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Molecular, clinical and peripheral neuropathy study of Tunisian patients with ataxia with vitamin E deficiency

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Ataxia with vitamin E deficiency is an autosomal recessive cerebellar ataxia caused by mutations in the α-tocopherol transfer protein coding gene localized on chromosome 8q, leading to lower levels of serum vitamin E. More than 91 patients diagnosed with ataxia with vitamin E deficiency have been reported worldwide. The majority of cases originated in the Mediterranean region, and the 744delA was the most common mutation among the 22 mutants previously described. We examined the clinical and molecular features of a large cohort of 132 Tunisian patients affected with ataxia with vitamin E deficiency. Of these patients, nerve conduction studies were performed on 45, and nerve biopsy was performed on 13. Serum vitamin E was dramatically reduced for 105 of the patients analysed. Molecular analysis revealed that 91.7% of the patients ($n = 121$) were homozygous for the 744delA mutation. Three other mutations were detected among the remaining patients (8.3%, $n = 11$) in the homozygous state. Two were previously reported (400C>T and 205-1G>T), and one was novel (553+1T>A). Age of onset was 13.2 ± 5.9 years, with extremes of 2 and 37 years. All described patients exhibited persistent progressive cerebellar ataxia with generally absent tendon reflexes. Deep sensory disturbances, pyramidal syndrome and skeletal deformities were frequent. Head tremor was present in 40% of the patients. Absence of neuropathy or mild peripheral neuropathy was noted in more than half of the cohort. This is the largest study of the genetic, clinical and peripheral neuropathic characteristics in patients with ataxia and vitamin E deficiency. The 744delA mutation represents the most common pathological mutation in Tunisia and worldwide, likely because of a Mediterranean founder effect. Our study led us to suggest that any patient displaying an autosomal recessive cerebellar ataxia phenotype with absent tendon reflexes and minor nerve abnormalities should first be screened for the 744delA mutation, even in the absence of a serum vitamin E measurement.

Keywords: AVED; nerve conduction; neuropathy; tendon reflexes; TTPA

Abbreviations: ARCA = autosomal recessive cerebellar ataxia; AVED = ataxia with vitamin E deficiency; LLN = lower limits of normal

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Introduction

Ataxia with vitamin E deficiency (AVED) was first described by Burck *et al*. (1981). The authors describe a 12-year-old boy with progressive cerebellar ataxia and a low serum vitamin E level (Burck *et al.*, 1981). Other cases were reported before 1993; Ben Hamida *et al*. (1993) described eight new patients belonging to two Tunisian consanguineous families. These patients displayed a clinical phenotype similar to Friedreich's ataxia, as well as a low serum vitamin E level. The defective gene was excluded from the Friedreich's ataxia locus on chromosome 9q (Ben Hamida *et al*., 1993*b*) and was localized to chromosome 8q by homozygosity mapping (Ben Hamida *et al*., 1993*a*). The responsible gene was cloned as the hepatic α-tocopherol transfer protein coding gene (*TTPA*; Arita *et al.*, 1995) and mutations were identified (Ouahchi *et al*., 1995). The TTPA protein is a carrier of RRR-α-tocopherol, the primary vitamin E isomer in plasma. In AVED, intestinal vitamin E absorption is normal, but mutations of TTPA impair the hepatic incorporation of α-tocopherol in very low density lipoprotein, resulting in low levels of plasma vitamin E (Traber *et al*., 1990). Similar to Friedreich's ataxia, AVED is generally characterized by early onset progressive gait and limb ataxia, dysarthria, generally absent tendon reflexes, deep sensory disturbances, pyramidal syndrome and skeletal deformities. Among >20 identified mutations, 744delA, found in the Mediterranean region, is considered to be the most commonly encountered mutant (Cavalier *et al*., 1998; Benomar *et al*., 2002; Mariotti *et al*., 2004; Marzouki *et al*., 2005; Bouhlal *et al*., 2008).

Here, we report a complete clinical and molecular study of a large cohort of 132 patients affected with AVED composed of one Libyan and 131 Tunisians.

Materials and methods

Patients

A large cohort of 132 patients belonging to 49 families was selected from the records of the Departments of Neurology and Molecular Neurobiology and Neuropathology of the National Institute of Neurology from 1975 to 2009. Most of the patients were referred to the Department of Neurology, as they presented with a clinical phenotype of autosomal recessive cerebellar ataxia (ARCA) with a presumed autosomal recessive inheritance. Other patients were selected among family members of the patients, symptomatic or asymptomatic, via a field survey. Additional patients were referred by other neurologists for molecular diagnosis.

Blood samples were drawn from all 132 patients for DNA analysis. Serum levels of vitamins A and E were assessed using high-performance liquid chromatography in 104 patients. All patients analysed had low serum vitamin E levels, ranging from 0 to 1.62 mg/l, with a mean of 0.41 ± 0.77 mg/l (normal range, 7–15 mg/l) (Fig. 1). Serum vitamin A levels were in the normal range for all patients, thus excluding abetalipoproteinaemia.

Among the 132 selected patients belonging to 49 families, only 77 (34 families) underwent a full neurological examination.

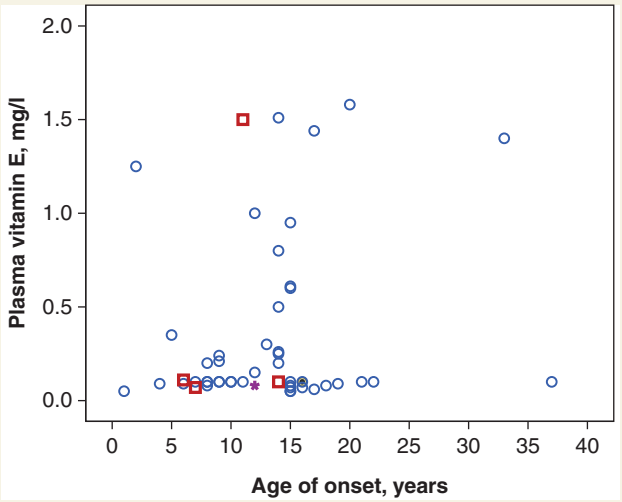

Figure 1 Serum vitamin E concentration according to age of onset in 51 AVED patients with the 744delA mutation (open circle), the 205-1G>T mutation (open square), the 553+1T>A mutation (asterisk) or the 400 C>T (filled circle) mutation. Vitamin E level: 0 to 1.6 (normal range: 8–15 mg/l).

Molecular analysis

Genomic DNA was extracted from peripheral blood leucocytes of all patients. First, a screen for the 744delA mutation was performed by direct sequencing of the fifth exon of the *TTPA* gene. If the target mutation was absent, direct whole gene sequencing was performed. All molecular tests were conducted using an ABI Prism 3130 genetic analyser (Applied Biosystems).

Nerve conduction studies and nerve and muscle biopsies

Nerve conduction studies were performed on 45 patients (25 families), and the superficial peroneal nerve were biopsied in 13 unrelated patients as described by Zouari *et al*. (1997). A fragment of the peroneus brevis muscle was also extracted in parallel to the nerve biopsy and in addition to undergoing the routine histoenzymatic analyses, sections were stained with haematoxylin and eosin.

Results

Molecular characteristics

Of the 34 families that were clinically evaluated, 29 were consanguineous. The 744delA mutation was detected in 91.7% of the screened patients ($n = 121$; 46/49 families). Two previously reported mutations (400C>T and 205-1G>T) were identified among four patients from two different families. A novel mutation was identified in seven other patients belonging to the same family (553+1T>A). All of these mutations were present in the homozygous state.

Clinical characteristics

One of the 77 clinically examined patients (from 34 families) was an asymptomatic 11-year-old boy with a low vitamin E serum level (0.49 mg/l). This individual was the youngest of three siblings, one of whom bore the 744delA mutation in the homozygous state.

The boy exhibited normal tendon reflexes with no evidence of neurological symptoms. Because of the low serum vitamin E level and the family history of AVED, he was included in the molecular study, which confirmed that he carried the same 744delA mutation in the homozygous state. Based on these findings, the boy began receiving 800 mg α-tocopherol per day, and since diagnosis, he remained asymptomatic for 6 years, up to his most recent physical examination in 2009.

Clinical features observed in the 76 remaining patients were largely consistent (Table 1). Although the majority were male, the sex ratio was not statistically significant (1.24). The mean age of these patients was 24.1 ± 10.6 years, with an age of onset ranging from 2 to 37 years. Gait impairment was the initial symptom initially detected in 93.4% of the patients ($n = 71$). Head tremor was present in conjunction with gait instability in 11 patients and was isolated at disease onset in two other patients. Dysarthria and hand clumsiness were also initially detected in 15.8% and 11.8% of the patients, respectively.

Throughout disease progression, cerebellar ataxia, gait ataxia and limb ataxia, predominately in the lower limbs, represented persistent features. Dysarthria was found in 61.8% of the patients ($n = 47$). Head tremor was present in 40.8% of the patients ($n = 31$). Deep sensory disturbances were present in 51 patients (67.1%). Babinski sign was present in 85.5% of patients ($n = 65$). Spastic gait was variable, transitioning from absent to significant independently of disease duration. The majority of the patients exhibited absent or weak reflexes of both the knee and the ankle (94.7%). Only one patient displayed brisk knee and ankle reflexes. Skeletal abnormalities, such as pes cavus, kyphoscoliosis and hammer toes, were apparent in more than half of the patients. Nystagmus was noted in seven patients. Critical impairment of visual acuity with night blindness and corresponding fundoscopic detection of retinitis pigmentosum was noted in three patients. Four patients presented with oculo-motor apraxia and two others with exotropia. Distal lower limb amyotrophy was present in 11 patients, among whom only one had additional upper limb amyotrophy. Urinary urgency was reported in 22.4% of the patients, while incontinence was reported in only 4%.

Table 1 Clinical data and phenotype-genotype correlation of 76 AVED patients

		All AVED 76 patients (34 families)	744dela 69 patients (31 families)	400 C>T 2 patients (1 family)	205-1G>T 1 patient (1 family)	553+1T>A 4 patients (1 family)
Age, years		24.1 ± 10.6	24.4 ± 10.9	10 and 18	26	18, 20, 23 and 27
Age of onset, years (range)		13.2 ± 5.9 (2–37)	13.4 ± 6.1 (2–37)	9 and 16	12	6, 7, 11 and 14
Initial symptom, *n* (%)	Ataxic gait	7 (93.4)	64 (92.8)	2	1	4
	Head tremor	13 (17.1)	13 (18.8)	0	0	0
	Dysarthria	12 (15.8)	12 (17.4)	0	0	0
	Hand clumsiness	9 (11.8)	7 (10.1)	1	0	1
Wheelchair-bound age, years (range; *n*)		28.4 ± 3.5 (22–33; 10)	28.4 ± 3.5 (22–33; 10)	–	–	–
Cerebellar syndrome, *n* (%)		76 (100)	69 (100)	2	1	4
Dysarthria, *n* (%)		47 (61.8)	40 (58.0)	2	1	4
Head tremor, *n* (%)		31 (40.8)	31 (40.8)	0	0	0
Knee or ankle reflexes, *n* (%)	Absent	72 (94.7)	65 (94.2)	2	1	4
	Weak	2 (2.6)	2 (2.6)	0	0	0
	Normal	1 (1.3)	1 (1.3)	0	0	0
	Brisk	1 (1.3)	1 (1.3)	0	0	0
Babinski sign, *n* (%)		65 (85.5)	59 (85.6)	1	1	4
Joint position error, *n* (%)		51 (67.1)	47 (68.1)	1	1	2
Pes cavus, *n* (%)		36 (50.0)	31 (44.9)	1	1	3
Scoliosis, *n* (%)		17 (22.4)	12 (17.4)	1	1	3
Hammer toes, *n* (%)		8 (10.5)	8 (11.6)	0	0	0
Retinitis pigmentosum, *n* (%)		3 (4.0)	3 (4.4)	0	0	0
Bladder disturbances, *n* (%)		13 (17.1)	10 (18.8)	1	0	2
Oculo-motor apraxia, *n* (%)		3 (4.0)	3 (4.4)	0	0	0
Lower limb amyotrophy, *n* (%)		11 (14.5)	11 (15.9)	0	0	0
Peripheral neuropathy: sensory/motor	Absent/mild, *n*	24/43	20/38	1/1	1/1	2/3
	Moderate, *n*	11/43	11/38	0/1	0/1	0/3
	Severe/no response, *n*	8/43	7/38	0/1	0/1	1/3

Absent = absence of neuropathy, all eight nerve conduction study parameters are normal; mild = at least one nerve conduction study parameter 70–100% of LLN; moderate = at least one nerve conduction study parameter 30–70% of LLN; severe = at least one nerve conduction study parameter <30% of LLN; no response = at least one nerve conduction study parameter non-measurable.

Ten patients carrying the 744delA mutation were wheelchair-bound within a time span of 8 to 19 years from the onset of the disease. These 10 patients belong to four unrelated families who were examined between 1977 and 1993. Because the disease remained unknown at that time, these patients were diagnosed for AVED at late ages, and hence, they have not received vitamin E supplementation. Vitamin E treatment (300 to 1000 mg/day) was introduced in all patients in the months or years before or after AVED diagnosis, making it difficult to estimate the true course of the disease. However, it is important to note that all patients who have been treated before the bedridden stage continue to walk independently. One of our wheelchair-bound patients with AVED treated with 800 mg/day vitamin E showed dramatic improvement within a few months and began walking with support.

Twenty-nine patients were evaluated using the ICARS (International Cerebellar Ataxia Rating Score) scale and attained an average score of 33.04 ± 15.30. The ICARS score divided by the disease duration (ICARS score/disease duration), reflecting the degree of disability, was extremely variable, differing from 0.75 to 25. Many of these patients had irregular treatment for several reasons, such as socio-economic level. However, two scenarios were compared to determine the variability of responses despite appropriate treatment. Two unrelated patients of 37 (Patient 1) and 15 years (Patient 2) with respective disease duration of 4 and 2 years, were treated with 800 mg of vitamin E daily. The initial ICARS score before treatment was 3 for Patient 1 and 35 for Patient 2. Despite the early treatment provided to each patient, there was significant worsening of Patient 1 and improvement of Patient 2. In fact, after respective treatment of 2 and 1 year, both ICARS scores of Patients 1 and 2 changed to 15. Indeed, ICARS score/disease duration increased for Patient 1 from 0.75 to 2.5 and decreased for Patient 2 from 17.5 to 5.

Nerve conduction studies

Nerve conduction studies were performed on 45 patients with AVED belonging to 25 families (Table 2). The values of the median and peroneal nerve motor conduction velocity, the median and peroneal nerve compound muscle action potential, the median and saphenous nerve sensory conduction velocity, and the median and saphenous nerve sensory action potential were schematically differentiated according to the lower limits of normal (LLN) for each parameter as follows: normal (⩾100% of LLN), mild (70–100% of LLN), moderate (30–70% of LLN), severe abnormalities (<30% of LLN) or no response (un-assessable motor conduction velocity or sensory conduction velocity).

For each parameter, normal values were detected in >50% of the patients. Mild abnormalities ranged from 16 to 42% of LNN for motor conduction velocity and from 7 to 25% for sensory conduction velocity. We detected a moderate decrease of compound muscle action potential in the peroneal or median nerve for some patients, as well as a reduction of sensory action potential in the median or saphenous nerve (Table 2). Nerve conduction studies were normal in only four patients (9%). Mild neuropathy (at least one parameter 70–100% of LLN) was present in 21 patients (47%). Twelve patients (27%) exhibited moderate neuropathy (at least one parameter 30–70% of LLN), and severe neuropathy (at least one parameter <30% or with no response) was noted in eight patients (17%).

Among the 41 patients with nerve conduction study abnormalities, ~88% displayed axonal neuropathy, whereas the remaining patients displayed no sensory responses. Sensorimotor neuropathy was observed in 42% of the patients. Neuropathy was either purely sensory (34%) or purely motor (24%) in the other patients. Severity was variable among siblings, and there was no evidence of a correlation between the genotype and the phenotype.

Table 2 Nerve conduction studies in 45 AVED patients

	Motor nerve conduction				Sensory nerve conduction			
	Median		Peroneal		Median		Saphenous	
	A (mV)	V (m/s)	A (mV)	V (m/s)	A (μV)	V (m/s)	A (μV)	V (m/s)
Normal	*⩾ 5*	*⩾ 52.3*	*⩾ 2.5*	*⩾ 41.8*	*⩾10*	*⩾ 43.5*	*⩾10*	*⩾ 41.3*
Number/total (%)	32/43 (74.4)	25/43 (58.1)	25/45 (55.6)	38/45 (84.4)	22/41 (53.7)	26/40 (65)	34/45 (75.6)	31/43 (72.1)
Mild abn.: 70-99% LLN	*3.6-4.7*	*45-52*	*1.8-2.3*	*35-41.5*	*7.2-9.8*	*30.5-42.5*	*7-9.6*	*29-41*
Number/total (%)	9/43 (20.9)	18/43 (41.9)	13/45 (28.9)	7/45 (15.6)	4/41 (9.8)	10/40 (25)	3/45 (6.7)	7/43 (16.3)
Total normal/mild abn.	41/43	43/43	38/45	45/45	26/41	36/40	37/45	38/43
Number/total (%):	(95.4)	(100)	(84.4)	(100)	(63.4)	(90)	(83.7)	(88.4)
Moderate abn. 30–70% LLN	*2.8-3.3*	-	*1.5-1.7*	-	*4-6.5*	-	*5.1-5.5*	*28.2*
Number/total (%)	2/43 (4.7)	0/43 (0)	5/45 (11.1)	0/45 (0)	10/41 (24.4)	0/40 (0)	3/45 (6.7)	1/43 (2.3)
Severe abn.: <30% LLN	-	-	*0.14-0.65*	-	*2.9*	-	*1*	-
Number/total (%)	0/43 (0)	0/43 (0)	2/45 (4.4)	0/45 (0)	1/41 (2.4)	0 (0)	1/45 (2.3)	0/43 (0)
Non-measurable Number/total (%)	0/43 (0)	0/43 (0)	0/45 (0)	0/45 (0)	4/41 (9.8)	4/40 (10)	4/45 (8.9)	4/43 (9.3)

A = amplitude; V = velocity, abn. = abnormalities.

Biopsy characteristics

Nerve and muscle biopsies were performed on 13 patients with AVED, 12 carrying the 744delA mutation and one carrying the 205-1G>T mutation. A trend toward fibre type grouping of the peroneus brevis muscle was the primary and persistent feature. Rare abnormalities, such as degenerated axons and some demyelinated segments of the superficial peroneal nerve, were noted. The density of large myelinated fibres (>8 μm) was slightly reduced, confirming the nerve conduction data (1108 ± 252/mm^2 in nerves from the 13 analysed patients compared to 2426 ± 653/mm^2 from the control subjects).

Other investigations

Lipid and fasting blood sugar levels displayed normal values in 104 and 36 examined patients with AVED, respectively. Cerebral MRI was normal in seven examined patients who were homozygous for the 744delA mutation. No abnormalities were revealed in a spine MRI performed on one patient. Cerebral CT was normal in 17 of 23 examined patients. Vermian atrophy and hemispheric cerebellar atrophy were observed in four and three patients, respectively. Electrocardiogram and echocardiogram were normal in 29 and 11 analysed patients, respectively. Only two siblings exhibited tachyarrhythmia and cardiomyopathy. One of them presented with severe cardiomyopathy with both left and right ventricular hypertrophy and dilatation. He was wheelchair-bound at the age of 25 years and died from heart failure at the age of 29 years. The clinical and pathological study of this patient was previously reported (Larnaout *et al*., 1997).

Discussion

Genetic characteristics and epidemiologic data

With a total of 132 patients belonging to 49 families, we report here the largest studied cohort of patients with AVED. In fact, ~91 AVED genetically confirmed patients, belonging to 64 families, have been previously reported worldwide.

The total of AVED-related mutations reaches 23 with the novel mutation described here. However, the frameshift 744delA mutation was clearly the most frequent *TTPA* mutation, detected in 73 families of the 106 reported worldwide to date, including 48 Tunisian families, of which five had already been reported (Ouahchi *et al*., 1995; Larnaout *et al*., 1997; Cavalier *et al*., 1998; Bouhlal *et al*., 2008). The same mutation was also described in 23 other Mediterranean families: 11 Moroccan (Ouahchi *et al*., 1995; Cavalier *et al*., 1998; Benomar *et al*., 2002; Roubertie *et al*., 2003; Marzouki *et al*., 2005), nine Italian (Ouahchi *et al*., 1995; Cavalier *et al*., 1998; Martinello *et al*., 1998; Angelini *et al*., 2002; Mariotti *et al*., 2004), one Algerian (Cavalier *et al*., 1998), one French (Ouahchi *et al*., 1995), and two Spanish (May Cabrero *et al*., 2000; Aparicio *et al*., 2001).

Interestingly, we identified the first three Mediterranean families with patients carrying *TTPA* mutations different than the 744delA frameshift. The 400C>T mutation reported in two siblings from Tataouine (south Tunisia) was previously described in two unrelated Canadian (Cavalier *et al*., 1998), one Italian (Cellini *et al*., 2002) and one Norwegian patient (Koht *et al*., 2009). The 205-1G>T splice-site mutation identified in two sisters from Gasserine (central west Tunisia) was only reported in one patient from France (Cavalier *et al*., 1998). The Canadian patients could be descendants of French ancestors, as expected for autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS), explaining the presence of these two mutations in Canada, France and Tunisia (El Euch-Fayache *et al*., 2003). The newly reported mutation 553+1T>A was detected in seven siblings from Djerba (south Tunisia).

Thus, the Mediterranean region contains the majority of patients with AVED, with the 744delA as the most common mutation. This suggests a strong Mediterranean founder effect, supporting the hypothesis of Ouahchi *et al*. (1995). The second most common mutation after 744delA is the 513insTT frameshift, found in Italy and in two American families of European descendance (Ouahchi *et al*., 1995; Hentati *et al*., 1996; Cavalier *et al*., 1998).

In Tunisia, the number of patients is extremely high and could reach seven per family. On the other hand, we noticed that all patients from the South Mediterranean region had homozygous mutations, which is not surprising. In fact Morocco, Tunisia, Algeria and Libya are all geographically and culturally related Arabic and Islamic countries with a high rate of inbreeding. Additionally, we noticed that the majority of the Tunisian patients with AVED are from rural areas and/or small underdeveloped towns. Tunisia is also the smallest country included in this study, with >85% consanguineous AVED families, which also might explain the high frequency and the founder effect of the 744delA mutation.

In the absence of consistent epidemiologic data, the rate of inbreeding appears to favour the emergence of AVED and ARCA in general from Tunisia (Ben Hamida *et al*., 1993*b*) compared with other countries, even Morocco, where the inbreeding rate was estimated to be 50% (Benomar *et al*., 2002; Marzouki *et al*., 2005). A unique epidemiological study of AVED was performed in Japan, where the T303G mutation was found in only 21 of 801 selected inhabitants of one Japanese island and was not detected in 150 unrelated individuals from Tokyo (Gotoda *et al*., 1995).

The different *TTPA* mutations reported worldwide are described in Table 3.

Clinical features

Most of the patients described here began suffering from the disease before the age of 20. Only seven patients had initial signs between the age of 21 and 37 years. Such late onset was rarely reported worldwide. Onset after the age of 30, even up to 59 years old, was reported in five Japanese patients homozygous for the T303G *TTPA* mutation (Gotoda *et al*., 1995; Yokota *et al*., 1997, 2000). Late age of onset is therefore not specific to any country or mutation, and in addition, intrafamilial variability was observed in one of the families described here. Cerebellar syndrome was a consistent finding in patients from Tunisia as well as those from Morocco (Benomar *et al*., 2002). In some rare cases, cerebellar syndrome can be mild to absent (Cavalier *et al*., 1998). Head tremor, which was present in

Table 3 AVED patients (*n* = 220, 106 families) reported worldwide and their 23 different pathologic TTPA mutations

Country	Tunisia	Libya	Morocco	Algeria	Italy	France	Spain	Japan	USA	Canada	German	Belgium	Albania	Netherlands	Norway	Portugal
Families *n* (110)	48	1	11	1	20	3	2	8	3	2	2	1	1	1	1	1
Patients *n* (220)	141	1	21	2	26	3	2	10	5	2	2	1	1	1	1	1
Homozygote%	100	100	100	100	52	34	100	91	40	50	100	100	100	0	0	100
744delA (%)	92	100	91	100	39	17	100									
553 + 1T > A (%)	5															
205-1G > C (%)	1,5					17										
400 C > T (%)	1,5				2					75					50	
513insTT (%)					39				50							
219insAT (%)					4											
486delT (%)			9		2				20	25						
306A > G (%)					7											
Gly246Arg (%)					5											
E141K (%)						33										
552 + A > T (%)						33										
H101Q (%)								50								
D64G (%)								5								
L183P (%)								20								
C > T5′UTR (%)								5								
T > CEx1 (%)								20								
R192H (%)									30							
552G > A (%)											50					
530AG→6 (%)											50					
A120T (%)												100			50	
R221W (%)													100			
R59W(%)														50		100
437delT (%)														50		
NI (%)					2											
First authors	Reported here Ouahchi 1995 Cavalier 1998 Bouhlal 2008	Reported here	Cavalier 1998 Roubertie 2003 Marzouki 2005	Cavalier 1998	Ouahchi 1995 Cavalier 1998 Mariotti 2004 Martinello 1998 Cellini 2002 Angelini 2002	Cavalier 1998 Anheim 2010	May Cabrero17 Aparicio 2001 May 2000	Gotoda 1995 Yokota 1987, 1996, 1997, 2000 Pang 2001 Usuki 2000 Shimohata 1998 Hoshino 1999 Tamaru 1999	Hentati 1996 Cavalier 1998	Ouahchi 1995 Cavalier 1998	Ouahchi 1995 Schuelke 1999	Cavalier 1998	Cavalier 1998	Ponten 2006	Koht 2009	Cavalier 1998

% = per cent of alleles with pathologic *TTPA* mutation, NI = not identified.

>40% of our patients, was reported at different rates, varying from 37% in patients studied by Cavalier *et al.* (1998), 44% in Mariotti *et al.* (2004) and 73% in Moroccan patients (Benomar *et al.,* 2002; Marzouki *et al*., 2005). Ankle and knee reflexes were absent or weak in >90% of our patients. Normal tendon reflexes were observed in only two of our patients, one of which was asymptomatic and in one symptomatic American family (Hentati *et al*., 1996; Cavalier *et al*., 1998). Brisk tendon reflexes, present in only one Tunisian patient, were similarly rarely reported worldwide [three patients in Morocco (Benomar *et al*., 2002) and two in Italy (Mariotti *et al*., 2004)]. Hammer toes were not described in previous reports and were present in 10% of our patients. Retinitis pigmentosum was rarely reported, and it was also a rare finding in our patients described here. However, independent of genotype, retinitis pigmentosum was a strikingly consistent finding in Japanese patients (Gotoda *et al*., 1995; Yokota *et al*., 1997; Shihomata *et al*., 1998; Hoshino *et al.* 1999; Usuki *et al.*, 2000; Pang *et al*., 2001). Ultimately, oculomotor apraxia and exotropia are potential findings that were first reported here. Complaints of urinary urgency and incontinence are unique to our patients. Cardiac involvement was also rare, occurring only in Moroccan and Tunisian patients. Several other symptoms, such as dystonia (Yokota *et al*., 1987; Cavalier *et al*., 1998; Schuelke *et al*., 2000; Angelini *et al*., 2002), myoclonus (Yokota *et al*., 1987; Angelini *et al*., 2002), tongue fasciculations (Martinello *et al*., 1998; Roubertie *et al*., 2003) and deafness (Shimohata *et al*., 1998; Usuki *et al*., 2000), are considered to be rare findings in patients with AVED and were not observed in our patients. Wheelchair-bound age reported for some of our patients was variable, as reported by Cavalier *et al.* (1998), and Mariotti *et al*. (2004) despite vitamin E treatment. In our patients, none were bedridden if treated with vitamin E. The course of the disease appears to be variable, however, with clear disability without vitamin E treatment. Supplementation of vitamin E in most instances allowed for stabilization of the disease. At least 12 patients who were monitored during 1 year of vitamin E oral administration showed mild improvement and maintained vitamin E levels in the normal range (Gabsi *et al*., 2001). However, after >1 year of treatment, the findings could be different, and worsening of symptoms could occur, as reported in 6 of 16 patients by Mariotti *et al*. (2004). These patients required >1000 mg/day of vitamin E. Two treated patients [one Italian (Mariotti *et al*., 2004) and one reported here] exhibited worsening of head tremor. Some improvement was observed in our patient after botulinum toxin injection into the splenius. One of our treated patients remained asymptomatic even though he had reached the age of onset of his sibling.

Despite this variability, vitamin E supplementation is likely the most effective treatment for confirmed AVED.

Neuropathy

More than half of Tunisian patients with AVED exhibited mild or no abnormalities in the nerve conduction study parameters. Moderate to severe nerve conduction study abnormalities could be detected. The neuropathy was axonal, as confirmed through nerve biopsy, and it was primarily sensorimotor or purely sensory. Muscle biopsy revealed consistent findings of lesion of the anterior horn, likely explaining the possible motor axon loss. All of the superficial peroneal nerve biopsies revealed mild abnormalities, displaying an absence of injury, at least in the distal portion. The mechanism of sensory nerve lesion was principally a neuronopathy because sensory loss was not restricted to the lower limbs, and either all motor nerves were normal or fewer than two nerves exhibited abnormal motor conduction study (Camdessanche *et al*., 2009). This was confirmed in one post-mortem patient for whom the posterior root ganglion displayed moderate cell loss with mild storage deposition (Larnaout *et al*., 1997).

Ataxia with vitamin E deficiency versus Friedreich's ataxia and other autosomal recessive cerebellar ataxias

The characteristics of our patients with AVED were similar in many aspects with those reported in the literature. A serum vitamin E level <2.5 mg/ml associated with a normal serum vitamin A level represents the main consistent feature of AVED. This form of vitamin E deficiency can be easily distinguished from abetalipoproteinaemia, in which the vitamin A level is also low. A significant decrease in serum vitamin E can also be detected in patients with Friedreich's ataxia, but with a lower threshold of 5 μg/ml (Gabsi *et al*., 2001; Feki *et al*., 2002). Therefore, we suggest that both vitamin E and vitamin A screening should be performed on any suspected ARCA patient.

Many characteristics are shared between AVED and Friedreich's ataxia, such as age of onset, presence of cerebellar syndrome with dysarthria, pyramidal syndrome, deep sensory disturbances, absence of tendon reflexes and skeletal deformities. However, head tremor, which is rarely encountered in patients with Friedreich's ataxia, was relatively frequent in patients with AVED. In contrast, cardiomyopathy, which is a common finding in patients with Friedreich's ataxia, was rarely detected in patients with AVED. Some other features are exclusively specific to one of the two diseases, including retinitis pigmentosum and dystonia for AVED and diabetes for Friedreich's ataxia (Dürr *et al*., 1996).

Unlike Friedreich's ataxia, where a consistently severe sensory axonal neuropathy is always observed (Zouari *et al*., 1997; Benomar *et al*., 2002; Santiago-Perez *et al*., 2007; Bouhlal *et al*., 2008), nerve conduction study results are normal or mildly abnormal in most patients with AVED. However, it is important to note that severe neuropathy does not exclude AVED, further complicating ARCA in its physiopathology. Moreover, sensorimotor neuropathy is a typical finding in ataxia-telangiectasia (Anheim *et al*., 2010) and tends to be severe in three other ARCA diseases: ARSACS (El Euch-Fayache *et al*., 2003), ataxia with oculo-motor apraxia type 1 (AOA1; Le Ber *et al*., 2003), and ataxia with oculo-motor ataxia type 2 (AOA2; Hammer *et al*., 2012). Molecular analysis of these ARCA is generally focused on other symptoms, such as a low level of IgA for ataxia-telangiectasia, brisk knee reflexes for ARSACS, a high level of cholesterol with hypoalbuminaemia for AOA1 and a high level of alpha fetoprotein for AOA2. In general, genetic diagnosis in all ARCA cases can be guided by the type and the severity of the neuropathy coupled with a set of clinical and paraclinical parameters.

General conclusions

In conclusion, AVED disorder is highly frequent in Tunisia compared with all previous genetic and epidemiological studies in the world, and it is even more frequent in the Mediterranean region. The 744delA mutation is the preponderant mutation identified worldwide, and a Tunisian founder effect is likely. A combination of consistent features, including very low level serum vitamin E and cerebellar syndrome, as well as inconsistent but frequent symptoms, such us abolished tendon reflexes, head tremor and absent or mild sensory neuropathy, distinguish it from Friedreich's ataxia and other ARCAs. Clinical symptoms, such as retinitis pigmentosum, are specific to AVED but can be present in different frequencies and are primarily correlated with geography rather than the genetic defect. Uncommon presentations of AVED, such as the presence of severe sensory neuropathy or dystonia, are possible and must be considered. Systematic molecular analysis, including screening for the 744delA mutation, should be offered in Tunisia, not only to treat the disease symptoms but, most importantly, to prevent onset through vitamin E supplementation to reduce the prevalence of the disease.

In general, for the heterogeneous group of ARCA, including different forms such as AVED, Friedreich's ataxia and others, molecular diagnosis should be performed regardless of the clinical and paraclinical symptoms, with a strong consideration of ethnic origins. More extensive epidemiological studies of AVED and other ARCA will provide greater understanding and knowledge about their frequencies and the possibilities for genetic analysis that can be offered to each region of the globe to limit their prevalence worldwide.

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