Case Report

Pathology of frontotemporal dementia with limb girdle muscular dystrophy caused by a *DNAJB6* mutation

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Introduction

Frontotemporal lobar degeneration (FTLD) is a heterogeneous group of disorders characterized by disturbances of behavior and personality and different types of language impairment with or without concomitant features of motor neuron disease or parkinsonism [1]. FTLD is classified into three categories: frontotemporal dementia (FTD), progressive non-fluent aphasia (PNFA), and semantic dementia (SD) [2]. Moreover, FTD is classified into two categories: pick type and motor neuron disease (MND) type. FTLD is also classified into four neuropathological categories: FTLD-tau pathology type, FTLD-TDP43 pathology type, FTLD-FUS pathology type, and FTLD-other type [2]. Clinical FTD-Pick type is associated with FTLD-tau, TDP, or FUS, and some FTLD is caused by various genetic mutations.

Limb girdle muscular dystrophy (LGMD) is a progressive myopathy with necrosis and regenerative changes in skeletal muscle. LGMD Type 1D is caused by mutations in the *DNAJB6* (DnaJ homolog subfamily B member 6) gene [3]. Muscle pathology shows aggregates of TDP-43 and DNAJB6, together with rimmed vacuoles.

Here, we describe the phenotype and pathology of a case with frontotemporal

dementia (FTD) and LGMD caused by a *DNAJB6* mutation.

Case report

A 61 year old man complained of progressive lower limb muscle weakness, similar to his 3 siblings. Muscle CT demonstrated fatty degeneration, and the patient was diagnosed as LGMD. Genetic analysis indicated a heterozygous missense mutation of c.279C>G (p. Phe93Leu) in *DNAJB6* (Family D-1 in the ref.3) [4]. At 75 years old, he became stubborn and angry toward his family, and was suggested to have a behavioral variant of FTD [2]. These symptoms gradually progressed, and brain magnetic resonance images showed frontal atrophy with mild temporal atrophy (Fig. 1A,B). However, hippocampal atrophy and cerebellar atrophy were not observed (Fig. 1C,D). He developed pneumonia frequently, and died of septic shock at 76 years old. No mutations were observed in *VCP, PRGN* and *MAPT* genes and no abnormal expansion of *C9ORF72* was observed, all of which are common causes of hereditary FTD [1].

Total brain weight was 1,338 g, and mild neuronal loss was observed in the frontal and temporal cortex. Axonal structure was preserved (Suppl. Fig.1. A), and no

neurofibrillary tangles, senile plaques, Pick bodies, or Lewy bodies were observed in the brainstem, basal ganglia, frontotemporal cortex or white matter (Suppl. Fig.1. B-D). No TDP-43, Tau or FUS reactivity was observed in the central nervous system (CNS) (Suppl. Fig.1. E-H). However, there was a severe reduction of DNAJB6 immunoreactivity in the frontal cortex (Fig. 1E) compared to samples from normal control (Fig. 1F), an ALS-dementia patient without underlying proteinopathy (Fig. 1G), a TDP-43 positive ALS patient (Fig. 1H), and a TDP-43 negative ALS patient (Fig. 1I). DNAJB6 staining in the thalamus was preserved (Fig. 1J), and was similar to that in normal control (Fig. 1K). While intranuclear inclusion bodies were not detected, ubiquitin-positive aggregates were observed in the frontotemporal cortex (Fig. 2A), some of which appeared co-localized with p62 (Fig. 2B). The expression of LC3 in this patient (Fig.2C) was reduced compared to that of normal control (Fig. 2D).

Discussion

DNAJB6 is highly enriched in the CNS and inhibits polyglutamine aggregation of huntingtin. In addition, DNAJB6 is a component of Lewy bodies [5]. The availability of DNAJ proteins, co-chaperones to the Hsp70 machine, may be a rate-limiting factor in

handling diseased proteins within the cell. DNAJ proteins can affect the aggregation of disease-causing proteins [6]. These reports indicate that DNAJB6 is important in the etiology of CNS neurodegenerative diseases. However, there are no reports of FTD associated with mutations in *DNAJB6*. Anti-DNAJB6 antibody recognized both wild type and mutant DNAJB6 proteins (Suppl. Fig. 2) in all samples. The tissue samples were obtained at autopsy performed at similar time intervals after death, and the samples were stained simultaneously. Therefore, the severe reduction of DNAJB6 in this patient is considered to be significant. Moreover, ubiquitin-positive aggregates were detected in the cerebral cortex, consistent with FTD. However, neither TDP-43, Tau nor FUS reactivity were observed in the CNS. Therefore, this patient was diagnosed as "FTLD-other", or FTD with ubiquitin-positive aggregates and without tau, TDP, and FUS pathology [2]. Previous studies suggest that DNAJB6 facilitates ubiquitin-dependent degradation substrates. Therefore, this *DNAJB6* mutation may be closely related to neurodegeneration. Previously, we reported that DNAJB6 accumulated in the cytoplasm where it co-localized with members of the autophagy complex [4]. In this study, we showed a severe reduction of DNAJB6 staining, and a

mild reduction of LC3 staining of the cerebral cortex in our patient. These results suggest that this patient showed autophagy dysfunction in the brain, as well as in skeletal muscle. Although p62 immunoreactive inclusions have been observed in FTLD cases, with an underlying *C9ORF72* mutation [1], we did not find any mutations in *VCP, PRGN* and *MAPT* genes and no abnormal expansion of *C9ORF72*. In addition, the accumulations of ubiquitin were co-localized with p62, which is a common component of neuronal protein inclusions, a major characteristic of neurodegenerative diseases [7] and a key molecule managing autophagic clearance of polyubiquitinated proteins. In this study, the reduction of LC3 and p62 staining suggests a loss of autophagy function by the *DNAJB6* mutation. Although there were mild reductions of DNAJB6 staining and aggregation of p62 in the cerebellum, aggregation of ubiquitin and reduction of LC3 staining were not observed. This patient showed no ataxia or cerebellar atrophy. These results suggest that the cerebellar functional loss may be milder than that of the frontotemporal lobe.

Some genes are known to be associated with multisystem proteinopathy. Mutations in *VCP* cause vacuolar myopathy and FTD, and influence autophagy, an important

mechanism of dementias including FTD. Moreover, the muscle pathology of LGMD1D has similarity with that of vacuolar myopathy. Loss of VCP activity leads to the accumulation of ubiquitinated proteins and impaired autophagy. These studies and our findings suggest that dysfunction of ubiquitination and autophagy may be a common mechanism between *VCP* and *DNAJB6* mutations.

Conclusions

We report a pathological study of a frontotemporal dementia patient with LGMD caused by a *DNAJB6* mutation. A missense mutation in *DNAJB6* may cause not only LGMD, but also FTD through the dysfunction of autophagy. Further study is necessary to determine whether LGMD and FTD are caused by the same pathomechanism. Functional analysis of DNAJB6 at the molecular level should provide therapeutic benefits for LGMD and dementia.

References

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Figure Legends

Figure 1. Brain MRI and immunoreactivity for DNAJB6

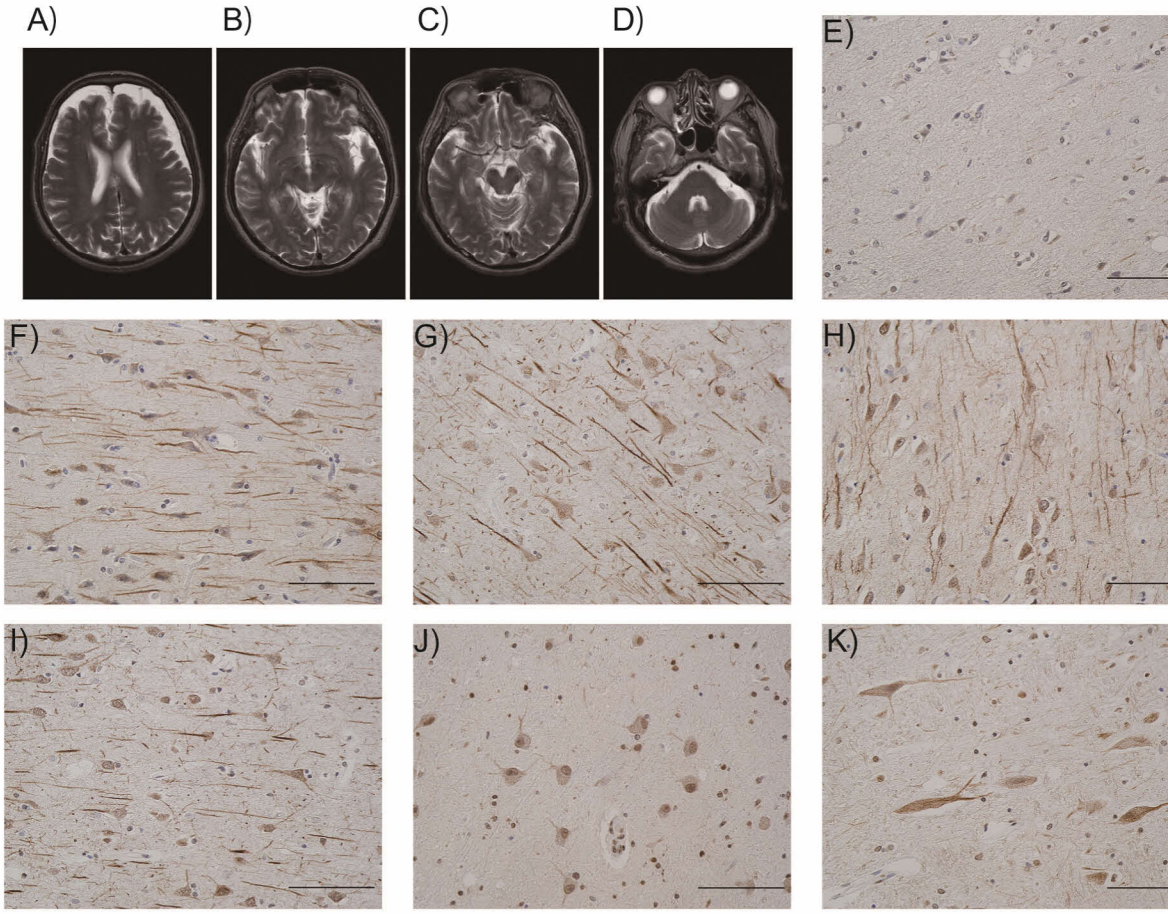
A-D: T2-weighted magnetic resonance images of the brain, showing bilateral frontotemporal atrophy, but not cerebellar atrophy.

E-K: Immunoreactivity for DNAJB6. Reduced DNAJB6 immunoreactivity in the left frontal lobe of this patient (E) is shown, compared with normal control (F), an ALS-dementia patient (G), a TDP-43 positive ALS patient (H) and a TDP-43 negative ALS patient (I). DNAJB6 immunoreactivity in the thalamus is not reduced (J) compared to normal control (K). Scale bar, 100 μm.

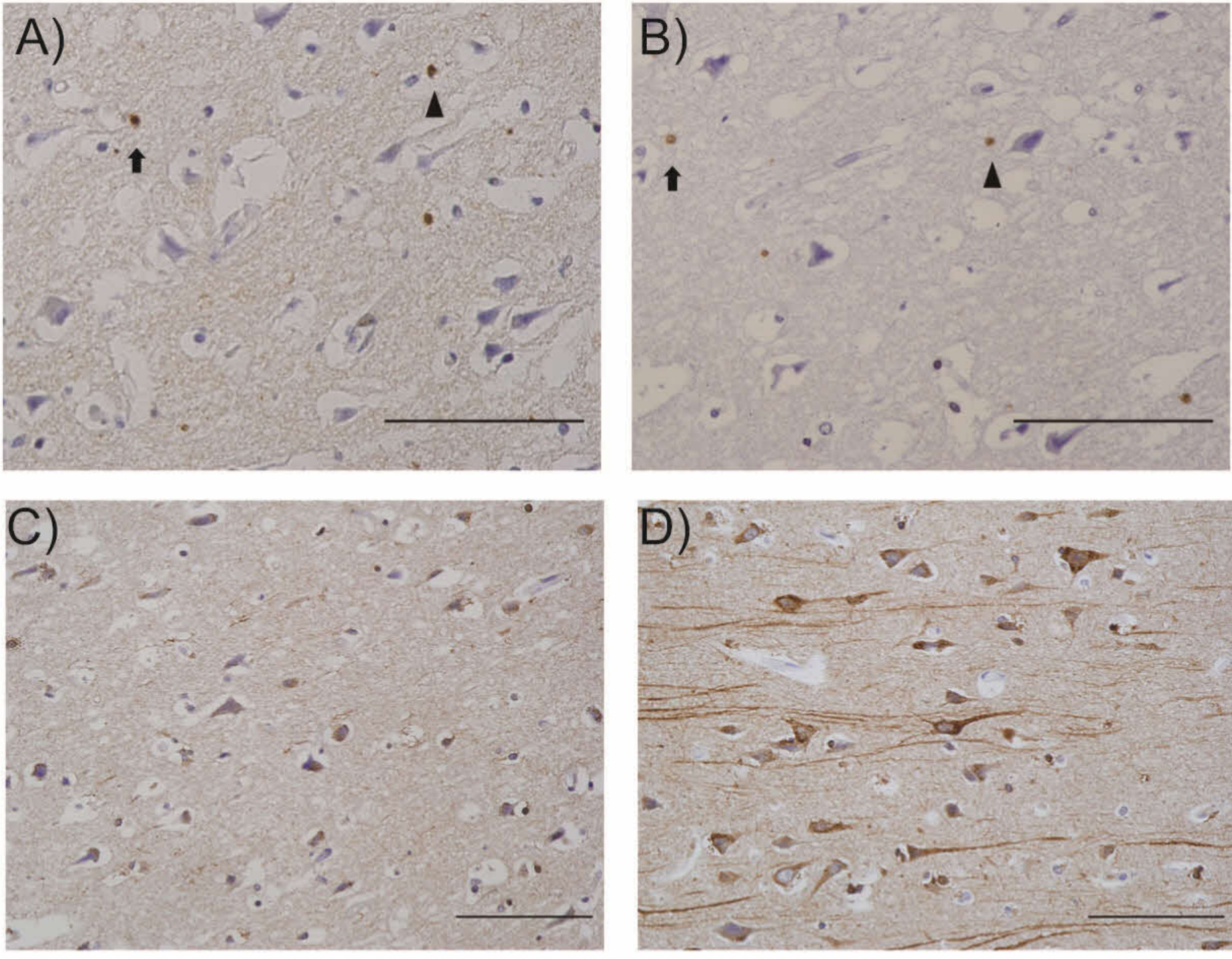
Figure 2. Immunoreactivity for p62 and LC3

Serial sections of grey matter in the left frontal lobe of this patient (A and B). A: Immunoreactivity for ubiquitin is observed in some aggregations. B: Some p62-immunoreactive aggregations are observed. Aggregations indicated by the arrow and arrow head seem to be co-localized. C and D: LC3 immunoreactivity in the gray matter in the left frontal lobe. The expression of LC3 immunoreactivity of this patient

(C) is reduced compared to that of normal control (D). Scale bar, 100μm



Yabe et al. Figure 1



Yabe et al. Figure 2