

CLINICAL CASE

Clinical and molecular findings in a patient with ataxia with vitamin E deficiency, homozygous for the c.205-1G>C mutation in the *TTPA* geneCarmen Esmer,¹ Abel Salazar Martínez,² Enrique Rentería Palomo,³ Antonio Bravo Oro³**ABSTRACT**

Background. Ataxia with vitamin E deficiency is a disorder caused by mutations in the *TTPA* gene. Common symptoms include ataxia, areflexia, head titubation, loss of proprioception, Babinsky sign, dysdiadochokinesia, retinitis pigmentosa and cardiomyopathy.

Case report. The patient was the first child of consanguineous parents. She presented at 10 years of age due to bilateral lower limb pain and numbness and difficulty in speech, writing and chewing. Physical examination showed dysarthria, diminished distal strength, hyperreflexia, positive Babinsky sign, decreased proprioception, pes cavus, dysmetria, dysdiadochokinesia and positive Romberg sign. Genetic screening for the Friedreich's ataxia gene resulted negative, α -tocopherol levels were low and *TTPA* gene sequencing detected the homozygous mutation c.205-1G >C in intron 1. Treatment was initiated with vitamin E, showing improvement of symptoms.

Conclusions. The presence of Friedreich's ataxia-like phenotype suggests the need to perform tests of plasma levels of α -tocopherol and the confirmatory genetic test. Treatment with vitamin E decreases symptoms in both affected and presymptomatic individuals. Few patients have been described in America, and our case showed a homozygous mutation outside of high-prevalence areas. Clinical findings of this patient and a previous case would indicate that the c.205-1G>C mutation is associated with severe symptoms.

Key words: alpha-tocopherol, recessive ataxia, *TTPA* gene, vitamin E.

INTRODUCTION

Since its identification by Evans and Bishop in 1922, knowledge on the actions of vitamin E in the maintenance of the neurological structure and function has increased considerably.¹ Vitamin E is liposoluble, prevents lipid oxidation in the membranes and is widely distributed in the brain and in the cerebellar cortex. There are several vitamin E isomers called α , β , γ and δ tocopherol. The isomer α -tocopherol is the most biologically active form. It is absorbed in the small intestine and is transported to the liver by chylomicrons where it binds selectively to very low density lipoproteins (VLDL) and are then released into the blood circulation.^{2,3} This vitamin

is found in almost all foods. Its deficiency in healthy individuals is uncommon. States of deficiency are found in patients with problems of poor lipid absorption (as a result of hepatic cholestasis, short intestine due to resection), or genetic disorders (such as cystic fibrosis, abetalipoproteinemia, and homozygous hypobetalipoproteinemia), causing a severe and progressive picture of spinocerebellar degeneration characterized by ataxia and areflexia with peripheral nerve damage.⁴⁻⁷

Ataxia due to deficiency of vitamin E (AVED; OMIM #277460) is an autosomal recessive inherited neurodegenerative disorder described for the first time by Burck et al. in 1981.⁸ The condition occurs due to the presence of mutations in the *TTPA* gene located on chromosome 8q13.1,

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which encodes the protein of the α -tocopherol transporter.^{9,10} Currently, in gene sequencing studies, 25 mutations have been identified, which may be missense, non-sense and with open reading frame mutations at the splice site and deletions.¹¹

The first manifestations are observed, on average, between 4 and 18 years of age in individuals without history of malabsorption disorders. The clinical picture is characterized by progressive ataxia, absence of myotactic reflexes, head tremor, loss of proprioception, Babinski, disdiadochokinesia, retinitis pigmentosa, decreased visual acuity and cardiomyopathy.^{12,13}

The prevalence is not well known but is considered to be low. Zortea et al. in an epidemiological study conducted in Italy found a prevalence of 3.5:1,000,000.¹⁴ The regions with a greater number of cases are North Africa and Italy where the disorder occurs because of the presence of a common mutation with founder effect c.744ofA.¹⁵ In addition, there are reports of cases in other countries of Europe, Asia and a smaller number in America where the rest of the mutations have been described.¹⁶ Clinical and molecular findings have been described in a case of ataxia due to vitamin E deficiency, homozygous for the mutation c.205-1G>C in the *TTPA* gene in which the clinical phenotype is not well characterized.

CLINICAL CASE

We present the case of an 11-year-old female Mexican mestizo patient with grandparents and great grandparents born in Mexico. Parents have a history of consanguinity (uncle-niece/third degree). The patient was the product of a fourth gestation with three living half-brothers (28, 24 and 23 years of age), and a 6-year-old brother. From the time she began to walk at 16 months, she had frequent falls and experienced isolated episodes of cramps during sleep. At 10 years of age she began to have pain and paresthesias in the lower extremities, mainly during walking. She was evaluated by orthopedic physicians. The walking disorder was noted. X-rays of the pelvis showed flattening of the femoral epiphyses and physical rehabilitation was recommended. In recent weeks the parents observed problems with writing, difficulty chewing and changes in the tone of her voice. On neurological examination, preserved mental functions were noted with dysarthric speech, normal fun-

dus of the eyes, normal cranial nerves, decreased distal strength 4/5 in all four extremities with marked weakness of the anterior tibia, generalized hyperreflexia, bilateral Babinski, sensitivity to touch and pain, normal temperature, reduced lower extremity decreased vibratory perception, adequate discrimination of the joint position, bilateral pes cavus, plantigrade walk without wide support, discrete dysmetria and disdiadochokinesia, and positive Romberg. Cardiopulmonary evaluation showed no abnormalities and abdomen was without organomegaly.

A spinal X-ray was performed and showed a mild scoliosis to the left of the thoracic spine. Nerve conduction velocity was normal. X-ray of the chest did not show cardiomegaly and magnetic resonance of the skull was without any alterations in the brain or in the gray or white matter. Ophthalmology and cardiology examinations were normal. Laboratory studies showed the following results: α -fetoprotein 1.02 ng/ml (0.00-13.60 ng/ml), cholesterol 121 mg/dl (<200 mg/dl), high-density lipoprotein cholesterol (HDLc) 40.0 mg/dl (>65 mg/dl), low-density lipoprotein cholesterol (LDL) 67.8 (100-129 mg/dl), triglycerides (TG) 66 mg/dl (<200 mg/dl). The patient was referred for genetic testing with the diagnostic suspicion of Friedreich ataxia (FRDA). After these tests, an analysis of the expanded GAA in the gene *FXN* was done, which was normal. α -tocopherol levels were determined and were 1.1 mg/l (5-12 mg/l); therefore, treatment with vitamin E was begun (400 IU daily). A month after initiating treatment, partial improvement was noted. The patient demonstrated greater stride in her step and greater safety when walking. Also, the symptoms such as dysarthria, dysmetria and disdiadochokinesia decreased.

Five mL of blood was obtained and placed in EDTA. Subsequently, DNA extraction was carried out according to normal techniques. The *TTPA* gene was analyzed with PCR (polymerase chain reaction) in highly purified genomic DNA. Unidirectional sequencing of the codifying regions was carried out (5 exons, 837 bp) including 20 bp of intronic DNA and the highly preserved regions of the exon-intron unions. A homozygote c.205-1G>C was identified by a change of a guanine for a cytosine in position -1 of the intron 1. This mutation occurred at the acceptor site of the splicing. Exon 2 skipped and created a shift in the reading frame and a stop codon in the protein after amino acid 68.

DISCUSSION

Hereditary ataxias are a group of pathologies with diverse etiologies. Advances in molecular biology have allowed for the identification of numerous responsible genes. The most recent classification is based on the inheritance pattern: autosomal dominant, autosomal recessive, X-linked and mitochondrial. Hereditary ataxias were previously grouped according to the disease mechanism as congenital, metabolic, degenerative or by the age of presentation with early initiation before the age of 20 years or with late appearance after the age of 20 years.^{17,18}

In this case, the family history of consanguinity and the clinical findings created the suspicion for an autosomal recessive variant of heredoataxia. Due to these findings, the principal differential diagnoses that were established were FRDA (OMIM #229300), abetalipoproteinemia (OMIM #200100) and Refsum disease (OMIM #266500), which primarily present progressive manifestations of the brain without atrophy, stem, tracts, spinocerebellar and other signs that occasionally allow for its clinical differentiation.¹⁹

One of the greatest difficulties in the clinical distinction of the diseases is that AVED and FRDA have important similarities such as progressive ataxia, dysarthria, absence of myostatic reflexes, Babinski and decrease in vibratory sensation and predominantly axonal sensitive neuropathy. Skeletal disorders such as scoliosis and pes cavus are more frequent in FRDA, whereas head tremor is more characteristic in AVED. Both diseases share non-neurological manifestations such as cardiomyopathy and diabetes, although these are more frequent in FRDA. Patients with AVED present with dystonia, cognitive deterioration and psychotic episodes at later ages and, for this reason, are not signs that would assist in the diagnosis during the pediatric age (Table 1).^{12,13}

The patient's clinical picture began within the age range described for the disease (4 to 15 years of age). Her initial symptom was gait difficulty, which at that time was seen as an alteration in proprioception due to posterior cord damage. Neurological examination showed an incipient picture of brain involvement without visual problems, hyporeflexia, dystonia, or head tremor. Up to this time there is no consensus for the diagnostic criteria of AVED. Evidence in the literature indicates that in the presence of a neurological phenotype similar to Friedreich

ataxia, a determination should be made of the plasma levels of α -tocopherol by means of a relatively easy and accessible study.¹⁹ Although there is no universal range of vitamin E levels and standardization methods of each laboratory vary, it is expected that the plasma concentration of α -tocopherol should be <1.7 mg/l in cases with AVED. Also, when there are symptoms of intestinal lipid malabsorption, abetalipoproteinemia is diagnosed.¹⁵

Other diagnostic studies such as neuroimaging are of limited usefulness as only half of the cases present with some degree of brain atrophy. On the other hand, neurophysiological studies are nonspecific because motor and sensory nerve conduction velocities are normal and somatosensory-evoked potentials may show an increase of the latencies nonspecific for the disease.²⁰

Most cases of AVED come from European countries located around the Mediterranean basin and are associated with blood relatives, which is common in diseases with low-frequency autosomal recessive inheritance.²¹ Few patients have been described in America. In this case, the role of consanguinity was evidenced on demonstrating a mutation in the homozygous state in a Mexican patient. This provides information of the profile of mutations that may be found outside the areas of highest prevalence of the disease.

The *TTPA* gene is, up to now; the only gene associated with AVED.²² Sequencing of the exons and exon/intron junctions detects mutations in 90% of the affected individuals. The most frequent mutations are c.744delA

Table 1. Clinical signs that differentiate AVED from FRDA

Clinical manifestation	FRDA	AVED
Pes cavus	+	Uncommon
Scoliosis	+	Uncommon
Peripheral neuropathy	+	Occasional
Muscle debilitation	+	—
Babinsky	+	(+)
Retinitis pigmentosa	—	(+)
Decreased visual acuity	Uncommon	(+)
Cardiomyopathy	+	(+)
Type 1 diabetes mellitus	+	(+)
Head tremor	Uncommon	+
Alteration of cardiac conduction	+	Uncommon
Psychotic episodes	—	+

+, generally present; (+), depends on the mutation; —, generally absent.
FRDA, Friedreich ataxia; AVED, ataxia due to deficiency of vitamin E.

in exon 5 in patients from North Africa and mutations c.513_514insTT, c.400C>T, c.486delT in families of Northern Europe.^{9,13} Among the 25 mutations described to date, at least six slightly pathogenic mutations are known. The one best characterized for its frequency, from the clinical point of view, is the c.303T>G mutation with symptomatology beginning after the age of 10 years, mild clinical course and higher frequency of retinopathy. Another 13 mutations such as c.744delA have a severe phenotype beginning before the age of 10 years and with a high risk for heart disease. The remaining six mutations do not have a defined phenotype. Among them is mutation c.125-1G>A.¹⁹

The only case previously described has a French origin and a heterozygous mutation composed of c.744delA/ c.125-1G>A. The symptoms began at the age of 2 years with a rapidly progressive clinical course with ataxia, dysarthria and arreflexia. At 13 years of age the patient lost the ability to walk and may be one of the patients with the earliest beginning of the clinical manifestations and the inability to walk, among those described by Cavalier et al.¹³ Our case is the first with homozygosity for the intronic mutation that also showed a severe clinical picture with early initiation of the manifestations but with a favorable response to vitamin E supplementation.

It is described that patients with a severe phenotype have mutations that affect splicing sites and generate premature stop codons, which markedly alter protein function. In contrast, heterozygous cases with an erroneous direction (change of one amino acid for another) in one of the mutant alleles produce a protein with some residual activity that favors the presence of less severe manifestations. Mutations that substitute one amino acid for another also present a severe phenotype when the important functional residues of the protein are affected.¹¹

AVED is the best example of neurological disorders caused by chronic vitamin E deficiency. The usual means of action are not yet understood, although there have been three main actions described: 1) its role as an antioxidant in the central nervous system and retina, 2) its regulation of the action of different enzymes, e.g., inhibits protein kinase C, 5-lipoxygenase and phospholipase A2 and activates protein phosphatase 2 and diacylglycerol-kinase²³ and 3) its regulation of genes involved in apoptosis in cell cycle regulation and receptor of lipoproteins.²⁴ The pathogenesis of the cerebellar condition appears to be caused

by two mechanisms: damage by free radicals and defects in the repair of damage to the single- or double-stranded DNA.¹¹

For this reason, treatment for the AVED consists of the administration of lifelong high doses of vitamin E so as to normalize plasma levels. Although we do not have studies that determine the optimal posology, it is recommended that between 800 and 1500 mg or 40 mg/kg daily of vitamin E in children be administered.²⁵ Some of the symptoms such as ataxia or cognitive deterioration may be reversible. However, in adult patients, proprioception disorders and gait alterations may be permanent.¹⁵ When treatment is not received, patients lose their ability to walk and require a wheelchair between 11 and 50 years of age due to the progression of the ataxia. In the case presented, after 2 years of receiving vitamin E at a dose of 800 IU daily, the patient showed improvement in language. There is no pain in the extremities and there is better coordination and she still persists with paresthesias. Romberg maneuver decreased considerably, without dysmetria or disdiadochokinesia. Treatment in presymptomatic individuals is successful because they do not develop manifestations of the disorder. Early detection is important among families with AVED.^{20,25} There remains much to learn about the actions of vitamin E in the central nervous system. In addition to its functions as an antioxidant, it may have anti-inflammatory and neuroprotective properties. Current studies have focused on demonstrating a beneficial effect of presymptomatic vitamin E supplementation, alone or as part of multivitamin supplementation on neuronal loss in diseases such as Alzheimer's disease and other types of dementia such as cognitive impairment due to age and amyotrophic lateral sclerosis, among others.^{26,27} For the etiologic diagnosis of hereditary ataxias in children, a systematic analysis of the neurological and non-neurological manifestations accompanying the data of cerebellar compromise is required. Although biochemical, neurophysiological and neuroimaging studies can be useful, sometimes only molecular analysis allows confirmation of the diagnosis of a specific condition. Suspicion of AVED is established in patients without data of intestinal malabsorption and neurological symptoms similar to FRDA.

It is essential to confirm the condition in the early stages of the disease because appropriate supplementation halts disease progression and, depending on the age, may

reduce some of the neurological symptoms. In some cases, complete remission is achieved.

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