Novel Biallelic Variant in the BRAT1 Gene Caused Nonprogressive Cerebellar Ataxia Syndrome

Yiming Qi1,2†, Xueqi Ji1,3†, Hongke Ding1,2, Ling Liu1,2, Yan Zhang1,2 and Aihua Yin1,2,3*

1Prenatal Diagnosis Center, Guangdong Women and Children Hospital, Guangzhou, China, 2Maternal and Children Metabolic-Genetic Key Laboratory, Guangdong Women and Children Hospital, Guangzhou, China, 3Clinical Medicine College, Guangzhou Medical University, Guangzhou, China

Recessive mutations in BRAT1 cause lethal neonatal rigidity and multifocal seizure syndrome (RMFSL), a phenotype characterized by neonatal microcephaly, hypertonia, and refractory epilepsy with premature death. Recently, attenuated disease variants have been described, suggesting that a wider clinical spectrum of BRAT1-associated neurodegeneration exists than was previously thought. Here, we reported a 10-year-old girl with severe intellectual disability, rigidity, ataxia or dyspraxia, and cerebellar atrophy on brain MRI; two BRAT1 variants in the trans configuration [c.1014A>C (p.Pro338=); c.706delC (p.Leu236Cysfs*5)] were detected using whole-exome sequencing. RNA-seq confirmed significantly decreased BRAT1 transcript levels in the presence of the variant; further, it revealed an intron retention between exon 7 and exon 8 caused by the synonymous base substitute. Subsequent prenatal diagnosis for these two variants guided the parents to reproduce. We expand the phenotypic spectrum of BRAT1-associated disorders by first reporting the pathogenic synonymous variant of the BRAT1 gene, resulting in clinical severity that is mild compared to the severe phenotype seen in RMFSL. Making an accurate diagnosis and prognostic evaluation of BRAT1-associated neurodegeneration is important for reproductive consultation and disease management.

Keywords: BRAT1, nonprogressive cerebellar ataxia syndrome, synonymous variant, intron retention, prenatal diagnosis

INTRODUCTION

Variations in BRAT1 (BRCA1-associated ataxia telangiectasia mutated activator 1) are initially recognized as the cause of lethal neonatal rigidity and multifocal seizure syndrome (RMFSL; OMIM#614498), which is characterized by neonatal microcephaly, intractable seizures, hypertonia, and early demise. Subsequently, neurodevelopmental disorder with cerebellar atrophy and with or without seizures (NEDCAS; OMIM#618056) caused by biallelic BRAT1 variants were reported and redefined the description of “lethality.” Recently, a milder clinical form that manifests as nonprogressive cerebellar ataxia (NPCA) was described in some childhood-onset patients (Mahjoub et al., 2019), suggesting that a wider phenotypic spectrum of BRAT1-associated neurodegeneration exists than was previously thought.

Physiological functions of the disease-causing gene BRAT1 are diverse (Fernandez-Jaen and Ouchi, 2016). It encodes a protein that interacts with the tumor suppressor gene BRCA1 at its
C-terminus and binds to ATM1, considering a master controller of the cell cycle signaling pathways required for cellular responses to DNA damage (Ouchi and Ouchi, 2010). BRAT1 can form a complex with an ATPase domain-containing protein, BRP1 (BRAT1 Partner 1), and prevent transcriptional silencing at methylated genomic regions (Zhang et al., 2016). It may also be involved in cell growth and apoptosis (Straussberg et al., 2015). BRAT1 deficiency secondary to biallelic BRAT1 mutations may increase the glucose metabolism, reduce the mitochondrial reactive oxygen species (ROS) concentration, deteriorate cell growth and migration, and induce neuronal atrophy (Puffenberger et al., 2012; Saunders et al., 2012; So and Ouchi, 2014; Wolf et al., 2015). The complexity and extensiveness of the BRAT1 gene function are the biological basis for the huge phenotypic heterogeneity. However, the exact mechanism by which variations in BRAT1 trigger neurodegeneration and to what extent a defect in ATM function contributes to this disease are unknown.

In this study, we first reported the clinical course of a proband with NPCA caused by novel compound heterozygous BRAT1 variants, which include a negligible pathogenic synonymous variant. Functional studies confirmed the effect of them on transcription. The result provided a theoretical basis and guidance for this family in reproductive genetic counseling and prenatal diagnosis. Furthermore, we summarized all published cases with BRAT1 variation, which provide insight into the clinical–genetic correlation and the pathophysiology of the disease.

**Clinical Report**

A 10-year-old girl who presented severe intellectual disability and poor motor ability was transferred to our genetics center for consultation due to the family’s reproductive plan.

The patient was the second child of nonconsanguineous parents of Chinese and had a healthy adolescent sister (pedigree in Figure 1A). There were unremarkable findings during her prenatal, perinatal, and neonatal courses. Physical examination at birth revealed a normal height, normal weight, and normal head 101 circumference (51 cm; 48th percentile).

However, global developmental delay was presented initially a few months later. She developed head control at 6 months and could not sit until 15 months. At 2.5 years, she could only babble, make consonant sounds, and communicate her needs by crying and gazing. After an individualized neurorehabilitation therapy, she was able to sit briefly and pull to stand at 3 years. One year later, she could stand independently for a few minutes and walk with a walker. At 5 years, she could say 5–10 word phrases that...
were dysarthric, identify people who were in constant contact with her, and follow simple commands. Meanwhile, behavior with episodes of impulsivity and irritability began to appear since then. She attended special schooling for rehabilitation training but with poor performance until last re-evaluation and was considered to have severe intellectual disability with a mental age of less than 3 years.

Until now, she could stand alone and walk slowly with limited support in a rigid- and broad-based gait with flexed arm posture (Supplementary Videos S1,2). She also presented slight dysmetria, which interferes in fine motor skills during the performance of tasks (Supplementary Video S3). Although her facial expression shows relative paucity (Figure 1B), no evident BRAT1-associated dysmorphisms, such as epicanthic folds, high arched palate, fifth finger clinodactyly, or single palmar crease, were observed (Figure 1C). Her cranial nerves were intact except pendular nystagmus. Motor exam has shown mild hypertonia and resistance during extension, but tendon reflexes were normal.

Cranial magnetic resonance imaging (MRI) spectroscopy was first performed at the age of 4, which recorded cerebellar atrophy (Figure 1D). In the following years, she showed stable cerebellar atrophy on serial neuro-MRI (Figure 1E). Electroencephalography was uniformly negative.

Laboratory examination included serum tests for alpha-fetoprotein (AFP), inborn errors of the metabolism, amino acids, very long-chain fatty acids, acylcarnitine and carnitine. Furthermore, Electroencephalography was uniformly negative.

Genetic Investigations
Genomic DNA was extracted using a Qiagen DNA blood mini kit (Qiagen GmbH, Hilden, Germany). Library preparation and target enrichment were performed using a SureSelectXT Clinical Research Exome kit (Agilent Technologies, Santa Clara, CA) according to the manufacturer’s specifications. Then, Trio WES was performed using 2 × 150 bp in the paired end mode of the NextSeq 500 platform (Illumina, San Diego, CA) to obtain an average coverage of above 110x, with 97.6% of target bases covered at least 10x. Sequence quality analysis and filtering of mapped target sequences were performed with the ‘varbank’ exome and genome analysis pipeline v2.1 as described previously (Vetro et al., 2020). Analysis of genetic results was based on the genomic variation database (http://dgv.tcgag.ca/dgv/app/home), DECIPHER database (https://decipher.sanger.ac.uk/), and OMIM database (http://www.ncbi.nlm.nih.gov/omim). The found variants were further verified by Sanger sequencing in fetuses and parents.

RESULTS
Genetic Findings
Whole-exome sequencing analysis identified compound heterozygous mutations in the BRAT1 gene, c.706delC (p.Leu236Cysfs*5), and a synonymous variant, c.1014A > C (p.Pro338 = ), which were confirmed by sanger sequencing and segregated with the disorder in her family (Figure 2A).

Neither both the variants were reported in public databases nor their functional impact was examined. The deletion variant c.706delC (p.Leu236Cysfs*5) in exon 5 was predicted to lead to a frameshift and to cause loss of the full-length protein (821 amino acids) due to truncation after the first 236 residues. It is predicted to be pathogenic (VarSome, https://varsome.com/ and ClinVar https://www.ncbi.nlm.nih.gov/clinvar/).

The other variant, c.1014A > C (p.Pro338 = ) in exon 7/8, is not conserved among species, and the amino acid pro at the position 338 of BRAT1 protein is not changed as well; however, varSEAK analysis predicts that the mutation may cause the classic splicing site c.1015 + 1 to be skipped (Figure 2B). Thus, RNA-seq was exerted to identify potential splicing defects associated with the variants in the NPCA case.

BRAT1 expression levels in RNA-seq showed a trend toward lower expression in heterozygous parents and the compound heterozygous proband and confirmed significantly decreased BRAT1 transcript levels in the presence of the variant (Figure 2C), consistent with NMD of the mutant transcript. Meanwhile, BRAT1 expression was higher in the synonymous variants’ father than in the frameshift variants’ mother, although the difference was not statistically significant (Figure 2C).

MATERIALS AND METHODS

Genetic Findings
Genomic DNA was extracted using a Qiagen DNA blood mini kit (Qiagen GmbH, Hilden, Germany). Library preparation and target enrichment were performed using a SureSelectXT Clinical Research Exome kit (Agilent Technologies, Santa Clara, CA) according to the manufacturer’s specifications. Then, Trio WES was performed using 2 × 150 bp in the paired end mode of the NextSeq 500 platform (Illumina, San Diego, CA) to obtain an average coverage of above 110x, with 97.6% of target bases covered at least 10x. Sequence quality analysis and filtering of mapped target sequences were performed with the ‘varbank’ exome and genome analysis pipeline v2.1 as described previously (Vetro et al., 2020). Analysis of genetic results was based on the genomic variation database (http://dgv.tcgag.ca/dgv/app/home), DECIPHER database (https://decipher.sanger.ac.uk/), and OMIM database (http://www.ncbi.nlm.nih.gov/omim). The found variants were further verified by Sanger sequencing in fetuses and parents.

RNA Sequence
Total RNA was extracted from peripheral blood samples using Qiagen blood RNA extraction kit 1 (Qiagen, United States); the procedures and standards were performed according to the manual. After complete property control of RNA and quality control of RNA concentration and purity are qualified, 1 μg of RNA is aspirated for mRNA library construction. The mRNA library was constructed using a TIANSeq Fast RNA Library Kit (Illumina, United States) according to the manufacturer’s instructions, where mRNA was purified and enriched from 1 μg of the total RNA samples and then fragmented about 250 bp, and the index adapter was added. Finally, using a high-fidelity enzyme amplifies the library. After quality control, the libraries were sequenced on an Illumina HiSeq 4,000 platform.

Chorionic Villus Sampling
Fetal samples were collected by chorionic villus sampling at 13 weeks of gestation. The procedure was performed using the transabdominal approach. Under aseptic conditions, an 18 or 20 gauge spinal needle was inserted into the placenta under continuous ultrasound guidance. A 20 cc syringe containing the collection media was attached to the end of the needle once the stylet is removed. Negative pressure is created, and the needle is moved up and down through the placenta, collecting the tissue (Jones and Montero, 2021).
Furthermore, RNA-seq analyses identified significantly increased retention of intron 7 of BRAT1 in the proband and the heterozygous father relative to the heterozygous mother and wild-type control samples (Figure 2D). It was presumed to be the effect of NM_152743.3 c.1014A>C (p.Pro338 = ) mutation. Altogether, these results suggest that both the variants participate in the pathogenesis of NPCA.

**Prenatal Diagnosis for Reproduction and Follow-Up**

The mother of the proband underwent prenatal diagnosis at 12 weeks of gestation during her third pregnancy using chorionic villus sampling. Fortunately, Sanger sequencing showed that the fetus inherited neither of the above sites (Figure 1A). Prenatal and neonatal courses of the fetus were uncomplicated. A male neonate 2,560 g in weight and 51 cm high was delivered at the term, without microcephaly (HC 348 mm). In the following years, his head circumference has grown consistently within the 95th centile. Other growth parameters, strength, reflexes, and sensation were normal until the last visit at 18 months.

**DISCUSSION**

A total of 30 patients with clinical manifestations ranging from RMFSL to NPCA had been identified as homozygous or compound heterozygous variants in BRAT1 (Table 1, Figure 1F). In BRAT1-related disorders, cerebellar hypoplasia seemed not congenital (Wolf et al., 2015; Srivastava et al., 2016; Celik et al., 2017). Clinical classifications depend on the rate of progression of atrophy after birth determined. Nearly 75% of them present with severe RMFSL, with almost all accompanied by multifocal or refractory epilepsy or even intrauterine jerks (Celik et al., 2017). Epilepsy occurs in 41.6% of NEDCAS/NPCA patients, mostly before 3 years of age. It had been reported that a few NPCA cases, which mapped to the SCA15 locus on chromosome 3pter, might develop spasticity or focal dystonia with increasing age (Dudding et al., 2004). However, in NPCA caused by BRAT1, including the case here, later-onset spasticity is extremely rare on long-term follow-up.

Divergence is much huger for the clinical–genetic correlation. It has been mentioned that the phenotypic spectrum of BRAT1-associated disorders is associated with the domain, localization,
| NO. | Gender | Variant | Effect | Pedigree | Reported condition | Associated clinical phenotypes | Electroencephalogram | Brain MRI | Ref |
|-----|--------|---------|--------|----------|--------------------|---------------------------------|----------------------|-----------|-----|
| 1   | M      | c.185T > A | p.Val62Glu | Sibling w/ NO.2 | 24 years’ old, graduated from college | Mid intellectual disability, ataxia, motor development delay, language delay, gaze-evoked nystagmus | Not offered | 9 years: stable isolated cerebellar atrophy | Mahjoub et al. (2019) |
| 2   | M      | c.185T > A | p.Val62Glu | Sibling w/ NO.1 | 7 years’ old | Mid intellectual disability, ataxic, motor development delay, dysarthria, gaze-evoked nystagmus | Not offered | 16 years: stable isolated cerebellar atrophy | Mahjoub et al. (2019) |
| 3   | F      | c.638dupA | p.Val214Glyfs*189 | Sibling w/ NO.4 | 10 years’ old | Hypotonia, microcephaly, dysmetria and truncal titubation, ataxic, intellectual disability, ataxia, and cerebellar atrophy, head circumference, global developmental delay, bilateral 5th finger clinodactyly | Not offered | Prominent cerebellar interfolial spaces, which remained unchanged from 2, 3 years | Srivastava et al. (2016) |
| 4   | F      | c.803+1G > C | Splice site | Sibling w/ NO.3 | 6 years’ old | Hypotonic, dysmetria, microcephaly, dysarthric speech and pendular nystagmus, global developmental delay, activity-induced tremor, bilateral 5th finger clinodactyly | Normal | Progressive enlargement of the cerebellar interfolial spaces, cerebellar atrophy | Srivastava et al. (2016) |
| 5   | F      | c.803+1G > C | p.Leu697fs*92 | Sporadic | 6 years’ old | Seizures, hypertonia, microcephalic, generalized axial and peripheral hypertonia and hyper-reflexia, motor development delay, language delay | Mainly left-sided temporoparietal epilepsy, absence of a posterior dominant rhythm | 3 mo: decreased myelination and thin corpus callosum | Mundy et al. (2016) |
|     |        | c.1925C > A | p.Ala642Glu |            |                    |                                 |                      | 3 years: right temporal lobe encephalomalacia and cerebellar and vermis hypoplasia |  |

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| NO. | Gender | Variant | Effect | Pedigree | Reported condition | Associated clinical phenotypes | Electroencephalogram | Brain MRI | Ref |
|-----|--------|---------|--------|----------|--------------------|--------------------------------|----------------------|-----------|-----|
| 6   | M      | c.1564G > A | p.Glu522Lys p.Val214Glyfs*189 | Sporadic 4.5 years’ old | Microcephaly, hypertonia, progressive encephalopathy never presented seizures | Normal | 19 and 48 mo: moderate progressive cerebellar atrophy | Fernandez-Jaen et al. (2016) |
| 7   | F      | c.638dupA c.638dupA | p.Val214Glyfs*189 | Sporadic 4 years 4 mo old | Right esotropia, mild optic nerve hypoplasia, with decreased visual acuity bilaterally, moderate appendicular rigidity, dyspraxia, global developmental delay, bilateral 5th finger clinodactyly | Showed frequent 3–4 Hz generalized spike and wave complexes (without clinical correlate) | 5 mo: normal 21 mo and 4 years 3 mo: enlargement of the cerebellar interfolial spaces compatible with cerebellar atrophy and mildly delayed myelination | Srivastava et al. (2016) |
| 8   | F      | c.419T > C c.1857G > A | p.Leu140Pro p.Trp619* | Sibling wih NO.15 4 years and 4 mo | Drug-resistant seizures, microcephaly, developmental delay | Multifocal epileptiform activity | Not offered | Smith et al. (2016) |
| 9   | F      | c.2125_2128delTTTG p.294dupA c.1825C > T | p.Phe709Thrfs*17 p.Leu99Thrfs*92 | Sporadic 3 years and 8 mo | Seizures, microcephaly, difficulty swallowing, visual impairment, nystagmus, ataxia, and frequent episodes of autonomic dysregulation axial hypotonia, appendicular hypertonia, global developmental delay, motor development delay | Normal | 3.5 years: progressive cerebellar and brainstem atrophy | Hanes et al. (2015) |
| 10  | F      | c.294dupA c.803G > A | p.Arg609Trp p.Leu99Thrfs*92 | Sporadic 20 mo of age | Febrile seizures, hypertonia, nystagmus, esotropia, arrested head growth P10, motor development delay, developmentally delayed | Not offered | Not offered | Oatts et al. (2017) |

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| NO. | Gender | Variant | Effect | Pedigree | Reported condition | Associated clinical phenotypes | Electroencephalogram | Brain MRI | Ref |
|-----|--------|---------|--------|----------|-------------------|--------------------------------|-----------------------|-----------|-----|
| 11  | M      | c.171delG | p.Arg268His, p.Glu67Aspfs*7 | Sporadic | 15 mo old | Seizures, hypertonia, microcephaly, axial hypotonia and symmetric hypertonia, intermittent asymptomatic bradycardia and hypothermia, non-epileptic apnea, chronic lung disease, dry skin | Episodes of focal electrographic status epilepticus | One d of life: normal structures but subtle nonspecific foci of the cerebral white matter | Srivastava et al. (2016) |
|     |        | c.419T>C | p.Leu140Pro | | | | | | |
| 12  | M      | c.638_639insA | p.Val214Glyfs*189 | Sporadic | Died at the age of 5 years and 9 mo due to respiratory insufficiency | Early onset epileptic encephalopathy postnatal, microcephaly, apnea, feeding problems, bradycardia, global developmental delay, maldescent testis, left-sided club foot, and left-sided pes adductus | Focal continuous spike discharges in the right more than in the left occipital region | Thin corpus callosum, dilated internal and external cerebrospinal fluid spaces, and delayed myelination | Horn et al. (2016) |
|     |        | c.1134+1G>A, c.1496+1G>A | Splice site | Sporadic | Died at 4 years 3 mo | Microcephaly, hypertonia, focal, multifocal motor seizures with clonic features, apnea, eye deviation to either side, cluster on awakening and drowsy, epileptic spasms, and tonic seizures | Multifocal epileptiform dischargesictal: migrating focal seizures; seizures arising from right central region, vertex, left central, left occipital, right temporal, and left temporal region; epileptic spasms and periodic spasms, hypsarrhythmia | One m 12 d: very small hemosiderin deposition within lateral ventricles and subarachnoid spaces from previous IVH. 7.5 m: prominent ventricles and extra-axial CSF spaces with associated white matter volume loss, nonspecific abnormal white matter signal | Scheffler et al. (2020) |
| NO. | Gender | Variant | Effect | Pedigree | Reported condition | Associated clinical phenotypes | Electroencephalogram | Brain MRI | Ref |
|-----|--------|---------|--------|----------|-------------------|-------------------------------|----------------------|----------|-----|
| 14  | F      | c.1498+1G > A  
   c.638dupA  
   c.638dupA | p.Val214Glyfs*189  
   Sibling* with NO. 25 | Died at the age of 17 mo because of respiratory failure | Epileptic seizures (eye blinking and myoclonus left hand) and hypertonia, microcephaly | Continuous abnormal background pattern and multifocal seizure activity | Two mo: normal | Wolf et al. (2015) |
| 15  | M      | c.1857G > A  
   c.1359_1361delCCT | p.Trp619*  
   p.Leu454del | Sibling with NO.8 | Drug-resistant seizures, microcephaly, and developmental delay | Multifocal epileptiform activity | 12 mo: severe generalized atrophy, hardly any myelination  
13 mo: global cerebral and cerebellar atrophy | Smith et al. (2016) |
| 16  | F      | c.1225_1228delTTTG  
   c.1315_1361delCCT | p.Phe709Thrfs*17  
   p.Leu454del | Sporadic | Died at 14 mo | Microcephaly, hypertonia, focal motor clonic seizures, migrating between hemispheres | Multifocal epileptiform discharges, discontinuous backgroundictal: migrating focal seizures from one region to another, most frequent onset from the right posterior quadrant, other onsets in the left posterior region and left frontocentral region | Three d: small right occipital subdural hemorrhage  
18 d: hemorrhage resolved | Scheffer et al. (2020) |
| 17  | M      | c.1395G > C  
   c.1313_1314delAG | p.Thr465Thr  
   p.Gln438fs | Sibling* | Died at the age of 12 mo | Polymorphic seizures and hypertonia, microcephaly | Generalized and focal sharp and spike waves | The myelination pattern was appropriate for the patient’s age, subarachnoid space was slightly widened | Szymańska et al. (2018) |
| 18  | F      | c.1310_1314delAG  
   c.964C > T  
   c.2284C > T  
   c.2230_2237dupAACACTGC  
   p.Gln322*  
   p.Gln762*  
   p.S747Tfs*36 | Sibling* with NO.27 | Died at 10 mo | Microcephaly, hypertonia, focal clonic seizures with apnea, tachycardia | Multifocal epileptiform discharges, discontinuous backgroundictal: migrating focal seizures; central, right occipital spread to the left occipital region, left temporal spread to the left hemisphere then the right hemisphere, bi-occipital onset | Two d: mild thinning of the corpus callosum  
2 m 10 d: mild thinning of the corpus callosum, increasing prominence of CSF spaces, likely ex vacuo dilatation | Scheffer et al. (2020) |
| 19  | M      | c.2230_2237dupAACACTGC  
   c.2284C > T | p.Gln762*  
   p.S747Tfs*36 | Sporadic | Died at the age of 10 mo | Drug-resistant seizures, hypertonia, microcephaly | 4–6 Hz theta background activity, bilateral frontotemporal sharp waves and 6–10 Hz alpha waves during clinical seizures | Initial: normal, 3 mo: cerebral and cerebellar atrophy and thinning of the corpus callosum | Celik et al. (2017) |

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| NO. | Gender | Variant | Effect | Pedigree | Reported condition | Associated clinical phenotypes | Electroencephalogram | Brain MRI | Ref |
|-----|--------|---------|--------|----------|-------------------|--------------------------------|----------------------|----------|-----|
| 20  | M      | c.2230_2237dupAACACTGC | c.1499-1G > T | p.Glu500Alafs*36 | Sporadic Died at the age of 7.5 mo | Seizures (myoclonic, tonic and clonic migrating focal), hypotonia, micrognathia, microcephaly, down-slanted palpebral fissures, myoclonic jerks, apnea, and bradycardia | Generalized epileptiform activity, migrating focal epileptiform activity, and background deceleration | Atrophic corpus callosum, hypomyelinisation, brainstem, and cerebellar vermis hypoplasia | Colak et al. (2020) |
|     |        | c.1499-1G > T |        |          |                   |                                |                      |          |     |
| 21  | M      | c.233G > C | p.Arg78Pro | sporadic | died at the age of 7 mo due to respiratory infection and malnutrition | Myoclonic seizures, paroxysmal convulsions, hypertonia, hyperactive deep tendon reflexes, small and asymmetrical frontal bones, overlapping cranial sutures, recurrent respiratory tract infections, dysphagia | Initial: more sharp wave discharges in the left forehead-parietal region than in the right forehead-parietal region, 2 mo: focal sharp wave discharges and spike and slow-wave complexes in the left forehead-temporal region | Brain magnetic resonance imaging indicated that the bilateral frontal and temporal subarachnoid space was widened, and the corpus callosum was thin | Li et al. (2021) |
| 22  | M      | c.233G > C | p.Leu391fs | Sibling with NO.23 | Died at the age of 6 mo due to cardiac arrest | Myoclonic seizures, hypertonia and contractures, arrested head growth, inability to swallow, and bouts of apnea-bradycardia, cardiac arrest | Bilateral epileptic activity with bilateral discharges | Normal | Straussberg et al. (2015) |
| 23  | F      | c.1173delG | p.Leu391fs | Sibling with NO.22 | Died at the age of 5 mo due to cardiac arrest | Myoclonic seizures, hypertonia and contractures, arrested head growth, inability to swallow, and bouts of apnea-bradycardia, cardiac arrest | Sharp waves and bilateral spikes predominantly over the right hemisphere | Normal | Straussberg et al. (2015) |
| 24  | F      | c.1173delG | p.Thr465Thr | Sporadic | Died at 10 weeks of age | Progressive encephalopathy with refractory seizures, hypertonia, episodic apnea, microcephaly, dysmorphic features | Diffuse encephalopathy, with frequent ictal activity from multiple cortical areas | Mid thinning of the corpus callosum and delayed myelination | Van Ommeren et al. (2018) |

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| NO. | Gender | Variant          | Effect             | Pedigree          | Reported condition                                      | Associated clinical phenotypes                                                                 | EEG                         | Brain MRI | Ref                  |
|-----|--------|-----------------|--------------------|-------------------|---------------------------------------------------------|-------------------------------------------------------------------------------------------------|-----------------------------|-----------|----------------------|
| 25  | M      | c.1396G > C     | p.Val214Glyfs*189  | Sibling<sup>a</sup> | Died at the age of 2 mo due to severe necrotizing enterocolitis grade III | Epileptic seizures (loss of consciousness, tonic posturing, myoclonus), hypertonia, microcephaly, mild hypotonia | Burst-suppression pattern, with long suppressions (10–15 s), multifocal negative sharp waves | Not offered | Wolf et al. (2015)   |
|     |        | c.638dupA       |                    |                   |                                                         |                                                                                                |                             |           |                      |
|     |        |                 | c.638dupA          |                   |                                                         |                                                                                                |                             |           |                      |
|     |        |                 |                    |                   |                                                         |                                                                                                |                             |           |                      |
| 26  | M      | c.1120G > T     | p.Glu374<sup>a</sup> | Sporadic          | Died at 2 mo                                            | Microcephaly, hypertonia, myoclonic seizures, focal clonic seizures migrating between hemispheres, excessive startle from day 1 | Multifocal epileptiform discharges, discontinuous background ictal: myoclonic seizures, clonic seizures, focal clonic movements, with migration from the right posterior occipital region to the left posterior region | 17 d: small subacute subdural hemorrhage along the tentorium with left parietal bone cephalhematoma | Scheffer et al. (2020) |
| 27  | F      | c.1120G > T     | p.Gln322<sup>a</sup> | Sibling with NO.18 | Died at 34 d                                            | Focal motor seizures, microcephaly, hypertonia                                                 | Multifocal epileptiform discharges, discontinuous background ictal: focal seizure migrating from the left central region to the right hemisphere | 3 d: asymmetric T2 signal in deep posterior parietal white matter bilaterally, small subdural hemorrhages in the posterior parietal region and posterior fossa, focal area of subarachnoid/pial hemorrhage in the posterior fossa adjacent to the tentorium on the right side 3 w: poor opercularization of Sylvian fissure in the frontotemporal region, hemorrhages unchanged | Scheffer et al. (2020) |
| 28  | M      | c.2284C > T     | p.Gln762<sup>a</sup> | Sporadic          | Died at 6 d old                                         | Intractable focal seizures, microcephaly, rigidity, apnea, and congenital heart disease         | Not offered                  | Not offered | Pourahmadiyan et al. (2021) |
|     |        | c.2041G > T     | p. E681X           |                   |                                                         |                                                                                                |                             |           |                      |

EEG = electroencephalogram.
MRI = serial magnetic resonance imaging of the brain.
d = day.
mo = month.
<sup>a</sup> In “Pedigree” = sibling with similar symptoms has died without exome sequencing.
type, and zygosity of the identified variant (13) (Dudding et al., 2004). According to Table 1, the variant type rather than the variant domain is closely related to the phenotype severity. For example, homozygous variants R78P and p.V62E, both located on the apoptosis-related N-terminal CID (cell death inducing DFF45-like effector) (Lugovskoy et al., 1999; Choi et al., 2017), caused typical RMFSL (Li et al., 2021) and mild NPCA (Lugovskoy et al., 1999), respectively. Complex mechanisms such as the mutation affecting one or more as unidentified activities of this protein (Kurosaki and Maquat, 2016) or associated with BRAT1-related pathways might be involved. It may modulate the severity rather than simply disrupt the mitochondrial function and the ATM kinase activity. As to the variant type, individuals with BRAT1 biallelic null variants usually lead to severe symptoms and are mostly fatal at the early stage. However, in biallelic BRAT1 missense variants, phenotypic variability is huge, ranging from RMFSL to NPCA. Interestingly, we noticed that in individuals with biallelic BRAT1 gene null variants, females always exhibited a milder phenotype than males (Mundy et al., 2016; Srivastava et al., 2016), even for siblings who carried the same variants. The special phenotypic divergence cannot be explained simply by variable penetrance or genetic backdrop heterogeneity. It is hypothesized that other sub-equivalent genes located on the X chromosome have some effects of the "female protective model" in neurodevelopmental disorders (Jacquemont et al., 2014). The mechanism is yet to be further confirmed.

The variant p. Leu236Cysfs*5 resulted truncation after the first 236 residues, and half of the protein was most likely to lose the function. The trans variant c.1014A > C translates to p. Pro338Pro, which has not been functionally silent. It generated an aberrant transcript with intron 7 retention by affecting the splicing accuracy. Intron retaining can reduce gene expression at the post-transcriptional level and thereby impose an additional level of gene regulation, such as the degradation of mRNA transcripts via nonsense-mediated decay (NMD) and the regulation of nuclear mRNA export (Low et al., 2015; Schmitz et al., 2017). Wild-type BRAT1 was diffusely expressed in the cytoplasm and the nucleus (Li et al., 2021). As-retained intron transcripts accumulate in the nucleus (Boutz et al., 2015), which might also reduce the amount of cytoplasmic BRAT1 available for the downstream events.

The poor efficiency of mRNA transcription, rather than completely abolished BRAT1 protein in a truncating genetic backdrop, made the BRAT1 protein synthesis in the early life insufficient to maintain the good development of the cerebellum, which may underlie the etiology of the mild, nonprogress phenotypic form of BRAT1-related neurodevelopmental disorders. c.1014A > C is the first pathogenic synonymous variant identified in the BRAT1 gene, which is associated with autosomal recessive NPCA. Before this, there was a synonymous substitution at c.1395G > C (p.Thr465 = ), which had been reported in the severe phenotypic form of RMFSL (24). Although the mechanism has not been clarified, it conjectured that there exist multiple exon skipping, mRNA degradation, and complete deletion of BRAT1 protein. Novel synonymous variants in BRAT1 should never be ignored as silent sound in diagnosis, which occasionally shed light on the underlying pathogenesis of the disease.

CONCLUSION

Our results not only broaden the mutation/phenotype spectrum of BRAT1 but also contribute to comprehend possible pathogenic mechanisms of BRAT1. It is beneficial to specific genetic counseling and timely perinatal management.

DATA AVAILABILITY STATEMENT

The datasets for this article are not publicly available due to concerns regarding participant/patient anonymity. Requests to access the datasets should be directed to the corresponding author.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s)’ and minor(s)’ legal guardian/next to kin for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors have materially participated in the study and manuscript preparation. YQ carried out all the data analyses, participated in the design of the work, and wrote the draft; XJ, HD, and YZ collected all clinical data; LL participated in molecular genetic test work; AY designed the work and manuscript preparation. YQ carried out all the data analyses, and minor(s)’ legal guardian/next to kin for the publication of any potentially identifiable images or data included in this article.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2022.821587/full#supplementary-material
