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ORIGINAL ARTICLE

Compound heterozygous loss-of-function variants in *BRAT1* cause lethal neonatal rigidity and multifocal seizure syndrome

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Abstract

Background: Lethal neonatal rigidity and multifocal seizure syndrome (RMFSL, OMIM 614498) is a rare autosomal recessive disease characterized by the onset of rigidity and intractable seizures at or soon after birth. The *BRAT1* has been identified to be the disease-causing gene for RMFSL. This study aimed to determine the underlying pathogenic mutations of a Chinese family with RMFSL and to confirm the effect of the splice-site mutation by reverse transcription analysis.

Methods: Detailed family history and clinical data were recorded, and peripheral blood samples were collected from all available family members. Whole exome sequencing (WES), Sanger sequencing, and bioinformatics analysis were performed to investigate the causative variants. The impact of the intronic variant on splicing was subsequently analyzed by RT-PCR analysis.

Results: We identified two compound heterozygous variants in the *BRAT1*, c.431-2A>G in intron 3 and c.1359_1361del(p.Leu454del) in exon 9 in the proband, one inherited from each parent. Furthermore, the 3′-splice site acceptor (c.431-2A>G) variant was found to activate a cryptic acceptor splice site, which resulted in the loss of 29 nucleotides and generation of a premature stop codon at code 180, producing a truncated BRAT1 (c.432_460del; p.Ala145Argfs*36).

Conclusions: This research identified two mutations in the *BRAT1* of one Chinese family with RMFSL. These data can aid in developing clinical diagnoses as well as providing genetic counseling and prenatal interventions to the family. These findings also expand our knowledge of the spectrum of *BRAT1* pathogenic variants in RMFSL syndrome.

KEYWORDS

BRAT1, compound heterozygous, lethal neonatal rigidity and multifocal seizure syndrome (RMFSL), splice site mutation, whole exome sequencing

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1 | INTRODUCTION

Lethal neonatal rigidity and multifocal seizure syndrome (RMFSL, OMIM 614498) is a severe autosomal recessive epileptic encephalopathy characterized by the onset of rigidity and intractable seizures at or soon after birth (Pourahmadiyan et al., 2021; Saitsu et al., 2014). Affected infants oftentimes fail to reach developmental milestones and die within the first months or years of life (Srivastava et al., 2016). Neonates diagnosed with RMFSL present with rigidity, microcephaly, myoclonic jerks, and multifocal refractory seizures, which together with apnea and bradycardia can lead to early death (Straussberg et al., 2015). Bilateral temporal and central spike activity, multifocal seizures, background slowing, and loss of posterior dominant rhythm are displayed on electroencephalograms (EEG) (Celik et al., 2017). Normal or abnormal frontal lobe hypoplasia and global atrophy are shown in brain magnetic resonance imaging (MRI). Neuropathology discloses gliosis and neuronal loss in the corticobasal region (Srivastava et al., 2016).

This syndrome is caused by homozygous or compound heterozygous biallelic loss of function variants in the *BRAT1* (OMIM 614506) coding for the BRCA1-associated ataxia telangiectasia mutated (ATM) activator 1 protein (Celik et al., 2017; Puffenberger et al., 2012; Saunders et al., 2012; Valence et al., 2019). The *BRAT1* maps to chromosome 7p22.3 and encodes three protein isoforms generated by alternative splicing (Smith et al., 2016). The *BRAT1* consists of 14 exons and encodes a 100 kDa protein with 821 amino acids. The BRAT1 localizes to both the nucleus and cytoplasm in normal human mammary epithelial cells (Saitsu et al., 2014). BRAT1 is essential for mitochondrial function, cell proliferation, and cell cycle progression by recruitment of growth factors (Horn et al., 2016). BRAT1 is also involved in regulating phosphorylation of ataxia telangiectasia (ATM) and DNA-dependent protein kinase (DNA-PK), as well as mediating the DNA damage response (Low et al., 2015; Reuter et al., 2018). Disruption of BRAT1 function in RMFSL has been proposed to cause dysfunction in the DNA damage response pathway and impair mitochondrial homeostasis (Mahjoub et al., 2019). In 2012, Puffenberger et al. reported the first RMFSL case with a homozygous variant in the BRAT1 (Puffenberger et al., 2012). Subsequently, *BRAT1* variants were found in patients with epilepsy of infancy with migrating focal seizures (EIMFS), neurodevelopmental disorder cerebellar atrophy and with or without seizures (NEDCAS), brain malformation and congenital ataxia (CA) as well as milder non-lethal clinical symptoms (Celik et al., 2017; Colak et al., 2020; Mundy et al., 2016; Oatts et al., 2017; Rim et al., 2018; Smith et al., 2016; Srivastava et al., 2016; Valence et al., 2019).

In the present study, we investigated a female infant born to non-consanguineous Chinese parents who presented with developmental delay, hypertonia, microcephaly, chronic lung disease, refractory seizures, and worsening episodic apnea, which led to intubation and eventually death at 10 months of age. Trio-based whole-exome sequencing (WES) and Sanger sequencing identified a novel splicing mutation and a heterozygous truncating mutation in *BRAT1* in the proband. In addition, we determined that the intronic mutation could lead to aberrant mRNA splicing by reverse transcription analysis.

2 | MATERIALS AND METHODS

2.1 | Ethics and consent statement

Informed written consents for the genetic analysis and publication of data were obtained from two adult participants and the legal guardians of children under age 18. This study was performed in accordance with the 2013 Helsinki Declaration and was approved by the Institutional Review Board (IRB) of the Beijing Jishuitan Hospital.

2.2 | Blood sample collection and DNA extraction

3–5 ml of peripheral blood samples were collected from all available participants using a sterile EDTA tube. Genomic DNA was then isolated from blood samples using the QIAamp DNA Blood Midi Kit (Qiagen) and measured using a Nanodrop 2000 spectrophotometer (Thermo Scientific).

2.3 | Whole exome sequencing (WES)

Whole-exome sequencing (WES) was performed in the proband and her parents (II-2, I-1, I-2) to detect variants. DNA was broken into fragments of 180–280 bp using an ultrasonoscope. Sequencing libraries were generated using an Agilent SureSelect Human All Exon V6 kit (Agilent Technologies), and the resultant fragments were sequenced on the HiSeq 2000 platform (Illumina) with the average 150× read depth.

2.4 | Bioinformatics analysis

Sequencing reads were aligned to the human reference genome sequence (UCSC Genome Browser GRCh37/

hg19) using the Burrows-Wheeler Aligner (BWA) software. Variants were filtered with minor allele frequency (MAF) > 1% in at least one of the available frequency databases including the Single Nucleotide Polymorphism Database (dbSNPs, https://www.ncbi.nlm.nih.gov/projects/SNP/), Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/), 1000 Genomes database (www.internationalgen-ome.org), Exome Aggregation Consortium (ExAC, http://www.exac.broadinstitute.org), and esp6500si_all. The Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php) and previous literature were used to determine if the identified variants are novel. The variants were then predicted for their effects by using the Mutation Taster (http://www.mutationtaster.org/), Polyphen-2 (http://genetics. bwh.harvard.edu/pph2), Sorting Intolerant From Tolerant (SIFT, http://provean.jcvi.org/index.php), Protein Variation Effect Analyzer (PROVEAN; http://provean.jcvi.org/seq_submit.php), and M-CAP (http://bejerano.stanford.edu/mcap/). Finally, the pathogenicity of variants was assessed using the American College of Medical Genetics and Genomics (ACMG) 2015 criteria (Richards et al., 2015).

2.5 | Confirmation of variants by sanger sequencing

To validate the candidate variants generated from WES, the target sites and their flanking sequences were amplified by PCR combined with Sanger DNA sequencing. Genomic DNA reference sequences of the *BRAT1* (NM_152743.4) gene were obtained from the University of California, Santa Cruz (UCSC) Genome Browser database (http://genome.ucsc.edu/). All primers for PCR were designed using the online tool Primer3 (http://primer3.ut.ee/) (Table S1). The PCR program was performed as followed: 95°C for 3 min; 94°C for 30 s, 58°C for 30 s, 72°C for 40 s (38 cycles); 72°C for 8 min. The PCR products were separated by electrophoresis using 2% agarose gel, and the target fragment was purified by the QIAquick Gel Extraction kit (Qiagen). Sequencing results from the Applied Biosystems 3730xl DNA Analyzer (Thermo Fisher Scientific) were aligned to the reference sequence using the CodonCode Aligner (version 6.0.2.6; CodonCode).

2.6 | RNA level analysis

Total RNA was extracted from peripheral blood samples of the proband's parent using the QIAamp RNA Blood Mini Kit (Qiagen). Complementary DNA (cDNA) was reverse-transcribed (RT) from total RNA using the PrimeScript™ RT reagent Kit with gDNA Eraser (RR047A, Takara Bio), according to the manufacturer's protocol. cDNA was amplified with 40 reaction cycles for sequencing analysis.

2.7 | T-Clone sequencing

T-Clone sequencing was used as Sanger sequencing of the cDNA results showed interlaced alleles. The purified PCR products with disrupted signals were linked to the pMD19-T vector. Each ligation reaction included 5 μl of purified PCR product, 4 μl of Solution I (Takara), and 1 μl of the pMD19-T vector. Following E. coli transformation, bacterial culturing and DNA Sanger sequencing were performed as previously described (Li et al., 2020).

2.8 | Molecular modeling and structural analysis

The amino acid sequences of the BRCA1-associated ATM activator 1 protein (encoded by BRAT1) were obtained from the NCBI Protein database (FASTA format). Multiple sequence alignments with different animals and conservative analyses were performed using the software MEGA (Version7; Institute for Genomics and Evolutionary Medicine, Temple University). 3D structures of normal and deletion mutants in the BRAT1 were generated by homology modeling using the SWISS-MODEL (http://swissmodel. expasy.org/). The interactions between the amino acid and the neighboring residues were exhibited and simulated by the PyMOL (Schrödinger, LLC; http://www.pymol.org/).

3 | RESULTS

3.1 | Clinical presentation

The female proband was born to nonconsanguineous normal parents as the second child after a 38-week pregnancy with a birth weight of 2455 g (−2.4SD) (Figure 1a). The first pregnancy of the proband's mother ended in spontaneous miscarriage (II-1) at 10 weeks for unknown reasons. The patient was born with symptoms of full-term small size, neonatal asphyxia, neonatal hypoxic ischemic encephalopathy, and neonatal hyperbilirubinemia. The apgar scores were 6, 7, and 8, at 1, 5, and 10 min, respectively. Clinical presentations of the proband included microcephaly, micrognathia, and small zygomatic arch (Figure 1b). Moreover, the proband presented low and weak crying, muscular hypertonia, respiratory tract infections, and dysphagia.

The 66-day-old proband visit the hospital for discontinuous cyanosis without obvious inducement for 7 h. The patient's condition worsened and was further hospitalized. A routine physical examination was performed in the hospital and recorded a body temperature of 36.7°C, a pulse of 140/min, a respiration rate of 40/min, a length of 50 cm (−3.5SD), a body weight of 3240 g (−3.8SD), and an occipito-frontal head circumference of 34 cm (−3.3SD). During hospitalization, the proband suddenly developed twitch symptoms with blue face, eyes staring, fisted hands, trembling in the flexion of the upper limbs, rigidity of the lower limbs, foaming at the mouth, heart rate reduction down to 50/min, and transcutaneous oxygen saturation at 60% with no obvious causes. Immediate treatments with oxygen, positive balloon pressure ventilation, cardiac compression, and pentobarbital sodium (10 mg/kg) were injected to stop the twitching. The convulsions gradually eased, the transcutaneous oxygen saturation increased to 95%, and the heart rate increased to 120/min. The paroxysmal convulsions lasted on average from 10 to 60 s and then stopped spontaneously. The patient's neurological symptoms progressed into tractable seizures with apneic episodes that eventually resulted in intubation and mechanical ventilation. Brain MRI and EEG results at 66 days of age were unremarkable. Ultrasonic examinations of the liver, bile, pancreas, spleen, bilateral adrenal glands, both kidneys, and ureters did not reveal obvious abnormalities.

Brain MRI indicated that the bilateral frontal and temporal subarachnoid space was widened at 6 months with small subdural effusion in the left frontal subarachnoid space. The EEG showed focal sharp wave discharges and spikes as well as slow-wave complexes in the left forehead-temporal region (Figure 1c). Despite supportive treatment, this infant died at 10 months old due to cardiopulmonary arrest. The infant and her parents were recruited for WES.

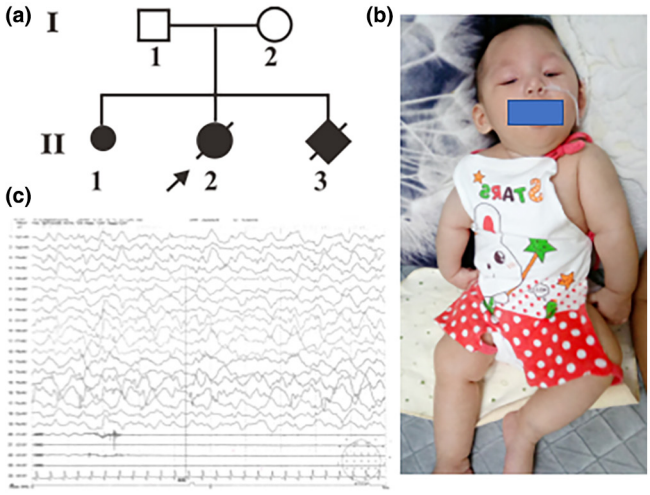

FIGURE 1 Pedigree and clinical features of the proband. (a) the black arrow denotes the proband. The open circles and squares represent unaffected females and males. Black filled circles and squares represent the affected members. (b) the dysmorphic features, microcephaly, micrognathia, and small zygomatic arch. (c) the EEG showed focal sharp wave discharges and spike and slow-wave complexes in the left forehead-temporal region when the proband was 6 months old.

3.2 | Compound heterozygous variant in *BRAT1* was identified

WES analysis revealed two likely pathogenic variants inherited from both parents in the *BRAT1* (NM_152743.4) in this proband. One novel heterozygous splice-site variant of maternal origin c.431-2A>G (Figure 2), which is located at the intron 3, was identified. Several bioinformatics analysis tools were used to predict the deleteriousness of this splice acceptor site mutation. Alternative Splice Site Predictor (ASSP) (http://wangcomputing.com/assp/index. html) revealed a score of 8.089 (Acceptor site cutoff: 2.2), and Spliceman (http://fairbrother.biomed.brown.edu/spliceman/ index.cgi) revealed a ranking of 76%. The pathogenicity of this splicing variant was classified as "pathogenic" (PVS1 + PM2 + PM3) according to the ACMG guidelines. The variant was not found in any publicly available databases. The other known heterozygous frameshift variant of paternal origin c.1359_1361del(p.

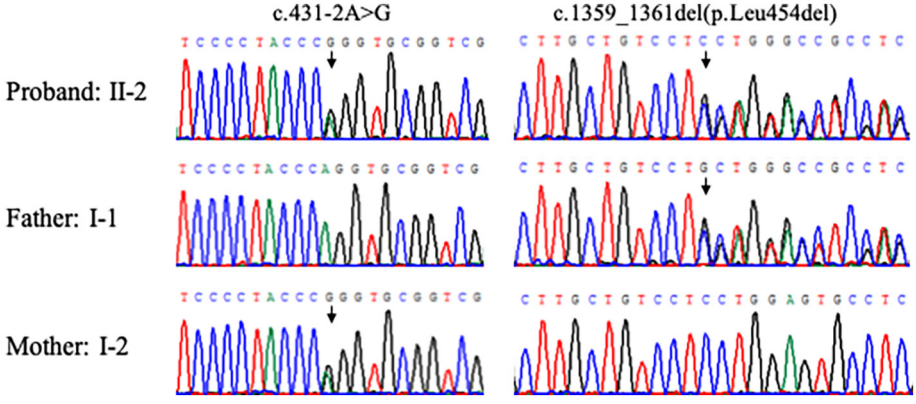

FIGURE 2 Results from genomic DNA sequencing showed compound heterozygous mutations of c.431-2A>G and c.1359_1361del(p.Leu454del) in the *BRAT1* in the patient. Parents of the proband were heterozygous carriers of these two variants.

Leu454del) was located in exon 9, which led to the leucine deletion at codon 454 (Figure 2). The frameshift variant of c.1359_1361del had been reported associated with RMFSL before (Valence et al., 2019). The frequencies of this variant were reported to be 0.0011% in ExAC and 0.00155% in gnomAD_ ALL. However, this variant was not found in the esp6500si_all or 1000g_EAS. The pathogenicity of this frameshift variant was classified as "pathogenic" (PVS1 + PM3 + PP1) according to the ACMG guidelines.

Molecular analysis indicated that the compound heterozygous variants were inherited from the unaffected parents, and her parents were heterozygotes for each of these two variants (Figure 2). Therefore, the heterozygous genotype was co-segregated with the RMFSL phenotypes in this family.

3.3 | mRNA analysis for the splicing variant

cDNA sequence combined with T-cloning analysis confirmed that the c.431-2A>G variant could result in aberrant splicing which gave rise to the skipping of 29 nucleotides in exon 4 (Figure 3). The loss of 29 nucleotides further caused the generation of a premature stop codon at code 180, producing a truncated BRAT1 (c.432_460del; p.Ala145Argfs*36) (Figure 3). Together this suggested that the c.431-2A>G variant in the *BRAT1* is responsible for the functional defect of the truncated protein.

3.4 | Bioinformatics analysis of the deletion variant

Conservation analysis indicated that p.L454 of the BRCA1-associated ataxia telangiectasia mutated (ATM) activator 1 protein amino acid sites were highly conserved (Figure 4a), which suggested it likely plays an important functional role. The wild-type and mutant BRAT1 protein showed that the alteration was in the 3D structures (Figure 4b). Comparison of the wild-type L454 was predicted to form hydrogen bonds with A451 and L457, while the deletion in the mutant variant caused steric hindrance to the formation of hydrogen bonds, which would alter its biological function (Figure 4b). The bioinformatics analysis further elucidated the pathogenicity.

4 | DISCUSSION

RMFSL is a rare autosomal recessive disorder with a broad spectrum of phenotypic, manifestations including microcephaly, rigidity, intractable focal seizures, bradycardia, and apnea (Balasundaram et al., 2021). Clinical features

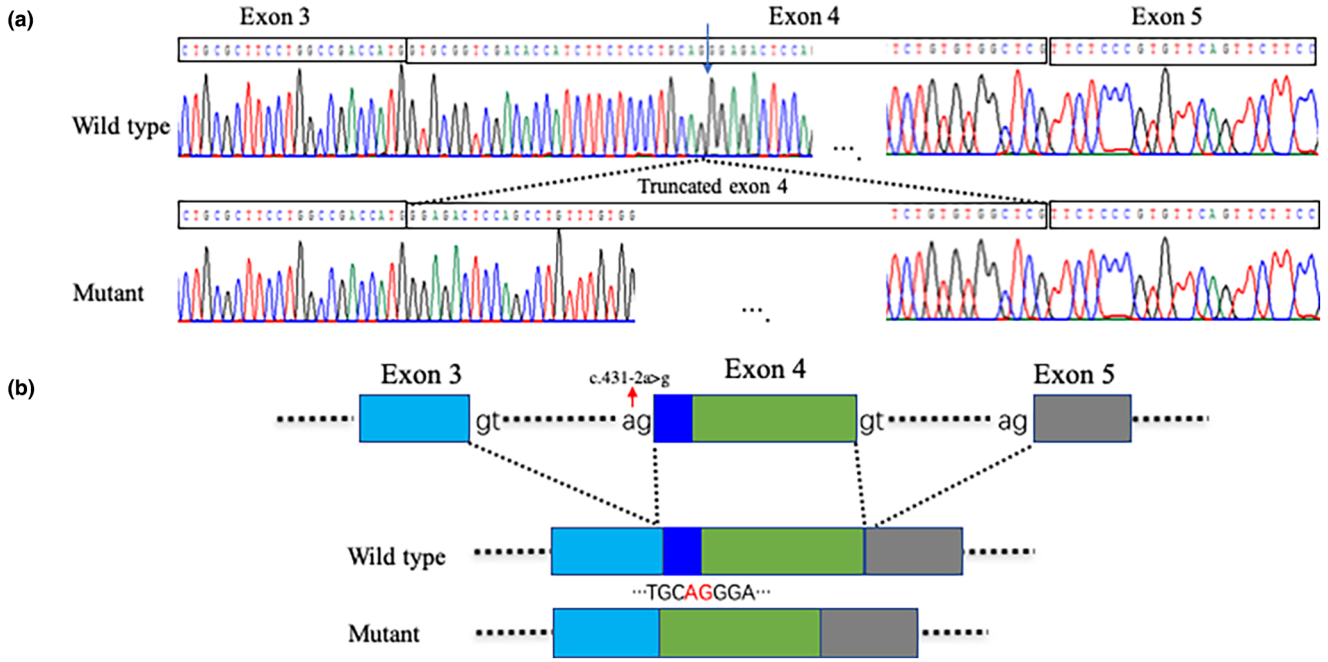

FIGURE 3 Identification of the splicing effect. The wild-type transcript and the mutant transcript of the mother (I-2) were separated using T-clone sequencing. (a) the T-clone sequencing result confirmed that the c.431-2A>G variant in the mutant transcript led to aberrant splicing, which gave rise to the skipping of 29 nucleotides in exon 4. (b) Schematic representation of splicing effect in this case. The dinucleotide in black indicates an intrinsic splicing donor or acceptor.

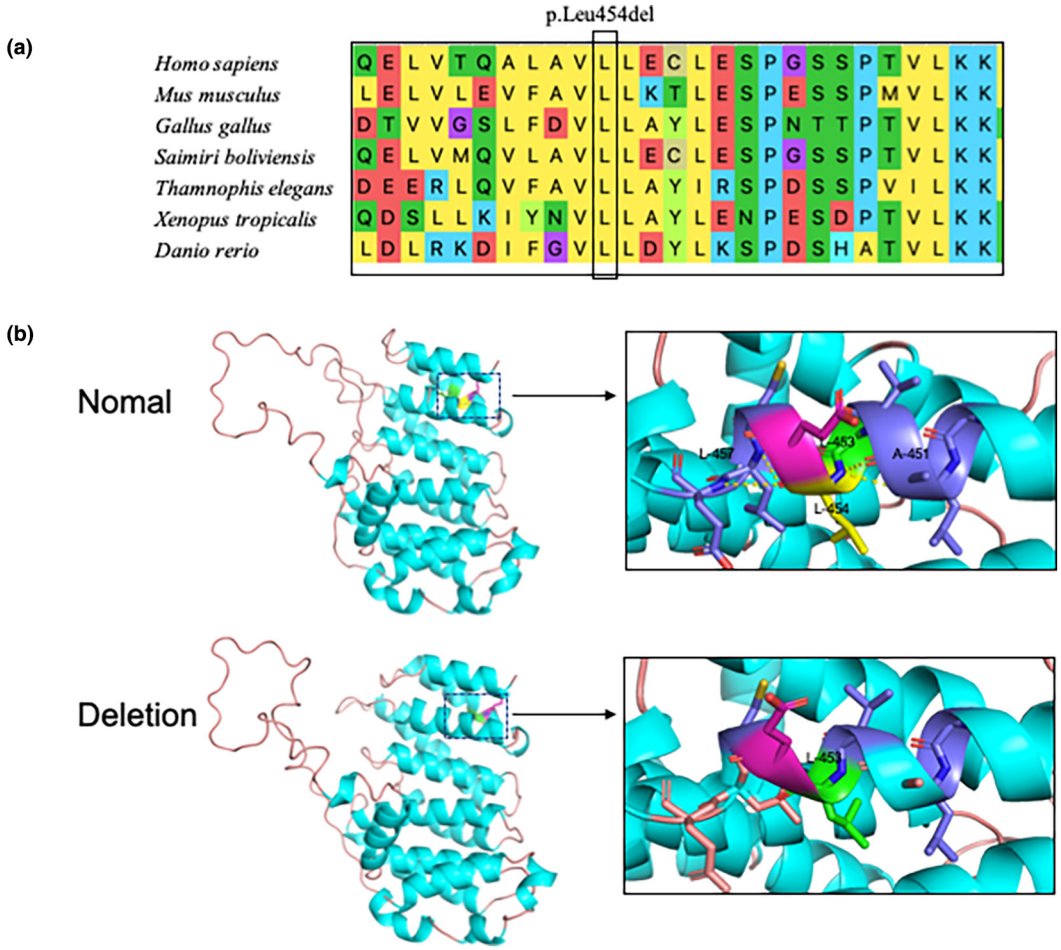

FIGURE 4 Bioinformatics analysis of the c.1359_1361del(p.Leu454del) deletion variant. (a) the residue p.Leu454 indicated in the box was evolutionarily conserved in different species. (b) the wild-type L454 was predicted to form hydrogen bonds with A451 and L457, while the deletion caused steric hindrance to the formation of hydrogen bonds.

appeared at or soon after birth and patients die within the first few months or years of life due to cardiopulmonary arrest (Pourahmadiyan et al., 2021).

In this study, we reported a female infant born to non-consanguineous parents who presented with hypertonia, dysmorphic features, progressive seizures, and worsening episodic apnea, which led to intubation and eventually death at 10 months of age. Except for pneumonia, the clinical features of the proband in this study were comparable to typical characteristics of RMFSL. Although RMFSL has been reported in different populations, to our knowledge, RMFSL is less common among the Chinese population with only four cases reported to date (Burgess et al., 2019; Li et al., 2021; Qi et al., 2022; Van Ommeren et al., 2018). In 2018, Van et al. described for the first time a female newborn affected by RMFSL with homozygous *BRAT1* mutation variant c.1395G>C born to non-consanguineous Chinese parents (Van Ommeren et al., 2018).

Here, we have identified compound heterozygous variants (c.431-2A>G; c.1359_1361del) in the *BRAT1* in the affected infant and confirmed a heterozygote status of the parents using WES followed by bioinformatic analysis. The deletion variant c.1359_1361del has previously been described in one patient, whereas the discovery of the splice-site variant c.431-2A>G is novel. In 2019, Burgess et al. also described a Chinese female with infant epilepsy of infancy with migrating focal seizures (EIMFS) caused by compound heterozygous: c.1359_1361del and c.1395G>C in the *BRAT1* (Burgess et al., 2019). This patient and our proband had the same c.1359_1361del mutation, yet their clinical features differed slightly. Compared to our patient, the patient in Burgess et al. had epilepsy, hypertonia, and hearing loss and was still alive at 13 months with a milder phenotype. In agreement with the previous findings, our study confirmed the pathogenicity of this mutation again (Burgess et al., 2019). Furthermore, the c.1359_1361del mutation frequency was extremely low in different databases, we, therefore, speculated that the variant in this proband may be explained by the founder effect. Of note, this variant has only been reported in Chinese cases of

RMSFL, raising the possibility that the frequency of this variant in the Chinese population may be higher.

Since the *BRAT1* was first reported as the causative gene of RMFSL (Puffenberger et al., 2012), 45 mutations in the *BRAT1* have been reported to date according to the Human Gene Mutation Database HGMD (http://www.hgmd.org/; Professional 2022.1) and the existing literature, including 20 missense/nonsense mutations, 8 splice mutations, 9 small deletions, 5 small insertions/duplications, and 3 gross deletions mutations (Figure 5, Table 1) (Capalbo et al., 2019; Colak et al., 2020; Fernández-Jaén et al., 2016; Hanes et al., 2015; Heide et al., 2020; Li et al., 2021; Na et al., 2020; Qi et al., 2022; Scheffer et al., 2020; Szymańska et al., 2018; Wu et al., 2021). In this study, we found a sixth splice site mutation c.431-2A>G (Colak et al., 2020; Horn et al., 2016; Srivastava et al., 2014; Stödberg et al., 2020; Rudolf et al., 2020). To determine the effect of this splicing variant, Sanger sequencing analysis of the cDNA covering exons 2–8 of the *BRAT1* showed that the 3′-splice site acceptor c.431-2A>G variant activated a cryptic acceptor splice site, which resulted in the loss of 29 nucleotides and generation of a premature stop codon at code 180, producing a truncated BRAT1 (c.432_460del; p.Ala145Argfs*36). Our research demonstrated the pathogenicity of this splice-site mutation. The family study has revealed that the *BRAT1* variant was co-segregated with the RMFSL phenotypes in this family. Compound heterozygous c.431-2A>G and c.1359_1361del variants in the *BRAT1* were identified to be the cause of RMFSL after comprehensive consideration of the clinical manifestations, co-segregation analysis, and cDNA sequencing result. As a result, a prenatal diagnosis (PND) was performed in the third pregnancy which indicated that the third pregnancy fetus (Figure 1a, II3) also carried the same compound heterozygous pathogenic variants. The parents decided to terminate the pregnancy based on the PND.

Previous reports have speculated a genotype–phenotype correlation among patients such that homozygous frameshift variants are associated with a more severe RMFSL phenotype, whereas in-frame deletions or missense variants outside of important domains in homozygous or compound heterozygous states are associated with a milder NEDCAS phenotype (Valence et al., 2019). We observed in the present patient that the identified splice variant and deletion variant resulted in a loss of protein function and led to a more severe form, furthering our understanding of this genotype–phenotype correlation.

To date, while no therapeutic modalities beyond supportive care have been proposed, initiatives to identify therapeutics for treatment of RMFSL may hold some promise for patients with *BRAT1* mutation (Colak et al., 2020). Continued efforts towards elucidating genotype–phenotype correlations of novel *BRAT1* mutation variants may facilitate more accurate prognostication and allow for more informed decision-making by clinicians.

Together these findings suggested that the RMFSL symptoms of the proband were attributed to the two compound heterozygous variants in the *BRAT1*. This study further expands our knowledge of the mutation spectrum of the *BRAT1* and provide solid evidence for future genetic counseling and prenatal gene diagnosis for the RMFSL family.

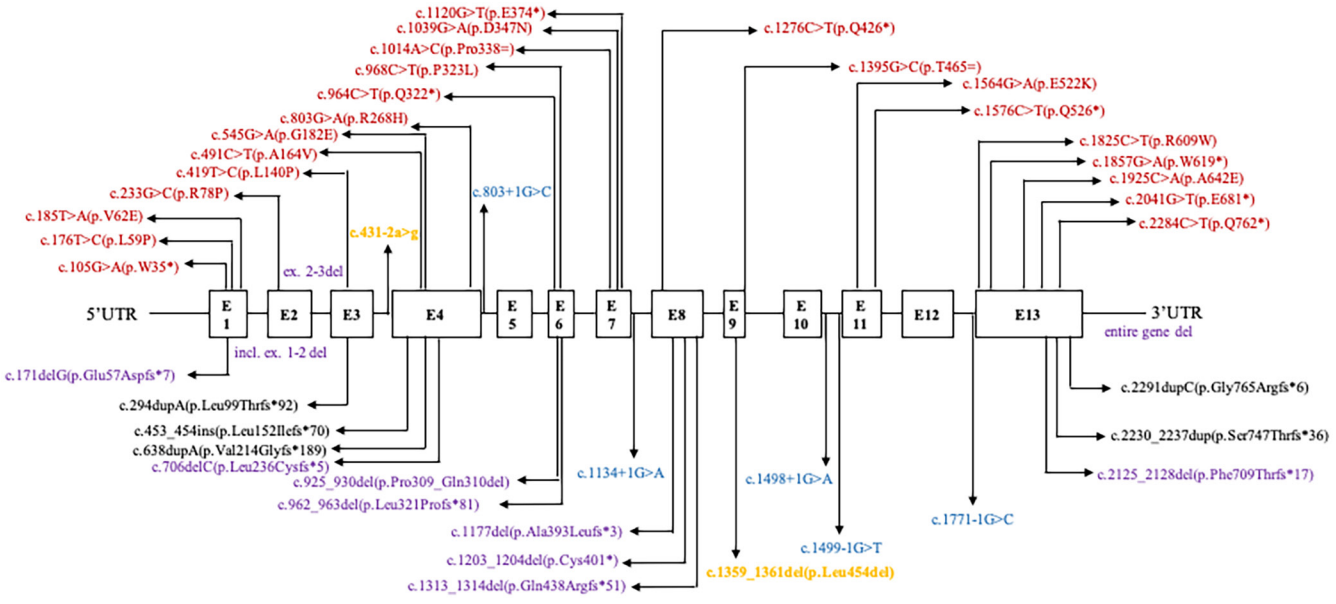

FIGURE 5 Mutation spectrums of the *BRAT1* gene. Black boxes represent the exons in each gene and lines represent the introns. Mutations are indicated by arrows, and the variants detected in the present work are marked in yellow. Variants with missense/nonsense mutations are in red. Splicing, deletion, and insertion variants are marked in blue, purple and black, respectively.

TABLE 1 List of *BRAT1* mutations in this study and reported in the literatures

Clinical phenotype	Variant type	Variant position	cDNA change	Protein alteration	References
Lethal neonatal rigidity & multifocal seizure syndrome	Gross deletions	Exon1-2	incl. ex. 1–2 deletion	—	Balasundaram et al. (2021) Cureus 13, e13600
Lethal neonatal rigidity & multifocal seizure syndrome	Missense mutation	Exon1	c.105G>A	p.Trp35*	Pourahmadiyan et al., 2021) Int J Neurosci, 1
Developmental delay, epilepsy, microcephaly, spasticity, hyperkinetic movements, dysautonomia & lung disease	Small deletion	Exon1	c.171delG	p.Glu57Aspfs*7	Srivastava et al. (2016) Am J Med Genet A 170, 2265
Ohtahara syndrome with hypertonia & microcephaly	Missense mutation	Exon1	c.176T>C	p.Leu59Pro	Saitsu et al. (2014) J Hum Genet 59, 687
Cerebellar ataxia	Missense mutation	Exon1	c.185T>A	p.Val62Glu	Mahjoub et al. (2019) Neurol Genet 5,
Lethal neonatal rigidity & multifocal seizure syndrome	Missense mutation	Exon2	c.233G>C	p.Arg78Pro	Li et al. (2022) Pediatr Res 91,565
West syndrome	Gross deletion	Exon2-3	ex. 2–3 deletion	—	Na et al. (2020) Brain Dev 42, 438
Hypertonia & seizures, neonatal onset	Small insertion	Exon3	c.294dupA	p.Leu99Thrfs*92	Mundy et al. (2016) Am J Med Genet A 170, 699
Developmental delay, epilepsy & microcephaly	Missense mutation	Exon3	c.419T>C	p.Leu140Pro	Srivastava et al. (2016) Am J Med Genet A 170, 2265
Lethal neonatal rigidity & multifocal seizure syndrome	Splicing	Intron4	c.431-2A>G	p.Ala145Argfs*36	Current study
Lethal neonatal rigidity & seizure syndrome	Small insertion	Exon4	c.453_454ins	p.Leu152Ilefs*70	Saunders et al. (2012) Sci Transl Med 4, 154ra135
Cerebellar ataxia	Missense mutation	Exon4	c.491C>T	p.Ala164Val	Valence et al. (2019) Genet Med 21, 553
Rolandic epilepsy, atypical	Missense mutation	Exon4	c.545G>A	p.Gly182Glu	Rudolf et al. (2020) Eur J Paediatr Neurol 27, 104
Lethal neonatal rigidity & seizure syndrome	Small insertion	Exon4	c.638dupA	p.Val214Glyfs*189	Puffenberger et al. (2012) PLoS One 7, 28936
Nonprogressive Cerebellar Ataxia Syndrome	Small deletion	Exon4	c.706delC	p.Leu236Cysfs*5	Qi et al. (2022) Front Genet 13, 821587
Inner retinal dystrophy	Missense mutation	Exon4	c.803G>A	p.Arg268His	Oatts et al. (2017) Ophthalmic Genet 38, 559
Lethal neonatal rigidity & seizure syndrome	Splicing	Intron4	c.803+1G>C	—	Srivastava et al. (2014) Ann Neurol 76, 473
Cerebellar ataxia	Small deletion	Exon6	c.925_930del	p.Pro309_Gln310del	Valence et al. (2019) Genet Med 21, 553
Ohtahara syndrome with hypertonia & microcephaly	Small deletion	Exon6	c.962_963delTC	p.Leu321Profs*81	Saitsu et al. (2014) J Hum Genet 59, 687
Epilepsy of infancy with migrating focal seizures	Nonsense mutation	Exon6	c.964C>T	p.Gln322*	Burgess et al. (2019) Ann Neurol 86, 821

TABLE 1 (Continued)

Clinical phenotype	Variant type	Variant position	cDNA change	Protein alteration	References
Cerebellar ataxia	Missense mutation	Exon6	c.968C>T	p.Pro323Leu	Valence et al. (2019) Genet Med 21, 553
Nonprogressive Cerebellar Ataxia Syndrome	Splicing	Exon7	c.1014A>C	p.Pro338=	Qi et al. (2022) Front Genet 13,821587
Rolandic epilepsy, atypical	Missense mutation	Exon7	c.1039G>A	p.D347N	Rudolf et al. (2020) Eur J Paediatr Neurol 27, 104
Epilepsy of infancy with migrating focal seizures	Missense mutation	Exon7	c.1120G>T	p.Glu374*	Burgess et al. (2019) Ann Neurol 86, 821
Epileptic encephalopathy, infantile & mitochondrial dysfunction	Splicing	Intron7	c.1134+1G>A	—	Horn et al. (2016) Am J Med Genet A 170, 2274
Lethal neonatal rigidity & seizure syndrome	Small deletion	Exon8	c.1177delG	p.Ala393Leufs*3	Straussberg et al. (2015) Eur J Paediatr Neurol 19, 240
Lethal neonatal rigidity & seizure syndrome	Small deletion	Exon8	c.1203_1204delTG	p.Cys401*	Capalbo et al. (2019) PLoS Genet 15, e1008409
Neurodevelopmental disorder with cerebellar atrophy, with/without seizures	Nonsense mutation	Exon8	c.1276C>T	p.Gln426*	Wu et al. (2021) Crit Care Med,
Intractable epileptic encephalopathy	Small deletion	Exon8	c.1313_1314delAG	p.Gln438Argfs*51	Szymańska et al. (2018) Folia Neuropathol 56,362
Lethal neonatal rigidity & multifocal seizure syndrome	Small deletion	Exon9	c.1359_1361del	p.Leu454del	Current study
Lethal neonatal rigidity & multifocal seizure syndrome	Splicing	Exon9	c.1395G>C	p.Thr465=	Van Ommeren et al. (2018) J Neuropathol Exp Neurol 77,1071
Epilepsy of infancy with migrating focal seizures	Splicing	Intron10	c.1498+1G>A	—	Burgess et al. (2019) Ann Neurol 86, 821
Migrating focal seizures and pontocerebellar hypoplasia	Splicing	Intron10	c.1499-1G>T	—	Colak et al. (2020) Acta Neurol Belg 120, 1425
Encephalopathy, progressive, autosomal recessive	Missense mutation	Exon11	c.1564G>A	p.Glu522Lys	Fernández-Jaén et al. (2016) Eur J Paediatr Neurol 20, 421
Infantile spasms	Nonsense mutation	Exon11	c.1576C>T	p.Gln526*	Rim et al. (2018) BMC Med Genomics 11, 6
Brain malformation	Splicing	Intron12	c.1771-1G>C	—	Stödberg et al. (2020) Epilepsia 61, 2486
BRAT1-associated neurodegenerative disorder	Missense mutation	Exon13	c.1825C>T	p.Arg609Trp	Hanes et al. (2015) Pediatr Neurol 53, 535
BRAT2-associated neurodegenerative disorder	Missense mutation	Exon13	c.1857G>A	p.Trp619*	Smith et al. (2016) Am J Med Genet A 170, 3033
Hypertonia & seizures, neonatal onset	Missense mutation	Exon13	c.1925C>A	p.Ala642Glu	Mundy et al. (2016) Am J Med Genet A 170, 699
Lethal neonatal rigidity & multifocal seizure syndrome	Missense mutation	Exon13	c.2041G>T	p.Glu681*	Pourahmadiyan et al. (2021) Int J Neurosci, 1

(Continues)

TABLE 1 (Continued)

Clinical phenotype	Variant type	Variant position	cDNA change	Protein alteration	References
BRAT3-associated neurodegenerative disorder	Small deletions	Exon13	c.2125_2128del	p.Phe709Thrfs*17	Smith et al. (2016) Am J Med Genet A 170, 3033
Lethal neonatal rigidity & seizure syndrome	Small insertion	Exon13	c.2230_2237dup	p.Ser747Thrfs*36	Celik et al. (2017) Epilepsy Behav Case Rep 8, 31
Epilepsy of infancy with migrating focal seizures	Nonsense mutation	Exon13	c.2284C>T	p.Gln762*	Burgess et al. (2019) Ann Neurol 86, 821
Rigidity and multifocal seizure syndrome	Small insertion	Exon13	c.2291dupC	p.Gly765Argfs*6	Heide et al. (2020) Genet Med 22, 1887
Neurodevelopmental disorder with cerebellar atrophy, with/without seizures	Gross deletion	Exon1-13	entire gene deletion	—	Wu et al. (2021) Crit Care Med,

5 | CONCLUSION

In conclusion, we first reported two mutations c.431-2A>G and c.1359_1361del(p.Leu454del) within the RMFSL-causing *BRAT1* in a Chinese family. Our research demonstrated the pathogenicity of this c.431-2A>G mutation and further contributed to a better understanding and establishment of the genotype–phenotype correlations in RMFSL.

AUTHOR CONTRIBUTIONS

SL performed the experiment and wrote the manuscript. SY, YZ, and YW collected the patient samples. CW and XJ designed and supervised this project. All authors performed critical reading and approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ETHICS STATEMENT

The present studies involving human participants were approved by the Institutional Review Board (IRB) of the Beijing Jishuitan Hospital, Beijing, China (11-2016). Written informed consent was obtained from all adult participants/legal guardians of minor, for publication of any potentially identifiable images or data included in this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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