

Novel mutation in the *MPZ* gene causes early-onset but slow-progressive Charcot–Marie–Tooth disease in a Russian family: a case report

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Abstract

Charcot–Marie–Tooth disease (CMT) is a genetically heterogeneous group of peripheral neuropathies most of which are associated with mutations in four genes including peripheral myelin protein-22 (*PMP22*), myelin protein zero (*MPZ*), gap junction protein beta 1 (*GJB1*) and mitofusin2 (*MFN2*). This current case report describes the clinical and genetic characteristics of a 6-year-old male proband. A physical examination revealed muscular hypotonia. He started walking on his own at 18 months. A nerve conduction study with needle electromyography revealed conduction block. A novel *MPZ* mutation (c.398C > T, p.Pro133Leu) was revealed in the proband. This mutation was also found in the 32-year-old father of the proband. The father had had deformity

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of the feet and distal muscle weakness since childhood. The novel p.Pro133Leu pathogenic mutation was responsible for early onset but slowly progressive CMT1B. We assume that this site is an intolerant to change region in the *MPZ* gene. This variant in the *MPZ* gene is an important contributor to hereditary neuropathy with reduced nerve conduction velocity in the Russian population. This case highlights the importance of whole exome sequencing for a proper clinical diagnosis of CMT associated with a mutation in the *MPZ* gene.

Keywords

Charcot-Marie-Tooth disease, *MPZ* gene, neuropathy

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Introduction

Charcot–Marie–Tooth (CMT) disease is an inherited peripheral neuropathy that has an estimated prevalence in the population of 1 in 1214.¹ It is a clinically and genetically heterogeneous group of disorders characterized by distal muscle weakness and atrophy leading to motor handicap. Sensory loss is another symptom of CMT.² The disease is characterized by orthopaedic problems (e.g. pes cavus, scoliosis, hip dysplasia), pain, sleep disturbances and muscle cramps.^{2–7}

Charcot–Marie–Tooth disease can be divided into several phenotypes as follows: (i) CMT1B with early/late onset and reduced nerve conduction velocity (NCV) (demyelinating type); (ii) CMT2I with a manifestation in adulthood and normal NCV (axonal type); (iii) Dejerine–Sottas syndrome (DSS) with infant-onset and reduced NCV.⁸ CMT1, CMT2 and autosomal dominant intermediate CMT are typically inherited in an autosomal dominant manner.⁹ CMT4 refers to autosomal recessive types and CMTX refers to X-linked types of CMT.¹⁰

The myelin protein zero (*MPZ*) gene is the major structural protein in peripheral nerve myelin and it is involved in myelin formation and the maintenance of myelin

integrity.^{11,12} *MPZ* mutations have been associated with several hereditary neuropathies.¹³ Due to the high level of heterogeneity of the phenotypic manifestations of *MPZ* mutations, it is difficult to identify any regularities for this gene.

This current case report describes a Russian family in which both the father and son have muscle weakness, foot deformities and gait abnormalities. Molecular analysis in the father and son identified a novel heterozygous variant c.398C>T (p.Pro133Leu) in exon 3 of the *MPZ* gene that segregated with the phenotype. This part of the gene is highly conserved between species and a new pathogenic variant in it can be the cause of the development of neuropathy. This family case series exemplifies the phenotypic variants that are based on mutations in the *MPZ* gene.

Case report

In July 2017, the proband, a 6-year-old boy from the Komi Republic, Russia, presented to the Department of Psychoneurology, State Institution Republican Children's Clinical Hospital, Syktyvkar, Komi Republic, Russia with gait abnormality and coordination impairment. His medical history showed that he was born from the

first pregnancy as a full-term baby with a birth weight of 3640 g. Apgar scores were 8 and 9 at the 1st and 5th min, respectively. A physical examination at birth revealed muscular hypotonia. He started walking on his own at the age of 18 months.

When the proband was 3 years old, a nerve conduction study with needle electromyography (EMG) of the distal parts of the medial and tibial nerves revealed conduction block. The EMG from the hand muscles revealed spinal and neural types of muscle electrogenesis damage. An orthopaedic examination revealed congenital flat valgus feet. Blood (including creatine phosphokinase) and urine tests were normal. Electroencephalography (EEG) data showed diffuse dysrhythmia but without foci of epileptic activity. Electrocardiography (ECG) revealed tachycardia and demonstrated no signs of cardiomyopathy. The presence of neuropathy was suggested, but investigations did not identify duplication of the peripheral myelin protein 22 (*PMP22*) gene.

After admission to hospital when he was 6 years old, the patient responded appropriately to the examination and did not have any cerebral symptoms. An orthopaedic examination identified poor posture. The neurological examination revealed muscle hypotonia, symmetrical reduction of knee and Achilles tendon reflexes, moderate reflexes from the hands and severe ataxia. There was instability in the Romberg's position. Magnetic resonance imaging of the brain and spinal cord could not be performed due to the increased motor activity of the child. During electromyoneurography (EMNG) of the motor fibres of the medial, fibular and tibial nerves, low amplitude M-waves were obtained from the tibialis anterior muscle on the right side with a conduction velocity 12 m/s (normal range 58–60 m/s). Conduction block along the examined nerves was detected. Developmental delay was not documented. A speech therapist detected dyslalia that

did not include speech impairment due to neurological factors. When the proband was 6 years old, *MPZ* gene sequencing revealed a novel heterozygous mutation c.398C > T (p.Pro133Leu) in the *MPZ* gene (NM_000530.6) (Figure 1).

The 32-year-old father of the proband had had deformity of the feet since childhood and did not start walking until the age of 4. By the age of 20, he had weakness in his legs and was diagnosed with neuralgic amyotrophy. A neurological examination performed at the age of 22 years revealed conduction block along nerves of the lower limbs (NCV 0 m/s in the lower limbs and 8–19 m/s in the upper limbs). EEG revealed insufficient organization of alpha activity in the form of a rhythm with amplitude up to 114 μ V, index up to 77% and frequency spread of 9.7–11.8 Hz. At the age of 31 years, he had pes cavus, weakness and numbness of his arms and legs, hypotrophy of the interosseous muscles of the hands and scoliosis of the thoracolumbar spine. ECG showed an incomplete right bundle branch block. Tendon reflexes were absent in his arms and legs, hypoaesthesia was observed on his arms from the elbows, and on his legs from the knees. During EMNG of the distal parts of the medial, fibular and tibial nerves, the M-waves were not obtained at maximum stimulation. The father was able to walk on his own. He has a healthy 9-year-old son from his first marriage.

As CMT is an autosomal dominant disease, to identify the genetic cause of CMT in this family whole exome sequencing (WES) was undertaken for the proband and Sanger sequencing for his parents. The WES (Genotek Limited, Moscow, Russia) was performed after informed consent from the patient's parents. Genomic DNA from peripheral blood samples was isolated using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). DNA libraries were prepared using a QIAseq FX DNA

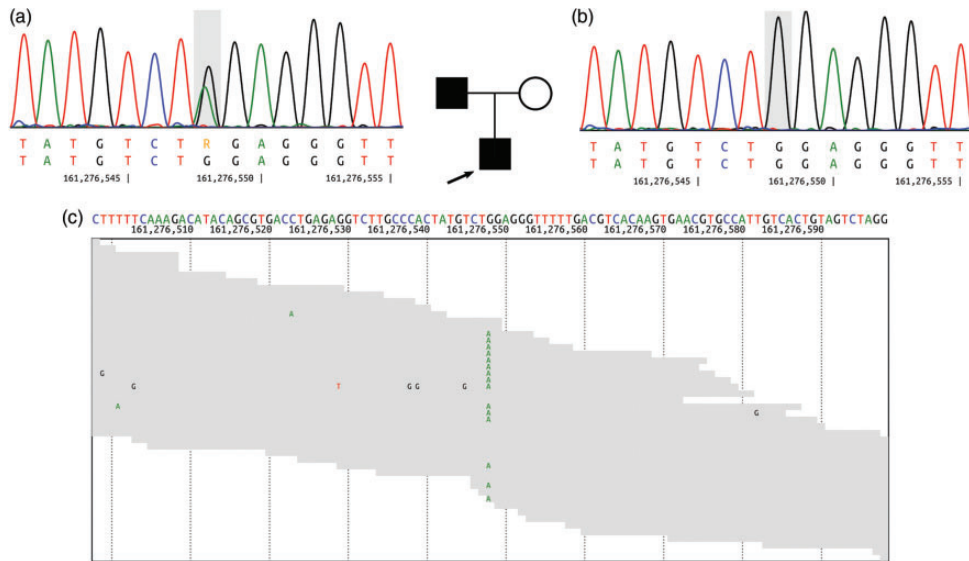


Figure 1. Pedigree of the 6-year-old male proband's family: (a) sequencing chromatogram of the proband's father showing heterozygous missense mutation c.398C>T in the myelin protein zero (*MPZ*) gene; (b) sequencing chromatograms of the proband's mother showed no mutation and (c) next-generation sequencing reads aligned against a reference genome hg19 (GRCh37.p13). The proband and his father were both heterozygous for the c.398C>T pathogenic variant. Circle – female, square – male, open symbols – unaffected, filled symbols – affected, arrow – proband. The bases in the grey frame are mutational sites. The colour version of this figure is available at: <http://imr.sagepub.com>.

Library Combinatorial Dual-Index Kit (Qiagen). The samples were enriched with SureSelect XT2 kit (Agilent Technologies, Santa Clara, CA, USA) and sequenced using a HiSeq 2500 sequencer (Illumina, San Diego, CA, USA), generating 2×100 base pair reads (see supplementary materials, Supplementary methods). After sequencing, 3'-nucleotides with read quality below 10 were trimmed using Cutadapt.¹⁴ Illumina raw reads were aligned to a reference genome hg19 (GRCh37.p13) using the Burrows–Wheeler Aligner Maximum Entropy Method. The aligned file was used for variant calling using a Genome Analysis Toolkit (GATK) according to GATK best practices.¹⁵ FastQC was used for data quality control. The proband and his father had a novel p.Pro133Leu substitution within the *MPZ* protein, which is a mutation (NM_000530.6:c.398C>T)

(Figure 1) that has not been identified previously in ExAC, 1000 Genomes Browser and in our 2000 in-house exomes. This variant was classified as 'likely pathogenic' according to the variant interpretation guidelines of The American College of Medical Genetics and Genomics as follows: three pathogenic moderate (PM) criteria (PM1, PM2, PM5); and three pathogenic supporting (PP) criteria (PP2, PP3, PP5).¹⁶ The variant was classified as a 'pathogenic' according to the predictive programs SIFT, PolyPhen2 and Mutation Taster (PolyPhen2 HumDiv score 1.00; PolyPhen2 Hum Var score 0.96; SIFT score 0.00; Mutation Taster score 1.0) (see supplementary materials, Supplementary methods). The mother of the proband is healthy and does not carry the mutation in the *MPZ* gene (Figure 1).

This case report conforms to CARE guidelines.¹⁷ The Ethics Committee of

Genotek Limited approved this research (no. 01/2019). The patient's parents provided written informed consent for the research and for publication of the clinical information and sequencing data.

Discussion

The diagnosis of CMT1B in the proband was confirmed by the age of manifestation, clinical examination and the presence of the *MPZ* gene mutation. Previously, the father of the proband had not been diagnosed, however a detailed medical history of the father revealed signs of neuropathy at the age of 20, which was later than in his son. He had similar symptoms to his son, presenting with muscle weakness and EMNG revealed conduction block. In the proband, the same new mutation resulted in manifestation at an early age. According to previous data, CMT1B is the most common type in the Russian population and there is a greater contribution of the *MPZ* mutations in reducing NCV.¹⁸ If CMT1B manifests at an early age, then there is a significant decrease in the NCV.¹⁹ If the disease manifests later, then there is a slight decrease in the NCV.¹⁹ A strategy of focused genetic testing for the CMT has been proposed.²⁰ This diagnostic algorithm is based on conduction velocity and severity of manifestation.²⁰ At a conduction velocity <15 m/s, the algorithm suggests initial *PMP22* duplication testing.²⁰ Mutations in the *MPZ* gene are assumed to occur in a smaller percentage of cases (e.g. 3.3% among 427 Taiwanese CMT patients).²¹ However, data suggests a greater contribution from *MPZ* mutations in reducing conduction velocity in the Russian population.¹⁸ This conclusion was supported by three heterozygous mutations in the *MPZ* gene that were previously described in ClinVar and were found in four Genotek Limited patients that belong to the Russian population: c.186C>G (p.Ile62Met), c.270C>A

(p.Asp90Glu), and c.235-1_235delGA (p.Ile79fs).

Myelin protein zero is a myelin sheath protein that is found in peripheral nerves, which consists of three domains: extracellular, intracellular and transmembrane.²² The intracellular domain ensures adhesion of the myelin sheath with the nerve axial cylinder and individual myelin fibres to each other.²³ The extracellular domain forms the structure of the myelin sheath and its compaction.²⁴ Numerous mutations leading to a change in the extracellular *MPZ* domain have been described previously that can give rise to highly variable clinical phenotypes.¹³ Therefore, a review of the literature was undertaken to investigate the effect of the *MPZ* mutations in this region on the clinical characteristics of CMT patients. According to the literature, mutations that lead to substitution on the extracellular domain of the *MPZ* protein had a significant effect on the CMT disease severity.²⁵ Despite this, no dependence of the NCV on the localization of the mutation within the extracellular domain of *MPZ* protein was found. Several patients were identified with a mutation in the *MPZ* gene that leads to an amino acid substitution in the protein structure,^{26,27} similar to that observed in the current case. Therefore, an additional comparison of this parameter was undertaken among the patients presented in this case report, which identified a mutation leading to a substitution at positions 123–143 of the *MPZ* protein. Unfortunately, for some patients, it was not possible to find any description of their symptoms of the disease in the published articles, which makes it impossible to make a detailed comparison.^{28–30} The phenotype of patients with CMT and DSS that have missense mutations in exon 3 of the *MPZ* gene varies from early to late onset of the disease with a different score of conduction velocity.²⁸ Exon 3 is highly conserved between species and mutation in



Figure 2. Analysis of the mutation and protein domains of myelin protein zero gene. The affected amino acid locates in the highly conserved amino acid region in different species of mammals. The arrow and red letters show the affected site. The colour version of this figure is available at: <http://imr.sagepub.com>.

this segment results in a severe form of CMT with a severe slowdown in the NCV (Figure 2). It was found that a similar amino acid substitution at codon 132 (p. Pro132Leu) resulted in the focally folded myelin with degenerative changes.³¹

Mutations in positions close to those described in this current case report were found in ClinVar and patients carrying these pathogenic variants had similar symptoms to those identified in the proband with the novel p.Pro133Leu substitution. A previous study described the p.Asn131Lys mutation in exon 3 of the *MPZ* gene, which is responsible for its adhesive properties.²⁶ The case of a 5-year-old affected boy was similar to that of the current proband.²⁶ He demonstrated delayed motor milestones, tremor of the fingers, weakness of the distal muscles, pes cavus in the lower limbs, bilateral moderate distal muscle wasting and weakness, his tendon reflexes were depressed and the NCV was 3.8 m/s.²⁶ The proband's father had the same symptoms but it was diagnosed at the age of 34 years.²⁶ The phenotypic expression in patients with CMT may depend on genetic, environmental factors or the number of normal genes in an affected patient.²⁷ This was confirmed with the phenotypic variability observed in a pair of genetically identical twins with CMT (Asp128Asn).²⁷ The proband had an earlier and more severe disease onset than his brother.²⁷

In conclusion, this current case report describes two cases of CMT in a Russian family. These two cases, a father and his son, demonstrate that the same mutation leading to the development of CMT1B can have different phenotypic manifestations even within the same family. This report highlights the importance of WES for a proper clinical diagnosis of the CMT associated with a mutation in the *MPZ* gene. Molecular genetic testing of the *MPZ* gene should be considered to confirm the diagnosis of CMT in patients with neuropathy. This case report also emphasizes the significance of WES in the molecular diagnosis of inherited Mendelian disorders with phenotypic heterogeneity.

Author contributions

Kozina A.A., Baryshnikova N.V., Ilinskaya A.Y., Kim A.A., Plotnikov N.A., Pogodina N.A., Surkova E.I., Shatalov P.A. and Ilinsky V.V. met the International Committee of Medical Journal Editors criteria for authorship. Kozina A.A., Baryshnikova N.V. and Shatalov P.A. collected, analysed and interpreted the clinical data. Ilinskaya A.Y., Kim A.A., Plotnikov N.A. and Pogodina N.A. contributed to sequencing data collection and carried out the mutation analysis. Kozina A.A., Baryshnikova N.V., Ilinskaya A.Y., Pogodina N.A., Surkova E.I. and Shatalov P.A. drafted the manuscript. Ilinsky V.V. guided the completion of this article, supervised and revised the manuscript for intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request. The novel mutation was approved by ClinVar (VCV000636232.1).

Declaration of conflicting interests


The authors declare that there are no conflicts of interest.

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Supplemental material

Supplemental material for this article is available online.

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