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**GFAP mutations, age at onset, and clinical subtypes in Alexander disease**

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**ABSTRACT**

**Objective:** To characterize Alexander disease (AxD) phenotypes and determine correlations with age at onset (AAO) and genetic mutation. AxD is an astroglialopathy usually characterized on MRI by leukodystrophy and caused by glial ﬁbrillary acidic protein (GFAP) mutations.

**Methods:** We present 30 new cases of AxD and reviewed 185 previously reported cases. We conducted Wilcoxon rank sum tests to identify variables scaling with AAO, survival analysis to identify predictors of mortality, and χ² tests to assess the effects of common GFAP mutations. Finally, we performed latent class analysis (LCA) to statistically define AxD subtypes.

**Results:** LCA identiﬁed 2 classes of AxD. Type I is characterized by early onset, seizures, macrocephaly, motor delay, encephalopathy, failure to thrive, paroxysmal deterioration, and atypical MRI features. Type II is characterized by later onset, autonomic dysfunction, ocular movement abnormalities, bulbar symptoms, and atypical MRI features. Survival analysis predicted a nearly 2-fold increase in mortality among patients with type I AxD relative to those with type II. R79 and R239 GFAP mutations were most common (16.6% and 20.3% of all cases, respectively). These common mutations predicted distinct clinical outcomes, with R239 predicting the most aggressive course.

**Conclusions:** AAO and the GFAP mutation site are important clinical predictors in AxD, with clear correlations to deﬁned patterns of phenotypic expression. We propose revised AxD subtypes, type I and type II, based on analysis of statistically deﬁned patient groups. *Neurology®* 2011;77:1287-1294

**GLOSSARY**

AAO = age at onset; AxD = Alexander disease; CNMC = Children’s National Medical Center; GFAP = glial fibrillary axial protein; IRB = institutional review board; LCA = latent class analysis.

Alexander disease (AxD)¹ often presents as a progressive astroglialopathy caused by dominant mutations in the glial ﬁbrillary acidic protein (GFAP) gene.² Pathogenic mutations are thought to confer cytotoxicity through gain-of-function mechanisms.³,⁴

Three age-dependent clinical subtypes—infantile, juvenile, and adult—have been adopted.⁵–⁷ Infantile AxD (birth to 2 years) is characterized by developmental delay, seizures, megalencephaly, and progressive deterioration, with increased severity in neonatal patients.⁸ Juvenile AxD (2–14 years of age) is characterized by hyperreﬂexia, bulbar symptoms, and ataxia, with preserved motor and cognitive function and milder progression than the infantile form. Adult AxD (late adolescence and beyond) has been described as being similar to the

References e1–e47 are available on the *Neurology®* Web site at www.neurology.org.

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juvenile form \(^9\) and is characterized by bulbar symptoms, ataxia, palatal myoclonus, and spastic paraparesis.

Early efforts to characterize age-dependent subtypes preceded the discovery of the role of \(\text{GFAP}\) in \(\text{AxD}\). The availability of \(\text{GFAP}\) sequencing has increased diagnostic accuracy across the lifespan, and patient samples have grown to allow statistical analysis of the relationships between age at onset (AAO), the \(\text{GFAP}\) genotype, and clinical outcomes.

In this study, we reviewed the clinical, radiologic, and genetic features of 215 patients with \(\text{AxD}\). We explored clinical outcomes as a function of AAO and \(\text{GFAP}\) mutation site, with emphasis on the most commonly affected amino acid residues, R79 and R239. In an effort to statistically characterize \(\text{AxD}\) subtypes, we used latent class analysis (LCA) to isolate clinically coherent patient groups with similar disease outcomes and investigated group difference in AAO and frequency of common \(\text{GFAP}\) mutations.

**METHODS**

**Standard protocol approvals, registrations, and patient consents.** Histories of 215 patients with \(\text{GFAP}\)-confirmed \(\text{AxD}\) were examined according to an institutional review board (IRB)-approved protocol. Informed consent was obtained from 30 unpublished patients evaluated and sequenced at Children’s National Medical Center (CNMC) with complete histories, imaging, and \(\text{GFAP}\) sequencing (appendix e-1 on the Neurology® Web site at www.neurology.org; for detailed patient information, see table e-1). In accordance with IRB regulations, we retrospectively analyzed data from 185 cases previously described in the literature (57 published patients with complete histories and imaging from collaborating physicians and 128 patients identified in the literature).

**Clinical and radiologic outcome measures.** Data sought included gender, AAO, survival, birth history, presence of antecedent encephalopathy or developmental delay at diagnosis (motor and cognitive development 25% delayed for age or clinical description consistent with this measure), seizures, failure to thrive (lack of normal height and weight gains), frequent emesis, bulbar symptoms (dysphonia, dysarthria, or dysphagia), motor disturbance, autonomic dysfunction (orthostatic hypotension, sphincter dysfunction, or urinary tract dysfunction), sleep disturbance, and history of paroxysmal deterioration (acute worsening associated with infection or trauma). On physical examination, macrocephaly (head circumference greater than 2 SD above normal for age and gender), ocular movement abnormalities, bulbar symptoms, palatal myoclonus, spasticity, dystonia, chorea, and ataxia were recorded. Summary scores for seizures, encephalopathy, bulbar symptoms, autonomic dysfunction, and motor dysfunction were generated and included all subjects with presentation or history of relevant phenotypes.

Where available (162 subjects), MRI findings were reviewed for \(\text{AxD}\) typical characteristics,\(^{10}\) including frontal predominance of white matter abnormalities, periventricular rim of low signal on T2/high signal on T1, signal abnormalities of basal ganglia or thalami, brainstem abnormalities, and contrast enhancement in the periventricular region, ventricular lining, frontal white matter, optic chiasm, brainstem, dentate nucleus, cerebellar cortex, fornix, basal ganglia, or thalami. Patients with available radiologic data who did not present with at least 4 of these 5 criteria were considered to have atypical radiologic presentations.\(^{10}\)

**Review of case histories from the literature.** A literature review targeted all published \(\text{GFAP}\) mutation-positive confirmed cases of \(\text{AxD}\) and was conducted in PubMed for articles published between January 2001 and July 2009 using \(\text{GFAP}\) and Alexander disease as search criteria, identifying 67 studies.\(^{11-41,45-47}\) Letters were sent to study authors, and detailed histories were received for 57 patients. Histories were coded to extract patients’ AAO, age at the time of study or patient’s death, presence or absence of clinical and radiologic features detailed above, and location of \(\text{GFAP}\) mutation.

**Statistical analysis.** Statistical analysis was performed using Stata (StataCorp, College Station, TX). To assess the relationship between AAO and clinical data, Wilcoxon rank sum tests were performed for each clinical and radiologic variable, and survival analysis was performed on all subjects with known values for AAO and last known age or age of death \((n = 171)\). Logistic regressions were conducted with phenotypic variables against mutated \(\text{GFAP}\) exons and protein domains. The high frequency of R79 and R239 mutations permitted \(\chi^2\) analyses for patients with these mutations to identify associated phenotypes. To eliminate orthogonality, patients carrying these mutations were removed and exon by phenotype logistic regression was repeated.

Finally, LCA was conducted in MPLus (Muthén & Muthén, Los Angeles, CA)\(^{36}\) to analyze clinical patterns. LCA is a person-centered, probability-based test for heterogeneous subpopulations in a target population.\(^{36-38}\) This model accommodates unequal degrees of variance for each cluster and can be tested using formal statistical approaches. Seven dichotomous variables judged to represent the phenotypic spectrum of \(\text{AxD}\) were included (seizures, autonomic dysfunction, encephalopathy, bulbar symptoms, history of paroxysmal deterioration, palatal myoclonus, and typical/atypical MRI). We limited our selection of variables to clinical parameters that may be expressed across the lifespan to avoid a definitional bias in our selection of variables. For example, macrocephaly, a predictor of early-onset \(\text{AxD}\), was not included because it is not seen in older individuals. Gait disturbance, which predicts late-onset \(\text{AxD}\), was not included because infantile patients would not express this trait by definition.

First, various LCA models were explored and compared, and Bayesian information criterion\(^{58-59}\) and Lo-Mendel-Rubin likelihood ratio tests\(^{60}\) were used to optimize the number of classes. Phenotypic variables were then classified into their most likely classes by estimated posterior probabilities, and quality of membership classification was assessed by average posterior probabilities and the entropy statistic. The prevalence of each class and the conditional probable incidence of each phenotypic variable in each class were assessed. In addition, we conducted post hoc analyses of onset age and incidence of R79 and R239 mutations across classes. Finally, we performed a Mantel-Cox survival analysis and Cox regression-based test for equality of survival curves to determine median survival periods and mortality rate ratios across classes.
RESULTS AAO predicts clinical and radiologic phenotypes. Clinical and radiologic features varied with AAO. The following clinical features predicted early onset: presentation with seizures, febrile seizures, motor delay, and cognitive delay; history of failure to thrive and paroxysmal deterioration; development of seizures and encephalopathy; and physical examination finding of macrocephaly. In some cases, such as motor and cognitive delay, these findings are probably consistent with the age at presentation. Clinical phenotypes predicting late onset were presentation with bulbar symptoms and autonomic dysfunction, development of autonomic dysfunction and gait disturbance, and physical examination findings of ataxia, dysarthria, dysphonia, ocular movement abnormalities, and palatal myoclonus (figure 1A, table e-2A). We did not replicate prior findings of male predominance in juvenile cases.\textsuperscript{e2}

Among radiologic features, early onset was associated with typical MRI features and with each criterion individually, except brainstem abnormalities. Late onset predicted atypical MRI features that were characterized most commonly by predominance of posterior fossa white matter abnormalities, brainstem atrophy, cerebellar atrophy, and spinal cord atrophy (figures 1B and e-1, table e-2B).

GFAP mutation analysis. GFAP mutations were identified in exon 1 (45.5% of cases), exon 3 (3.3% of cases), exon 4 (27.2% of cases), exon 5 (1.8% of cases), exon 6 (16.0% of cases), exon 7 (<1% of cases), and exon 8 (7.5% of cases). No mutations have been identified in exons 2 and 9. These mutations were distributed across the following GFAP domains: N-terminal head domain (<1% of cases), coil 1A (43.7% of cases), coil 1B (4.2% of cases), linker 12 (<1% of cases), coil 2A (23.7% of cases), linker 2 (<1% of cases), coil 2B (13.0% of cases), and C-terminal tail (13.5% of cases). No mutations have been identified in the linker 1 region, and small sample sizes for linker 12 and linker 2 mutations precluded genotype-phenotype analysis for these regions. Within the CNMC sample, 7 novel mutations were identified (table 1). A total of 78 unique GFAP mutations causing amino acid changes in the coding region of the gene have been identified (table e-3). In addition, 43.6% of mutations occurred in CpG methylation sites, accounting for 68.7% of cases. Mutations occurred disproportionately at residues R239 (20.3% of cases) and R79 (16.6% of cases). The next most common mutations affected R88 (7.9% of cases) and R416 (5.6% of cases). Thus, more than half (50.7%) of subjects had mutations at 1 of these 4 residues. All other mutations occurred in fewer than 4% of cases.

R79 mutations predicted early onset (mean ± SE 1.90 ± 0.70; figure e-2) and increased presentation with seizures, febrile seizures, motor delay, cognitive delay, and development of gait disturbances relative to patients with non-R79 mutations (table e-4A). R239 mutations also predicted early onset (1.00 ± 0.12; figure e-2), presentation with seizures, febrile seizures, motor delay, and cognitive delay; development of seizures, focal motor complaints, and encephalopathy; and a history of frequent emesis, failure to thrive, and paroxysmal deterioration (table e-4B). Contrary to prior findings,\textsuperscript{e2} R239 mutations did not segregate by gender. However, R239H patients presented earlier (0.48 ± 0.14 years; p = 0.007, 2-tailed t test) than R239C patients (1.28 ± 0.17 years), although no phenotypic differences were found among these patients. Similar analyses of R88 and R416 patients did not identify significant genotype-phenotype correlation nor was either mutation associated with AAO (R88: p = 0.210; R416: p = 0.692). When R79 and R239 patients were excluded, no variables localized to individual GFAP exons, whereas failure to thrive, frequent emesis, and encephalopathy remained associated with coil 2A mutations (n = 7, p < 0.05, logistic regression).

LCA. Bayesian information criterion and Lo-Mendel-Rubin likelihood ratio tests indicated that the 2-class model optimally fit the data and that it was superior to both the 1-class and 3-class models (table 2). Within the 2-class model, average posterior probabilities of correct class membership assignment were high: 0.96 for class 1 and 0.90 for class 2, significantly higher than the cutoff value of 0.70.\textsuperscript{e41} Further, the entropy value of 0.772 indicates that the 2-class model provides clear classifications\textsuperscript{e42,e43} (table 3).

On the basis of the pattern of conditional probabilities for each phenotypic variable (table e-5), we define class 1 as type I AxD, with increased likelihood of seizures, encephalopathy, paroxysmal deterioration, and typical MRI features and class 2 as type II AxD, with increased likelihood of autonomic dysfunction, bulbar symptoms, and palatal myoclonus. Post hoc $\chi^2$ tests of physical examination findings vs class membership further revealed that type I is associated with macrocephaly (p < 0.0001) and that type II is associated with ocular movement abnormalities (p = 0.005). The estimated prevalence rate of types I and II AxD (i.e., the probabilities of being assigned to specific latent classes) were 60% and 40%, respectively.

AAO and incidence of common GFAP mutations varied across the latent classes. Patients with type I AxD showed earlier onset (1.74 ± 0.29 years; p < 0.0001, 2-tailed t test) and higher incidence of R79 (0.25 on average; p < 0.0001, LCA) and R239 (0.31 on average; p < 0.0001, LCA) mutations. Patients...
with type II AxD showed later onset (21.64 ± 2.35 years; \( p < 0.0001 \), 2-tailed \( t \) test) and relative paucity of R79 (0.08 on average; \( p < 0.0001 \), LCA) and R239 (0.04 on average; \( p < 0.0001 \), LCA) mutations (figure e-3, A and B).

Finally, survival analysis revealed significant differences in postonset survival periods between patients with type I and type II AxD (for Kaplan-Meier survival curves, see figure e-4). Mantel-Cox comparison revealed a mortality rate ratio of 1.93 for type I AxD (\( n = 108 \)) relative to type II AxD (\( n = 63 \)), with a median survival of 14.0 ± 1.8 years for type I and 25.0 ± 2.1 years for type II. Relative hazards assigned by a Cox regression-based test for equality of survival curves were 1.51 for type I and 0.62 for type II (\( p = 0.0063 \)). The survival differences between patients with type I and type II AxD remained significant when R79 and R239 cases were removed.

Mean AAO for clinical (A) and radiologic (B) features showing significant association with AAO. In B, the 4 of 5 MRI criteria are from van der Knaap et al.\textsuperscript{10}: Frontal predominance, frontal predominance of white disturbances; Periventricular rim, periventricular rim of low signal on T2/high signal on T1; Basal ganglia/thalamus: signal abnormality of basal ganglia or thalamus; Contrast enhancement: contrast enhancement in periventricular region, ventricular lining, frontal white matter, optic chiasm, brainstem, dentate nucleus, cerebellar cortex, fornix, basal ganglia, or thalami. The fifth van der Knaap criterion, brainstem abnormalities, did not show significant age effects and is not displayed. Bars represent mean AAO among individuals with positive identification of clinical/radiologic feature, error bars represent ±1 SEM. All comparisons were significant at the \( p < 0.01 \) level. For additional statistics, see table e-2, A and B.

Figure 1  Age at onset (AAO) associated with Alexander disease (AxD) clinical outcomes
We confirm that AAO is, in itself, a powerful predictor of phenotypic patterns and disease course in AxD. Our findings offer statistical support to past studies demonstrating age-dependent variation in the clinical features of AxD but highlight the need for revisions to the precise definition of clinical subtypes. LCA model fitting was robustly significant for 2 patient classes but failed to converge on a 3-class model, suggesting that AxD is characterized by 2, not 3, clinical subtypes.

Importantly, our LCA model did not include age as a variable, and assessment of age differences across patients with type I and type II AxD was conducted a posteriori to test the validity of previously defined age constructs. Post hoc analysis revealed onset age differences across the 2 identified classes, with patients with type I AxD tending to present within the first 4 years of life and patients with type II AxD presenting across the lifespan. With the exception of ataxia and dysphagia, each age-sensitive outcome measure identified by rank-sum test was differentially expressed across type I and type II AxD. In addition, both R79 and R239 mutations showed higher prevalence within patients with type I relative to type II AxD. Taken together, these data converge on a clinical portrait of 2 phenotypically distinct variants of AxD (table 4). Type I AxD is characterized by early AAO, seizures, encephalopathy, paroxysmal deterioration, failure to thrive, developmental delay, and hallmark radiologic features. In contrast, type II AxD manifests across the lifespan and is character-
ized by autonomic dysfunction, bulbar symptoms, ocular movement abnormalities, and palatal myoclonus and is largely without neurocognitive or developmental deficits. It has been suggested that juvenile and adult AxD subtypes share common features, and our findings suggest that these forms are indeed not clinically distinct, both fitting well in the model of type II AxD.

It is important to note that despite the high quality of fit, our LCA model predicts trends and does not allow for type classification in each case with absolute certainty. Post hoc cluster analysis of our LCA findings suggests that type I AxD tends to manifest by age 4 and that type II tends to manifest beyond age 4. These age cutoffs, however, are not definitive, and age must be considered in the context of clinical, radiologic, and genetic data before a definitive type assignment can be made. Of the 113 patients with age data that LCA assigned to type I, 12 presented after age 4. Likewise, of the 72 patients assigned to type II, 20 presented at or before age 4. Although it is possible that some early-onset cases assigned to type II had not developed the full complement of clinical manifestations at the time of case report and that further monitoring would have revealed a more type I–like clinical pattern, many unambiguously fell into the type II category. Overall these results suggest that AxD clinical manifestations occur along a spectrum of severity and type and that this spectrum is closely associated with AAO.

Our findings support prior suggestions that severity is increased in early-onset AxD relative to postinfantile presentations, with survival analysis revealing reduced median survival and nearly a 2-fold increase in relative incidence of mortality among patients with type I relative to type II.

Most GFAP mutations occur with low frequency throughout the coding region. We found, however, that more than half of all patients had mutations affecting 1 of 4 amino acids in the GFAP peptide sequence (R79, R88, R239, and R416). The frequency of these mutations highlights the potential utility of screening for mutations affecting these residues, preempting whole-gene sequencing in many individuals with suspected cases. We confirm prior genotype-phenotype correlation studies of R79 and R239 mutations but failed to find an association of clinical features with R88 or R416. Novel mutations continue to be identified and we have identified 7 novel GFAP mutations within the 30 patients evaluated at CNMC (table 1).

Studies have identified AxD-causing mutations throughout the GFAP coding region and suggested that their pathogenicity may arise from various mechanisms. R239 mutations predict severe manifestations and affect the coil 2A domain of GFAP. Preliminary analysis of clinical variance across GFAP domains suggests that coil 2A may be particularly sensitive to genetic insult. Non-R239 mutations in the coil 2A domain predicted features robustly associated with R239 mutations, although the limited sample size highlights the need for confirmation in future studies. We failed to identify a previously reported association between coil 2B mutations and a markedly fulminant course. It is possible that our analysis lacked the resolution to isolate phenotypic associations within specific regions of each domain. Targeted analysis with larger sample sizes may reveal statistically robust associations between phenotypes and particular disruptions in GFAP structure.

Despite robust genotype-phenotype correlation for R79 and R239, other mutations do not associate with defined phenotypes. Nearly half of patients studied carry a mutation occurring in 5 or fewer cases. Given the low prevalence of AxD, analyses of these infrequent mutations will be underpowered for a considerable period of time. In addition, until recently, the clinical heterogeneity type II AxD reduced diagnostic accuracy, and this form is probably underrepresented in the literature. A cluster of mutations associated with late onset has been identified in a region spanning coil 1B and coil 2A. Whereas our findings do not identify type II–associated mutations, future studies may reveal genetic signals associated with this subtype.

Overall, these findings confirm AAO and R79/R239 genotype as powerful predictors of clinical manifestations and outcomes in AxD. We propose revisions to subtype classification that reflect the lack of statistical distinction between juvenile and adult forms. Although each case of AxD must be considered in the unique context of the patient’s clinical history, we believe that high-throughput screening for common mutations in patients with suspected cases can improve diagnostic efficiency and that type I/type II AxD categorization affords a statistically sound predictive clinical tool.

**AUTHOR CONTRIBUTIONS**

M. Prust: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, statistical analysis, and study supervision. Dr. Wang: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, and statistical analysis. Dr. Morizono: drafting/revising the manuscript, analysis or interpretation of data, and acquisition of data. Dr. Messing: drafting/revising the manuscript and analysis or interpretation of data, and statistical analysis. Dr. Brenner: drafting/revising the manuscript, analysis or interpretation of data, and acquisition of data. Dr. Gordon: drafting/revising the manuscript, study concept or design, acquisition of data, and study supervision. Dr. Hartka: analysis or interpretation of data, contribution of vital reagents/tools/patients, and acquisition of data. A. Sokhol: drafting/revising the manuscript, analysis or interpretation of data, acquisition of data, and study supervision. Dr. Schiffmann: drafting/revising the manuscript and acquisition of data. H. Gordish-
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