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Alexander Disease: A novel mutation in GFAP leading to epilepsy partialis continua

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Abstract

Alexander disease is a genetically-induced leukodystrophy, due to dominant mutations in the glial fibrillary acidic protein (GFAP) gene, causing dysfunction of astrocytes. We have identified a novel GFAP mutation, associated with a novel phenotype for Alexander disease. A boy with global developmental delay and hypertonia was found to have a leukodystrophy. Genetic analysis revealed a heterozygous point mutation in exon 6 of the GFAP gene. The guanine-to-adenine change causes substitution of the normal glutamic acid codon (GAG) with a mutant lysine codon (AAG) at position 312 (E312K mutation). At the age of four years, the child developed epilepsy partialis continua, consisting of unabating motor seizures involving the unilateral perioral muscles. *Epilepsia partialis continua* has not previously been reported in association with Alexander disease. Whether and how the E312K mutation produces pathologic changes and clinical signs that are unique from other Alexander disease-inducing mutations in GFAP remain to be determined.

Introduction

Alexander disease is a degenerative disease of the central nervous system that primarily affects children (Srivastava and Naidu, 2015). The disease is classified among the leukodystrophies, because progressive loss of cerebral myelin has been recognized as a major component of pathogenesis ever since the time of disease discovery. However, Alexander disease is not a primary disease of myelin, oligodendrocytes, or axons. Instead, Alexander disease is an “astrogliopathy”—a disease of astrocytes. The disease is due to mutations in the glial fibrillary acidic protein (GFAP) gene, which encodes a type III intermediate filament predominantly found in astrocytes within the CNS.

The mutations of Alexander disease confer their cytotoxic effects through a toxic gain-of-function mechanism (Messing et al., 2012). Abnormal GFAP protein aggregates with several additional proteins to form intracellular inclusion bodies, known as Rosenthal fibers, within astrocytes. The mechanism by which Rosenthal fibers produce dysfunction and degeneration of the nervous system is unknown, but the altered GFAP protein disturbs the normal interaction between astrocytes and oligodendrocytes, resulting in demyelination. The altered GFAP likewise disturbs the normal interaction between astrocytes and neurons, resulting in manifold signs of cerebral dysfunction.

Clinical manifestations of Alexander disease often include developmental delay, megalencephaly, bulbar symptoms, ataxia, seizures, and neurologic deterioration. The diagnosis is based on clinical findings and confirmed by MRI scan, which often shows extensive cerebral white matter abnormalities, and by genetic testing, which reveals a heterozygous pathogenic mutation in the GFAP gene (Srivastava and Naidu, 2015).

Virtually all patients with Alexander disease have GFAP mutations, and no other genetic or non-genetic cause of Alexander disease is known. However, multiple sites within the GFAP gene can be mutated, leading to Alexander disease (Messing et al., 2012). In addition, it is becoming increasingly evident that genotype-phenotype correlations exist. Different sites and types of GFAP mutations have been associated with differences in age of disease onset, type and severity of symptoms, and radiologic features (Prust et al., 2011).

Furthermore, astrocytes might not all be equally affected by GFAP mutations. Regional differences in astrocyte physiology exist across the brain (Bonthius and Steward, 1993; Schitine et al., 2015). Thus, astrocytes in some regions may be differently affected by certain GFAP mutations than astrocytes in other regions. Here, we describe a novel GFAP mutation associated with a previously undescribed clinical problem – epilepsy partialis continua. This patient's unique clinical findings might reflect regional differences in astrocyte physiology and reaction to disease.

Case Report

The patient was originally the 3250 gram male product of a full-term, uncomplicated pregnancy, born in Iowa to Spanish-speaking parents who were non-consanguineous and had recently emigrated from Mexico. The labor and delivery were unremarkable. He thrived as a newborn with Apgar scores of 8 at both one minute and five minutes of life. Head circumference at birth was 34.5 cm (50th percentile). His newborn screen for congenital adrenal hyperplasia, hypothyroidism, galactosemia, phenylketonuria, biotinidase deficiency, and hemoglobinopathies was negative.

He seemed healthy until age five months, when he was referred to the child neurology clinic for evaluation of developmental delay. He was not yet rolling over, would smile spontaneously but not responsively, and did not babble. He was also having frequent episodes of dysphagia, in which he would cough and gag during bottle feedings.

On examination at age five months, his vital signs were all normal. Head circumference was 41.0 cm (10th percentile). His anterior fontanelle was open, with a diameter less than 1 cm. He had poor head control, would not turn his head to a voice or rattling sound, and would not grasp an object. He would make eye contact with examiners, but would not track objects visually. Muscle tone was moderately increased in the lower extremities bilaterally, but normal in the upper extremities. Muscle stretch reflexes were normal throughout. The remainder of the physical examination was normal.

Family history was negative for any close relatives with developmental or neurological disorders. He had no siblings.

For evaluation of his developmental delay and lower extremity hypertonia, he underwent an MRI scan of the brain, which revealed marked prominence of the cerebral cortical sulci and extra-axial CSF space, along with enlarged ventricles. The white matter of the cerebral hemispheres was diffusely abnormal, with T1 hypointensity and T2 hyperintensity throughout the subcortical and deep white matter and in the internal and external capsules (Figure 1). In addition, the brain stem was diffusely small. The results suggested a leukodystrophy with secondary cerebral atrophy.

Laboratory testing to identify the specific leukodystrophy ensued. Genetic and/or metabolic tests for X-linked adrenoleukodystrophy, Krabbe's disease, Canavan's disease, metachromatic leukodystrophy, Pelizaeus-Merzbacher disease, organic acidopathies, and Tay-Sachs disease were all negative or normal.

DNA testing of a blood sample for Alexander disease revealed a point mutation in exon 6 of the GFAP gene. The sample was heterozygous for a guanine-to-adenine change, which causes substitution of the normal glutamic acid codon (GAG) with a mutant lysine codon (AAG) at position 312. This Glu312Lys (E312K) mutation in GFAP had not previously been reported, but is predicted to alter the structure and/or function of the GFAP protein since it induces a non-conservative amino acid change from an acidic residue (glutamic acid) to a basic residue (lysine).

The E to K substitution generated scores of <0.05 and 0.992 when analyzed using SIFT (<http://sift.jcvi.org/>) and PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/index.shtml>) tools respectively, both of which predicted that the lysine substitution at the 312 position is potentially damaging. Further analysis of the sequence using the Mutation Taster algorithm (<http://www.mutationtaster.org/>) predicted that the E312K mutation is a disease-causing change. Analysis of the E312K mutation using the MUpro program (<http://www.ics.uci.edu/~baldig/mutation>) predicted that the mutation would likely increase stability of the GFAP protein.

To determine whether the E312K mutation arose *de novo* or was inherited, testing for the mutation was performed on the patient's parents. No GFAP mutation was found in either parent, strongly suggesting that the mutation had arisen *de novo* (though the possibility of germline mosaicism remained). These results, when combined with the clinical course and neuroimaging studies, confirmed the diagnosis of Alexander disease, due to a previously unreported mutation in the GFAP gene.

At six months of age, the patient developed recurrent generalized tonic-clonic seizures, for which he was treated with phenobarbital, which greatly improved the frequency and duration of the seizures. However, he remained developmentally stalled, and, over the course of the next several months, became diffusely spastic and hypertonic. He also developed increasingly frequent episodes of choking and gagging while eating. His swallowing difficulties were accompanied by poor weight gain, which necessitated placement of a gastric tube at 17 months.

Throughout the second and third years of life, he remained diffusely hypertonic and developed contractures in all four extremities, despite physical therapy. His muscle stretch

reflexes became difficult to elicit. He acquired microcephaly, with a head circumference below the 5th percentile. Over this same time period, he became increasingly less responsive to external stimulation. He occasionally had exacerbations of his generalized seizures, which abated with increased doses of phenobarbital.

At the age of four years, he developed a constantly present rhythmic right-sided perioral twitching movement, with a frequency of approximately 3 Hz, consistent with a continuous focal seizure. He was treated with a dose of lorazepam, which temporarily terminated the focal seizure. However, the seizure activity returned within 24 hours. An EEG revealed a slow background with arrhythmic delta activity, but no epileptiform activity. The EMG lead in the right temporal region revealed rhythmic EMG artifact with a frequency that coincided with the perioral movements. Diagnosed with *epilepsia partialis continua*, he was treated with carbamazepine, in addition to phenobarbital, which decreased the intensity of the perioral movements. Nevertheless, the focal seizure continued. Because the focal seizures did not appear to be causing him any distress, the decision was made to treat him conservatively with carbamazepine and phenobarbital, rather than to launch trials of alternative anticonvulsants.

At each of his subsequent biannual appointments over the course of the next five years, he continued to have *epilepsia partialis continua*, manifesting as right-sided perioral twitching. His last follow up appointment occurred at the age of ten years, at which time he was microcephalic (head circumference 47 cm, < 1st percentile), minimally responsive to external stimuli, would not track objects visually, was diffusely hypertonic, and had continuous right-sided perioral twitching.

Discussion

This case report reveals a novel mutation in the GFAP gene that is associated with the novel phenotype of *epilepsia partialis continua* in Alexander disease. Alexander disease was initially recognized in 1949, when Dr. William Alexander described a 16-month-old boy with a degenerative disease, whose signs included megalencephaly, hydrocephalus, and psychomotor regression (Alexander, 1949). Neuropathologic analysis revealed abundant eosinophilic inclusion bodies within the cytoplasm of astrocytes, leading Alexander to speculate that this disease may represent a primary disorder of astrocytes. Proof that astrocytes are at the epicenter of this disease came half a century later, when Messing and co-workers discovered that Alexander disease was due to missense mutations in the gene for glial fibrillary acidic protein (GFAP), the principal intermediate filament of astrocytes (Brenner et al., 2001). Human clinical data and laboratory studies involving transgenic mice demonstrated that the disease was due to a toxic gain of function mechanism, in which mutated GFAP accumulates within astrocytes and aggregates with several other proteins to form inclusion bodies, known as Rosenthal fibers (Tomokane et al., 1991; Goldman, 1988).

Analysis of GFAP mutations from patients with Alexander disease has revealed that disease-inducing mutations can occur throughout the coding region of the GFAP gene. It was initially thought that disease-inducing mutations at any site on the GFAP gene lead to equivalent disease. More recently, however, genotype-phenotype correlations have begun to

emerge. In particular, the R79 and R239 mutations often lead to early-onset disease, and the R239 mutation consistently induces a notably severe clinical course (Prust et al., 2011).

Furthermore, a particular mutation at the E312 site of GFAP can induce a neurological disease that differs markedly in its clinical manifestations from those of most patients with Alexander disease (Nam et al., 2015). A G to T change converts a glutamate GAG codon to a nonsense TAG stop codon, resulting in termination of the nascent mRNA and the deletion of 121 amino acids. Despite this major truncation, the E312 mutant GFAP protein still disrupts the formation of normal filaments, acts via a toxic gain of function, produces Rosenthal fibers, and causes a leukodystrophy. This E312ter mutation can induce a unique clinical phenotype, which includes headache and short-term memory disturbances, with onset in adulthood (Nam et al., 2015; Brenner and Messing, 2015).

Interestingly, the GFAP mutation in our patient likewise occurred at the E312 site and is associated with a different unique phenotype. In our patient, however, the mutation did not produce an internal stop codon, but instead a missense mutation that leads to the replacement of an acidic amino acid (glutamate) with a basic amino acid (lysine). The GFAP protein consists of a central four-part alpha-helical rod segment, flanked by random coils at the N-terminal head and C-terminal tail (Eng et al., 2000). The E312 site is within the fourth rod domain. The rod domains are all highly conserved, and a premature termination or acid-to-base amino acid substitution at E312 would be expected to substantially alter the protein's structure and function. Thus, it is not surprising that mutations at E312 in GFAP induce Alexander disease. But why these mutations at E312 were associated with unusual phenotypes in these two patients is unknown, since other mutations in the same rod domain do not seem to similarly alter the phenotype.

The unique phenotype in our patient is *epilepsia partialis continua* – an unabating focal seizure manifesting as continuous twitching of unilateral perioral muscles. The focality of the seizures must reflect focal neuropathology and suggests that the mutation in our patient is creating pathophysiological changes in one part of the brain that are different from those elsewhere. We speculate that the E312K GFAP mutation affects astrocytes differently in different brain locations, reflecting regional differences among astrocytes.

Classically, astrocytes have been divided into the protoplasmic type, which are found in gray matter, and the fibrous type, which reside in white matter (Wilkin et al., 1990). For many years, it was believed that this was the only distinction among astrocyte populations. In recent decades, however, evidence has begun to emerge of regional differences among astrocytes. For example, we have shown that astrocytes in the cerebral cortex and subfields of the hippocampus differ in their responses to spreading depression and reverberatory seizures (Bonthius and Oswald, 1993; Bonthius et al., 1994; 1995). Others have demonstrated regional differences among astrocytes in the expression of cytoskeletal elements and neuropeptides, and in the uptake of neurotransmitters (Wilkin et al., 1990). Regional differences in astrocytes have been observed in a variety of disease states, including Alzheimer's Disease, amyotrophic lateral sclerosis, and major depression (Schitine et al., 2015). However, in many of these situations, it has been unclear whether the regional differences in astrocytes are due to intrinsic differences in the astrocytes themselves

or to regional differences in neuronal pathology. Because Alexander disease is a primary disease of astrocytes, it may be an excellent disorder in which to investigate astrocyte–neuron interactions and the basic neuroscience of regional astrocytic heterogeneity.

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Figure 1. MRI scan of the brain obtained at age five months reveals evidence of a leukodystrophy

A. T1-weighted image in the horizontal plane reveals hypointense signaling throughout the subcortical and deep white matter of the cerebral hemispheres. The ventricular system and the extra-axial cerebrospinal fluid collections are enlarged. Cortical sulci are prominent, consistent with diffuse cortical atrophy.

B. T2-weighted image in the horizontal plane reveals hyperintense signaling throughout the subcortical and deep white matter, as well as in the external and internal capsules. The results are consistent with a leukodystrophy.

C. T1-weighted image in the mid-sagittal plane reveals a diffusely small brain stem, consistent with a neurodegenerative process.