed a *de novo* heterozygous missense variant (NM_005273.3: c.229G>A). (H) Amino

228 acid alignments between *GNB2* and *GNB1* were analyzed by ClustalW tool. The amino
229 acid sequences of the two proteins are identical in this interval. The identified GNB2
230 variant is highlighted in upper line, and the previously reported variants of GNB1 are
231 highlighted in the lower line (Brett et al., 2017; Hemati et al., 2018; Petrovski et al., 2016;
232 Szczałuba et al., 2018)
233
234

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Supplemental material list

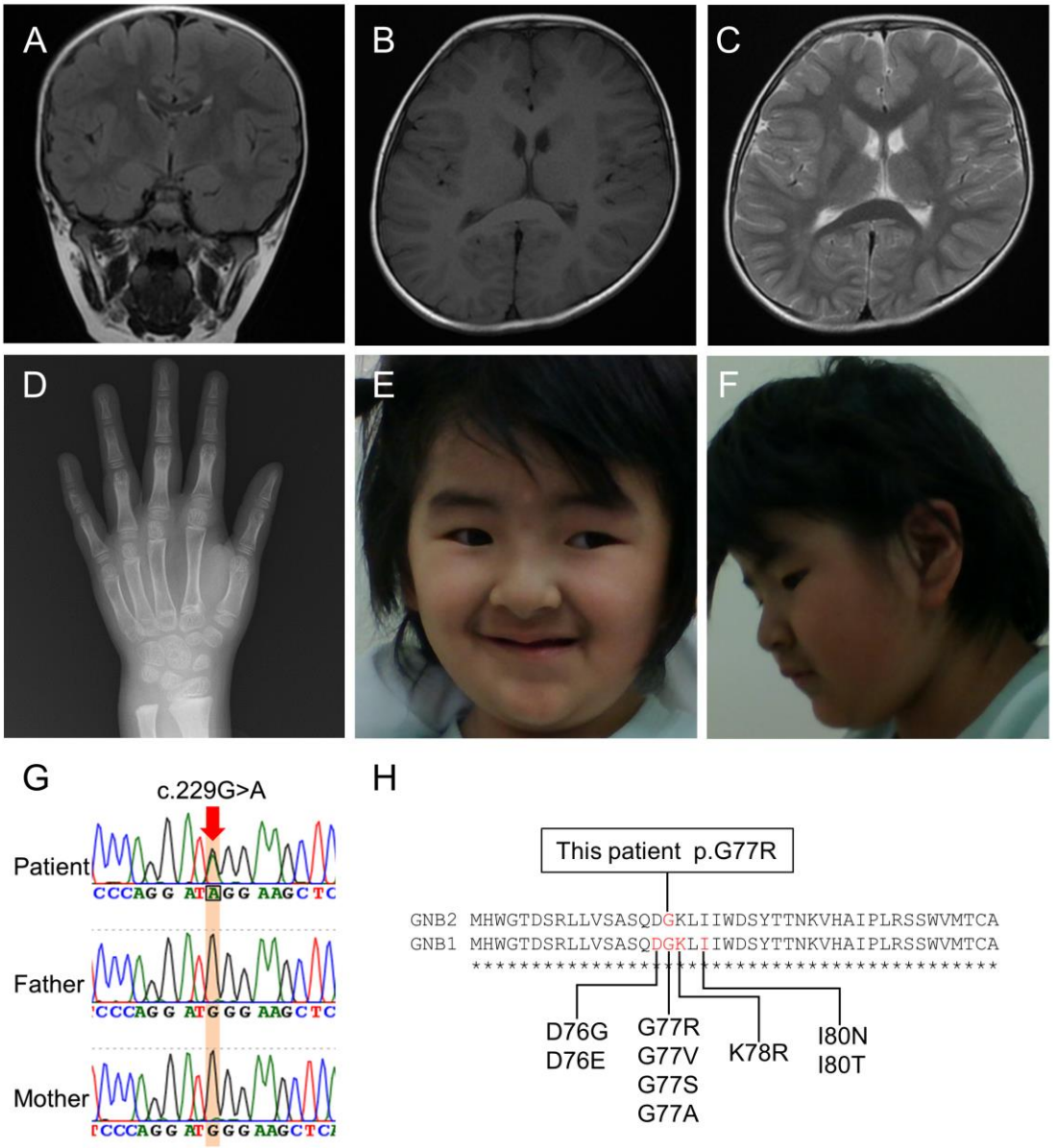
1. Supplemental data

Supplemental Table S1. Candidate variants identified by Trio-WES

Supplemental Table S2. Clinical findings of cases with *GNB2* and *GNB1* variants

Supplemental Figure S1. The structure of the G protein heterotrimer Gi alpha 1 beta 1 gamma 2

2. Supplemental Statement. Permission to publish identifiable pictures


A
B
C
D
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G
c.229G>A
Patient
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Father
CCCAGG ATGGG AAGCTC
Mother
CCCAGG ATGGG AAGCTC
H
This patient p.G77R
GNB2 MHWGTDSRLLVSASQDGKLIIWDSYTTNKVHAIPLRSSWVMTCA
GNB1 MHWGTDSRLLVSASQDGKLIIWDSYTTNKVHAIPLRSSWVMTCA
D76G
D76E
G77R
G77V
G77S
G77A
K78R
I80N
I80T

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341

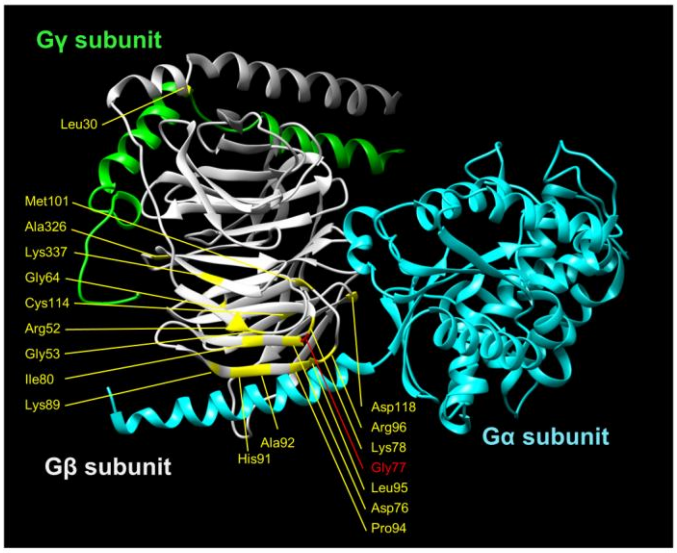
Table S1. Candidate variants identified by Trio-WES

Chr	Gene	Variant	Origin	dbSNP138	gnomAD EAS	SIFT	PP2 HVAR	CADD phred	M-CAP	GERP	Phast Cons	Grantham score
7	*GNB2*	NM_005273.3:c.229G>A, p.(Gly77Arg)	de novo	−	−	0	0.921	31	0.108	5.27	1	125

Table S2. Clinical findings of cases with *GNB2* and *GNB1* variants. (Brett et al., 2017; Hemati et al., 2018; Lohmann et al., 2017; Petrovski et al., 2016; Steinrücke et al., 2016; Szczałuba et al., 2018)		
Case	*GNB2* varint	*GNB1* variants
	n=1	n=46
Global developmental delay	Yes	46/46 (100%)
Growth delay	No	10/46 (22%)
Muscle hypotonia	Yes	34/46 (74%)
Intellectual disability	Yes (DQ 49)	32/46 (70%)
Seizures	No	23/46 (50%)
Microcephaly	No	1/33 (3%)
Craniosynostosis	No	3/ 13 (23%)
Cerebellar hypoplasia	No	3/46 (7%)
Abnormal myelination	No	9/46 (20%)
Ophthalmoplegia	No	4/46 (9%)
Nystagmus	No	16/46 (35%)
Strabismus	Yes Exotropia	8/33 (24%)
Ataxia	No	4/43(9%)
Chorea	No	3/43(7%)
Dystonia	No	8/46 (17%)
Abnormal EEG	No	18/33 (55%)
Abnormal ECG	No	NA
Abnormal echocardiogram	No	2/18 (11%)
Dysmorphic findings	Yes	24/34 (71%)
Autistic behavior	No	3/31(10%)

ECG Electrocardiogram, EEG Electroencephalogram, NA not assessed or not available

Figure S1



Supplemental Figure S1.
The structure of the G protein heterotrimer Gi alpha 1 beta 1 gamma 2 is shown based on a crystal structure (PDB: ID 1GP2) (Gα, cyan; Gβ, grey; Gγ, green). Previously reported substituted residues of the GNB1 are indicated in yellow (Brett et al., 2017; Hemati et al., 2018; Lohmann et al., 2017; Petrovski et al., 2016; Steinrücke et al., 2016; Szczałuba et al., 2018). An affected residue p.Gly77 in GNB2, which is homologous to the p.Gly77 of GNB1, is indicated in red.