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Genomic copy number variations at 17p13.3 and epileptogenesis

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Summary Deletion of the terminal end of 17p is responsible for Miller-Dieker syndrome (MDS), which is characterized by lissencephaly, distinctive facial features, growth deficiency, and intractable seizures. Using microarray-based comparative genomic hybridization, 3 patients with epilepsy were revealed to have genomic copy number aberrations at 17p13.3: a partial *LIS1* deletion in a patient with isolated lissencephaly and epilepsy, a triplication of LIS1 in a patient with symptomatic West syndrome, and a terminal deletion of 17p including *YWHAE* and *CRK* but not *LIS1* in a patient with intractable epilepsy associated with distinctive facial features and growth retardation. In this study, it was suggested that the identified gain or loss of genomic copy numbers within 17p13.3 result in epileptogenesis and that triplication of *LIS1* can cause symptomatic West syndrome.

Introduction

The terminal end of the short arm of chromosome 17 is crucial for neurodevelopment and deletion of this region is associated with Miller-Dieker syndrome (MDS), a congenital malformation syndrome consisting of typical lissencephaly and distinctive facial features. Patients with MDS also show growth deficiency, severe developmental delays, and intractable seizures (Dobyns et al., 1991). MDS results from chromosomal disruption, including cytogenetically visible or submicroscopic deletions of the 17p13.3 region, which includes *LIS1*, a key indicator of MDS (Dobyns et al., 1993; Reiner et al., 1993). *LIS1* encodes PAFAH1B1 and participates in neural migration, disruption of which is responsible for lissencephaly. Indepen-

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dent *LIS1* deletions or nucleotide alterations in its coding exons cause isolated lissencephaly without growth deficiency or distinctive facial features (Cardoso et al., 2000). This finding indicates that the clinical manifestations associated with MDS patients, such as growth deficiency and dysmorphic features, are likely derived from other genes included in the 17p13.3 region. Genotype—phenotype correlation studies in patients with deletions in the terminal region of 17p revealed that *LIS1* deletion is responsible for lissencephaly and that combined deletion of *LIS1* and *YWHAE* results in severer lissencephaly and a distinctive facial appearance, the hallmarks of MDS (Cardoso et al., 2003).

Recent revolutionary technological advances in molecular cytogenetics have enabled us to identify submicroscopic chromosomal abnormalities including gain or loss of genomic copy numbers (Emanuel and Saitta, 2007). Such genomic copy number variations (CNV) have only recently been identified using microarray-based comparative genomic hybridization (aCGH), and the incidence of such abnormalities seems to be more frequent than was thought prior to the human genome project (Shaffer et al., 2007). Genomic duplications are of particular interest because many submicroscopic duplications have been shown to be related to neurological disorders, including developmental delay and epilepsy (Lee and Lupski, 2006). Small genomic deletions and duplications have also been reported in 17p13.3 (Bi et al., 2009; Haverfield et al., 2009; Mei et al., 2008; Mignon-Ravix et al., 2009; Roos et al., 2009; Sreenath Nagamani et al., 2009).

In this study, we identified three types of genomic CNVs in the chromosome 17p13.3 region in 3 patients with epilepsy. This result implicates the dose effects of the genes in the 17p terminal region in epilepsy.

Materials

After obtaining informed consents from the patients' families based on the permission approved by the ethical committee of the institution, peripheral blood samples were obtained from 300 patients with psychomotor developmental delay and/or epilepsy, which included 10 patients with early infantile epileptic encephalopathy, 43 patients with West syndrome, 2 patients with Lennox-Gastaut syndrome, 12 patients with symptomatic generalized epilepsy, 14 patients with symptomatic partial epilepsy, and 5 patients with other types of epilepsy.

Methods

aCGH analysis was performed using the Human Genome CGH Microarray 105A chip (Agilent Technologies, Palo Alto, CA, USA), according to the manufacturer's protocol (Shimojima et al., 2009). Genomic DNAs were extracted from peripheral blood using a QIAquick DNA extraction kit (Qiagen, Hilden, Germany), and genomic copy numbers were determined using CGH Analytics software version 3.5 (Agilent Technologies).

To confirm the genomic copy number variations identified by aCGH, fluorescent *in situ* hybridization (FISH) analysis was performed as described (Shimojima et al., 2009). To confirm whether the identified genomic copy number variations were de novo or not, parental samples were also obtained and analyzed.

The wild-type genomic sequence of *LIS1* exons 9—11 was amplified by long PCR using LA-Taq (Takara, Otsu, Japan), a forward primer designed to anneal within intron 8 (5′-CAGTGCTGTGCTATAACTGCACTATC-3′), and a reverse primer designed to anneal within exon 9 (5′-CACTGGCAGGTGTATACTATCAGATAC-3′), according to the manual provided by the manufacture. Then, the 2867-bp amplicon was cloned into the p-GEM T-vector® (Promega, Madison, WI, USA), and the resulting plasmid was used as a probe for FISH analysis. The bacterial artificial chromosome (BAC) clones mapped to chromosome 17p13.3 (Table 1) were selected from an in silico library (UCSC Human Genome Browser, March 2006, http://genome.ucsc.edu).

Fiber-FISH analysis was performed to determine the directionality of the repeated segments as described elsewhere (Shimojima et al., 2009).

Results

Molecular and cytogenetic analysis

In patient 1, a loss of genomic copy number was identified by aCGH. The deletion was comprised of a 294-kb region of chr17 (2,522,672—2,816,939), including the last 5 exons of *LIS1* (exons 7—11) and the neighboring *KIAA0664* and *GARNL* (Fig. 1). FISH analysis using an originally cloned plasmid probe containing the predicted deletion sequence confirmed the deletion of one copy of *LIS1* (Fig. 2A). The fact that neither parent had the *LIS1* deletion (data not shown) confirmed it as a de novo deletion in patient 1.

Table 1 Summary of FISH analyses.

Clone	Band	Start[a]	End[a]	Patient 1	Patient 2	Patient 3	Coverage genes
RP11-629C16	17p13.3	373,082	560,333	NT	NT	Deletion	
RP11-356I18	17p13.3	707,755	880,135	NT	NT	Deletion	
RP11-294J5	17p13.3	1,146,211	1,299,309	NT	NT	Deletion	*YWHAE*, *CRK*
RP11-380H7	17p13.3	2,026,967	2,250,500	NT	Duplication	NT	
RP11-135N5	17p13.3	2,312,022	2,492,178	NT	Triplication	NT	*LIS1*
CTD-2576K4	17p13.3	2,492,176	2,643,505	NT	Triplication	NT	*LIS1*
Plasmid[b]	17p13.3	2,528,949	2,530,730	Deletion	NT	NT	*LIS1*
RP11-1D5	17p13.1	7,918,567	8,082,208	Marker	Marker	NT	
RP11-153A23	17q25.3	73,516,547	73,694,284	NT	NT	Marker	

NT: not tested.
[a] Genomic position is according to the May 2006 human reference sequence (Build 2006).
[b] Originally constructed plasmid probe.