

Ataxia with vitamin E deficiency associated with deafness

Bülent Kara^{1,2}, Abdullah Üzümcü³, Oya Uyguner³, Rasim Özgür Rosti³, Ayça Koçbaş³
Meral Özmen¹, Hülya Kayserili³

Departments of ¹Pediatrics, and ³Medical Genetics, İstanbul University İstanbul Faculty of Medicine, İstanbul, and ²Department of Pediatrics, Kocaeli University Faculty of Medicine, Kocaeli, Turkey

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Ataxia with vitamin E deficiency (AVED) is a rare autosomal recessive disorder, usually with a phenotype resembling Friedreich ataxia, caused by selective impairment of gastrointestinal vitamin E absorption. Vitamin E supplementation improves symptoms and prevents disease progress. In North Africa and Southern Europe, AVED is as common as Friedreich ataxia. There are no reported cases from Turkey. We herein report a 16-year-old Turkish girl with AVED, who was found to have total deletion of the TTPA gene as well as sensorineural deafness, and we present her follow-up data after vitamin E therapy.

Key words: ataxia with vitamin E deficiency (AVED), α -tocopherol transfer protein, autosomal recessive neurodegenerative ataxias, deafness.

Autosomal recessive cerebellar ataxias are a heterogeneous group of neurological disorders involving both central and peripheral nervous systems¹. They are classified according to the major site of degeneration, which can be the cerebellum or the spinal cord¹. Friedreich ataxia (FRDA) is the most frequent of the autosomal recessive spinal cord ataxias¹. Ataxia with vitamin E deficiency (AVED) is a rare autosomal recessive disorder, usually with a phenotype resembling FRDA, caused by selective impairment of gastrointestinal absorption of vitamin E². Gastrointestinal absorption of lipids is normal; however, incorporation of vitamin E into very low density lipoproteins (VLDLs) secreted by the liver is impaired due to the lack of α -tocopherol transfer protein (α -TTP). The gene coding for the α -TTP was localized to 8q13³. AVED, which is as frequent as FRDA in North Africa, is one of the treatable causes of ataxia⁴. Vitamin E supplementation might stop further progression of the disease and typical symptoms can be averted if the treatment is started early in life⁵. It is recommended that in all patients with ataxia of unknown cause, vitamin E deficiency should be excluded⁴.

Ataxia with vitamin E deficiency cases in the literature are reported from North Africa, Southern Europe, Central and Northern Europe

and Japan, in decreasing order. To the best of our knowledge, there are no reported cases from Turkey.

We here report a 16-year-old Turkish girl with AVED, who also has sensorineural deafness, and we present her follow-up data after vitamin E therapy.

Case Report

A 16-year-old girl was presented due to difficulty in walking and slurring of speech. Her walking pattern was normal until seven years of age. She then had problems in keeping her balance while walking. She gradually started stumbling and even falling down. She had difficulty in tying her shoe laces and buttoning her clothes. A gradual decline in her hearing started at the age of 10 years. She had been using a hearing apparatus since 11 years of age for profound sensorineural hearing loss. Her walking and fine motor movements had gradually deteriorated in the last three years.

The patient was born of first-degree cousins following an uneventful pregnancy (Fig. 1). Her family history revealed a similarly affected sister, who experienced difficulty in walking and articulate speech at 12 years of age. She is 25 years old and has been non-ambulatory

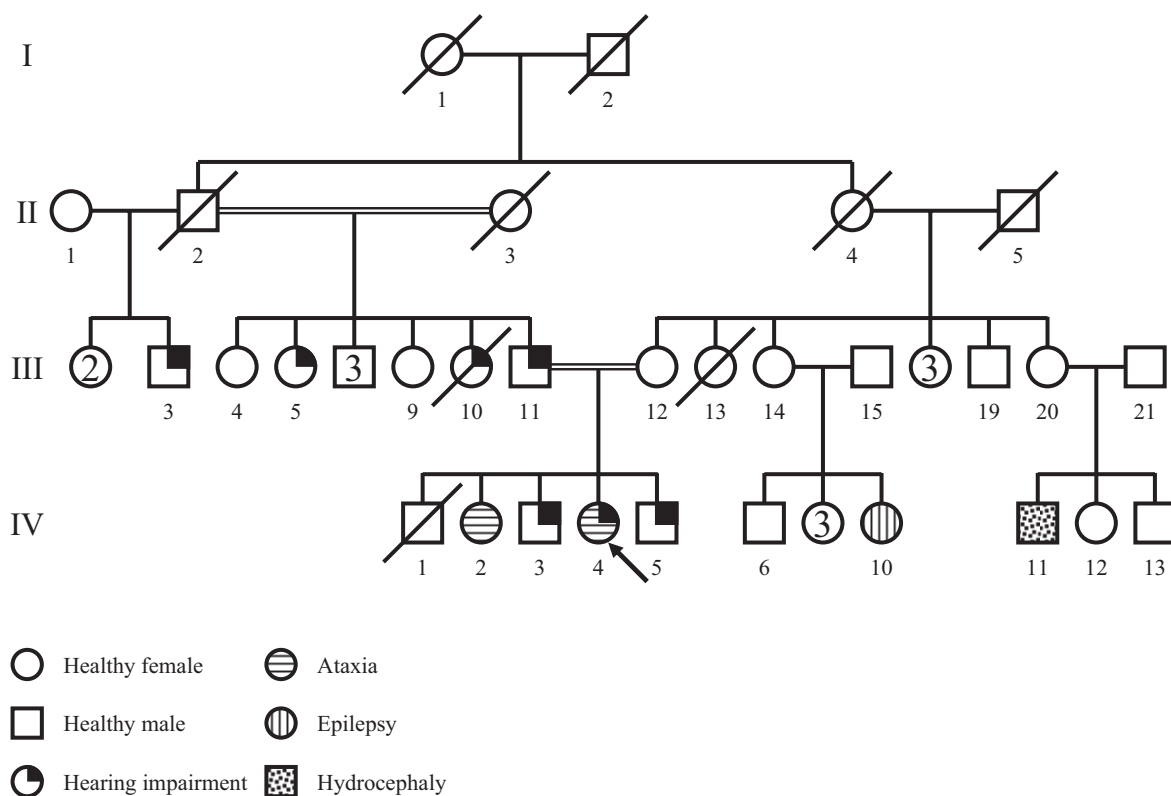


Fig. 1. Pedigree of the family.

for two years, but has no hearing loss. Her brother, father, paternal grandfather and one of her half-brothers all have sensorineural hearing deficiency, with no additional cerebellar signs.

On physical examination, her weight was 50 kg (10-25 p), height 162.5 cm (50-75 p) and head circumference 56 cm (90-97 p). She had mild scoliosis and kyphosis. Cognitive functions were normal. Cranial nerves were intact. Deep tendon reflexes were absent. No pathological reflexes were present. Her gait was observed to be ataxic. She had slurred speech due to dysarthria with lack of dysmetria and dysdiadokinesia. Neither superficial nor profound sensory loss was noted. Her strength was 5/5 in all muscles. No involuntary movements were observed. She was able to walk unaided. She could converse using complex sentences but due to her sensorineural hearing loss she had problems in comprehension. Presently, she has toilet control. She is attending high school but her academic status is unsatisfactory.

Whole blood count, biochemical analysis, and urine analysis were normal. Her ceruloplasmin, vitamin B₁₂, folate and alpha-fetoprotein (AFP) levels were within normal ranges. Tandem-mass spectrometry (MS) and organic acid analysis in urine were both within physiological limits. Electromyography (EMG) and ophthalmologic evaluation were normal. Cranial magnetic resonance imaging (MRI) and echocardiography were unremarkable. Audiometric analysis revealed bilateral sensorineural hearing loss. FRDA gene analysis revealed normal repeat numbers excluding Friedreich ataxia as the etiological cause. Serum vitamin E levels were very low in two independent measurements (0.4 mg/L and 1 mg/L; normal value: 6-15 mg/L). Serum lipid electrophoresis was normal (alpha 43.6%, pre-beta 14.9%, beta 41.2%, chylomicron 0.3%). Stool fat excretion was normal. The patient was diagnosed as AVED.

Oral vitamin E 800 mg/day was initiated twice daily. Three months after the initiation of the therapy, the patient was reevaluated,

and no improvement in neurological findings was observed, even though the parents had observed that she could walk while carrying a glass in her hand, a function she could not perform before the initiation of therapy.

DNA was isolated from peripheral blood lymphocytes of the tested individuals following the approval of the informed consent (Mammalian DNA Isolation Kit, Roche; Istanbul). Exclusion of 35delG mutation in the GJB2 gene in the index and in one of the hearing impaired sibs, IV:3 and IV:4 (Fig. 1), respectively, was performed using polymerase chain reaction (PCR)-mediated site-directed mutagenesis followed by digestion with BsiYI⁶.

Five coding exons of TTPA gene were amplified with STR marker, D12S1630 (www.gdb.org), as control in each, by touch down duplex PCR. The reaction was carried out in a final volume of 25 μ l containing 100 ng of genomic DNA, 1x PCR buffer with (NH₄)₂SO₄, 1.5 mM MgCl₂, 200 μ M dNTP, 320 μ M of each forward and reverse primers of TTPA (Table I), 200 μ M of each D12S1630 primers and 0.75 U of Taq polymerase (MBI Fermentas; Istanbul) with an initial denaturation at 94°C for 4 min, followed by 34-38 cycles of denaturation at 94°C for 30 s, annealing at 62°C-52°C for 30 s, extension at 70°C for 60 s, and a final extension at 70°C for 7 min using a DNA Engine PTC-200 Thermal Cycler (MJ Research, Elips; Turkey).

Analysis of duplex PCR products on 2% agarose gel revealed no bands for exons of TTPA gene, but a single band for STR marker for the index, and two bands for control samples, one for TTPA gene and the other for STR marker (Fig. 2). This result showed

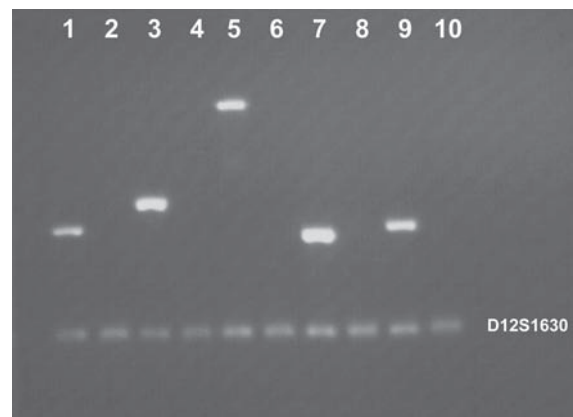


Fig. 2. Total deletion of TTPA gene of the index. Products obtained from duplex PCRs for each exon of TTPA gene (exon 1, lanes 1, 2; exon 2, lanes 3, 4; exon 3, lanes 5, 6; exon 4, lanes 7, 8; exon 5, lanes 9, 10) and STR marker D12S1630 in a healthy control (lanes 1, 3, 5, 7, 9) and in the index patient (lanes 2, 4, 6, 8, 10).

indirectly that the total coding sequence of the TTPA gene was deleted in the index patient. Further analysis will be required to determine the deletion breakpoint of the gene, which would be important for enlightenment of the molecular pathophysiology.

Discussion

The first case of inherited isolated vitamin E deficiency was reported in 1981 by Burck et al.⁷ Ben Hamida et al.⁸ reported eight affected individuals from two large Tunisian pedigrees in 1993, and delineated this disease as AVED. It was shown that mutations in the α -TTP gene were responsible for the phenotype⁹⁻¹¹.

The first clinical signs of AVED appear commonly around 10 years of age (range: 2 to 52 years), but most patients have had onset after the first

Table I. Intronic Primers Used for Amplification of the Exons of the TTPA Gene

Exon number	Primer name	Sequence in 5'-3' direction	Length of PCR product
1	TTPA-1F	GGAGAGTGGTTGTGCGAC	404 bp
	TTPA-1R	CGGCAGGGGTCAGATATC	
2	TTPA-2F	CCCCTGTCACCTCAGATC	480 bp
	TTPA-2R	GCCCAAATTCCTAAAGATGAA	
3	TTPA-3F	CTTGCCTGCCTGTTCTT	770 bp
	TTPA-3R	CTTCCTCTTAGCATTGTTGAAT	
4	TTPA-4F	GTGACAGTGAAGTGAAGCCA	384 bp
	TTPA-4R	GGGCAGGTACGAGGAAAG	
5	TTPA-5F	CCTTGTTTTATGAGGTTGATTT	402 bp
	TTPA-5R	CCTGTGTTGCTCATGTGTCAG	

two decades^{1,12}. In the early stages of the disease, hyporeflexia, decreased proprioception, decreased vibratory sense, distal muscle weakness, night blindness and preserved cognition are the prominent clinical findings⁴. Subsequently, neurologic symptoms progress and patients can develop truncal and limb ataxia, positive Romberg's sign, diffuse muscle weakness, upward gaze nystagmus and rarely retinitis pigmentosa. In the latter stages of the disease, dysphagia, dysarthria, cardiac arrhythmias, ophthalmoplegia, blindness and dementia may develop⁴. In the presence of affected relatives, hyporeflexia and/or loss of vibration sense in an individual may be indicative for an early diagnosis of AVED. If there is no other diagnosed case in the family, the diagnosis of cerebellar ataxia is ascertained after ataxia has become evident. In our case, although the sister had similar clinical findings, the diagnosis was established nine years after the onset of symptoms.

Diagnosis of AVED is ascertained by showing low serum vitamin E levels in the absence of fat malabsorption¹. Serum vitamin E levels were shown to be well below the normal range, as <2.5 mg/L and often <1 mg/L (normal range 6-15 mg/L). Vitamin E levels are found to be within the lower ranges of normal in carriers¹. Red blood cell morphology is normal. Molecular analysis is not a must for confirming the clinical diagnosis. Several features of AVED overlap with those of FRDA. In our case, excluding FRDA by normal repeat numbers by molecular analysis and showing that serum levels of vitamin E were low on two different measurements led us to the diagnosis of AVED. Her sister, who had similar findings, also had a serum vitamin E level <2 mg/L. The absence of fat in the stool and normal serum lipid electrophoresis also excluded vitamin E deficiency due to fat malabsorption.

Ataxia with vitamin E deficiency is caused by mutations in the gene for α -TTP, localized on chromosome 8q13, which has 5 exons encoding a 278-amino acid protein. To date, 19 mutations in total, including point and small insertion/deletion mutations, were reported in TTPA genes (Human Genome Mutation Database, <http://www.hgmd.cf.ac.uk>). We report for the first time the total deletion of the TTPA gene in a patient with AVED. Since the breakpoints are currently unknown, we can not perform control studies to determine

the carrier frequency of total TTPA gene deletion. It has been observed that truncating TTPA gene mutations are associated with a severe form of the disease, while some of the missense mutations are associated with milder clinical presentations¹³. The alleles harboring truncating mutations are likely to be null via non-sense mediated mRNA decay. Since it leads to absence of any coding mRNAs transcribed, total deletion of the TTPA gene is expected to be associated with a severe phenotype, as was the case in our patient.

In AVED patients, the administration of vitamin E in divided daily doses was noted to stop the progression of neurologic signs and symptoms and even to produce some amelioration of neurologic abnormalities¹². Cessation of supplementation in these patients caused a rapid decrease in plasma α -tocopherol to deficient concentrations within days¹⁴. Therefore, patients must receive continuous supplementation. Daily recommended doses are at least 600 mg in adults and 300 mg in children taken in divided doses of RRR- α -tocopherol along with fat-containing nourishments¹⁴. It is recommended that the serum vitamin E levels be monitored periodically and doses are set forth accordingly. RRR- α -tocopherol 800 mg was initially administered twice a day in our patient. Three months after initiation of the therapy, the patient was reevaluated. Although no evident amelioration of symptoms was observed at neurological examination, the parents had noticed that she could walk while carrying a glass in her hand, a function she could not perform before the therapy was started, providing a hint of the effectiveness of therapy.

The presence of three deaf individuals (2 males, 1 female) in the same sibship born to the first-degree cousin marriage, the father also being affected, led us to consider autosomal recessive sensorineural deafness in the family. Common mutation 35 del G in the GJB2 gene, which is responsible for 25% of autosomal recessive sensorineural deafness in Turkey, was excluded¹⁵. In the view of the fact that the father was also affected, which may implicate that deafness could also be autosomal dominantly inherited, further molecular testing is planned. The rational outcome, regardless of the inheritance model, is that deafness in this family is inherited as a separate entity other than AVED, which can be deduced by the

fact that only one of the two AVED patients displayed deafness and only one of the seven deaf members is affected with AVED.

In Mediterranean countries, taking into account the reported cases, the incidence of AVED is similar to that of FRDA. Although there are no other AVED cases reported from Turkey, the fact that Turkey is a Mediterranean country, in which autosomal recessive diseases have a high incidence due to the high rate of consanguineous marriages, makes it probable that many other AVED cases are present, but are undiagnosed or misdiagnosed. To the best of our knowledge, we herein report the first AVED case from Turkey and would like to emphasize the importance of early diagnosis of AVED since there is a chance of cure with early intervention.

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