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Original article

High prevalence of genetic alterations in early-onset epileptic encephalopathies associated with infantile movement disorders

Yu Kobayashi [a], Jun Tohyama [a,b,*], Mitsuhiro Kato [c,d], Noriyuki Akasaka [a], Shinichi Magara [a], Hideshi Kawashima [a], Tsukasa Ohashi [a], Hideaki Shiraishi [e], Mitsuko Nakashima [f], Hirotomo Saitsu [f], Naomichi Matsumoto [f]

[a] *Department of Child Neurology, Nishi-Niigata Chuo National Hospital, Niigata, Japan*
[b] *Niigata University Medical and Dental Hospital, Niigata, Japan*
[c] *Department of Pediatrics, Yamagata University Faculty of Medicine, Yamagata, Japan*
[d] *Department of Pediatrics, Showa University School of Medicine, Tokyo, Japan*
[e] *Department of Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo, Japan*
[f] *Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan*

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Abstract

Objective: Recent studies have elucidated causative roles for genetic abnormalities in early-onset epileptic encephalopathies (EOEE). Accompanying characteristic features, in addition to seizures, have also been suggested to provide important clues for an early and accurate genetic diagnosis of affected patients. In this study, we investigated the underlying genetic causes in patients with EOEE associated with infantile movement disorders.

Methods: We examined 11 patients with EOEE and involuntary movements (nine with West syndrome and two with nonsyndromic epileptic encephalopathy). All showed severe developmental delay, cognitive impairment, and involuntary movements such as chorea, ballism, dyskinesia or myoclonus, and hand stereotypies. We performed whole-exome sequencing of 10 patients, while the other patient underwent high-resolution melting analysis of candidate EOEE genes.

Results: We identified mutations in *CDKL5*, *SCN2A*, *SETD5*, *ALG13*, and *TBL1XR1* in seven patients with West syndrome, and in *SCN1A* and *GRIN1* in the two patients with unclassified epileptic encephalopathy. All mutations were validated as *de novo* events. The genetic cause was undetermined in the remaining two patients.

Conclusions: We found pathogenic mutations in seven genes, in nine of 11 patients with EOEE and involuntary movements. Although the results of our study are preliminary because of the small number of patients, they nevertheless suggest that specific accompanying phenotypes such as hyperkinetic movements or hand stereotypies could be important in narrowing the disease spectrum and identifying causative genetic abnormalities.

Keywords: Epileptic encephalopathy; West syndrome; Involuntary movement; Hyperkinetic movement; Hand stereotype; Whole-exome sequencing

* Corresponding author at: Department of Child Neurology, Nishi-Niigata Chuo National Hospital, 1-14-1 Masago, Nishi-ku, Niigata 950-2085, Japan. Tel.: +81 25 265 3171; fax: +81 25 231 2831.
E-mail address: jtohyama@masa.go.jp (J. Tohyama).

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1. Introduction

Early-onset epileptic encephalopathies (EOEEs) are a heterogeneous group of disorders characterized by intractable seizures and unremitting interictal paroxysmal epileptiform activity that consequently impair neurodevelopmental outcomes during the first year of life [1,2]. After the initial identification of mutations for cryptogenic West syndrome and Ohtahara syndrome in *ARX*, *CDKL5*, and *STXBP1* genes [3–7], advanced technologies have revealed that mutations in growing numbers of epilepsy genes are involved in various EOEE syndromes. EOEEs therefore arise from highly heterogeneous genetic causes, so understanding genotype–phenotype correlations will be useful for appropriate diagnosis and disease management.

Although several diagnostic approaches have been proposed for EOEEs [2,8,9], the diagnosis of an unspecific epilepsy phenotype is a challenging task [10]. While relatively rare, involuntary movements are sometimes observed in EOEEs. It has been reported that *ARX* mutations are associated with dystonia [11], while *STXBP1* mutations with choreo-ballistic movements, generalized tremors, and dystonia [12–14]. It is also well known that patients with *CDKL5* or *FOXG1* mutations show a Rett-like phenotype with hand stereotypies and autistic features [5,15]. These numerous reports suggest that accompanying characteristic features in EOEE patients, in addition to seizures, can provide important clues about the disease phenotype, leading to accurate genetic diagnosis.

In this study, we focused on patients with EOEE accompanied by involuntary movements such as hyperkinetic movements and hand stereotypies. We investigated the underlying genetic causes by high-resolution melting analysis or whole-exome sequencing (WES).

2. Subjects and methods

2.1. Patients

Patients with epilepsy were recruited from Nishi-Niigata Chuo National Hospital (Niigata, Japan) between 2007 and 2013. The inclusion criteria were as follows: onset within 1 year after birth, frequent epileptic seizures including spasms, severe developmental delay, cognitive impairment, and accompanying involuntary movements such as chorea, dyskinesia, ballism, and/or hand stereotypies. Epilepsy types were determined by an epileptologist (J.T., Y.K., N.A., S.M., H. K., T.O., or H.Sh) on the basis of clinical history, imaging, and electroencephalographic findings in accordance with epilepsy classifications of the International League Against Epilepsy, 2010 [1,16]. Prior to this study, all male patients were analyzed for *ARX* mutations by Sanger sequencing. Patients who possessed *ARX* mutations or who were diagnosed with Rett syndrome and carried an *MECP2* mutation were excluded from this study.

A total of 11 patients were included: nine patients with West syndrome and two patients with nonsyndromic epilepsy (epileptic encephalopathy: EE). The experimental protocols were approved by the Institutional Review Board of Yokohama City University School of Medicine, Yamagata University Faculty of Medicine, and Nishi-Niigata Chuo National Hospital. Peripheral blood samples were obtained from family members after obtaining their written informed consent. The work described was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

2.2. Genetic testing

We performed WES in 10 of the 11 patients. Briefly, genomic DNA was captured using a SureSelect Human All Exon v4 or v5 Kit (Agilent Technologies, Santa Clara, CA, USA) in nine patients or a SeqCap EZ Exome Library v2.0 (Roche NimbleGen, Madison, WI, USA) in one patient, then was sequenced on a HiSeq2000 (Illumina, San Diego, CA, USA) with 101-bp paired-end reads. Exome data processing, variant calling, and variant annotation were performed as previously described [17]. The depth of coverage of the WES analysis is shown in Supplemental Table 1. In seven patients (patients 1, 2, 6, 7, 8, 10 and 11), we performed trio-based WES and focused on *de novo* variants [18–20]. In three patients (patients 3, 5 and 9), we performed proband-only WES and searched for mutations in known epilepsy genes including *ARX*, *KCNT1*, *KCNQ2*, *SCN1A*, *SCN2A*, *SCN8A*, *STXBP1*, *SPTAN1*, *GNAO1*, *GRIN1*, *FOXG1*, *QARS*, *EEF1A2*, *PIGA*, *CDKL5*, *SLC35A2*, *CASK*, *PCDH19* and *MECP2*. In patient 4, we performed high-resolution melting analysis for *STXBP1*, *SPTAN1*, *KCNQ2*, and *SCN2A*. All mutations were validated as *de novo* events by Sanger sequencing.

2.3. Reverse transcription (RT)-PCR

A lymphoblastoid cell line (LCL) was established from patient 8 carrying a *SETD5* mutation. RT-PCR using total RNA extracted from the LCL was performed as previously described [21]. Briefly, total RNA was extracted using an RNeasy Plus Mini kit (Qiagen, Tokyo, Japan) from the LCL with or without incubation in 30 μM cycloheximide (CHX; Sigma, Tokyo, Japan) for 4 h, and 2 μl of cDNA was used as a template for PCR. The primers used were ex16-F (5′-ATTCGC TTTGGCTCACCCTTTATCC-3′) and ex17-R (5′-CA GAGGCCGAAGTAACTTGGTGACA-3′). PCR products were electrophoresed on a 10% polyacrylamide