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Association of TRMT2B Gene Variants with Juvenile Amyotrophic Lateral Sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, which is characterized by progressive degeneration of motor neurons and demonstrates high clinical heterogeneity and complex genetic architecture. We identified a variation within *TRMT2B* (c.1356G > T; p.K452N) to be associated with ALS in a family comprising two patients with juvenile ALS. Then, two missense variations and one splicing variation were identified in 10 ALS patients in our cohort with 910 ALS patients, and three more variations were identified in a publicly ALS database (ALSdb) including 2800 ALS patients. Functionally, we detected a decrease of mitochondrial complex I activities in patients originated Epstein-Barr virus–transformed lymphoblastoid cell lines due to decreased number of mitochondria and lower expression of ND1 in mitochondria. Further, we detected increasing ROS but decreased p62 expression alteration within patients. In conclusion, we identified a novel ALS-associated gene, *TRMT2B*, and broaden the clinical and genetic spectrum of ALS.

Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease characterized by progressive degeneration of upper and lower motor neurons, leading to muscular weakness and atrophy [1]. The incidence of ALS varies among different populations, ranging from 0.8–1.2 to 2.1–3.8 per 100 000 person-years in China and Europe, respectively [2]. Accumulating evidence indicates that the genetic background, environmental exposure, and aging are risk factors implicated in the pathogenesis of ALS [3, 4]. Genetically, approximately 10% of ALS cases are familial, and over 40 genes have been directly linked to ALS through next-generation sequencing.[5]

ALS typically occurs in individuals aging between 42 and 65 years, invariably leading to death due to respiratory failure 3–4 years after the onset [1, 6]. Juvenile ALS (JALS), a rare form of ALS, is defined as age at onset less than 25 years [7]. Some patients of familial ALS (fALS) subtypes, such as ALS2, ALS4, ALS5, ALS6, and ALS16, are juvenile onset, and most of these cases inherit in an autosomal recessive pattern. Compared with adult-onset ALS, JALS is more frequently recognized to be have a genetic origin suggesting that studies on JALS may provide an opportunity to identify more novel causative genes[7]. Till now, several genes, including *SPTLC1*[8], *FUS*[9], *ALS2*[10], *SETX*[11], *SPG11*[12], *SIGMAR1*[13] *etc.* have been identified in JALS. These disease causative genes underlying JALS also play a role in adult-onset ALS[8]. The identification of these genes has been important in disclosing the molecular mechanisms underlying ALS; however, the etiology is not fully illustrated, and further genetic research may discover novel disease-associated genes and provide new clues to the disease pathogenesis.

Mitochondrial malfunctions play a pivotal role in the development of ALS[14–16]. Mitochondria produces energy for cellular biochemical reactions, which are essential for the survival of motor neurons, and is implicated in the process of generating reactive oxygen species (ROS). The changes occurring in the mitochondrial respiratory chain enzymes and cell death-related proteins and the following activation of programmed cell death pathways have been implicated in ALS pathophysiology[17]. Haploinsufficiency of *C9ORF72* causes impairment of respiratory complex I assembly in patients with ALS [18].

In this study, we identified a novel ALS-associated gene, *TRMT2B*, in a JALS family and discovered additional *TRMT2B* variants in large cohorts of patients with ALS. Functional analysis revealed that *TRMT2B* might participate in the pathogenesis of ALS by impairing the functional activity of mitochondria.

Methods

Patients and clinical analysis

A family (XY003) that included two members who had ALS was recruited in Hunan Province, China (Fig. 1A). The two patients and their unaffected sister and parents underwent full physical and clinical examinations, including electromyography, electrocardiography, brain MRI, cognitive function evaluation, and blood biochemistry tests.

In this study, 910 cases with ALS from China were enrolled for mutation screening of the candidate genes[19]. This ALS cohort included 753 ALS patients that we have reported previously [6]. Among these patients, pathogenic in *C9ORF72* and *ATXN2* were excluded. Patients' demographic characteristics are listed in Table 1. *TRMT2B* gene variants were also analyzed in an independent publicly available ALS cohort (ALSdb, alsdb.org/) comprising 2,800 sporadic ALS cases subjected to whole exome sequencing (WES). For controls, 104,068 exomes of non-neuro individuals in the gnomAD v2 database were analyzed.

Table 1
Demographic characteristics of ALS patients

	ALS cohort
No of ALS patients	910
Juvenile ALS	14
Family history(number)	68
Male (number, rate%)	597, 65.5%
Age at onset (years)	53.75 ± 11.28

Karyotype analysis and whole genome genotyping

Karyotype analysis was performed on peripheral blood lymphocytes using the G-banding method as described before earlier [20]. Genomic DNA was extracted from peripheral blood of all participants [21].Whole genome genotyping was performed using Illumina ASA 750K Chip. Genotype analysis was carried out

using Illumina GenomeStudio Genotyping Module (V2011.1); the CNV gap value was set as 100 M.

WES

Samples of the two patients and their unaffected parents were subjected to WES as described [6, 22]. As the family might be inherited in an X-linked or autosomal recessive pattern, thus, we used different filtering pipelines. Rare damage variants (RDVs) that fulfilled the following criteria were included for further analysis: (1) rarity: heterozygous variants with a minor allele frequency (MAF) less than 0.1% in the 1000 Genome Project and gnomAD; (2) nonsynonymous substitutions, indels, and putative splice site variants; (3) variant pathogenicity predicted by at least 2 of 11 *in silico* tools; (4) for the X-linked pattern, all consensus variations in the X chromosome were extracted from both patients in the family; for autosomal recessive inheritance pattern, all consensus homozygous or compound heterozygous variations were extracted from both patients in the family. The samples of the healthy sister and parents were included for co-segregation analysis.

Further screening of candidate variants was performed on the samples of the 910 patients with ALS in our cohort using WES [6].

Mutant mRNA expression and qPCR

Lymphoblastoid cells were isolated from blood samples and immortalized using Epstein-Barr virus [21]. Total RNA was extracted as described before[21]. PCR was performed with primers TRMT2B-F/R (Supplemental Table S1) using Premix (Takara). The amplified product was inserted into the T-vector (Takara) and sequenced. qPCR was performed with Actin and ND1 primers (Supplemental Table S1) using TB Green® Premix (Takara). qPCR was performed on QuantStudio (Invitrogen).

Plasmid construction and cell transfection

TRMT2B cDNA (NM_001167972.2) was amplified by PCR using primers TRM-F/R (Supplemental Table S1). Premix (Takara) was used to amplified target sequenced and inserted into the PCDNA3.1(+) plasmid using T4 ligase (Thermo Scientific). Mutated *TRMT2B* cDNA was amplified using specific primers (Supplemental Table S1) and cloned as described above. HEK293 cells were transfected according to the manufacturer's instructions.

Immunoblotting and immunofluorescence

For immunoblotting, lymphoblastoid cells from the patients and normal participants were lysed in RIPA buffer. Protein Electrophoresis were conducted as described before[21].The expression of TRMT2B and p62 was analyzed using antibodies; anti-β-actin antibodies were used for loading control.

For immunofluorescence, HEK293 cells, culturing in DMEM 10% FBS (Invitrogen), were plated on collagen-coated cover slips, fixed with 4% ice-cold paraformaldehyde. The procedures of immunofluorescence were identical as described before[23]. Images were analyzed by confocal microscopy (ZEISS). The antibodies used in this study are listed in Supplemental Table S2.

Activities of mitochondrial complexes I and III

In each sample, 5×10^6 lymphoblastoid cells were used for mitochondria extraction. The activity of mitochondrial complex I was assessed by the NADH oxidation rate measured based on absorbance at 340 nm. The activity of mitochondrial complex III was assessed by the rate of cytochrome C increase based on absorbance at 550 nm. The assays were performed using the Mitochondrial complex I and III activity kits, respectively (Solarbio).

Flow cytometry detection of ROS

Cells were incubated with dihydroethidium (DHE; Applygen) diluted in PBS (1:1000) for 30 min at 37 °C, washed in PBS three times to remove the residual probe, and analyzed by flow cytometry using FACSCanto II (BD). The data were evaluated using the FlowJo software.

Transmission electron microscope

Lymphoblastoid cells were obtained and incubated in ice 2.5% glutaraldehyde solution for 1 hours and then washed with ice PB Buffer for 5 times. 2% osmic acid were added into each sample and then washed with ice ddH2O for 5 times. Samples were replaced with ethanol and embed with SPI-Pon 812. Leica EM UC7 ultramicrotome were used to obtain 70nm slice and classical staining with 2% uranyl acetate and lead citrate were performed. All the Transmission electron microscope (TEM) images were captured using HITACHI 7700.

Statistical analysis

Statistical analysis was performed using SPSS 25.0. Association analysis of RDVs across the *TRMT2B* gene was performed in the ALS cohort using Fisher's exact test for each allele. Unpaired *t*-test was conducted using Prism. P-value of 0.05 indicated statistical significance.

Results

Clinical characteristics of patients with JALS

The proband of the XY003 family (Fig. 1A) was a patient in his 30s that initially developed weakness of the lower limbs at the age of childhood. Before the age of ten years, weakness of the upper limbs occurred. He also showed delayed cognitive and motor developmental milestones, and his school performance was worse than the averages. At the age of 20s, he suffered from decrease in the visual acuity of the left eye. He complained of progressive exacerbation of limb weakness and but not of dysphagia or dysarthria. The detailed medical record was not available. On admission, the patient had a small stature, which was below the genetic expectation, and deformity of hands and feet (Supplemental Figure S1A–C) and showed low activity endurance and ability to exercise.

A neurological examination revealed obvious muscle weakness and atrophy of the four extremities, facial muscle bundle tremor, and normal eye movement but incomplete eye closure. The proband also showed slight increase of muscle tension in the lower limbs and stiffness of ankle joints, which induced ankle clonus. He showed cognitive decline (executive dysfunction and memory impairment). The proband's younger brother (XY003.P2) showed the same lower limb weakness at the age of childhood and similar symptoms at the age of 20s (Fig. 1D and Supplemental Figure S1D–F). The detailed clinical information was described in Table 2. Their sister and parents had no neurological symptoms or signs.

Table 2
Clinical features of JALS patients carrying TRMT2B variants

Clinical feature	Patient 1	Patient 2
ID in family	XY003	XY003
Amino acid change	K452N	K452N
Sex	Male	Male
Age at onset(years)	< 5	< 5
Age at evaluation(years)	30s	20s
BMI	22.89	16.65
Hand deformities	Flexion contracture deformity	Flexion contracture deformity
Foot deformities	Pes cavus; Hallux valgus; Ankle stiffness	Pes cavus; Hallux valgus
Walking	Abnormal	Normal
Atrophy	four extremities	four extremities
Weakness	Generalized	Generalized
Reflexes	Normal	Normal
UMN signs	Ankle clonus; hypertonia	hypertonia
Tongue	Fasciculations	NA
Jaw jerk	Present	Present
Dysarthria	Normal	Normal
Dysphagia	Normal	Normal
Respiratory	Normal	Normal
Cognition		
MMSE score	27/30	NA
ECAS score	68/	NA
Sensory	Normal	Normal
Additional features	Visual damage	Visual damage, scapular winging
Neurophysiology		
Motor	Reduced CMAP, chronic, neurogenic reinnervation, ongoing denervation	Reduced CMAP, chronic neurogenic reinnervation, ongoing denervation
Sensory	Normal	Normal

Nerve conduction studies revealed obvious reduction in compound action potential (CAMP) in the lower and upper limb nerves of the proband, but nerve conduction velocity (NCV) was normal, whereas sensory NCV studies did not detect abnormalities. The electromyogram of the proband showed chronic neurogenic reinnervation enlarged long-duration motor unit potentials (MUPs) in the cervical and lumbar spinal cord regions, and ongoing denervation (moderate spontaneous potentials in lower limb muscles). Left visual evoked potential (VEP) indicated obvious reduction in the amplitude of the P100 component. The proband's younger brother also showed chronic neurogenic reinnervation in the cervical and lumbar regions and ongoing denervation in the thoracic region (moderate spontaneous potentials); he also had the same VEP reduction as the proband. The proband and especially his younger brother had increased rest levels of creatine kinase (349 and 1,099 U/L, respectively, *vs.* normal level of 50–310 U/L). In both patients, blood lactate was normal before exercise and slightly increased (less than twice) after exercise. Blood levels of ammonia, glucose, and lipids and thyroid function were all normal. No obvious abnormalities were revealed by brain MRI (Supplemental Figure S2), ECG, and echocardiography.

Identification of TRMT2B **variants associated with JALS**

We analyzed all coding and splice variants that were identical in the X chromosomes of the two affected patients. After functional filtering, we identified a hemizygous variation c.1356G > T (p.K452N, NM_001167972.2) in the *TRMT2B* gene. Sanger sequencing revealed segregation of the c.1356G > T mutation

with the phenotypes in the XY003 family (Fig. 1B). The mother of the proband was heterozygous for this variant (Fig. 1B). The c.1356G > T mutation in *TRMT2B* has not been reported in 1000 Genomes Project or gnomAD databases and is highly conserved among species (Fig. 1C).

As *TRMT2B* is located on the X chromosome and the unaffected mother of the proband was a heterozygous carrier, we conducted TA cloning to determine the presence of the mutated *TRMT2B* mRNA in lymphoblastoid cells of the mother. The results revealed only 25% (4/16) of mutant mRNA in lymphoblastoid cells from the proband's mother, which was less than the expected rate of 50%.

The karyotypes of the patients and their mother were normal (Supplemental Figure S3). CNV analysis on two patients also showed no consensus abnormities (Supplemental Figure S4). We also performed analysis based on an autosomal recessive hereditary pattern; however, only two genes fulfilling the analysis criteria were found, and none of them were associated with potentially neurodegenerative processes (Supplemental Table S3).

Screening TRMT2B **variants in patients with ALS**

To explore the role of variation in the *TRMT2B* gene in ALS, we evaluated the occurrence of *TRMT2B* variants in a cohort of 910 patients with ALS. In 10 of them, we identified two missense variants (c.250C > G, L84V and c. 1344T > G, F448L) and one splice variant (c.539-3T > C) of the *TRMT2B* gene, which were rare or absent in gnomAD. Both missense variants were predicted to be damaging (Table 3). Notably, the L84V variant was identified in seven unrelated patients with sporadic ALS, who had typical clinical features of adult-onset ALS without vision impairment; the mean age at onset was 57.22 ± 7.96 years (range 42–66 years). Of note, three of the six patients who underwent cognitive tests had cognitive impairment (50%).

Table 3
Variations identified within ALS patients

Sample ID	Sex	Age of onset	Gene	Chr	Position	Location	cDNA change	AA alteration	Mutation type	rsID	MAF in gnomAD
XY003.P1 [a]	M	1	*TRMT2B*	X	100273992	Exon12	c.1356G > T	p.K452N	missense	/	/
XY003.P2 [a]	M	1	*TRMT2B*	X	100273992	Exon12	c.1356G > T	p.K452N	missense	/	/
M6783	M	66	*TRMT2B*	X	100296359	Exon3	c.250C > G	p.L84V	missense	rs201296426	0.0006019(119/197698)
M7966	F	63	*TRMT2B*	X	100296359	Exon3	c.250C > G	p.L84V	missense	rs201296426	0.0006019(119/197698)
S001433	F	65	*TRMT2B*	X	100296359	Exon3	c.250C > G	p.L84V	missense	rs201296426	0.0006019(119/197698)
S003418	F	42	*TRMT2B*	X	100296359	Exon3	c.250C > G	p.L84V	missense	rs201296426	0.0006019(119/197698)
S003943	M	50	*TRMT2B*	X	100296359	Exon3	c.250C > G	p.L84V	missense	rs201296426	0.0006019(119/197698)
S004576	M	56	*TRMT2B*	X	100296359	Exon3	c.250C > G	p.L84V	missense	rs201296426	0.0006019(119/197698)
S004913	M	50	*TRMT2B*	X	100296359	Exon3	c.250C > G	p.L84V	missense	rs201296426	0.0006019(119/197698)
M36116	F	58	*TRMT2B*	X	100290675	Exon7	c.539-3T > C	/	splicing	/	/
S003640	M	65	*TRMT2B*	X	100274004	Exon12	c.1344T > G	p.F448L	missense	rs374183741	0.00007793(16/205319)
/ [c]	F	/	*TRMT2B*	X	100276156	Exon9	c.1000C > T	p.R334W	missense	rs145089500	0.00001637(3/183278)
/ [c]	/	/	*TRMT2B*	X	100276212	Exon9	c.944G > T	p.R315L	missense	rs145912589	0.0001268(26/205046)
/ [c]	/	/	*TRMT2B*	X	100292017	Exon5	c.484C > G	p.R162G	missense	rs141694732	0.00002923(6/205301)

[a] patients identified in a juvenile ALS family

[b] The silico tools for predicting variants were (1) PolyPhen2 HDIV (polymorphism phenotyping version 2 human diversity), (2) PolyPhen2 HVAR (polymorphis human variation), (3) SIFT (sorting intolerant from tolerant), (4) PROVEAN (Protein Variation Effect Analyzer), (5) GERP++(genomic evolutionaryrate profiling) annotation dependent depletion), (7) LRT (likelihood ratio test), (8) FATHMM (functional analysis through hidden Markov models), (9) M-CAP (Mendelian clin pathogenicity), (10) MutationTaster, and (11) MutationAssessor.

[c] data extracted from ALSdb

Among 104,068 non-neuro individuals in the gnomAD v2 database (47,831 females and 56,237 males), a total of 494 rare putative pathogenic *TRMT2B* variants from WES data were identified in *TRMT2B* that fulfilled the same RDVs analysis criteria. The results of gene burden testing revealed *TRMT2B* variations as a risk factor for the disease ($p = 0.003$ by Fisher's exact test).

In an independent ALS cohort (ALSdb), we identified three additional rare deleterious *TRMT2B* variants, R334W (c.1000C > T), R315L (c.944G > T), and R162G (c.484C > G) (Table 3); however, no clinical data were available online.

TRMT2B **Variations Impair Mitochondrial Function**

As *TRMT2B* encodes a methyltransferase localized to mitochondria, we tested the activity of mitochondrial complexes I and III in lymphoblastoid cells from the two patients, their heterozygous mother, healthy sister of the XY003 family and one unrelated man. The activities of complex I in the patients were lower than those in the other tested individuals ($p = 0.0251$ by unpaired *t*-test), including in the carrier mother ($p = 0.0356$ by unpaired *t*-test). Although there was no significant difference between the three controls (carrier mother, healthy sister, and the unrelated man), however, the average value of carrier mother is lower than the other controls. No differences were observed in the activities of complex III (Fig. 2A and Supplemental S5).

Further, we detected the expression of ND1 which was the core subunit of the mitochondrial complex I. Comparing with controls, we discovered that lower expression of ND1 in patients in both mRNA level (Fig. 2B, p = 0.0331 by unpaired *t*-test) and protein level (Fig. 2C, p = 0.0465 by unpaired *t*-test and Supplemental Figure S7A). TEM results also showed decreasing numbers of mitochondria (Fig. 3A and B, $p < 0.0001$ by unpaired *t*-test) and increasing size of mitochondria within patients' lymphoblastoid cells (Fig. 3C and D, $p < 0.0001$ by unpaired t-test). Analysis of ROS production indicated that it was increased in lymphoblastoid cells of the patients (Fig. 4A and Supplemental Figure S6; $p < 0.0001$ by unpaired *t*-test). Evidences above suggested that *TRMT2B* vairations might impair mitochondrial function.

We also observed a decrease in the expression of p62 (Fig. 4B and Supplemental FigureS7B), an autophagic cargo adapter, in the patients, whereas that of TRMT2B remained unchanged (Fig. 4B). Immunocytochemistry analysis of HEK293 cells indicated that TRMT2B was co-localized with the mitochondrial marker TOM20 and that the mutated TRMT2B proteins were also located within mitochondria (Supplemental FigureS8).

Discussion

In this study, we identified an ALS-associated gene, *TRMT2B*, by analyzing a JALS family patients and sporadic ALS. The proband of the family showed progressive muscular weakness and atrophy, cognitive impairment, and deformity of the four extremities and impaired vision. Neurological examination revealed signs of both upper and lower motor neuron lesions, and neurological electrophysiology analysis showed extensive chronic and ongoing neurogenic damage, indicating damage of anterior horn cells in the spinal cord. After excluding other neurological diseases, the patient was diagnosed with familial ALS and the ALS-Plus syndrome according to the revised El Escorial criteria 2000 [19, 24]. The ALS-Plus has been introduced to characterize patients with ALS, who have non-motor symptoms such as oculomotor disorder, sensory disturbances, and cerebellar and extrapyramidal disorders [24, 25]. As the proband had visual damage and deformity of the four extremities, he was also considered to have ALS-Plus. Thus, our results extended the phenotype of the ALS-Plus syndrome.

As differential diagnosis, spinal muscular atrophy (SMA) plus and mitochondrial encephalomyopathy were excluded. First, UMN signs did not support the diagnosis of SMA plus[26]. Second, the diagnosis of mitochondrial encephalomyopathy was not established, according to un-elevated level of lactate, electrophysiological chronic and ongoing neurogenic changes rather than myogenic changes, and normal brain MRI. Therefore, we thought that JALS plus was the most appropriate diagnosis.

TRMT2B, which is located on the X chromosome, encodes tRNA methyltransferase 2 homolog B. Although the functional activity of TRMT2B is still not fully understood, it is considered responsible for m5U methylation in mitochondrial tRNA and rRNA[27], which is pivotal to tRNA stability and maturation [28], and it has been shown that *TRMT2B* inactivation reduces the activity of respiratory chain complexes I, III and IV[29]. The dysregulation of tRNA post-transcriptional modifications has been proved to be associated with neurodegenerative diseases [28]. *TRMT61B*, which belongs to the same gene family as *TRMT2B*, is differentially expressed in patients with Alzheimer's disease [30]. Other *TRMT* gene family members, *TRMT1* and *TRMT10A*, also play a role in the pathogenesis of neurological disorders such as Alzheimer´s disease and intellectual disability [31, 32]. Interestingly, previous study indicates that mice with the mutated *Trmt2b* gene have abnormal physical strength [33]. In this study, we disclosed the association between *TRMT2B* and ALS by identifying multiple variations within ALS patients (Fig. 5).

Both patients with JALS had lower mitochondrial complex I activity and ND1 expression, indicating that *TRMT2B* variants might cause ALS through dysregulation of mitochondrial genes expression. The altered size and numbers of mitochondria were also observed in patients, which indicated that biogenesis of mitochondria might also be affected. In this study, we also observed increased ROS levels in the patients with JALS. Dysfunction of the electron transport chain disturbs oxygen metabolism in mitochondria and deregulates ROS generation, creating redox imbalance, which results in cell and tissue damage due to protein carbonylatation, tyrosine nitration, decreased ATP synthesis, and impaired DNA repair [34]. A growing body of research data strongly suggests that imbalanced ROS production is a major factor in the onset and progression of several neurodegenerative disorders such as ALS, Alzheimer's disease, and Parkinson's disease[35]. ALS-associated genes, including *TARDBP*, *C9ORF72*, and *SOD1* have been reported to play a role in the etiology of ALS by causing mitochondrial dysfunction and inducing ROS accumulation [36, 37].

We also observed that increased ROS production corresponded with the downregulation of p62 expression in patients with ALS. According to previous studies, p62 encoded by *SQSTM1* is associated with ALS onset through ROS generation and selective autophagy, and *SQSTM1* variants have been identified in

patients with ALS [38, 39]. Thus, we hypothesize that *TRMT2B* might contributes to the onset of ALS by impairing mitochondrial function by altering ND1 expression, inducing ROS production, and downregulating p62 expression.

TA cloning and sequencing results revealed that only 25% of *TRMT2B* mRNA had the c.1356G > T substitution in the unaffected carrier mother, suggesting that a large portion of TRMT2B protein could be unaffected. The activity of mitochondrial complex I in the carrier mother remained at a normal level and was higher than that in the patients, however, the average value of carrier mother was lower than other two normal controls although without statistical significance (Supplemental Figure S4), and ROS production (Supplemental Figure S5) and p62 expression (Supplemental Figure S6) of carrier mother were unchanged. Together, these results indicate that the accumulative effect caused by the lower activity of mitochondrial complex I in the carrier mother did not reach the threshold required for ALS onset. We hypothesize that the alterations in the functional activity of TRMT2B caused by variations that we identified in patients with adult-onset ALS, especially in women, might contribute to the pathogenesis of ALS through time-dependent accumulation.

Because of technical limitations, we were unable to test mitochondrial tRNA methylation through isotope labeling of tRNA or methylation of rRNA; alternative methods will be used in our future study. We also could not examine the activity of mitochondrial complexes and ROS production in patients with sporadic ALS carrying mutated *TRMT2B* because of a lack of lymphoblastoid cell lines. In further studies, we will focus on the functions of mutated TRMT2B and verification of accumulated effects.

In conclusion, we identified a novel JALS-associated gene, *TRMT2B*, which might contribute to the pathogenesis of ALS through impairment of mitochondrial function.

Declarations

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Competing Interests The authors report no competing interests.

Author Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Yanling Liu and Xi He. The first draft of the manuscript was written by Junling Wang and Zhengmao Hu, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability Datasets analyzed in this study are not publicly available. Further information about the datasets is available from the senior author (J.W. and Z.H.) on reasonable request.

Ethics approval This study was approved by the Ethics Committee of Xiangya Hospital, Central South University. The approve number is 202103191.

Consent to participate All participants provided written informed consent to the study.

Consent to publish The authors affirm that human research participants provided informed consent for publication of the images in Figure 1D and Figure S1.

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Figures

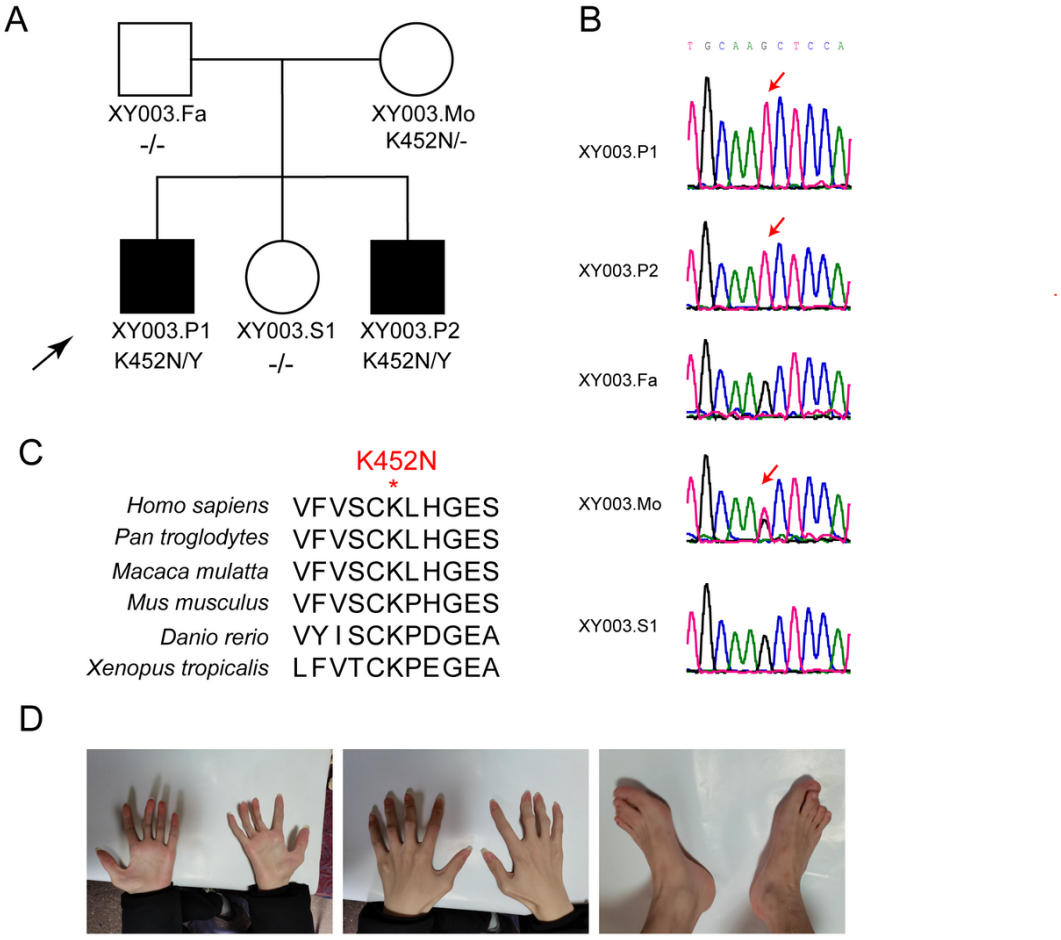

Figure 1

***TRMT2B* was identified to be associated in a JALS family. (A)** a JALS family recruited from Hunan, China, the black arrow indicated the proband. **(B)** *TRMT2B* c.1356G>T;p.K452N variation were co-segregated in this family. **(C)** K452 on TRMT2B protein were highly conserved among species; **(D)** Patients showed deformity of hands and feet.

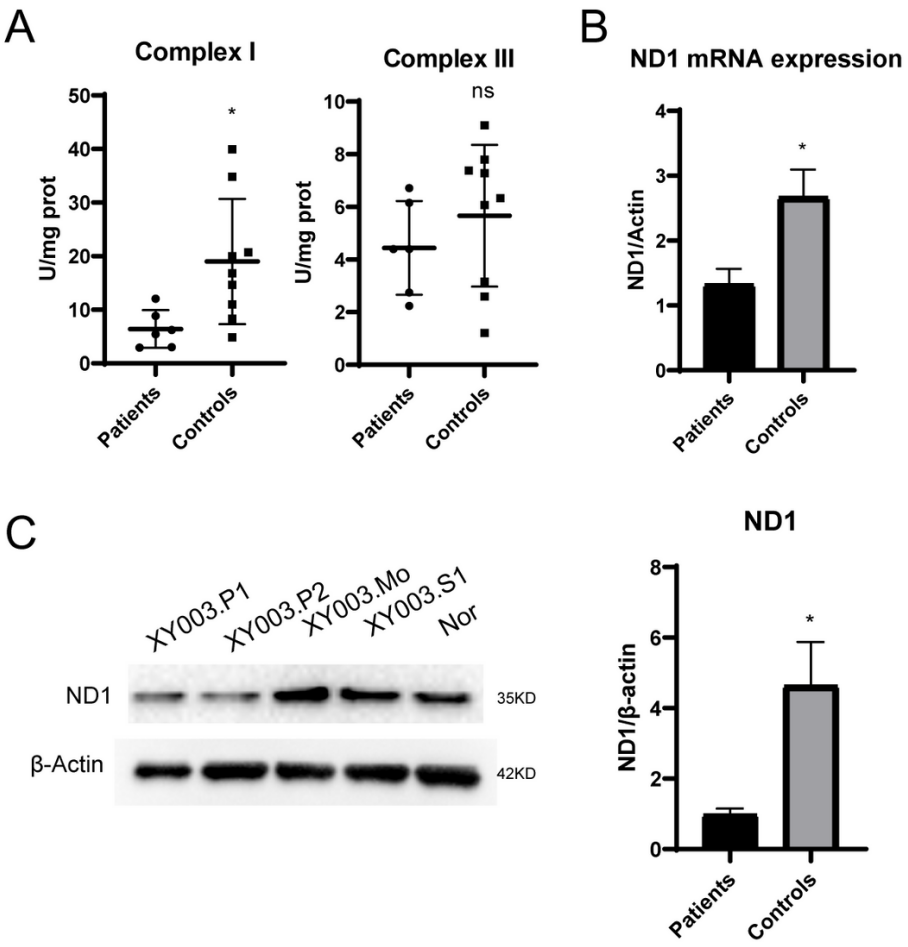

Figure 2

Altering mitochondrial function in patients' lymphoblastoid cells. (A) Mitochondrial complex I activities were decreased in patients, however, Mitochondrial complex III activities remained unchanged. **(B)** Lower mRNA expression of ND1 in patients originated lymphoblastoid cells. **(C)** Lower protein expression of ND1 in patients originated lymphoblastoid cells (left) and gray density analysis of WB lanes (right).

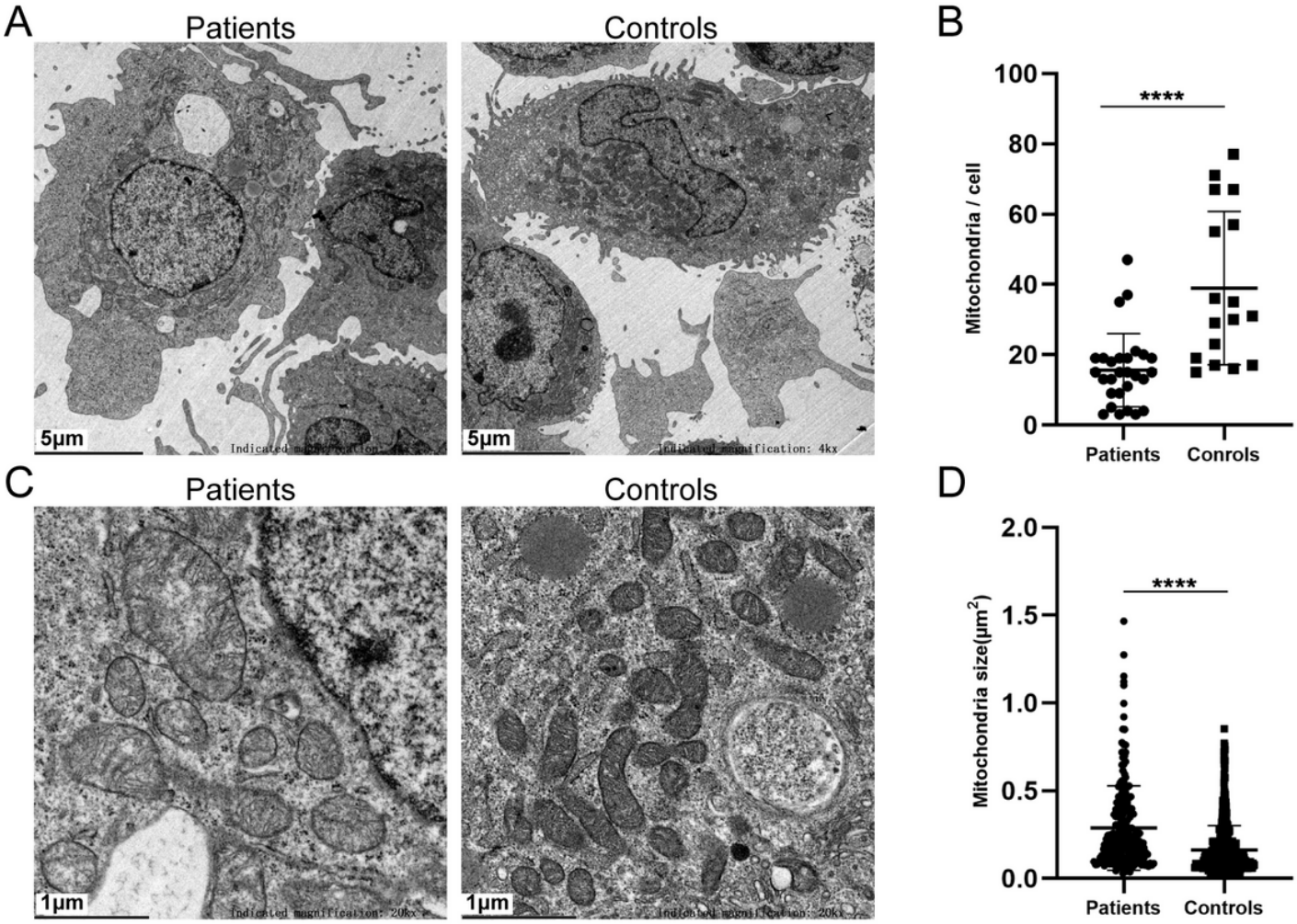

Figure 3

Reduced number and increased size of mitochondria in patients' lymphoblastoid cells. (A) TEM images showed reduced numbers of mitochondria in patients. **(B)** TEM images of lymphoblastoid cells from XY003.P1 (N=13), XY003.P2 (N=15), XY003.S1 (N=7) and unrelated control (N=10) were included in analysis. The numbers of mitochondria were reduced in patients ($p < 0.0001$ by unpaired *t*-test). **(C)** TEM images showed increasing size of mitochondria in patients. **(D)** TEM images of mitochondria from XY003.P1 (N=120), XY003.P2 (N=100), XY003.S1 (N=185) and unrelated control (N=274) were included in analysis. The size of mitochondria were increased in patients ($p < 0.0001$ by unpaired *t*-test).

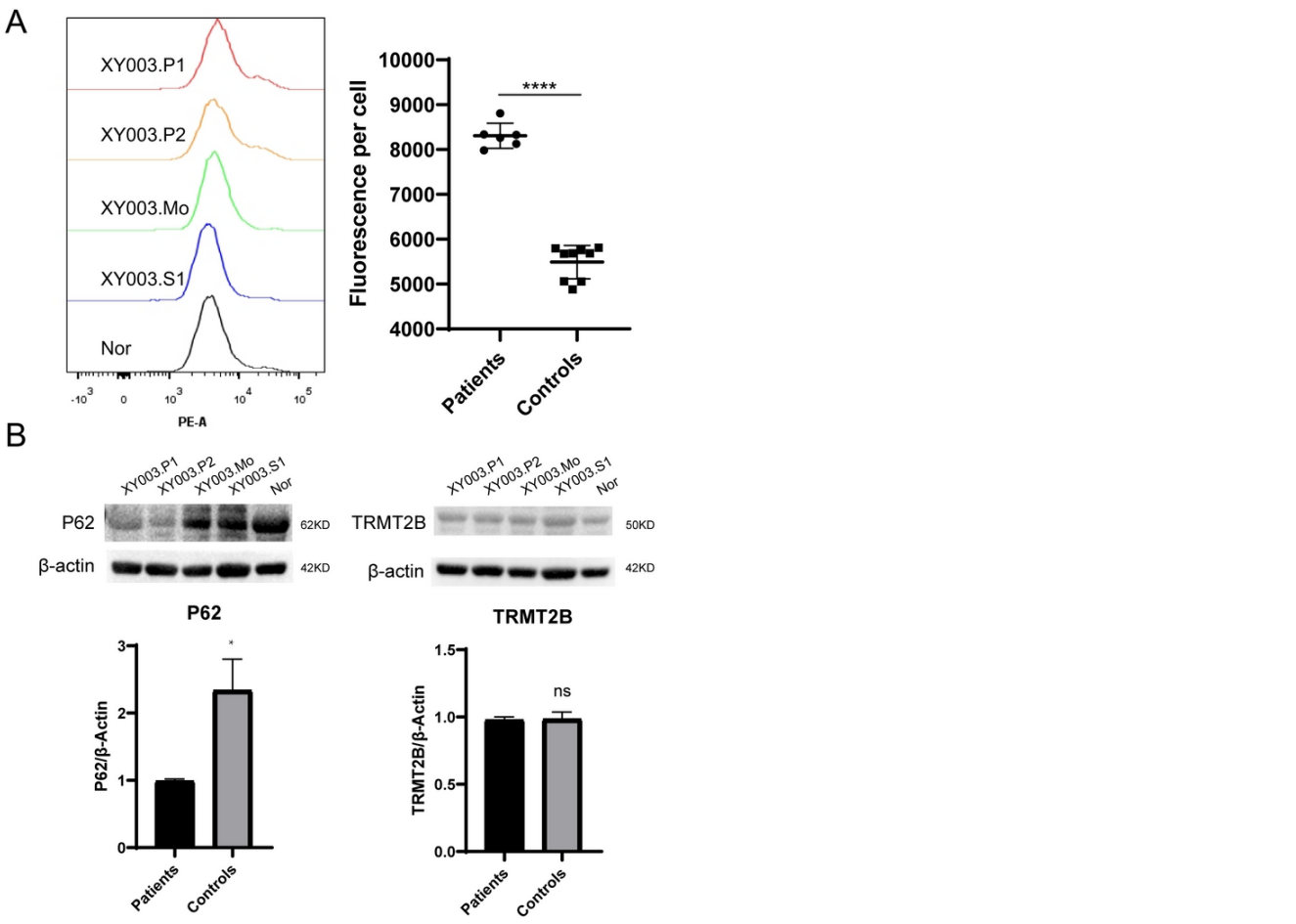

Figure 4

Increasing ROS levels were observed in patients' lymphoblastoid cells. (A)

ROS levels were increased in patients. **(B)** The expression of P62 decreased in patients (left), however, TRMT2B expression remained unchanged (right).

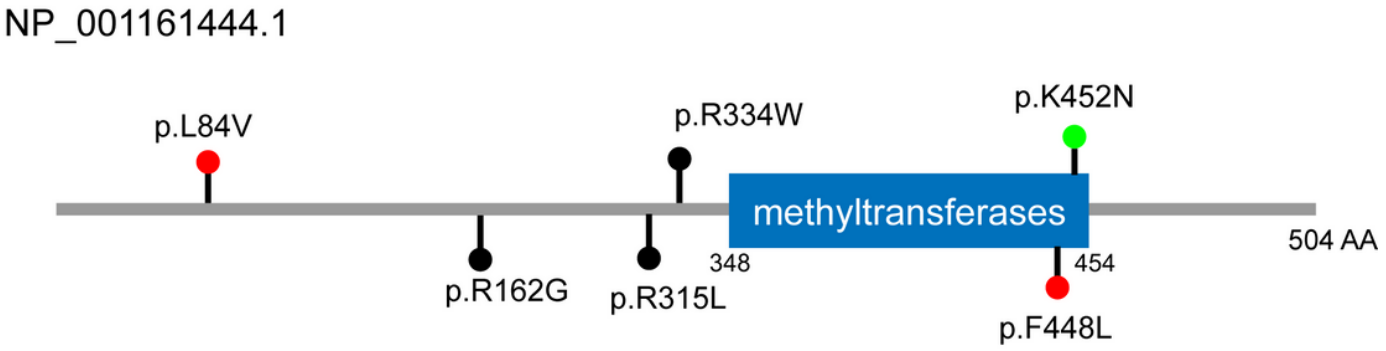

Figure 5

***TRMT2B* variations identified in ALS patients.** Green bottom displayed the variation we identified in JALS family, red bottom displayed variations identified within 910 sporadic cases, black bottom displayed variations identified in ALSdb; the methyltransferase domain of TRMT2B ranging from 348V to 454R amino acid in the full-length protein.

Supplementary Files

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