Case reports

Patient 1

This 3-years-old Russian girl was born at term. The Apgar scores at 1 and 5 minutes were eight. Her birth weight and head circumference were within normal limit. From 2\textsuperscript{nd} day of life on she was very quiet, sleepy and had poor suck but did develop eye contact and eye tracking in the following weeks. Flexor spasms occurring in prolonged clusters and lip smacking were observed at 2 months of age. First electroencephalography (EEG) at 5 months of age showed multifocal spikes without burst-suppression pattern. When spasms persisted, EEG repeatedly showed hypsarrhythmia. Oral prednisone controlled the spasms: however, partial complex seizures that presented as lip smacking accompanied by movement arrest developed at 6 months of age. She was treated by valproic acid and clonazepam but these attacks were still observed on a daily basis at 1 year of age when we first saw the patient. On the examination, no dysmorphic features were recognized. She showed no eye contact or consistent eye tracking, and was unable to roll over, crawl or pull to sit, and reach for toys, but showed excessive repetitive hand-into-mouth stereotypes. Ophtalmoplegia, nystagmus or extra pyramidal movements were not recognized. She showed acquired microcephaly [head circumference was 43 cm (below 3 percentile)] and diffuse hypotonia. Basic metabolic workup including electrolytes, liver function tests, renal function tests, lactate, pyruvate, ammonia, serum amino acids, urine organic acids, very-long-chain fatty acids, isoelectric focusing of transferrin, carnitine, acyl carnitines and cerebrospinal fluid test for glucose, protein and neurotransmitters were all within normal ranges. EEG showed that background consists of mixture of theta and delta waves in moderate amplitude.
with no posterior predominance or hemispheric asymmetry and intermittent multifocal spikes. During a 30 minutes’ recording 4 electrographic seizures were recorded: originating from both hemispheres independently consisting of rhythmic buildup of high amplitude theta activity mixed with spikes lasting 20-40 seconds each and none show spreading to the other hemisphere (Supplementary Figure 2). Brain magnetic resonance imaging (MRI) showed thin corpus callosum at 5 months of age, and progressive cerebral atrophy and delayed myelination at 10 months (Figure 2). No cerebellar atrophy was seen.

**Patient 2**

This 13-months-old Japanese girl, the first child of healthy and non-consanguineous parents, was born after an uneventful 40-week gestation. Her birth weight is 2,716 g (-1.2 standard deviation [SD]). At days 7 and 9, she presented with tonic upward movements of the bilateral upper limbs that ceased within 10-20 seconds. These episodes were accompanied by transient breath-holds and facial flushing. EEG was evaluated as normal (data not available). The tonic movements recurred once or twice a day from days 10 to 14, while her general activity was unaltered between each episode.

She was transferred to our hospital at day 21 because of recurrent attacks of bilateral tonic seizures with poor recovery of consciousness and general activity for 2 hrs. Acute infusion of phenobarbital of 20 mg/kg per day briefly aborted the seizure. Her height, weight and head circumference at day 30 were 51 cm (-1.7 SD), 3.47 kg (-2.2 SD) and 35 cm (-1.5 SD), respectively. She was afebrile and her fontanel was softly flat. No facial or limb anomaly was observed. Her motor activity was poor whereas other neurological findings were unremarkable. Laboratory examination showed a negative
TORCH study and normal blood cell counts and biochemistry, including electrolytes, liver enzyme, creatine kinase, and ammonium levels. Metabolic disorders were ruled out with blood gas analyses, tandem mass-spectrometry, quantitative amino acid analysis, and gas chromatography for urinary organic acids. Cerebrospinal fluid test excluded the diagnosis of infectious disorders and encephalitis with nucleated cell counts (1 /µl) and total protein (92 mg/dl).

An EEG recording at day 23 showed that focal sharp waves were frequently evoked from the central region of the right hemisphere. These paroxysmal discharges evolved very often into focal 5-6Hz slow wave activity or slow-wave bursts diffused to the affected side of hemisphere (Supplementary Figure 3). Phenobarbital was constantly maintained at the concentration of 50-60 µg/ml, which appeared to be effective for the next 2 weeks. However, she had a transient increase in tonic seizures, which occurred in clusters from day 39 to 42. The EEG at day 39 clearly demonstrated the migrating patterns of epileptiform discharges that arose in the right frontal region, diffused into the right hemisphere, and eventually moved to the left side (Figure 2), resembling the EEG findings in migrating partial seizures of infancy. In spite of antiepileptic drug polytherapy, intractable daily seizures were observed. She showed severe developmental delay and severe chorea developed in her late infancy. At 14 months of age, she was unable to control her head or utter meaningful words.

No cranial anomaly or parenchymal lesions were found on the computed tomography (CT) and MRI of the brain at day 22. Notably, single photon emission computed tomography disclosed the marked decrease in perfusion of the whole brain (data not shown). Follow up MRI at 14 months of age showed cerebral atrophy with delayed myelination (Figure 2)
**Patient 3**

The 12-year-old Japanese girl was the second child of healthy and non-consanguineous parents. She was born at 39 weeks of gestation by normal vaginal delivery. Her birth weight, length, and occipitofrontal circumference were 2,950 g (0.0 SD), 47 cm (-1.2 SD) and 31.2 cm (-1.5 SD), respectively. Developmental delay and generalized hypotonia were recognized at 4 months of age. At 5 months of age, she visited our hospital for medical investigation. Laboratory investigation for metabolic disorders and conventional cytogenetic studies were normal. EEG and brain MRI showed normal findings. She was diagnosed with athetotic cerebral palsy of unknown cause.

Her development was severely delayed. Involuntary movement developed at 4 years of age. Athetotic movement of arms was exaggerated after orthopedic surgery at 8 years. She developed status dystonicus. Her dystonia was refractory to traditional pharmacological therapy. She was treated in the intensive care unit with sedative treatment. EEG showed no epileptic discharge which was unchanged in her involuntary movement. At 12 years of age, she was attacked by the second episode of status dystonicus without specific cause, and again admitted to intensive care unit. She progressed to a bedridden state with tracheostomy and enterostomy. Neurological examination revealed spasticity of all extremities. She is unable to control her head and sit without support. Purposeful hand skills were not obtained. Eye contact was poor. Although she showed severe intellectual diability, she understood simple word and changed her facial expression. At present, her height was 149 cm (-0.2 SD), weight was 30.5 kg (-1.8 SD) and head circumference was 53 cm (-0.3 SD). MRI at 12 years
showed no apparent abnormalities (Figure 2).

Patient 4

The 18-year-old Japanese female was the second child of healthy and non-consanguineous parents. She was born at 39 weeks of gestation without asphyxia by normal vaginal delivery. Her birth weight was 2,886 g (-0.2 SD). Initial development was normal as she showed head control and rolling over at 3 and 7 months of age, respectively. She was able to sit with hand support at 11 months of age, but did not obtain standing or walking thereafter. Hypotonia was observed at 11 months of age, and spasticity of bilateral lower extremities appeared at 3 years of age. She had no meaningful word until 5 years of age when she could show simple word. She developed chorea of extremities at 5 years, which was accompanied by fever, vomiting, and acidosis. Her chorea disappeared when she became afebrile, but relapsed by infection or fever. She underwent surgery for fracture of left femur at 10 years of age, which temporarily worsened her chorea. Complex partial seizures with fever and afebrile complex partial seizures were observed at 10 and 11 years of ages, respectively, but EEG showed no abnormalities. Since 10 years of age, developmental regression was recognized. She lost head control, sitting, and meaningful words. She showed eye pursuit. Laboratory examination showed normal blood cell counts and biochemistry, including lactic acid and pyruvate. Brain MRI showed progressive atrophy of cerebrum, cerebellum, and brain stem, and thin corpus callosum (Figure 2).
Supplementary Figure 1. Structural details of the variant sites.

The crystal structures of Ga-containing complexes: the GDP-bound inactive Gaβγ heterotrimer (PDB code 1GG2) (a), the nucleotide-free Gaβγ heterotrimer in complex with an agonist-occupied monomeric β2 adrenergic receptor (β2AR) (PDB code 3SN6) (b), the transition-state GTP analogue (GDP+AlF₄⁻)-bound Gaq in complex with its effector phospholipase C-β (PLCβ) (PDB code 3OHM) (c), and the GTP analogue (GTPγS)-bound Gas in complex with the catalytic domains of adenylyl cyclase (AC) (PDB code 1AZS) (d) are shown in the same colors as in Figure 1 except for the switch I region in light-blue, with close-up views of the squared regions. Guanine nucleotides are depicted as orange sticks. In the close-up views, the side chains of Ala227 and Lys271 are shown as spheres. Black dotted lines represent hydrogen bonds in c and d.
Supplementary Figure 2. Ictal EEG recordings of patient 1 at one year of age.

EEG (14-21 electrode system) recording. (A) Ictal activity of rhythmic spiking intermixed with high amplitude theta waves over the right temporo-occipital region during a partial complex seizure. (B) In another similar clinically presented seizure, ictal activity of rapid rhythmic spikes intermixed with high amplitude theta waves was localized over the left temporo-occipital region representing the bilateral origin of her seizures.
Supplementary Figure 3. An EEG recording of patient 2 with natural sleep on day 23

A representative trace of EEG shows the typical form of epileptiform discharges on admission. Red arrows indicate the location and the appearance of sharp waves from the right centro-parietal region. The right-sided rhythmic slow waves that led to an ictal pattern are red-squared. The scale bar at bottom shows the duration (1 sec) and the amplitude (50 µV).
Table S1. Characterization of *GNAO1* variants

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<tr>
<th>Variant</th>
<th>Inheritance</th>
<th>dbSNP137</th>
<th>ESP6500 data</th>
<th>In-house database</th>
<th>SIFT</th>
<th>Polyphen2</th>
<th>MutationTaster</th>
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SIFT (http://sift.jcvi.org/): scores of less than 0.05% indicate substitutions that are predicted as intolerant.
PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/): HumVar scores are evaluated as 0.000 (most probably benign) to 0.999 (most probably damaging).
MutationTaster (http://www.mutationtaster.org/): rapid evaluation of DNA sequence alterations. The alterations are classified as disease causing or polymorphism.
ESP6500, 6500 exomes sequenced by National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project (http://evs.gs.washington.edu/EVS/)