Original Article

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Identification of a rare homozygous *SZT2* variant due to uniparental disomy in a patient with a neurodevelopmental disorder

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Summary **Because biallelic *SZT2* variants have been reported in patients with neurodevelopmental disorders associated with various degrees of developmental delay, intractable seizures, and distinctive features; this condition is recognized as an autosomal recessive disorder. Previously, eleven patients have been reported and most of them have compound heterozygous *SZT2* variants, leading to premature termination. In these patients, all reported variants were unique and there were no common pathogenic variants identified. In this study, we identified a paternal uniparental disomy of chromosome 1 in a patient with a neurodevelopmental disorder associated with severe intellectual disability, intractable epilepsy, autistic features, distinctive features, and transient macrocephaly. This resulted in homozygous patterns through chromosome 1. Among the variants in chromosome 1, a rare *SZT2* variant, NM_015284.3:c.6553C>T (p.Arg2185Trp), was selected as a powerful candidate variant in this patient. Although the clinical features of this patient are relatively milder than that reported previously, it may be derived from genetic heterogeneity. This is the first report of a homozygous missense *SZT2* variant.**

Keywords: Monosomy rescue, high forehead, loss-of-heterozygosity (LOH)

1. Introduction

Patients with neurodevelopmental disorders often show triad features with intellectual disability, autistic features, and epilepsy (*1-3*). Previous large-scale studies of patients with undiagnosed rare neurodevelopmental disorders showed the predominance of de novo mutations in genes that encode for molecules involving in neuronal functions (*4-6*). In such cases, haploinsufficiency and/or loss-of-function of the genes are suggested as the major mechanisms and only hetero-allelic involvement can cause the disorders. Compared to the prevalence of these cases, recessive disorders are rare because bi-allelic involvements are necessary for development of this condition (*7*). Prevalent autosomal recessive disorders are often caused by homozygous alterations due to common variants within ethnic groups. Consanguinity can also result in a homozygous gene status. As rare cases, uniparental disomy can also cause homozygous patterns.

In this study, we identified a rare homozygous variant of the seizure threshold 2 gene (*SZT2*) in a patient with a severe neurodevelopmental disorder presenting with triad features. A microarray testing revealed that uniparental disomy (UPD) was the mechanism of disease in this patient. This is the first report of UPD associated with S*ZT2* involvement in a neurodevelopmental disorder.

2. Materials and Methods

2.1.*Methods*

This study was performed in accordance with the Declaration of Helsinki and approved by the Tokyo Women's Medical University ethics committee. Blood

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samples were obtained from the patient and his parents after receiving informed consent.

Genomic DNA was extracted using a QIAamp DNA extraction kit (QIAGEN, Hilden, Germany). Next generation sequencing (NGS) was performed using a TruSight One v1.0 sequencing panel (Illumina, San Diego, CA) and Agilent SureSelect v5 (Agilent Technologies, Santa Clara, CA) according to previously described methods (*8,9*). The extracted data was annotated and filtered by VariantStudio (Illumina) and SureCall v4 (Agilent Technologies) software, respectively. Chromosomal microarray testing was performed using an Agilent microarray CGH+SNP 180K (Agilent Technologies), according to previously described methods (*10*). Standard Sanger sequencing was performed using a Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and a 3130 Genetic Analyzer (Applied Biosystems). The primer sets (forward; 5'-AGCATCCTTCCCAGACTCAG-3', reverse; 5'-GGGCAAAGGTACATATAGGGG-3') were designed using the UCSC genome browser (*https://genome.ucsc.edu/*).

2.2. *Patient's descriptions*

A 15-year-old Japanese boy was delivered at 39 weeks and 2 days of gestation by emergency caesarean section due to prolapse of the umbilical cord. The patient's parents are healthy and non-consanguineous. At the time of the patient's birth, the father and mother were 41 and 36 years old, respectively. There are two healthy brothers at 21 and 19 years of age. The patient's Apgar scores were 8 and 9 at 1 and 5 minutes, respectively. There is no remarkable family history of neurological diseases. His birth weight was 3,310 g (75~90th percentile), length was 52.0 cm (97th percentile), and occipitofrontal circumference (OFC) was 35.5 cm (90~97th percentile). Although his neonatal course was uneventful, he showed mildly delayed motor milestones with walking at 2 years and language development was notably delayed. At 4.5 years old, his OFC was 56.0 cm (> 97th percentile). This indicated post-natal macrocephaly. At 10 years, the first epileptic attack occurred. Although several anti-epileptic drugs have been prescribed, seizure attacks were noted several times per year, indicating intractable epilepsy. Electroencephalography showed multi-focal spikes or spikes and waves predominantly in the frontal lobes. Routine laboratory tests including a complete blood count, biochemical tests (including lactate, pyruvate and ammonia), and thyroid function test, were unremarkable. Brain magnetic resonance imaging showed no abnormalities. Conventional chromosomal G-banding showed normal male karyotype of 46,XY.

At present, his height is 173.5 cm (75~90th percentile), his weight is 100 kg (> 97th percentile), and his OFC is 57.0 cm (50~75th percentile), indicating obesity due to over-eating but not macrocephaly. Toilet training is established and he can remove clothing; however, he cannot dress by himself. Because his motor skills are not strong enough to allow the use chopsticks, he eats by using a spoon or with his hands. The patient uses no meaningful words and he seldom uses gestures for communication. He is also irritable and often has unwarranted temper tantrums. Together, these observations were recognized as autistic features. There are no dysmorphic features, excluding high forehead. He still has epileptic attacks, which are classified as complex partial seizures.

3. Results

To identify the underlying genetic cause of the disorder, NGS using a TruSight One sequencing panel was initially performed using a trio of samples derived from the patient and his parents. Although no strong candidate variants were identified, most of the variants in chromosome 1 showed homozygous patterns. Microarray analysis showed no genomic copy number aberration. Alternatively, loss-of-heterozygosity (LOH) throughout chromosome 1was identified (Figure 1A). Haplotypes in chromosome 1 were compared between the patient and his parents and it was confirmed that both copies of chromosome 1 in the patient were derived from his father, confirming paternal UPD.

The underlying homozygous variants in chromosome 1 were suspected as a mechanism of disease causation. For more detailed analysis, whole-exome sequencing was performed and the homozygous variants in chromosome 1 were analyzed in detail. Finally, variants were manually filtered by functional relevance to the clinical findings and a homozygous variant in *SZT2*, NM_015284.3(SZT2_v001):c.6553C>T (p.Arg2185Trp), was selected as a possible candidate. This variant has since been registered in dbSNP database (*https://www.ncbi.nlm.nih.gov/SNP/*) as rs765848129. However, the allele frequency is shown as 0.001% (1/121,308). The Exome Aggregation Consortium database (ExAC) also includes this variant with a frequency of 0.000008243. This variant is not observed in the Human Genetic Variation Database (HGVD; *http://www.hgvd.genome.med.kyoto-u.ac.jp/*), which contains genetic variants identified by exome sequencing of 1,208 Japanese individuals (*11*). These findings suggest that the incidence of this variant is extremely low. The functional consequences of the *SZT2* variant were annotated through wANNOVAR (*http://wannovar.wglab.org/*). As a result, CADD_phred score was 34, suggesting that this variant is deleterious (Supplemental Table S1, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=30*). Standard Sanger sequencing confirmed that this variant was derived

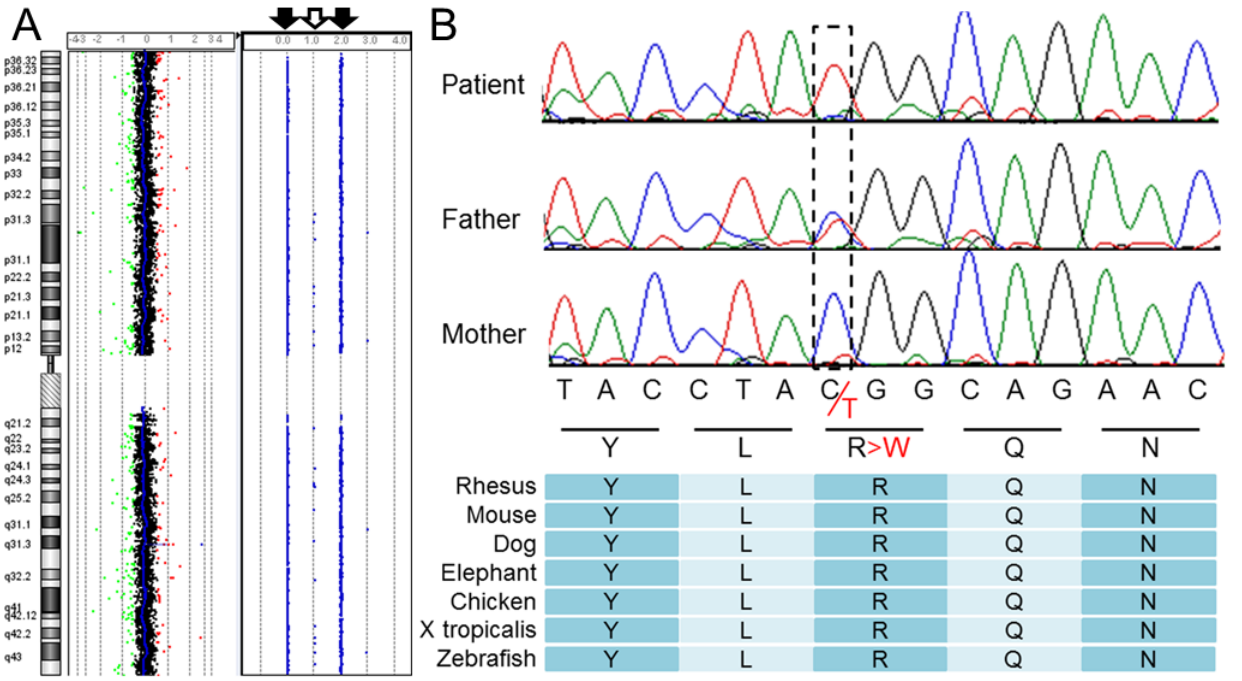

Figure 1. Results of genomic analyses. (A) Chromosomal microarray testing. The schematic representation of chromosome 1 data constructed by Agilent Genomic Workbench (Agilent Technologies) obtained through CGH+SNP array. In the CGH view (left), no genomic copy number aberration is shown. In the SNP view (right), almost all probes show homozygosity (black arrows) and very small numbers of probes show heterozygous patterns (a white arrow; these would be analytical errors). **(B)** Results of Sanger sequencing. Electropherograms of Sanger sequencing show the homozygous variant (indicated by a dotted rectangle) in the patient. This variant is identified in the patient's father as heterozygous but not in the mother. The affected amino acid is conserved among various species (lower panel).

from the patient's father who had this variant in the heterozygous state (Figure 1B).

4. Discussion

SZT2 is highly expressed in the brain, primarily in the parietal-frontal cortex, hippocampus, and dorsal root ganglia (*12*). Bi-allelic *SZT2* variants were first identified in two independent patients with early-onset epileptic encephalopathies (*13*). Both unrelated patients showed common facial features, severe developmental delay with hypotonia, and refractory seizures associated with secondary generalization. Following this report, additional nine patients have been reported (*14-17*). The clinical features of all reported patients are summarized in Table 1. Neurodevelopmental delay associated with intractable epilepsy, distinctive features, and dysmorphic findings of the corpus callosum are all common features of these patients (Table 1). Although the patient discussed in this report showed no abnormality in the findings of brain MRI, he fulfilled most of the other described features including developmental delay, intractable epilepsy, and distinctive features such as a high forehead. Macrocephaly is often observed in patients with *SZT2* variants. The present patient showed no macrocephaly at the last examination; however, it was transiently observed at 4.5 years of age. Thus, macrocephaly may be typically observed in these patients during childhood.

Motor developmental delay observed in this patient was milder than that reported in previous patients. Autistic features, which have never been reported previously, are additional characteristics for this patient. These characteristics may be related to the type of *SZT2* substitutions. Previously, 16 types of *SZT2* variants have been reported. Amongst these variants, 10 are related to premature termination. This indicates that loss-of-function would be the major mechanism and patients with *SZT2* loss-of-function mutations exhibited severe neurological symptoms. Compared to a loss-of-function variant, familial cases with in-frame *SZT2* mutations showed milder manifestations (*14*). The variant identified in this study contains a single nucleotide alteration leading to a missense substitution and is already registered in the dbSNP database. However, the frequency is extremely low. Because *SZT2* related phenotypes could be caused by bi-allelic involvement, the theoretical incidence of bi-allelic *SZT2* involvements will be low. Therefore, we concluded that the homozygous state of this rare *SZT2* variant would be disease causing.

The nine previously reported patients showed unique variants and there was no recurrent variant

Table 1. Summary of the clinical information of the present patient and previously reported patients with SZT2 involvements

Items	Present patient	2018 Nakamura *et al.*	2018 Tsuchida *et al.*			2016 Vanderver *et al.*	2016 Venkatesan *et al.*	2013 Falcone *et al.*	2013 Basel-Vanagaite *et al.*	
Age	13y	4y	4y	2y	5y	7y	3y	18y, 10y, 7y	10y	9y
Gender	Male	Female	Female	Male	Femal	Female	Male	Males	Female	Male
Genotype	Homozygous	Compound heterozygous	Compound heterozygous	Compound heterozygous	Compound heterozygous	Compound heterozygous	Compound heterozygous	Homozygous	Homozygous	Compound heterozygous
1st	c.6553C>T (p.R2185W)	c.896dup (p.Y2866Lfs*42)	c.3700_3716del (p.N1234Afs*35)	c.3497dup (p.E1317Gfs*4)	c.7303C>T (p.R2435T)	c.5499del (p.F1834Sfs*47)	c.3509_3512del (p.T1170Rfs*22)	c.4202_4204del (p.F1401del)	c.73C>T (p.R25*)	c.1496G>T (p.S499I)
2nd		c.2930-17_2930-3 delinsCTCGTG	c.5482del (p.G1829Vfs*52)	c.2929+1G>A (p.L939Dfs*19)	c.8162C>G (p.S2721C)	c.6916G>A (p.G2306R)	c.9703C>T (p.R3235*)			c.2092C>T (p.Q698*)
Epilepsy	Intractable epilepsy	+	EOEE	EOEE	EOEE	Refractory epileptic encephalopathy	EOEE	None	EOEE	EOEE
Motor development	No delay	Walk unassisted	Bedridden	Bedridden	Bedridden	NA	Walk	NA	Sit with support	Bedridden
Language development	A few words	No meaningful word	No speech	No speech	No speech	NA	No speech	Speech delay	No speech	No speech
Cognition		Recognize speaking				NA	NA			No social interaction
Distinctive features										
High forehead	+	+	+	+	-	NA	+	-	+	+
Hypertelorism	-	+	-	-	-	NA	+	-	NA	-
Macrocephaly	-	+	-	-	-	+	+	+	+	-
Brain MRI	-	Thick and short CC	Thick and short CC	Thick and short CC	Volume loss of CC	Myelination deficit/mild cerebeller atrophy	Heterotopia/ abnormal gryal formation	-	Thick and short CC	Thick and short CC

y, years; EOEE, Early-onset epileptic encephalopathy; NA, not available; MRI, magnetic resonance imaging; CC, corpus callosum.

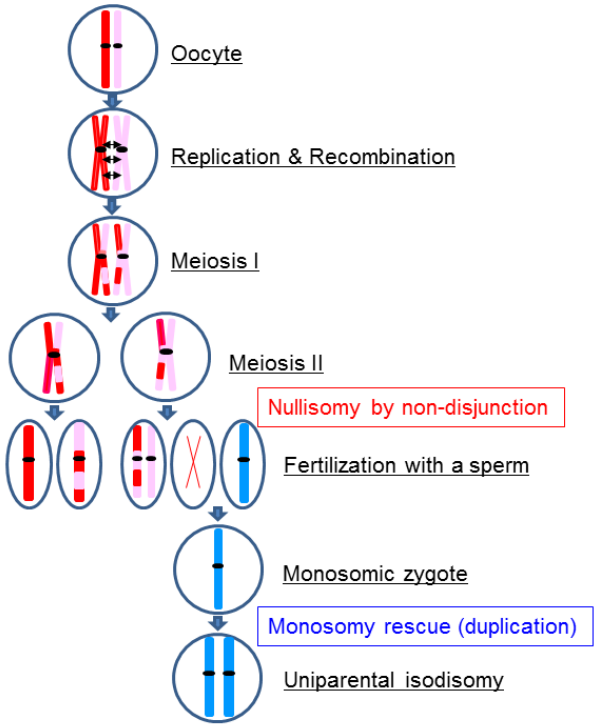

Figure 2. Representation of the suspected mechanism of monosomy rescue. Monosomic zygote is created by the fertilization of a nullisomic oocyte derived from meiotic non-disjunction in maternal meiosis II. As a monosomic embryo cannot survive, the paternally derived chromosome will be duplicated for compensation.

amongst them. Furthermore, only two families showed homozygosity. This indicates that bi-allelic involvements in the patients are incidental. *SZT2* is located on 1p34.2 and the observed homozygosity was caused by paternal UPD of chromosome 1 in the present patient. Homozygous variants induced by UPD are rare, but there are many cases of UPD induced neurological disorders (*10,18*).

From the genotypes and results of the CGH+SNP microarray for chromosome 1, we determined that the present patient did not show heterozygous region in chromosome 1. This finding suggested UPD, which describes disomy where both chromosomes are inherited from a single parent. UPD causes autosomal recessive disorders when the indicated parent carries pathogenic variants. UPD can be divided into two subtypes, the first is hetero-UPD (hUPD). In this subtype, two different homologous chromosomes are inherited from a single parent. The second is iso-UPD (iUPD), in which a single homologous chromosome is duplicated from a single parent. If the UPD was caused by trisomy rescue, heterozygous regions will be observed as evidence of homologous recombination through meiosis (Figure 2). However, the CGH+SNP array showed iUPD of chromosome 1 with LOH in the all regions. Thus, complete homozygosity throughout chromosome 1 indicates that monosomy rescue would be the mechanism (Figure 2). When a nullisomic oocyte that arose from meiotic non-disjunction in maternal meiosis II is fertilized with a monosomic sperm, the zygote becames monosomic. Because the monosomic embryo cannot survive, the paternally derived chromosome will be duplicated for compensation. The mother of the patient was relatively old (36 years) at the time of the patient's delivery. Thus, monosomy 1 may have been caused by chromosomal non-disjunction in the oocyte.

In conclusion, we identified UPD of chromosome 1 in a patient with neurological disorder. Owing to that, the rare missense *SZT2* variant, located in 1p34.2, was identified as a homozygous pattern. This is the first report of bi-allelic involvement of *SZT2* by a missense substitution and this may be related to milder phenotype of *SZT2*-related neurodevelopmental disorder.

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