BRIEF COMMUNICATION

Identification of a pathogenic *RNU4-2* variant in patients with mitochondrial disease: Broadening the spectrum of non-coding RNA gene variants in mitochondrial dysfunction

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Mitochondrial diseases are characterized by impaired energy production due to mitochondrial dysfunction. Despite advances in sequencing technologies, many cases remain genetically undiagnosed. We report two cases of mitochondrial disease harboring identical de novo variant in the non-coding RNA gene *RNU4-2*, previously associated with neurodevelopmental disorders. Re-analysis of whole genome sequencing data from 357 patients ascertained as possibly having mitochondrial disease (see Methods: Supplementary Data S1) identified two cases with a pathogenic *RNU4-2* variant (GRCh38: chr.12:120291839: T > TA; NR_003137.2: n.64_65insT). Both patients exhibited decreased oxygen consumption rates and clinical features including developmental delay, microcephaly, short stature. This study provides the first evidence linking *RNU4-2* variant to mitochondrial disease, expanding the phenotypic spectrum associated with this gene. Our findings highlight the importance of re-analyzing genomic data and considering non-coding RNA gene variants in mitochondrial disease diagnostics, potentially improving the diagnosis of previously unsolved cases.

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INTRODUCTION

Mitochondrial diseases are inherited disorders characterized by impaired energy production due to mitochondrial respiratory chain dysfunction [1, 2]. Despite advances in next-generation sequencing, more than half of mitochondrial disease cases remain genetically undiagnosed, partly due to variant in non-coding regions undetectable by whole exome sequencing (WES). Recent studies have shown that mutations in the non-coding RNA gene *RNU4-2* account for approximately 0.4% of all cases with a neurodevelopmental disorders (NDD) [3–5]. This discovery was made through whole-genome analysis, which captures non-coding as well as coding regions. *RNU4-2* encodes a small nuclear RNA (snRNA) that is a critical component of the major spliceosome, the cellular machinery responsible for removing introns from pre-mRNA [6, 7]. Specifically, *RNU4-2* forms part of the U4/U6 snRNA duplex, with the variant -prone region mapping to the T-loop and Stem III of this structure [8–10]. The variant -prone region in the T-loop is highly conserved, underscoring its functional importance. The discovery studies found that *RNU4-2* disorder accounts for about 0.4% of all NDD cases and that a single base insertion in the region accounts for approximately 75% of *RNU4-2* cases. Mitochondrial diseases often present with neurological symptoms, particularly in childhood-onset cases. Leigh syndrome, a representative clinical type, is characterized by developmental regression and specific brain lesions [11, 12]. Other mitochondrial diseases such as MELAS and MERRF also present with various neurological symptoms [13]. The nervous system's high metabolic demands make it particularly susceptible to energy metabolism disorders. In this report, we reanalyzed whole genome sequencing (WGS) data from 357 mitochondrial disease patients, identifying two cases with the pathogenic *RNU4-2* variant. This discovery expands the phenotypic spectrum associated with *RNU4-2* variant and suggests a potential link between this non-coding RNA gene and mitochondrial function. Our findings emphasize the importance of reanalyzing WGS data and considering non-coding RNA gene variants in mitochondrial disease diagnostics. This study provides the first evidence linking *RNU4-2* variant to mitochondrial disease, potentially improving the diagnosis of previously unresolved cases and opening new avenues for understanding the genetic basis of mitochondrial disorders.

CLINICAL REPORT

Two patients with suspected mitochondrial disease were identified in our cohort. Patient 1, born at 39 weeks' gestation weighing 2540 g, presented with symptomatic epilepsy, developmental delay, gray matter heterotopia, short stature, and microcephaly. Additional

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features included gastric volvulus and, as evidenced by reduced oxygen consumption rate (OCR) (Supplementary Data S2). This patient exhibited features including developmental delay, hyperlactatemia, and microcephaly. Patient 2 presented with intellectual disability, autism spectrum disorder, global developmental delay, gross motor delay, fine motor delay, microcephaly, hypotonia, drooling, and absence of meaningful words. Additionally, the patient exhibited features of developmental delay, hyperlactatemia, microcephaly. This patient also exhibited reduced OCR. (Table 1) (Supplementary Data S2) (Supplementary Data S3). Whole genome sequencing (WGS) analysis identified an identical heterozygous variant in the *RNU4-2* gene (GRCh38:chr.12:120291839:T > TA; NR_003137.2(*RNU4-2*):n.64_65insT) in both Patient 1 and Patient 2 (Fig. 1). This variant is located in the T-loop region of the *RNU4-2* gene and corresponds to the recently reported variant associated with neurodevelopmental disorders. To confirm the origin of the variant, we performed Sanger sequencing validation. For Patient 1, blood samples were available from both parents, allowing for a complete family trio analysis. The results confirmed that the variant was absent in both parents, establishing it as a de novo variant in Patient 1. In contrast, for Patient 2, only the maternal blood sample was available. Sanger sequencing confirmed the absence of the variant in the mother, ruling out maternal inheritance. However, due to the unavailability of the father's sample, we could not definitively confirm whether the variant was a complete de novo variant in Patient 2. These findings suggest that variants in the *RNU4-2* gene may represent a novel cause of neurodevelopmental disorders associated with mitochondrial dysfunction (Supplementary Data S4). Notably, the observation of decreased mitochondrial oxygen consumption rates in both patients strongly indicates a potential link between this variant and mitochondrial function. The identified variant aligns with the recently reported causative variant for *RNU4-2*-related neurodevelopmental disorder, expanding the clinical spectrum of this newly described condition. Furthermore, these cases provide new evidence suggesting that variant in non-coding RNA genes can potentially cause mitochondrial diseases, bridging the gap between neurodevelopmental disorders and mitochondrial dysfunction.

Table 1. Patient clinical information

	Patient1	Patient2
Age of onset	9 months	0 day
Alive/dead	Alive (2y4m)	Alive (2y11m)
Lactate/Pyruvate(mM) (<1.8/ < 0.1)	3.78/0.12	NR
Enzyme Diagnostics	Normal	Normal
Enzyme assay ComplexI	76.6%	74.7%
Enzyme assay ComplexII	114.6%	52.0%
Enzyme assay ComplexII+III	96.1%	79.3%
Enzyme assay ComplexIII	151.1%	151.0%
Enzyme assay ComplexIV	92.3%	55.6%
Oxygen Consumption Rate Glucose	63.0%	61.0%
Oxygen Consumption Rate Galactose	79.0%	77.0%
Hypothyroidism	+	−
Short stature	+	+
Microcephaly	+	+
Epilepsy	+	−
Developmental Delays	+	+
Ectopic gray matter	+	−
Mental retardation	−	+
Autism spectrum disorder	−	+
Hypotension	−	+

The Clinical information of Patient 1 and Patient 2 are summarized. Age, enzyme activity, oxygen consumption rate (OCR), and clinical symptoms of each patient are described. Enzyme activity is considered decreased when <40% of normal control mean in cell lines or <30% in tissue samples. OCR is considered significantly reduced when <71.6% of normal control mean ($p < 0.05$)

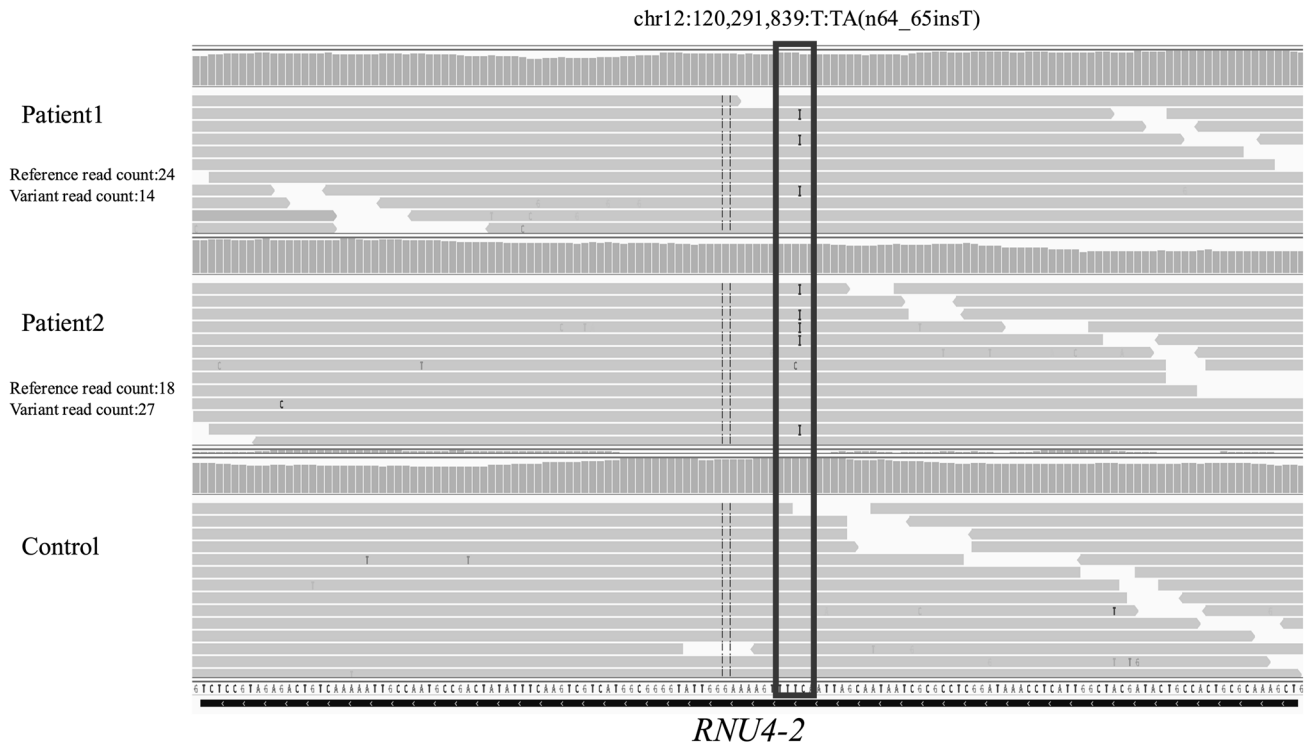

Fig. 1 Identification of *RNU4-2* variant in two patients with mitochondrial disease. The identified heterozygous insertion variant (GRCh38:chr.12:120291839:T > TA; NR_003137.2:n.64_65insT) is a BAM file showing

DISCUSSION

In this study, we identified two cases of patients suspected of mitochondrial disease with identical de novo variant in the *RNU4-2* gene, a crucial component of the spliceosome. Our findings raise the hypothesis that variant in non-coding RNA genes may contribute to mitochondrial diseases phenotypes, expanding our understanding of their genetic etiology. Recently, there have been reports of variant in genes related to the spliceosome. Notably, variant in the *RNU4-2* gene have been reported to cause neurodevelopmental disorders. While these findings highlight the importance of spliceosome-related genes in neurological disease, the association between RNU4-2 variants and mitochondrial dysfunction remains hypothetical and requires further investigation [14, 15]. The decreased OCR observed in both patients may suggest a potential link between *RNU4-2* variant and mitochondrial dysfunction. In support of this, we observed aberrant splicing within exon 1 of *NDUFV1*, a gene critical for mitochondrial complex I function, in one patient (Supplementary Data S5). However, this splicing abnormality was present only in a minority of transcripts, suggesting a limited impact, and the precise mechanism by which RNU4-2 variants affect mitochondrial function remains unclear. Conventional RNA-seq, which only detects mature mRNAs, is limited in its ability to observe splicing abnormalities directly. Future studies using techniques like nascent RNA-seq, capable of detecting immature mRNAs, could provide more accurate insights into how *RNU4-2* variant affect mitochondria-related gene expression. Our findings have implications for the diagnostic approach to mitochondrial diseases. Considering variant in non-coding regions like *RNU4-2*, which are undetectable by conventional WES, may help explain previously undiagnosed cases. This study has several limitations, including the small number of cases identified. In addition, the evidence for mitochondrial dysfunction in our patients is limited: the observed decrease in OCR was mild rather than marked. There is also a potential risk of selection bias, as our cases were identified from a cohort of patients suspected of mitochondrial disease. Further research is needed to determine the frequency of *RNU4-2* variant in mitochondrial diseases and to elucidate the full spectrum of associated phenotypes. Despite these limitations, our study raises the hypothesis that spliceosome-related gene variants, such as those in *RNU4-2*, may be associated with mitochondrial dysfunction. However, further studies are required to validate this possible link and to determine its clinical significance. Our findings underscore the need for continued research into the relationship between spliceosome-related genes, mitochondrial disease, and neurodevelopmental disorders.

Although this study reveals a potential link between *RNU4-2* variants and mitochondrial dysfunction, the unique clinical diversity of mitochondrial disease and the plethora of potential causative genes precludes defining specific phenotypic differences associated with *RNU4-2* mutations at this time. Because of the large number of causative genes in mitochondrial diseases, there is a wide range of inheritance forms, and clarification of the causative genes is required to obtain accurate information. Another factor that makes the diagnosis of mitochondrial disease difficult is the diversity of causative genes.

DATA AVAILABILITY

The data sets generated and/or analyzed in the current study are available from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS

KN and YK wrote the manuscript. AI, TO, MN, AO, KM and YO provided the clinical information KN, YK, YY, AS and NM analyzed the data. All authors discussed the results and commented on the manuscript.

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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/s10038-025-01356-8.

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