CLINICAL/SCIENTIFIC NOTE OPEN ACCESS

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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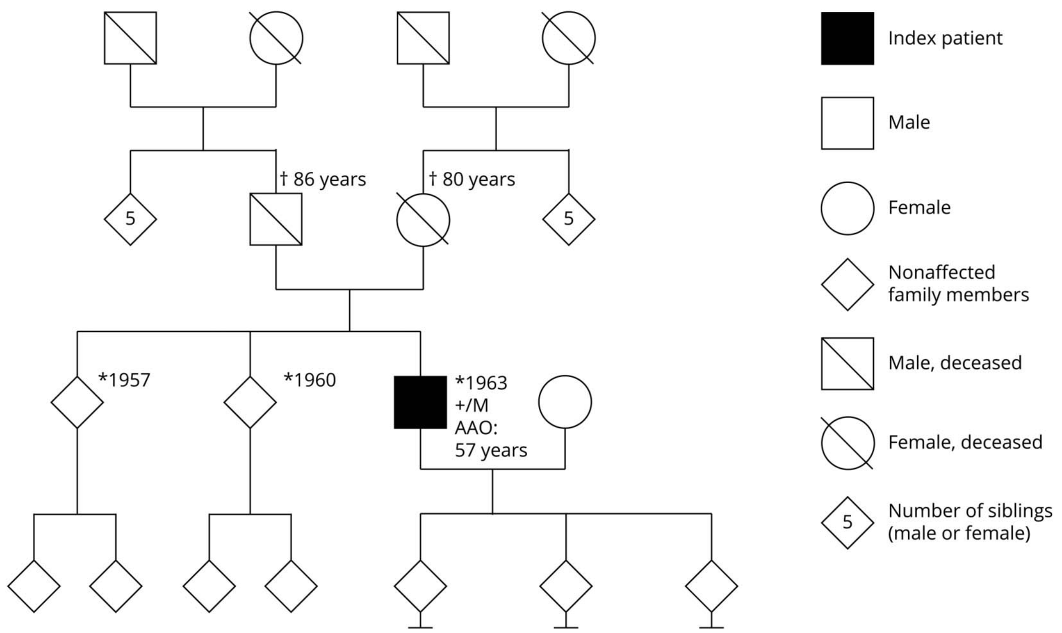
Figure 1 Patient's Pedigree



Patient's pedigree carrying the missense mutation in *GFAP*: c.173T>C; p.L58P. The pedigree has been modified for confidentiality (diamonds). Wild type (+) and mutated (M) allelic forms of *GFAP*. AAO = age at onset.

Alexander disease (AD) is a rare, mostly sporadic, disorder of the CNS with degeneration of astrocytes.[1] Adult-onset AD (AOAD) is more heterogeneous with nonspecific neurologic symptoms, mainly bulbar dysfunction, pyramidal signs, cerebellar ataxia, and palatal myoclonus.[2]

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.[3] Neuropathologically, AD is defined as having intracytoplasmic eosinophilic inclusions in astrocytes.[2] Autopsy shows leukodystrophy and atrophy of the lower brainstem and upper cervical cord.[1,2]

Mutations in the glial fibrillary acid protein gene (*GFAP*) are linked to AD.[4] Its mutations lead to alterations in the protein, thereby causing accumulation and aggregation of precipitates of misfolded GFAP proteins.[4]

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).

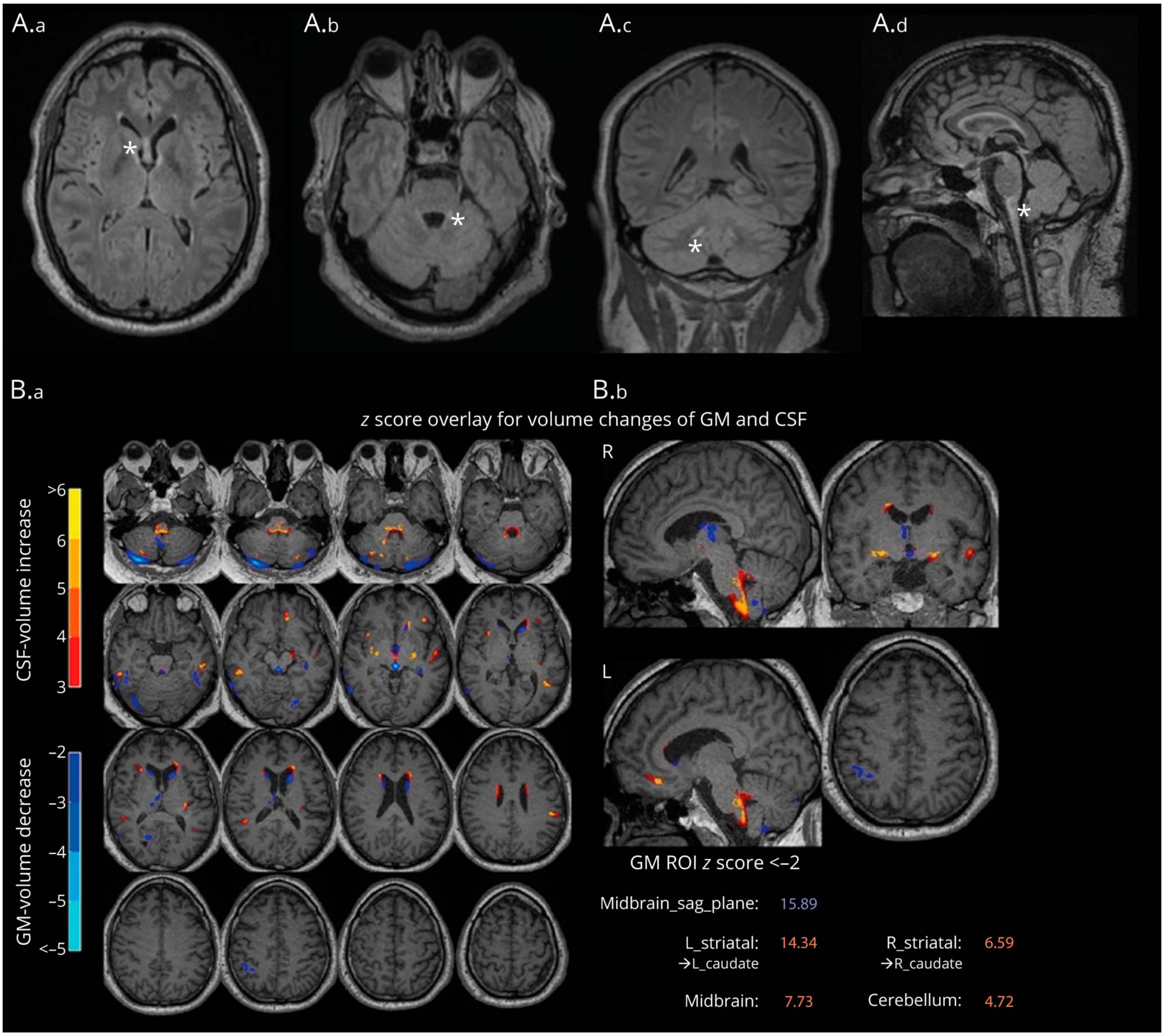
A clinical examination showed nystagmus, cerebellar dysarthria, an ataxic gait pattern, and dysmetria of the upper and lower extremities. In addition, the patient experienced mild bradykinesia on the right side, rigidity of the right upper extremity, brisk muscle reflexes, and positive pyramidal signs.

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.[5] Several in silico analyses have predicted that the p.L58P substitution is likely to be pathogenic.[6]

To test whether this new mutation c.173T>C; p.L58P in *GFAP* affects intermediated 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

Figure 2 MRI and Automated Brain Volumetry Analysis of the Patient



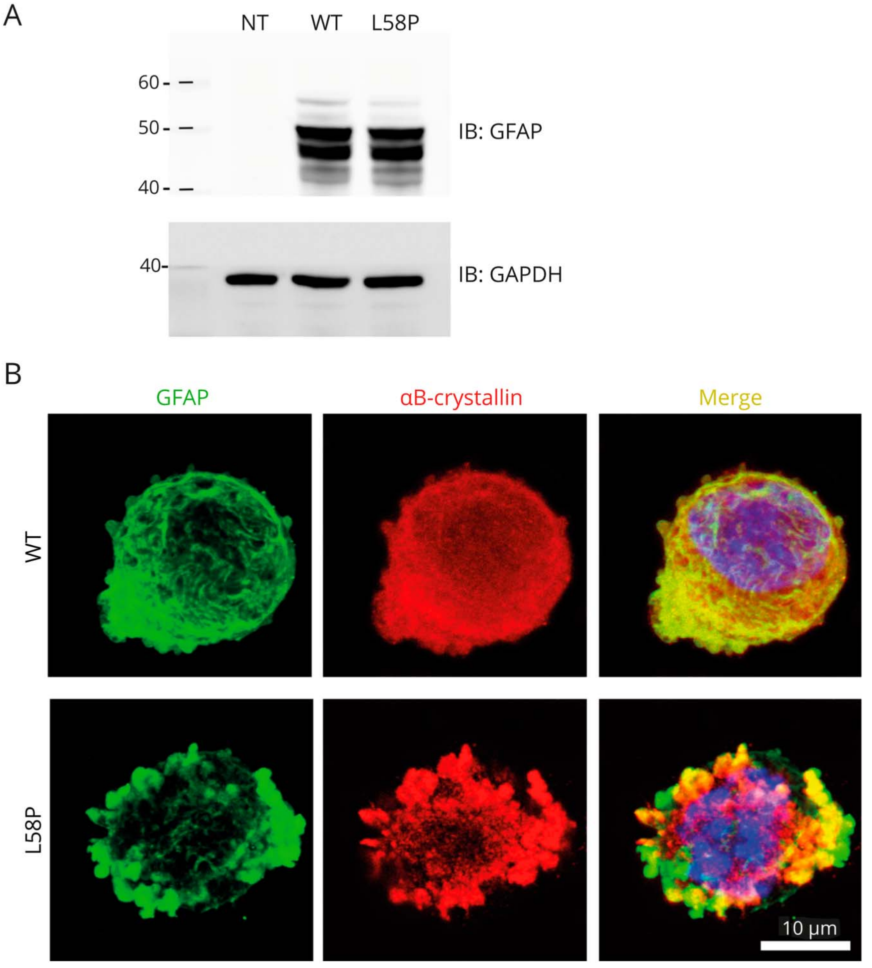
(A.a–d) Brain MRI T2-weighted fluid-attenuated inversion recovery sequences in the axial plane (A.a, A.b), coronal plane (A.c) and sagittal plane (A.d) showing hyperintensities periventricular (*) (A.a), in the left cerebellar peduncle (*) (A.b), and dentate nuclei (*) (A.c). The typical tadpole sign signaling spinal cord atrophy is shown in (*) (A.d). (B.a–b) Automated brain volumetry analysis using the software VEOmorph (VEObrain GmbH). In the results of the automated, combined voxel and region (CVR)-based whole-brain volumetry using 3-dimensional T1-weighted MR images, regional volume increase in CSF and regional volume decrease in gray matter (GM) are superimposed in red to yellow and in light blue to dark blue (depending on the according *z* score) onto the patient's individual brain MRI in the transverse, sagittal, and coronal orientation (B.a). Besides the visually detectable atrophy of the medulla oblongata, additional volume abnormality is shown in the midbrain, cerebellum, and striatal regions of both hemispheres (B.b). Abnormal areas are defined based on a volume change of at least 2 SDs (*z* score 2) in comparison with an age-matched and sex-matched healthy control group. Egger K. *Neuroimage Clin*. 2018; doi: 10.1016/j.nicl.2018.09.013.

or *GFAP*-L58P. 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. The GFAP containing aggregates in the cells transfected with *GFAP*-L58P gene were positive for 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;

Figure 3 Western Blot and Immuncytochemistry of the Transfected HeLa Cell Lines



(A) Representative Western blot performed in lysates from HeLa cells transfected with plasmids encoding human GFAP-WT or mutant GFAP-L58P (GFAP: mAb #3670 cell signaling 1:1000, GAPDH: sc-25778 Santa Cruz, 1:1000). (B) Representative confocal images showing HeLa cells transiently transfected with plasmids encoding human wild-type GFAP or mutant GFAP-L58P and labeled with GFAP antibody (mAb #3670 cell signaling 1:300, green fluorescence). The image shows that wild-type GFAP assembled in filament networks, whereas mutant L58P formed dot-like aggregates. Cells were costained with αβ-crystallin antibody (sc-137129 Santa Cruz 1:100, red fluorescence) in cells transfected with mutant *GFAP*-L58P, and αβ-crystallin formed dot-like aggregates that colocalized with the GFAP signal. Images are representative of 50 analyzed cells from 3 independent experiments. DAPI is indicated in blue in the merge images on the right.

p.L58P) in *GFAP* was found and categorized as a 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.

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Disclosure

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Maria Regoni, MSc	Division of Neuroscience, San Raffaele Scientific Institute; Vita-Salute San Raffaele University, Milan, Italy	Analysis or interpretation of data
Karl Egger, Dr med	Department of Radiology, Tauernklinikum Zell am See, Academic Teaching Hospital of the Paracelsus University Salzburg, and Medical University of Vienna, Austria	Analysis or interpretation of data
Elias Kellner, Dr med	Department of MR Physics, Medical Center, University of Freiburg, Faculty of Medicine, University of Freiburg, Germany	Analysis or interpretation of data
Cornelius Deuschl, Dr med	Institute of Diagnostic and Interventional Radiology and Neuroradiology, University Hospital Essen, Germany	Drafting/revision of the article for content, including medical writing for content; and analysis or interpretation of data
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Appendix *(continued)*

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Stephan Klebe, Prof Dr med	Department of Neurology, Essen University Hospital, Germany	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data

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