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A novel *delins* mutation in the α-*TTP* gene in a family segregating ataxia with isolated vitamin E deficiency

Running title: A complex mutation in the α-*TTP* gene

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ABSTRACT

Ataxia with isolated vitamin E deficiency (AVED) is a rare autosomal recessive neurodegenerative disease due to mutations in the α-*TTP* (α-tocopherol transfer protein) gene. In AVED, the biochemical hallmark is the low plasmatic levels of vitamin E and in most of patients, vitamin E supplementation allows a stabilization of the neurological conditions. We have investigated the genetic cause of ataxia and reduced levels of vitamin E, and apolipoproteins A1 (apoA1) and B (apoB) in a 16-year-old patient. Results revealed that our propositus is a compound heterozygote for the c.227_229delinsATT/c.744delA mutations in the α-*TTP* gene, each inherited from one of the two parents. His sister is also a compound heterozygote for both mutations and she presents a biochemical pattern similar to that of his brother. After receiving the vitamin E supplementation, plasmatic levels of vitamin E and apoA1 have been normalized in the propositus. The detected mutations would justify the undetectable levels of vitamin E, but would not explain the also decreased levels of the apolipoproteins, as neither that after treatment with vitamin E, the levels of apoB do not become normal. These findings suggest that other genes may play a role in producing this atypical biochemical profile.

ABBREVIATIONS

α-TTP	α-tocopherol transfer protein
apo	apolipoprotein
AVED	ataxia with isolated vitamin E deficiency
bp	base pair
delins	deletion-insertion
FHBL	familial hypobetalipoproteinemia
GUCY2D	guanylate cyclase 2D

INTRODUCTION

Ataxia with isolated vitamin E deficiency (AVED; MIM 277460) is a rare autosomal recessive neurodegenerative disease, mostly detected in the Mediterranean basin. AVED is due to mutations in the $\alpha\text{-}TTP$ (α-tocopherol transfer protein) gene placed on chromosome 8q (1). In North-African and South-Italian populations, the most frequent mutation responsible for the disease is the c.744delA mutation, while in AVED families of North European origin the c.513insTT mutation has been often characterised (1, 2). Since the $\alpha\text{-}TTP$ gene was first identified as the defective gene in this disease near 18 different mutations in the $\alpha\text{-}TTP$ gene have been reported and they are placed along the entire gene (1-8).

Clinical features include age at onset before 20 years gait and limb ataxia, dysarthria, lower limb areflexia, loss of vibration and positional sense, and bilateral extensor plantar reflexes. The deficit reduces the capacity to incorporate α-tocopherol (the most biologically active form of vitamin E) into very low density lipoproteins secreted by the liver and therefore into plasma and tissues. In fact, in AVED the sole and primary biochemical abnormality is very low levels of vitamin E in plasma (<3 mg/L; reference values 3-15 mg/L), in the absence of intestinal fat malabsorption and/or abetalipoproteinemia (9). The vitamin E supplementation allowed a stabilization of the neurological conditions in most of the patients (10) what suggests that a prompt genetic characterisation of AVED may promote an early effective treatment of the disease.

METHODS

Patients. The propositus is currently a 16 year-old male, born of unrelated parents, and without any familial history of epilepsy, mental retardation, or neurodegenerative disorders. The first symptoms were observed at the age of 3 years: he showed

progressive ataxic gait. Physical examination showed that he was well nourished but hypotonic. All senses including positional and vibratory were normal. Lower limbs reflexes were weak whereas upper limbs reflexes were normal and symmetric. Romberg's test was negative and he has a slight head and intention tremor. Brain magnetic resonance imaging (MRI) showed no signs of anomaly. Electrophysiological examination of sural and peroneal nerves showed normal action potentials and conduction velocities. Visual and trunk evoked potentials (VEP and TEP) were normal, but somato-sensorial evoked potentials (SSEP) findings were consistent with an alteration of the sensory way in the spinal cord (data not shown). Cardiac examination and electrocardiogram were normal. Funduscopy (eye exam) was also normal. Hematological and biochemical tests were normal except for the vitamins A and E, and apolipoproteins A1 (apoA1) and B (apoB) (Table). Patient is currently receiving treatment with high doses of vitamin E. Plasmatic levels have been normalized (Table) and neurological symptoms have been stabilized. The 12-years-old sister did not refer any symptom except for a light head titubation and intention tremor. MRI was normal and plasmatic vitamin E, apoA1 and apoB levels were reduced (Table), suggesting that she could also be affected. She is currently receiving vitamin E supplementation. The propositus and his family were aware of the investigative nature of the studies and gave their consent. Investigations were approved by the Scientific Committees of both Hospital of Mérida and Instituto de Biomedicina de Valencia.

Mutation analysis. Genomic DNA from the patient, his sister and his parents was obtained by standard methods. Mutation analysis of the α-TTP gene was performed by direct sequencing of each exon and their exon-intron boundaries of purified PCR products (Qiagen, Hilden, Germany) amplified using primers as described elsewhere (7) in an ABI Prism 3130xl autoanalyzer (Applied Biosystems, Foster City, CA).

RESULTS

Clinical and biochemical data, mainly the extremely low levels of plasmatic vitamin E, pointed towards the diagnosis of AVED. The analysis of the α-TTP gene revealed that the patient was a compound heterozygote for the mutations c.227_229delinsATT (p.W76_R77delinsYX) in exon 2 and c.744delA (p.E248fs) in exon 5 (Figure 1). The deletion-insertion (delins) change consists of three consecutive novel heterozygous missense mutations what predict a truncated protein. Healthy parents were heterozygous carriers: the mother carried the peculiar c.227_229delinsATT mutation and the father harboured the so frequent frameshift c.744delA mutation. Both changes were also analysed in his sister. Results showed that she was also a compound heterozygote for the α-TTP p.W76_R77delinsYX/p.E248fs mutations.

DISCUSSION

The α-TTP c.744delA mutation is the most common alteration in AVED patients from the Mediterranean basin, accounting for 68% of the mutant alleles (1, 2). By contrast, the α-TTP c.227_229delinsATT mutation is a novel heterozygous triple missense mutation that consists of three base pairs (bp) deletion, GGA, and three bp insertion, ATT, and generates a premature stop codon (p.W76_R77delinsYX). The α-TTP protein is a member of the family of lipid-binding protein containing two CRAL-TRIO domains, pfam03765 (residues 11-83) and pfam00650 (residues 89-275). The substrate of the α-TTP protein, the α-tocopherol, is bound in a hydrophobic pocket that is constructed by residues belonging to the second domain of the α-TTP. Consequently, a protein with the p.W76_R77delinsYX mutation could not be functionally active.

Many mutations of different types have been described along the α-TTP gene (*The Human Gene Mutation Database*, HGMD,

http://www.hgmd.cf.ac.uk/ac/index.php; Figure 2), although only one small delins

mutation is known: c.530_532delAGinsGTAAGT (1). For this reason, the complex α-

TTP c.227_229delinsATT mutation is itself a striking finding. The HGMD contains

57,047 mutations belonging to 2,183 genes (release March 2008). The most common

types of mutation causing human genetic disease are missense and nonsense

mutations (57.13%), whereas the delins (1.47%) is an uncommon type of mutation

(11). Delins appear to represent combined microdeletion/microinsertion events that

give rise to the replacement of one or more bp by others, not necessarily the same

number. Sorted complex mutations and also small delins mutations have been

described in many different genes. A three bp delins mutation, c.2540_2542delinsTCC

(p.Q847_K848delinsLQ), has been described in the *GUCY2D* (guanylate cyclase 2D)

gene, which is responsible of a cone-rod dystrophy (12). The *GUCY2D* gene appears to

be prone to complex missense mutations such as the p.E837_R838_T839delinsDCM

mutation (13) and the p.E837_R838delinsDS mutation (14). It has been suggested that

the hypermutability of CpG dinucleotides could be the cause of these complex

mutations (14). By contrast, the *GUCY2D* c.2540_2542delinsTCC change is not placed

in a CpG rich region, as neither the c.227_229delinsATT mutation in the α-*TTP*.

The rich CpG sequences are not the only hotspot regions with a high degree of

mutability. Overrepresentation of some penta-, tetra-, and trinucleotide motifs in the

vicinity of delins have been described (15). In fact, an accurate analysis of the close

sequence to the α-*TTP* c.227_229delinsATT mutation shows that some of these motifs

are present, such as the WNRGT, TARGY, TARGY and TARSY. In the α-*TTP* gene other

delins mutation has been reported, c.530_532delinsGTAAGT (1), and also some of

these motifs, TAGSY and TARSY, are present. Moreover, Krawczak and Cooper (16)

identified a consensus sequence (TGRRKM), present in several micro-deletion hotspots

that are found to be strikingly similar to putative DNA polymerase α arrest sites. This

consensus sequence is also present where the α-TTP c.227_229delinsATT mutation

occurs. Based on the types of repeat involved, several mechanisms of mutation have

been reported to explain the delins changes. In a delins, there is an initial mutational

event being followed by a partially successful attempt at restoring the original

sequence and the complexity of a given fragment should remain more or less the same

as a net consequence of the combined delins event (15). This point is confirmed in the

α-TTP c.227_229delinsATT mutation, as the overall complexity of the sequence is not

extremely modified.

Patients with mutations in the α-TTP gene have impaired ability to incorporate

α-tocopherol, the most biological active form of vitamin E, into lipoproteins secreted by

the liver (17). Because both mutations generate a premature stop, no functionally

active protein is expected to be present in the patient's cells what would explain the

undetectable levels of vitamin E. Interestingly, apolipoproteins levels are also

decreased in our propositus and moreover, it is striking that apoA1 levels, but not

apoB, becomes normal after treatment with vitamin E (Table). This is not a frequent

association in AVED patients. One plausible explanation could be that other genes are

involved. Because of the so low levels of apoB in both sibs, a concomitant familial

hypobetalipoproteinemia (FHBL; OMIM 107730) or even, abetalipoproteinemia (ABL;

OMIM 200100) were suspected. FHBL patients have reduced levels of apoB and LDL-C

and have mutations in the APOB gene. ABL patients show low levels of total

cholesterol, VLDL, LDL and an almost complete absence of apoB, and present

mutations in the MTP gene. We searched for mutations in both APOB and MTP genes

in the propositus, but no changes were found. Maybe, other still unidentified genes

linked to FHBL (18) could explain the biochemical profile in both sibs. Recently, the

PCSK9 gene that cosegregates with hypercholesterolemia (OMIM 603776) has been

proposed as a candidate gene in FHBL (19), although searching for mutations in the

PCSK9 gene did not yield any positive result in our patient. In any case, it is tempting to speculate that other genes may play a role in the atypical biochemical profile detected in our patients.

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Figure 1. **(A) Family tree and mutation inheritance**. The mutations carried by each individual have been indicated. Healthy parents carried one of the two detected mutations, c.744delA or c.227_229delinsAT, in a heterozygous state and both siblings had the two identified mutations in heterozygosis. A black arrow indicates the proband. **(B) Electrophoregrams of the two identified mutations in the α-*TTP* gene.** On the left, the electrophoregrams corresponding to a healthy control and to the proband with the c.227_229delinsAT mutation in heterozygosis. Three red arrows point at the three consecutive changes: [227G>A + 228G>T + 229A>T]. On the right, the electrophoregrams corresponding to a healthy control and to the proband with the c.744delA mutation in heterozygosis. A red arrow shows where the frameshift starts.

Figure 2. **Distribution of the identified α-*TTP* gene mutations.** We have identified two mutations in our proband: the novel c.227_229delinsATT (p.W76_R77delinsYX) mutation in exon 2 and the so frequent c.744delA (p.E248fs) frameshift in exon 5.