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Refining the phenotype associated with *GNB1* mutations: clinical data on 18 newly identified patients and review of the literature

Running title: Refining the phenotype associated with *GNB1* mutations

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Abstract:

De novo germline mutations in *GNB1* have been associated with a neurodevelopmental phenotype. To date, 28 patients with variants classified as pathogenic have been reported. We add 18 patients with *de novo* mutations to this cohort, including a patient with mosaicism for a *GNB1* mutation who presented with a milder phenotype. Consistent with previous reports, developmental delay in these patients was moderate to severe, and more than half of the patients were non-ambulatory and non-verbal. The most observed substitution affects the p.Ile80 residue encoded in exon 6, with 28% of patients carrying a variant at this residue. Dystonia and growth delay were observed more frequently in patients carrying variants in this residue, suggesting a potential genotype-phenotype correlation. In the new cohort of 18 patients, 50% of males had genitourinary anomalies and 61% of patients had gastrointestinal anomalies, suggesting a possible association of these findings with variants in *GNB1*. In addition, cutaneous mastocytosis, reported once before in a patient with a *GNB1* variant, was observed in three additional patients, providing further evidence for an association to *GNB1*. We will review clinical and molecular data of these new cases and all previously reported cases to further define the phenotype, and establish possible genotype-phenotype correlations.

Keywords: *GNB1*, whole exome sequencing, developmental disabilities, seizures, hypotonia, mastocytosis, mosaicism

Introduction:

Missense, splice-site and frameshift variants in the *GNB1* (guanine nucleotide-binding protein, beta 1) gene have been associated with a broad neurological phenotype [Brett et al., 2017; Lohmann et al., 2017; Petrovski et al., 2016; Steinrucke et al., 2016; Szczaluba et al., 2017]. *GNB1* encodes Gβ1, a G protein β subunit, which forms heterotrimeric complexes with subunits α and γ that are encoded by the *GNA* and *GNG* gene families, respectively. G proteins are responsible for signaling from transmembrane G protein-coupled receptors (GPCRs) to a variety of intracellular and membrane effector proteins [Syrovatkina et al., 2016].

The phenotype associated with germline *GNB1* variants was first described by Petrovski et al., [2016]. Thirteen patients with germline *de novo* missense variants were described in this paper. The reported phenotype included global developmental delay (13/13), hypotonia (11/13), seizures (10/13), ophthalmological anomalies (9/13), and persistent growth delay (6/13).

In the same year, Steinrucke et al., [2016] reported on an additional patient with a novel germline *de novo* missense variant in *GNB1* with dystonia, hypotonia, and intellectual disability. Shortly thereafter, Brett et al., [2017] reported a novel germline *de novo* missense variant in a patient with acute lymphoblastic leukemia, as well as global developmental delay, hypotonia, dystonia, cleft palate and left convergent strabismus.

More recently, Lohmann et al., [2017] reported on 16 patients with *GNB1* mutations, including the patient reported by Steinrucke et al., [2016]. Twelve patients had missense variants, two had frameshift variants, and two had splice site variants. Three of the detected missense variants were deemed to be possibly benign based on functional studies assessing the trimeric G protein complex formation and signaling by BRET-based cellular assays [Lohmann et al., 2017]. Among the 12 cases with functionally confirmed pathogenic variants in the BRET

assay, excluding the patient previously reported by Steinrucke et al., [2016], 12 had global developmental delay, seven had seizures, and five had eye anomalies [Lohmann et al., 2017]. Seven of these patients had *de novo* variants, and three had a variant that was inherited in an autosomal dominant manner. Mode of inheritance could not be determined for two cases. No definite genotype-phenotype correlation was established. Very recently, Szczaluba et al., [2017] reported on an additional patient with a novel germline *de novo* missense variant in *GNB1* who presented with major developmental delay, hypotonia, and cutaneous mastocytosis.

In this paper, we describe 18 additional patients with pathogenic *de novo* variants in *GNB1*, bringing the total number of known patients to 46. Our cohort includes a patient with mosaicism for a *GNB1* mutation who presented with a milder phenotype. We will review clinical and molecular data of these new cases (Results section and Tables 1, 2 and 4), and compare our cases to all previously reported cases (Discussion section and Table 3), with the goal of further defining the phenotype associated with germline *GNB1* variants and establishing genotype-phenotype correlations.

In addition to previously described features such as developmental delay, hypotonia and seizures, there is evidence of an increased risk for mastocytosis and cleft palate in individuals with *GNB1* variants, as well as genitourinary anomalies in males.

Material and Methods:

Patients 1, 2 and 3 were identified in the Laboratory of Personalized Genomic Medicine (PGM) at Columbia University Medical Center. Patients 4, 5, 7, 8, 9, 10, 13, 15, 16, 17 and 18 were ascertained via direct contact made by clinicians and/or families with the authors of the Petrovski et al., [2016] paper. Patients 12 and 14 were ascertained via contact with clinical

laboratory GeneDx. DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources) was also utilized to contact physicians of patients 6 and 11 [Firth et al., 2009].

Trio whole exome sequencing was performed for 18 families to identify candidate variants using standard methods. Informed consent was obtained from each family prior to testing, and approval of the ethics board was obtained in each institution. Detailed description of materials and methods used in each institution is available in the Supplementary Materials section.

Somatic whole exome sequencing was performed on skin biopsy samples from the monozygotic twin patients (Patients 1 and 2) with cutaneous mastocytosis with special attention to the *KIT* and *JAK2* genes. Full details regarding methods used are available in the Supplementary Materials section.

Relevant papers describing patients with *GNB1* variants were identified via thorough literature review that included PubMed (https://www.ncbi.nlm.nih.gov/pubmed), OMIM (Online Mendelian Inheritance of Man, http://www.omim.org/), and HGMD (Human Gene Mutation Database, http://www.hgmd.cf.ac.uk/ac/index.php).

Results:

Molecular Findings:

A *de novo* variant in the *GNB1* gene was detected in each of the 18 patients we have described in this report (GenBank: NM_002074.4). Previously reported variants observed in our cohort included p.Ile80Thr (in eight patients), p.Leu95Pro (in two patients), p.Asp118Gly (in two patients), and p.Lys78Arg (in one patient) [Brett et al., 2017; Lohmann et al., 2017;

Petrovski et al., 2016; Steinrucke et al., 2016]. Novel variants included p.Cys114Tyr, p.Ala92Asp, p.Lys89Arg, p.Gly53Glu, and p.Gly77Arg, which were each observed in one patient. The p.Cys114Tyr variant was found in 29 of 163 reads for Patient 3, representing an 18% allelic fraction and suggesting somatic mosaicism in this patient. Detailed variant information is included in Tables 1 and S1 (Supplementary Material).

Distribution of case-reported GNB1 missense variants:

The distribution of variants observed in all patients (our cohort plus previously reported cases) is shown in Figures 1 and 2. The gnomAD Missense Tolerance Ratio (MTR) data for the overall intolerant *GNB1* gene is available in Figure 1A. Using all 45 case-reported missense variants (those reported in this paper and in previous publications) and MTR based on standing variation data in the gnomAD database, version 2.0 [Traynelis et al., 2017], we found that 38 of the 45 (84%) case-ascertained missense variants reside among the 50% most missense intolerant sequence of the overall intolerant *GNB1* gene (Binomial exact test comparing to 50% median expectation; $p = 3.1x10^{-6}$). These results highlight the regionalization of pathogenic *GNB1* missense variants among *GNB1* sequence known to least tolerate missense variation in the general population.

Phenotypic Findings:

Phenotypic details are provided for the 18 new patients in our cohort in Tables 1 and 2 and are discussed below. Clinical assessment was performed from age two to 16 years. Full clinical summaries for these patients are available in the supplemental material. Identical twins (Patients 1 and 2) have been counted as two separate patients in the evaluation of clinical features because they present with slightly different phenotypes.

Please refer to Table 3 and the Discussion section for a review of phenotypic findings across papers.

Pregnancy, delivery and neonatal period

In seven patients, pregnancy and labor were uncomplicated and delivery was via normal vaginal route (Table 1). Pregnancy for Patient 6 was complicated by hydrops, but labor and delivery were uncomplicated. Nine of 18 patients (56%) were delivered via C-section; either elective, due to breech presentation (in two patients), fetal bradycardia (in two patients), decreased fetal movements (in one patient), lack of progression of labor (in one patient), or maternal vaginal bleeding (in one patient). The neonatal period was without severe complications for most patients in our cohort, but poor feeding and weight gain in the neonatal period were reported in Patients 4, 5, 6, 7, 13, 16 and 17.

Neurodevelopment

All patients in our cohort had moderate to severe neurodevelopmental deficits (Table 2). Patients were above two years of age at time of assessment. Overall, all patients except for Patient 3 had motor delay (17 of 18, or 94%). The Gross Motor Function Classification System (GMFCS) was used to further characterize motor delay (Table 2) [Palisano et al., 1997; Rosenbaum et al., 2002]. Except for Patient 14 (who walked with poor balance at 2.5 years) and Patient 3 (see below), none were able to walk independently. None of the patients in our cohort exhibited developmental regression.

All the patients in our cohort exhibited some degree of speech delay, with 13 of 18 (72%) being nonverbal at an age range of two years to 12 years. Expressive language was more severely affected than receptive language in the five patients who acquired speech, with vocabulary limited to a few words.

From a developmental standpoint, Patient 3 was the least severely affected patient in our cohort. Her milder phenotype could be attributed to potential somatic mosaicism. She started walking and talking at 12 months of age and was able to walk without assistance. Her language development was more affected than her motor development: her speech was limited to 30-40 words and was able to communicate only in short phrases at seven years old. She receives speech and language therapy in school through a special education class.

Brain MRI studies have been conducted on all 18 patients in our cohort, and various abnormalities were detected in 11 patients (Table 1). Abnormal or delayed myelination, abnormal corpus callosum, cerebral volume loss, and ventriculomegaly were each observed in at least two patients.

Six of 18 (33%) patients in our cohort had a history of epilepsy, with an age of onset of two to 10 years. Patient 13, who is currently 5 years old, had only one seizure episode at two years. Patient 16 had EEG epileptiform abnormalities but no clinical seizures.

Neuromuscular system

Moderate to severe hypotonia was the most common clinical feature observed in infancy, and was present in 15 out of 18 (83%) patients in our cohort. In addition to the persistence of hypotonia, our data demonstrates that hypertonia and spasticity may develop over time. These patients tend to show low axial tone but high appendicular tone. We observed this pattern in eight patients (55%), some of whom had developed hamstring shortening, valgus deformity of the feet, and contractures.

In addition, we observed dystonia in four of 18 (22%) patients in our cohort. Patients 4, 5 and 8 were noted to have both spasticity and dystonia.

Growth

Seven of 18 patients (38%) in our cohort had poor feeding and weight gain in the neonatal period. However, there were no patients with persistent growth delay (with both weight and height at least two standard deviations below the mean at the time of their last measurements). Patient 4 is underweight (-3.2 SD) but has normal height, and was therefore not considered to have growth delay.

Craniofacial

Consistent with observations from previous publications, we did not observe distinctive dysmorphic features in our cohort. Most patients either lacked or had very mild features with no commonalities. Two of 18 patients in our cohort were born with a cleft palate. We did not observe other types of craniofacial abnormalities in our cohort.

Vision and hearing

Nine of 18 (50%) patients had abnormal vision. Eight patients had strabismus, nystagmus, or both. In addition to nystagmus, Patient 16 was noted to have possible optic atrophy, cortical visual impairment, and was legally blind. Patient 6 was also noted to have cortical blindness.

Hearing impairment was observed in two patients in our cohort: bilateral sensorineural hearing loss in Patient 16 and unilateral sensorineural hearing loss in Patient 14. Additionally, Patient 14's brain MRI was significant for hypoplasia of the right cochlear nerve.

Cardiovascular

Two of 18 (11%) patients had congenital cardiovascular defects: Patient 6 had a ventricular septal defect (VSD) and Patient 9 had a duplicated superior vena cava.

Gastrointestinal

Eleven of 18 (61%) patients presented with gastrointestinal symptoms, with recurrent constipation in seven (38%). Cyclical vomiting, gastroesophageal reflux, and distended abdomen with cramps were each observed in at least two patients.

Genitourinary

Genitourinary anomalies were detected in four of the eight male (50%) patients in our cohort: three had undescended testes, and one had bifid scrotum in addition to duplicated collecting system.

Hematologic/immunologic system

We identified cutaneous mastocytosis, also known as mast cell disease, in three patients in our cohort: identical twins (Patients 1 and 2) and Patient 13. Somatic whole exome sequencing on fibroblasts from Patients 1 and 2 did not identify any additional qualifying variants or regions of loss of heterozygosity. No somatic variants in *JAK2* and *KIT* were detected. Refer to Supplementary Section for additional details.

Discussion:

The distribution of variants observed in all 46 patients (our cohort plus previously reported cases) is shown in Figures 1 and 2. The substitution of the isoleucine residue at amino acid position 80 was the most commonly observed variant in our cohort and previously reported patients, with 13 of 46 (28%) patients carrying either p.Ile80Thr or p.Ile80Asn. These substitutions affect a Gβ residue important for inhibition of calcium channels, activation of potassium channels, activation of phospholipase C-β2, and Gα binding [Ford et al., 1998; Petrovski et al., 2016]. Overall, 10 variants identified affect a residue coded for in exon 6, and 11 variants affect a residue coded in exon 7 (Figure 1B). Most germline mutations identified so far

reside in exons 6 and 7 of the gene, which encode the majority of residues pertinent for the interaction between Gα and Gβγ in the G protein complex (Fig 1A- MTR plot) [Ford et al., 1998]. Only three variants reported so far affect a residue coded for by exon 5 (Figure 1B). The region of exons 6 and 7 encoding residues 76 to 118 represents only 12.6% of the total *GNB1*-coding sequence, further suggesting the importance of this region for Gβ1 function (Table S1, Supplementary Material). Moreover, 17 of 46 patients (36%) have a variant that overlaps with one of the five recurrent oncogenic amino-acid residues (Lys57, Lys78, Ile80, Lys89 and Met101) reported by Yoda et al., [2015]. All five of these residues are located along the Gβ protein surface that interacts with Gα subunits and downstream effectors (Figure 2).

Table 3 provides a summary of clinical findings of patients in our cohort as well as previously reported patients. All patients (46/46 or 100%) had developmental delay. Severe neurodevelopmental deficit, marked by an inability to walk independently, was present in 14 of 29 (48%) patients for whom this skill was documented. The patient reported by Brett et al., [2017] started walking independently at nine years of age, suggesting that patients younger than nine years could potentially improve with intensive physical therapy over time and gain independent ambulation in the future. Speech delay was a common finding across cohorts (30 of 46, or 65%). In patients for which this skill was documented, 18 of 30 (60%) were nonverbal and 12 of 30 (40%) had speech delay. Improved communication with sign language was documented in two patients with normal hearing, highlighting the potential benefit of sign language in these patients.

Seizures have been reported in 23 of 46 (50%) of patients across studies. Table 4 summarizes available data on epilepsy manifestation in the six new patients and those reported by Petrovski et al., [2016], as more detailed information regarding seizures was available for this

cohort. A variety of epilepsy types were reported, including generalized, focal, mixed as well as infantile spasms. Consistent with previous reports, abnormalities on brain MRI (24 of 46, or 52%) and hypotonia (36 of 46, or 78%) were common findings in all cohorts (Table 3). Overall, eight of 46 (17%) patients with *GNB1* variants have developed dystonia. Of these patients, five (62%) carry a p.Ile80 substitution, consistent with the possible genotype-phenotype correlation initially suggested by Petrovski et al., [2016].

The non-neurologic and growth phenotypes seen in patients with *GNB1* variants were not consistently documented across publications. Poor feeding and weight gain in the neonatal period were present in 11 of 20 (55%) patients across studies. Of these 11 patients, eight outgrew their feeding difficulties and poor weight gain. Overall, persistent growth delay was reported in 10 out of 46 (21%) of all patients. Petrovski et al., [2016] suggested a possible genotype-phenotype correlation between a p.Ile80 substitution and growth delay in their cohort. In our cohort, among seven patients who exhibited growth delay in the neonatal period, two carry the p.Ile80Thr variant. Overall, from the 13 patients reported with a p.Ile80 substitution across papers, five (38%) showed persistent growth delay. In contrast, only five of 33 (15%) patients with all other variants combined were reported with growth delay.

The presence of dysmorphic features was documented in 24 of 34 (70%) of patients, but taken together these features are inconsistent. Five of 34 (14%) patients across studies were born with a cleft palate. Abnormal vision (strabismus, nystagmus, optic atrophy and cortical visual impairment) were reported in 27 of 46 (58%) patients, making these features common across all studies. We observed a high percentage of patients in our cohort (11 of 18, or 61%) with gastrointestinal problems such as recurrent constipation, cyclical vomiting, gastroesophageal reflux, and distended abdomen with cramps. These problems were not reported in previous

publications. Genitourinary anomalies were present in six of 17 (35%) males for whom this information was available. Sensorineural hearing loss was present in three of 46 (6.5%) patients in this report, a figure that is far greater when compared to the approximate prevalence of hearing loss of more than 40 decibels (dB) of one per 1000 children in population-based studies of children in Europe and North America (https://www.cdc.gov/ncbddd/hearingloss/data.html). Cardiovascular defects were observed in only 2 of 18 (11%) patients in our cohort. This frequency is higher than the 1% incidence reported in the general pediatric population [Hoffman and Kaplan 2002] and therefore may be linked to *GNB1* variants.

Cutaneous mastocytosis has been reported in four of 34 (11%) patients across studies. The three patients with mastocytosis in the current cohort (patients 1, 2 and 13) carry the p.Ile80Thr variant. This variant was highlighted by Yoda et al., [2015] as one of eight recurrent mutations associated with hematologic transformation identified in tumor sequencing collections. Yoda et al., [2015] also showed that the mutant p.Ile80Thr protein had reduced binding to Gα subunits by using tandem affinity-purification and mass-spectrometry analyses. The addition of these three patients to the one patient reported previously by Szczaluba et al., [2017], provides further evidence for a link between mastocytosis and *GNB1* due to G protein beta subunit activation and abnormalities in GPCR downstream signaling [Szczaluba et al., 2017]. The exact prevalence of mastocytosis in the general population has not been established, but a recent population-based Danish study estimated the prevalence to be approximately 1 per 10,000, or 0.01% [Cohen et al., 2014]. Although the number of cases of mastocytosis from all *GNB1* studies combined is small, it yields a prevalence of 3 of 46, or 6.52%, which is greater than the estimate from the study of Cohen et al. (2014).

Brett et al., [2017] reported on a patient with germline *de novo* p.Gly77Ala variant with acute lymphoblastic leukemia (ALL). Somatic mutations affecting the same residue have been previously detected by Yoda et al., [2015] in patients with hematologic malignancies. This report provided further support for the concern raised by Petrovski et al., [2016] regarding the possibility of the association of germline *GNB1* mutation carriers and an increased risk for hematologic malignancies. While we saw no cases of malignancies among our cohort, we agree that until additional information is available, surveillance for hematologic malignancies via periodic blood counts should be considered in follow up of *GNB1* mutation carriers.

Patient 3 in our cohort was found to carry the p.Cys114Tyr variant in 18% of reads from a blood sample. There are, however, interpretation challenges surrounding this specific finding as *GNB1* has also been described as a mitotically mutable oncogene [Yoda et al., 2015]. As we were unable to obtain and test an alternate tissue sample from Patient 3, interpreting causality of this specific *GNB1* somatic mutation is more challenging than other disease genes due to the possibility that somatic mutation could potentially reflect a blood-restricted hematopoietic clonal expansion.

In this paper, we excluded the three patients reported in Lohmann et al., [2017] with considered possibly benign variants based on functional studies. The BRET results presented in that paper showed variable alteration of coupling to dopamine D1 receptor associated with $G\alpha_{olf}/G\beta1/G\gamma7$, with some mutants showing reduced BRET response following D1R activation. However, the relevance of $G\alpha_{olf}$ and $G\gamma7$ is limited mainly to the striatum where they regulate ADCY5 and motor functions [Corvol et al., 2001; Fuchs et al., 2013; Schwindinger et al., 2003]. Thus, it may be premature to judge as benign the three variants that did not affect the BRET response, as they may have effects on other GPCR/$G\alpha\beta\gamma$ combinations. However, since no

definite evidence regarding the pathogenicity of those three variants exists at this point, we decided to exclude those patients.

Conclusion:

We report on 18 new patients found to have a pathogenic variant in *GNB1*, raising the total number of reported patients with *GNB1* variants to 46 (excluding patients with variants that were deemed benign by Lohmann et al., [2017]). Our data provides further evidence for the correlation between *GNB1* gene mutations and severe neurodevelopmental delay.

The most common features seen in these patients are global developmental delay (100%), abnormal muscle tone that can manifest as early hypotonia evolving to hypertonia and spasticity (78%), abnormal vision (58%), and epilepsy (50%). Dystonia has been reported in 17% of patients across studies. In addition, in our new cohort of 18 patients, we found 61% of patients with gastrointestinal symptoms and 50% of males with genitourinary anomalies, raising the possibility of an association with *GNB1* in these patients. Hearing loss affected a small number of patients in this cohort, with a frequency that is higher than the general pediatric population.

We observed dystonia and poor growth to be more common in carriers of the p.Ile80 substitutions. Even though the number of patients in this report is not sufficient to draw any definite conclusions, these observations suggest a genotype-phenotype correlation. Finally, our cohort included three patients with mastocytosis, two of them identical twins. The addition of these three patients to the one reported by Szczaluba et al., [2017] provides further evidence for a link between *GNB1* and mastocytosis.

We hope this summary of data on *GNB1* germline variants will be useful for physicians who care for patients with *GNB1* variants, families of children with *GNB1* variants, and investigators interested in research on *GNB1*.

Table 1: Review of systems in our cohort of 18 patients

Symptoms	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Mutation (GRCh37; GenBank: NM_002074.4)	Chr1:1737942A>G c.239T>C p.Ile80Thr	Chr1:1737942A>G c.239T>C p.Ile80Thr	Chr1:1735947C>T c.341G>A p.Cys114Tyr	Chr1:1736004A>G c.284T>C p.Leu95Pro	Ch1:1737942A>G c.239T>C p.Ile80Thr
Ethnicity	Caucasian	Caucasian	Hispanic	Caucasian	Caucasian
Gender, Age	F, 6yrs	F, 6yrs	F, 7yrs	F, 2yrs	F , 8.5yrs
Prenatal complications/ birth history	twin pregnancy/ elective C-section	twin pregnancy/ elective C-section	No	decreased fetal movement/ C-section	maternal vaginal bleeding due to a uterine polyp/C-section
Infantile muscular hypotonia	Yes	Yes	No	Yes	Yes
Feeding difficulties	No	No	No	Yes	Yes
Growth delay: both height (Ht) and weight (W) below -2 SD	No	No	No	No	No
Microcephaly (below -2 SD)	No	No	No	No	No
Macrocephaly (above +2 SD)	No	No	No	No	Yes: (+2.5 SD)
Global developmental delay	Yes	Yes	Yes	Yes	Yes
Dysmorphic features	myopathic facies with drooling, frontal bossing	myopathic facies with drooling, frontal bossing	No	low set ears, cleft palate, micrognathia	short neck, broad nose, curly hair
Hypotonia	Yes	Yes	No	Yes: axial	Yes: axial
Hypertonia, spasticity, and complications	No	No	No	Yes: hypertonia in lower limbs	Yes: hypertonia in limbs, spasticity, and lateral deviation of foot
Dystonia and complications	No	No	No	Yes: abnormal posture of the body	Yes
Epilepsy	No	No	tonic seizures with stiffening and staring spells	No	3yrs: intermittent starring episode; 8yrs: status epilepticus (generalized clonic movement with right eye deviation)
EEG	Normal	Normal	3yrs: right and left temporal epileptiform discharges during sleep (T7-T8) and left temporal slowing	Normal	1 yr.: normal; 6yrs: centroparietal rt+lt epileptiform activity with significant increase during sleep(ESES)
MRI	2 yrs: abnormal or delayed myelination, pattern suggestive of sequelae of prior ischemic, inflammatory, or infectious etiologies, dysmorphic corpus callosum, mild cerebral volume loss	10 months: normal	3.5 yrs: mild bifrontal dysmorphic gyri and mild cerebral volume loss	Normal	6 months: relative thickness of the corpus callosum and some prominence of the frontal and temporal horns of the lateral ventricles; 6 yrs: relative thickness of the corpus callosum and hippocampus and mild hyperintensity of the posterior periventricular regions
Abnormal vision	No	No	No	horizontal and vertical nystagmus	nystagmus, improving with age and strabismus
Cardiovascular anomalies	No	No	No	No	No
Renal anomalies	No	No	No	No	No
Genital anomalies	No	No	No	No	No
Skin anomalies	multiple hyperpigmented lesions-mastocytosis	multiple hyperpigmented lesions-mastocytosis	No	No	No
Gastrointestinal disorder	constipation	constipation	No	gastroesophageal reflux, colic and constipation	No
Additional features			autism spectrum disorder	hypersensitivity to acoustic stimuli, maternally inherited 2/3 toe syndactyly	drooling, articulation problems

Symptoms	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Mutation (GRCh37; GenBank: NM_002074.4)	Chr1:1736013G>T c.275C>A p.Ala92Asp	Chr1:1737948T>C c.233A>G p.Lys78Arg	Chr1:1737942A >G c.239T>C p.Ile80Thr	Chr1:1737952C>G c.229G>C p.Gly77Arg	Chr1:1735935T>C c.353A>G p.Asp118Gly
Ethnicity	Caucasian	Caucasian	Hispanic	Caucasian	Caucasian
Gender, Age	M, deceased at 4yrs 2months	M, 12yrs	M, 5yrs	M, 2yrs	F, 2yrs
prenatal/ birth Complications	hydrops, congenital ascites	38weeks: fetal bradycardia; 29 weeks: bleeding and partial placenta abruption/ C-section	conceived by IVF/ C-section at 37.5 weeks, reason for C-section unknown	breech presentation /C-section	No
Infantile muscular hypotonia	Yes	Yes	Yes	unknown: presented to clinic at 4 months of age	Yes
Feeding difficulties	Yes	Yes: Poor suck, slow eater, G-tube dependent	Yes	No	No
Growth delay: both height (Ht) and weight (W) below -2 SD	No	No	No	No	No
Microcephaly (below -2 SD)	No	No	No	No	No
Macrocephaly (above +2 SD)	Yes (+2 SD)	Yes (+2.5 SD)	No	No	No
Global developmental delay	Yes	Yes	Yes	Yes	Yes
Dysmorphic facial features	plagiocephaly, thin lips, mild hypertelorism, little and slow growing hair	prominent forehead, long face	No	prominent philtrum, bilateral thumb abduction	small and flat anterior fontanelle, frontal prominence
Hypotonia	Yes	Yes	Yes	Yes	Yes
Hypertonia, spasticity, and complication	No	No	Yes: mild lower limbs	Yes: mild lower limbs	No
Dystonia and complication	No	No	Yes: athetoid posturing in hands, equinovarus posture in feet	No	No
Epilepsy	No	No	tonic, spasms and myoclonic generalized seizures	No	No
EEG	No	8yrs: normal	3yrs: normal; 4.5yrs: presence of Ictal activity during sleep, and wake characterized by high waves and generalized slow waves	Normal	Normal
MRI	mild dilatation of the frontal horns of the lateral ventricles	19 months: normal	3.5yrs: delayed myelination with abnormal FLAIR signal in the posterior periventricular white matter, suggestion of prominence of the cerebellar dentate nucleus on T2 and FLAIR studies	9 months: prominent large ventricles and general atrophy in addition to abnormal signal pattern from the dopaminergic system and low levels of isolated homovanilic acid in cerebrospinal fluid	Normal
Abnormal vision	cortical blindness	No	No	congruent gaze deviation in all directions, periodic vertical and horizontal nystagmus, continuous symmetric/asymmetric eye movements, improved ability to hold gaze for longer at 2yrs	No
Cardiovascular anomalies	VSD	No	No	duplicated superior vena cava	No
Renal anomalies	duplicated collecting system	No	No	No	concern for hyperoxaluria type 1
Genital anomalies	bifid scrotum	No	No	No	No
Skin anomalies	prominent scalp veins, excess sweat, lack of redundant skin on eyelids	No	No	No	No
Gastrointestinal disorder	vomiting and constipation, PEG fed	cyclical vomiting	possible GERD	abdominal cramps	chronic constipation
Additional features	tongue tie, persistent fetal finger pads, aspiration pneumonia			hepatic vein anomaly	

Symptoms	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15
Mutation (GRCh37; GenBank: NM_002074.4)	Chr1:1737942A >G	Chr1:1737942A >G	Chr1:1737942A >G	Chr1:1737915T>C	chr1:1747240 C > T
	c.239T>C	c.239T>C	c.239T>C	c.266A>G	c.158G>A
	p.Ile80Thr	p.Ile80Thr	p.Ile80Thr	p.Lys89Arg	p.Gly53Glu
Ethnicity	Caucasian	African-American, Caucasian	Caucasian	Middle-East	Middle-East
Gender, Age	M, 12yrs	F, 16yrs	M, 5yrs	F, 4yrs	M, 2yrs
prenatal/ birth Complications	fetal bradycardia, umbilical cord wrapped around neck/ C-section	No	No	No	No
Infantile muscular hypotonia	Yes	Yes	Yes	No	Yes
Feeding difficulties	No	No	Yes	No	No
Growth delay: both height (Ht) and weight (W) below -2 SD	No	No	No	No	No
Microcephaly (below -2 SD)	No	No	No	No	No
Macrocephaly (above +2 SD)	No	Yes (+2.8 SD)	No	No	No
Global developmental delay	Yes	Yes	Yes	Yes	Yes
Dysmorphic facial features	No	minimal macroglossia, slender distal digits	tall broad forehead, triangular face, Dental malocclusion, high arched palate	frontal bossing	two whorls hair pattern, mildly hypoplastic nipples, diastema, thin upper lip, pronounced anti-helix, long eye lashes, long flat philtrum
Hypotonia	Yes	Yes: proximal and appendicular	Yes	No	Yes: axial
Hypertonia, spasticity, and complications	No	Yes: mild contractures, valgus deformity	No	No	Yes: hypertonia and spasticity in lower limbs
Dystonia and complications	No	Yes: mild dystonic positioning of fingers	No	No	No
Epilepsy	absences since childhood, about 10 per day; Tonic-clonic since 11 years	No	one episode at 2yrs	No	No
EEG	11yrs: infrequent, brief, independent right and left frontal / fronto-central sharp wave discharges suggestive of multi-focal epilepsy	No	2yrs: normal	Normal	No
MRI	11months: Increased fluid in subarachnoid spaces, especially in fronto-parietal region	1yr and 3yrs: minimal perisylvian polymicrogyria	9 months: mild prominence of the lateral ventricles; Spinal MRI at 9 months: mild prominence of the central canal of the spinal cord	Hypoplasia of the right cochlear nerve, stenotic ipsilateral cochlear aperture and inferior internal auditory canal, thickened crista falciformis. Signal increase in the medial cerebellar white matter. Possible hypoplastic olfactory apparatus.	Normal
Abnormal vision	rotatory nystagmus with electrodiagnostics suggestive of ocular albinism	astigmatism, strabismus	right sided nystagmus; abnormal ERG: possible rod-cone dystrophy	No	No
Cardiovascular anomalies	No	No	No	No	No
Renal anomalies	No	No	No	No	No
Genital anomalies	bilateral undescended testes	No	undescended testes	No	No
Skin anomalies	No	large right abdominal pigmented nevus	Multiple small almost darker foci that were almost like café-au-lait-like in color across his abdomen-Mastocytosis.	café-au-lait macules	mild skin redundancy in hands
Gastrointestinal disorder	No	constipation, incontinence	distended abdomen	No	No
Additional features	asthma, rocks head constantly, hip dysplasia	scoliosis, occasional tics, osteopenia	white blood cell electron microscopy suggestive of inclusion bodies	repetitive and self-mutilation behaviors, profound unilateral sensorineural hearing loss in the right ear	brisk tendon reflexes

Symptoms	Patient 16	Patient 17	Patient 18
Mutation (GRCh37; GenBank: NM_002074.4)	Chr1:1736004A>G	Chr1:1735935T>C	Chr1:1737942A >G
	c.284T>C	c.353A>G	c.239T>C
	p.Leu95Pro	p.Asp118Gly	p.Ile80Thr
Ethnicity	Caucasian	Caucasian	Caucasian
Gender, Age	F, 2yrs	F, 2yrs	M, 10yrs
prenatal/ birth Complications	failure to progress/ C-section	breech presentation/ C-section	No
Infantile muscular hypotonia	Yes	Yes	Yes
Feeding difficulties	Yes	Yes	No
Growth delay: both height (Ht) and weight (W) below -2 SD	No	No	No
Microcephaly (below -2 SD)	No	No	No
Macrocephaly (above +2 SD)	No	No	No
Global developmental delay	Yes	Yes	Yes
Dysmorphic facial features	cleft palate, low set ears, anteverted nares, plagiocephaly, slight asymmetry of the face with horizontal and slightly broad eye brows, long curly eye lashes, cupid bow shaped upper lip, depressed nasal bridge, lymphedema of the dorsal aspects of both feet	upward slanting eyes, small chin, delayed tooth eruption, ear overfolded upper helix	right sided occipital flatness, brachycephaly
Hypotonia	Yes	Yes: axial	Yes
Hypertonia, spasticity, and complications	Yes: lower extremity hypertonia	No	Yes: hypertonia in extremities
Dystonia and complications	No	No	No
Epilepsy	No: no significant clinical seizures have been noted	No	Yes
EEG	6months: significant spike and slow wave activity during sleep, suggesting a generalized epileptiform disorder; 8months and 10months: gradual improvement in epileptiform discharges, noted alongside a reduction in jerky movements	No	4 yrs and 9yrs: focal epileptiform activity with important persistence in wakefulness that is strikingly activated in NREM sleep
MRI	Normal	Normal	1yr and 4yrs: bilateral and symmetric hyperintensity in the periventricular white matter atrial and occipital and frontal parasagittal
Abnormal vision	possible optic atrophy and cortical visual impairment, nystagmus, legal blindness	No	strabismus, nystagmus, hypermetropia
Cardiovascular anomalies	No	No	No
Renal anomalies	urine tract infections due to retention of urine when distressed	No	No
Genital anomalies	No	No	Cryptorchidism
Skin anomalies	No	café-au-lait macule	No
Gastrointestinal disorder	gastroesophageal reflux, recurrent constipation	No	No
Additional features	bilateral sensorineural hearing loss, otomastoid opacification, decreased reflexes, club foot	congenital hip dysplasia, anterior fontanel open at 20 months, neonatal teeth	deep tendon reflexes, stereotypic behaviors such as rolling body and rubbing hands

Table 2: Review of developmental milestones in our cohort of 18 patients

Symptoms	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15	Patient 16	Patient 17	Patient 18
Mutation (GRCh37; GenBank: NM_002074.4)	Chr1:173794 2A>G	Chr1:173794 2A>G	Chr1:1735947C >T	Chr1:173600 4A>G	Ch1:173794 2A>G	Chr1:173601 3G>T	Chr1:173794 8T>C	Chr1:1737 942A >G	Chr1:173795 2C>G	Chr1:173593 5T>C	Chr1:1737 942A >G	Chr1:1737 942A >G	Chr1:1737 942A >G	Chr1:173791 5T>C	chr1:1747240 C > T	Chr1:173600 4A>G	Chr1:173593 5T>C	Chr1:1737 942A >G
	c.239T>C	c.239T>C	c.341G >A	c.284T>C	c.239T>C	c.275C>A	c.233A>G	c.239T>C	c.229G>C	c.353A>G	c.239T>C	c.239T>C	c.239T>C	c.266A>G	c.158G>A	c.284T>C	c.353A>G	c.239T>C
	p.Ile80Thr	p.Ile80Thr	p.Cys114Tyr	p.Leu95Pro	p.Ile80Thr	p.Ala92Asp	p.Lys78Arg	p.Ile80Thr	p.Gly77Arg	p.Asp118Gly	p.Ile80Thr	p.Ile80Thr	p.Ile80Thr	p.Lys89Arg	p.Gly53Glu	p.Leu95Pro	p.Asp118Gly	p.Ile80Thr
Gender, Age	F, 6yrs	F, 6yrs	F, 7yrs	F, 2yrs	F , 8.5yrs	M, deceased at 4yrs 2months	M, 12yrs	M, 5yrs	M, 2yrs	F, 2yrs	M, 12yrs	F, 16yrs	M, 5yrs	F, 4yrs	M, 2yrs	F, 2yrs	F, 2yrs	M, 10yrs
Motor developmental delay	Yes	Yes	No	Yes	Yes: walks with a walker only short distances	Yes	Yes: mostly in wheelchair	Yes: Weak, poor head control, postural instability	Yes: poor head control, unable to sit	Yes: not able to sit with support	Yes: sat independently at 22 months	Yes	Yes	Yes	Yes: 14 months: sits but not stable	Yes	Yes: could sit unassisted but does not stand	Yes
Onset of Ambulation	Non ambulatory	Non ambulatory	12months	Non ambulatory	2 yrs	Non ambulatory	5.5yrs: uncoordinated walk with broad based gait	3.8 years: walks with a reverse walker	Non ambulatory	Non ambulatory	Non ambulatory	Age of onset unknown: able to take 10 steps with gait trainer	5yrs: learning to walk with a gait trainer	2.5 years: poor balance	28 months: walks with help on tip toes	Non ambulatory	Non ambulatory	Non ambulatory
GMFCS	V	V	I	V	IV	V	IV	III	V	V	V	IV	IV	III	III	V	V	V
Speech and language delay	Yes	Yes	Yes: 30-40 words, speech often not meaningful, echolalic	Yes	Yes: expressive language delay, speaks in sentences with articulation problems	Yes	Yes: vocalizes and cries only	Yes: able to vocalize and cry only	Yes: babbles, but no words	Yes	Yes: no words, unable to indicate needs, limited understanding	Yes	Yes	Yes: speaks only a few words	Yes: speaks few words, no sentences at 28 month	Yes	Yes: minimal sounds, no words	Yes
Onset of speech	No words	No words	12months	No words	1.5yrs	No words	No words	No words	No words	No words	No words	4yrs: few words, nasal speech	No words, few sign language	age of onset unknown: few words	2.6yrs: few words	No words	No words	No words
Cognitive delay	Yes	Yes	Yes: Special education class	Yes	Yes: has relative preservation of her non-verbal cognitive functions	Yes	Yes: severe intellectual disability, not able to communicate needs	Yes	Yes	NA	Yes	Yes	Yes	Yes significant cognitive delay	Yes: at 23 month DQ-59-61	NA	Yes: at age 20 months developed at a 9-10months level	Yes

Table 3: Comparison of symptoms across all reported patients

Symptoms	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15	Patient 16	Patient 17	Patient 18	New patients	Petrovski et al,2016	Lohmann et al, 2017	Brett et al, 2017	Steinrücke et al, 2016	Szczaluba et al, 2017	Total
Global developmental delay	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	18/18	13/13	12/12	Yes	Yes	Yes	46/46 (100 %)
Abnormal muscle tone	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	16/18	12/13	4/12	Yes	Yes	Yes	36/46 (78%)
Epilepsy	No	No	Yes	No	Yes	No	No	Yes	No	No	Yes	No	Yes	No	No	No	No	Yes	6/18	10/13	7/12	No	No	No	23/46 (50%)
Abnormal EEG	No	No	Yes	No	Yes	No	No	Yes	No	No	Yes	Not done	No	No	Not done	Yes	Not done	Yes	6/15	11/13	Not known	Not known	No	Yes	18/30 (60%)
Abnormal MRI	Yes	No	Yes	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	No	No	No	Yes	11/18	5/13	7/12	Yes	No	No	24/46 (52%)
Abnormal vision	No	No	No	Yes	Yes	Yes	No	No	Yes	No	Yes	Yes	Yes	No	No	Yes	No	Yes	9/18	9/13	8/12	Yes	No	No	27/46 (58%)
Growth delay: both height (Ht) and weight (W) below -2 SD	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	0/18	6/13	3/12	Yes	No	No	10/46 (21%)

Microcephaly (below -2 SD)	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	0/18	1/13	Not known	No	Not known	No	1/33 (3%)
Macrocephaly (above +2 SD)	No	No	No	No	Yes	Yes	Yes	No	No	No	No	Yes	No	No	No	No	No	No	4/18	3/13	Not known	No	Not known	No	7/33 (21%)
Dysmorphic features	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	15/18	6/13	Not known	Yes	Yes	Yes	24/34 (70%)

Table 4: Summary of Epilepsy manifestation in our cohort as well as Petrovski et al. (2016) cohort

Symptoms	Patient 3	Patient 5	Patient 8	Patient 11	Patient 13	Patient 18	Individual 2, Petrovski et al. (2016)	Individual 3, Petrovski et al. (2016)	Individual 5, Petrovski et al. (2016)	Individual 6, Petrovski et al. (2016)	Individual 7, Petrovski et al. (2016)	Individual 8, Petrovski et al. (2016)	Individual 10, Petrovski et al. (2016)	Individual 11, Petrovski et al. (2016)	Individual 12, Petrovski et al. (2016)	Individual 13, Petrovski et al. (2016)
Mutation (GRCh37; GenBank: NM_002074.4)	Chr1:1735947C >T	Ch1:1737942 A>G	Chr1:1737942 A>G	Chr1:1737942 A>G	Chr1:1737942 A>G	Chr1:1737942 A>G	chr1: g.1737948T >C	chr1: g.1737942 A>T	chr1: g.1735987T >C	chr1: g.1718817 C>T	chr1: g.1737953 A>C	chr1: g.1735987 T>C	chr1: g.1737942 A>G	chr1: g.1737942 A>G	chr1: g.1737942 A>T	chr1: g.1736004 A>G
	c.341G >A	c.239T>C	c.239T>C	c.239T>C	c.239T>C	c.239T>C	c.233A>G	c.239T>A	c.301A>G	c.976G>A	c.228T>G	c.301A>G	c.239T>C	c.239T>C	c.239T>A	c.284T>C
	p.Cys114Tyr	p.Ile80Thr	p.Ile80Thr	p.Ile80Thr	p.Ile80Thr	p.Ile80Thr	p.Lys78Arg	p.Ile80Asn	p.Met101Val	p.Ala326Thr	p.Asp76Glu	p.Met101Val	p.Ile80Thr	p.Ile80Thr	p.Ile80Asn	p.Leu95Pro
EEG	3yrs: right and left temporal epileptiform discharges during sleep (T7-T8) and left temporal slowing	1 yr.: Normal; 6yrs: centroparietal rt+lt epileptiform activity with significant increase during sleep (ESES)	3yrs: Normal; 4.5yrs: presence of Ictal activity during sleep, and wake characterized by high waves and generalized slow waves	11yrs: infrequent, brief, independent right and left frontal / fronto-central sharp wave discharges suggestive of multi-focal epilepsy	2yrs: Normal	4 yrs and 9yrs: focal epileptiform activity with important persistence in wakefulness that is strikingly activated in NREM sleep	6 months: hypsarrhythmia, VEEG captured infantile spasms	15 months: generalized subcortical alterations; 3 years: generalized epileptiform process	7, 10, and 11 months: modified hypsarrhythmia; 2 years: normal; 3 years: multifocal and 2 Hz GSW	14 months: Normal; 5 years: L and R posterior temporal epileptiform activity	burst suppression	4 years: Normal; 11 years: diffuse slowing and rare right temporal spikes	2 years: generalized paroxysmal epileptiform activity, myoclonic seizure with generalized paroxysmal polyspike slow-wave activity	9 years: multifocal spikes and generalized background slowing	4 years: high amplitude background with occipital slowing, multifocal epileptiform discharges	4 months: multifocal epileptiform discharges from both posterior quadrants, right temporoparietal sharp waves
Epilepsy	tonic-like seizures with stiffening and staring spells	3yrs: intermittent starring episode; 8yrs: status epilepticus (generalized clonic movement with right eye deviation)	tonic, spasms and myoclonic generalized seizures	absences since childhood, about 10 per day; tonic-clonic since 11 years	one episode at 2yrs	Yes	infantile spasms	9 months: generalized epilepsy	infantile spasms, absence seizures, Non-convulsive status epilepticus, focal seizures	1 year: febrile status epilepticus followed by afebrile focal convulsive seizures and absence and atonic seizures; 14 years: focal hyperkinetic sleep-related seizures	hemiclonic seizure associated with hypoglycemia (<20), went into complex partial status	11 years: onset of tonic-clonic seizures	2 years: onset of head drops and myoclonic seizures	9 years: onset of focal seizures	4 years: onset of focal seizures	4 months: tonic posturing of the arms and occasional generalized upper-body myoclonus
Well controlled	Yes	No	No	Yes	NA	No	Yes	unknown	unknown	unknown	unknown	Yes	unknown	Yes	unknown	Yes
Treatment	anti-epileptics x 1 year (Trileptal 5.5 mg bid)	Sultiam, valproic acid	MAD diet at 3:1 ratio, Locasamide, Valproate Magnesium, Clonazepam	levetiracetam	None	valproic acid, sultiam, and levetiracetam	oral prednisolone	unknown	unknown	unknown	anti-convulsant therapies	valproic acid and lamotrigine	unknown	onfi and Trileptal	unknown	pyridoxal-5-phosphate

Conflict of interest disclosures:

David Goldstein, PhD is a founder of and holds equity in Pairnomix and Praxis, serves as a consultant to AstraZeneca, and has research supported by Janssen, Gilead, Biogen, and UCB. Slavé Petrovski, PhD is an employee of AstraZeneca, and serves on the advisory board and is an equity holder of Pairnomix. Sophie Colombo serves as a consultant to Pairnomix. Francisca Millan, PhD, Amanda Singleton, MS, CGC, and Megan T. Cho, MS, CGC are employees of GeneDx laboratories. Other authors have no conflicts of interest to declare.

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Web resources:

1000 genomes: http://www.internationalgenome.org/

Catalogue of Somatic Mutations in Cancer (COSMIC): http://cancer.sanger.ac.uk/cosmic

Centers for Disease Control and Prevention: https://www.cdc.gov/ncbddd/hearingloss/data.html

dbSNP: https://www.ncbi.nlm.nih.gov/projects/SNP/

Deciphering Developmental Disorders (DDD): http://www.sanger.ac.uk/science/collaboration/deciphering-developmental-disorders-ddd

ExAC Browser: http://exac.broadinstitute.org/

Ensembl genome assembly GRCh37: http://grch37.ensembl.org/Homo_sapiens/Info/Index

Ensembl Variant Effect Predictor (VEP): http://grch37.ensembl.org/Homo_sapiens/Tools/VEP

GeneDx ClinVar variant classification: http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/

Gross Motor Function Classification System (GMFCS): https://www.cerebralpalsy.org.au/what-is-cerebral-palsy/severity-of-cerebral-palsy/gross-motor-function-classification-system/

HGMD: http://www.hgmd.cf.ac.uk/ac/index.php

Missense Tolerance Ratio (MTR): http://mtr-viewer.mdhs.unimelb.edu.au/

OMIM: http://www.omim.org/

Pubmed: https://www.ncbi.nlm.nih.gov/pubmed

The Human Gene Mutation Database (HGMD): http://www.hgmd.cf.ac.uk/ac/index.php

Figure 1 legend: *GNB1* MTR plot

A: An MTR = 1 (blue dashed line) represents neutrality (i.e., observe the same proportion of missense variants in the window as expected based on the underlying sequence context). Red segments of MTR plot achieved exome-wide FDR < 0.10 for a significance test of a window's deviation from MTR = 1. Black dashed line (gene-specific median MTR), brown dashed line (gene-specific 25th percentile MTR) and orange dashed line (gene-specific 5th percentile MTR). Data available at http://mtr-viewer.mdhs.unimelb.edu.au/. Across the *GNB1* MTR landscape, the current 45 case-ascertained missense variants are denoted by red stars.

B: Demonstrates the distribution of variants across exons

Figure 2 legend: *GNB1* tertiary structure

Molecular representation of a heterotrimeric G protein (Gα, white; Gβ, green; Gγ, blue) is based on a crystal structure. The recurrently affected p.Ile80 residue is indicated in red. The Gβ side chains residues affecting 4 and 3 patients each are indicated in orange, residues affecting 2 patients each are in yellow, and residues affecting one patient each are in white.

Table 1: Review of systems in our cohort of 18 patients

Symptoms	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Mutation (GRCh37; GenBank: NM_002074.4)	Chr1:1737942A>G c.239T>C p.Ile80Thr	Chr1:1737942A>G c.239T>C p.Ile80Thr	Chr1:1735947C>T c.341G>A p.Cys114Tyr	Chr1:1736004A>G c.284T>C p.Leu95Pro	Ch1:1737942A>G c.239T>C p.Ile80Thr
Ethnicity	Caucasian	Caucasian	Hispanic	Caucasian	Caucasian
Gender, Age	F, 6yrs	F, 6yrs	F, 7yrs	F, 2yrs	F , 8.5yrs
Prenatal complications/ birth history	twin pregnancy/ elective C-section	twin pregnancy/ elective C-section	No	decreased fetal movement/ C-section	maternal vaginal bleeding due to a uterine polyp/C-section
Infantile muscular hypotonia	Yes	Yes	No	Yes	Yes
Feeding difficulties	No	No	No	Yes	Yes
Growth delay: both height (Ht) and weight (W) below -2 SD	No	No	No	No	No
Microcephaly (below -2 SD)	No	No	No	No	No
Macrocephaly (above +2 SD)	No	No	No	No	Yes: (+2.5 SD)
Global developmental delay	Yes	Yes	Yes	Yes	Yes
Dysmorphic features	myopathic facies with drooling, frontal bossing	myopathic facies with drooling, frontal bossing	No	low set ears, cleft palate, micrognathia	short neck, broad nose, curly hair
Hypotonia	Yes	Yes	No	Yes: axial	Yes: axial
Hypertonia, spasticity, and complications	No	No	No	Yes: hypertonia in lower limbs	Yes: hypertonia in limbs, spasticity, and lateral deviation of foot
Dystonia and complications	No	No	No	Yes: abnormal posture of the body	Yes
Epilepsy	No	No	tonic seizures with stiffening and staring spells	No	3yrs: intermittent starring episode; 8yrs: status epilepticus (generalized clonic movement with right eye deviation)
EEG	Normal	Normal	3yrs: right and left temporal epileptiform discharges during sleep (T7-T8) and left temporal slowing	Normal	1 yr.: normal; 6yrs: centroparietal rt+lt epileptiform activity with significant increase during sleep(ESES)
MRI	2 yrs: abnormal or delayed myelination, pattern suggestive of sequelae of prior ischemic, inflammatory, or infectious etiologies, dysmorphic corpus callosum, mild cerebral volume loss	10 months: normal	3.5 yrs: mild bifrontal dysmorphic gyri and mild cerebral volume loss	Normal	6 months: relative thickness of the corpus callosum and some prominence of the frontal and temporal horns of the lateral ventricles; 6 yrs: relative thickness of the corpus callosum and hippocampus and mild hyperintensity of the posterior periventricular regions
Abnormal vision	No	No	No	horizontal and vertical nystagmus	nystagmus, improving with age and strabismus
Cardiovascular anomalies	No	No	No	No	No
Renal anomalies	No	No	No	No	No
Genital anomalies	No	No	No	No	No
Skin anomalies	multiple hyperpigmented lesions-mastocytosis	multiple hyperpigmented lesions-mastocytosis	No	No	No
Gastrointestinal disorder	constipation	constipation	No	gastroesophageal reflux, colic and constipation	No
Additional features			autism spectrum disorder	hypersensitivity to acoustic stimuli, maternally inherited 2/3 toe syndactyly	drooling, articulation problems

Symptoms	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Mutation (GRCh37; GenBank: NM_002074.4)	Chr1:1736013G>T	Chr1:1737948T>C	Chr1:1737942A >G	Chr1:1737952C>G	Chr1:1735935T>C
	c.275C>A	c.233A>G	c.239T>C	c.229G>C	c.353A>G
	p.Ala92Asp	p.Lys78Arg	p.Ile80Thr	p.Gly77Arg	p.Asp118Gly
Ethnicity	Caucasian	Caucasian	Hispanic	Caucasian	Caucasian
Gender, Age	M, deceased at 4yrs 2months	M, 12yrs	M, 5yrs	M, 2yrs	F, 2yrs
prenatal/ birth Complications	hydrops, congenital ascites	38weeks: fetal bradycardia; 29 weeks: bleeding and partial placenta abruption/ C-section	conceived by IVF/ C-section at 37.5 weeks, reason for C-section unknown	breech presentation /C-section	No
Infantile muscular hypotonia	Yes	Yes	Yes	unknown: presented to clinic at 4 months of age	Yes
Feeding difficulties	Yes	Yes: Poor suck, slow eater, G-tube dependent	Yes	No	No
Growth delay: both height (Ht) and weight (W) below -2 SD	No	No	No	No	No
Microcephaly (below -2 SD)	No	No	No	No	No
Macrocephaly (above +2 SD)	Yes (+2 SD)	Yes (+2.5 SD)	No	No	No
Global developmental delay	Yes	Yes	Yes	Yes	Yes
Dysmorphic facial features	plagiocephaly, thin lips, mild hypertelorism, little and slow growing hair	prominent forehead, long face	No	prominent philtrum, bilateral thumb abduction	small and flat anterior fontanelle, frontal prominence
Hypotonia	Yes	Yes	Yes	Yes	Yes
Hypertonia, spasticity, and complication	No	No	Yes: mild lower limbs	Yes: mild lower limbs	No
Dystonia and complication	No	No	Yes: athetoid posturing in hands, equinovarus posture in feet	No	No
Epilepsy	No	No	tonic, spasms and myoclonic generalized seizures	No	No
EEG	No	8yrs: normal	3yrs: normal; 4.5yrs: presence of Ictal activity during sleep, and wake characterized by high waves and generalized slow waves	Normal	Normal
MRI	mild dilatation of the frontal horns of the lateral ventricles	19 months: normal	3.5yrs: delayed myelination with abnormal FLAIR signal in the posterior periventricular white matter, suggestion of prominence of the cerebellar dentate nucleus on T2 and FLAIR studies	9 months: prominent large ventricles and general atrophy in addition to abnormal signal pattern from the dopaminergic system and low levels of isolated homovanilic acid in cerebrospinal fluid	Normal
Abnormal vision	cortical blindness	No	No	congruent gaze deviation in all directions, periodic vertical and horizontal nystagmus, continuous symmetric/asymmetric eye movements, improved ability to hold gaze for longer at 2yrs	No
Cardiovascular anomalies	VSD	No	No	duplicated superior vena cava	No
Renal anomalies	duplicated collecting system	No	No	No	concern for hyperoxaluria type 1
Genital anomalies	bifid scrotum	No	No	No	No
Skin anomalies	prominent scalp veins, excess sweat, lack of redundant skin on eyelids	No	No	No	No
Gastrointestinal disorder	vomiting and constipation, PEG fed	cyclical vomiting	possible GERD	abdominal cramps	chronic constipation
Additional features	tongue tie, persistent fetal finger pads, aspiration pneumonia			hepatic vein anomaly	

Symptoms	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15
Mutation (GRCh37; GenBank: NM_002074.4)	Chr1:1737942A >G	Chr1:1737942A >G	Chr1:1737942A >G	Chr1:1737915T>C	chr1:1747240 C > T
	c.239T>C	c.239T>C	c.239T>C	c.266A>G	c.158G>A
	p.Ile80Thr	p.Ile80Thr	p.Ile80Thr	p.Lys89Arg	p.Gly53Glu
Ethnicity	Caucasian	African-American, Caucasian	Caucasian	Middle-East	Middle-East
Gender, Age	M, 12yrs	F, 16yrs	M, 5yrs	F, 4yrs	M, 2yrs
prenatal/ birth Complications	fetal bradycardia, umbilical cord wrapped around neck/ C-section	No	No	No	No
Infantile muscular hypotonia	Yes	Yes	Yes	No	Yes
Feeding difficulties	No	No	Yes	No	No
Growth delay: both height (Ht) and weight (W) below -2 SD	No	No	No	No	No
Microcephaly (below -2 SD)	No	No	No	No	No
Macrocephaly (above +2 SD)	No	Yes (+2.8 SD)	No	No	No
Global developmental delay	Yes	Yes	Yes	Yes	Yes
Dysmorphic facial features	No	minimal macroglossia, slender distal digits	tall broad forehead, triangular face, Dental malocclusion, high arched palate	frontal bossing	two whorls hair pattern, mildly hypoplastic nipples, diastema, thin upper lip, pronounced anti-helix, long eye lashes, long flat philtrum
Hypotonia	Yes	Yes: proximal and appendicular	Yes	No	Yes: axial
Hypertonia, spasticity, and complications	No	Yes: mild contractures, valgus deformity	No	No	Yes: hypertonia and spasticity in lower limbs
Dystonia and complications	No	Yes: mild dystonic positioning of fingers	No	No	No
Epilepsy	absences since childhood, about 10 per day; Tonic-clonic since 11 years	No	one episode at 2yrs	No	No
EEG	11yrs: infrequent, brief, independent right and left frontal / fronto-central sharp wave discharges suggestive of multi-focal epilepsy	No	2yrs: normal	Normal	No
MRI	11months: Increased fluid in subarachnoid spaces, especially in fronto-parietal region	1yr and 3yrs: minimal perisylvian polymicrogyria	9 months: mild prominence of the lateral ventricles; Spinal MRI at 9 months: mild prominence of the central canal of the spinal cord	Hypoplasia of the right cochlear nerve, stenotic ipsilateral cochlear aperture and inferior internal auditory canal, thickened crista falciformis. Signal increase in the medial cerebellar white matter. Possible hypoplastic olfactory apparatus.	Normal
Abnormal vision	rotatory nystagmus with electrodiagnostics suggestive of ocular albinism	astigmatism, strabismus	right sided nystagmus; abnormal ERG: possible rod-cone dystrophy	No	No
Cardiovascular anomalies	No	No	No	No	No
Renal anomalies	No	No	No	No	No
Genital anomalies	bilateral undescended testes	No	undescended testes	No	No
Skin anomalies	No	large right abdominal pigmented nevus	Multiple small almost darker foci that were almost like café-au-lait-like in color across his abdomen-Mastocytosis.	café-au-lait macules	mild skin redundancy in hands
Gastrointestinal disorder	No	constipation, incontinence	distended abdomen	No	No
Additional features	asthma, rocks head constantly, hip dysplasia	scoliosis, occasional tics, osteopenia	white blood cell electron microscopy suggestive of inclusion bodies	repetitive and self-mutilation behaviors, profound unilateral sensorineural hearing loss in the right ear	brisk tendon reflexes

Symptoms	Patient 16	Patient 17	Patient 18
Mutation (GRCh37; GenBank: NM_002074.4)	Chr1:1736004A>G	Chr1:1735935T>C	Chr1:1737942A >G
	c.284T>C	c.353A>G	c.239T>C
	p.Leu95Pro	p.Asp118Gly	p.Ile80Thr
Ethnicity	Caucasian	Caucasian	Caucasian
Gender, Age	F, 2yrs	F, 2yrs	M, 10yrs
prenatal/ birth Complications	failure to progress/ C-section	breech presentation/ C-section	No
Infantile muscular hypotonia	Yes	Yes	Yes
Feeding difficulties	Yes	Yes	No
Growth delay: both height (Ht) and weight (W) below -2 SD	No	No	No
Microcephaly (below -2 SD)	No	No	No
Macrocephaly (above +2 SD)	No	No	No
Global developmental delay	Yes	Yes	Yes
Dysmorphic facial features	cleft palate, low set ears, anteverted nares, plagiocephaly, slight asymmetry of the face with horizontal and slightly broad eye brows, long curly eye lashes, cupid bow shaped upper lip, depressed nasal bridge, lymphedema of the dorsal aspects of both feet	upward slanting eyes, small chin, delayed tooth eruption, ear overfolded upper helix	right sided occipital flatness, brachycephaly
Hypotonia	Yes	Yes: axial	Yes
Hypertonia, spasticity, and complications	Yes: lower extremity hypertonia	No	Yes: hypertonia in extremities
Dystonia and complications	No	No	No
Epilepsy	No: no significant clinical seizures have been noted	No	Yes
EEG	6months: significant spike and slow wave activity during sleep, suggesting a generalized epileptiform disorder; 8months and 10months: gradual improvement in epileptiform discharges, noted alongside a reduction in jerky movements	No	4 yrs and 9yrs: focal epileptiform activity with important persistence in wakefulness that is strikingly activated in NREM sleep
MRI	Normal	Normal	1yr and 4yrs: bilateral and symmetric hyperintensity in the periventricular white matter atrial and occipital and frontal parasagittal
Abnormal vision	possible optic atrophy and cortical visual impairment, nystagmus, legal blindness	No	strabismus, nystagmus, hypermetropia
Cardiovascular anomalies	No	No	No
Renal anomalies	urine tract infections due to retention of urine when distressed	No	No
Genital anomalies	No	No	Cryptorchidism
Skin anomalies	No	café-au-lait macule	No
Gastrointestinal disorder	gastroesophageal reflux, recurrent constipation	No	No
Additional features	bilateral sensorineural hearing loss, otomastoid opacification, decreased reflexes, club foot	congenital hip dysplasia, anterior fontanel open at 20 months, neonatal teeth	deep tendon reflexes, stereotypic behaviors such as rolling body and rubbing hands

Table 2: Review of developmental milestones in our cohort of 18 patients

Symptoms	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15	Patient 16	Patient 17	Patient 18
Mutation (GRCh37; GenBank: NM_002074.4)	Chr1:17379 42A>G	Chr1:17379 42A>G	Chr1:173 5947C >T	Chr1:17360 04A>G	Ch1:17379 42A>G	Chr1:17360 13G>T	Chr1:17379 48T>C	Chr1:173 7942A >G	Chr1:17379 52C>G	Chr1:17359 35T>C	Chr1:173 7942A >G	Chr1:173 7942A >G	Chr1:173 7942A >G	Chr1:17379 15T>C	chr1:174 7240 C > T	Chr1:17360 04A>G	Chr1:17359 35T>C	Chr1:173 7942A >G
	c.239T>C	c.239T>C	c.341G >A	c.284T>C	c.239T>C	c.275C>A	c.233A>G	c.239T>C	c.229G>C	c.353A>G	c.239T>C	c.239T>C	c.239T>C	c.266A>G	c.158G>A	c.284T>C	c.353A>G	c.239T>C
	p.Ile80Thr	p.Ile80Thr	p.Cys114 Tyr	p.Leu95Pro	p.Ile80Thr	p.Ala92Asp	p.Lys78Arg	p.Ile80Thr	p.Gly77Arg	p.Asp118Gly	p.Ile80Thr	p.Ile80Thr	p.Ile80Thr	p.Lys89Arg	p.Gly53 Glu	p.Leu95Pro	p.Asp118Gly	p.Ile80Thr
Gender, Age	F, 6yrs	F, 6yrs	F, 7yrs	F, 2yrs	F , 8.5yrs	M, deceased at 4yrs 2months	M, 12yrs	M, 5yrs	M, 2yrs	F, 2yrs	M, 12yrs	F, 16yrs	M, 5yrs	F, 4yrs	M, 2yrs	F, 2yrs	F, 2yrs	M, 10yrs
Motor developmental delay	Yes	Yes	No	Yes	Yes: walks with a walker only short distances	Yes	Yes: mostly in wheelchair	Yes: Weak, poor head control, postural instability	Yes: poor head control, unable to sit	Yes: not able to sit with support	Yes: sat independently at 22 months	Yes	Yes	Yes	Yes: 14 months: sits but not stable	Yes	Yes: could sit unassisted but does not stand	Yes
Onset of Ambulation	Non ambulatory	Non ambulatory	12months	Non ambulatory	2 yrs	Non ambulatory	5.5yrs: uncoordinated walk with broad based gait	3.8 years: walks with a reverse walker	Non ambulatory	Non ambulatory	Non ambulatory	Age of onset unknown: able to take 10 steps with gait trainer	5yrs: learning to walk with a gait trainer	2.5 years: poor balance	28 months: walks with help on tip toes	Non ambulatory	Non ambulatory	Non ambulatory
GMFCS	V	V	I	V	IV	V	IV	III	V	V	V	IV	IV	III	III	V	V	V
Speech and language delay	Yes	Yes	Yes: 30-40 words, speech often not meaningful, echolalic	Yes	Yes: expressive language delay, speaks in sentences with articulation problems	Yes	Yes: vocalizes and cries only	Yes: able to vocalize and cry only	Yes: babbles, but no words	Yes	Yes: no words, unable to indicate needs, limited understanding	Yes	Yes	Yes: speaks only a few words	Yes: speaks few words, no sentences at 28 month	Yes	Yes: minimal sounds, no words	Yes
Onset of speech	No words	No words	12months	No words	1.5yrs	No words	No words	No words	No words	No words	No words	4yrs: few words, nasal speech	No words, few sign language	age of onset unknown: few words	2.6yrs: few words	No words	No words	No words
Cognitive delay	Yes	Yes	Yes: Special education class	Yes	Yes: has relative preservation of her non-verbal cognitive functions	Yes	Yes: severe intellectual disability, not able to communicate needs	Yes	Yes	NA	Yes	Yes	Yes	Yes significant cognitive delay	Yes: at 23 month DQ-59-61	NA	Yes: at age 20 months developed at a 9-10months level	Yes

Table 3: Comparison of symptoms across all reported patients

Symptoms	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15	Patient 16	Patient 17	Patient 18	New patients	Petrovski et al,2016	Lohmann et al, 2017	Brett et al, 2017	Steinrücke et al, 2016	Szczaluba et al, 2017	Total
Global developmental delay	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	18/18	13/13	12/12	Yes	Yes	Yes	46/46 (100 %)
Abnormal muscle tone	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	16/18	12/13	4/12	Yes	Yes	Yes	36/46 (78%)
Epilepsy	No	No	Yes	No	Yes	No	No	Yes	No	No	Yes	No	Yes	No	No	No	No	Yes	6/18	10/13	7/12	No	No	No	23/46 (50%)
Abnormal EEG	No	No	Yes	No	Yes	No	No	Yes	No	No	Yes	Not done	No	No	Not done	Yes	Not done	Yes	6/15	11/13	Not known	Not known	No	Yes	18/30 (60%)
Abnormal MRI	Yes	No	Yes	No	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	No	No	No	Yes	11/18	5/13	7/12	Yes	No	No	24/46 (52%)
Abnormal vision	No	No	No	Yes	Yes	Yes	No	No	Yes	No	Yes	Yes	Yes	No	No	Yes	No	Yes	9/18	9/13	8/12	Yes	No	No	27/46 (58%)
Growth delay: both height (Ht) and weight (W) below -2 SD	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	0/18	6/13	3/12	Yes	No	No	10/46 (21%)

Microcephaly (below -2 SD)	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	0/18	1/13	Not known	No	Not known	No	1/33 (3%)
Macrocephaly (above +2 SD)	No	No	No	No	Yes	Yes	Yes	No	No	No	No	Yes	No	No	No	No	No	No	4/18	3/13	Not known	No	Not known	No	7/33 (21%)
Dysmorphic features	Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	15/18	6/13	Not known	Yes	Yes	Yes	24/34 (70%)

Table 4: Summary of Epilepsy manifestation in our cohort as well as Petrovski et al. (2016) cohort

Symptoms	Patient 3	Patient 5	Patient 8	Patient 11	Patient 13	Patient 18	Individual 2, Petrovski et al. (2016)	Individual 3, Petrovski et al. (2016)	Individual 5, Petrovski et al. (2016)	Individual 6, Petrovski et al. (2016)	Individual 7, Petrovski et al. (2016)	Individual 8, Petrovski et al. (2016)	Individual 10, Petrovski et al. (2016)	Individual 11, Petrovski et al. (2016)	Individual 12, Petrovski et al. (2016)	Individual 13, Petrovski et al. (2016)
Mutation (GRCh37; GenBank: NM_002074.4)	Chr1:1735947C >T	Ch1:1737942 A>G	Chr1:1737942 A>G	Chr1:1737942 A>G	Chr1:1737942 A>G	Chr1:1737942 A>G	chr1: g.1737948T >C	chr1: g.1737942 A>T	chr1: g.1735987T >C	chr1: g.1718817 C>T	chr1: g.1737953 A>C	chr1: g.1735987 T>C	chr1: g.1737942 A>G	chr1: g.1737942 A>G	chr1: g.1737942 A>T	chr1: g.1736004 A>G
	c.341G >A	c.239T>C	c.239T>C	c.239T>C	c.239T>C	c.239T>C	c.233A>G	c.239T>A	c.301A>G	c.976G>A	c.228T>G	c.301A>G	c.239T>C	c.239T>C	c.239T>A	c.284T>C
	p.Cys114Tyr	p.Ile80Thr	p.Ile80Thr	p.Ile80Thr	p.Ile80Thr	p.Ile80Thr	p.Lys78Arg	p.Ile80Asn	p.Met101Val	p.Ala326Thr	p.Asp76Glu	p.Met101Val	p.Ile80Thr	p.Ile80Thr	p.Ile80Asn	p.Leu95Pro
EEG	3yrs: right and left temporal epileptiform discharges during sleep (T7-T8) and left temporal slowing	1 yr.: Normal; 6yrs: centroparietal rt+lt epileptiform activity with significant increase during sleep (ESES)	3yrs: Normal; 4.5yrs: presence of Ictal activity during sleep, and wake characterized by high waves and generalized slow waves	11yrs: infrequent, brief, independent right and left frontal / fronto-central sharp wave discharges suggestive of multi-focal epilepsy	2yrs: Normal	4 yrs and 9yrs: focal epileptiform activity with important persistence in wakefulness that is strikingly activated in NREM sleep	6 months: hypsarrhythmia, VEEG captured infantile spasms	15 months: generalized subcortical alterations; 3 years: generalized epileptiform process	7, 10, and 11 months: modified hypsarrhythmia; 2 years: normal; 3 years: multifocal and 2 Hz GSW	14 months: Normal; 5 years: L and R posterior temporal epileptiform activity	burst suppression	4 years: Normal; 11 years: diffuse slowing and rare right temporal spikes	2 years: generalized paroxysmal epileptiform activity, myoclonic seizure with generalized paroxysmal polyspike slow-wave activity	9 years: multifocal spikes and generalized background slowing	4 years: high amplitude background with occipital slowing, multifocal epileptiform discharges	4 months: multifocal epileptiform discharges from both posterior quadrants, right temporoparietal sharp waves
Epilepsy	tonic-like seizures with stiffening and staring spells	3yrs: intermittent starring episode; 8yrs: status epilepticus (generalized clonic movement with right eye deviation)	tonic, spasms and myoclonic generalized seizures	absences since childhood, about 10 per day; tonic-clonic since 11 years	one episode at 2yrs	Yes	infantile spasms	9 months: generalized epilepsy	infantile spasms, absence seizures, Non-convulsive status epilepticus, focal seizures	1 year: febrile status epilepticus followed by afebrile focal convulsive seizures and absence and atonic seizures; 14 years: focal hyperkinetic sleep-related seizures	hemiclonic seizure associated with hypoglycemia (<20), went into complex partial status	11 years: onset of tonic-clonic seizures	2 years: onset of head drops and myoclonic seizures	9 years: onset of focal seizures	4 years: onset of focal seizures	4 months: tonic posturing of the arms and occasional generalized upper-body myoclonus
Well controlled	Yes	No	No	Yes	NA	No	Yes	unknown	unknown	unknown	unknown	Yes	unknown	Yes	unknown	Yes
Treatment	anti-epileptics x 1 year (Trileptal 5.5 mg bid)	Sultiam, valproic acid	MAD diet at 3:1 ratio, Locasamide, Valproate Magnesium, Clonazepam	levetiracetam	None	valproic acid, sultiam, and levetiracetam	oral prednisolone	unknown	unknown	unknown	anti-convulsant therapies	valproic acid and lamotrigine	unknown	onfi and Trileptal	unknown	pyridoxal-5-phosphate