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CASE REPORT

Identification of a Novel Homozygous Mutation in *MTMR2* Gene Causes Very Rare Charcot–Marie–Tooth Disease Type 4B1

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Background: Charcot–Marie–Tooth disease (CMT) is a heterogeneous group of disorders involving peripheral nervous system. Charcot–Marie–Tooth disease 4B1 (CMT4B1) is a rare subtype of CMT. CMT4B1 is an axonal demyelinating polyneuropathy with an autosomal recessive mode of inheritance. Patients with CMT4B1 usually manifested with dysfunction of the motor and sensory systems which leads to gradual and progressive muscular weakness and atrophy, starting from the peroneal muscles and finally affecting the distal muscles. Germline mutations in *MTMR2* gene causes CMT4B1.

Material and Methods: In this study, we investigated a 4-year-old Chinese boy with gradual and progressive weakness and atrophy of both proximal and distal muscles. The proband's parents did not show any abnormalities. Whole-exome sequencing and Sanger sequencing were performed.

Results: Whole-exome sequencing identified a novel homozygous nonsense mutation (c.118A>T; p.Lys40*) in exon 2 of *MTMR2* gene in the proband. This novel mutation leads to the formation of a truncated MTMR2 protein of 39 amino acids instead of the wild-type MTMR2 protein of 643 amino acids. This mutation is predicted to cause the complete loss of the PH-GRAM domain, phosphatase domain, coiled-coil domain, and PDZ-binding motif of the MTMR2 protein. Sanger sequencing revealed that the proband's parents carried the mutation in a heterozygous state. This mutation was absent in 100 healthy control individuals.

Conclusion: This study reports the first mutation in *MTMR2* associated with CMT4B1 in a Chinese population. Our study also showed the importance of whole-exome sequencing in identifying candidate genes and disease-causing variants in patients with CMT4B1.

Keywords: Charcot–Marie–Tooth disease, *MTMR2* gene, novel mutation, homozygous, whole-exome sequencing

Background

The Charcot–Marie–Tooth (CMT) disease is an inherited and major form of peripheral neuropathy. CMT is a heterogeneous group of disorders with axonal and demyelinating neuropathies which affect the motor and sensory systems.[1] CMT is genotypically and phenotypically extremely heterogeneous, with a worldwide prevalence of 1:2500–3300.[2] In addition, CMT patients were usually characterized by the progressive weakness and atrophy of distal muscles, majorly involving the legs.[2,3] CMT usually follow either an autosomal dominant or an autosomal recessive or X-linked recessive modes of inheritance.[4] CMT4B is a severe, juvenile-onset, autosomal recessive neuropathy with demyelination and myelin out-folding. CMT4B1 is classified into three subgroups; namely, CMT4B1 (OMIM# 601382), CMT4B2 (OMIM# 604563) and CMT4B3 (OMIM# 615284).[4] Mutations in myotubularin-related protein 2 (*MTMR2), MTMR13* and *MTMR5* causes CMT4B1, and CMT4B2 and CMT4B3, respectively.[5–11] CMT4B1 is extremely rare and till date,

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only 42 CMT4B1 patients has been reported, mostly from countries with a high frequency of consanguineous marriages.[4,12] The incidence rate of CMT4B1 is <1/1,000,000 world-wide (https://www.orpha.net/consor/cgi-bin/OC_Exp.php?Expert=99955andlng=EN).

Germline mutations in *MTMR2* cause CMT4B1. *MTMR2* gene is located in the long arm of chromosome 11.[13] *MTMR2* gene is comprised of 15 exons and encodes Myotubularin-related protein 2 (MTMR2) protein.[6,13,14] MTMR2 is a member of a large myotubularin family, majorly involved in the modulation of cellular membrane trafficking.[14] MTMR2 protein is a phosphatase protein with a phosphoinositol headgroup.[12] MTMR2 protein is an important member of the phosphoinositol (PI) 3-phosphatases family and involved in specific dephosphorylation of two lipids, namely, phosphatidylinositol 3-phosphate and phosphatidylinositol 3,5-bisphosphate which regulate the membrane trafficking.[6]

MTMR2 is highly expressed in both the motor and sensory neurons. *MTMR2* plays a significant role in regulating membrane homeostasis in Schwann cell myelination.[5,15] *MTMR2* associated CMT4B1 is a rarely reported form of muscular dystrophy, and the genotype-phenotype correlation has also been recently investigated.[4,12,16] To date, no therapeutic treatment has been developed for patients with CMT4B1.[17–20]

Here, we report the case of a 4-year-old Chinese boy with gradual and progressive weakness and atrophy of both the proximal and distal muscles in both legs. Whole-exome sequencing identified a novel homozygous nonsense mutation (c.118A>T; p.Lys40*) in *MTMR2* that resulted in the formation of a truncated MTMR2 protein. The present study reports the first mutation in the *MTMR2* gene associated with CMT4B1 in a Chinese population.

Materials and Methods

Ethics Statement

The ethics committee of Xi'an People's Hospital (Xi'an Fourth Hospital), Xi'an, China approved the present study in accordance with the recommendations of the Declaration of Helsinki.

Subjects

Herein, we report the case of a 4-year-old Chinese boy clinically diagnosed with CMT4B1 from a non-consanguineous Han Chinese family at Xi'an People's Hospital (Xi'an Fourth Hospital), Xi'an, China (Figure 1). All samples were collected from the proband and proband's parents at the Xi'an People's Hospital (Xi'an Fourth Hospital), Xi'an, China. Written informed consent was obtained from the parents of the proband for their participation in this study and publication of this case report.

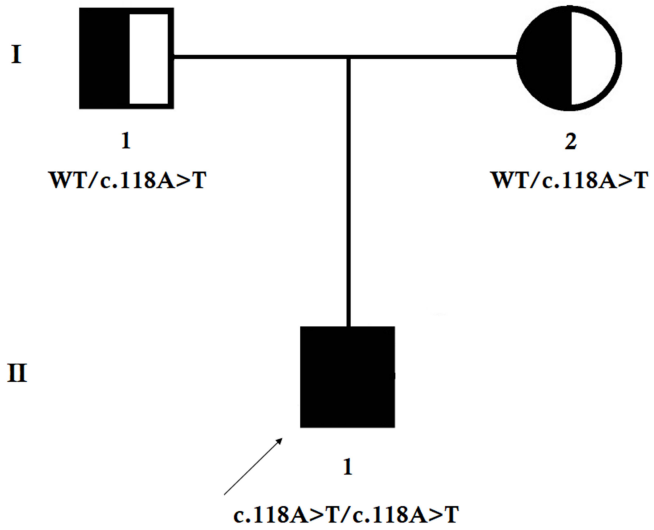

Figure 1 Pedigree of the family. The filled symbol indicates the patient, and the half-filled symbols show the unaffected healthy parents. The arrow points to the proband.

Patient and Clinical Description

Here, we report the case of a 4-year-old Chinese boy with gradual and progressive weakness and atrophy of both the proximal and distal muscles in both legs (Figure 1). A clinical examination of the proband was performed for neuromuscular disorders. In addition, nerve conduction velocity (NCV) tests were performed on the proband to understand the demyelinating neuropathy. Physical tests; routine blood tests; and thyroid, liver, and kidney function tests were performed. Serum electrolyte levels and muscle creatine kinase levels were also evaluated. Electrophysiological studies have been performed to diagnose demyelinating sensorimotor polyneuropathy clinically. The proband had no family history of neuromuscular disorder. Hence, clinical diagnosis, genetic screening, and further counselling of this proband and his parents are recommended.

Karyotyping Analysis

In order to understand the structural abnormalities of chromosomes of this proband, we performed standard G-banding karyotyping.[21] The G-banding karyotype was performed with analysis of metaphase chromosome from temporary lymphocyte cultures of peripheral blood of the proband. First, we prepared slides with small droplets of cell suspension and dried them. Then, these slides were incubated in 2XSSC (0.3 M sodium chloride plus 0.03 M trisodium citrate) at 60 °C for 90 min. Subsequently, all slides were transferred to 0.9% NaCl solution at room temperature. Each slide was then rinsed in fresh NaCl solution and stained with trypsin-Giemsa solution for 4–6 minutes. The slides were then rinsed in running water and transferred to fresh buffer. Finally, the slides were rinsed with a fresh buffer and dried.

Chromosomal Microarray Analysis (CMA)

In order to identify copy number variations (CNV) in this proband, we performed chromosome microarray analysis (CMA).[21] CMA was performed with the Affymetrix CytoScan HD array to identify CNVs in this proband. CMA data analysis was performed using Chromosome Analysis Suite software (version 3.1). CMA data are presented as minimum coordinates in the NCBI37/hg19 genome assembly. Variants were evaluated according to their phenotypes using standard in silico tools. The CMA results were analyzed and interpreted using public genomic databases (UCSC, OMIM, DGV, DECIPHER, and CLINGEN). We set the copy number threshold to 10 kb and marker count to ≥50.

Whole-Exome Sequencing and Identification of Candidate Variant

Whole-exome sequencing of the proband was performed. Proband peripheral blood was collected, and genomic DNA was extracted according to the manufacturer's instructions. Proband genomic DNA was subjected to whole-exome sequencing.[21,22] First, exome sequences were captured using Agilent SureSelect version 6 (Agilent Technologies, Santa Clara, CA, USA) to prepare the sequencing library. Next, whole-exome sequencing was performed using this enriched sequencing library using the Illumina HighSeq 4000. Sequencing reads obtained after whole-exome sequencing were aligned with GRCh37.p10 using the Burrows–Wheeler Aligner software (http://bio-bwa.sourceforge.net) (version 0.59). Subsequently, the Burrows–Wheeler aligned reads were realigned locally using the GATK IndelRealigner (https://gatk.broadinstitute.org). Next, GATK Base Recalibrator (https://gatk.broadinstitute.org) was used for base quality recalibration of the Burrows–Wheeler aligned reads. GATK Unified Genotyper (https://gatk.broadinstitute.org) was used to identify single-nucleotide variants (SNV) and insertions or deletions (indels). The identified variants were annotated using the consensus coding sequence database (20,130,630) of the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/CCDS/CcdsBrowse.cgi). We then performed both image analysis and base calling using the Illumina pipeline. Indexed primers were designed for data fidelity surveillance. Next, clean sequencing reads were aligned with the human reference genome (hg19) (https://www.ncbi.nlm.nih.gov/datasets/genome) using SOAP aligner (soap2.21) (http://soap.genomics.org.cn) software. Finally, consensus sequences were assembled, and genotype calling was performed using SOAPsnp (v1.05) (http://soap.genomics.org.cn) software.

Bioinformatics Data Analysis and Interpretation

We collected variants obtained from whole-exome sequencing and selected variants with minor allele frequency <0.01 in public databases [dbSNP (https://www.ncbi.nlm.nih.gov), HapMap (https://www.genome.gov), 1000 Genome Database (http://www.internationalgenome.org) and our in-house database for 30,000 Chinese Han samples].[23–25] We used all these public databases to identify the minor allele frequencies (MAF) of genetic variations in different populations. Based on MAF values, we can understand the possible pathogenicity of the identified variants in a specific case. All the reported variants associated with monogenic disorders are listed in the Human Gene Mutation Database (HGMD, www.hgmd.cf.ac.uk/). Therefore, we can interpret whether the identified genetic variant is novel or previously reported to cause any disease by comparing with a database.[26] Next, we used the Online Mendelian Inheritance in Man (OMIM, https://www.omim.org) database to understand the genotype–phenotype correlation or information of our identified genetic variant. OMIM contains information of more than 16,000 genes associated with Mendelian disorders.[27] Then, we used the Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org) database to confirm the possible pathogenicity of identified genetic variants by comparing their frequencies in different populations. ExAC comprises exome sequencing data from large-scale sequencing projects from different populations.[28] After that, we used the Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org) to confirm the possible pathogenicity of the identified genetic variants by comparing their frequencies in different populations. The gnomAD contains both genome and exome sequencing data from large-scale genome or exome sequencing projects from different populations.[29] Hence, we interpreted the pathogenicity of the variant identified in our study.

Lastly, variants were interpreted according to the variant interpretation guidelines of the American College of Medical Genetics and Genomics (ACMG).[30] Here, we selected all the heterozygous, homozygous and compound heterozygous variants based on the gene function and disease association information provided by OMIM and published literature. In addition, "Mutalyzer 2" software was used to confirm the expression of the variant according to the HGVS rules.[31] The quality control (QC) data of the whole-exome sequencing are described in Table 1. Bioinformatic data analysis and interpretation were schematically presented in Figure 2.

Table 1 The Quality Control Data of Whole Exome Sequencing of the Proband

	Proband
Raw reads (mapped to hg19)	9,489,764
Raw data yield (Mb)	851.86
Reads mapped to target region	5,986,759
Reads mapped to flanked 100 bp region	6,168,797
Data mapped to target region (Mb)	489.88
Data mapped to flanked 100 bp region (Mb)	496.79
Length of target region	895,998
Length of flanked 100 bp region	977,687
Number of covered bases on target region	802,343
Coverage of target region	99.89%
Number of covered bases on flanked 100 bp region	988,789
Coverage of flanked 100 bp region	99.97%
Average sequencing depth of target region	590.58
Average sequencing depth of flanked 100 bp region	486.80

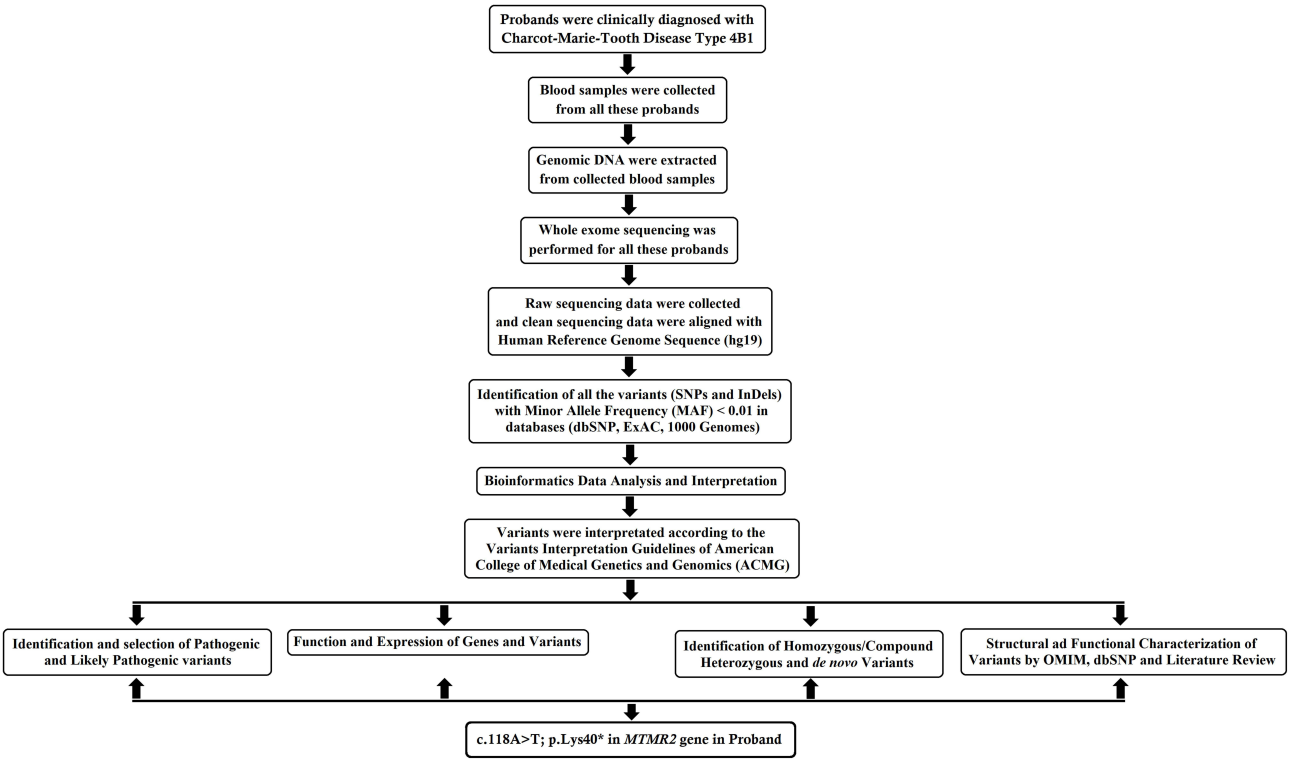

Figure 2 Bioinformatics Data Analysis and Interpretation of Whole Exome Sequencing.

Sanger Sequencing

The variant identified in the proband by whole-exome sequencing was confirmed by Sanger sequencing. Therefore, we performed Sanger sequencing of the proband and his parents to validate the variants identified by whole-exome sequencing. We then designed primers for candidate loci based on the reference genomic sequences of the human genome from GenBank. Next, we synthesized primers (Invitrogen, Shanghai, China) and performed a polymerase chain reaction (PCR) by using an ABI 9700 Thermal Cycler. Then, we directly sequenced the PCR products with an ABI PRISM 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA). Finally, we analyzed the sequencing data using the DNASTAR SeqMan (DNASTAR, Madison, Wisconsin, USA).

Sanger sequencing validated the identified heterozygous variants by whole-exome sequencing using the following primers: F1, 5'-AGTGCCTGGTGTAGTGGTTTT-3', R1, 5'- TACTCCATCAGTATCTCCTGCTAA-3'. The reference sequence NM_016156 from *MTMR2* was used in the present study.

In silico Analysis

Mutation Taster (http://mutationtaster.org/) was used to analyze the identified variant in the proband.[32]

Results

Clinical Report

In this study, we investigated a 4-year-old Chinese boy with gradual and progressive weakness and atrophy of both the proximal and distal muscles in both legs. The proband's parents were phenotypically normal (Figure 1).

A 4-year-old Chinese boy was enrolled in this study. He was the first and only child of the non-consanguineous Chinese parents. The patient was clinically diagnosed with a CMT4B1. He presented with gradual and progressive motor and sensory polyneuropathy with developmental delay since he was 2-years old. He had motor delay before the age of 2 years and was unable to walk. The proband was unable to walk without assistance. The proband also manifested mild scoliosis. The proband's voice was abnormal, probably due to vocal cord palsy. Physical examination revealed weakness

and atrophy of both proximal and distal limb muscles. No tendon reflexes were observed in the upper or lower extremities of the proband. Moreover, the patient was diagnosed with normal cognition, without facial weakness. The proband did not report pes cavus or any foot deformity. The proband did not present with any deafness or visual abnormalities. We observed a normal sensation level and delayed autonomic function in the proband. The detailed clinical characteristics of patients with CMT4B1 are shown in Table 2.

In addition, nerve conduction velocity (NCV) test of the proband showed severe demyelinating neuropathy (Table 3). No abnormalities were identified in routine blood tests or functional tests of the thyroid, liver, or kidneys. Serum electrolyte levels and muscle creatine kinase levels were normal. Severe demyelinating sensorimotor polyneuropathy was identified by electrophysiology. No abnormalities were identified in the NCV tests of the proband's parents.

The Charcot–Marie–Tooth neuropathy score (CMTNS) test was performed on the proband. The CMTNS is a reliable and conventional method to assess signs, symptoms, and neurophysiology for measuring the impairment of motor and sensory nerve systems in patients with genetic neuropathies.[33] Each assessment parameter was scored on a range of 0–4-point scales. According to CMTNS, CMT4B1 patients are classified into three categories: mild (CMTNS < 10), moderate (CMTNS = 11–20), or severe (CMTNS > 20).[34] The proband's CMTNS score was 9. Hence, our proband was classified as having mild CMT4B1. The results are presented in Table 4.

The proband did not present respiratory insufficiency or chest deformities. No dysarthria, dysphagia, or hypophonia were found in the proband.

Table 2 Clinical Characteristics of the Proband with CMT4B1

Findings	Proband
Gender	Male
Age	4 years
Age of onset	2 Years
Growth development	Delayed
Muscles strength (MRC grade)	Upper limbs: Proximal: 5/5; Distal: 4/5 Lower limbs: Proximal: 4/5; Distal: 3/5.
Deep tendon reflex (DTR)	Absent
Scoliosis	Yes
Gait	Walk with support
Facial weakness	No
Cognition	Normal
Skeletal deformities	Normal
Foot deformity	No
Sensory ataxia	No
Touch and Pain sensation	Normal
Vibration sense	Normal
Position sense	Normal
Vocal cord paresis	Yes
Visual activity	Normal
Respiratory difficulties	No
Family History	Proband's Parents: Nonconsanguineous and phenotypically normal.

Table 3 Nerve Conduction Study (NCV) of the Proband

	Test	Patient's value	Reference value
Right side	**Median nerve**		
	Distal latency (ms)	7.1	<4.3
	Compound muscle action potential (mV)	0.2	>6.5
	Motor conduction velocity (m/s)	14	>49.9
	Sensory nerve action potential (μV)	2	>7
	Sensory conduction velocity (m/s)	20	>47.4
	Ulnar nerve		
	Distal latency (ms)	4.4	<3.5
	Compound muscle action potential (mV)	2.2	>7.2
	Motor conduction velocity (m/s)	17.7	>48.6
	Sensory nerve action potential (μV)	2.6	>7
	Sensory conduction velocity (m/s)	22.6	>40.8
	Tibial nerve		
	Distal latency (ms)	10.8	<5.1
	Compound muscle action potential (mV)	2.9	>12.3
	Motor conduction velocity (m/s)	20.8	>42
	Sural nerve		
	Sensory nerve action potential (μV)	4.4	>7
	Sensory conduction velocity (m/s)	8.8	>37.8

Table 4 CMT Neuropathy Score (CMTNS) for the Patient with CMT4B1

CMTNS Parameter	Score (Max)	Score (Mean)	Score (Min)
Sensory symptoms	0	0	0
Strength in arms	0	0	0
Vibration sense in arms and legs	0	0	0
Pin sensibility	0	0	0
Motor symptoms in arms	0	0	0
Motor symptoms in legs	2	1.9	1
Strength in legs	1	0.57	0
Compound muscle action potential	2	1.8	1
Sensory nerve action potential	4	2.8	1
Total score	9	7.07	3

Karyotype and Chromosomal Microarray Analyses

Karyotype revealed a normal chromosomal structure in the proband (46, XY) (Figure 3). No pathogenic copy number variations (CNVs) were identified by CMA analysis (Figure 4).

Whole-Exome Sequencing Identified a Novel Mutation in the MTMR2 Gene

Whole-exome sequencing and Sanger sequencing identified a novel homozygous transversion (c.118A>T) in exon 2 of *MTMR2* (Figure 5) in the proband. This mutation leads to the formation of a premature stop codon which finally results in the formation of a truncated (p.Lys40*) MTMR2 protein of 39 amino acids compared to the wild-type MTMR2 protein of 643 amino acids. This mutation causes complete loss of all domains (the PH-GRAM Domain, Phosphatase Domain, Coiled-coil Domain and PDZ-Binding Motif) of the MTMR2 protein. Sanger sequencing confirmed that this mutation was also present in the proband's father and mother but in a heterozygous state.

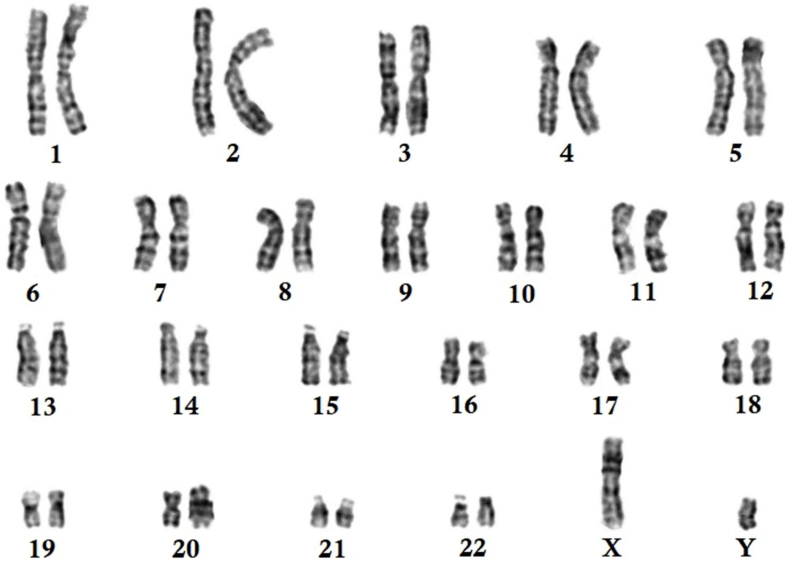

Figure 3 Karyotype Analysis. Karyotype analysis showed a normal chromosomal structure in the proband (46, XY).

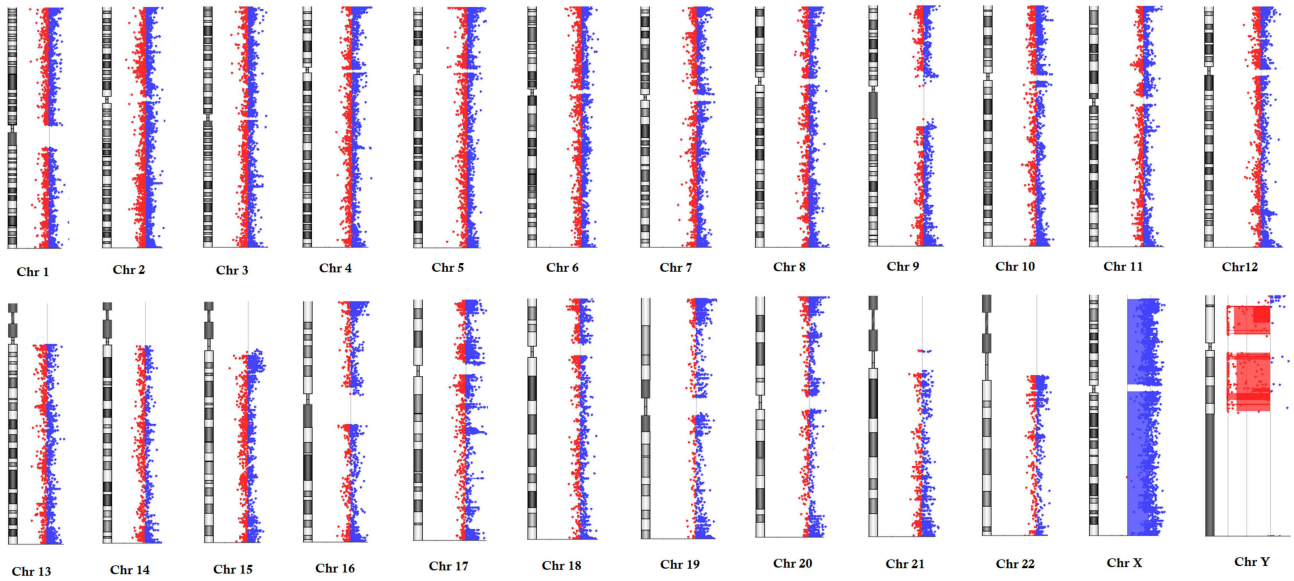

Figure 4 Chromosome Microarray Analysis (CMA). No pathogenic copy number variations (CNVs) were identified by CMA.

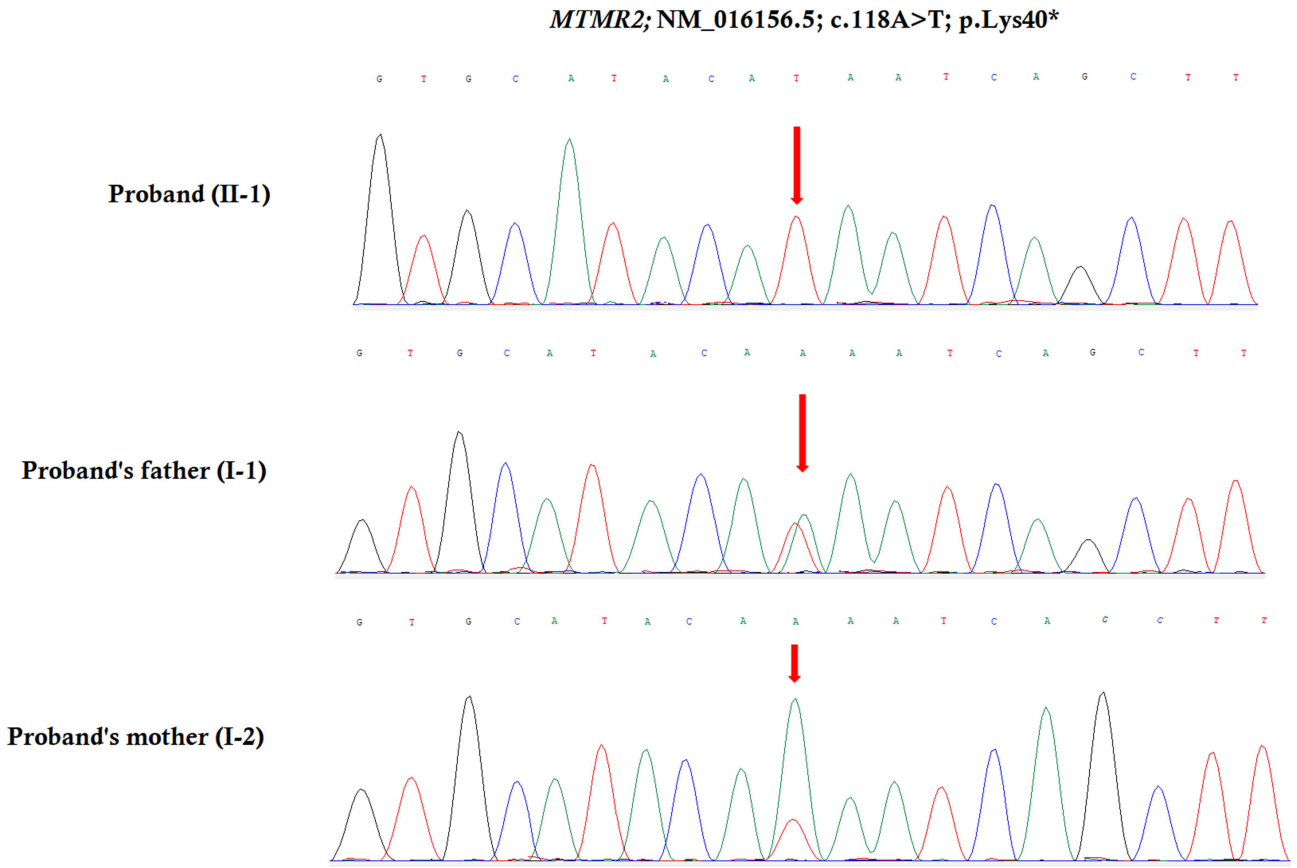

Figure 5 Sanger Sequencing. Partial DNA sequences in the *MTMR2* by Sanger sequencing of the proband and his parents [NM_016156]. Arrows point to the mutation.

This mutation was not detected in the 100 ethnically matched healthy controls. This mutation was also not present in the Human Gene Mutation Database (HGMD, www.hgmd.cf.ac.uk/), Online Mendelian Inheritance in Man (MIM, (https://www.omim.org), our in-house database consisting of ~30,000 Chinese Han samples, ExAC, dbSNP, and the 1000 Genome Database.

Selection of Potential Variants Identified by Whole-Exome Sequencing of the Patient was listed in Table 5.

In silico Analysis

The variant (c.118A>T, p.Lys40*) of *MTMR2* gene was predicted as a "disease causing" variant.[32]

Table 5 Selection of Potential Variants Identified by Whole Exome Sequencing of the Patient

Gene	Transcript	cDNA Alteration	Protein Alteration	Location	Zygosity	Impact	Interpretation
MTMR2	NM_016156	c.118A>T	p.Lys40*	Exon 2	Homozygous	Loss-of-function Variant.	Homozygous variant in *MTMR2* gene causes Charcot–Marie–Tooth disease 4B1 (CMT4B1) [MIM# 601,382] **Hence, this variant can cause the disease in this patient.**
PRX	NM_181882	c.381G>C	p.Leu127Leu	Exon 6	Heterozygous	Missense Variant. This variant is located at the last base of Exon 6. So, it could have an effect in splicing of PRX mRNA.	Homozygous variant in *PRX* gene causes Charcot–Marie–Tooth disease 4F (CMT4F) [MIM# 614,895]. **As the identified variant is heterozygous (CMT4F is an Autosomal Recessive Disease), so we discarded this variant for this case.**

(*Continued*)

Table 5 (Continued).

Gene	Transcript	cDNA Alteration	Protein Alteration	Location	Zygosity	Impact	Interpretation
FGD4	NM_139241	c.1119G>A	p.Trp373*	Exon 8	Heterozygous	Loss-of-function Variant.	Homozygous variant in *FGD4* gene causes Charcot–Marie–Tooth disease 4H (CMT4H) [MIM# 609,311]. **As the identified variant is heterozygous (CMT4H is an Autosomal Recessive Disease), so we discarded this variant for this case.**
SBF1	NM_002972	c.1138C>T	p.Gln380*	Exon 11	Heterozygous	Loss-of-function Variant.	Homozygous variant in *SBF1* gene causes Charcot–Marie–Tooth disease 4B3 (CMT4B3) [MIM# 615,284]. **As the identified variant is heterozygous (CMT4B3 is an Autosomal Recessive Disease), so we discarded this variant for this case.**
SH3TC2	NM_024577	c.1000A>G	p.Met334Val	Exon 8	Heterozygous	Missense Variant. This variant is located at the second last base of Exon 8. So, it could have an effect in splicing of SH3TC2 mRNA. Lastly, this variant is a known variant (rs139653980) and high heterozygous frequency in ExAC database.	Homozygous variant in *SH3TC2* gene causes Charcot–Marie–Tooth disease 4C (CMT4C) [MIM# 601,596]. **As the identified variant is heterozygous (CMT4C is an Autosomal Recessive Disease), so we discarded this variant for this case.**
TRIM2	NM_015271	c.606G>C	p.Arg202Ser	Exon 5	Heterozygous	Missense Variant. This variant is located at the first base of Exon 5. So, it could have an effect in splicing of TRIM2 mRNA.	Homozygous variant in *TRIM2* gene causes Charcot–Marie–Tooth disease, axonal, 2R (CMT2R) [MIM# 615,490]. **As the identified variant is heterozygous (CMT2R is an Autosomal Recessive Disease), so we discarded this variant for this case.**

Discussion

In the present study, we investigated a 4-year-old Chinese boy with a CMT4B1. We identified a novel homozygous nonsense (c.118A>T, p.Lys40*) mutation in *MTMR2* in the proband. Therefore, it was a *loss-of-function* (LOF) mutation. According to the variant interpretation guideline of the American College of Medical Genetics and Genomics (ACMG), this variant is categorized as a "likely pathogenic" variant [absent in population database (PM2) + predicted null variant in a gene where LOF is a known mechanism of disease (PVS1) + truncated/altered region is critical to protein function (PVS1) + variant removes >10% of protein (PVS1) + co-segregation of disease associated variants from parents to offspring (PP1)].[30] Till now, very few mutations of this gene have been reported, and we are reporting for the first time the *loss-of-function* mutation in the *MTMR2* gene in a Chinese family with autosomal recessive CMT4B1.

The MTMR2 protein is an enzyme involved in the modification of signaling molecules and regulates intracellular transportation of lipids and proteins.[35,36] The MTMR2 protein also regulates cell proliferation, differentiation, autophagy and cytokinesis.[35] In addition, MTMR2 protein is also playing a key role in cytoskeletal and cell junction dynamics.[36]

In the present study, we identified a novel homozygous nonsense mutation in the N-terminal domain of MTMR2 (Figure 6). Previous studies have also reported that nonsense mutations in the N-terminal domain of the MTMR2 protein cause the CMT phenotype.[3] Nonsense mutation at the N-terminal domain of MTMR2 protein causes loss of the C-terminal coiled- coil domain which is involved in homodimerization of the MTMR2 protein.[37] Additionally, C-terminal domain of the MTMR2 protein also contains a PDZ-binding motif which is involved in myelin homeostasis.[38] Therefore, identification of nonsense mutations in the N-terminal domain of MTMR2 protein is very important for patients with CMT4B1.[39,40]

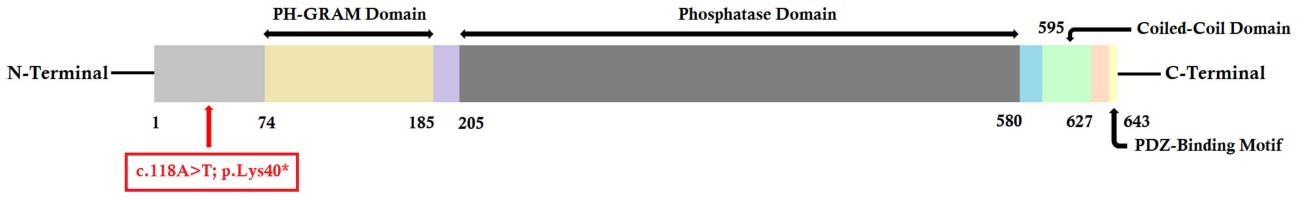

Figure 6 Schematic presentation of MTMR2 protein structure with all the domains and their location. Red box showed the identified mutation in our study. Red arrow denotes the mutation location.

In the present study, the identified mutation resulted in the formation of a truncated MTMR2 protein with 39 amino acids. However, the wild-type MTMR2 protein consists of 643 amino acids. Therefore, this mutated MTMR2 protein lacks 604 amino acids compared with the wild-type MTMR2 protein. Hence, owing to this mutation, the structural loss of the wild-type MTMR2 protein also exerts a negative effect on its function of MTMR2 protein which finally results in CMT type 4B1.[41]

CMT4B1 is a rare and severe disorder that manifests as motor and sensory polyneuropathy.[13] A patient with CMT4B1 usually presents with gradual and progressive weakness and atrophy of the distal muscle with depressed tendon reflexes and severe sensory loss.[5] Our studied patient also presented with these phenotypes. However, owing to the unavailability of the proband, we were unable to perform distal limb and neuromuscular magnetic resonance imaging (MRI) of the proband. This was the only limitation of the present study. In some patients with CMT4B1, vocal cord paresis, chest deformities, facial weakness, and claw hands were also identified.[35,36] In rare cases, patients with CMT4B1 were also reported to have optic neuritis and cervical cord schwannoma.[38–40] Previous reports, together with our present findings, strongly emphasize the association between *MTMR2* mutations and CMT4B1 disorder.[42]

In addition, as CMT4B1 is a very rare disorder with extreme phenotypic heterogeneity, it is quite challenging for the clinicians to clinically diagnose the patients with CMT4B1 accurately.[43,44] Therefore, genetic screening by whole-exome sequencing is the most significant way for timely and proper clinical diagnosis of patients with CMT4B1.[1] Therefore, a whole-exome sequencing test is recommended for rapid and accurate molecular genetic screening of CMT4B1 patients.[1]

Charcot–Marie–Tooth diseases (CMT) are the major groups of genetic neuromuscular disorders with extreme genotypic and phenotypic heterogeneity. Till date, no therapeutic strategies or effective treatment have been established for patients with CMTs. However, disease management for CMT patients usually done by rehabilitation, occupational therapy and treatment for relieving pain with regular follow-up based on the severity of disease. Recently, in a review, Okamoto et al, 2023, showed that continuous development in gene therapy or genome editing techniques are also becoming the major therapeutic strategies used for most common form of CMTs (CMT1A, CMT1B, CMTX1, and CMT2A).[45] In addition, Beloribi-Djefaflia et al, 2023, also reported that application of gene therapies as one of the most potential future therapeutic strategies for patients with CMTs.[46] Moreover, management of pediatric or childhood CMTs is also expanding a new horizon for the establishment of effective therapeutic strategies for CMTs.[47] In 2024, Alberti et al reported the pathogenesis and therapeutic perspectives for CMT2A.[48] The data from national CMT registry of Italy showed the disease epidemiology, clinical symptoms, genetic distribution and disease progression among CMT patients in Italy followed by recruiting CMT patients for future clinical trials.[49] Stavrou et al also showed the recent development of gene therapies and application of gene therapies as a therapeutic strategy for patients with CMTs.[50] Lastly, Bolino et al described the recent advances or development of viral-mediated delivery-based gene therapies, alternative delivery systems based molecular therapies, and pharmacological therapies for patients with CMT at both the preclinical and clinical levels.[51]

In conclusion, we described a Chinese boy with CMT4B1. Whole-exome sequencing revealed a novel homozygous nonsense mutation in *MTMR2* in the proband. The parents of the proband also carried this mutation in a heterozygous state. Our findings expand the mutational spectrum of *MTMR2* that is associated with CMT4B1. The present study also describes the significance of whole-exome sequencing for the accurate and timely clinical diagnosis of patients with CMT4B1.

Data Sharing Statement

All data generated or analyzed during this study are included in this published article.

Ethics Approval and Consent to Participate

The ethics committee of Xi'an People's Hospital (Xi'an Fourth Hospital), Xi'an, China approved the present study in accordance with the recommendations of the Declaration of Helsinki. The genomic DNA samples from the 100 normal healthy individuals were collected from our hospital's sample bank (Xi'an People's Hospital (Xi'an Fourth Hospital), Xi'an, China). The hospital's sample bank (The Xi'an People's Hospital (Xi'an Fourth Hospital) Sample Bank, Xi'an People's Hospital (Xi'an Fourth Hospital), Xi'an, China) collected the genomic DNA samples from the 100 normal healthy individuals after getting written informed consent. The ethics committee of The Xi'an People's Hospital (Xi'an Fourth Hospital) (approval no. 2023-D-187881; Xi'an, China) approved the use of the genomic DNA samples from 100 normal healthy individuals from the hospital's sample bank (The Xi'an People's Hospital (Xi'an Fourth Hospital) Sample Bank, Xi'an People's Hospital (Xi'an Fourth Hospital), Xi'an, China) for the present study.

Consent for Publication

All patients for whom clinical or molecular data were used in this study provided informed consent for publication.

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests in this work.

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