Letter to the Editor

Association of genomic deletions in the \textit{STXBP1} gene with Ohtahara syndrome

To the Editor:

Ohtahara syndrome (OS) is characterized by early-onset of seizures, suppression-burst patterns on electroencephalogram (EEG), and severe psychomotor retardation (1–3). \textit{De novo} mutations in the \textit{STXBP1} gene, including various point mutations and one complete deletion, have been found in about one-third of Japanese cases of cryptogenic OS (4–6). However, the clinical spectrum of \textit{STXBP1} mutations can be applied to other pathologies. For instance, in one study, \textit{STXBP1} abnormalities including a microdeletion were detected in approximately 10\% of patients (5/49) with early-onset epileptic encephalopathy that did not fit into a specific epilepsy syndrome (7). Other studies have also detected \textit{de novo} \textit{STXBP1} mutations in 2 of 95 individuals with mental retardation and non-syndromic epilepsy (8), in addition to the detection of a \textit{de novo} partial deletion in a child with epilepsy and autistic features (9, 10). On the basis of these findings, extensive genetic testing including copy number analysis of \textit{STXBP1} should be considered in children with early-onset seizures. However, the use of high-resolution copy number analysis of \textit{STXBP1} thus far has been limited.

In this study, we performed customized array comparative genomic hybridization (aCGH) analysis, in which a total of 27,026 probes covering the \textit{STXBP1} locus (UCSC coordinates, May 2006: Chr9: 129,350,808–129,558,072 bp) were distributed with 5-bp spacing except for repeating element regions (Roche NimbleGen, Tokyo, Japan). Among the 28 patients with cryptogenic OS tested, we found pathogenic \textit{de novo} deletions in two patients (7.1\%), where one 4.6-kb deletion included only exon 4, and the other 2.85-Mb one involved the entire \textit{STXBP1} gene (Table 1).

Patient 1506, a product of unrelated healthy parents, had no problems in the perinatal period. Tonic seizures with a flexion of the upper extremities started at 32 days of age, and frequent myoclonic seizures subsequently appeared. On the basis of suppression-burst pattern on EEG, the patient was diagnosed as having OS or early myoclonic encephalopathy (EME), which is another epileptic syndrome showing suppression-burst pattern on EEG (11). As OS and EME have common features, they can be difficult to distinguish (2, 3). Brain magnetic resonance imaging (MRI) revealed normal neuroanatomy. High-dose phenobarbital was able to effectively reduce the frequency of seizures. Customized aCGH and breakpoint polymerase chain reaction (PCR) analyses detected a \textit{de novo} 4635-bp deletion involving exon 4 of the \textit{STXBP1} gene (Fig. 1a–c). The presence of a 2-bp microhomology at the deletion junction suggested non-homologous recombination leading to a

<table>
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<th>Patient</th>
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<th>Start (bp)</th>
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<th>Size (bp)</th>
<th>Genes</th>
<th>Inheritance</th>
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<td>2231</td>
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<td>131,869,806</td>
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<td>70 RefSeq genes including STXBP and SPTAN1</td>
<td>De novo</td>
<td>Paternalb</td>
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</table>

aCGH, array comparative genomic hybridization; OS, Ohtahara syndrome.

aNo informative markers were available within the 4.6-kb region corresponding to the deletion.

The detection of two microdeletions using microarray. (a) A customized array comparative genomic hybridization (aCGH) profile of STXBP1 locus in patient 1506. x- and y-axis show the genomic location from the p telomere of chromosome 9 (UCSC coordinates, May, 2006) and log2 (Cy3/Cy5 signal ratio) values (green dots ≥0.25; −0.25 < black dots < 0.25; red dots ≤−0.25), respectively (top panel). A close up view of the aCGH profile along with maps of the STXBP1 exons (blue rectangles), showing the deletion of exon 4 (bottom panel). (b) Polymerase chain reaction (PCR) analysis of the family of patient 1506. Primers flanking the deletion amplified both 6398- and 1763-bp products from the wild type and deletion alleles, respectively, of the patient. However, the patient’s parents had only a 6398-bp product, indicating the presence of a de novo deletion (Neg, negative control which contained no template DNA). (c) The deletion junction sequence. The top, middle and bottom strands show the proximal, deleted, and distal sequences, respectively. The two overlapping nucleotides are colored in red. (d) The 2.7M array profile clearly showed a 2.85-Mb deletion at 9q33.3-34.11 found in patient 2231 (top panel). A total of 70 RefSeq genes, including STXBP1 and SPTAN1, were mapped within the deletion (bottom panel). (e) The breakpoint PCR analysis of the family of patient 2231. Primers flanking the deletion successfully amplified a 2430-bp product from the patient, indicating that the deletion occurred de novo (Neg, negative control that contained no template DNA). (f) The deletion junction sequence. The top, middle and bottom strands show the proximal, deleted and distal sequences, respectively. The three overlapping nucleotides are colored in red. The PCR conditions and primer sequences are available on request.
rearrangement (Fig. 1c) (12). The deletion of exon 4 was also confirmed by reverse transcriptase-PCR (Fig. S1, Supporting Information).

Patient 2231 was born at term after in vitro fertilization and embryo transfer. The body weight at birth was 2134 g (−2.4 SD), height 44.5 cm (−2.3 SD), and head circumference 32.0 cm (−0.8 SD). Multiple anomalies including cleft lip and palate, ventricular septal defect, overlapping fingers, and small penis were noted. G-banded chromosomal analysis was normal. The patient had an onset of sudden crying at 1 week of age followed by a cluster of epileptic spasms with suppression-burst pattern on EEG at 1 month. A brain MRI at 2 months showed a thin corpus callosum and relatively small cerebellum. After treatment with antiepileptic drugs proved ineffective, a ketogenic diet reduced the frequency of seizures. At 19 months, he showed spastic quadriplegia and profound intellectual disability at the level of a 2-month old. Customized aCGH, subsequent whole-genome 2.7M Array (Affymetrix, Santa Clara, CA), and breakpoint PCR analyses found a de novo 2.85-Mb microdeletion including STXBP1 and SPTAN1 (13) (Fig. 1d–f). The presence of a 3-bp homology at the deletion junction further suggested non-homologous recombination leading to the rearrangement (Fig. 1f).

In conclusion, our high-resolution copy number analysis in STXBP1 locus revealed a 4.6-kb deletion encompassing only exon 4, which strongly suggests that copy number analysis covering all STXBP1 exons should be recommended as a genetic test for children with early-onset seizures.

Supporting Information
The following Supporting information is available for this article:
Fig. S1. Examination of the mutated transcripts in lymphoblastoid cell lines derived from the patient 1506. (a) Reverse transcriptase polymerase chain reaction (RT-PCR) analysis of the patient with an exon 4 deletion relative to a normal control. A schematic representation of the transcript from exons 3 to 6 of STXBP1 is indicated (top). The exons and primers are depicted as boxes and arrows, respectively. Two PCR products were amplified from the patient’s cDNA: the upper was a wild-type (WT) transcript and the lower was the deleted mutant (middle). Only a single WT amplicon was detected in the control. The mutant amplicon was significantly increased by 30 μM cycloheximide (CHX) treatment for 4 h compared to dimethyl sulfoxide treatment as a vehicle control. RT (+): with reverse transcriptase, RT (−): without reverse transcriptase as a negative control. The sequence of the smaller amplicon clearly demonstrated exon 4 deletion (bottom). (b) Quantitative analysis of the nonsense-mediated mRNA decay (NMD) inhibition by CHX based on the data shown in (a).
*p = 0.0023 by unpaired two tailed Student’s t-test. Averages of duplicated experiments using two distinctive RNA samples are shown with error bars (SD). The mutant transcript lacking exon 4 created a premature stop codon at position 64, and suffered from degradation by NMD in the patient’s lymphoblastoid cells. PCR conditions and the primer sequences are available on request.

Additional Supporting information may be found in the online version of this article.

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Letter to the Editor

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