Journal of the Neurological Sciences xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Short communication

A novel ferritin light chain mutation in neuroferritinopathy with an atypical presentation ☆

Katsuya Nishida [a,*], Holly J. Garringer [b], Naonobu Futamura [a], Itaru Funakawa [a], Kenji Jinnai [a], Ruben Vidal [b,**], Masaki Takao [c,d]

[a] Department of Neurology, National Hospital Organization Hyogo-Chuo National Hospital, 1314 Ohara, Sanda 669-1592, Japan
[b] Department of Pathology & Laboratory Medicine, Indiana University School of Medicine, 635 Barnhill Drive MS A174, Indianapolis, IN 46202, USA
[c] Department of Neuropathology (The Brain Bank for Aging Research), Tokyo Metropolitan Geriatric Hospital, 35-2 Sakae-cho, Itabashi City, Tokyo 173-0015, Japan
[d] Tokyo Metropolitan Institute of Gerontology, 35-2 Sakae-cho, Itabashi City, Tokyo 173-0015, Japan

ARTICLE INFO

Article history:
Received 12 February 2014
Received in revised form 17 March 2014
Accepted 31 March 2014
Available online xxxx

Keywords:
Ferritin
Neurodegeneration
Hereditary ferritinopathy
Iron
Brain

ABSTRACT

Neuroferritinopathy or hereditary ferritinopathy is an inherited neurodegenerative disease caused by mutations in *ferritin light chain* (*FTL*) gene. The clinical features of the disease are highly variable, and include a movement disorder, behavioral abnormalities, and cognitive impairment. Neuropathologically, the disease is characterized by abnormal iron and ferritin depositions in the central nervous system. We report a family in which neuroferritinopathy begins with chronic headaches, later developing progressive orolingual and arm dystonia, dysarthria, cerebellar ataxia, pyramidal tract signs, and psychiatric symptoms. In the absence of classic clinical symptoms, the initial diagnosis of the disease was based on magnetic resonance imaging studies. Biochemical studies on the proband showed normal serum ferritin levels, but remarkably low cerebrospinal fluid (CSF) ferritin levels. A novel *FTL* mutation was identified in the proband. Our findings expand the genetic and clinical diversity of neuroferritinopathy and suggest CSF ferritin levels as a novel potential biochemical marker for the diagnosis of neuroferritinopathy.

1. Introduction

Neuroferritinopathy or hereditary ferritinopathy [17] is an autosomal dominant movement disorder caused by mutations in the *ferritin light chain* (*FTL*) gene on chromosome 19q13.3. Neuropathologically, the disease is characterized by the abnormal depositions of iron and ferritin in the brain, particularly in the basal ganglia. Thus far, six different mutations in exon four of the *FTL* gene have been reported, all affecting the FTL polypeptide C-terminus [6,7,11,16,18,23]. Clinically, the disease presents as a middle-age-onset chorea and dystonia. Clinical presentation may also include extrapyramidal and pyramidal tract signs as well as cerebellar ataxia, dysautonomia, cognitive decline, and psychiatric symptoms; however, the clinical presentation is highly variable both within and between families [2,3,5,14,21,25]. Several *in vitro* and *in vivo* studies (reviewed in [17]) have implicated at least two key toxic mechanisms in the pathogenesis of the disease: abnormal iron metabolism and generation of free radicals, and abnormal ferritin aggregation. These two mechanisms may be acting together to lead to neurodegeneration and thus to the progression of the disease. The mutant FTL polypeptide can cause deregulation of cellular iron metabolism (ferritin loss of function), oxidative stress, and overproduction of ferritin polypeptides (a positive feedback loop), while excess iron and ferritin could trigger the formation of ferritin aggregates, which may physically interfere with normal cellular functions (gain of a toxic function) [24].

Herein, we report the identification of a novel mutation in the *FTL* gene in a Japanese family with neuroferritinopathy, and highlight the utility of T2-weighted magnetic resonance imaging and biochemical studies as potential biomarkers for the diagnosis of the disease.

2. Subjects and methods

2.1. Case report

The proband, a 44-year-old, right-handed Japanese female, presented initially with chronic headaches at the age of 42. She was allergic to milk, wheat, and eggs. There was no history of anoxia at birth or carbon monoxide poisoning and no family history of neurodegenerative disorders or

☆ Full financial disclosures of all authors: This study was supported by grants from the National Institute of Neurological Disorders and Stroke (NS050227 and NS063056 (RV)), and by grants for Research on Measures for Intractable Diseases (H24-nanchi-ippan-063) and the Comprehensive Brain Science Network (MT).

* Correspondence to: K. Nishida, Department of Neurology, National Hospital Organization Hyogo-Chuo National Hospital, 1314 Ohara, Sanda 669-1592, Japan.
** Correspondence to: R. Vidal, Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, 635 Barnhill Drive MSB A136, Indianapolis, IN 46202, USA. Tel.: +1 317 274 1729; fax: +1 317 278 6613.
E-mail addresses: nishida@hch.hosp.go.jp (K. Nishida), rvidal@iupui.edu (R. Vidal).

http://dx.doi.org/10.1016/j.jns.2014.03.060

consanguineous marriage (Fig. 1A). At the age of 43, she demonstrated psychiatric disturbances such as emotional lability, and was diagnosed with panic disorder at a mental health clinic. At the age of 44, she experienced difficulty in speaking and walking as well as clumsiness in her left arm. Neurological examination showed emotional incontinence, mild cognitive decline (Full Scale Intelligence Quotient = 83, Verbal Intelligence Quotient = 84, Performance Intelligence Quotient = 84), slurred speech, bilateral hyperextensibility and hypotonus, left-sided cerebellar ataxia, hyperreflexia, and extensor plantar response. Cranial nerve examination showed slow eye saccades and involuntary movement of tongue. Rigidity, spasticity, tremor, dystonia, chorea, and parkinsonism were not observed initially. Her gait was unsteady (not wide-based); she exhibited some difficulty during tandem gait. Gait disturbance gradually progressed, and her gait became increasingly unsteady with a tendency to fall. Limb weakness, sensory disturbance, and bladder or rectal disturbances were not observed. Orthostatic hypotension was detected by the head-up tilt test (blood pressure: 121/79 mm Hg in the supine, 90/63 mm Hg in the standing position with dizziness). Several months after the initial presentation, oromandibular, orolingual, and left dominant arm dystonia, tongue dyskinesia, tongue wiggling movements, tongue biting, and dysphagia developed. Her serum ferritin levels were 20 ng/mL (normal range, 5–204 ng/mL). Serum levels of iron, copper, ceruloplasmin, hemoglobin, and vitamin E were normal. Routine hematological studies and thyroid function studies were also normal. No tumor markers or autoimmune antibodies such as anti-glutamic acid decarboxylase and anti-gliadin were detected. An abdominal computed tomography (CT)/MRI showed the presence of a left ovarian cyst; however, tests for paraneoplastic antibodies such as anti-Hu and anti-Yo in serum and CSF were negative. Analysis of CSF revealed a remarkably low ferritin level (<1.00 ng/mL, normal range: 6.68 ± 0.93 ng/mL). Several medications, including trihexyphenidyl, benzodiazepine, valproate, and muscle relaxants, were attempted; however, they did not improve the patient's symptoms. Herbal medicine (Shakuyaku-kanzo-to) relieved the left finger hyperextensibility due to dystonia.

2.2. Methods

MRI, laboratory tests including measurement of serum ferritin levels and genetic analysis were performed on the proband and her family (parents and brother). CSF ferritin levels were also measured on the proband. Brain MRI was performed using a 1.5-Tesla system (GyroscanAchieva; Philips Medical Systems, Best, The Netherlands). T1-weighted (TR = 600 ms, TE = 12 ms), T2-weighted (TR = 4423 ms, TE = 100 ms), and T2*-weighted (TR = 640 ms, TE = 23 ms) sequences were acquired in the transverse plane. In the proband, susceptibility-weighted imaging (SWI) based on 3-dimensional T1-weighted fast field echo (3DT1FFE) and ^{123}I-iodoamphetamine (IMP) single photon emission tomography (IMP-SPECT) were also performed.

After informed consent was obtained, genomic DNA was extracted from a 500 μL saliva aliquot collected with the Oragene Discover Collection Kit (DNAgenotek, Ottawa, Canada) using prepIT-C2D Genomic DNA MiniPrep Kit (Oragene) in accordance with the manufacturer's instructions. PCR amplification was performed on 0.15 μg of genomic DNA to amplify all four exons of the *FTL* gene using the oligonucleotide primer pairs: Exon 1 (352 bp) F: 5′-ACGTCCCCTCGCAGTTCGGCGG-3′ and R: 5′-GGAGGTGCGCAGCTGGAGG-3′; Exon 2 (327 bp) F: 5′-GGTAAACA GAGGGCGGAGTC-3′ and R: 5′-ACCGAACTCAATCTCCCAGA-3′; and Exons 3 & 4 (660 bp) F: 5′-TGTAGGTTTAGTTCTATGTG-3′ and R: 5′-AAGCCCTATTACTTTGCAAG-3′; at 2 mmol of each oligonucleotide, 200 μmol dNTPs, and 1.5 mM $MgCl_2$ in a 50 μL reaction solution, and cycled for 35 cycles of 94 °C for 30 s, 45 °C for 45 s, and 72 °C for 45 s. DNA fragments were separated on a 1% agarose gel and visualized by ethidium bromide staining, and the corresponding bands excised and purified using the GeneJET Gel Extraction Kit (Thermo Scientific, Lithuania). DNA sequencing was performed in both directions as described [23] using CEQ 8000 GeXP Genetic Analysis System and Software (Beckman Coulter). Amplification products of exons 3 and 4 were also subcloned into pCR 2.1 vector (Invitrogen, Carlsbad, CA) and transformed into One Shot® TOP10 Chemically Competent *E. coli* cells (Invitrogen) according to the manufacturer's protocol. Recombinant plasmid DNA was isolated from 10 clones of different PCR reactions and sequenced in both directions as described [23].

3. Results

3.1. Imagining studies

Brain MRI of the proband showed symmetrical hyperintense areas surrounded by hypointense areas in the bilateral posterior globus pallidus and putamen at age 42 (Fig. 1B). At age 44, brain CT revealed symmetrical low density areas in the bilateral basal ganglia (Fig. 2A). T1-weighted MRI images demonstrated cortical atrophy of the cerebrum and cerebellum. T2-weighted MRI revealed symmetrical hyperintense areas surrounded by hypointense areas in the bilateral posterior globus pallidus and putamen, whereas T2*-weighted and SWI-MRI revealed hypointense areas in the globus pallidus, putamen, thalamus, red nucleus, dentate nucleus, and cerebral cortex (Figs. 2 and 3). No spinal cord lesions were observed. ^{123}I-iodoamphetamine single photon emission tomography (IMP-SPECT) revealed bilateral mild hypoperfusion of the cerebellum and right hypoperfusion of the basal ganglia (Fig. 4). Cardiac ^{123}I-metaiodobenzylguanidine (MIBG)

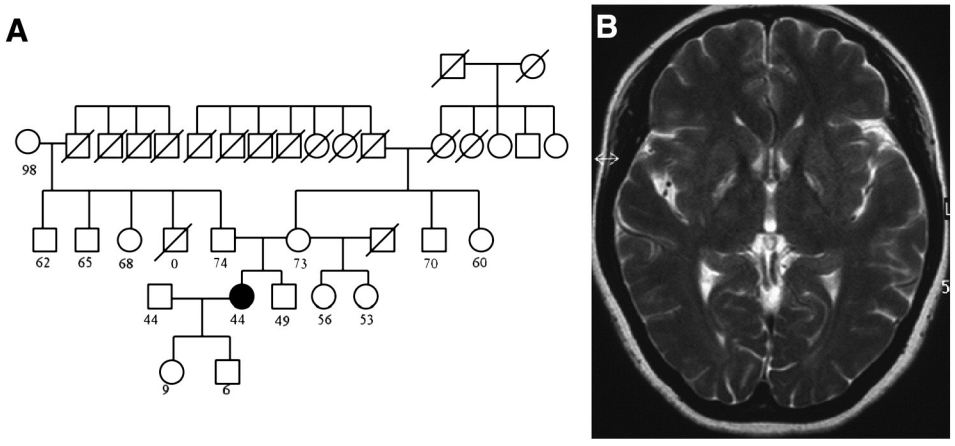

Fig. 1. A. Pedigree of the family. The square symbols represent males and the circle symbols represent females. The filled symbol represents the proband. The symbol "/" represents deceased individuals. The numbers under the symbols represent current age. B. MRI of the proband at the age of 42, before onset of neurological symptoms.

Please cite this article as: Nishida K, et al, A novel ferritin light chain mutation in neuroferritinopathy with an atypical presentation, J Neurol Sci (2014), http://dx.doi.org/10.1016/j.jns.2014.03.060