BRIEF COMMUNICATION

Novel *ITPA* variants identified by whole genome sequencing and RNA sequencing

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Approximately 80% of rare diseases have a genetic cause, and an accurate genetic diagnosis is necessary for disease management, prognosis prediction, and genetic counseling. Whole-exome sequencing (WES) is a cost-effective approach for exploring the genetic cause, but several cases often remain undiagnosed. We combined whole genome sequencing (WGS) and RNA sequencing (RNA-seq) to identify the pathogenic variants in an unsolved case using WES. RNA-seq revealed aberrant exon 4 and exon 6 splicing of *ITPA*. WGS showed a previously unreported splicing donor variant, c.263+1G>A, and a novel heterozygous deletion, including exon 6. Detailed examination of the breakpoint indicated the deletion caused by recombination between Alu elements in different introns. The proband was found to have developmental and epileptic encephalopathies caused by variants in the *ITPA* gene. The combination of WGS and RNA-seq may be effective in diagnosing conditions in proband who could not be diagnosed using WES.

Journal of Human Genetics (2023) 68:649–652; https://doi.org/10.1038/s10038-023-01156-y

Identifying the differentiating disease based on symptoms and general examination is often difficult in rare intractable diseases. Comprehensive genetic testing is usually essential to make a definitive diagnosis of an incurable disease. In recent years, whole-exome sequencing (WES) and whole-genome sequencing (WGS) have aided in determining the molecular cause of rare and undiagnosed diseases. WES has enabled the diagnosis of conditions in ~35% of the patients [1]. However, the large majority of these patients have undiagnosed conditions. Variants in deep intronic/intergenic regions are usually undetectable because they are not covered by WES. On the contrary, WGS provides comprehensive information on the human genome, including deep intronic/intergenic regions. The technique has been reported to be appropriate for increasing the diagnostic rates of rare disorders [2, 3]. RNA-seq could detect aberrant expression [4], aberrant splicing, and mono-allelic expression, which makes it a powerful method for variant interpretation. We performed WGS and RNA-seq on a case that could not be diagnosed using WES, which revealed a novel splicing variant and a deletion of ~3.4 kb via recombination between two Alu elements in *ITPA*. Variants in *ITPA* are known to cause developmental epileptic encephalopathy 35 (MIM:616647). In addition to neurological symptoms, the family had recurrent episodes of dilated cardiomyopathy caused by *ITPA* deficiency.

The proband (II-3) was the third child of nonconsanguineous parents. She was born at the 39th gestational week via spontaneous vaginal delivery with a birth weight of 2565 g (−1.6 SD), a body length of 48.8 cm (−0.3 SD), and an occipito-frontal circumference of 32.5 cm (−0.6 SD). The Apgar score was 8/9. She had hypotonia, mild feeding difficulties, and refractory epilepsy at birth. At 11 months of age, the proband had not yet acquired a fixed neck. She is now 6 years old and has refractory epilepsy associated with severe developmental delay and requires percutaneous endoscopic gastronomy for nutritional support. Her symptoms are similar to those of dilated cardiomyopathy, with the left ventricular ejection fraction being ~45% that has been stabilized by β-blocker treatment. Her two elder brothers had a similar presentation. The eldest brother was born at 40 weeks of gestational age with a birth weight of 3392 g (0.7 SD). He exhibited myotonia, feeding difficulties, and poor weight gain. Febrile seizures began at 10 months of age, followed by refractory epilepsy at 14 months of age. The second brother was born at 39 weeks of gestational age with a birth weight of 2730 g (−1.1 SD). He was diagnosed with congenital hypotonia at birth and, then at 4 months of age, began having involuntary movements, seizures, and epileptic gaze deviations. Finally, her eldest brother and the second brother died of dilated cardiomyopathy at 5 and 3 years of age, respectively.

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Received: 26 February 2023 Revised: 20 April 2023 Accepted: 21 April 2023
Published online: 29 May 2023

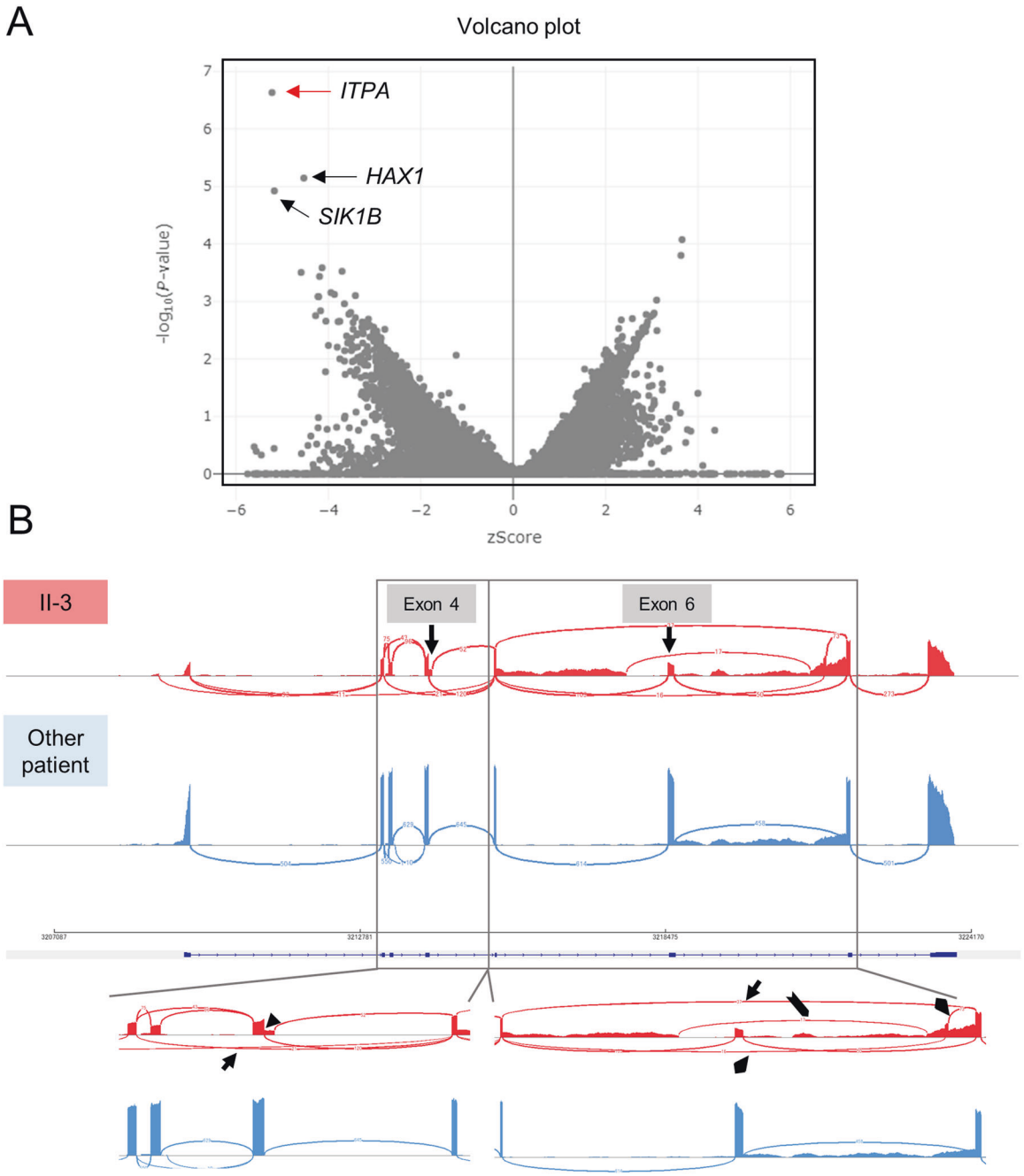

Fig. 1 Abnormal expression of *ITPA* revealed using RNA-seq. **A** Volcano plot at gene level from OUTRIDER analysis. RNA-seq is performed on 35 patients with suspected mitochondrial disease (undiagnosed cases) including the proband. The RNA-seq data of proband was compared with those of 34 cases. We used the patient population as a control and extracted outlier genes by using OUTRIDER software. *ITPA, HAX1* and *SIK1B* were genes that were significantly downregulated. **B** IGV Sashimi plot of RNA-seq data of II-3 and control at the whole *ITPA* gene (upper panel). At the bottom are zoomed-in views of exon 4 and exon 6. The arrows indicate exon skipping, and the arrowhead indicates exon extension. Diamond-shaped arrows indicate abnormal splicing events. The chevron arrow shows the location of the deletion

The disease-causing variants could not be determined by the trio-WES analysis. Initially, we performed RNA-seq using the proband's fibroblast cell (F1431). RNA-seq analysis using OUTRIDER software [4] revealed decreased expression of *ITPA* (Fig. 1A). We first found exon elongation on the 3' side of exon 4 (Fig. 1B), and exon skipping of exon 3 and 4 were detected as well (Fig. 1B and Supplementary Fig. 1). We observed a decrease in the read depth of exon 6 when compared with the other exons (Fig. 1B). In addition to exon 6 depth reduction, exon skipping and several abnormal splicing events were also detected (Fig. 1B). The Sashimi plot revealed skipping of exon 6 and other connections indicating abnormal splicing in the middle of introns (Fig. 1B and Supplementary Fig. 1). By performing reverse transcription-PCR in patient cells with suppressed nonsense-mediated mRNA decay, we found that these aberrant transcripts undergo degradation (Supplementary Fig. 2). A decrease in the expression of *HAX1* and *SIK1B* was also observed (Fig. 1A). The pathogenic heterozygous variant c.256C>T (p.Arg86Ter) in *HAX1* (NM_006118.4) was identified from WGS. *HAX1* has been reported to cause Neutropenia, severe congenital 3, autosomal recessive (MIM: 610738). The blood tests showed mild decrease in neutrophils (1585 cells/μl: neutropenia is defined as less than 1500 cells/μl) that might be caused by the heterozygous variant in *HAX1*. *SIK1B* encodes a putative serine/threonine-protein kinase

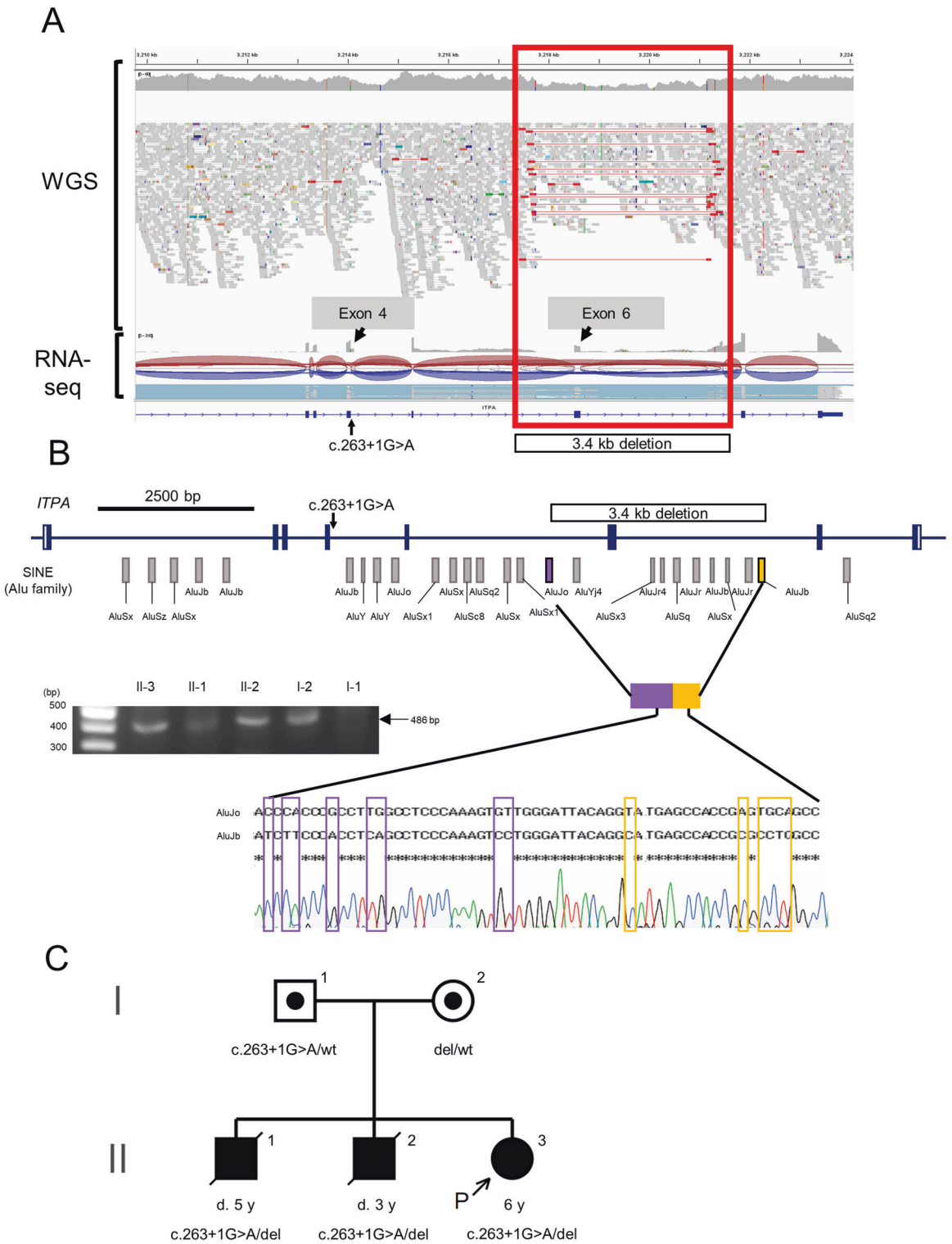

Fig. 2 Variants in *ITPA* revealed using WGS. **A** The deletion around exon 6 is indicated by IGV. Red indicates paired reads in distant regions. **B** The region surrounding exon 6 was amplified using PCR and sequenced using Sanger sequencing. The deletion was probably caused by recombination between AluJo (purple) in intron 5 and AluJb (orange) in intron 6. The Alu sequences were present in a mixed state, with purple derived from AluJo and orange from AluJb. **C** Pedigree of the family. Affected members are indicated with filled symbols. Individuals labeled with dots are carriers. The arrow indicates the index proband

protein, but its function is unknown. No variant in *SIK1B* has been detected by WGS. An association between *ITPA* deficiency and decreased expression of these genes has not been reported to date.

We performed WGS to search for deep intronic and intergenic variants. Analysis of structural variation (SV) and copy number variation (CNV) from the WGS data using Manta and Lumpy identified a heterozygous deletion of 3.4 kbp containing exon 6 (Fig. 2A, B). Furthermore, we found a heterozygous variant c.263+1G>A (NM_033453.4), in *ITPA*, which is likely to be involved in the extension of exon 4. The integrative genomics viewer (IGV)

screen snapshot showed a prominent decrease in depth around intron 5, exon 6, and intron 6. Read pairs showing deletions at the paired-end were also detected.

Trio Sanger sequencing confirmed that c.263+1G>A is of paternal origin (Fig. 2C). The PCR of a deletion region containing exon 6 revealed short fragments in DNA from the proband and the mother, which confirmed NC_000020.11: g.(3217736_3217747)_(3221159_3221170)del. The two elder brothers who died of dilated cardiomyopathy had the same genotype as the proband. To determine the detailed sequence of the breakpoint, PCR fragments were analyzed using Sanger sequencing, which revealed that the deletion was caused by homologous recombination between AluJo and AluJb (Fig. 2B).

We performed a multi-omics analysis for the proband whose condition could not be diagnosed using WES and finally identified compound heterozygous variants in *ITPA*. *ITPA* encodes inosine triphosphate pyrophosphohydrolase (ITPase) that hydrolyzes inosine triphosphate (ITP) and deoxyinosine triphosphate. Variants in *ITPA* cause developmental epileptic encephalopathy 35 (DEE35) [5] or Inosine triphosphatase deficiency [6]. DEE35 is a rare neurodegenerative disease characterized by developmental delay, microcephaly, feeding difficulties, early-onset intractable seizures, subsequent psychomotor retardation, and early childhood fatality [7]. In this family, siblings with *ITPA* mutations have died of dilated cardiomyopathy. Cardiac involvement has been noted in 28.6% of patients with *ITPA* deficiency, and dilated cardiomyopathy is the most common cause of cardiac involvement [7]. Furthermore, dilated cardiomyopathy has been reported in *ITPA* gene-disrupted mouse [8, 9]. Thus, in this proband and her brothers, dilated cardiomyopathy is thought to be caused by variants in *ITPA*. The other clinical features of the proband were consistent with those of DEE35.

Our analysis identified a novel Alu recombination-mediated deletion (ARMD). Deletions due to ARMD have been reported to account for 0.3% of human genetic disorders [10]. To the best of our knowledge, this is the first report of ARMD with DEE35. Such ARMDs are thought to have been missed by previous genetic testing because Alu is present in introns, which are difficult to cover with WES and panel sequencing. We must actively promote genetic testing methodologies to identify Alu-mediated recombination, such as ARMD. A recent study reported that capture-seq and long-read sequencing specifically examine recombination between Alu or L1 [11]. We believe that improving the accuracy of methods for detecting CNV and SV from WGS data and applying techniques to specifically detect transposable elements such as Alu, for the genetic testing of diseases are necessary to improve diagnostic accuracy. RNA-seq enables more effective identification of genes showing aberrant expression and abnormal splicing, which is handy when combined with WGS. In our previous studies, Alu-mediated deletions have been identified using RNA-seq and WGS [12, 13]. These methods should be considered for WES-negative cases to detect pathogenic variants missed by WES.

DATA AVAILABILITY

Raw data are available from the corresponding author upon reasonable request. Some genomic information that could be used to identify individuals cannot be shared due to ethical constraints.

REFERENCES

1. Clark MM, Stark Z, Farnaes L, Tan TY, White SM, Dimmock D, et al. Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases. npj Genom Med. 2018;3:1–10.
2. Kremer LS, Bader DM, Mertes C, Kopajtich R, Pichler G, Iuso A, et al. Genetic diagnosis of Mendelian disorders via RNA sequencing. Nat Commun. 2017;8:1–11.
3. Cummings BB, Marshall JL, Tukiainen T, Lek M, Donkervoort S, Foley AR, et al. Improving genetic diagnosis in Mendelian disease with transcriptome sequencing Genotype-Tissue Expression Consortium. Sci Transl Med. 2016;5209:1–12.
4. Brechtmann F, Mertes C, Matusevičiūtė A, Yépez VA, Avsec Ž, Herzog M, et al. OUTRIDER: a statistical method for detecting aberrantly expressed genes in RNA sequencing data. Am J Hum Genet. 2018;103:907–17.
5. Kevelam SH, Bierau J, Salvarinova R, Agrawal S, Honzik T, Visser D, et al. Recessive ITPA mutations cause an early infantile encephalopathy. Ann Neurol. 2015;78:649–58.
6. Sumi S, Marinaki AM, Arenas M, Fairbanks L, Shobowale-Bakre M, Rees DC, et al. Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency. Hum Genet. 2002;111:360–7.
7. Scala M, Wortmann SB, Kaya N, Stellingwerff MD, Pistorio A, Glamuzina E, et al. Clinico-radiological features, molecular spectrum, and identification of prognostic factors in developmental and epileptic encephalopathy due to inosine triphosphate pyrophosphatase (ITPase) deficiency. Hum Mutat. 2022;43:403–19.
8. Handley MT, Reddy K, Wills J, Rosser E, Kamath A, Halachev M, et al. ITPase deficiency causes a martsolf-like syndrome with a lethal infantile dilated cardiomyopathy. PLoS Genet. 2019;15:1–23.
9. Behmanesh M, Sakumi K, Abolhassani N, Toyokuni S, Oka S, Ohnishi YN, et al. ITPase-deficient mice show growth retardation and die before weaning. Cell Death Differ. 2009;16:1315–22.
10. Kim S, Cho C-S, Han K, Lee J. Structural variation of Alu element and human disease. Genom Inf. 2016;14:70–7.
11. Pascarella G, Hon CC, Hashimoto K, Busch A, Luginbühl J, Parr C, et al. Recombination of repeat elements generates somatic complexity in human genomes. Cell. 2022;185:3025–3040.e6.
12. Kishita Y, Shimura M, Kohda M, Fushimi T, Nitta KR, Yatsuka Y, et al. Genome sequencing and RNA-seq analyses of mitochondrial complex I deficiency revealed Alu insertion-mediated deletion in NDUFV2. Hum Mutat. 2021;42:1422–8.
13. Yépez VA, Gusic M, Kopajtich R, Mertes C, Smith NH, Alston CL, et al. Clinical implementation of RNA sequencing for Mendelian disease diagnostics. Genome Med. 2022;14:1–26.

ACKNOWLEDGEMENTS

We thank the family for their participation in the study.

AUTHOR CONTRIBUTIONS

NO and YK wrote the manuscript. NO and YK performed the experiments. NO, YK, KRN, TF and YY analyzed the data. MN, HS, AT, EK, YS, KI and KM acquired clinical information. YK, AO, KM, and YO supervised the study. All authors discussed the results and commented on the manuscript.

FUNDING

This work was supported by a grant for the Practical Research Project for Rare/Intractable Diseases from AMED to KM, YO, and AO (Fund ID: JP21ek010946, 22ek0109485, JP22ek0109468, JP19ek0109273), Program for Promoting Platform of Genomics based Drug Discovery to YO (Fund ID: JP22kk0305015) and JSPS KAKENHI JP19H03624 to YO.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the regional Ethics Committees of Juntendo University, Saitama Medical University, Chiba Children's Hospital, and Kindai University. Written informed consent was obtained from the parents. All methods were performed by relevant guidelines and regulations.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s10038-023-01156-y.

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