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An infantile case of Alexander disease unusual for its MRI features and a GFAP allele carrying both the p.Arg79His mutation and the p.Glu223Gln coding variant

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Alexander disease (AxD) (MIM 203450) is a rare progressive neurological disorder caused by mutation of the *GFAP* gene (2), with variable clinical features (reviewed in 1). The most common infantile form usually presents before 2 years of age as a megalencephalic leukodystrophy with psychomotor retardation, seizures, and early death. In contrast, later-onset forms are more variable, with predominant bulbar signs and a slower progression. MRI criteria have been identified for diagnosis of infantile cases (9). The pathologic hallmark of AxD is the accumulation within astrocyte cytoplasm of protein aggregates primarily composed of glial fibrillary acidic protein (GFAP) and small stress proteins (1).

We report here an infantile case of AxD that is unusual both for its MRI features and for being heterozygous for a *GFAP* allele carrying a *de novo* p.Arg79His mutation and a paternally inherited p.Glu223Gln coding change. This female patient had delayed psychomotor development, and presented with seizures at 9 months of age. From the age of 8 years, progressive dysarthria, dysphagia, and ambulatory difficulty were observed. At the age of 12 years, neurological examination showed severe mental retardation, poor dysarthric speech, spasticity, Babinski sign, and ankle clonus. Cranial circumference and an ophthalmologic examination were normal. Extensive laboratory metabolic testing including lactate, pyruvate, urine organic acid, plasma amino acids, plasma very long chain fatty acids and lysosomal enzymes gave normal results.

Brain MRI showed diffuse white matter alteration without frontal predominance. A periventricular rim of decreased signal intensity on T2-weighted images was appreciable; medulla oblongata, dentate nuclei and peridentate cerebellar white matter showed signal alterations. Lateral ventricles were enlarged and the corpus callosum was very thin. Mean diffusivity was reduced in periventricular and fronto-parietal white matter. Postcontrast

enhancement was absent. A repeat MRI performed 3 years later showed severe brainstem and cerebellar atrophy (Fig. 1A-D). The neurological picture slowly deteriorated, and she suddenly died at 17 years. No autopsy was performed.

Negative results of widespread metabolic screening for known causes of leukodystrophies prompted us to consider a diagnosis of Alexander disease. After written consent, PCR products of DNA from peripheral leukocytes were sequenced (2), revealing heterozygous p.Arg79His (c.236G>A) and p.Glu223Gln (c.667G>C) coding changes. Both clinically unaffected parents tested negative for the p.Arg79His mutation; however, the father was positive for the p.Glu223Gln change. Using a PCR cloning procedure (6), it was determined that the p.Arg79His and p.Glu223Gln coding changes in our patient were on the same chromosome. Thus the patient inherited a normal GFAP allele from her mother, and inherited from her father the p.Glu223Gln allele which also bore a *de novo* p.Arg79His mutation. This is consistent with prior findings that *de novo* AxD mutations arise primarily in the paternal germ line (6).

The MRI findings for our patient are unusual in that they meet only two of the five standard criteria proposed (9) for an MRI-based diagnosis of infantile AxD (periventricular rim and brainstem lesions) and meet another only partially (leukodystrophy without frontal predominance). Our patient also displayed atrophy of the cerebellum and severe thinning of the brainstem and corpus callosum. The first two findings are encountered for later onset forms of AxD, but are rare for the infantile form (1). The last, severe thinning of the corpus callosum, is a quite common feature in several genetic neurodegenerative disorders, most notably spastic paraplegia with thin corpus callosum (e.g., see 8,11), but to our knowledge has not previously been described in AxD. The development of these changes in a later stage of the disease suggests that they may be part of the evolution of brain lesions in p.Arg79His patients. Unfortunately, details are lacking for the progression of the MRI abnormalities for the other p.Arg79His cases. However, we can surmise that unlike our patient, the majority initially had typical MRI features since MRI diagnosis was the basis for their selection for molecular genetic analysis (4,7). The presence in our patient of clinical and MRI features characteristic of both the infantile and adult forms of AxD further illustrates that the prototypical forms of the disease are linked by a continuum of cases with overlapping features.

Both the p.Arg79His and the p.Glu223Gln coding changes have been previously associated with AxD patients (2,3). The p.Arg79His mutation is considered causative for AxD due to its *de novo* appearance, whereas the role of p.Glu223Gln is unclear (1). It has now been associated with three AxD cases (3, 5, this report), but in each instance was also found in a parent. It was initially thought to be disease-causing because it was the sole GFAP alteration in a patient reported to have adult onset AxD (3). However, the diagnosis for this patient is equivocal; his MRIs are not suggestive of the disorder (M. van der Knaap, personal communication), and the interpretation of his clinical signs was complicated by hypertension, diabetes and alcoholism. p.Glu223Gln was subsequently found in an infantile patient whose clinical signs and MRIs were typical of AxD (5). However, this patient also had a *de novo* Y366H mutation, suggesting it was responsible for the disease. Although the p.Glu223Gln change was reported absent in 150 control chromosomes (3) and its only record in the NCBI human SNP database (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp&cmd=search&term=human+GFAP>) is from a previously reported case (3; B. Lane, personal communication), due to its presence in the unaffected parents we were prompted to test an additional 400 chromosomes obtained from 200 healthy subjects of Italian origin unrelated to our patient. DNA from peripheral blood was analyzed for the presence of the coding change by digestion of a PCR product with restriction enzyme HpyCH4V, whose recognition site is generated by the p.Glu223Gln (c.667G>C) coding change. One of the controls was found to be heterozygous, suggesting p.Glu223Gln is a rare variant rather than being disease causing.

Functional studies were performed to further test the possible role of p.Glu223Gln. The polymerization properties of GFAP containing the p.Arg79His and p.Glu223Gln single coding changes as well as the two together were investigated by transient transfection of SW13vim⁻ cells. Wild type GFAP predominantly produced thin, highly arborized filament networks which were variably interspersed with thicker, rope-like bundles of filaments (Fig. 2, upper left). A similar range of patterns was produced by p.Glu223Gln GFAP, but with a greater frequency of cells that contained thicker filaments (Figure 2, upper right; note the left hand side of the panel). In contrast, cells expressing p.Arg79His GFAP failed to form filaments; instead they displayed a diffuse pattern of cytoplasmic staining, with small cytoplasmic and/or peri-nuclear aggregates also being present in about 10% of the cells (Fig. 2, lower left). The p.Arg79His/p.Glu223Gln double mutant produced the same pattern as p.Arg79His, but nearly all cells contained aggregates, and the aggregates were often larger and more numerous (Fig. 2, lower right).

Altogether, four observations indicate that p.Glu223Gln is a rare variant rather than being disease causing: it has not arisen *de novo* in any of the three instances in which it has been observed in patients, none of the parents who harbour the mutation has shown signs of AxD, the disease course of our patient was substantially similar to that of others who had p.Arg79His as their sole GFAP coding change (1), and we have found it in a normal control. Despite this negative evidence, our cell transfection data do indicate that the p.Glu223Gln change has an effect on GFAP polymerization, albeit a modest one; and given its rarity among controls it is surprising that it has been associated with three independent patients with neurological disease. It is also possible that a compound p.Glu223Gln mutation could have a greater effect when the two mutations are present on different alleles, a situation which did not hold for our patient and so was not tested here. Thus overall the data point strongly to p.Glu223Gln being a rare variant, but the possibility remains that it can contribute to disease but with low penetrance.

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Figure 1. Axial T2 weighted images showing periventricular (A), medulla oblongata (B), and dentate nuclei (C) hyperintense lesions. Sagittal T1 weighted image showing a very thin corpus callosum, cerebellum and brainstem atrophy (D).

Figure 2.

Expression patterns of wild type and mutant GFAPs. Plasmids expressing the mutant GFAPs were constructed from pcDNA3.1-hGF(WT), transiently transfected into SW13vim⁻ cells and the cells stained 48 hours later for GFAP using methods similar to those previously described (5). An independent repetition of the transfections produced comparable results.