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Ataxia with Isolated Vitamin E Deficiency: Heterogeneity of Mutations and Phenotypic Variability in a Large Number of Families

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Summary

Ataxia with vitamin E deficiency (AVED), or familial isolated vitamin E deficiency, is a rare autosomal recessive neurodegenerative disease characterized clinically by symptoms with often striking resemblance to those of Friedreich ataxia. We recently have demonstrated that AVED is caused by mutations in the gene for α-tocopherol transfer protein (α-TTP). We now have identified a total of 13 mutations in 27 families. Four mutations were found in $\geqslant$2 independent families: 744delA, which is the major mutation in North Africa, and 513insTT, 486delT, and R134X, in families of European origin. Compilation of the clinical records of 43 patients with documented mutation in the α-TTP gene revealed differences from Friedreich ataxia: cardiomyopathy was found in only 19% of cases, whereas head titubation was found in 28% of cases and dystonia in an additional 13%. This study represents the largest group of patients and mutations reported for this often misdiagnosed disease and points to the need for an early differential diagnosis with Friedreich ataxia, in order to initiate therapeutic and prophylactic vitamin E supplementation before irreversible damage develops.

Introduction

Two forms of autosomal recessive ataxia caused by vitamin E deficiency have been described. The first form to be identified was abetalipoproteinemia (OMIM 200100 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?200100]), in which gastrointestinal absorption of lipids is impaired owing to the failure of formation of chylomicrons and the absence of very-low-density lipoproteins (VLDLs) and low-density lipoproteins (Kayden and Traber 1991). This results in severely reduced levels of serum vitamin E. This condition is due to mutations in the gene coding for the microsomal triglyceride transfer protein (Sharp et al. 1993). In the second form, familial isolated vitamin E deficiency (FIVE; OMIM 277460 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?277460]), gastrointestinal absorption of lipids is normal but incorporation of vitamin E into VLDLs secreted by the liver is impaired (Traber et al. 1990, 1993). In normal individuals, the latter function accounts for the efficient recycling of plasma vitamin E (1.4 $\pm$ 0.6 pools/d) that otherwise is rapidly eliminated (Traber et al. 1994). FIVE has been described in rare cases, studied during the period 1981–89 (Burck et al. 1981; Laplante et al. 1984; Harding et al. 1985; Krendel et al. 1987; Stumpf et al. 1987; Yokota et al. 1987; Kohlschütter et al. 1988; Sokol et al. 1988; Trabert et al. 1989). The description, reported in 1993, of eight affected individuals from two large Tunisian pedigrees (Ben Hamida et al. 1993*b*) initiated the identification of many other patients from North Africa, where this condition appears to be much more frequent. In the original study of Tunisian families, the clinical presentation was very similar to the severe Friedreich ataxia (OMIM 223300 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?223300]) phenotype, and the condition was named "ataxia with vitamin E deficiency" (AVED; OMIM 277460 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?277460]). We localized the defective gene in proximal 8q by linkage analysis, homozygosity mapping, and linkage-disequilibrium analysis (Ben Hamida et al. 1993*a;* Doerflinger et al. 1995). The gene coding for the α-tocopherol transfer protein (α-TTP) was localized independently to 8q13 (Arita et al.

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1995). The identification of mutations in the α-TTP gene confirmed that deficiency of vitamin E is the sole cause of the neurological symptoms in AVED. All North African and southern Italian patients studied were homozygous for the same frameshift mutation, 744delA (Ouahchi et al. 1995). Five other mutations were found in isolated families of north European and of Japanese origin (530AG→6 and 513insTT [Ouahchi et al. 1995], 486delT and R192H [Hentati et al. 1996], and H101Q [Gotoda et al. 1995]). We report here the identification of seven additional mutations in families of very diverse origins. A comparison of all the mutations reported to date, with the clinical presentation and progression of the disease before initiation of α-tocopherol supplementation, documents a correlation between the severity of the disease and the type of mutation.

Subjects and Methods

Subjects

Twenty-seven AVED families, comprising 41 patients, were included in the study. Essential criteria were very low vitamin E (<2.5 mg/liter) and neurological symptoms in the absence of fat malabsorption or abetalipoproteinemia. The numbering of families 1–16 is according to the study by Ouahchi et al. (1995). Families 3 and 4 (referred by M. Ben Hamida) and 9 and 17 (referred by A. Benomar) belong to large series of Tunisian and Moroccan families, respectively, and are not part of this study. Clinical descriptions were reported elsewhere for families 13 (Burck et al. 1981; Kohlschütter et al. 1988), 18 (Laplante et al. 1984), 14 (Sokol et al. 1988), 16 (Trabert et al. 1989), 1 and 2 (Ben Hamida et al. 1993*b*), 8 (Amiel et al. 1995), and 28 (Martinello et al., in press), and mutation was reported for 10 families (Ouahchi et al. 1995). In addition to the 27 families analyzed here, molecular and clinical data from 6 families were compiled from the literature (Gotoda et al. 1995; Hentati et al. 1996; Yokota et al. 1996, 1997).

Cloning of α-TTP Genomic Fragments

Approximately 10^6 clones from a λ GEM12 human genomic library (provided by Dr. J. M. Garnier) were screened with the 765-bp *Eco*RI cDNA fragment, corresponding to exons 1–4 and part of exon 5 (Arita et al. 1995), which was labeled by the random-priming method. One positive clone for exon 1 and one positive clone for exon 2 were isolated and were digested by *Eco*RI. The *Eco*RI fragments that contained an exon were subcloned by pBluescript (Stratagene) and were sequenced with cDNA-derived primers (Arita et al. 1995), followed by sequencing with intronic primers on the other strand.

SSCP Analysis

Primers used for amplification of exons 3–5 have been reported (exons a–c in Ouahchi et al. 1995). Primers designed for amplification of the 5′ UTR and of exons 1 and 2 are as follows, with the annealing temperature and $MgCl_2$ and dimethyl sulfoxide (DMSO) concentrations for PCR given in parentheses: 5′ UTR1, D-GAG GCT GCC AAG GAG GCA and R-GAA GCC ACT GAT GTT CAA ACAC, and 5′ UTR2, D-AAT CAT CCC AAC TAA CTT TGA CAT and R-GCC TCT GCC ATG CCC GC (59°C, 1.5 mM $MgCl_2$, 5% DMSO); exon 1a, D-TGC GGC CGC AGC AGC and R-CGG GCG CGC AGG AACC (60°C, 1 mM $MgCl_2$, 5% DMSO); exon 1b, D-AAG CTG GCG TCC CGCT and R-TGA GGT GCG CAC TGC CG (58°C, 1.5 mM $MgCl_2$, 5% DMSO); and exon 2, D-TTA CCA TGT ATG CCA TTT GTA and R-AGG GAA CAC AAC TGA ACT GGA (52°C, 1.5 mM $MgCl_2$, no DMSO). The "D" and "R" labels indicate primers in the coding and the noncoding direction, respectively. Exons were amplified in the presence of $\alpha[\text{-}^{32}P]$dCTP, as described elsewhere (Duclos et al. 1994). Two microliters of PCR products was diluted in 10 μl of 10 mM EDTA and 0.1% SDS, and 2 μl of the dilution was mixed in 8 μl 95% formamide, 20 mM EDTA, 0.05% xylene cyanol, and 0.05% bromophenol blue. DNA was denatured for 5 min at 80°C and was kept on ice for ⩾2 min. Four microliters of the mix was loaded onto 10% glycerol–6% polyacrylamide gels. The nondenaturing gels were run at 20 W for 8 h at room temperature or at 5 W overnight at 4°C. Gels were transferred to Whatman 3MM paper, were dried under vacuum, and were exposed overnight to X-AR Kodak film, with an intensifying screen.

Sequencing of Variant Bands

Variant bands were excised from dried SSCP gel, soaked overnight in 100 μl bidistilled water, 5 μl of which was reamplified by PCR. PCR fragments then were purified with the Geneclean kit (Bio101) and were precipitated by ethanol. Fragments were sequenced with a *Taq* cycle sequencing kit (Applied Biosystems), by use of fluorescent dideoxynucleotides and one of the PCR primers. Reaction products were run on an automated DNA sequencer (model 373A; Applied Biosystems). All sequences on both strands were determined.

Microsatellite Genotyping

Microsatellite genotyping with markers linked to the AVED locus has been described elsewhere (Doerflinger et al. 1995). Haplotypes were constructed on the basis of alleles from at least one parent or from two children and the patient's wife, and no recombination was detected (data not shown).

Table 1

5′ UTR and Flanking Intron Sequences of the First Two Exons of the α-TTP Gene

Exon	Sequence
Promoter and 5′ UTR	GAGGCTGCCAAGGAGGCATTATGGAATGCGTGTTTTAGAATGCATGCTGTAATC CAGTGCGAGGCGAGGGGTCATCAGTCTGGAATCTGATGGAAGGAGAAGGGA GAGGGCTCAGTAGCGCGTCGGCCACTGGCCATTAAAGGCTCGTTCTAAGGC TCCTTTCAGCTCCAAGATTGCAAGAAGGAAGGAAGAATGGAAGGAAGAGAG AGGGAGGAAGGAAAAAGAAAAAAAAAAAGATTCTTTAGTAGATCGTAAAAA TCATCCCAACTAACTTTGACATCGTTTCCTCGGTGTTTGAACATCAGTGGC TTCCCTCGCTGGGAAAAAGCTGGGGTCCCTAACTAAAGCTGCTAAACAGGT GGCTAGCACCCGTGGGGACACTGTGCGGTTTCCTTTAAGAGGCAGCTCTGC TCGCCGCGGGTCCGGCGTGCGAGGCCGGAGACCGCCCTGCCAGGCTGGAGA GTGGTTGTGCGACGGGCAGCGGCCGCAGAGGCGGGGCCGAGGGTGGCGCGG GGCGCGCGCTTTTCCCGGGTGCCCGGGTAGCTGCGGCCGCAGCAGCGG CGGCGGGCAtg 3
1 Donor	203 ggGTAAGCGTGCGTGCCCAGGGCGCTCGGCAGTGCGCACCTCACCCGC CCCGTCCCCCGCCCCACGACCCCGGATATCTG
2 Acceptor	TTA CCATGTATGCCATTTGTAGACAATTTTAATTTTATTTTTCAGtt 206
2 Donor	357 cgGTAAGTCATACACAACTCTTTTTTTTTTCTCCTCTCCTCCAGTTCA GTTGTGTTCCCT CCCCTCTCCTCTTCTCTCTTTCTTCCATTTCTTCCTTGA ATACCATATAGAAAGCTTCAATGCATTCATCTTTAGGAA

NOTE.—The first two or last two nucleotides of the exons are given in lowercase letters, and their position is indicated by a number preceding or following the cDNA sequence (Arita et al. 1995). Sequences used for design of the PCR primers are underlined. The 5′ UTR, exon 1, and flanking intron 1 sequences have been submitted to Genbank (accession AF031323; http://www.ncbi.nlm.nih.gov/Web/Genbank/), as well as have the exon 2 and flanking intron sequences (accession AF031324).

Statistical Analysis

Means were compared by use of the Kruskal/Wallis test (modified Student's *t*-test), and frequencies were compared by use of the χ^2 test. Means are given with SDs.

Results

In order to search for mutations in the 5′ portion of the coding sequence, we completed the determination of the genomic organization of the α-TTP gene. Two independent λ genomic clones were isolated with a 5′ cDNA probe (Arita et al. 1995), and each clone contained a different exon that was distinct from the three terminal exons reported elsewhere (Ouahchi et al. 1995). Sequencing of the exons indicated that the transcript is encoded entirely by the five exons. The 5′ UTR and the intron sequences flanking exons 1 and 2 are given in table 1. The exon-intron structure had been established independently by Hentati et al. (1996). Primers were designed to amplify the 5′ UTR and exons 1 and 2, from genomic DNA of the patients. Because of its length and high G+C content, exon 1 was amplified in two overlapping fragments.

Table 2

Novel Mutations in the α-TTP Gene of AVED Patients

Exon	Nucleotide Change	Effect on Coding Sequence	Corresponding Amino Acid in Rat α-TTP, h-CRALBP, and SEC14p[a]
1	175C→T	R59W	R, R, R
2	205-1G→C	Skipping of exon 2 and frameshift after R68	…
2	306A→G	No change on G102 (possible splice-site activation)	…
2	358G→A	A120T	S, E, N
3	400C→T	R134X, protein truncation	…
3	421G→A	E141K	E, E, Q
4	661C→T	R221W	R, R, K

[a] For the missense mutations only (h-CRALBP denotes human CRALBP; and SEC14p denotes yeast SEC14 protein).

Mutations were searched, by SSCP, in the 5′ UTR and in all five exons, followed by direct sequencing of the variant band. The mutations described elsewhere—744delA, 513insTT (initially described erroneously as 513insTC) (Ouahchi et al. 1995), and 486 delT (Hentati et al. 1996)—were found in one or more additional families. Seven novel mutations were found (table 2 and fig. 1). Two mutations, one nonsense (R134X) and one acceptor–splice-site mutation (205-1G→C), predict a major disruption of α-TTP synthesis. One family (21) with a 513insTT mutation on one chromosome presented a 306A→G change on the other chromosome. This mutation leaves invariant the glycine residue at position 102. However, it was never found in 80 unrelated normal chromosomes, and no other variation was detected in the coding sequence bearing the 306A→G var-

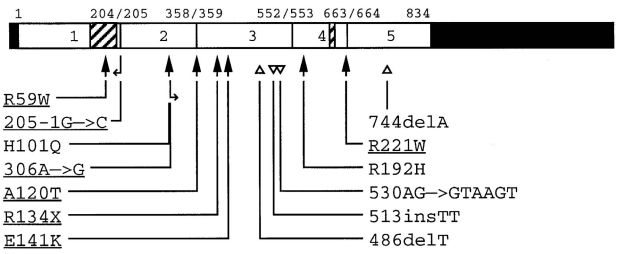

Figure 1 Distribution of α-TTP mutations. Unblackened boxes indicate coding sequences, and blackened boxes indicate 5′ and 3′ (partial) UTRs. Hatched areas indicate highly conserved domains in CRALBP and SEC14 (Arita et al. 1995). The nucleotide positions of the exon boundaries are indicated above the bar, and the positions of mutations are indicated below the bar. Novel mutations described in this study are underlined. The deletions and insertions are represented by upward-pointing and downward-pointing triangles, respectively; splice mutations are represented by a left-pointing (acceptor site) or a right-pointing (potential donor site) arrow; and nonsense and missense mutations are represented by upward-pointing arrows.

iation, suggesting that it might be disease causing, possibly by activation of a cryptic splice site (A/GTCCT→G/GTCCT). The remaining four mutations were missense mutations (table 2). Comparison of the mutated amino acids to the corresponding amino acids in the related *cis* retinaldehyde binding protein (CRALBP) and yeast SEC14 protein (Arita et al. 1995) revealed that three missense mutations were nonconservative substitutions (R59W, E141K, and R221W) that altered highly conserved amino acids (identical in CRALBP and identical or with a conservative change in SEC14) (table 2). In contrast, the A120T mutation is a semiconservative substitution of an amino acid that is not conserved in CRALBP, SEC14, or even rat α-TTP (Arita et al. 1995). We also confirmed the presence of the missense mutation R192H on the maternal chromosome of family 14 (Hentati et al. 1996). These four missense mutations and the nonsense mutation were changes of a CpG dinucleotide into TpG or CpA. Both the R192H mutation and the previously described H101Q missense mutation (Gotoda et al. 1995) are semiconservative substitutions of amino acids that are not conserved in CRALBP and SEC14.

Mutation in the α-TTP coding sequence was not found in four independent patients with isolated low vitamin E deficiency and Friedreich ataxia–like symptoms, even after direct sequencing of all exons and of the 5′ UTR. Analysis with closely linked markers (Doerflinger et al. 1995) showed that one of these patients (family 15), from a first-degree consanguineous marriage, was homozygous for eight contiguous markers encompassing the α-TTP locus (not shown), therefore suggesting linkage to the AVED locus (odds of 16:1 in favor of linkage). The three other patients were neither from consanguineous parents nor homozygous for the linked markers. It seems unlikely that they carry two distinct mutations not detected by SSCP of the coding and flanking intronic sequences, and, therefore, they may suffer from another vitamin E deficiency that may or may not be inherited. Haplotype analysis also was used to test whether identical mutations found in independent families have distinct or common origins. The haplotype linked with the 513insTT mutation in families 14 and 21 was identical over four contiguous markers, which is evidence in favor of a common ancestor. The haplotypes linked with the R134X mutation in two Canadian families (20 and 31) were different, suggesting recurrent, independent mutations, which is not unexpected for a change in a CpG dinucleotide.

In order to correlate the mutations with disease severity, the clinical features of the patients were compiled (table 3; features of the four patients with no identified mutation also are given, for comparison). The large amount of data collected from patients with documented mutations (total of 43 patients in 29 families), including the 6 patients reported by other groups, provided the opportunity to assess the diagnostic criteria for AVED and to explore phenotypic variability. Age at onset was within the range of 2–52 years, but onset at age >20 years was reported in only seven cases. In general, most patients fulfilled diagnostic criteria corresponding to Friedreich ataxia (Harding 1981; Dürr et al. 1996), including cerebellar ataxia, dysarthria, reduced or absent deep tendon reflexes, and vibratory-sense disturbances (table 4). However, cardiomyopathy, an important criterion for Friedreich ataxia, was found in only eight patients, with two cases documented by echocardiography and six cases by electrocardiography alone ($\chi^2 = 21$; $P < .0005$, for the difference between AVED and Friedeich ataxia). On the other hand, head titubation was found in 11 (28%) of 39 cases for which information was available, but it usually is not found in Friedreich ataxia patients ($\chi^2 = 37$; $P < .0005$). Four patients (13%) who did not have head titubation had dystonia. Four patients had marked retinopathy, and two had moderately reduced amplitude on an electroretinogram. Amplitude of median sensory-nerve conduction was normal in six patients, was reduced in five patients, and was undetectable in three patients (data not shown). No case, of the 16 for which information was available, had diabetes (data not shown). Age when wheelchair bound or age at last examination when still ambulatory also is given in table 3, even though persistence of ambulation is dependent largely on when vitamin E–supplementation therapy was initiated. In one case (family 18), ambulation was lost just prior to initiation of treatment and was recovered thereafter.

Clinical findings were compared between two groups of families—families in which the patients had two trun-

cating mutations (frameshift or nonsense) and families in which at least one mutation was a missense mutation or the silent 306A→G mutation (table 3). In the first group, which mostly included patients with the 744delA mutation, the mean age at onset of the disease was 9 ± 5 years, whereas in the second group the mean age at onset was 22 ± 16 years. In the second group, the mean age at onset for patients with nonconservative missense mutations (R59W, E141K, and R221W), which affected amino acids conserved during evolution, was 10 ± 3 years and was not different from the mean age at onset associated with truncating mutations. The three semiconservative mutations not conserved in related proteins (R192H, A120T, and H101Q) and the potential mutation 306A→G appeared to be associated with a milder presentation of the disease, with later onset (mean age 29 ± 15; $P < .01$, for the difference between the group with truncating mutations and that with semiconservative mutations) and/or no aggravation over 5 years. Patients in families 14 (R192H), 21 (306A→G), and 24 (A120T) had normal, increased, and partially decreased deep tendon reflexes, respectively, whereas all others, including the four patients with missense mutation H101Q, had abolished deep tendon reflexes. Interestingly, retinopathy was associated with the H101Q mutation (present in three of four patients), whereas it was very rarely associated with the other mutations.

Discussion

FIVE was first described byBurck et al.(1981), and, despite the small number of cases reported initially, phenotypic variability appeared to be very great, ranging from severe Friedreich ataxia–like presentation (AVED) (Stumpf et al. 1987; Ben Hamida et al. 1993*b*) to mild neurological impairment (Sokol et al. 1988) and very late onset of disease (Yokota et al. 1987). We and others have found that AVED is due to mutations in the α-TTP gene (Gotoda et al. 1995; Ouahchi et al. 1995; Hentati et al. 1996). In this article, we report the identification of seven novel mutations. In addition, mutation-identification and clinical records were obtained for 37 patients in 23 families. This represents the largest group of AVED patients collected, and the compilation of these data, plus data from six other patients with documented mutations reported elsewhere (Gotoda et al. 1995; Hentati et al. 1996; Yokota et al. 1996, 1997), allowed us to explore the clinical overlap and differences from Friedreich ataxia, with which AVED is often confused. Age at onset appeared to be very broad, being within the range of 2–52 years, but most patients had onset at age $\leqslant 20$ years. Several features are shared with Friedreich ataxia, including cerebellar ataxia, loss of deep tendon reflexes, vibratory-sense disturbances, dysarthria, muscle weakness, and Babinski sign. However, cardiomyopathy is significantly rarer in AVED than in Friedreich ataxia ($P < .0005$), whereas head titubation (28% of patients; $P < .0005$) and dystonia (13% of patients) appeared to be specific to AVED. The clinical differences between Friedreich ataxia and AVED may point to subtle differences in pathological pathways; yet, recent results indicate that Friedreich ataxia is also related to oxidative stress (Babcock et al. 1997; Foury and Cazzalini 1997; Koutnikova et al. 1997), against which vitamin E presumably has a major protective role.

The 744delA mutation is clearly the most frequent mutation, with a founder effect originating from North Africa (Ouahchi et al. 1995). However, one North African patient (family 31) was homozygous for another mutation, 486delT, which also was found in two families, one from Canada (western Ontario; family 20) and the other from the United States (Hentati et al. 1996), suggesting a very wide geographic distribution. Three other mutations were found in two or more independent families. Mutation 513insTT was found in four families—two Italian families (21 and 28) and two American families with European ancestry (Danish [family 14] and German) (Hentati et al. 1996). We also found the R134X mutation in two independent Canadian families, one from Québec (family 18) and the other from western Ontario (family 20). However, linked haplotypes suggested the occurrence of two independent CpG mutations resulting in the R134X mutation. Finally, the H101Q mutation was found in four independent Japanese families but appears to be restricted to Japan.

The large number of patients and mutations collected in this study permitted clinical/molecular correlation of AVED. For the few patients who were compound heterozygotes for a frameshift mutation and a missense mutation (including the 306A→G mutation, which has an uncertain pathogenicity), we assumed that clinical status was driven by the missense mutation, since the latter is likely to allow production of some partially functional protein. The severity of the disease clearly can be modulated by different, nongenetic factors including the amount of vitamin E in the daily diet and the time of initiation and dosage of vitamin E supplementation, once the biochemical diagnosis has been made. However, the phenotype associated with the three semiconservative missense mutations (R192H, A120T, and H101Q) and the potential 306A→G mutation appears to be milder than that seen in the majority of cases. The partial loss of function associated with mutations R192H and H101Q is corroborated by the results of previous studies, which used deuterated forms of α-tocopherol stereoisomers (RRR and SRR) (Traber et al. 1993). In the study of the function of the hepatic α-TTP in normal humans, a marked preference for the RRR stereoisomer over the SSR form of α-tocopherol was found. The ability to discriminate between the isomers also was dem-

Table 3

Correlation between Genotype (Grouped by Mutation) and Clinical Findings

Mutation Type and Family[a]	Geographic Origin	Consanguinity	Mutation	Vitamin E Level[b] (mg/liter)	Age at Onset (years)	Clinical Features[c]											
						WC (years)	P[d]	CA	D	TR[e]	VSD	WL	B	HT	CM	Dyst	R[f]
Truncating:																	
1	Tunisia	Yes	744delA/744delA	.5	8	<27	+	+	+	a	+		+	−	+		−
				.6	8	<25	+	+	+	a	+		+	−	−		−
				…	9	Died at 21	+	+	+	a	+		+	−			−
2	Tunisia	Yes	744delA/744delA	.9	18	<30	+	+	+	a	+		+	−	−		−
				.3	12	<28	+	+	+	a	+		+	−	−		−
				.5	15	<23	+	+	+	a	+		+	−	+		−
				.5	10	<30	+	+	+	a	+		+	−	+		−
				.6	6	28	+	+	+	a	+		+	−	+		−
5	Italy	Yes	744delA/744delA	2	13	>50	+	+	+	a	+		+	−	+	−	−
				1.5	12	>44	+	+	+	a	+	+/−	+	+	+	−	−
7	Tunisia	Yes	744delA/744delA	<1	3	16	+	+	−	a	+	−	−	−	−	+	−
8	Morocco	Yes	744delA/744delA	.6	7	13	+	+	+	a	−	−	−	−	−	+	+
				1.3	6	>7	na	+	+	a	−	−	−	−	−	−	
10	Italy	Yes	744delA/744delA	.8	13	>24	+	+	+	a	+	+	+	−	−	−	−
16	Italy		744delA/744delA	<1	19	>24	+	+	−	a	+	+	+	+	−		
19	Algeria	Yes	744delA/744delA	.5	3	>23	+	+	+	a	+	+	+	−	−	−	−
				.9	12	>13	na	+/−	−	a	+	−	+	−	−	−	−
23	Tunisia	Yes	744delA/744delA	<1	4	>15	+	+	+	a	+/−	+	+	−	−	−	−
29	Italy	No	744delA/744delA	.25	12	15	+	+	+	a	+	+	+	−	−	−	−
30	Morocco	Yes	744delA/744delA	.5	15	>23	+	+	−	a	+	−	−	−	−	−	−
12	France	No	744delA/205-1G→C	1.2	2	13	+	+	+	a	+	−	−	−	−	−	+/−
13	Germany	Yes	530AG→6/530AG→6	<1	3	>27	+	+/−	+/−	a	+	−	−	−	−		
18	Canada	Yes	R134X/R134X	<1	5	11	+	+		a	+	+	−	−	−	−	−
20	Canada	No	R134X/486delT	<2	6	17	+	+	+	a	+	+/−	+	+	−	−	−
31	Morocco	Yes	486delT/486delT	<1	8	>9	na	+	+	a	−	−	−	−	+/−	−	−
Hentati	United States		486delT/486delT	<1.1	7	17	+	+	+	a	+	−	+		−	+	−
Hentati	United States (Germany)	No	513insTT/513insTT	1.0	3	20	+	+	+	a	+	+	+	+	−	−	−
28	Italy	No	513insTT/513insTT	<2.1	5	21	+	+	+	a	+	+	+	+	−	−	−

Missense:																	
6	Albania	Yes	R221W/R221W	…	10		+	+			−		+		−		
				…	14		+	+			−		+		+		
				…	12		+	+			−		+		−		
11	France	Yes	E141K/E141K	1.3	8	>28	+	+	+/−	a	+	+/−	−	+	−	−	−
26	Portugal	Yes	R59W/R59W	.3	7	>17	+	+	+	a	+	−	−	+	−	−	−
14	United States	No	R192H/513insTT	1.8	6	>35	+	+	+	n	+	−	−	+	−	−	
	(Denmark and			1.8	27	>39	−	−	−	n	+	−	−	−	−	−	
	England)			1.2	>21	>33	na	+/−	−	n	+	−	−	−	−	−	
21	Italy	No	306A→G?/513insTT	1.2	…	>31	−	+	+/−	i	+	−	+	+/−	−	−	−
				3.3	16	>28	+	+	−	i	+	−	+	+	−	−	−
24	Belgium	Yes	A120T/A120T	1	21	>24	+	+	−	d	+	−	−	−	−	−	+/−
Gotoda	Japan	No	H101Q/H101Q	1.2	52	>72	+	+	+	a	+	−	−	−	−	−	+
Yokota	Japan	Yes	H101Q/H101Q	1.5	38	>47	+	+	+	a	+	−	−	−	−	−	+
Yokota	Japan	No	H101Q/H101Q	1.0	30	>55	+	+	−	a	+	−	−	−	−	+	+
Yokota	Japan	No	H101Q/H101Q	2.3	52	>59	+	+	+	a	+	−	−	+	−	−	−
Undetected:																	
15	Puerto Rico	Yes	?/?	<1	13	21	+	+	+	a	+	+	+	−	−		−
22	France	No	?/?	.5	38	>53	+	+	+	a	+	+	+	+	−		
25	Italy	No	?/?	<1	62	>65	+	+	+	d	+	+	−	+/−	−	−	−
27	Italy	Yes	?/?	2.2	11	>16	−	+	+	n	+	+	−	+	−	−	−

[a]"Hentati" indicates families from the study by Hentati et al. (1996); "Gotoda" indicates those from the study by Gotoda et al. (1995); and "Yokota" indicates those from the studies by Yokota et al. (1996, 1997).

[b]Normal level 8.7 ± 3.7 μg/ml.

[c]WC = age when first in wheelchair; P = progressive worsening over 5 years; CA = cerebellar ataxia; D = dysarthria; TR = tendon reflexes in the lower limbs (knees and ankles); VSD = vibration-sense disturbances; WL = weakness in legs; B = Babinsky sign; HT = head titubation; CM = cardiomyopathy; Dyst = dystonia; and R = retinopathy. A plus sign (+) indicates the presence of a symptom; a minus sign (−) indicates the absence of a symptom.

[d]na = not applicable.

[e]n = normal; i = increased; d = decreased; and a = abolished.

[f]A plus sign (+) indicates presence of retinitis pigmentosa; a plus and a minus sign (+/−) indicates moderately reduced amplitudes on electroretinogram.

Table 4

Compared Frequency of Clinical Signs between Friedreich Ataxia and AVED Patients

	Frequency among (%)		
	Friedreich Ataxia Patients		AVED Patients from This Study (n = 43)[a]
Clinical Sign	From Dürr et al. 1996 (n = 140)[a]	From Harding 1981 (n = 115)[a]	
Gait and limb ataxia	99	99	98
Dysarthria	91	97	77 (n = 39)
Lower-limb areflexia	87	99	85 (n = 40)
Loss of vibratory sense	78	73	86
Extensor plantar reflexes	79	89	58
Muscle weakness in lower limb	67	88	35 (n = 31)
Head titubation	0[b]	...	28 (n = 39)
Cardiomyopathy	63 (n = 75)	...	19 (n = 42)
Diabetes or impaired glucose tolerance	32 (n = 61)	10	0 (n = 16)

[a] Unless otherwise noted in parentheses after the corresponding frequency.
[b] Data from A. Dürr (personal communication).

onstrated in perfused monkey livers in vitro. Patients with R192H or H101Q mutations were still able to preferentially incorporate the natural RRR stereoisomer into VLDL—to a lesser extent than normal subjects—and were labeled "discriminators" (table 5).

These patients contrasted with other patients who had a complete loss of the capacity to preferentially incorporate the natural α-tocopherol stereoisomer into VLDL (labeled "nondiscriminators"). In four of these patients, the mutations have been characterized, and they are homozygous for severe truncating mutations (530AG→GTAAGT, 744delA, 486delT, and R134X) (table 5). Interestingly, they all are associated with a severe, early-onset form of the disease. All other truncating mutations and the nonconservative missense mutations (R59W, E141K, and R221W) also seemed to be associated with the severe form of the disease, suggesting that they also result in complete loss of function, although the patients were not studied for their ability to discriminate between RRR and SRR isomers of α-tocopherol. This points to an important role for amino acids R59, E141, and R221, which are invariant or conserved in the related CRALB and SEC14 proteins (Arita et al. 1995). All cases with cardiomyopathy were associated with frameshift or R221W (one case) mutations.

Of particular interest was the fact that retinitis was associated more highly with the H101Q mutation (Yokota et al. 1996) than with other mutations. The H101Q mutation is also associated with a mild phenotype and very late onset. It is conceivable that, in the case of the H101Q mutation, the presence of retinitis is associated with older age of the patient (onset of visual symptoms occurred at age >42 years [Yokota et al. 1987, 1996]) and longer disease duration. One patient homozygous for the 744delA mutation (the first child of family 8) showed early onset of visual impairment and retinal pigments (Amiel et al. 1995). At least two other families with isolated vitamin E deficiency and early onset of symptoms had abnormal fundoscopy, described as yellowish-white spots of the peripheral retina (Rayner et al. 1993; Shorer et al. 1996). Mutations of the α-TTP gene in these two families have not been reported yet.

Our experience has shown that, in AVED patients, there is no limitation to or difficulty with the absorption of vitamin E by the intestinal tract (Kayden and Traber 1993). The administration of vitamin E supplements in divided doses daily has resulted in cessation of progression of the neurological symptoms and signs and in amelioration of established neurological abnormalities, in a number of patients (Kohlschütter et al. 1988; Yokota et al. 1997). Our experience is that, for adults, the administration of 800 mg RRR α-tocopherol twice daily, with meals that contain fat, results in plasma α-tocopherol

Table 5

Correlation between Genotype and Discrimination among Stereoisomers of α-Tocopherol

Family[a]	Patient[b]	Mutation	Vitamin E Level (mg/liter)	Age at Onset (years)	Discrimination Status[c]
Hentati	4	513insTT/513insTT	1.0	3	Nondiscriminator
18	8	R134X/R134X	<1	5	Nondiscriminator
13	6	530AG→6/530AG→6	<1	3	Nondiscriminator
16	9	744delA/744delA	<1	19	Nondiscriminator
14	1	R192H/513insTT	1.8	6	Discriminator
14	2	R192H/513insTT	1.8	27	Discriminator
Gotoda	7	H101Q/H101Q	1.2	52	Discriminator

[a] "Hentati" indicates a family from the study by Hentati et al. (1996); "Gotoda" indicates a family from the study by Gotoda et al. (1995);

[b] Numbering is according to Traber et al. (1993). The discrimination status of patient 9 is described in the "note added in proof" in the study byTraber et al. (1993).

[c] RRR and SRR α-tocopherol stereoisomer discrimination, from Traber et al. (1993).

levels that are at or above the normal range. This dose is far below our recommended dose for patients with abetalipoproteinemia, which is 150 mg RRR α-tocopherol/kg body weight daily, since in abetalipoproteinemia the malabsorption and lipoprotein abnormalities make transfer into the CNS extremely difficult.

Despite the differences between the clinical presentation of AVED patients and that of Friedreich ataxia patients, 19 (of 38) AVED patients initially were diagnosed as having Friedreich ataxia, several years before serum vitamin E measurement was undertaken, resulting in late initiation of vitamin E supplementation. The significant number of new cases reported in this study indicates that AVED, originally thought to represent only a very small proportion of all recessive ataxias, is not so rare, stressing again the importance of not missing the diagnosis of this treatable condition, in order to institute therapy promptly.

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