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Ataxia with Vitamin E Deficiency: Refinement of Genetic Localization and Analysis of Linkage Disequilibrium by Using New Markers in 14 Families

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Summary

Ataxia with vitamin E deficiency (AVED) is an autosomal recessive disease characterized clinically by neurological symptoms with often striking resemblance to those of Friedreich ataxia. This disorder has been reported previously as familial isolated vitamin E deficiency. We have mapped recently the AVED locus to a 5-cM confidence interval on chromosome 8q by homozygosity mapping in six Mediterranean families. We have now analyzed six new and two previously described families and demonstrate genetic homogeneity despite important clinical variability and wide geographic origins. Analysis of nine new tightly linked microsatellite markers, including four characterized in this study, revealed a predominant but not unique mutation in northern African populations, where this condition is more frequent. Haplotype analysis but also classical recombinations allowed us to refine the AVED position to a 1-cM interval. A YAC contig over this interval was constructed from marker STSs and YAC fingerprint data, in order to facilitate the search of the AVED gene.

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Introduction

We have reported recently that autosomal recessive vitamin E deficiency (AVED) is associated with a Friedreich ataxia–like phenotype in six Mediterranean families (four of them from Tunisia), and we have used homozygosity mapping (Lander and Botstein 1987) in these consanguineous families to map precisely the AVED locus to a 5-cM region in chromosome 8q (Ben Hamida et al. 1993*a*, 1993*b*). It was previously known that secondary vitamin E deficiency (in abetalipoproteinemia and cholestatic liver disease) can be associated with neurological symptoms including ataxia (Muller et al. 1983). Furthermore, 11 cases of isolated vitamin E deficiency had been reported, from 1981 to 1989, who presented neurological symptoms of varying severity, with often striking resemblance to those of Friedreich ataxia (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). These cases were sporadic, with the exception of a sibship of three suggesting autosomal recessive inheritance and where vitamin E deficiency was associated with a rather mild phenotype (Sokol et al. 1988). We have now reanalyzed this family and the first reported sporadic case of isolated vitamin E deficiency, and, by taking homozygosity by descent into account, we demonstrate that these are also linked to the AVED locus on chromosome 8q, extending the range of both clinical phenotype and geographic origin of patients with AVED. Six new families have been identified that confirm that the disease is particularly frequent in populations of northern African origin. Four new microsatellite markers that have been isolated from the YAC contig that we described elsewhere (Ben Hamida et al. 1993*b*)

and five new markers from the Généthon map (Gyapay et al. 1994) allowed us to significantly refine the localization of AVED, on the basis of both analysis of recombination events and linkage-disequilibrium analysis. Our results indicate the presence of a predominant but not unique mutation in northern African populations and suggest that phenotypic variability is related to mutation heterogeneity.

Subjects, Material, and Methods

Subjects

Fourteen families with vitamin E deficiency were available for study. Families 1–6 have been described elsewhere (Ben Hamida et al. 1993*a*, 1993*b*), as have families 8 (Amiel et al., in press), 13 (Burck et al. 1981; Kohlschütter et al. 1988), and 14 (Sokol et al. 1988). The index case in all families presented with neurological symptoms including cerebellar ataxia and severe isolated vitamin E deficiency. All sibs were tested for serum vitamin E, since some individuals were too young to present neurological symptoms. In particular, a sixth child in family 4 was included in the study because she had a serum vitamin E level of 0.47 mg/liter (normal 9 ± 4 mg/liter) and no clinical symptoms at age 8 years. Detailed clinical description of patients in families 9–12 will be published elsewhere.

Isolation and Sequencing of the Microsatellite Repeats

YACs 1 (751h7) and 16 (602h8) (Ben Hamida et al. 1993*b*) were digested by *Eco*RI or *Xba*I and were subcloned in λZAPII (Stratagene). Subclones were screened in duplicate, with a $(CA)_n$:$(TG)_n$ repeat probe (Pharmacia LKB Biotechnology) labeled by nick-translation and with a total human genome probe labeled by random priming. Positive clones were isolated by secondary screening, and filters were subsequently rehybridized with D8S260 and D8S510 PCR fragments labeled by random priming. Clones positive for $(CA)_n$ probe and negative for D8S260 and D8S510 were excised from λZAPII into plasmids, according to the manufacturer's (Stratagene) instructions, and were further subcloned as *Sau*3A fragments in the *Bam*HI site of pBluescript (Stratagene). Colonies were gridded and screened with a $(CA)_n$ probe. Positive clones were sequenced with a *Taq* cycle-sequencing kit (Applied Biosystems) using fluorescent dideoxynucleotides and vector primers. Reaction products were run on an automated DNA sequencer (model 373A; Applied Biosystems).

Microsatellite Analysis

Primer sequences of markers D8S1177, D8S1178, and D8S1225–D8S1228 are given in table 1. Other markers used in this study have been published previously (Ben Hamida et al. 1993*b*; Gyapay et al. 1994). D8S512, D8S553, and D8S533 segments correspond to markers AFM206xh4, -326te5, and -088xb3, respectively (Gyapay et al. 1994). Microsatellite polymorphisms were typed by PCR amplification of 200 ng of genomic DNA in a 25-μl volume, as described by Sirugo et al. (1992), with one primer end-labeled with ^{32}P. Allele frequencies were determined from the normal chromosomes of parents. Haplotype frequencies were calculated by multiplying allele frequencies, with the exception of marker D8S510, which showed moderate linkage disequilibrium with D8S1228 and was not used in the calculations.

Microsatellite Mapping on YACs

YACs isolated with probe D8S260 (Ben Hamida et al. 1993*b*), including three (356g9, 252d8, and 335c12) that were further characterized, were used to map the microsatellite sequences. The PCR fragments were labeled by random priming, hybridized with total human genomic DNA (100 μg/ml) in 400 μl of 6 × SSC at 65°C for 90 min, in order to block the $(CA)_n$:$(TG)_n$ sequences, and hybridized to pulse-field blots of *Mlu*I-, *Not*I-, and *Bss*HII-digested YAC clones (Ben Hamida et al. 1993*b*).

Results

Isolation of New Microsatellite Markers

We have reported elsewhere (Ben Hamida et al. 1993*b*) the construction of a ≥800-kb YAC contig that encompasses D8S260 and D8S510, two of the three markers that showed complete cosegregation with AVED in the six families studied (including complete homozygosity in all affected patients). In order to refine the genetic analysis, we set out to isolate new highly polymorphic markers from the region. Four new $(CA)_n$:$(TG)_n$ repeats—ND6, ND9, ND10, and ND12—were isolated and sequenced. All four clones contained repeats with ≥15 uninterrupted AC repeats that were found to be polymorphic (table 1). They were mapped by hybridization to pulse-field blots of the various YACs digested with rare-cutter restriction enzymes, the result of which is shown in figure 1*b*. Five other microsatellites tightly linked to this region were obtained from Généthon (Gyapay et al. 1994; G. Gyapay and J. Weissenbach, unpublished results). The genetic map of the region is shown in figure 1*a*. The Généthon microsatellites were used to screen the MegaYAC CEPH library. The positive YACs were assembled into contigs, by means of information from the first-generation contig map in particular (Cohen et al. 1993). A single contig was obtained from D8S1177–D8S512 with level 1 tiling paths and a single level 3 tiling path between D8S510 and D8S1178 (table 2). We thus dispose now of 12 highly polymorphic markers over a 3-cM region, including 6 markers in a cloned 500-kb region. Such a density

Table 1

Sequence of Primers Used for PCR Amplification

D8 Segment	Marker	Length of Amplified DNA (bp)	No. of Alleles	Repeat Sequence	PCR Primers[a]	Annealing Temperature (°C)
1225	ND6	150	8	$(AC)_{15}$	5′ TCTGACTACACCCCTTACAT 3′ 5′ CTCCTTATGTAAATTATTGAGAAG 3′	58
1226	ND9	104	9	$(CA)_{22}$	5′ GTGTGAATGACATCAGCCTA 3′ 5′ AGTCTTTCACTTCTTGCTTG 3′	49
1227	ND10	174	9	$(CA)_{23}$	5′ ACTGACAAAGGATTTCCTGG 3′ 5′ AATCCCACTTCTGAGTAGCT 3′	54
1228	ND12	176	5	$(CA)_{16}TA(CA)_8$	5′ ACTATTCTTTCCCTAAAACA 3′ 5′ TTGGATGACTTGATGTTCCC 3′	48
1117	a152yc2	136	6	$(CA)_n$	5′ TGAAATCATGTGACCCC 3′ 5′ AAAAAGTAAACATTTAGATTGCC 3′	53
1178	184xb10	275	5	$(CA)_n$	5′ AGCTACTTGGNAGGCTGA 3′ 5′ AACATTGGGAATGGGTTATT 3′	53

[a] CA strand primer is listed as the first.

favors both precise mapping of recombination events and efficient linkage-disequilibrium analysis.

Analysis of New Families: Confirmation of Genetic Homogeneity

Six new families with ataxia and vitamin E deficiency were identified (families 7–12; fig. 2). Five families were consanguineous, four of which originated from the Mediterranean basin. All results were consistent with linkage to the AVED locus. The most important contribution to linkage confirmation came from the observation that patients from consanguineous families were homozygous for all markers tested. There is 1 in 16 chance that the affected child from a first-cousin marriage is homozygous in any region of the genome, and thus the equivalent lod score (Z) is 1.2. Family 8 is large enough, with several affected children, to yield a significant additional Z value of 2.05. The evidence is lower in family 11, where homozygosity by chance is 1 in 4 (brother-sister mating), and the equivalent Z value is only 0.6. The nonconsanguineous family (family 12) had four children, including a single affected who was heterozygous for five tightly linked markers, of eight markers tested. The Z value in favor of linkage is thus only 0.38. The two younger healthy sibs had vitamin E levels suggestive of heterozygote carrier status (3.2 and 4.5 mg/liter, respectively; normal, 9 ± 4 mg/liter), in complete agreement with haplotype segregation (not shown), giving additional support for linkage.

We also analyzed two families previously described as having the isolated vitamin E deficiency syndrome. The single proband in family 13 is in fact the first described case of the disease (Burck et al. 1981). The parents originate from the North Frisian Islands in the North Sea and are not aware of recent consanguinity. The patient is, however, homozygous for all 10 markers tested, an event with a probability of $\sim 10^{-5}$ (this is approximate, since we could not test the allele and haplotype frequencies in the normal population of this island). Distant consanguinity is therefore much more likely than homozygosity by chance. Since second-degree consanguinity can be ruled out, the equivalent Z value is >1.8. The last family (family 14), described originally by Sokol et al. (1988), is characterized by a mild phenotype in the three patients. They are all identical for the chromosome 8 markers tested, and the healthy sister is predicted to be a heterozygote carrier (total Z value of 1.33 at $\theta = 0$), but the patients are heterozygous for two completely different haplotypes, unlike 12 of the 13 other families studied. This suggests that the patients are compound heterozygotes for two different mutations at the AVED locus, as in family 12.

Analysis of Recombination and Linkage Disequilibrium

The previously studied families showed six recombinations between AVED and the proximal markers D8S285 and D8S166, including four recombinant haplotypes based on consanguinity (Ben Hamida et al. 1993*b*). The analysis with the new microsatellites allowed precise mapping of the divergence point of two recombinant haplotypes between markers ND6 and D8S260, which are <40 kb apart (families 3 and 4 in table 3). This defines ND6 as the new proximal marker, within the region cloned in YAC 16 (602h8). Of the

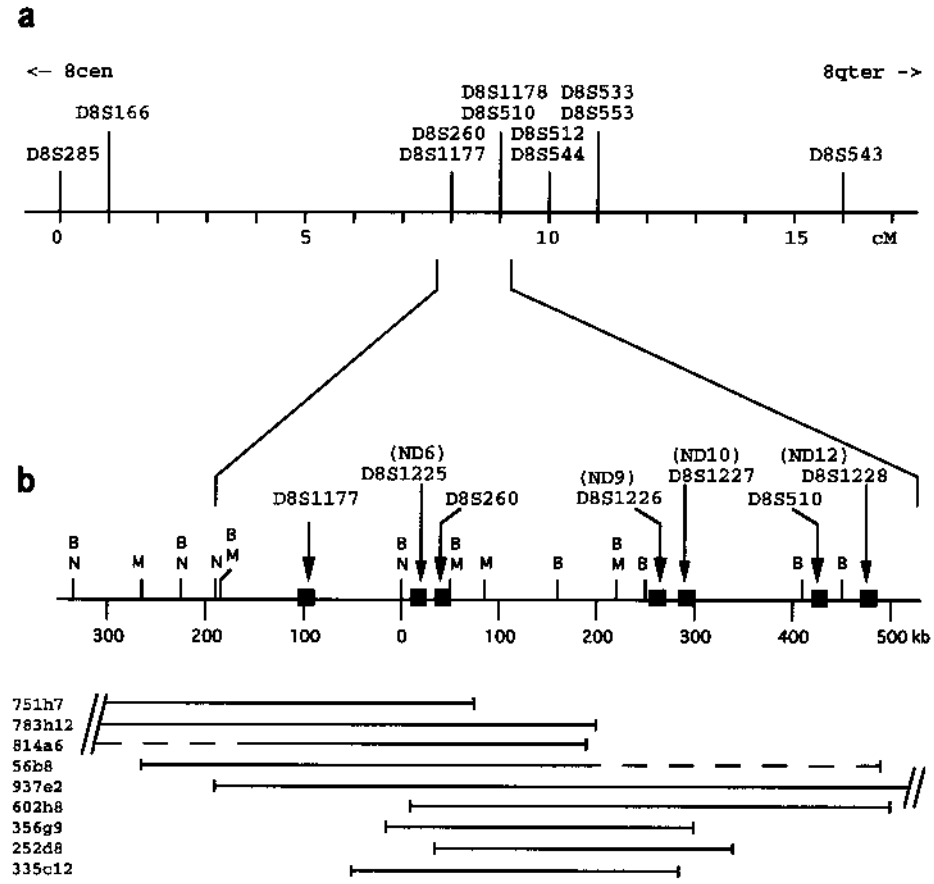

Figure 1 Genetic and physical map around loci D8S260 and D8S510. *a*, 16-cM genetic map of the markers used in this study (according to Gyapay et al. [1994] and authors' unpublished results). *b*, Rare-cutter restriction map around loci D9S260 and D9S510, showing the positions of the new markers ND6, ND9, ND10, and ND12 and of D8S1177. N = *Not*I; B = *Bss*HII; and M = *Mlu*I. The scale is in kilobases. YACs used for pulse-field mapping are shown at the bottom. Dashed lines indicate portions of YACs that are not on chromosome 8 (Ben Hamida et al. 1993*b*).

five recombinations previously identified with the distal locus D8S543 (Ben Hamida et al. 1993*b*), a single one was still recombinant with marker D8S553 (family 1 in table 3), markers D8S1178, D8S544, D8S512, and D8S533 being not informative in the critical parent. More important, a recombination was seen in the third patient of family 14 with marker D8S544 (D8S512 was not informative in the critical parent, but D8S1178 was). Taken together, these results define a 2-cM candidate region for AVED, between ND6 and D8S544.

Analysis of extended haplotypes provided support for the demonstrated candidate interval for AVED. A common haplotype extending over eight consecutive markers was found in five families: two of the five Tunisian families, the two Moroccan families, and the Sicilian family (table 3). Allele 1 of marker ND10 was found

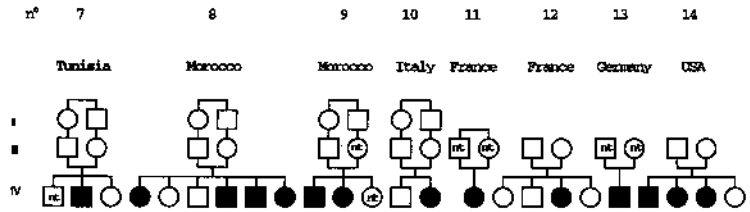

Figure 2 Pedigrees of the six new families with AVED and of the two families described elsewhere (one each by Burck et al. [1981] and Sokol et al. [1988]). Generation-III and -IV individuals were genotyped, with the exception of the individuals labeled "nt." Family number and geographic origin are given at the top.

Table 2

YAC Contig Map from D8S1177 to D8S512

	8q STS and Fingerprint[a]							
YAC	D8S1177	D8S260	D8S510	FP1	FP2	D8S1178	D8S544	D8S512
751h7	nt	+						
783h12	+	+						
793b8	nt	+						
814a6	nt	+						
956b6	nt	+						
937b12	nt	+						
937e2	+	+	+					
602h8		+	+	nt	nt		nt	nt
817b1	nt		+					
908h6	nt		+					
973e6			+					
918d7	nt		+	+				
925g7			+	+				
955d11	nt		+	+				
955e1	nt		+	+				
752c9	nt			+	+			
964b7	nt			+	+			
942e7	nt				+			
947c6	nt				+			
950d11	nt				+	+		
904a10	nt				+	+		
921g9	nt					+		
962g12	nt					+	+	
810e10	nt						+	
825b8	nt						+	
958d8	nt						+	
953c6	nt						+	+
754b4	nt							+
911c9	nt							+
938e8	nt							+
952e6	nt							+

[a] FP1 and FP2 represent groups of YACs sharing a similar fingerprint pattern (Bellane-Chantelot et al. 1992; Cohen et al. 1993). With the exception of YAC 602h8, YACs that were not analyzed by fingerprint are not included in this table.

nt = not tested; and a blank space indicates a negative result.

Note that D8S1178 is closer to D8S544 than to D8S510 on the YAC contig map, unlike the situation on the genetic map (fig. 1).

exclusively on the affected chromosomes of the five families, including the second Moroccan family where only five of the eight markers were analyzed, and was never found on normal chromosomes. Given the high informativeness (table 4) and the independent assortment, on normal chromosomes (not shown), of six of these markers, the probability of finding this haplotype by chance is $<10^{-4}$ (3×10^{-4} and 4×10^{-4} for haplotypes in families 8 and 9, respectively). This strongly suggests that all these families share a common mutation. The divergence of this haplotype at D8S1178 in the Moroccan family 8 would place AVED proximal to this marker. It is, however, not possible to exclude the possibility that this divergence is due to a microsatellite mutation at D8S1178. The common haplotype also shows divergence at D8S544 in family 4 and, between family 1 and 5, at D8S512. It is unlikely that the three haplotype divergences are all due to microsatellite mutation, since the three markers show moderate variability (table 4) and are therefore relatively stable.

The Tunisian family 2 showed a disease-associated haplotype reminiscent of the "North African" founder haplotype. Highest match was obtained with the Sicilian family 5, over five consecutive loci, from D8S510 to D8S512. However, the significance of this similarity is rather poor, because of the high frequency of the alleles (table 4) and the multiple comparison tests performed with the other "North African" core haplotypes. More interesting is the similarity between the French family (family 11) haplotype and the maternal haplotype of the

Table 3

Haplotype and Recombination Analysis in 14 AVED Families

Family	Country	Cousinship[a]	$Z(\theta = 0)$[b]	8q Marker[c]														
				D8S285	D8S166	D8S1177	D8S1225 ND6	D8S260	D8S1226 ND9	D8S1227 ND10	D8S510	D8S1228 ND12	D8S1178	D8S544	D8S512	D8S553	D8S533	D8S543
13	Germany	N	>1.8			9	12	6	5	3	4	4	5	2	4			
						9	12	6	5	3	4	4	5	2	4			
14	United States	N	1.33			2	11	4	9	3	5	3	2	4	2			
						9	8	6	7	3	3	4	4	4/1	1			
11	France	0	.6			9	1	7	7	3	3	4	4	4	2			
						9	1	7	7	3	3	4	4	4	2			
12	France	N	>.37				8	8	7	11	2	3	2	3	2			
							8	8	8	4	5	6	2	4	2			
10	Italy	1	1.32				10	4	4	2	1	6	2	2	2			
							10	4	4	2	1	6	2	2	2			
7	Tunisia	1	1.32			3	12	5	1	8	6	3	2	2	2			
						3	12	5	1	8	6	3	2	2	2			
6	Albania	1.5	2.69	2	5	9	4	8	4	3	5	2	4	2	2	6	5	7
				2	5	9	4	8	4	3	5	2	4	2	2	6	5	7
3	Tunisia	1.5	5.7	4	6	9	12	5	7	10	6	1	2	4	2	4	4	5
				3	4	9	2	5	7	10	6	1	2	4	2	4	4	5/1
		2		3	4	9	2	5	7	10	6	1	2	4	2	4	4	5
2	Tunisia	1	4.46	3/4	4/9	3	1	5	1	8	3	4	2	2	2	7	3	12
				3	4	3	1	5	1	8	3	4	2	2	2	7	3	12/1
5	Italy (Sicily)	2	2.36	2	6	9	12	8	4	1	3	4	2	2	2	1	4	12
				3	5	9	12	8	4	1	3	4	2	2	2	1	4	7
1	Tunisia	2	2.73	3	7	9	12	8	4	1	3	4	2	2	1	17/9	4	12/4
				9	5	9	12	8	4	1	3	4	2	2	1	17	4	12
4	Tunisia	N	4.33	4	9	9	12	8	4	1	3	4	2	4	2	1	5	2
		1		2	11	3	2	8	4	1	3	4	2	4	2	4	6	7
				9	3	9	12	8	4	1	3	4	2	4	2	4	6	7
9	Morocco	1	1.8					8		1	3	4	2	4	2			
								8		1	3	4	2	4	2			
8	Morocco	1	3.25	9	6	9	12	8	4	1	3	4	4	2	1	9	4	1
				9	6	9	12	8	4	1	3	4	4	2	1	9	4	1

[a] The degree of cousinship is given for each consanguinity loop; N = no known consanguinity; 0 = brother-sister mating.

[b] The frequency of the disease-linked haplotype was assumed to be 10^{-4}.

[c] Two alleles separated by a slash indicate phase-known recombinations, with the second allele being recombinant. Recombinations of marker D8S544 in family 11 and of marker D8S533 in family 1 are of particular interest for AVED mapping. Parental haplotypes associated with the disease are indicated by lines: haplotypes that recombined with a known consanguinity loop are boxed by a dashed line, and haplotypes common to unrelated families are boxed by a solid line.

Table 4

Allele Frequencies for 8q Markers on Normal Chromosomes

	Allele Number[a]/ Frequency									
	D8S1177	D8S1225 (ND6)	D8S260	D8S1226 (ND9)	D8S1227 (ND10)	D8S510	D8S1228 (ND12)	D8S1178	D8S544	D8S512
	1/ .16	0/ .11	1/ .03	2/ .11	2/ .10	1/ .07	1/ .14	1/ .13	1/ .10	1/ .43
	2/ .05	2/ .21	3/ .03	3/ .14	3/ .52	2/ .11	2/ .18	2/ .33	2/ .52	2/ .43
	4/ .05	8/ .11	4/ .07	4/ .36	4/ .07	3/ .43	3/ .25	3/ .42	3/ .03	3/ .09
	5/ .05	10/ .11	5/ .21	5/ .11	5/ .07	5/ .28	4/ .36	4/ .08	4/ .31	4/ .04
	8/ .05	12/ .46	6/ .17	6/ .11	7/ .07	6/ .11	6/ .07	5/ .04	5/ .03	
	9/ .63		7/ .17	7/ .07	8/ .10					
			8/ .28	8/ .07	11/ .07					
			9/ .03	9/ .04						
Total no.[b]	19	28	29	28	29	28	28	24	29	23
Heterozygosity:										
Present study	.57	.71	.81	.80	.69	.71	.75	.69	.62	.62
CEPH pedigrees[c]	.56		.81			.67		.74	.65	.61

[a] Increases as number of CA repeats decreases. Some alleles were only found on AVED chromosomes (fig. 2).
[b] Total no. of independent chromosomes analyzed.
[c] From Gyapay et al. (1994).

American family. The two haplotypes are identical over six consecutive loci, and the common segment has an estimated frequency of ~0.02%. The American mother has ancestry of Welsh and English origin, but no French ancestry was known. The common haplotype segment suggests that the AVED locus is between D8S260 and D8S512, confirming the recombination-based candidate region (table 3).

Discussion

We have characterized four new highly informative microsatellite markers in a 500-kb region around D8S260 and D8S510. These were used, together with markers from the Généthon map, to analyze linkage, homozygosity, and linkage disequilibrium in a total of 14 families: the 6 families that we had studied previously, 6 newly identified families, and 2 of the first described families with the isolated vitamin E deficiency syndrome. All families showed segregation consistent with the chromosome 8q localization. In 12 families, support for this localization is based on the homozygosity for at least eight consecutive microsatellites, in addition to classical linkage information for the largest families. The Z values in favor of linkage at $\theta = 0$ for the two nonhomozygous families are 1.33 and >0.38. This indicates that a single locus is implicated in families originating from very different geographic locations: the Mediterranean basin, an island in the Baltic Sea, and the United States. The AVED locus was initially localized by homozygosity mapping in six families from the Mediterranean basin, with proved consanguinity (subsequently demonstrated for family 6; V. Mokini, personal communication). Homozygosity was also found in six of the eight other families. Interestingly, one of the two families that show heterozygosity in patients, for totally different haplotypes, had been described elsewhere as having a mild phenotype (a 27-year-old sib even denied having neurological symptoms) (Sokol et al. 1988). Detailed biochemical analysis of these patients showed a preserved α-tocopherol stereoisomer discrimination capacity, by the liver, into secreted VLDL, that was not found in three other cases studied, including the patient in family 13 (Traber et al. 1993). This discrimination is putatively a function of the hepatic α-tocopherol transfer protein (αTTP). These data suggest that at least one of the two mutations at the AVED locus that segregate in this family is a functionally mild one.

Analysis of recombination events allowed us to define ND6 and D8S510 as new proximal and distal markers, respectively, within a region cloned in YACs. The finding of a common extended haplotype of seven consecutive markers—in five families originating from Tunisia, Morocco, and Sicily—that diverge beyond D8S1178 strongly suggests that a single mutation accounts for a substantial proportion of cases of northern African origin (Sicily was conquered, in the 9th century, by Muslims originating from Tunis) and that D8S1178 might be the closest distal marker. This allows a substantial refinement of the candidate region for AVED, from the initial 5-cM confidence interval to the 1-cM interval between ND6 and D8S1178, and illustrates the power of a very dense set of microsatellites for analysis of founder effects (Sirugo et al. 1992; Hästbacka et al. 1993; Rodius et al. 1994). Haplotype analysis suggests that at least two less frequent mutations occur in Tunisia and that seven other mutations occur elsewhere. The

haplotype shared between families 11 and 14 indicates a localization distal to D8S260. One Tunisian family shares a small common subhaplotype (D8S510–D8S1178) with the major "northern African" haplotype. This may not be significant, since this subhaplotype was also found on normal chromosomes. However, a common origin cannot be excluded, and a definitive conclusion would require the isolation of new markers for this region. This would be of some importance, since support for a common origin would suggest a localization in the small region where the haplotypes are identical.

This study reinforces our conclusion (Ben Hamida et al. 1993*a*, 1993*b*) that isolated vitamin E deficiency is an important cause of the Friedreich ataxia–like phenotype in northern African populations but that it appears to be very rare in France. Of four families detected in France, two are of northern African origin, and, at a diagnostic center for neurological disease, where, for the past 5 years, plasma vitamin E was systematically tested in patients with ataxia, one family with AVED (family 11) was detected (A. Brice, unpublished observations). Whereas 11 cases had been reported in the literature before we started our work, we have now identified a total of 32 additional cases, 26 of which either have a northern African origin or carry the "northern African" haplotype. A systematic study of this disease in this population is warranted, especially since early detection of vitamin E deficiency before the onset or progression of the neurodegenerative process would allow implementation of vitamin E supplementation therapy that can either stabilize neurological function or prevent neurological dysfunction (Kohlschütter et al. 1988; Sokol et al. 1988; Kayden 1993).

The refinement of the localization of AVED will facilitate positional cloning strategies. Biochemical studies of patients showed abnormal liver incorporation of tocopherol derivatives into VLDL (Traber et al. 1990, 1993). The gene coding for the hepatic tocopherol transfer protein (Mowri et al. 1981; Murphy and Mavis 1981; Behrens and Madere 1982; Sato et al. 1991) is an obvious candidate for AVED. The corresponding rat cDNA has recently been cloned (Sato et al. 1993). It will be important to see if the human gene is located on chromosome 8q.

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