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ORIGINAL ARTICLE

A Japanese case of cerebellar ataxia, spastic paraparesis and deep sensory impairment associated with a novel homozygous *TTC19* mutation

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Mitochondrial complex III (CIII) deficiency comprises a group of complex and heterogeneous genetic disorders. *TTC19* mutations constitute a rare cause of CIII deficiency and are associated with neurological disorders in childhood and adulthood. Herein, we describe a 27-year-old Japanese man with cerebellar ataxia, spastic paraparesis, loss of deep sensation, mild frontal lobe dysfunction and transient psychiatric symptoms. Brain magnetic resonance imaging showed cerebellar atrophy and bilateral high-intensity signals in the inferior olives and regions adjacent to periaqueductal gray matter, on T2-weighted images. On whole-exome sequencing, we detected a novel homozygous frameshift mutation c.157_158dup [p.Pro54Alafs*48] in *TTC19*. Mitochondrial enzyme assays confirmed mild impairment of CIII enzymatic activity in lymphoblasts, which was consistent with *TTC19*-related CIII deficiency. His symptoms and radiological findings demonstrated an early stage or mild form of this disease, and further clarify the characteristics of patients with rare *TTC19* mutations.
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INTRODUCTION

Mitochondrial complex III (CIII) deficiency is a rare disease that belongs to a heterogeneous group of neuromuscular and multisystemic disorders. CIII is a multiprotein enzyme complex located in the mitochondrial inner membrane, which catalyzes the transfer of electrons from reduced coenzyme Q to cytochrome *c* with a concomitant pump of protons to the inner membrane.[1] CIII is composed of 10 nuclear-encoded subunits and 1 mitochondrial-encoded subunit.[2] Mutations in either nuclear or mitochondrial genes can be the cause of CIII deficiency. Among these genes, mutations in nuclear genes, such as *UQCRB* [MIM 191330],[3] *UQCRQ* [612080],[4] *UQCRC2* [191329],[5] *CYC1* [123980],[6] *BCS1L* [603647],[7] *TTC19* [613814],[8] *UQCC2* [614461],[9] *UQCC3* [no MIM number][10] and *LYRM7* [615831],[11] and in the mitochondrial gene *MT-CYB* [MIM 516020][12] are currently known to cause CIII deficiencies. *UQCRB*, *UQCRQ*, *UQCRC2* and *CYC1* encode components of CIII itself, whereas *BCS1L*, *TTC19*, *UQCC2*, *UQCC3* and *LYRM7* produce mitochondrial assembly factors. Recently, homozygous or compound heterozygous mutations of *TTC19*, which encodes tetratricopeptide repeat domain 19, have been found to be the cause of a neurological disorder presenting with various degrees of progressive encephalopathy and ataxia.[8,13–15] Herein, we report a 27-year-old Japanese man presenting with progressive cerebellar ataxia, spastic paraparesis, loss of deep sensation, mild frontal lobe dysfunction and transient psychiatric symptoms, along with a previously undescribed homozygous frameshift mutation in *TTC19*.

SUBJECTS AND METHODS

Clinical information and a blood sample were taken from the man after obtaining written informed consent. The experimental protocols were approved by the Institutional Review Board of Yokohama City University School of Medicine.

To identify a causative mutation, whole-exome sequencing was performed on the patient's DNA. Three micrograms of genomic DNA were processed using the SureSelect Human All Exon Kit (51 Mb; Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. The captured DNA was sequenced using a HiSeq2000 sequencer (Illumina, San Diego, CA, USA).

Mitochondrial fractions were prepared from lymphoblasts derived from the patient and control subjects. Activities of respiratory chain complexes CI, CII, CIII and CIV were assayed as described previously.[16–18] Enzyme activities are expressed as % of mean normal control activity relative to the levels of protein or to the activities of citrate synthase (CS) or CII.

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RESULTS

Case report

The present case is a 27-year-old man, who is the second of two children from non-consanguineous healthy parents originating from the same area. Other than showing a slight delay in the acquisition of gait, his psychomotor development was normal with no clinically relevant complaints, until the age of 17 years. It was then that he became aware of a slight speech difficulty. At 23 years of age, he started to experience difficulty in walking. One year later, because his gait disturbance had progressed and he was falling frequently, he visited a local doctor. Brain magnetic resonance imaging (MRI) indicated subtle cerebellar atrophy. Around that time he became unable to walk unaided and he also gradually developed psychiatric symptoms such as depression and irritability. At the age 25, his parents had him admitted to a psychiatric hospital because of his violent behavior. Following short-term treatment with carbamazepine and nitrazepam, his psychiatric symptoms were well controlled and he was transferred to our hospital. After withdrawal of the medications, neurological examination revealed a disturbance of smooth pursuit eye movements, slight dysarthria, mild limb ataxias, pronounced truncal ataxia, spasticity of the lower extremities and increased deep tendon reflexes. He had a moderate loss of vibratory sense and a severe loss of position sense in the lower extremities, with *pes cavus* and hammer toe deformities. He exhibited attentional impairment and psychomotor deteriorations. His Mini-Mental State Examination (MMSE) score was 28/30, and his Frontal Assessment Battery (FAB)[19] score was 13/18, indicating mild frontal lobe dysfunction. Laboratory biochemistry results were normal, including levels of vitamin B_1, vitamin B_{12}, vitamin E and very long chain fatty acids, as was a cerebrospinal fluid study. His lactate and pyruvate levels in blood were 8.7 mg dl^{-1} (normal range: 4–16 mg dl^{-1}) and 0.71 mg dl^{-1} (normal range: 0.3–0.9 mg dl^{-1}), respectively. Serum antibody for Human T lymphotropic virus type 1 was negative. Activity of galactocerebrosidase, glucosidase and β-hexosaminidase in leukocytes was normal. Blood amino-acid analysis revealed no apparent abnormality. Neoplastic, autoimmune, thyroidal and rheumatic diseases were excluded by the appropriate tests. Brain MRI showed mild cerebellar atrophy (Figure 1a), and symmetrical high-intensity signals in the inferior olives and lesions adjacent to periaqueductal gray matter, on T2-weighted images (Figures 1b and c). Supratentorial lesions, such as white matter changes, basal ganglia lesions, cortical brain atrophy or structural brain anomalies were not detected by MRI. Magnetic resonance spectroscopy (MRS) could not detect a clear lactate doublet in the basal ganglia (Figure 1d). MRIs of the whole spine showed no abnormality. ^{99m}Tc-ethyl cysteinate dimer single-photon emission computed tomography demonstrated reduced perfusion in the cerebellum and bilateral occipital cortices (Figure 1e). Nerve conduction studies indicated a motor axonal neuropathy of the lower extremities, but no apparent sensory neuropathy. The patient was negative for genetic alterations associated with Friedreich ataxia, spinocerebellar ataxia (SCA) 1, SCA2, SCA3 (Machado–Joseph disease), SCA6, SCA7, SCA12, SCA17 and dentatorubro-pallidoluysian atrophy. We diagnosed him as having a rare form of SCA or a complicated form of spastic paraparesis. At 27 years of age, his MMSE score was 30/30, indicating that his cognitive functions remained stable without medications. At this point, his verbal intelligence quotient (IQ) was 85, performance IQ was 71 and total IQ was 76 on the Wechsler Adult Intelligent Scale-Third Edition. Other neurological findings also showed no apparent progressions.

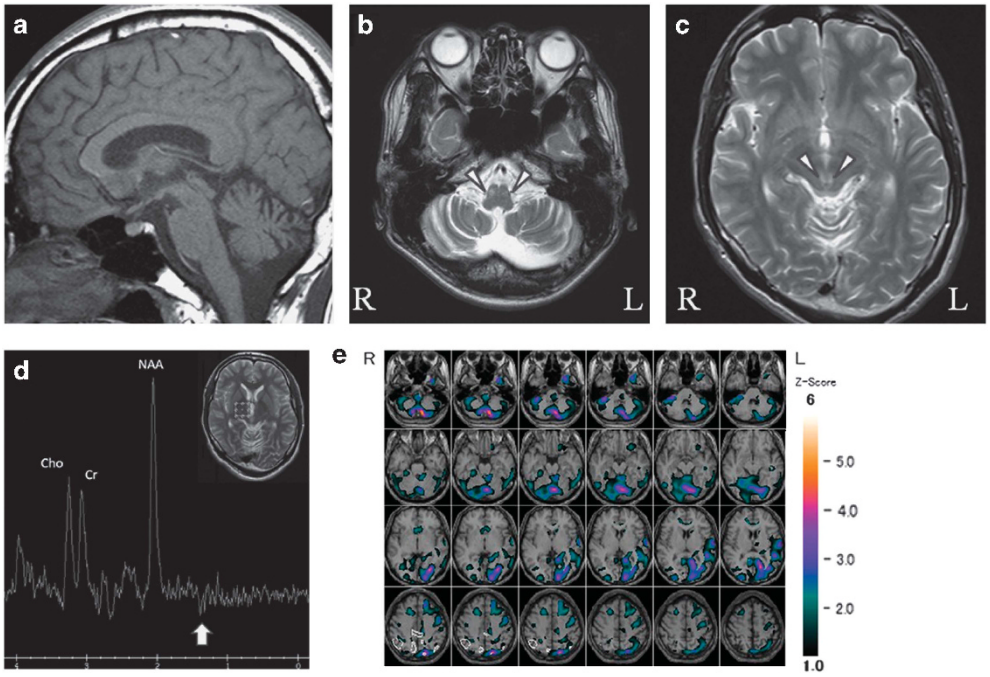

Figure 1 (**a**–**c**) Brain MRI of the patient at 25 years of age. A midsagittal section of brain on a T1-weighted image (**a**), and axial sections of medulla oblongata (**b**) and midbrain (**c**) on a T2-weighted image are shown. Arrowheads indicate hyper-intense signal in the inferior olives (**b**) and in the midbrain (**c**). (**d**) Proton MRS obtained from the thalamic area, at a magnetic field of 3 Tesla (echo time 144 ms). A clear lactate peak, which should be present at 1.3 p.p.m. (arrow), could not be detected. Cho, choline; Cr, creatine; NAA, *N*-acetyl aspartate. (**e**) ^{99m}Tc-ECD SPECT images at 25 years of age. The *Z*-score maps displayed on an anatomically standardized MRI template are shown.[27]

His elder sister has not recognized any difficulties, and has not undergone any radiological and electrophysiological examinations. However, on neurological examination, she showed slight truncal ataxia and a slight impairment of position sense in the lower extremities, with *pes cavus* deformity (Table 1).

Exome sequencing

Among the detected variants, 2873 were located in exons or splice sites (within 2 bp of the boundary), and were unregistered or registered as uncommon single-nucleotide polymorphisms with minor allele frequency <1% in dbSNP135. We examined whether these mutations were present in known SCA or hereditary spastic paraplegia-related genes.[20] A novel homozygous frameshift mutation, c.157_158dup [p.Pro54Alafs*48] (NM_017775.3), was identified in *TTC19* (17p12, [MIM 613814]). Sanger sequencing with an ABI 3500xL (Life Technologies, Carlsbad, CA, USA) confirmed c.157_158dup homozygosity in the patient and his sister, and heterozygosity in the parents (Figure 2a). No mutations were detected by Sanger sequencing in the

Table 1 Clinical and laboratory findings in patients with *TTC19* mutations

Reference		*Ghezzi et al.*[8]			*Nogueira et al.*[13]				*Atwal*[15]	*Morino et al.*[14]	*The present report*	
Ethnic origin		*Italian*			*Portuguese*				*Hispanic*	*Japanese*	*Japanese*	
Sex	F	M	F	M	M	M	M	F	M	F	M	F
Age at onset	5	10	5	43	27	12	15	34	1	31	25	29
Nucleotide changes		c.656T>G		c.517C>T		c.963_966del			c.964_967del/ c.577G>A	c.829C>T	c.157_158dup	
Amino-acid changes		p.Leu219*		p.Gln173*		p.Ala321Alafs*8			p.Trp186*/ p.Gly322-Metfs*8	p.Glu277*	p.Pro54Alafs*48	
Symptoms at onset	Learning disability, gait ataxia	Learning disability, gait ataxia	Regression of language, gait ataxia	Weakness of the four limbs	Mood disorder, gait ataxia	Compulsive lying	Aggressive behavior	Avoidance behavior	Developmental delay, regression of language	Dysarthria	Mood disorder, gait ataxia	No subjective symptoms
Clinical symptoms												
Cognitive impairments	NA	+	NA	NA	+	+	+	+	NA	+	+	NA
Psychiatric disturbances	NA	NA	NA	NA	+	+	+	+	NA	NA	+	–
Gait ataxia	+	+	+	+	+	+	+	+	NA	+	+	+
Spasticity	NA	NA	NA	+	+	+	+	NA	NA	NA	+	–
Enhanced tendon reflexes	+	NA	NA	NA	+	+	+	+	NA	+	+	–
Deep sensory impairment	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	+
Peripheral neuropathy	NA	NA	+	+	+	NA	+	NA	NA	NA	+	NA
Pes cavus	NA	NA	NA	NA	NA	NA	NA	NA	NA	+	+	+
MRI												
Cerebellar atrophy	+	NA	+	NA	+	+	+	+	NA	+	+	NA
Supratentorial lesions	+	NA	NA	+	+	+	+	NA	+	NA	–	NA
Hyperintense inferior olives	+	NA	+	NA	+	+	+	+	NA	+	+	NA
Hyperintense mid brain	NA	NA	+	NA	NA	+	NA	NA	NA	NA	+	NA
MRS												
Lactate peak	+	NA	NA	+	NA	NA	NA	NA	NA	NA	unclear	NA
Hypoperfusion in SPECT	NA	NA	NA	Frontoparietal lobe	NA	NA	NA	NA	NA	NA	Cerebellum and occipital lobe	NA

Abbreviations: F, female; M, male; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NA, no data available; SPECT, single-photon emission computed tomography.

other exons of *TTC19*. The mutation was undetected in 408 'in-house' Japanese control exomes.

Mitochondrial enzyme activity

As shown in Table 2, his residual CIII activity relative to CS activity was 74.6% in cultured lymphoblasts. CIII activity relative to CII activity was reduced to 48.5%, indicating impairment of his CIII activity. A previous report showed that residual CIII activity relative to CS activity was 46–89% in cultured cells from patients with a homozygous *TTC19* mutation, whereas in muscles from the same patients, CIII activity relative to CS activity was 14–19%.[8] A milder defect of CIII enzymatic activity was also observed in cultured cells (for example, lymphoblasts or fibroblasts) compared with that in solid tissues (for example, muscle or liver) in other mitochondrial disorders, such as CIII deficiency with *BCS1L* mutations.[2,7] Thus, the mild decrease of CIII activity in lymphoblasts of the patient was consistent with *TTC19*-related CIII deficiency.

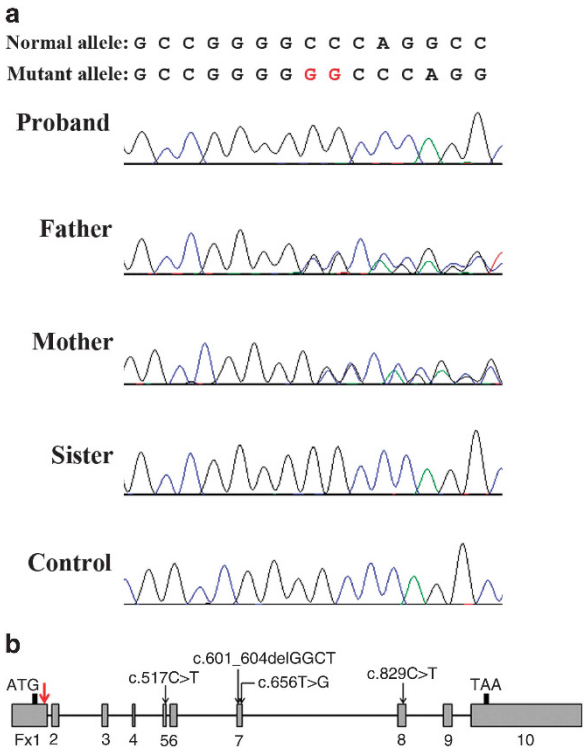

Figure 2 (**a**) Electropherograms of the patient (proband), his parents, his sister and normal control showing the mutation. Red letters indicate the inserted sequence. The patient and his sister are homozygous for the mutation, whereas the parents are heterozygous for the mutation. (**b**) Schematic presentation of *TTC19* mutations. The red arrow indicates the location of the mutation in the patient.

Table 2 Activities of respiratory chain complexes in lymphoblasts

	Co I	*Co II*	*Co II+III*	*Co III*	*Co IV*	*CS*
% of normal	84.6	145.2	153.7	70.4	102.7	94.4
CS ratio (%)	89.5	153.8	162.8	74.6	108.8	
Co II ratio (%)	58.2		105.8	48.5	70.7	

Abbreviations: Co I, complex I; Co II, complex II; Co III, complex III; Co IV, complex IV; CS, citrate synthase.
Enzyme activities are expressed as % of mean normal control activity relative to protein levels (% of normal), CS activities (CS ratio) and Co II activities (Co II ratio).

DISCUSSION

In this report, we describe in detail the clinical symptoms of a patient with a novel homozygous frameshift mutation, c.157_158dup [p.Pro54Alafs*48], in *TTC19*. As three unrelated Italian kindreds with homozygous *TTC19* mutations were first reported in 2011,[8] 10 patients from six families with *TTC19* mutations have been identified.[13–15] The age of disease onset is variable, ranging from 13 months to 42-year old. Progressive cerebellar ataxia and cognitive impairment (mental retardation and/or intellectual deterioration) are the common symptoms, whereas the severity, progression and prognosis of the disease vary even between patients from the same family.[8,13] When the progression of symptoms was slow, patients were diagnosed as possibly having SCA, spastic paraplegia or psychiatric disorders.[13,14] When the progression was relatively rapid, patients were diagnosed as having metabolic disorders, even Leigh syndrome.[15] Our case showed essentially similar clinical manifestations to cases diagnosed as having SCA. In previous reports, all patients with *TTC19* mutations showed cognitive impairment to a greater or lesser extent. Our patient had transient psychiatric symptoms, but cognitive decline was relatively mild compared with the previous cases. Brain MRI of the patient showed mild cerebellar atrophy and bilateral high-intensity signals in the inferior olives, and lesions adjacent to periaqueductal gray matter on T2-weighted images, but no apparent supratentorial lesions. In previous reports, the patients with *TTC19* mutations showed cerebellar atrophy with abnormal high-intensity signals in the inferior olives, caudate, putamen, cerebellar dentate nucleus and/or medial midbrain, on T2-weighted imaging.[8,13–15] Supratentorial brain lesions appeared to be detected in the cases with severe cognitive impairment. Among these abnormalities, cerebellar atrophy and bilateral T2 high-intensity lesions in the inferior olives were detected in all of the reported cases, including in our case. Because an abnormal lactate peak was detected in two out of two cases with *TTC19* mutations, whose ^{1}H-MRS information was available,[13] we performed ^{1}H-MRS in our patient but no lactate peak was apparent. ^{1}H-MRS detection of lactate in the brain parenchyma is regarded as a more precise indicator of cerebral lactic acidosis than blood or cerebrospinal fluid lactate levels.[21,22] The result may indicate that cerebral lactic acidosis in the present case was relatively milder than that in the previous cases. Although not previously described in patients with *TTC19* mutations, the current case showed severe loss of deep sensation in the lower extremities; however, a spondylotic myelopathy or sensory neuropathy was not detected. Disturbance of deep sensation is possibly a symptom of this disease, but might not have been fully recognized because of severe cognitive impairment in the previous cases. Our patient, as in previous cases, had pronounced truncal ataxia and gait disturbance despite relatively mild cerebellar atrophy on MRI, which may be explained by severe loss of deep sensation in the lower extremities. Alternatively, lesions in the inferior olives, which are known to produce truncal ataxia,[23] might have contributed to the exacerbation of his gait disturbance. Considering the clinical symptoms and radiological findings, we consider that our patient may be in the early stage of this disease or have a mild form. It will be necessary to monitor his symptoms and examination findings over time.

CIII deficiencies are rare, but they cause a broad spectrum of symptoms and display tissue-specific lesion formation in humans.[24] Although the functions of TTC19 are still poorly characterized, TTC19 is thought to be an important factor in early CIII assembly. Mutations of *BCS1L*, another factor involved in CIII assembly, are known to cause a wide range of phenotypes, from highly restricted pili torti and sensorineural hearing loss (Björnstad syndrome) to profound

multisystem organ failure (GRACILE syndrome and CIII deficiency).[25] Although a molecular basis for the phenotypic differences was identified in these cases,[25] disease severity varied even within the family members harboring the same mutation,[26] suggesting that alternative unknown determinants of disease severity may exist. Because all of the reported *TTC19* mutations were nonsense or frameshift mutations, loss of function of *TTC19*, which leads to impairment of CIII assembly, is thought to be the cause of the disease. Given that the p.Pro54Alafs*48 mutation in our case was the most N-terminally located of all the reported mutations (Figure 2b), and that the clinical manifestations are mild in comparison with previous cases,[8,13–15] genotype–phenotype correlations cannot be explained simply by the retaining of partial *TTC19* function. Moreover, in the cell lines, we could not detect a clear difference in CIII activity between the present case and severe cases.[8] We speculate that phenotypic determinants, other than *TTC19* genotype (for example, environmental factors or genotypes of other genes), exist in patients with *TTC19* mutations. We assessed exome data of the patients for single-nucleotide variants (SNVs) in nuclear-encoded CIII component genes (*CYC1*, *UQCRC1*, *UQCRC2*, *UQCRFS1*, *UQCRB*, *UQCRQ*, *UQCRH*, *UQCR10* and *UQCR11*) and assembly factor genes (*UQCC1*, *UQCC2*, *UQCC3*, *BCS1L* and *LYRM7*), which may modify the disease phenotype, but we found no functional SNVs in these genes.

In conclusion, our case carrying a novel homozygous frameshift mutation in *TTC19* indicated that severe loss of deep sensation might be an unrecognized symptom of this disease. More cases are needed to further understand the crucial factor(s) determining the disease phenotype.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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