Adult-Onset Alexander Disease: New Causal Sequence Variant in the GFAP Gene

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Abstract

Objectives
Alexander disease (AD) is a rare disorder of the CNS. Diagnosis is based on clinical symptoms, typical MRI findings, and mutations in the glial fibrillary acid protein (GFAP) gene. In this case study, we describe a new mutation (p.L58P) in GFAP that caused a phenotype of adult-onset AD (AOAD).

Methods
In our outpatient clinic, a patient presented with cerebellar and bulbar symptoms after brain concussion. We used MRI and performed next-generation exome sequencing (NGS) to find mutations in GFAP to diagnose AD. The mutation was then transfected into HeLa cell lines to prove its pathogenicity.

Results
The brain MRI finding showed typical AD alterations. The NGS found a heterozygous variant of unknown significance in GFAP (c.173T>C; p.L58P). After transfecting HeLa cell lines with this mutation, we showed that GFAP-L58P formed pathogenic clusters of cytoplasmic aggregates.

Discussion
We have found a new mutation that causes AOAD. We recommend that AOAD is included in the diagnostic workup in adult patients with gait ataxia and cerebellar and bulbar symptoms in association with a traumatic head injury.

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Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

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Alexander disease (AD) is a rare, mostly sporadic, disorder of the CNS with degeneration of astrocytes. Adult-onset AD (AOAD) is more heterogeneous with nonspecific neurologic symptoms, mainly bulbar dysfunction, pyramidal signs, cerebellar ataxia, and palatal myoclonus.

Typical MRI findings are T2-hyperintensities of the periventricular white matter and atrophy of the spinal cord (tadpole sign), as well as contrast enhancement of the medulla oblongata and the spinal cord. Neuropathologically, AD is defined as having intracytoplasmic eosinophilic inclusions in astrocytes. Autopsy shows leukodystrophy and atrophy of the lower brainstem and upper cervical cord.

Mutations in the glial fibrillary acid protein gene (GFAP) are linked to AD. Its mutations lead to alterations in the protein, thereby causing accumulation and aggregation of precipitates of misfolded GFAP proteins.

Case

We report a 57-year-old male patient who experienced a progressive gait disorder, clumsiness, generalized muscle weakness, and intermittent position-independent vertigo. The patient first reported symptoms after a mild traumatic brain injury approximately 1 year before. Later, dysarthria and dysphagia also occurred. Autonomic dysfunction such as orthostatic hypotension and bowel or bladder dysfunction was not present. A family history of neurologic or neurodegenerative disorders was negative (Figure 1).

A clinical examination showed nystagmus, cerebellar dysarthria, an ataxic gait pattern, and dysmetria of the upper and lower extremities. In addition, the patient experienced mild bradykinesia on the right side, rigidity of the right upper extremity, brisk muscle reflexes, and positive pyramidal signs.

An MRI of the brain, immediately after the brain injury and 1 year before symptom onset, displayed atrophy of the medulla oblongata and cervical myelon (tadpole sign). A follow-up brain MRI revealed progressive T2-hyperintensities surrounding the fourth ventricle, symmetrical in the dentate nucleus and in the putamina, together with the tadpole sign (Figure 2A). In line with these findings, an automated brain volumetry analysis using VEOmorph software (VEObrain GmbH, Freiburg, Germany) detected atrophic changes in the medulla oblongata (Figure 2B).

Next-generation exome sequencing (NGS) identified a heterozygous variant of unknown significance in GFAP (c.173T>C; p.L58P). This variant substitutes an evolutionary, highly conserved amino acid, but the present substitution was not found in 125,748 GnomAD exomes and 15,708 GnomAD genomes. Several in silico analyses have predicted that the p.L58P substitution is likely to be pathogenic.

To test whether this new mutation c.173T>C; p.L58P in GFAP affects intermediate filament network formation, we introduced the point mutation in the plasmid encoding human GFAP (OriGene Technologies, SC118873 by mutagenesis (QuikChange XL Site-Directed Mutagenesis Kit, Agilent)). HeLa cells were transfected with either GFAP-WT.
Forty-eight hours after transfection, cell lysates were analyzed by Western blot to demonstrate the plasmid expression (Figure 3A), and cell monolayers were immunostained with an antibody against GFAP. Fluorescence images showed that wild-type GFAP assembled into bundled filaments that extended throughout the cytoplasm, whereas GFAP-L58P formed clusters of cytoplasmic aggregates (Figure 3B). Because cytoplasmic inclusions within astrocytes of patients with AD also contain the chaperones αB-crystallin, we costained the cells with an antibody against αB-crystallin. Written informed consent was obtained from the patient.

**Discussion**

Owing to the primarily nonspecific clinical symptoms of cerebellar ataxia, bulbar symptoms, and positive pyramidal signs, only the brain MRI led to the suspected diagnosis of AOAD. Using NGS, the heterozygote variant (c.173T>C; or GFAP-L58P gene were positive for αB-crystallin. Written informed consent was obtained from the patient.
p.L58P) in GFAP was found and categorized as variant of unknown significance. In vitro experiments demonstrated that this variant represented a novel mutation that affected the formation of the intermediate filament network and confirmed the diagnosis of AOAD.

Our patient stated that he experienced gait ataxia, clumsiness, and vertigo after minor brain concussion due to an accident. A correlation between AOAD and traumatic head injuries has been described before, with a latency between trauma and symptom onset of up to 10 years.7-9 Considering that severe symptoms may appear many years later, these incidences might be underrated. Similar to dystonia, we hypothesize a second-hit theory in the emergence of AOAD.

AD must be considered as a differential diagnosis in adult patients with new ataxia, bulbar symptoms, and leukodystrophy and the tadpole sign in brain MRI. Furthermore, anamnestic hints for traumatic head injuries exposing the disease onset must be taken into account. The second-hit theory is an interesting concept in the emergence of AOAD that should be considered in upcoming research.

**Acknowledgment**

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