Homozygous UBA5 Variant Leads to Hypomyelination with Thalamic Involvement and Axonal Neuropathy

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Abstract

The enzyme ubiquitin-like modifier activating enzyme 5 (UBA5) plays an important role in activating ubiquitin-fold modifier 1 (UFM1) and its associated cascade. UFM1 is widely expressed and known to facilitate the post-translational modification of proteins. Variants in UBA5 and UFM1 are involved in neurodevelopmental disorders with early-onset epileptic encephalopathy as a frequently seen disease manifestation. Using whole exome sequencing, we detected a homozygous UBA5 variant (c.895C>T p. [Pro299Ser]) in a patient with severe global developmental delay and epilepsy, the latter from the age of 4 years. Magnetic resonance imaging showed hypomyelination with atrophy and T2 hyperintensity of the thalamus. Histology of the sural nerve showed axonal neuropathy with decreased myelin. Functional analyses confirmed the effect of the Pro299Ser variant on UBA5 protein function, showing 58% residual protein activity. Our findings indicate that the epilepsy currently associated with UBA5 variants may present later in life than previously thought, and that radiological signs include hypomyelination and thalamic involvement. The data also reinforce recently reported associations between UBA5 variants and peripheral neuropathy.

Keywords

► UBA5
► UFM1
► hypomyelination
► neuropathy

Introduction

The enzyme ubiquitin-like modifier activating enzyme 5 (UBA5) plays an important role in the activation of ubiquitin-fold modifier 1 (UFM1) and its associated cascade. UFM1 is widely expressed and known to facilitate the post-translational modification of proteins. While the UFM1 system has long been implicated in a variety of non-neurological pathological processes, in recent years research established its involvement in neurodevelopmental...
disorders.\textsuperscript{6–15} Bilalelic variants in \textit{UBA5} or \textit{UFM1} have been described in cases with encephalopathy, intellectual disability, movement disorders, epilepsy, and neuropathy. Neuroimaging findings in these cases are variable and mostly consist of cerebral and cerebellar atrophy.\textsuperscript{6–14} Delayed myelination, thalamic involvement and (only for types, de number of published cases and the variability of their phenotypes, defining clinical and neuroimaging characteristics remains challenging.

\textbf{Patient and Methods}

\textbf{Clinical Presentation}

This female patient, now 12 years old, is the first child of nonconsanguineous parents. There was no family history of neurological disorders. Because of preeclampsia, delivery was induced at 38 weeks' gestational age. Birth weight was 2,710 g. The patient was admitted to hospital for 10 days due to hypoglycemia, feeding problems, and hyperexcitability. At age 3 months, persistent general muscular hypertonia and opisthotonus were noted. At 5 months, development was delayed, with absent visual contact, axial hypotonia with impaired head control and elevated tone of her legs. Feeding was difficult due to slow and uncoordinated sucking. Electroencephalogram, ophthalmologic evaluation, and visual evoked potentials were normal, as well as extensive metabolic investigations. Repeat EEG at 12 months showed normal background activity with interspersed theta activity and hyperventilation. At age 3, persistent general muscular hypertonia and opisthotonus were noted. At 5 months, development was delayed, with absent visual contact, axial hypotonia with impaired head control and elevated tone of her legs. Feeding was difficult due to slow and uncoordinated sucking. Electroencephalogram, ophthalmologic evaluation, and visual evoked potentials were normal, as well as extensive metabolic investigations. Repeat EEG at 12 months showed normal background activity with interspersed theta activity and hyperventilation.

\textbf{Histopathology}

Samples were acquired through open sural nerve biopsy at age 2 years 10 months. CD68, PLP (myelin proteolipid protein), hematoxylin and eosin (HE), neurofilament (medium and heavy molecular weight, NE14), and Kluver–Barrera stains were performed by using standard protocols.

\textbf{Results}

\textbf{Genetic Testing}

Using WES, a homozygous variant in \textit{UBA5}, NM_024818.4: c.895C > T p.(Pro299Ser) (\textbf{\textit{UBA5}}) was detected in the patient. Both parents were heterozygous carriers. Frequency of this variant in healthy controls of gnomAD v2.1.1 (https://gnomad.broadinstitute.org/) is low: 6 alleles (heterozygous) out of 282508. The variant c.895C > T p. (Pro299Ser) classified as likely pathogenic (PS3, PM1, and PM2) according to the American College of Medical Genetics criteria (see InterVar Web Resources).\textsuperscript{18}

\textbf{Functional Data}

The Pro299Ser variant showed a significantly delayed activity after 2 minutes of incubation (\textit{\textbf{\textit{UBA5}}}, with 58% of remaining thiolation activity compared with the wild-type UBA5 protein (p < 0.05, t-test; \textbf{\textit{\textit{UBA5}}}). We then investigated...
whether the \textit{UBA5} variant affects the \textit{UFM1} conjugation activity to \textit{UFC1} (\textit{Fig. 2B}). After 3 minutes, the p.Pro299Ser variant conjugate band reached a mean relative density of 54\% ($p < 0.01$, t-test) (\textit{Fig. 2C}) and therefore had a significantly delayed conjugation activity compared with the wild-type \textit{UBA5}. Modelling of p.Pro299Ser depicts its location right at the interface with \textit{UFM1} (\textit{Fig. 2D}).

Histopathology
Neurofilament and HE stain showed thickened, thinned, and degenerated axons with mild activity in the NF and NE14 stains (\textit{Fig. 3A, C, D, F, and G}). Kluver–Barrera stain showed interrupted and decreased myelin presence (\textit{Fig. 3B and E}). CD68 stain revealed scattered macrophages in and around nerve cells. Electron microscopy analyzing myelin fiber

\textbf{Fig. 1} Brain MRI findings at 1, 2 and 4 years of age. Scans at age 19 months (top row) from left to right: One T2 weighted sagittal image and three T2 weighted axial images. MRIs at age 2.5 and 4 years (middle and bottom row, respectively) each from left to right: One T1 weighted sagittal image and three T2 weighted axial images. The MRI at 19 months shows a diffuse hyperintense T2 signal in the cerebral white matter, indicating significant lack of myelin. Hyperintense T2 signals are also seen in the lateral part of the thalamus (arrowhead). Thalamic volume is reduced. Volume and signal of the basal ganglia are normal. There is cerebellar atrophy. At age 2.5 years, MRI shows a stable T2 hyperintense white matter signal. The thalami are slightly smaller than on the previous MRI. At age 4 years, MRI shows pronounced cerebral atrophy. Signal of the left occipital cortex is elevated. White matter hyperintense T2 signal is stable.
cross-sections revealed disproportionately thin (compared with the axonal diameter) and split myelin sheaths, as well as myelin figure debris between the sheath layers.

**Discussion**

In this report, we describe a female patient with a homozygous *UBA5* variant with severe global developmental delay, prominent peripheral neuropathy, and epilepsy from age 4 years. Brain magnetic resonance imaging (MRI) showed severe myelin deficit without significant cerebral atrophy in the first 2.5 years, followed by global atrophy. In addition, there was prominent thalamic involvement with a T2 hyperintense stripe and decreased volume of the thalamus.

When we identified the homozygous *UBA5* variant in our patient, there was only one report on a sib pair with a much milder clinical presentation including progressive ataxia and cerebellar atrophy who were found to carry two biallelic *UBA5* variants, one of which a nonsense mutation. We therefore interpreted the missense variant found in the current case as probably not disease causing, as our patient presented with a clinically severe phenotype. When WES at another institution did not lead to a diagnosis, we reevaluated our own data. As *UBA5* variants were by then described in other patients with severe early onset encephalopathy, we considered the variant found in our patient as possibly disease causing.

In vitro studies could confirm its functional consequences by demonstrating impaired UFM1 conjugation.

What can we learn from this case? Perhaps the most important lesson is to reconsider results which seem not fitting or unexplainable at the time. New insights may lead to revised interpretation of genetic variants or MRI findings.

The second lesson is that clinical presentations may greatly vary in severity, which makes careful descriptions of neurological findings and imaging abnormalities essential to understand the full scope of possible manifestations and also to facilitate diagnosis in new potential cases. This is especially true (and challenging) for ultra-rare disorders where few patients are scattered among different centers.

Brain MRI findings in *UBA5*-related cases are variable and may even be normal in infancy. The most frequently described neuroimaging signs so far were cerebral and/or cerebellar atrophy. Delayed myelination was also seen in several cases, all younger than 7 months, with the majority showing only mildly delayed myelination. All published MR imaging data of older patients did not show this sign. This contrasts with the severe myelin deficit classified as hypomyelination in our patient. In one respective case each, thalamic
Fig. 3  Sural nerve histopathology. Tissue was obtained at age 2 years 10 months. (A) hematoxylin and eosin stain shows a longitudinally cut nerve segment with variability of axonal thickness and myelin pallor. (B, E) Kluver periodic acid Schiff for myelin shows global paucity of myelin (longitudinal and cross sections). (C, D, F, G) Stains against the phosphorylated (NF) and nonphosphorylated neurofilaments (NE14) confirms segmental variability of axonal thickness resembling in places axonal spheroids. (H–J) Electronic microscopy images show thin and split myelin sheaths, with myelin figure debris between the sheath layers. Magnifications 1,900, 9,300, and 30,000, respectively; all images at 120 kV.
atrophy and T2 hyperintensity were present.\textsuperscript{13} Interestingly, a promoter variant in \textit{UFM1} has previously been described to lead to hypomyelination and basal ganglia involvement.\textsuperscript{12} \textit{UBA5} and \textit{UFM1} closely interact,\textsuperscript{1,2} and the findings reported here support their importance for normal myelination.

Histopathological findings confirmed axonal neuropathy in the sural nerve. This substantiates that peripheral neuropathy is part of the \textit{UBA5} associated spectrum, and this manifestation should be actively investigated also in young, severely affected children.\textsuperscript{7} The paucity of myelin in the sural nerve highlights the potential importance of \textit{UBA5} for myelination. Whether the underlying pathological process consists of primary hypomyelination or a lack of myelin secondary to severe neuroaxonal damage is difficult to ascertain. Axonal damage can lead to a lack of myelin and vice versa.\textsuperscript{21} The severe early encephalopathy in patients with \textit{UBA5} variants and also epilepsy as one of the prominent symptoms in most patients argues for a primarily neuronal involvement with secondary hypomyelination, as is the case for \textit{UFM1}-associated disease. Why some variants clearly do impair myelination and others apparently not is not fully understood.

Our analyses confirmed functional effects of the Pro299Ser variant. It was previously noted that clinical severity of \textit{UBA5}-associated disorders might correlate with residual \textit{UBA5} activity.\textsuperscript{8} Cases with null alleles or a missense variant with strongly abolished protein function had early onset refractory epileptic encephalopathy, while some cases with higher residual protein activity presented with developmental delay but no epilepsy.\textsuperscript{8,12} In our case, the relatively high residual \textit{UBA5} activity might explain the late onset of epilepsy, but contrasts with the severe neurological presentation from early infancy.

In conclusion, \textit{UBA5}-associated disorders may lead to (secondary) brain hypomyelination and characteristic thalamic involvement and prominent peripheral neuropathy, in addition to severe developmental delay and epilepsy.

Note
M.S.V.D.K has a patent P112686CA00, therapeutic effects of Guanabenz treatment in vanishing white matter pending to VU University Medical Center. Amsterdam Leukodystrophy Center is a member of the European Reference Network for Rare Neurological Disorders, project ID 739510.

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Conflict of Interest
None declared.

References