Association between homozygous c.318A>G mutation in exon 2 of the EIF2B5 gene and the infantile form of vanishing white matter leukencephalopathy

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Abstract

Background: Vanishing white matter disease is one of the most frequent leukodystrophies in childhood with autosomal recessive inheritance. A mutation in one of the genes encoding the five subunits of the eukaryotic translation initiation factor 2 (EIF2B5) is present in 90% of the cases. The diagnosis can be made by clinical and neuroradiological findings and molecular tests. Case report: We describe a thirteen-month-old male with previous normal neurodevelopment, who was hospitalized for vomiting, hyperthermia, and irritability. On examination, cephalic perimeter and cranial nerves were normal. Hypotonia, increased deep tendon reflexes, generalized white matter hypodensity on head tomography were found. Fifteen days after discharge, he suffered a minor head trauma presenting drowsiness and focal seizures. Magnetic resonance showed generalized white matter hypointensity. Vanishing white matter disease was suspected, and confirmed by sequencing of the EIF2B5 gene, revealing a homozygous c.318A>T mutation in exon 2. Subsequently, visual acuity was lost and cognitive, and motor deterioration was evident. The patient died at six years of age due to severe pneumonia. Conclusions: This case contributes to the knowledge of the mutational spectrum of VWM in Mexican patients and allows extension of the phenotype associated to this mutation.

Key words: Vanishing white matter disease. EIF2B5 gene. Magnetic resonance. Leukodystrophy.

Asociación entre la mutación homocigota c.318A>T en el exón 2 del gen EIF2B5 y la forma infantil de la leucoencefalopatía con sustancia blanca evanescente

Resumen

Introducción: La leucoencefalopatía con sustancia blanca evanescente es una de las leucodistrofias más frecuentes. Generalmente inicia en la infancia y presenta un patrón de herencia autosómica recesiva. El 90% de los casos manifiesta mutaciones en uno de los genes que codifican para los cinco subunidades del factor de iniciación eucariótica 2 (EIF2B5). El diagnóstico se realiza por las manifestaciones clínicas, hallazgos en la resonancia magnética cerebral y estudios moleculares confirmatorios. Caso clínico: Paciente masculino de 13 meses con neurodesarrollo previo normal. Antecedente de
Convulsivas. Posteriormente mostró deterioro cognitivo, motor y pérdida de la agudeza visual. Falleció a los 6 años por homocigota c.318A>T en el exón 2. El paciente requirió múltiples hospitalizaciones por hipertermia y descontrol de crisis.

Conclusiones: Este caso contribuye a conocer el espectro de mutaciones que se presenta en pacientes difficulty-to-treat seizures, coma, apnea, microcephaly, decreased fetal movements. At birth, patients present with feeding difficulties, vomit, hypotonia, decreased fetal movements. At birth, patients present with feeding difficulties, vomit, hypotonia, difficult-to-treat seizures, coma, apnea, microcephaly, cataract, organomegaly, renal hypoplasia, ovarian dysgenesis, joint contractures and death before one year of age. The childhood form manifests in the first year of life with irritability, stupor, seizures, motor neuroregression, recurrent infections; usually, patients die before two years of age. There is a late childhood form that presents in two ways: some patients develop ataxia, intention tremor, dysmetria between one and 5 years of age, with a previous period of normal motor and cognitive development, some with motor and mild language retardation. Other cases present with coma after a traumatic brain injury (TBI) or fever, and even though recovery exists, patients develop progressive motor deterioration with gait problems, signs of upper motor neuron disease, dysarthria, seizures, feeding problems and optic nerve atrophy. In these patients, cognitive abilities seem to be preserved, and they later enter a phase of stabilization until they die in a period of 1 to 5 years. The juvenile form starts between 5 and 15 years of age, with slowly progressive spastic diplegia that alternates with periods of stability, and even motor improvement; patients have a long survival. In the adult form, cognitive impairment, transient episodes of optic neuritis, hemiparesis, intense headaches predominate. It presents as secondary amenorrhea due to ovarian failure in women often referred as ovarioleukodistrofia. Recent publications have emphasized the presence of movement disorders, such as dystonia and myoclonus, which characterize late onset presentations, but occasionally childhood forms with opsoclonus-myoclonus have been described.

Neuroradiological abnormalities are a constant, but they vary according to age and can be present in asymptomatic patients. The study of choice is a brain magnetic resonance (MR). In the neonatal forms, only brain immaturity can be observed, characterized by thick gyri, and white matter with a high-water content. In the childhood forms, the hemispheric white matter is


Introduction
Vanishing White matter disease (VWM, OMIM#603896), also known as childhood ataxia with central nervous system hypomyelination, is one of the most frequent leukodystrophies in Caucasians. The first case was described by W. Eicke in 1962, in a 36-year-old woman with secondary amenorrhea that, after a mild traumatic brain injury, developed progressive neurological deterioration until her death. VWM is a hereditary disease with an autosomal recessive pattern of inheritance. In 90% of the cases, it is associated with mutations in one of the five genes—EIF2B1 (12q24.3), EIF2B2 (14q24), EIF2B3 (1p34.1), EIF2B4 (2p23.3), EIF2B5 (3q27)—in charge of codifying the five subunits (α, β, γ, δ, ε), which form the eukaryotic translation initiation factor 2 (eIF2B). Its main function is to initiate translation of the messenger RNA to polypeptides and its regulation in stress states. The factor is activated through cycles of phosphorylation and dephosphorylation coupled with guanosine diphosphate (GDP)/guanosine triphosphate (GTP) in its subunit GEF (guanine nucleotide exchange factor). Cellular stress states that promote phosphorylation of eIF2α inhibit reactivation by eIF2B; its low intracellular concentration limits reactivation cycles, decreasing global protein synthesis. Although it can be found in different cell lines, glial cells present increased vulnerability to stress while neurons and other cells of the central nervous system (CNS) are protected. This vulnerability leads to alteration of myelin production, characteristic of this illness.

In 1997, van der Knapp et al. defined VNW according to clinical and neuroradiological criteria. The prenatal form of the disease presents with oligohydramnios and decreased fetal movements. At birth, patients present with feeding difficulties, vomit, hypotonia, difficult-to-treat seizures, coma, apnea, microcephaly, cataract, organomegaly, renal hypoplasia, ovarian dysgenesis, joint contractures and death before one year of age. The childhood form manifests in the first year of life with irritability, stupor, seizures, motor neuroregression, recurrent infections; usually, patients die before two years of age. There is a late childhood form that presents in two ways: some patients develop ataxia, intention tremor, dysmetria between one and 5 years of age, with a previous period of normal motor and cognitive development, some with motor and mild language retardation. Other cases present with coma after a traumatic brain injury (TBI) or fever, and even though recovery exists, patients develop progressive motor deterioration with gait problems, signs of upper motor neuron disease, dysarthria, seizures, feeding problems and optic nerve atrophy. In these patients, cognitive abilities seem to be preserved, and they later enter a phase of stabilization until they die in a period of 1 to 5 years. The juvenile form starts between 5 and 15 years of age, with slowly progressive spastic diplegia that alternates with periods of stability, and even motor improvement; patients have a long survival. In the adult form, cognitive impairment, transient episodes of optic neuritis, hemiparesis, intense headaches predominate. It presents as secondary amenorrhea due to ovarian failure in women often referred as ovarioleukodistrofia. Recent publications have emphasized the presence of movement disorders, such as dystonia and myoclonus, which characterize late onset presentations, but occasionally childhood forms with opsoclonus-myoclonus have been described.

Neuroradiological abnormalities are a constant, but they vary according to age and can be present in asymptomatic patients. The study of choice is a brain magnetic resonance (MR). In the neonatal forms, only brain immaturity can be observed, characterized by thick gyri, and white matter with a high-water content. In the childhood forms, the hemispheric white matter is
affected symmetrically and diffusely in T1, T2 and FLAIR sequences (fluid-attenuated inversion recovery), with an intensity that is similar to cerebrospinal fluid (CSF). A fine mesh of remaining tissue can be observed within the affected areas, with a radiated appearance, and in the semioval center a speckled pattern can be detected in sagittal and coronal slices in T1 and FLAIR; in this stage, atrophy cannot be observed. In later stages the presence of cystic lesions observed in the FLAIR sequence is common. Spectroscopy of these areas show similarities with the CSF, with a decrease in normal metabolites and the presence of lactate and glucose. Cerebellar atrophy varies from mild to severe; the vermis is the first structure to be affected and signal alterations in the midbrain, pons, and occasionally in the spine can be observed. In adults with slow progression of the disease, cortico-subcortical atrophy can also be seen\(^1,2,8\).

In this work, we describe the case of the childhood form of VWM caused by a homozygous mutation c.318A>T in the exon 2 of the \(\text{EIF2B5}\) gene. This case contributes to the knowledge regarding the spectrum of mutations that occurs in Mexican patients, which allows broadening the associated phenotype of this mutation.

**Clinical case**

Thirteen-month-old male patient, young parents, healthy, non-consanguineous. Product of a first full-term pregnancy, without any signs of perinatal asphyxia, normal motor development. He was taken to the emergency room because of vomit, intermittent hyperthermia, irritability, and oral intolerance during the previous fifteen days. At admission, he presented with hyperthermia and periods of somnolence with irritability. On physical examination by the pediatric neurologist, he was found to be irritable, with a normal cephalic perimeter, normal cranial nerves, and fundus, decreased tone in the extremities and increased deep tendon reflexes, without any meningeval signs or evidence suggestive of increased intracranial pressure, abdomen without any organomegaly and no abnormal skin findings. A head computed tomography showed generalized white matter hypodensity. A brain MR showed alterations of signal intensity in the white matter in both cerebral hemispheres on T1 and T2 weighted images. (Figures 1-3). The “U” fiber was not compromised, and there were remains of white matter in the central portions, especially in the semioual centers, and the corona radiata at the periatral and frontal level (Figures 2 and 3). On T1 weighted images, atrophy of the corpus callosum was observed with signs of hypomyelination (Figure 1). On T2 weighted images, the brain cortex was discretely atrophied, while the basal ganglia and brain stem were normal (Figure 3).

Metachromatic leukodystrophy was discarded based on normal enzymatic activity. The patient was discharged without gait recovery. During the following fifteen days, he presented with focal complex seizures along with fever, an abnormal electroencephalogram with focal paroxysmal activity; he was started on magnesium...
and the corona radiata are detected. Images of remaining white matter in the semioval centers of the cortex due to atrophy can be observed. Several hemispheres, respecting the gray matter, although thinning practically all the white matter of both cerebral T2 axial images. Alteration in the myelination of the nervous system; they comprise about thirty entities.

Figure 3. T2 axial images. Alteration in the myelination of practically all the white matter of both cerebral hemispheres, respecting the gray matter, although thinning of the cortex due to atrophy can be observed. Several images of remaining white matter in the semioval centers and the corona radiata are detected.

valproate. After a week, he suffered a mild traumatic brain injury. His motor deterioration worsened, and the episodes of somnolence and irritability with vomit and hypotonia recurred. VWM was suspected, and sequencing of the EIF2B5 gene was indicated. A homozygous c.318A>T mutation in the exon 2 was reported causing a change of a leucine for a phenylalanine at the position 106 of the protein (pLeu106Phe). A heterozygous state in both parents was confirmed. Molecular studies were done at the VU University Medical Center’s laboratory, in Amsterdam.

Admissions due to hyperthermia continued, with episodes of uncontrolled seizures and exacerbations of cognitive impairment. At 2 years of age, a gastroscope tube was placed due to swallowing difficulties. In his last evaluation, at 6 years, he presented with occasional controlled seizures on magnesium valproate and topiramate, a complete loss of visual acuity due to optic nerve atrophy and spasticity in all four extremities. The patient received physical therapy, orthosis, baclofen orally and application of botulinum toxin. He finally died at age six due to severe community-acquired pneumonia.

Discussion

Diseases that affect the white matter are a challenge in the field of both genetics and pediatric neurology. They have a wide clinical spectrum, with presentation ranging from birth to the adult age. They have been classified in two groups with the goal of facilitating diagnosis of these diseases: the classical forms, which are hereditary conditions that mainly affect glial cells, with or without affection of myelination of the peripheral nervous system; they comprise about thirty entities. The genetically determined leukoencephalopathies, which affect the white matter secondarily by affecting neurons, the vasculature or due to systemic diseases. Among the leukodystrophies, one of the most frequent is VWM.

The present case had an early-childhood onset, with clinical characteristics that evolved according to what has been described for this disease, episodes of neurologic deterioration associated with a TBI and fever. According to this phenomenon, it has been observed that normal oligodendrocytes show hyperactivation of kinases such as GCN2, PERK, PKR and HRI, which phosphorylate eIF2, which inhibits eIF2B in animal models, thus restricting GEF activity and decreasing the reactivation capacity without altering its functions. eIF2B deficiency increases PERK signaling which, by interacting with mutated eIF2B, inhibits protein synthesis in oligodendrocytes and alters its function in stress conditions.

In patients with acute neurological deterioration during or after a febrile process, acute disseminated encephalomyelitis or infectious encephalitis should be suspected. When deterioration is subacute or chronic, mitochondrial disease, such as pyruvate dehydrogenase deficiency, pyruvate carboxylase deficiency and Aicardi-Goutières syndrome should be part of the differential diagnosis. In X-linked adrenoleukodystrophy, as well as in VWM, neurological deterioration is associated with a TBI. Nerve optic atrophy is observed in Canavan’s disease, cerebrotendinous xanthomatosis, and peroxisomal diseases.

Analysis of clinical manifestations, mainly neuroradiological, allowed identification of VWM among these demyelinating diseases. MR findings in this patient are compatible with the imaging criteria for VWM since demyelination affects both cerebral hemispheres, it shows myelin remains at the semiomial centers, corona radiate, frontal lobes and periatral regions, compromise of the corpus callosum and discrete atrophy of the cerebral cortex. A normal electroencephalogram is expected at disease onset and progressive slowing of basal rhythms with multifocal paroxysmal activity. Visual and auditory evoked potentials are initially normal, with a progressive increase in latency until they disappear. Unlike metachromatic leukodystrophy, nerve conduction velocities and electromyography are normal.

The main neuropathological findings in VWM are cystic degenerations, reactive gliosis, astrocytes with abnormal morphology and scarce myelin.

Initially, Dietrich et al. associated a loss of white matter with dysfunction of astrocytes and their progenitor.
cells due to an absence of expression of the glial fibrillary acidic protein (GFAP) mediated by an interference RNA of the EIF2B5 gene\(^2\). Later, Dooves et al. found an accumulation of hyaluronic acid, which inhibits proliferation of astrocytes during gliosis. Glial cell affection extends to structures like the cerebellum and retina, explaining some patients’ cerebellar and visual dysfunction, including the present case\(^2\)

More than 160 mutations have been reported in the five subunits of the eIF2B complex: 64.7% in the eIF2B5 gene, 17% in the eIF2B4 gene, followed by 15% in eIF2B2. In eIF2B5, most amino acid substitutions, such as pArg113His, which is the most frequent in the adult form\(^4\). The mutation found in this patient is the second most frequent and consists of a change of leucine for phenylalanine at the position 106. It was reported initially in a heterozygous state by Leegwater et al.\(^2\). Later, another heterozygous case of a girl with ataxia and hemiparesis was reported\(^2\).

In the literature, there have been two other homozygous cases described with a less rapid and severe evolution\(^2\). Some mutations, such as pVal309Leu, consistently show a more severe phenotype while the homozygous state for the mutation p.Thr91Ala can present in asymptomatic adults and also in early-onset cases\(^2\). The p.Arg113His mutation is localized near to the one presented in this case, with a less severe course, reflecting that there is no clear correlation between the effect of the mutation over GEF exchange activity of eIF2B and the severity of clinical manifestations\(^2\).

Two cases of Mexican patients with VWM were identified after a bibliographical search: one, that debuted at 14 months without a molecular study, and other with a p.Arg299His homozygous abnormality (c.896G>A) of the EIF2B5 gene. In both cases, manifestations were very similar to the patient herein reported. Patients showed a previously normal neurological development, motor and cognitive deterioration associated with an infectious process, without full recovery of the lost developmental milestones. In the second case, the patient shows a newly-onset neurological deterioration after a TBI\(^2\). Description of the present case contributes to knowledge of the clinical and molecular epidemiology of this disease. Probably, the fact that both parents are carriers without evidence of consanguinity or endogamy reflects the important frequency of the mutation in Mexico.

Currently, there is no specific treatment for VWM, only interventions to treat seizure, spasticity and feeding difficulties. It is recommended to avoid stressful situations that would lead to deterioration, such as infections, fever, contact sports, although this is not enough to prevent progression of the disease\(^2\).

VWM is one the most common leukodystrophies with a worldwide case distribution. In our country, it is probably underdiagnosed, specifically the late and atypical forms of the disease.

The clinical course supports the diagnostic suspicion. Neuroimaging findings are usually characteristic, but DNA studies contribute to confirming the diagnosis. The reported case presented with a previously described mutation, with some clinical discrepancies between the previously described individuals, so a definitive conclusion about the phenotype-genotype correlation of this genetic abnormality is not possible. Given that there is no specific treatment, genetic counseling and informing the family about the natural history of the disease is an important part of the clinical management. When the status of the healthy heterozygous carrier is confirmed in both parents, there a risk of recurrence of 25% in each pregnancy. In this case, reproductive options include a preimplantation diagnosis, prenatal diagnosis, and gamete donation from one of the parents and adoption.

**Ethical disclosures**

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this study.

**Confidentiality of data.** The authors declare that they have followed the protocols of their work center on the publication of patient data.

**Right to privacy and informed consent.** The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

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**Conflicts of interest**

The authors declare no conflicts of interest.
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