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SHORT COMMUNICATION

A girl with West syndrome and autistic features harboring a *de novo TBL1XR1* mutation

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Recently, *de novo* mutations in *TBL1XR1* were found in two patients with autism spectrum disorders. Here, we report on a Japanese girl presenting with West syndrome, Rett syndrome-like and autistic features. Her initial development was normal until she developed a series of spasms at 5 months of age. Electroencephalogram at 7 months showed a pattern of hypsarrhythmia, which led to a diagnosis of West syndrome. Stereotypic hand movements appeared at 8 months of age, and autistic features such as deficits in communication, hyperactivity and excitability were observed later, at 4 years and 9 months. Whole exome sequencing of the patient and her parents revealed a *de novo TBL1XR1* mutation [c.209 G>A (p.Gly70Asp)] occurring at an evolutionarily conserved amino acid in an F-box-like domain. Our report expands the clinical spectrum of *TBL1XR1* mutations to West syndrome with Rett-like features, together with autistic features.
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TBL1XR1 at 3q26.32 encodes transducin β-like 1 X-linked receptor 1, a co-repressor of nuclear hormone transcription factors that is required for β-catenin–Tcf-mediated Wnt signaling.[1–3] Recently, two *de novo TBL1XR1* mutations were found in 2 of 2446 patients with autism spectrum disorders, suggesting an association between *TBL1XR1* mutations and autism.[4,5] Here, we present a third case with a *de novo TBL1XR1* mutation, showing West syndrome, Rett-like and autistic features.

CASE REPORT

This report concerns a 5-year-old girl who is the offspring of unrelated healthy Japanese parents. She was born at 39 weeks of gestation without asphyxia after an uneventful pregnancy. Her birth weight, birth length and head circumference were 3088 g (+0.3 standard deviation (s.d.)), 51.1 cm (+1.0 s.d.) and 34.0 cm (+0.5 s.d.), respectively. She showed social smiling and head control at 3 and 4 months of age, respectively. Then at 5 months she developed a series of spasms occurring 5–6 times a day, when her head control became unstable. Electroencephalography at 7 months of age showed hypsarrhythmia patterns (Figure 1a), which led to a diagnosis of West syndrome. Brain magnetic resonance imaging showed no structural brain anomalies (Figures 1a and c). Administration of adrenocorticotropic hormone therapy only temporarily reduced the frequency of spasms.

On examination at 7 months of age, the patient's weight, height and head circumference were 8720 g (+1.2 s.d.), 69.5 cm (+0.9 s.d.) and 42 cm (−0.6 s.d.), respectively. Mild dysmorphic features were observed, including a long palpebral fissure, thick eyebrows and downturned corners of the mouth. The patient showed no eye fixation and pursuit, as well as no social smile. Her muscle tone was mildly hypotonic. Despite being given extensive treatments including adrenocorticotropic hormone therapy in combination with valproic acid, nitrazepam, vitamin B6, topiramate, clonazepam and clobazam, she continued to exhibit frequent subtle tonic seizures with eyelid opening. At 8 months of age, stereotypical hand movements appeared that resembled hand-washing.

Laboratory examination revealed elevated serum levels of several components: lactic acid (39.9 mg dl^{-1}, compared with a normal range of 5.0–20.0 mg dl^{-1}), pyruvate (2.79 mg dl^{-1}, normal range 0.3–0.9 mg dl^{-1}) and alanine (1447 nmol ml^{-1}, normal range 180–470 nmol ml^{-1}). However, following vitamin B1 treatment, both pyruvate and alanine serum levels returned to normal. The levels of lactic acid and pyruvate in cerebrospinal fluid appeared normal (14.6 and 0.65 mg dl^{-1}, respectively). Respiratory-chain enzymes, using muscle homogenates and fibroblasts, and mitochondrial DNA sequence analysis were all normal, and pathological examination of muscle specimens revealed no specific findings. Repeated examination of both serum lactic acid and pyruvate levels also showed no abnormalities.

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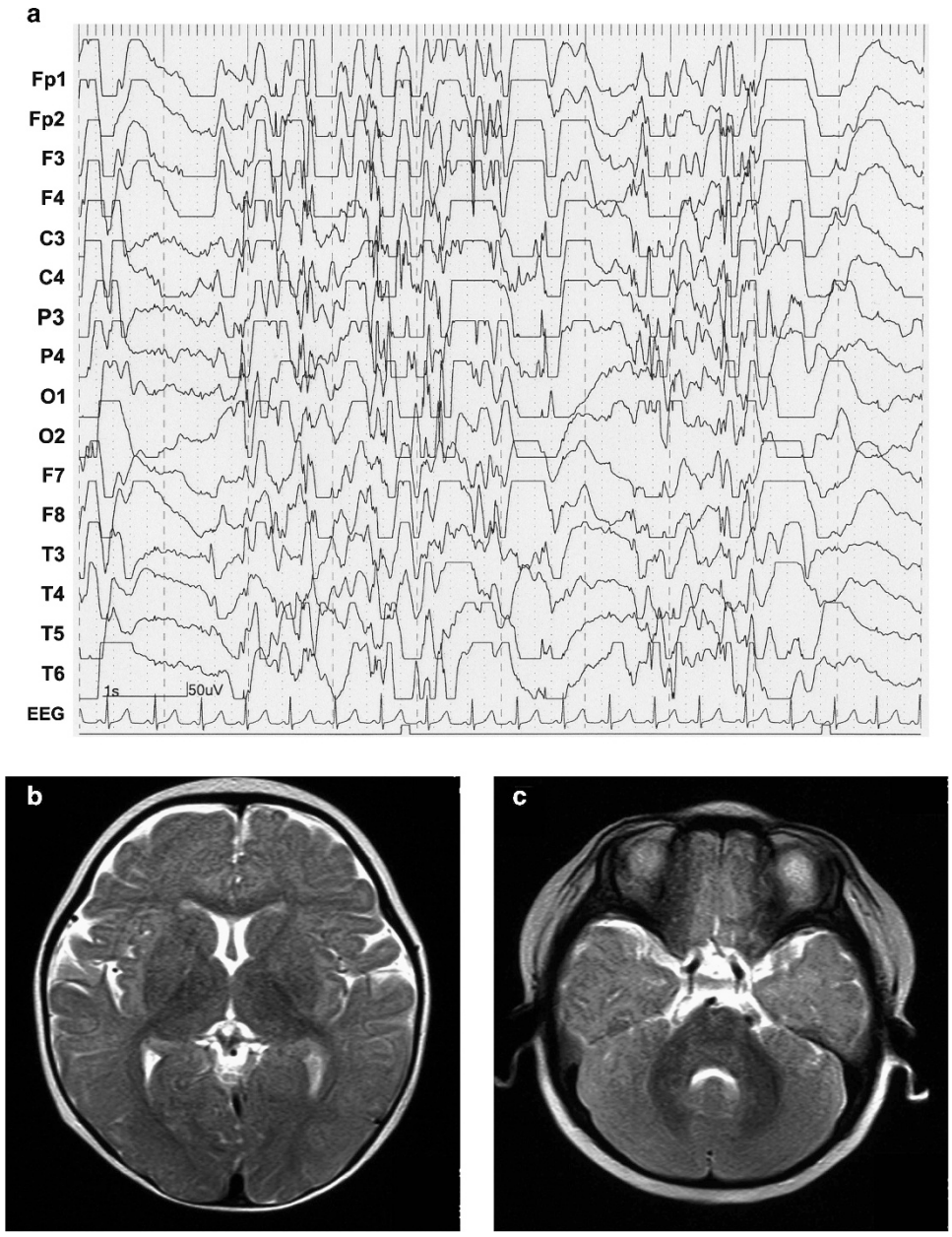

Figure 1 Electroencephalogram (EEG) and brain magnetic resonance imaging in the patient at 7 months of age. (**a**) Interictal EEG showed high-amplitude multifocal spikes with irregular slow waves consistent with a finding of hypsarrhythmia. T2-weighted axial images through the basal ganglia (**b**) and the cerebellum (**c**) showed no abnormalities.

After contracting a fever at the age of 1 year and 10 months, her seizures were controlled using a combination therapy of topiramate, valproic acid, clobazam and vitamin B1. However, the patient still could not speak any meaningful words at 4 years and 9 months of age but could walk with support; she had a developmental quotient of 13. At this age she still showed stereotypic hand movements as well as autistic features such as deficits in communication, hyperactivity and excitability.

RESULTS AND DISCUSSION

G-banded karyotyping was normal (46,XX). No pathological copy number aberrations were detected by the 2.7M Array (Affymetrix, Santa Clara, CA, USA). Whole exome sequencing of the patient and her parents was performed. Genomic DNA of blood leukocytes was captured using the SeqCap EZ Exome Library v2.0 (Roche NimbleGen, Madison, WI, USA), and sequenced with on HiSeq2000 (Illumina, San Diego, CA, USA). Variants were detected as previously described.[6] Variants with a Phred-like consensus quality score of >100 were considered as candidate variants. We found a total of four *de novo* candidate variants, in which two mutations were further validated as *de novo* by Sanger sequencing: *SELPLG* NM_001206609.1: c.794C>T (p.Thr265Met) and *TBL1XR1* NM_024665.4:c.209G>A (p.Gly70Asp). The other two variants were transmitted from her mother, demonstrating that the two variants were falsely uncalled in the mother. Neither of the two *de novo* mutations was found in the 6500 National Heart, Lung, and Blood Institute exomes nor in our

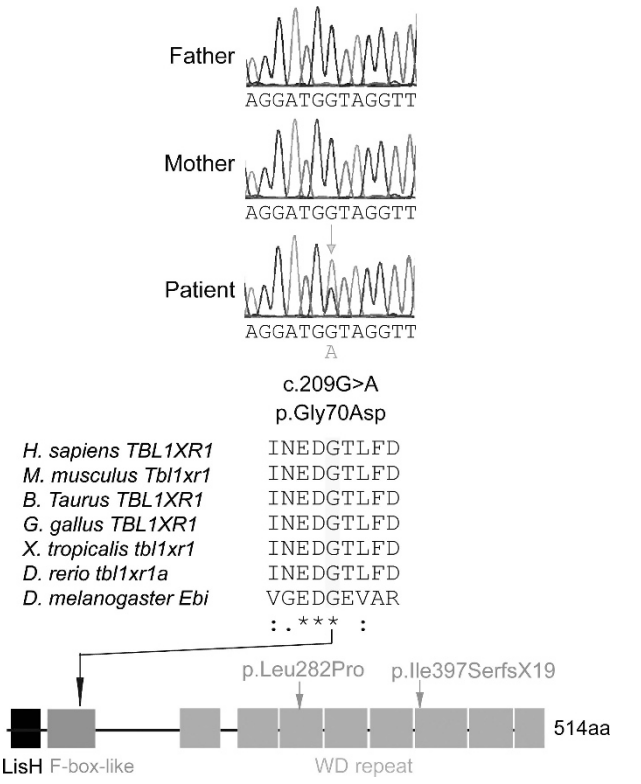

Figure 2 *De novo TBL1XR1* mutation. The c.209G>A (p.Gly70Asp) mutation occurred *de novo* at an evolutionarily conserved amino acid in an F-box-like domain. Multiple amino-acid sequences of TBL1XR1 proteins were aligned with tools available on the CLUSTALW web site. Two previously reported mutations (p.Leu282Pro and p.Ile397SefsX19) are highlighted in red. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

575 in-house control exomes. Both mutations were predicted as damaging by SIFT and PolyPhen2. However, MutationTaster classified only p.Gly70Asp in *TBL1XR1* as damaging whereas p.Thr265Met in *SELPLG* was predicted as a polymorphism. We found no recessive mutations in known early-onset epileptic encephalopathy genes including *SLC25A22*, *PNPO*, *PNKP* and *PLCB1*.[7] All experimental protocols used were approved by the Institutional Review Board of Yokohama City University School of Medicine.

SELPLG encodes P-selectin glycoprotein ligand 1, for which a knock-out mouse study showed that *Selplg* is required for leukocyte adhesion and rolling.[8,9] No neurological abnormalities were reported, suggesting that the *SELPLG* mutation is less likely to be involved in the phenotype of this patient.

TBL1XR1, also denoted as *TBLR1*, is required for β-catenin–Tcf-mediated Wnt signaling.[1,2] Mutations in *TCF4*, an essential mediator of Wnt signaling, have been shown to cause Pitt–Hopkins Syndrome, which presents with severe intellectual disability, seizures and stereotypic movements.[10,11] This suggests that the β-catenin–Tcf-mediated Wnt pathway of signaling is essential for normal brain function. Moreover, two *de novo TBL1XR1* mutations (p.Leu282Pro and p.Ile397SerfsX19) were found in 2 of 2446 patients with autism spectrum disorders.[4,5] In our case, the p.Gly70Asp mutation occurred in an evolutionarily conserved amino acid within an F-box-like domain (Figure 2). Indeed, the F-box-like domain of TBLR1 (TBL1XR1) is essential for a high affinity interaction between TBL1XR1 and SMRT, a co-repressor of nuclear hormone receptors.[3] This implies that p.Gly70Asp may affect this interaction. Therefore, the evidence suggests that the p.Gly70Asp mutation may cause a West syndrome phenotype with Rett-like and autistic features.

The role of *TBL1XR1* mutations was also investigated in 280 epileptic patients. High-resolution melting analysis revealed that three rare missense variants (p.Ala116Ser, p.Gly405Glu and p.Asn407Ser) were present in three patients. Since they were predicted as benign by PolyPhen-2, these mutations are unlikely to be pathogenic. These data suggest that *TBL1XR1* mutations are rarely involved in epileptic patients.

In conclusion, we describe a Japanese girl with a *de novo TBL1XR1* mutation that is predicted as pathogenic. Our report suggests that the clinical spectrum of *TBL1XR1* mutations includes autistic features as a core phenotype, as well as presenting with West syndrome and Rett-like features.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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