

# Activation of a Cryptic Splice Site of *GFAP* in a Patient With Adult-Onset Alexander Disease

Eiichiro Amano, MD, Tomokatsu Yoshida, MD, PhD, Ikuko Mizuta, MD, PhD, Jun Oyama, MD, PhD, Shingo Sakashita, MD, PhD, Syunsuke Ueyama, MD, Akira Machida, MD, PhD, and Takanori Yokota, MD, PhD

**Correspondence**

Dr. Amano  
eiichiroamano1122@hotmail.co.jp

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## Abstract

### Background and Objective

Alexander disease (ALXDRD) is an autosomal dominant neurologic disorder caused by mutations in the glial fibrillary acidic protein (*GFAP*) gene and is pathologically defined by Rosenthal fiber accumulation. Most mutations are exonic missense mutations, and splice site mutations are rare. We report a very-late-onset autopsied case of adult-onset ALXDRD with a novel splice site mutation.

### Methods

Genetic testing of *GFAP* was performed by Sanger sequencing. Using autopsied brain tissues, *GFAP* transcript analysis was performed.

### Results

The patient presented mild upper motor neuron symptoms in contrast to the severe atrophy of spinal cord and medulla oblongata. The patient had c.619-1G>A mutation, which is located in the canonical splice acceptor site of intron 3. The brain RNA analysis identified the r.619\_621del (p.Glu207del) mutation, which is explained by the activation of the cryptic splice acceptor site in the second and third nucleotides from the 5' end of the exon 4.

### Discussion

*GFAP* gene expression analysis is necessary to clarify the effects of intronic mutations on splicing, even if they are in canonical splice sites. This case showed a much milder phenotype than those in previous cases with missense mutations at Glu207, thereby expanding the clinical spectrum of ALXDRD with Glu207 mutation.

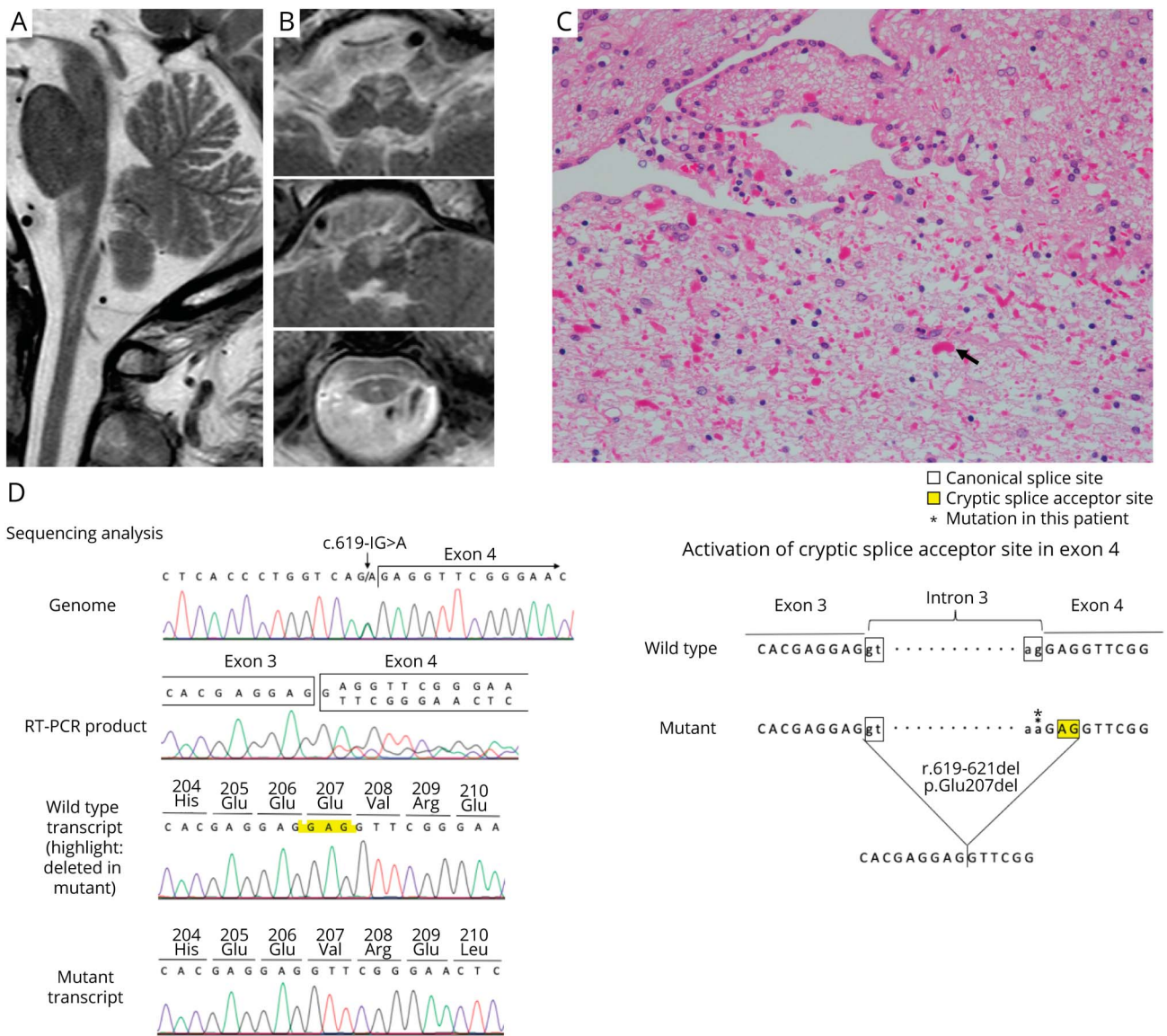
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From the Department of Neurology and Neurological Science, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (E.A., T. Yokota); Department of Neurology, Tsuchiura Kyodo General Hospital (E.A., A.M.), Ibaraki; Department of Neurology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine (T. Yoshida, I.M.); Department of Radiology, Tokyo Medical and Dental University (J.O.); Department of Pathology, Tsuchiura Kyodo General Hospital (S.S.), Ibaraki; Division of Pathology, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center (S.S.), Chiba; and Department of Gastroenterology, Tsuchiura Kyodo General Hospital (S.U.), Ibaraki, Japan.

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(A) Sagittal section on T2-weighted MRI revealed abnormal high intensities in the ventral medulla oblongata. (B) Axial sections on T2-weighted MRI revealed ventral-dominant abnormal intensities and atrophy of the medulla oblongata and upper cervical spine. (C) Rosenthal fibers were widely identified via hematoxylin-eosin staining (magnified at 200 $\times$ ). Periventricular lesion underlying the choroid plexus of the lateral ventricle. The arrow indicates one of the Rosenthal fibers. (D) Sequencing analysis of genomic DNA identified heterozygous c.619-1G>A mutation (left panel). Cloning and sequencing of RT-PCR product revealed GAG (r.619\_621del, p. Glu207del) deletion. These findings suggested that c.619-1G>A led to activation of a cryptic splice acceptor site in the second and third nucleotides from the 5' end of exon 4, generating p. Glu207del (right panel). RT-PCR = reverse transcription-PCR.

Alexander disease (ALXDRD) is an autosomal dominant neurologic disorder caused by mutations in the glial fibrillary acidic protein (*GFAP*) gene and is pathologically defined by Rosenthal fiber accumulation.<sup>1</sup> The clinical course of adult-onset ALXDRD is heterogeneous and sometimes mild or even asymptomatic despite the remarkable atrophy of the brainstem and spinal cord on MRI.<sup>2</sup> Most causative mutations are exonic missense ones, and very few splice site mutations have been reported.<sup>3</sup> When elucidating the effect of mutations in the splice site, expression analysis using RNA extracted from CNS tissues is required because the expression of *GFAP* is specific to astrocytes.

Here, we describe an autopsied case of adult-onset ALXDRD with a novel splice site mutation presenting with elderly-onset mild upper motor neuron symptoms in contrast to the severe atrophy of the spinal cord and medulla oblongata. This mutation is located in the canonical splice acceptor site, and brain RNA analysis identified a three-base deletion, which is explained by the activation of the cryptic splice acceptor site.

### Data Availability

Anonymized data not published within this article will be made available to qualified investigators.

## Ethics Approval

The Ethics Committee of Kyoto Prefectural University of Medicine approved the genetic testing.

## Consent to Participate

Before the patient died, written informed consent was obtained from the patient for the genetic testing. The written informed consent for the autopsy study was obtained from the family of the patient based on the patient's own will before death.

## Consent for Publication

Before the patient died, written informed consent was obtained from the patient for the publication of the result of genetic testing in anonymized fashion. The written informed consent of publication of the autopsy study in anonymized fashion was also obtained from the family of the patient based on the patient's own will before death.

## Case Report

A 76-year-old man with neither medical history nor family history of neurologic diseases presented with progressive diplopia and ptosis in his left eye. Neurologic examination revealed complete ophthalmoplegia, loss of light reflex, and ptosis in the left eye, indicating palsies of cranial nerves III, IV, and VI. Mild spastic gait with bilateral positive Babinski reflex and increased deep tendon reflex in all limbs was observed. He could walk without assistance, and no other neurologic abnormalities were observed.

Brain MRI revealed orbital schwannoma extending from the left orbital apex and superior orbital fissure toward the orbital cavity. In addition, systemic screening revealed gastric adenocarcinoma with multiple metastases in para-aortic lymph nodes. Unexpectedly, brain and spinal MRI revealed bilateral abnormal high intensities in the ventral medulla oblongata, a mild periventricular hyperintensity lesion, and marked spinal cord atrophy (Figure, A and B). These findings are characteristic of adult-onset ALXDRD,<sup>1</sup> and Sanger sequencing analysis of *GFAP* disclosed a novel heterozygous c.619-1G>A mutation.

The patient died of multiple metastases of adenocarcinoma and pneumonia 6 months after diagnosis. Autopsy revealed marked atrophy of the medulla and spinal cord. Fibrotic astrocyte degeneration, Rosenthal fiber deposition, and myelin loss were widespread in the medulla, spinal cord, and periventricular locations underlying the lateral ventricles and fourth ventricle (Figure C), which was pathologically compatible with ALXDRD.<sup>4</sup> Neurons in the anterior horn and cerebral cortex were relatively reserved. The orbital lesion was schwannoma, which was confirmed by the typical palisading with positive S-100 staining and negative GFAP staining immunohistochemistry and considered not to be related to ALXDRD.

Because c.619-1G>A is located in the canonical splice acceptor site of intron 3, we initially expected that this mutation would result in exon 4 skipping. Indeed, a previous study of a patient

with a heterozygous c.619-3C>G mutation showed exon 4 skipping in approximately 10% of the transcripts.<sup>3</sup> However, analysis of RNA from the autopsied brain tissue of the present patient showed deletion of GAG (r.619\_621del) (Figure D). The reverse transcription-PCR product was cloned, and 54 individual clones in total were sequenced. The number of wild-type and mutant clones was 34 and 20, respectively. These findings suggested that c.619-1G>A caused activation of the cryptic splice acceptor site in the second and third nucleotides from the 5' end of exon 4 (Figure D), in approximately 37% (20/54) of the transcripts. r.619\_621del results in loss of Glu207, which is located in the  $\alpha$ -helical segment 1B. Loss of Glu207 is believed to be pathogenic because the missense mutations at Glu207, p.Glu207Gln,<sup>5</sup> p.Glu207Lys,<sup>6</sup> and p.Glu207Val<sup>7</sup> have been previously reported in patients with ALXDRD. Patients with p.Glu207Gln and p.Glu207Lys were juvenile onset, whereas the patient with p.Glu207Val presented symptoms including dysphagia and dysarthria at age 52 years, earlier onset than the present patient with p.Glu207del. These suggest that the broad clinical spectrum of mutations at Glu207 and p.Glu207del may be related to milder phenotype than missense mutations at Glu207. As the number of cases with Glu207 mutation is limited, further study is necessary to address the phenotypic heterogeneity of the mutations at Glu207.

In conclusion, we identified a novel ALXDRD-causing *GFAP* mutation in the splice acceptor site of intron 3, leading to activation of a cryptic splice site in exon 4. This splicing error resulted in very-late-onset pyramidal signs, which were milder than expected from the severe atrophy of spinal cord and medulla caused by astrocyte degeneration. *GFAP* gene expression analysis using CNS tissues was necessary to clarify the effect of intronic mutations on splicing.

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## Disclosure

No relevant competing interest was declared by the authors. Go to [Neurology.org/NG](http://Neurology.org/NG) for full disclosures.

## Publication History

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## Appendix Authors

Name	Location	Contribution
Eiichiro Amano, MD	Department of Neurology and Neurological Science, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan; Department of Neurology, Tsuchiura Kyodo General Hospital, Ibaraki, Japan	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data

Continued

## Appendix (continued)

Name	Location	Contribution
<b>Tomokatsu Yoshida, MD, PhD</b>	Department of Neurology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data
<b>Ikuko Mizuta, MD, PhD</b>	Department of Neurology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data
<b>Jun Oyama, MD, PhD</b>	Department of Radiology, Tokyo Medical and Dental University, Tokyo, Japan	Analysis or interpretation of data
<b>Shingo Sakashita, MD, PhD</b>	Department of Pathology, Tsuchiura Kyodo General Hospital, Ibaraki, Japan; Division of Pathology, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center, Chiba, Japan	Analysis or interpretation of data
<b>Syunsuke Ueyama, MD</b>	Department of Gastroenterology, Tsuchiura Kyodo General Hospital, Ibaraki, Japan	Analysis or interpretation of data

## Appendix (continued)

Name	Location	Contribution
<b>Akira Machida, MD, PhD</b>	Department of Neurology, Tsuchiura Kyodo General Hospital, Ibaraki, Japan	Analysis or interpretation of data
<b>Takanori Yokota, MD, PhD</b>	Department of Neurology and Neurological Science, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan	Study concept or design and analysis or interpretation of data

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