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Short communication

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

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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

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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)

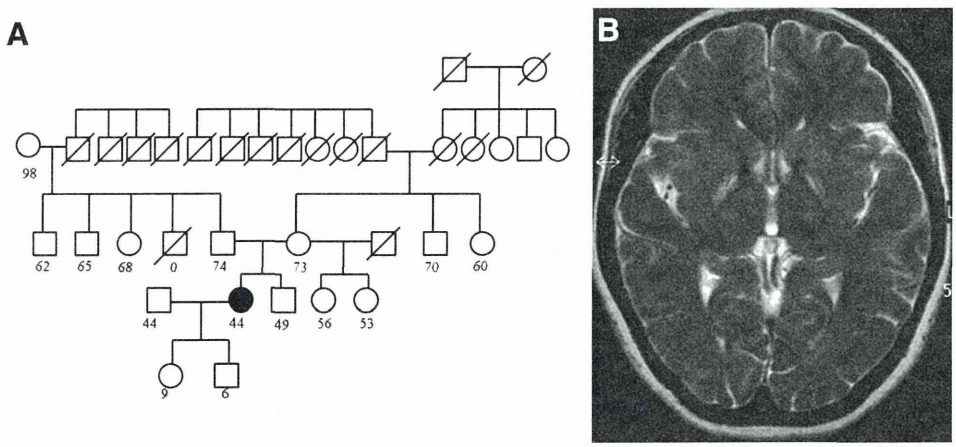

Fig. 1. A. Pedigree of the family. The square symbols represent males and the circle symbols represent females. The filled symbol represents the proband. The symbol "/" represents deceased individuals. The numbers under the symbols represent current age. B. MRI of the proband at the age of 42, before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

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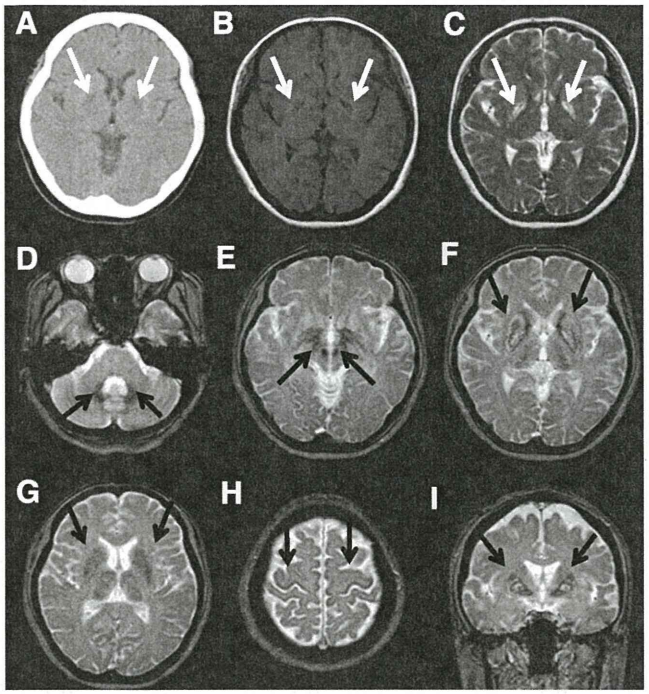

Fig. 2. CT and MRI scans of the proband at the age of 44. A. Axial head CT showing symmetrical hypodense lesions in the basal ganglia, bilaterally. B. T1-weighted axial MRI image showing symmetrical hypointense areas in the basal ganglia, revealing the degenerative and partially cystic nature of the lesions. C. T2-weighted axial MRI image showing symmetrical hyperintense areas encircled by hypointense areas in the bilateral posterior globus pallidus and putamen. D–H. T2*-weighted axial MRI images showing hypointense areas in the dentate nucleus (D), red nucleus (E), globus pallidus, putamen, caudate, thalamus (F, G), and cerebral cortex (H), suggesting increased deposition of ferritin in multiple nuclei. I. T2*-weighted coronal MRI image showing a hypointense rim and a hyperintense center reminiscent of the "eye of the tiger" sign in the basal ganglia.

uptake was not decreased (H/M ratios for early phase: 2.70 and delayed phase: 2.78).

3.2. Ferritin measurements

Serum ferritin values in the proband, her parents and brother were in the normal range (5–204 ng/mL). CSF ferritin levels in the proband were remarkably low (<1.00 ng/mL, normal range: 6.68 ± 0.93 ng/mL).

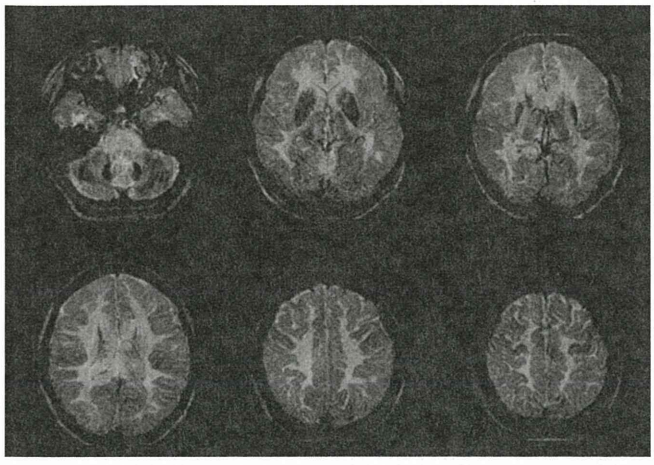

Fig. 3. Axial susceptibility-weighted images (SWI) show hypointense areas in the globus pallidus, putamen, caudate, thalamus, red nucleus, dentate nucleus, and motor cortex.

3.3. Genetic analysis

The sequence of exons 1, 2 and 3 of the *FTL* gene and adjacent intronic sequences were found to be normal. Gel migration analysis of a PCR product comprising exons 3 and 4 of *FTL* revealed the presence of two amplification products of 660 and 676 bp in the proband, whereas the control sample showed only the 660 bp product (Fig. 5A). DNA sequencing of exon 4 of the *FTL* gene in the proband revealed a novel mutation in one allele of the *FTL* gene, c.468_483dupTGGCCCGGAGGCTGGG (Fig. 5B). The position of the mutation was near to that of previously reported Japanese [18] and Italian [22] mutation, c.469_484dupGGCCCGGAGGCTGGGC, shifted one nucleotide to the 5′ of the previously reported mutation sequence. To confirm the presence of the mutation found in the proband, the PCR products of the amplification of exons 3 and 4 were subcloned and recombinant plasmid DNA isolated and sequenced in both directions. Sequence analysis confirmed the presence of the 16 bp insertion in the mutant allele (Fig. 5B). As a result, the predicted mutant polypeptide (p.Leu162TrpfsX24) and the previously reported mutant polypeptide (p.Leu162ArgfsX24) [18,22] have different amino acids at codon 162 (tryptophan in p.Leu162TrpfsX24 and arginine in p.Leu162ArgfsX24) but identical sequence and length of the C-terminus of the mutant protein (Fig. 5C). The mutation was not observed in the proband's parents or her brother, who had normal serum ferritin levels and unremarkable MRI. Genetic analyses for mutations associated with spinocerebellar degeneration, such as spinocerebellar ataxias (*SCA1*, *SCA2*, *SCA3*, *SCA6*, *SCA7*, *SCA8*, *SCA10*, *SCA12*, *SCA17*), and dentatorubral-pallidoluysian atrophy, were negative. No mutations were observed in the pantothenate kinase 2 (*PANK2*) gene associated with pantothenate kinase-associated neurodegeneration (*PKAN*).

4. Discussion

We report a Japanese female with neuroferritinopathy harboring a novel mutation in exon 4 of the *FTL* gene. The proband by age 42 presented with chronic headaches, developing during the next two years progressive orolingual and arm dystonia, dysarthria, cerebellar ataxia, pyramidal tract signs, and psychiatric symptoms. Previously reported individuals with neuroferritinopathy show dystonia and dysarthria although our proband did not present initially with dystonia; she gradually exhibited progressive orolingual and limb dystonia over several months. It has been previously reported that the clinical presentation of the disease may be highly variable not only between families but also within a family [2–5,14,21,24,25], suggesting that a heterogeneous presentation may be a characteristic of neuroferritinopathy. Although dystonia and dysarthria are the main manifestations of this condition, the disease cannot be diagnosed only on the basis of clinical symptoms alone because many other clinical features such as pyramidal signs, cerebellar ataxia, dysautonomia, cognitive impairment, and psychiatric symptoms may be observed in affected individuals.

The MRI findings in patients with neuroferritinopathy change with disease progression according to clinical stage. In the early clinical stage, hypointense lesions in the basal ganglia are observed on T2*-weighted images and SWI, particularly in the globus pallidus and putamen [12]. With disease progression, the T2* signal loss extends to the dentate nucleus, red nucleus, substantia nigra, thalamus, caudate nucleus, and cerebral cortex [19]. In later stages, hyperintense lesions in the basal ganglia reflecting neuronal loss and gliosis due to iron deposition and ferritin accumulation can be seen on T2-weighted images. In our case, hypointense lesions on T2* images indicative of widespread abnormal iron deposition were observed in the globus pallidus, putamen, caudate, thalamus, red nucleus, dentate nucleus, and motor cortex. In addition, T2-weighted images revealed mild cerebral and cerebellar cortical atrophy and symmetrical hyperintense areas surrounded by hypointense areas in the bilateral posterior globus pallidus and putamen. The present individual has not revealed the bilateral large cavities observed in advanced-stage disease, and

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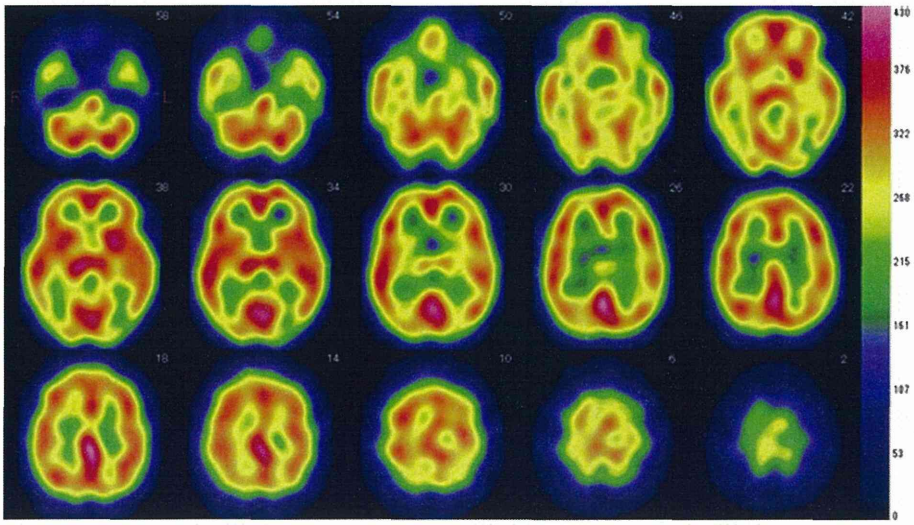

Fig. 4. ^{123}I-iodoamphetamine single photon emission tomography (IMP-SPECT) shows hypoperfusion in the bilateral cerebellum and right basal ganglia.

interestingly, in our case, brain MRI showed the hypointensity in the basal ganglia on T2-weighted MRI images before symptom onset (Fig. 1B). A previous study of young carriers of the c.460InsA mutation (6–36 years old) showed the presence of hypointense signals on T2*-weighted images indicative of iron deposition [10], strongly suggesting that iron deposition may actually begin several decades before the appearance of clinical symptoms. Coronal T2*-weighted images showed a combination of a hypointense rim and hyperintense center in the basal ganglia called the "eye of the tiger" sign, which reflects iron deposition in the basal ganglia on both T2-weighted MRI and T2*-weighted MRI. This sign has been considered to be pathognomonic of PKAN, but it has also been observed in other neurodegenerative diseases such as cortical–basal ganglionic degeneration, multiple system atrophy, progressive supranuclear palsy, pure akinesia with gait freezing [9], and neuroferritinopathy [13]. These studies point out a substantial degree of overlap between neuroferritinopathy and other NBIA diseases in terms of presentation on T2*-weighted images and SWI. Thus, the diagnosis of neuroferritinopathy may not be made solely on the basis of neuroimaging. Brain perfusion studies in the proband (IMP-SPECT) showed asymmetric hypoperfusion of the cerebellum and the basal ganglia with predominance on the right side. This finding is likely to reflect the laterality of neurological symptoms.

Low serum ferritin levels were initially reported in individuals with the c.460InsA mutation [6]; however, low serum ferritin levels are not always observed in individuals with neuroferritinopathy [4,6,7,14,16,18,20–22,25]. Serum ferritin levels in our premenopausal female patient

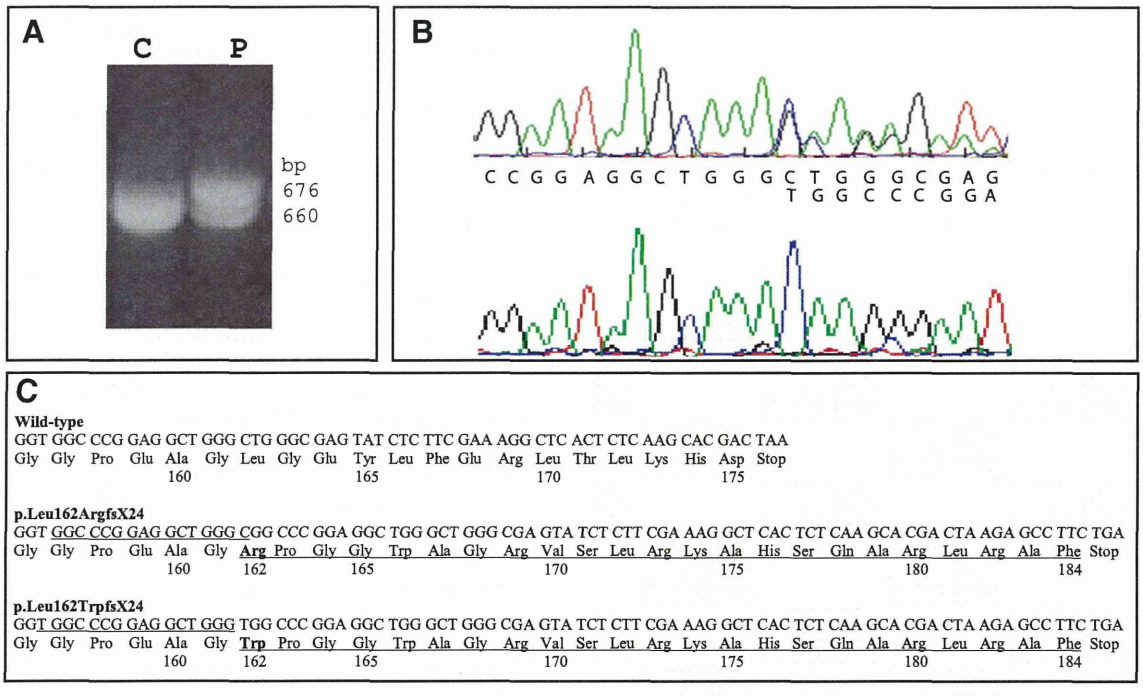

Fig. 5. Genetic analyses of the *FTL* gene in the proband. A. Analysis of PCR products containing exons 3 and 4 of the *FTL* gene by agarose gels shows that the proband (P) has an additional band (676 bp) in addition to the normal amplification product of 660 bp observed in control individuals. B. Direct sequencing of genomic DNA from the proband (top) and cloned DNA containing the mutant allele (bottom) clearly showing the beginning (thymine) of the 16-nucleotide duplication (c.468_483dupTGGCCCGGAGGCTGGG). Wild-type nucleotide sequence is indicated on top and the duplicated sequence on bottom. C. Wild-type sequence (top) and mutant sequences (bottom) of the *FTL* gene showing the 16-bp duplication. The duplicated nucleotide sequences are underlined. The p.Leu162ArgfsX24 was previously reported in Japanese [18] and Italian [22] affected individuals. The predicted C-terminal amino acid sequences are indicated. Amino acid 162 is in bold.

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were 20 ng/mL (normal range, 5–204 ng/mL), and thus not found to be significantly decreased compared to normal range. Interestingly, CSF ferritin levels in our patient (<1.00 ng/mL) were significantly lower than normal levels in control individuals (6.68 ± 0.93 ng/mL), and also lower than ferritin levels in patients with restless leg syndrome (below 3.5 to 4.1 ng/mL), a disease associated with lower CSF ferritin levels [8,15]. Further studies will demonstrate whether the measurement of CSF ferritin levels could be used as a novel biomarker for neuroferritinopathy.

Patients carrying the mutation c.469_484dupGGCCCGGAGGCTGGGC have been described in Japan [18] and Italy [22]. This mutation is similar to the one presented here, with one nucleotide difference that causes a single amino acid change between both mutant ferritins at codon 162 (Fig. 5C); however, we have observed a significant degree of variability in the presentation of the disease between the affected individuals from the Japanese family (tremor was the main characteristic symptom) [18], the Italian patient (behavioral changes and tremor) [22] and our case. This difference may not be explained at the protein level, since the general structure of the spherical protein shell seems to be maintained in mutant ferritin as was seen by X-ray crystallography [1]. All known mutations associated with the disease may have a similar effect: the disruption of the 4-fold pores, making the pores unstable and leaky, and the gradual aggregation of ferritin and iron into a precipitate mediated by the unraveled and extended mutant C-termini (reviewed by [17]). Further research will be needed to understanding the effect of amino acid variations between mutant C-terminal sequences in the pathogenic process of the disease and the role of genetic and environmental factors in the disease.

In conclusion, our findings expand the genetic and phenotypic diversity of neuroferritinopathy. The observed clinical features of the disease in this family further imply the importance of testing for *FTL* mutations in patients with distinctive MRI findings. Further studies will determine the clinical significance of low levels of CSF ferritin and the potential value of determining CSF ferritin levels for the diagnosis of the disease.

Conflict of interest

None.

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特集　鉄と神経疾患

ニューロフェリチノパチーの病理*

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はじめに

ニューロフェリチノパチー(neuroferritinopathy)はきわめて稀な常染色体優性遺伝性の疾患である．剖検症例も限られている．呼び名としてneuro-とついているが，剖検症例の検討では全身臓器にも特徴的な病理所見を認めることから，フェリチノパチー(ferritinopathy)がよいとする立場もある．

われわれは，本邦におけるニューロフェリチノパチーの実態を明らかにするために，厚生労働科学研究費補助金(難治性疾患等克服研究事業)により，「神経フェリチン症の実態調査と診断基準の構築に関する研究班(H24-難治等(難)－一般-063)代表：高尾昌樹」を立ち上げて，検討を行っているところである[1]．特に，図1に示すような本症の診断指針を作成し，全国の主たる施設などへのアンケート調査なども施行し，また，疑わしい症例に関しては研究班における診断支援を行ってきた．平成24年度報告書作成時点では，遺伝子変異を確認できている確定症例は論文報告例も含め4例であったが，今後症例の増えることも考えられる[1)~3)](その後も検討症例の追加を行い新規遺伝子変異なども発見はされているが，ニューロフェリチノパチーではないことが確認された症例もあり，本症の診断には遺伝子診断が必須である)．特に，本邦では現時点で剖検を施行したことが確認できた症例はなく，既存のライブラリーにも本症を示唆する症例を現段階では認めていない．したがって，ここで述べる神経病理所見は，すべて諸外国の報告例によるものである．すなわち自身が経験した症例[4]，および論文を通しての記載以外によりどころがないので，それらをまとめたものをここに記載している．特に，原著報告以外に，本疾患の神経病理所見に関しては本誌2010年(73巻2号)にも記載させていただいたが，それ以降の新規病理症例の報告はないので[5]，以前の記載と内容が一致する点もあることをご理解いただきたい．

過去の報告例に関して

詳細は本誌の「ニューロフェリチノパチーの臨床」で述べられると思うが，過去の報告の中でいくつかの注意点を記載する．2005年にMacielらにより報告された症例は[6]，他の既報告例での遺伝子変異がフェリチン軽鎖遺伝子のエクソン4に存在することに対して，エクソン3に存在すること，また母親にも同一の遺伝子変異がみられ

* Pathology of neuroferritinopathy.

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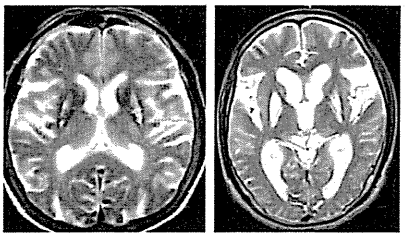
概念：神経フェリチン症は，フェリチン軽鎖遺伝子変異により，変異フェリチンと正常フェリチンからなる封入体が，神経細胞やグリア細胞を中心に蓄積し，多彩な錐体外路徴候，錐体路徴候，認知障害など多彩な神経症候を長期にわたり認める．頭部MRIで両側基底核の変性所見（添付図）（特に病期が進行すると嚢胞性変化をきたす）が特徴的である

臨床症候
1．振戦，小脳失調，錐体路徴候，錐体外路徴候，認知障害を認める
2．20歳代から発症することがある（一部10歳代での発症もある）
3．1の症状は，数十年にわたり，緩徐に，さまざまな程度で出現する
4．家族歴があることがあるので，慎重に確認する

画像診断
1．頭部MRIのT2強調画像で，大脳基底核に中心が高信号，周囲が低信号の病変を認め（2例の参考画像を呈示した），周囲の低信号病変はT2*強調画像あるいは磁化率強調画像（SWI）で明瞭となることがある（→パントテン酸キナーゼ関連神経変性症に含まれるNBIA1などと異なり，神経フェリチン症では淡蒼球，被殻，視床，歯状核など広範に低信号となることが多いとされている）
（参考：頭部CTだけしか施行できない場合，基底核に対称性の低吸収域を認めることが考えらる）



病理診断
1．神経病理学的にフェリチンの神経細胞内，グリア細胞内，神経細胞内での沈着を確認する

遺伝子診断
1．フェリチン軽鎖遺伝子変異が確認できればほぼ確定できる

その他
1．血清フェリチン値の低下（文献的には20 ng/ml以下となっている可能性が高い）

ヘモグロビン値，血清鉄は正常であることが一般的である

図1　神経フェリチン症の診断指針（試案）（文献[1]より引用）

MRIでも異常所見があるとされているが，臨床的に無症候であることから，ニューロフェリチノパチーであるかどうか，特にこの変異が疾患関連性であるかどうかは十分な検討が必要であると考えられている．また，2012年にShahらにより，臨床症候と画像所見に関して，ニューロフェリチノパチーが考えられる症例報告は[7]，その後，本総説の共著者Vidalにより直接Shahらへ問い合わせがなされ，フェリチン軽鎖遺伝子には変異を認めなかったことが確認されているので，他の疾患であることが考えられる．したがって，Shahらの報告の画像所見はニューロフェリチノパチーのものではない[7]．

このように，ニューロフェリチノパチーはわれわれの作成した診断指針（図1）でも，特徴的な画像所見を認めた場合に診断を疑うとしているが，確定診断には遺伝子診断が必須であろう．また，血清フェリチン値の低下を診断補助項目として検討されているが[8]，正常値の幅が大きいこと，医療機関，検査機関による違いなど，まだ考慮されなければならない点が多く，結論をだすにはまだ症例数が少ないものと考えられる．

神経病理所見

神経病理所見がフェリチン軽鎖遺伝子変異の確認とともに報告されているのは，*c.442dupC*，*c.460dupA*，*c.497_498dupTC* mutations[4][9]~[13]の3家系の中で剖検が施行された症例に限られている．われわれは，*c.497_498dupTC* mutationsの症例に関して，報告する機会を得てきた[1][4][5][9][10][14]．

特に遺伝性疾患の場合は，同一遺伝子における異なる部位の変異，あるいは同一の変異でも家系内においてすら，臨床病理学的には表現形の差が常に経験される．今後，症例が蓄積された場合，ここに述べたような病理学的所見と異なるものが発見されてくる可能性もある．また，全身臓器における検討は十分に行われていない．本総説で提示した神経病理所見はすでに論文で報告した症例と同一のものであるが，病理写真はすでに報告したもの，あるいは未報告のもの

を含めて呈示したので，既報告論文と比較をしていただきたい[4]．

*c.497_498dupTC*変異

本遺伝子変異はフランス家系で発見され，臨床病理所見が報告されたものである[9)4)15)]．20歳代ごろから始まる姿勢時振戦が特徴で，その後，安静時振戦，小脳症状，歩行障害，構音障害，パーキンソニズム，認知機能障害を認め，最終的には，小脳症状，パーキンソニズム，不随意運動，皮質下認知症をさまざまに組み合わさった状態となる．また，錐体路徴候，眼球運動障害，うつ状態も認めることがある．発症年齢は20～50歳代と幅広く，全経過が最長で30年程度までになった症例もある．したがって，臨床症候は比較的緩徐進行性の疾患であると考えられる．

神経病理所見

1．肉眼所見

論文に報告した症例は[4]，脳重は1,120 g で，大脳半球はびまん性に萎縮していた．特に前頭葉の萎縮が強かった．割面では，側脳室は軽度拡大し，尾状核と小脳は軽度萎縮していた．被殻には小さい嚢胞性変化を認めた．黒質の色素沈着は軽度の脱失を認めた．脳主幹動脈には明らかな異常はなかったが，血管内皮面が白色に肥厚している所見を認めた．

2．顕微鏡的所見

神経細胞の脱落は，大脳皮質，扁桃体，視床，黒質，青斑核においては軽度であった．同部位においてはグリオーシスも軽度であった．一方，尾状核，被殻，淡蒼球においては，神経細胞の脱落は高度であった．特に被殻においては，小嚢胞性変化(1.5 mm程度)を多数認め，全体として大きな嚢胞を形成するかのようにみえた．大脳白質においては，ミエリン染色でいわゆる髄鞘の淡明化を認め，有髄線維の脱落が示唆された．同部位におけるグリオーシスは中等度であった．小脳においては，皮質の神経細胞脱落は比較的軽度であったが，小葉間白質においてグリオーシスが中等度認められた．

特徴的でかつ印象的な所見は，主に核内と一部の胞体内にみられる封入体であった．この封入体はグリア細胞と一部の神経細胞に広範かつ高度に認められた(図 2)．核内封入体はヘマトキシリンエオシン染色で均質なきわめて淡い好酸性を呈し，鉄染色でも均質な淡い青色に染められた．染色法を選択することで，2 価鉄と 3 価鉄の両者が含まれることが確認された．ボディアン染色では，典型的な嗜銀性は示さず，PAS，アルシアンブルー，髄鞘染色でも陽性所見を示さなかった．チオフラビン S を用いた蛍光染色でも，封入体は蛍光性を示さなかった．通常のホルマリン固定，パラフィン包埋後，ヘマトキシリンエオシン染色標本で測定した封入体のサイズは 2～35 μmであった．多くの場合，核内封入体は核内全体を占拠し，核内クロマチンなどの構造物を核膜へ押しやられたような形態を呈した．その結果と思われるが，封入体を有する核は通常の核よりもサイズが大きくみえた．大脳皮質では，核内封入体は神経細胞の周囲にある小細胞の中や，脳実質血管周囲のグリア細胞にも認められた(図 2)．

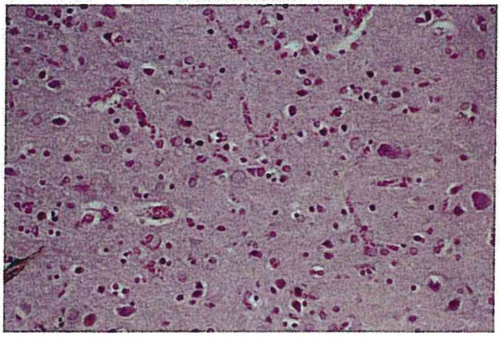


図 2　前頭葉皮質(FTL498-499InsTC症例)
グリア細胞の多くは核内(一部は細胞体内と考えられる)に，多数の淡い均質な封入体を認める．それぞれの核におけるクロマチンは核膜にむかって押しやられたような所見を呈する(HE染色，20×，以下倍率は対物レンズの倍率)．

肉眼的に嚢胞性変化を認めた本例の尾状核，被殻，淡蒼球は，封入体を最も多数認めた部位であった．この部位では，封入体の数はきわめて多く，核内にも認めるが，細胞質内や，それ以上に細胞外にも多数認め，封入体ではなく球状の沈着物といえる．一部の巨大な球状沈着は，小さい球状沈着物あるいは封入体の集合したもののようにみえた．また，核内封入体と比較し

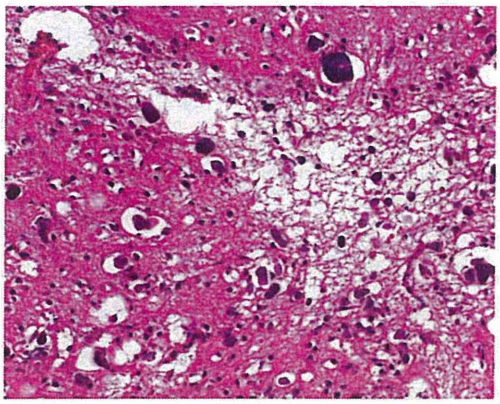


図3　被殻(FTL498-499InsTC症例)
図2と比較すると実質に大小さまざまな球状で強い好酸性を有する沈着物が多数に認められ，基質が嚢胞状に変性し空胞を呈している(HE染色，20×，文献[5]の図2と同一)．

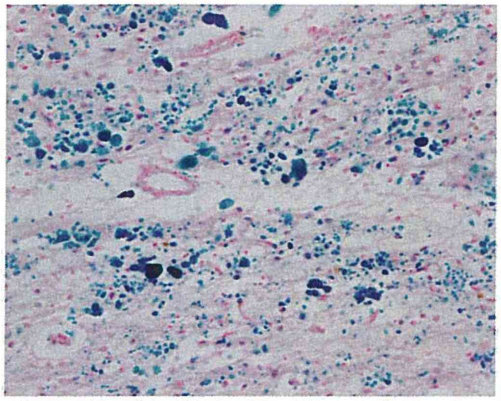


図4　被殻(FTL498-499InsTC症例)
図3と同一部位．鉄染色(3価鉄)で封入体(沈着物)は青色に染められる(Perl's染色，20×，文献[5]の図3と同一)．

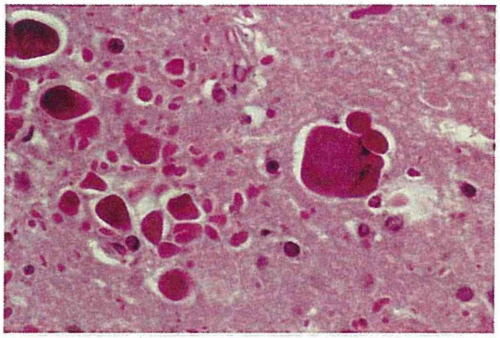


図5　被殻(FTL498-499InsTC症例)
好酸性の強い，さまざまな大きさと形態をした沈着物が多数認められる(HE染色，63×)．

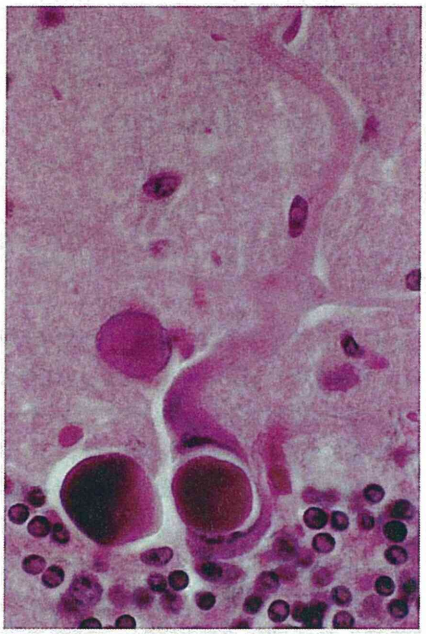


図6　小脳(FTL498-499InsTC症例)
小脳Purkinje細胞核内，顆粒細胞核内に好酸性の封入体を認める(HE染色，40×)．

てその色調は好酸性が強く，一部は好塩基性であった．さらに，多数の沈着物のために基底核の正常構造は破壊されていた(図3～5)．

小脳ではグリア細胞や神経細胞内，特に，Purkinje細胞や顆粒細胞の核内に多くの封入体を認めた．Purkinje細胞の封入体はその細胞体内や神経突起の中にも認めた．封入体はしばしば単一ではなく多数の封入体を同時に認めることもあった(図6)．小脳グリア細胞の中では特にGolgi細胞に多くの封入体を認めた．

大脳白質や小脳白質においてアストロサイトやオリゴデンログリアの核内に封入体を認めた．しかし，形態だけで，それがアストロサイトかオリゴデンログリアかどうかを区別することは必ずしも容易ではなかった．動脈や静脈の血管内皮細胞あるいは外膜の核内にも封入体を認めた．脳実質の血管周囲腔に存在する単核細胞の核内にも，封入体を同定できることがあった．脳室脈絡叢細胞の核内にも多数の封入体を認めた．脳室上衣細胞の核内には検討した範囲では明らかな封入体を認めなかった．

フェリチンに対する抗体を用いた免疫染色では，グリア細胞と神経細胞内封入体をすべての