A novel mutation in the GFAP gene expands the phenotype of Alexander disease

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ABSTRACT

Background Alexander disease, an autosomal dominant leukodystrophy, is caused by missense mutations in GFAP. Although mostly diagnosed in children, associated with severe leukoencephalopathy, milder adult forms also exist.

Methods A family affected by adult-onset spastic paraplegia underwent neurological examination and cerebral MRI. Two patients were sequenced by whole exome sequencing (WES). A candidate variant was functionally tested in an astrocytoma cell line.

Results The novel variant in GFAP (Giall Fibrillary Acidic Protein) N-terminal head domain (p.Gly18Val) cosegregated in multiple relatives (LOD score: 2.7). All patients, even those with the mildest forms, showed characteristic signal changes or atrophy in the brainstem and spinal cord MRIs, and abnormal MRS. In vitro, this variant did not cause significant protein aggregation, in contrast to most Alexander disease mutations characterised so far. However, cell area analysis showed larger size, a feature previously described in patients and mouse models.

Conclusion We suggest that this variant causes variable expressivity and an attenuated phenotype of Alexander disease type II, probably associated with alternative pathogenic mechanisms, that is, astrocyte enlargement. GFAP analysis should be considered in adult-onset neurological presentations with pyramidal and bulbar symptoms, in particular when characteristic findings, such as the tadpole sign, are present in MRI. WES is a powerful tool to diagnose atypical cases.

INTRODUCTION

Missense gain-of-function mutations in GFAP are the only known cause of Alexander disease, a rare neurodegenerative disorder pathologically defined by white matter degeneration and the presence of characteristic Rosenthal fibres (intranuclear inclusions in astrocytes).1,2 In infantile cases (Alexander disease type I), patients present developmental delay, macrocephaly, seizures and progressive encephalopathy, leading to death within the first decade. MRI shows leukoencephalopathy without brainstem abnormalities.2,3 In later-onset cases (Alexander disease type II) present wide phenotypic variability, with symptoms such as ataxia, spastic paraparesis, palatal tremor, abnormal ocular movements, and bulbar or pseudobulbar symptoms.4 Additional neurological signs such as dystautonomia, urinary disturbances and sleep disorders are often described.5 Atypical features, including scoliosis, mild cognitive deficit, parkinsonism, seizures, peripheral neuropathy or microcoria, have been reported.6–8 MRI shows little cerebral white matter involvement and is characterised by atrophy and signal intensity changes in the brainstem.9

Although most GFAP mutations occur de novo, adult-onset Alexander disease has also been described in familial cases with autosomal dominant transmission.7 In this work, we report a family affected by ocular movement abnormalities and mild signs of pyramidal involvement, in which a rare variant of the GFAP gene was found by whole exome sequencing (WES). Based on clinical data and functional studies, we suggest that this variant is less deleterious than the vast majority of Alexander disease mutations, giving rise to an attenuated clinical phenotype.

RESULTS

A 46-year-old Caucasian woman (figure 1, patient II:3) presented with a 2-year history of spasticity and lower limb weakness. Cranial and cervical MRI was initially reported as normal. She denied a history of neurological disease in her family, except for a maternal cousin (patient II:5) who had ‘gait problems’. She also mentioned that her 16-year-old son (patient III:4) had frequent falls and mild difficulties in running starting at 9 years old, similar to her cousin’s son (patient III:5). After ruling out acquired causes, we tested spastic paraparesis genes (SPG3, SPG4, SPG10 and SPG11) and ABCD1 gene (X linked adrenomyeloneuropathy), with negative results. Genetic tests for hereditary ataxias were also negative. In a final attempt to elucidate this disease, we included the family in a research protocol and carried out WES on patients II:3 and III:4. WES analysis revealed five rare variants shared by both patients. Only one variant cosegregated in all four affected relatives (II:3, II:5, III:4 and III:5). A heterozygous missense variant in the GFAP gene, p.Gly18Val. Mutations in this gene cause Alexander disease (OMIM #203450), an autosomal dominant leukodystrophy with described adult presentations.1,4 This variant was not previously associated with Alexander disease, nor was it present in databases of control individuals (1000 Genomes, ExAC (Exome Aggregation Consortium) and gnomAD (Genome Aggregation Database). Segregation analysis indicated that this variant was also carried by two asymptomatic family members (I:2 and II:2).
due to spastic paraparesis. Patients II:3 and II:5, aged 49 and 48 years old at first examination, needed unilateral support to walk. Patients II:3 and II:5 referred urinary disturbances; urodynamic study of patient II:3 confirmed detrusor overactivity. All patients showed abnormalities in ocular movement, with gaze-evoked nystagmus without ptosis, diplopia or alterations in saccadic pursuit; brisk tendon reflexes/hyper-reflexia; extensor plantar responses; and Hoffman sign. Patients II:2, II:3, III:4 and III:5 presented mild scoliosis.

An exhaustive MRI re-evaluation of patient II:3 revealed signal changes and medullary atrophy. Brain and spinal cord MRI studies were then extended to patients II:2, II:5, III:4 and III:5. All patients showed a mild signal change in T2/Fluid-attenuated inversion recovery sequences in the brainstem, specifically in the medulla and cervical spinal cord. This is illustrated by MRIs of patient II:3, in which signal change is visible in the midbrain (figure 1B), the medulla (figure 1C) and the spinal cord (figure 1D). Furthermore, patients II:3, II:5, III:4 and III:5 showed the characteristic ‘tadpole sign’: some degree of atrophy in the cerebellum, medulla and spinal cord with a well-preserved pontine base, markedly characteristic of Alexander disease (illustrated in figure 1E). The paucysymptomatic patient II:2 had no atrophy nor tadpole sign, but showed signal change in the medulla and spinal cord (figure 1F,G). Magnetic resonance spectroscopy was carried out in patients II.2, II.3, II.5 and III.4, with voxels centred in the area of signal and morphological abnormality. All patients showed highly elevated levels of myoinositol and choline with a decreased total N-acetylaspartate in the pontomedullary junction (figure 1H,I, online supplemental figure 1), a feature described in Alexander disease. Radiological findings are summarised in online supplemental table 2 and illustrated in figure 1 and online supplemental figure 1.

This GFAP gene variant (chr17:42992802C>A GRCh37; NM_001131019: c.53G>T; p.Gly18Val), found in all affected family members, was located in GFAP’s N-terminal head domain (Glia1 Fibrillary Acidic Protein), which plays an important role in self-assembly process. This is the most N-terminal variant ever described. However, this residue is not strongly conserved in evolution, missense predictors were not conclusive, and no other pathogenic variants are known in the vicinity. When considering all genotyped individuals, this variant reached a maximum LOD (logarithm of odds) score of 2.7 (odds of ~500 to 1 supporting linkage of this locus to the disease). By applying the American College of Medical Genetics criteria for variant interpretation to assess this nucleotide change, we reached a classification of VUS (variant of unknown significance), and thus decided to functionally validate this variant using a transfection assay to test the capacity of the GFAP protein carrying p.Gly18Val to induce protein aggregation in the astrocytoma cell line U251-MG (online supplemental methods). We used two GFAP-EGFP (Enhanced Green Fluorescent Protein) control constructs, one containing the wild-type GFAP sequence and the second incorporating the p.Arg239Cys mutation, a widely used positive control for GFAP protein aggregation. As described elsewhere, transfection of the wild-type (WT) construct showed large inclusions in ~20% of transfected cells, both after 24 hours or 48 hours of transfection. Cells transfected with the p.Arg239Cys-mutated construct showed the same large inclusions, but also dot-like clumps or aggregates, as reported, which in some cases were distributed around the cell and in other cases converged and formed large aggregates near the cell nucleus, in particular at 48 hours after transfection (figure 2A). In contrast, after transfecting the p.Gly18Val-mutant construct, we did not observe aggregates similar to the p.Arg239Cys construct, but

We therefore decided to clinically re-evaluate all family members and found abnormalities in oculomotor movements and pyramidal involvement in both two patients. In conclusion, this family showed variable disease expressivity among four patients exhibiting clear signs of disease and two paucysymptomatic individuals who presented alterations in neurological examination but had no complaints nor symptoms (patients I:2 and II:2). The age of clinical onset ranged from 9 to 46 years, and age at diagnosis ranged from 16 to 73 years. Clinical findings are summarised in online supplemental table 1, and online supplemental video 1 shows movement abnormalities in patient III:5.

In patients II:3, II:5, III:4 and III:5, symptoms at disease onset included asymmetric proximal lower limb weakness due to pyramidal involvement, which was associated with proximal upper limb weakness in patient II:5. All four subjects had gait difficulties

Figure 1 Family tree and genotype data for the p.Gly18Val variant and brain and cervical MRI and MRS of patients II:3 and II:2. (A) Family tree and genotype data for the p.Gly18Val variant. Square: male; circle: female; diagonal black line: deceased; black-filled symbol: affected individual; white-filled symbol: clinically healthy; question mark: unknown status; syringe symbol: blood sampled individual; asterisk: individual sequenced. (B–E) Brain MRI of patient II:3, symptomatic. (B,C) Axial fluid-attenuated inversion recovery (FLAIR) shows signal change in the midbrain (B, arrow) and subpial involvement in both two patients. In conclusion, this family

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We present a family affected by a dominantly inherited neurological disease, characterised by mild to moderate late-onset cerebellar and pyramidal signs, showing signal abnormalities or atrophy in the brainstem and spinal cord, in whom we identified a candidate variant in GFAP using WES, segregating even in asymptomatic individuals. Clinical re-evaluation of all family members combined with functional validation of the novel variant ultimately led to a definitive diagnosis of familial Alexander disease type II.

Clinically, Alexander disease type II presents with cerebellar ataxia, pyramidal involvement, bulbar symptoms and palatal tremor. It is accompanied by variable MRI findings, although most cases present the ‘tadpole sign’. In this family, four patients showed clear signs of cerebellar dysfunction, with mild ataxia, alteration of ocular movements and spastic paraparesis with hyper-reflexia and extensor plantar responses, and two patients were paucisymptomatic, presenting mild alterations on neurological examination, namely scoliosis, nystagmus, diplopia, hyper-reflexia and the Babinski sign. MRIs of all symptomatic patients showed notable atrophy of the spinal cord and medulla, in contrast to what was observed in the less affected patients, who presented mild signal changes in the trunk and less atrophy. We nonetheless wish to emphasise that all patients except one (patient II:2) presented the previously mentioned tadpole sign. Moreover, MRS (Magnetic Resonance Spectroscopy) on patients II.2, II.3, II.5 and III.4 showed a metabolite profile suggesting hypertrophy of astrocytes as previously discussed, consistent with neuroaxonal degeneration. This underscores our in vitro findings showing size enlargement of astrocytes.

Indeed, no dot-like clumps or protein aggregates were found for the p.Gly18Val GFAP construct in our functional study, in contrast to most other pathogenic variants described in the literature. We also detected lesser inclusions than the WT construct, in particular when transfected with lesser amounts of plasmid. However, we detected an increased size in p.Gly18Val-transfected cells when compared with the WT and p.Arg239Cys constructs. Astrocyte hypertrophy is a known consequence of GFAP mutations in Alexander disease, as observed in mouse models and patient necropsies. It is possible that astrocyte hypertrophy has been historically overlooked for other mutations in vitro due to the strong specificity of GFAP aggregates. We thus propose considering astrocyte hypertrophy as an additional criterion of pathogenicity in the functional evaluation of unreported variants.

Although we did not have access to brain biopsies from these patients, the absence of strong pathological signs in MRI and milder clinical manifestations in this family are compatible with the results of the aggregation assay for p.Gly18Val. This expansion of the clinical spectrum of Alexander disease suggests that other adult-onset neurological cases with overlapping ataxia and pyramidal involvement may be caused by pathogenic GFAP variants. Therefore, screening of this gene would be recommended in the presence of those symptoms and abnormal findings in MRI, even when these are subtle. An exhaustive and systematic clinical exploration of family members with milder forms or an absence of overt symptoms is recommended, since it may lead to the identification of clinically unnoticed cases. This work underscores the usefulness of WES to identify paucisymptomatic or atypical cases, and proposes its implementation as first-tier test for neurogenetic conditions with adult presentations, with the goal of improving disease management and genetic counselling.

**Acknowledgements.** We are grateful to the family members involved for sharing their data. The Neurometabolic Diseases Lab is a member of the Undiagnosed Diseases Network International (UDNI). We thank CERCA Program/Generalitat de Catalunya for institutional support. We also thank Juanjo Martínez and Cristina Guílera for excellent technical assistance and Asociación Española contra la Leucodistrofia (ALE-ELA España).

**Contributors** CC, EV, SF and AP designed and conceptualised the study. CC, EV, VV, AS, AP, E, CH, MR and NL analysed and interpreted the data. CC, EV, VV and AP drafted the manuscript. All authors critically revised the manuscript.
Funding This study was supported by the Centre for Biomedical Research on Rare Diseases (CIBERER) (AC114-759), Hisperion Foundation, and the Secretariat for Universities and Research of the Ministry of Business and Knowledge of the Government of Catalonia (2017SGR1206) to AP, and Instituto de Salud Carlos III (PI14/00581) (co-funded by the European Regional Development Fund, ERDF, a way to build Europe) and la Marató de TV3 (345/C/2014) to CC and AP. EV was funded by a grant from Ministerio de Economía, Industria y Competitividad (Juan de la Cierva Programme FJC-2016-28811). SF was funded by Instituto de Salud Carlos III (Miguel Servet Programme CPI16/0016), and MR and NL were funded by CIBERER.

Competing interests None declared.

Patient consent for publication Obtained.

Ethics approval The research project was approved by the Clinical Research Ethics Committee of the Bellvitge University Hospital (PRO17/14).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data are in the submitted paper.

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