Adult Alexander Disease With Autosomal Dominant Transmission

A Distinct Entity Caused by Mutation in the Glial Fibrillary Acid Protein Gene

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Background: Infantile and juvenile forms of Alexander disease are well characterized and are caused by de novo mutations in the glial fibrillary acid protein (GFAP) gene. In contrast, the adult form of the disease has been rarely described, and the etiology of this variant remains unknown.

Objective: To characterize the clinical phenotype and identify the gene causing an autosomal dominant form of adult Alexander disease.

Methods: We identified a large kindred segregating clinical features compatible with adult Alexander disease in an autosomal dominant fashion. A neurological examination was performed on all affected members of this family. Most of these patients also underwent magnetic resonance imaging of the brain and a polysomnographic study. The diagnosis was confirmed pathologically in 2 individuals. We screened all coding regions of the GFAP gene in affected individuals by means of direct sequencing and single-stranded conformational polymorphisms analysis.

Results: We found a novel D78E mutation in GFAP in all affected individuals. This mutation was not detected in more than 100 control subjects. Clinical and radiological features of affected individuals were clearly different from those of patients with the infantile and juvenile forms of the disease. The most consistent finding was the presence of bulbar signs. In addition, sleep disturbance (mainly sleep apnea), symptoms of dysautonomia, and dysmorphism were found in all affected individuals. In younger patients, magnetic resonance imaging showed T2 signal abnormalities in the medulla compatible with an area of demyelination. In contrast, in older patients, we found marked atrophy of the medulla without signal abnormalities. None of the affected individuals exhibit signs of demyelination of the cerebral white matter.

Conclusions: The present study is the first demonstration of a mutation in GFAP that causes an autosomal dominant form of Alexander disease and establishes the existence of the adult variant. Clinical evaluation in individuals carrying mutation in the GFAP gene allowed a better definition of this heterogeneous clinical syndrome and will help increase its recognition in neurological practice.

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erogeneous and includes bulbar, pyramidal, and cerebellar signs. The evolution is protracted, and some patients may remain asymptomatic. Although Alexander disease with adult onset seems to be sporadic in most cases, familial segregation, suggesting an autosomal dominant mode of transmission, has been reported.13,14 Whether adult-onset Alexander disease represents a distinct entity or a fortuitous association of variable signs with nonspecific pathological findings is still debated.15,16 Furthermore, the etiology of the adult form of Alexander disease remains to be determined. In this study, we report a novel D78E mutation in GFAP in a large family with segregation of adult-onset Alexander disease in an autosomal dominant fashion.

METHODS

CLINICAL CHARACTERIZATION OF THE FAMILY

An interview was performed by 3 investigators (E.S., H.M, and P.C.) on all family members (n=26). A neurological examination was performed on all but 3 affected individuals (II:2, II:9, and III:3, deceased at the time of the study). Magnetic resonance imaging of the brain was obtained in 5 affected individuals. Many affected members of this family (n=5) also underwent a polysomnographic study as part of a sleep disturbance investigation. All these examination results were reviewed by a specialist in sleep disorders (J.M.).

PATHOLOGY

A complete neuropathological examination was performed by one of us (F.B.) for individual II:6. The brain was fixed with formalin, and sections of paraffin-embedded tissue were stained with hematoxylin-eosin and combined Luxol fast blue and hematoxylin-eosin for myelin. Selected tissue sections were immunostained for GFAP, neurofilament protein, and ubiquitin. For individual III:3, an autopsy was performed in another hospital, and only 1 brainstem section was available. This paraffin-embedded hematoxylin-eosin–stained tissue section was also examined by one of us (F.B.).

MUTATION SCREENING

All 26 living members of this family signed informed consents. We collected blood samples from these individuals, and genomic DNA was isolated from leukocytes by standard methods. The genomic organization of the human GFAP gene was determined by aligning sequences from GFAP messenger RNA (Genbank accession NM_0002055) to the corresponding genomic sequence on chromosome 17 (GenBank accession NT_010763). Primers were designed to amplify 300– to 500–base pair fragments from genomic DNA to screen all coding portions of the gene. Portions of the GFAP gene were amplified by means of polymerase chain reaction in 1 affected individual (III:7) and sequenced by means of a DNA sequencer (ABI 3700; Applied Biosystems, Foster City, Calif). Primers GFA-3,5'-GGGGCACCCTCAATGCTGGCTTCAAG-3' and GFA-4,5'-GTTCGGCCCTCCTCGAGCCTCT-3' were used to amplify exon 1. The D78E mutation found in exon 1 was confirmed by sequencing individuals III:1, III:7, III:8, and IV:1 and 2 healthy control subjects. The segregation of the D78E mutation in the family and the screening of additional controls were assessed by means of single-stranded conformational polymorphism analysis using primers GFA-3 and GFA-4.

LINKAGE ANALYSIS

We calculated 2-point logarithm of odds (LOD) scores of the D78E mutation using M-link of the linkage program,17,18 assuming autosomal dominant inheritance with 90% penetrance, gene frequency of 0.0001, and mutated allele frequency of 0.01.

RESULTS

CLINICAL PHENOTYPE

Many individuals are affected in this large pedigree, with an autosomal dominant mode of inheritance (Figure 1). All affected members of this family exhibit a similar phenotype (Table). However, the clinical phenotype varies in severity, ranging from mild neurological symptoms to more severe symptoms. The most consistent neurological finding was the presence of bulbar signs, which could constitute any combination of dysphagia (n=7), dysphonia (n=4), and dysarthria (n=3). Aspiration pneumonia secondary to bulbar dysfunction was thought to be responsible for the death of 2 individuals (III:3 and II:6, at 30 and 67 years of age, respectively). Cerebellar ataxia was present in 4 individuals, but palatal myoclonus was found in only 1 affected individual. Pyramidal signs, in the form of hyperreflexia and mild weakness of lower limbs, were found in 3 individuals.

Unexpectedly, we observed sleep disturbances in all affected individuals, consisting of sleep apnea or hypopnea (n=5), increased rapid eye movement sleep time (n=1), and the occurrence of sudden-onset rapid eye movement sleep during multiple sleep latency testing (n=1). Furthermore, all affected patients had symptoms of dysautonomia consisting of severe constipation that necessitated medical treatment (mineral oil, laxative, and/or enema) (n=7) and bladder dysfunction (n=1). In 2 individuals (II:6 and II:14), severe dysautonomia with paroxysmal episodes of hypothermia and orthostatic hypotension was observed. In addition, all affected members of this family share dysmorphic features, including progressive kyphosis, arched palate, and short neck (Figure 2A). Finally, sev-
Although the overall phenotype is variable among affected individuals, we were able to identify some features common to all affected individuals of this family. Bulbar signs were the most reliable signs of the disease. Also, all affected individuals exhibit mild to severe signs of dysautonomia, sleep disturbance, and dysmorphic features.

Several individuals (n=4) exhibit symptoms or signs compatible with an epileptic disorder, ie, one individual has intractable epilepsy, and another had repeated episodes of loss of consciousness associated with urinary incontinence that may represent seizures. Electroencephalograms showed epileptic activity without obvious clinical seizures in 2 other individuals.

The age of onset of the disease was difficult to determine, especially for individuals with mild symptoms. In affected individuals, a similar pattern of evolution was observed. The first symptoms consisted of severe constipation starting at 5 to 10 years of age, followed by sleep disturbance after 20 years of age. The onset of other neurological symptoms was more variable, but usually occurred in the third or the fourth decade of life (Table). More severe signs of dysautonomia (hypothermia and orthostatic hypotension) were observed in 2 individuals after 50 years of age.

### MRI FINDINGS

Magnetic resonance imaging of the brain was obtained in 5 affected individuals. In 2 individuals (IV:1 and III:7), MRI was performed early in the evolution of the disease (at 14 and 34 years of age, respectively). Both individuals had signal abnormalities in the medulla compatible with areas of demyelination (Figure 2C), as well as mild atrophy of the brainstem and the cervical spinal cord. In 2 individuals at a later stage of the disease, marked atrophy of the medulla was observed without any signal abnormalities (Figure 2B). In these older patients, atrophy of the cervical spinal cord (n=2) or the cerebellum (n=1) was also observed. Magnetic resonance imaging did not reveal demyelination of the cerebral white matter in any of the affected patients. Finally, for individual III:8 with intractable epilepsy, MRI showed pachygyria of the frontal and parietal lobes.

### PATHOLOGY

A complete neuropathological examination was performed for individual II:6. The fixed brain weighed 1380 g. On macroscopic examination, there were no obvious lesions of the white matter of the cerebral hemispheres, except for a grayish discoloration localized at the tip of the frontal horns. There was no apparent gyral atrophy. However, severe atrophy of the medulla was observed (Figure 3A). Transverse sections of the brainstem confirmed bulbar atrophy associated with grayish discoloration and cavitation of the white matter involving the inferior olivary nuclei and the pyramids. Similar lesions were found in the cerebellar white matter in the vicinity of the dentate nucleus.

Microscopic examination of the lesions in the vicinity of the frontal horn of the lateral ventricles showed variable pallor of the myelin associated with numerous Rosenthal fibers and extensive gliosis. Rosenthal fibers and gliosis were also found in the white matter around the temporal and occipital horns of the lateral ventricles, in the CA4 region of the hippocampus, the optic chiasma, and in the hypothalamus, particularly within the supraoptic nucleus and the ventrolateral region. In the medulla, numerous Rosenthal fibers and gliosis were found in the tegmental zone (Figure 3B). However, the loss of myelin around inferior olivary nuclei and within the pyramids was associated with less abundant deposition of Rosenthal fibers. In contrast, the white matter medial to the cerebellar dentate nucleus was severely depleted of myelin and packed with Rosenthal fibers. Finally, Rosenthal fibers were seen in the spinal gray and white matters. For individual III:3,
a single brainstem section was available for examination and numerous Rosenthal fibers were also found.

**MOLECULAR FINDINGS**

Sequence analysis determined that affected individual III:7 was heterozygous for a C-to-A substitution in exon 1, predicted to change a GAC (aspartic acid) to a GAA (glutamic acid) codon at position 78 of the human GFAP complementary DNA (Figure 4A). This amino acid lies in the rod domain of the predicted protein (Figure 4B) and is conserved in GFAP and in other homologous proteins (eg, desmin and vimentin) from different species. This D78E variation was detected in all of the affected patients (n=5) available for testing and in none of the unaffected members (n=21) of the family. The only exception is individual III:1, who carries the mutation but has no obvious neurological symptoms. However, because of ethical considerations, having established her carrier status after she completed an interview that did not include a physical examination, we were not able to perform a complete neurological examination. Therefore, we cannot exclude that this individual at 30 years of age indeed has a mild form of the disease. We did not find this D78E variation in more than 200 control chromosomes. Linkage analysis using this mutation as the marker for the disease provides an LOD score of 3.1 at theta 0. We did not find mutations in the other exons of the GFAP gene.

**COMMENT**

Adult Alexander disease is a distinct entity. The infantile and juvenile forms of Alexander disease are well-defined syndromes, usually classified in the group of leukodystrophies. However, the clinical presentation of the adult variant of the disease seems very heterogeneous, and few
common features emerge from these reports.\textsuperscript{11-14} Moreover, Rosenthal fibers, which are the pathological hallmark of the disease, have been found in asymptomatic individuals.\textsuperscript{15} On the basis of these observations, the existence of adult Alexander disease has been questioned.\textsuperscript{15,16}

In this study, we report a novel D78E mutation in the GFAP gene that causes adult Alexander disease transmitted in an autosomal dominant fashion. Several lines of evidence suggest that D78E is the pathogenic mutation in this family. First, the mutation segregates with the disease with an LOD score of 3.1; second, the D78E mutation was not found in more than 200 control chromosomes; and third, the mutation involves a conserved residue located in segment 1A of the rod domain of the protein. This segment has been shown to be important in the polymerization of intermediate filaments and could be critical in the pathogenesis of Alexander disease.\textsuperscript{21} Fourth, this region has been previously identified as a hot spot for spontaneous mutations in infantile and juvenile forms of Alexander disease.\textsuperscript{9,10} Therefore, the identification of the D78E mutation in GFAP as the cause of the disease in this large kindred establishes the existence of the adult variant of Alexander disease as a distinct entity. To our knowledge, this is the first demonstration that a mutation in GFAP is causing an inheritable form of Alexander disease. In addition, careful examination of individuals carrying the D78E mutation allowed a better definition of the clinical and radiological features that are associated with the adult variant of the disease.

**CLINICAL AND RADIOLOGICAL FEATURES OF ADULT ALEXANDER DISEASE**

Despite some variability, detailed examination of affected individuals carrying the D78E mutation in the GFAP gene revealed many common neurological features. First, all affected individuals presented with bulbar signs, which were the most reliable and objective neurological signs. Previous studies of adult Alexander disease reported ataxia, palatal myoclonus, and pyramidal signs in many affected individuals.\textsuperscript{11-14} However, in our study, these neurological signs were notably absent in about half of the affected members, and the age of onset of these neurological symptoms was variable. In turn, detailed examination of affected individuals revealed clinical features that were common to all affected individuals, but that have been rarely or never reported in patients with Alexander disease. Among these signs, we observed sleep disturbance in all affected individuals, in the form of sleep apnea and rapid eye movement sleep abnormalities. Also, all of these individuals have an arched palate, a short neck, progressive kyphosis, and dysautonomia. Overall, these clinical features are clearly different from the infantile and juvenile forms of the disease.

In contrast to the infantile form of Alexander disease, the evolution was protracted in all members of this family with adult onset of neurological symptoms. The only exception is individual IV:1, who presented with a rapidly progressive and severe form of the disease with juvenile onset. This observation suggests that the adult and juvenile variants of Alexander disease represent a biological continuum rather than distinct entities. In older affected individuals, the clinical phenotype varies in severity, but the same pattern of evolution was observed. The first symptoms consisted of dysautonomia starting during childhood, followed by sleep disturbance in early adulthood, and then bulbar symptoms, with or without other neurological signs, that could eventually lead to premature death. At the other extreme of this phenotype, 1 individual (III:1) carries the D78E mutation, but seems to be asymptomatic at 30 years of age. This finding may be compatible with incomplete penetrance of the GFAP mutation. However, since we were unable to perform a complete evaluation in this individual, we cannot exclude that she is at an early stage of the illness.

Magnetic resonance imaging of the brain in affected individuals also revealed striking differences between the infantile and adult forms of Alexander disease. Thus, in contrast to the infantile and juvenile forms of Alexander disease, MRI did not show demyelination of cerebral white matter. In older patients, MRI showed

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**Figure 4.** Location of the D78E mutation in relation to critical domains of glial fibrillary acid protein (GFAP). A, Sequence analysis of polymerase chain reaction products amplified from genomic DNA of a normal control subject and an affected individual using GFA-3 and GFA-4 primers. The C-to-A substitution detected in the heterozygous patient results in a conservative amino acid change (D78E). B, This amino acid lies in the rod domain of the protein and is conserved in GFAP as well as in other homologous proteins (eg, desmin and vimentin) from different species (not shown). The boxes indicate the α-helical subdomains within the rod domain of GFAP. Segment 1A has been shown to be important in the polymerization of intermediate filaments and could be critical in the pathogenesis of Alexander disease. This functional domain was already known to be a hot spot for spontaneous mutations in juvenile and infantile forms of the disease. Previously described mutations in this and other domains are shown in this diagram. C248A indicates a cytosine to adenine (C→A) substitution at position 248 of the GFAP complementary DNA (cDNA). This substitution will cause a GAC (aspartic acid) to GAA (glutamic acid) codon change at position 78 of the GFAP cDNA. The number-letter combinations (B) refer to the rod domains of the GFAP protein. There are 4 rod domains: 1A, 1B, 2A, and 2B.
a marked bulbar and spinal cord atrophy, without sig-
nal abnormalities, whereas in younger individuals we ob-
served a T2 hypersignal in the medulla that presumably
represents a focal area of demyelination, along with a mild
degree of brainstem and cervical cord atrophy. We specu-
late that this abnormal signal represents an early stage
of the disease, with later evolution toward degeneration
of brainstem structures and more severe atrophy.

We were able to correlate radiological and patho-
logical findings in only 1 individual (II:6). We found
pathological evidence of myelin loss throughout the brain,
but the MRI did not show a corresponding T2 hypersig-
nal. In this individual, although Rosenthal fibers were
found in several brain areas, they were clearly predomi-
nating in the tegument of the medulla and, to a lesser
extent, in the hypothalamus. Thus, it appears that the
adult form of Alexander disease preferentially involves
the gray matter of the hypothalamus, brainstem, and, spini-
al cord and presents with a slowly progressive neuro-
logical disturbance. In contrast, rapidly progressive de-
mynelination of the cerebral white matter predominates
in infantile and juvenile cases. Why a missense muta-
tion in the same critical domain of the GFAP results in
such different clinical phenotypes between the adult and
infantile variants of the disease is intriguing and de-
serves future attention.

Because of the protracted course of the disease com-
bined with mild symptoms observed in many individu-
als even later in the stage of the disease, many cases of
Alexander disease with adult onset are probably over-
looked in clinical practice. The cluster of slowly evolv-
ing bulbar signs, sleep disturbances, and dysautonomia
associated with brainstem atrophy should raise the sus-
picion for late-onset Alexander disease. The absence of
white matter changes on brain imaging does not pre-
clude the diagnosis. Screening for GFAP mutations in criti-
cal domains should facilitate diagnosis in suspected cases.
Further studies of additional cases with a mutation in the
GFAP gene will be needed to clarify and expand the clinical
phenotype of adult Alexander disease and help in-
crease its recognition by clinicians.

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