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Clinical manifestations and gene mutation in a case of Machado-Joseph disease★

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Abstract

This study reports a case of a 75-year-old female Machado-Joseph disease patient exhibiting unstable walking and inaccurate hand holding for 8 months, which progressively worsened. Physical examination on admission showed cerebellar ataxia and a history of hypertension. Cranial MRI demonstrated cerebellar and brain stem atrophy. Gene analysis showed abnormal amplification of the CAG trinucleotide repeat in exon 10 of the ataxin-3 (ATXN3) gene, resulting in 70–81 CAG repeats in the patient, with a significant positive family history.

Key Words

machado-joseph disease; clinical characteristics; imaging; molecular genetics; spinocerebellar ataxia-3 gene; gene mutation; exon 10; spinocerebellar ataxia; nervous system disease

Research Highlights

- (1) This study reports a case of an elderly female patient with Machado-Joseph disease, characterized by unstable walking and inaccurate hand holding for 8 months. Physical examination on admission showed cerebellar atrophy, brainstem atrophy and cerebellar ataxia.
- (2) Machado-Joseph disease was determined by MRI and gene analysis.
- (3) Gene mutation (CAG repeat expansion) in the patient in this study was located in exon 10 of the ataxin-3 gene on chromosome 14.

Abbreviations

MJD, Machado-Joseph disease; SCA3/MJD, spinocerebellar ataxia-3/Machado-Joseph disease

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INTRODUCTION

Machado-Joseph disease (MJD) is an autosomal dominant multisystem neurodegenerative disorder that is a form of dominantly-inherited spinocerebellar ataxia^[1]. In 1972, Nakano *et al* identified a family of Portuguese ancestry inhabiting New England with autosomal dominant

hereditary ataxia. Subsequently, an increasing number of families with this disease were discovered worldwide^[2-5]. MJD has been identified in five continents, and many of these families do not have Portuguese ancestry. The majority of patients are affected with ataxic gait at approximately the age of 40, involving various nervous system regions, although the regions principally affected are the

cerebellum, brainstem and spinal cord^[3-4]. Genetic linkage analysis of the autosomal dominant hereditary form of MJD in the family localized the gene to 14q32.1, named SCA-3^[6-7]. Spinocerebellar ataxia-3/Machado-Joseph disease (SCA3/MJD) is a major genotype, accounting for about 50% of hereditary ataxia. Sequencing revealed CAG repeat expansion in MJD, with the mutation affecting exon 4^[8]. After expansion, the number of CAG repeats increases to between 61 and 89, while that in a healthy person is between 12 and 41. This study reports a case of an MJD patient with a CAG repeat expansion in exon 10.

CASE REPORT

General presentation

History of present illness: a female patient aged 75 years old, suffering from unstable walking and inaccurate hand holding for 8 months. Eight months before admission, the patient experienced unstable walking without obvious inducement and sometimes fell down, similar to being drunk. During the last 6 months before admission, she exhibited inaccurate hand holding. During the last three months before admission, she experienced limb numbness. During the last half month before admission, she felt dizziness, without nausea, vomiting, head distention, disturbance of consciousness, urinary and fecal incontinence, and tinnitus or deafness. She was diagnosed as having an aging brain using cranial CT in the outpatient clinic of Fengxian Central Hospital. She was hospitalized for further treatment. Since onset, the patient had normal appetite, normal sleep, and normal urine and stool, and did not display signs of influenza or fever.

History of past illness: hypertension, maximum 160/90 mmHg (1 mmHg = 0.133 kPa); taking *Zhenjujiangya* tablets.

Family history: four sisters; two had similar diseases and died from cardiovascular disease.

Physical examination

On admission observations were as follows: body temperature, 36.7°C; heart rate, 69 beats/minute; blood pressure, 135/85 mmHg; respiration, 20 times/minute; showing normal heart and lung function; normal abdomen examination.

Nervous system examination revealed: clear mind, equivocal language, dialogue relevant to the subject, normal orientation and possessiveness, mask-like face,

two pupils of the same size with a diameter of 0.3 cm, light reflex, horizontal nystagmus, normal visual acuity, without hemianopia; symmetrical nasolabial fold, normal lifting of the soft palate, positive pharyngeal reflex; limb muscle force of grade IV, gear wheel-like increase of limb muscle tension, radial periosteal hyperreflexia, patellar tendon hyperreflexia, with physical reflex, bilateral positive Babinski sign; inaccurate nose touch, inaccurate heel-knee-tibia test, sensory disturbance of limb extremities; positive Romberg's sign; tuning fork hypopallesthesia of both limbs.

Auxiliary examination

Auxiliary examination showed normal blood examination, electrolyte, cardiac troponin I, myoglobin and creatine kinase-MB isoenzyme, clotting mechanism, hepatic function, renal function, blood glucose, glycosylated hemoglobin, myocardial enzyme, blood lipid, homocysteine, stool, thyroid function and tumor index. Transcranial Doppler sonography revealed an increased blood flow resistance index. Brainstem auditory evoked potential was normal. Nerve conduction velocity: the motor conduction velocity of the right median nerve was slightly low. The sensory conduction velocities of the median and ulnar nerves were normal. The amplitude of the motor conduction velocity of the right common peroneal nerve was low. The wave form of the sensory conduction velocity of the left superficial peroneal nerve was dispersing. The frequency of the F wave of the median nerve was low. The latency of the F wave of the ulnar nerve was normal. Hoffmann's reflex of the bilateral tibial nerve was within the normal range.

MRI examination

Using 1.5 T permanent magnet magnetic resonance equipment (Phillips, Amsterdam, the Netherlands), the parameters of the spin-echo sequence were as follows: axial, sagittal T1 weighting (repetition time = 500 ms, echo time = 30 ms) and axial T2 weighting (repetition time = 5 000 ms, echo time = 102 ms), 5–8 mm gap, matrix 256 × 256. The plane through the midbrain aqueduct and the fourth ventricle at the cerebellomedullary cistern was designated the measurement plane. On the workstation, the following parameters were measured using computer function keys^[9]: (A) the distance between the midpoint of the anterior border of the cerebral peduncle and the midpoint of the midbrain aqueduct; (B) shortest distance between the midpoint of the anterior border of the pons and the floor of the fourth ventricle; (C) the distance between the midpoint of the anterior and posterior borders of the medulla oblongata; (D) shortest distance between the floor and top of the fourth ventricle; (E) shortest

anteroposterior diameter of the pontine cistern; (F) anteroposterior diameter of the medullar cistern (shortest distance between the midpoint of the anterior border of the medulla oblongata and the clivus). Accuracy of the above-described indices was 0.1 mm. Each parameter was measured twice, and an average value was obtained (Table 1).

Table 1 MRI measurement results of brain tissue-related indices in patients with Machado-Joseph disease

Index	Measurement value	Normal value
Transverse diameter of the fourth ventricle (cm)	1.86	1.10–1.64
Anteroposterior diameter of the fourth ventricle (cm)	1.06	0.22–0.86
Ventriculo-cranial ratio of transverse cerebral ventricles	0.21	0.09–0.15
Ventriculo-cranial ratio of anterior and posterior cerebral ventricles	0.18	0.03–0.11
Anteroposterior diameter of medulla oblongata (cm)	0.86	Less than 1.0

Normal values according to the criteria of Koehler *et al* [10].

MRI displayed cerebellar and brainstem atrophy (Figure 1).

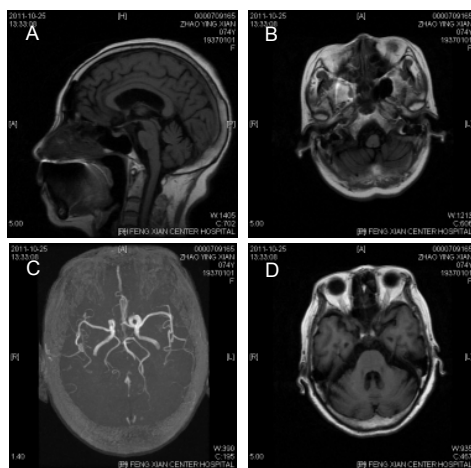


Figure 1 MRI manifestation of a Machado-Joseph disease patient.

- (A) MRI showing obvious thinning of the superior extremity of the medulla-spinal cord, as well as cerebellar and brainstem atrophy.
- (B) MRI showing cerebellar atrophy.
- (C) Magnetic resonance angiography showing vascular sclerosis, vascular stiffness, sparse branching and disordered distribution.
- (D) MRI showing an enlarged fourth ventricle.

Gene analysis

A total of 3 mL of ulnar vein blood was separately

obtained from the MJD patient and her son, treated with ethylenediamine tetraacetic acid anticoagulant, and stored at 4°C in the Molecule Room of Huashan Hospital. Total DNA was extracted, and the SCA3/MJD gene was amplified by PCR. Primer sequences were in accordance with reference 11 as follows: upstream: SCA3-F, 5'-CCAgTgACTACTTTg ATTCg-3'; downstream: SCA3-R, 5'-TggCCTTTCACA TggATgTg-3'. Primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Total reaction volume was 25 µL: DNA extract 2 µL, 20 mM forward and reverse primers each 0.5 µL, 5 U/L TaqDNA polymerase 0.2 µL, 25 mM dNTP 10 µL, 10 × buffer solution (MgCl₂ 15 mM) 4 µL, R110 and R6G fluorescein-labeled dUTP 0.25 µL. These reagents were purchased from Perkin-Elmer, Fremont, CA, USA. Double distilled water was used as required. Using a PCR thermocycler (9600 DNA thermal cycler, Perkin-Elmer, USA), cycling conditions were 3 minutes at 95°C, 40 seconds at 94°C, 50 seconds at 56°C, 40 seconds at 72°C for 30 cycles, followed by 7 minutes at 72°C. An Abiprism™ 310 genetic analyzer was utilized for capillary electrophoresis and fragment length analysis using the following parameters: electrophoretic medium, POP-4 gel; electrophoresis condition, 15 kV for 24 minutes; capillary, 47 cm (length) × 50 µm (inner diameter). Using the capillary tube method for Stp-PCR, the reaction was run on a low melting point agarose gel, followed by column purification. PCR products were concentrated using a cryogenic vacuum apparatus. Results revealed an abnormal number of CAG repeats and mutation of the SCA3/MJD gene. Agarose gel electrophoresis showed an abnormal amplified band (Figure 2).

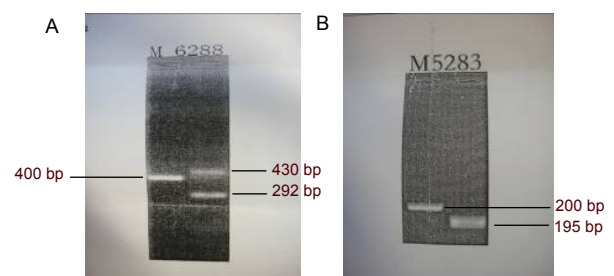


Figure 2 Results of 2% agarose gel electrophoresis of PCR products of spinocerebellar ataxia-3/Machado-Joseph disease (SCA3/MJD) gene CAG trinucleotide repeat sequencing.

M: Marker; 6288, 5283: number of DNA specimen. (A) SCA3 patient with a PCR-amplified fragment of 430 bp, with a CAG repeat number of 47. (B) A healthy person from the same family, PCR amplification of 192 bp fragment, with a CAG repeat number of 14.

By Stp-PCR sequencing, the CAG repeat number was found to be 77 in the mutated allele, which exceeded the

normal range (Figure 3).

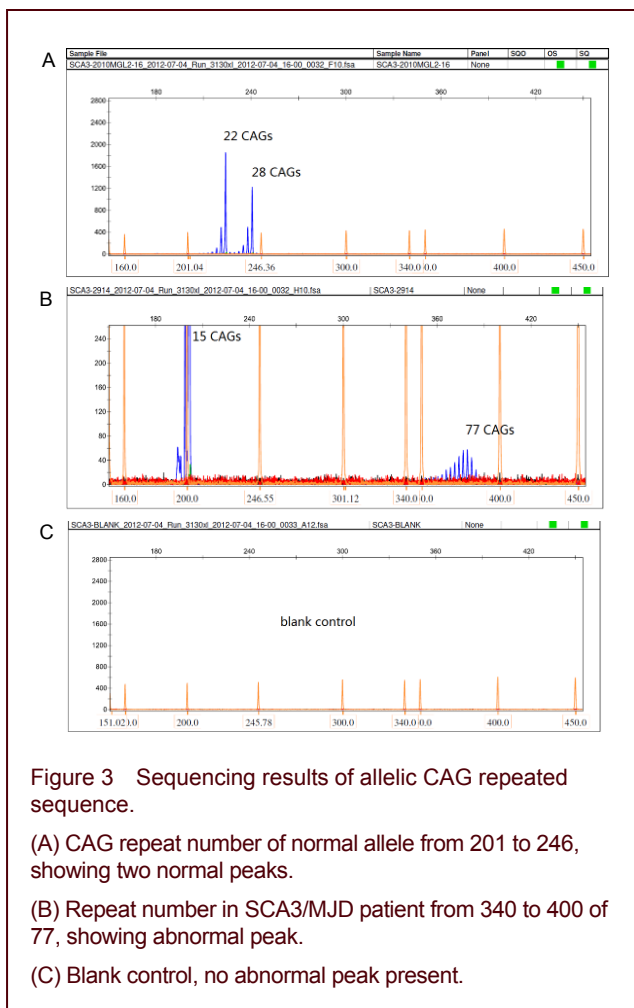


Figure 3 Sequencing results of allelic CAG repeated sequence.

(A) CAG repeat number of normal allele from 201 to 246, showing two normal peaks.

(B) Repeat number in SCA3/MJD patient from 340 to 400 of 77, showing abnormal peak.

(C) Blank control, no abnormal peak present.

Discharge diagnosis

(1) MJD; (2) hypertension, grade three, high risk.

DISCUSSION

MJD is an autosomal dominant hereditary ataxia^[11-12], which was first identified in Portuguese Azoreans^[13]. MJD accounts for 42–48% of autosomal dominant hereditary ataxias^[14]. Amplification of the trinucleotide CAG is the main cause of MJD^[15].

Clinical features

Persons aged 1–73 years can display signs of SCA-3/MJD, although onset mainly occurs at middle age, with an average age of onset of 37.4 ± 14.1 years^[16]. Average survival is 20 years. The average onset age in China is 33.9 ± 9.5 years^[12].

MJD affects the motor nerve axon and myelin sheath, but not cerebral cortex or cerebellar cortex neurons, in the central nervous system. MJD mainly involves the (1)

globus pallidus internus, subthalamic nucleus and substantia nigra; (2) red nucleus, cerebellar peduncle and dentate nucleus; (3) brainstem motor nucleus (nucleus of oculomotor nerve, nucleus of abducent nerve, nucleus of facial nerve and nucleus of hypoglossal nerve); (4) spinal cord Clarke nuclei and direct cerebellar tract and spinal cord anterior horn motor cells. The degeneration of these structures affects muscle tension, resulting in extrapyramidal symptoms, dysarthria, ocular movement defects and ataxia.

Harding^[17] divided SCA-3/MJD into three types: type I, mainly dysmyotonia-extrapyramidal symptoms, pyramidal sign and ocular myopathy; type II, mainly cerebellar signs and pyramidal signs; type III, mainly distal symmetrical amyotrophy and cerebellar signs, obvious peripheral nerve disease signs, including myasthenia, amyotrophy and dysesthesia. Ocular myopathy, facial and tongue muscle spasm and exophthalmos are characteristic manifestations of SCA-3/MJD. Rosenberg^[18] added type IV: onset at old age, significant Parkinson's signs, accompanying ataxia, distal amyotrophy and sensory deprivation. Some type IV patients have been diagnosed as having Parkinson's disease^[19]. Most cases do not exhibit symptoms of only one type; most show significant overlap with the other types. Thus, it is likely that patients at different stages of the disease exhibit clinical symptoms that vary over time^[20]. The patient in this study was characterized by unstable walking and inaccurate hand holding for 8 months. For the last three months, she experienced limb numbness. Nerve electrophysiology detected damage to the peripheral nerve and revealed that this case exhibited peripheral neuropathy. Therefore, this case can be classified as type III MJD; the clinical manifestation was diverse.

MRI characteristics

Cranial MRI reveals cerebellar and brainstem atrophy. The following changes are detected: cerebellar afferent fiber, efferent nerve fiber, frontal lobe, temporal lobe and pallidal atrophy; reductions in superior cerebellar peduncle width, pallidal transverse diameter, pons anteroposterior diameter and transverse diameter; and cerebellar vermis, frontal lobe and temporal lobe atrophy. Pons transverse fibers in T2-weighted images and proton-weighted images display high signal changes in half of patients, and the dorsolateral putamen in T2-weighted images in a few patients display low signal changes^[21]. Cerebellar vermis and brainstem atrophy were not only associated with onset age, but also with CAG amplification^[22]. In addition to cerebellar and brainstem atrophy, SCA-3/MJD patients exhibit significant fourth ventricle expansion^[8]. Cranial MRI

in this study revealed cerebellar and brainstem atrophy, as well as fourth ventricle expansion, consistent with a previous study^[23].

Molecular genetics

Takiyama *et al*^[24] and Kawaguchi *et al*^[11] used linkage analysis and *in situ* chromosome hybridization. The MJD gene is located on the long arm of chromosome 14, 14q32.1. PCR analysis showed that MJD patients are heterozygous for the number of CAG repeats. The cDNA sequence of the MJD-1 gene was cloned, which revealed that MJD is a neurodegenerative disease caused by CAG trinucleotide repeat expansion in the MJD-1 protein coding region^[25].

The MJD-1 gene is composed of 1 776 bases (bp), containing a long open reading frame. A CAG repeat is located near the 3' end of the coding region, which encodes a polyglutamine sequence^[25-26]. Ataxin-3, the protein product of the MJD-1 gene, contains this polyglutamine tract. When ataxin-3 contains an expanded polyglutamine tract, as in MJD, the protein forms neuronal intranuclear inclusions, resulting in neurodegeneration and death^[27-28].

The number of CAG repeats in the MJD-1 gene varies between 12 and 41 in healthy individuals, with the most common number of repeats being 41. The number of CAG repeats in the MJD-1 gene is heterozygously amplified in SCA-3/MJD patients, with the number of repeats between 61 and 89^[28]. A previous study^[29] found the CAG mutation in exon 4, but in this study, we identified the mutation in exon 10. This indicates that the MJD gene mutation is polymorphous, which might be associated with the varied clinical manifestations. The number of CAG repeats in the MJD-1 gene is strongly associated with clinical features. Studies^[30-31] concerning SCA-3/MJD family in different regions demonstrated that (1) The number of abnormally-amplified CAG repeats is inversely associated with onset age and disease severity. That is, the greater the number of CAG repeats, the earlier the onset age and the more severe the clinical symptoms. (2) The CAG repeat number varied among the different phases. Increased repeat number is more common than decreased repeat number. This provides a reasonable explanation for anticipation in SCA-3/MJD patients. That is, anticipation is due to an increase in the number of trinucleotide repeats. (3) The number of CAG repeats is associated with the occurrence of some clinical symptoms and signs. For example, Takiyama *et al*^[24] found that the number of CAG repeats is positively associated with exophthalmos and pyramidal signs.

The correlation of major clinical symptoms and signs with CAG repeat number is not consistent in different families. Consequently, CAG repeat number may only be a genetic marker of the disease^[14, 32]. However, there are other factors influencing disease phenotype. Thus, we cannot use CAG repeat number as a predictive index of SCA-3/MJD clinical manifestations.

Differential diagnosis

MJD in the female patient enrolled in this study was insidious in onset, with a slow progression. Her initial symptoms were unstable walking, inaccurate hand holding for 8 months, with family history. Imaging demonstrated cerebellar and brainstem atrophy. DNA analysis revealed abnormal amplification of the CAG trinucleotide repeat in exon 10. MJD should be distinguished from the following diseases: (1) multiple system atrophy: frequently in middle-aged and elderly persons; insidious onset, slow progression, unstable walking, limb disturbance. Imaging changes as follows: cerebellar and brainstem atrophy; nervous system lesion; no abnormal SCA3-ataxin3 protein. (2) Parkinson's disease: frequently in middle-aged and elderly persons; limb trembling, rigidity, hypokinesia, limb disturbance. Imaging changes as follows: low signal on T1-weighted images, high signal on T2-weighted images in the substantia nigra of the midbrain; no abnormal SCA3 by DNA analysis.

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