

Frequency and phenotypic spectrum of ataxia with oculomotor apraxia 2: a clinical and genetic study in 18 patients

Isabelle Le Ber,^{1,2} Naïma Bouslam,^{2,8} Sophie Rivaud-Péchoux,² João Guimarães,⁹ Ali Benomar,⁸ Céline Chamayou,³ Cyril Goizet,⁶ Maria-Ceù Moreira,⁷ Sandra Klur,⁷ Mohamed Yahyaoui,⁸ Yves Agid,^{1,4} Michel Koenig,⁷ Giovanni Stevanin,² Alexis Brice^{1,2,5} and Alexandra Dürr^{2,5}

¹Fédération de Neurologie, ²INSERM U289, ³Centre du Langage, ⁴Centre d'Investigation Clinique and ⁵Département de Génétique, Cytogénétique et Embryologie, Hôpital Pitié-Salpêtrière AP-HP, Paris, ⁶Service de Génétique, Centre Hospitalo-Universitaire, Bordeaux, ⁷Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/Université Louis-Pasteur, Illkirch, CU de Strasbourg, France, ⁸Laboratoire de Neurogénétique, Service de Neurologie, Hôpital des Spécialités, Rabat, Morocco and ⁹University Department of Neurology, Hospital de Egas Moniz, Lisboa, Portugal

Correspondence to: Alexandra Dürr, MD, PhD, INSERM U289 and Département de Génétique, Cytogénétique et Embryologie, Hôpital Pitié-Salpêtrière, 47, boulevard de l'Hôpital, 75651 Paris Cedex 13, France
E-mail: durr@ccr.jussieu.fr

Summary

Ataxia with oculomotor apraxia type 2 (AOA2) is a newly described autosomal recessive cerebellar ataxia (ARCA) defined by genetic location to 9q34 of three families sharing gait ataxia, oculomotor apraxia and/or elevated α -foetoprotein (AFP) levels. We have evaluated 77 families with progressive non-Friedreich ARCA and have identified six families with a phenotype suggestive of AOA2. Linkage was confirmed in all six families, with a maximal lod score of 5.91 at D9S1830. We report the first detailed phenotypic study, including neuropsychological, oculographic and brain imaging investigations, in the largest series of AOA2 patients yet recruited. The mean age at onset was 15.1 ± 3.8 years. Sensory motor

neuropathy (92%) and choreic or dystonic movements (44%) were frequent. Oculomotor apraxia was observed in 56% of patients and characterized by increased horizontal saccade latencies and hypometria. AFP levels were elevated in 100% of the families, making it a useful biological marker. This study shows for the first time that AOA2 can be found in Europe, North Africa and the West Indies, and its relative frequency represents ~8% of non-Friedreich ARCA, which is more frequent than ataxia telangiectasia and ataxia with oculomotor apraxia type 1 (AOA1), in our series of adult patients. In adults, AOA2 may be, therefore, the most frequent cause of ARCA identified so far, after Friedreich's ataxia.

Keywords: cerebellar ataxia; ocular motor apraxia; AOA1; AOA2; α -foetoprotein

Abbreviations: AOA1 = ataxia with oculomotor apraxia type 1; AOA2 = ataxia with oculomotor apraxia type 2; ARCA = autosomal recessive cerebellar ataxia; A-T = ataxia telangiectasia; APTX = aprataxin gene; ATM = ataxia-telangiectasia mutated gene; AFP = α -foetoprotein; CA = cerebellar ataxia; CK = creatine phosphokinase; CVLT = California Verbal Learning Test; FA = Friedreich's ataxia; MADRS = Montgomery and Asberg Depression Rating Scale; MDRS = Mattis Dementia Rating Scale; MMSE = Mini-mental State Examination; VOR = vestibulo-ocular reflex; WCST = Wisconsin Card Sorting Test

Received September 22, 2003. Revised November 21, 2003. Accepted November 22, 2003. Advance Access publication January 21, 2004

Introduction

Hereditary autosomal recessive cerebellar ataxias (ARCAs) are rare diseases. The most common is Friedreich's ataxia

(FA) that accounts for 30–40% of the cases in Caucasian populations. Other aetiologies, including ataxia telangiect-

tasia (A-T), autosomal recessive spastic ataxia of Charlevoix–Saguenay and ataxia with vitamin E deficiency, are less frequent. A subgroup of ARCA associated with oculomotor apraxia but different from A-T has been identified (Aicardi *et al.*, 1988; Barbot *et al.*, 2001). Oculomotor apraxia was described initially in children affected by congenital oculomotor apraxia as the inability to generate volitional horizontal saccades with a characteristic compensatory headthrusting and synkinetic blinking (Cogan, 1953). It is better described as intermittent saccade failure rather than a true apraxia (Harris *et al.*, 1996; Shawkat *et al.*, 1996).

A-T is characterized by an early age at onset, the association of cerebellar ataxia (CA), oculomotor apraxia, telangiectasias, recurrent infections, cancers and markedly elevated α -foetoprotein (AFP) level. The patients usually become wheelchair-bound in their early teens (Woods *et al.*, 1992; Stankovic *et al.*, 1998). The *ATM* gene codes for a protein involved in DNA double strand break repair (Stavitsky *et al.*, 1995; Laake *et al.*, 2000; Campbell *et al.*, 2003). Approximately 10% of A-T patients have mutations causing a milder phenotype ('A-T variants') that differs from the typical phenotype by a later age at onset, a longer disease duration or the absence of one characteristic feature of the disease (Gilad *et al.*, 1998). In addition, two families presenting an A-T-like phenotype ('A-T-like syndrome') have mutations in the *hMRE11* gene which maps proximal to ataxia-telangiectasia mutated gene (*ATM*) (Stewart *et al.*, 1999).

Based on linkage studies, ataxia with oculomotor apraxia recently was found to encompass at least two types, type 1 (AOA1) (Moreira *et al.*, 2001a) and type 2 (AOA2) (Bomont *et al.*, 2000; Németh *et al.*, 2000). AOA1, located to 9p13, is characterized by the association of CA with cerebellar atrophy on MRI, frequent choreic movements at onset which regress with the course of the disease, oculomotor apraxia, severe peripheral neuropathy, occasional mild mental retardation, hypercholesterolaemia and hypoalbuminaemia (Bomont *et al.*, 2000; Tachi *et al.*, 2000; Moreira *et al.*, 2001a; Shimazaki *et al.*, 2002; Le Ber *et al.*, 2003; Tranchant *et al.*, 2003). The defective gene, aprataxin gene (*APTX*), mapped to 9p13, was found to code for a new histidine-triad protein, named aprataxin, possibly involved in DNA single strand break repair (Date *et al.*, 2001; Moreira *et al.*, 2001b). All mutations identified so far are located in exons 5, 6 and 7 (Date *et al.*, 2001; Moreira *et al.*, 2001b).

In 2000, Németh and colleagues, and Bomont and colleagues independently reported linkage to 9q34 of autosomal recessive ataxia resembling A-T but with a later age at onset, without the extra-neurological features of A-T (Watanabe *et al.*, 1998; Németh *et al.*, 2000). These results were based on the study of a large Pakistani family with oculomotor apraxia but normal serum AFP level (Németh *et al.*, 2000) and a large family with elevated AFP level but no oculomotor apraxia (Watanabe *et al.*, 1998; Bomont *et al.*, 2000). The subsequent identification of families with both

oculomotor apraxia and elevated serum AFP level suggested that this represents a single new entity, named AOA2, and allowed the critical region to be narrowed to a 4 cM interval (Moreira *et al.*, 2002).

We have screened a large population of non-FA progressive ARCA families on a clinical and biological basis and have identified six new families with a phenotype suggestive of AOA2 and evidence of linkage to this locus. We report a detailed phenotypic study of this series of AOA2 patients, which is the largest examined to date. Neuropsychological, oculo-graphic, biological and brain imaging investigations, as well as molecular analyses were performed.

Material and patients

Patient recruitment

We studied a population of 77 families (154 patients) with progressive ARCA of various origins, most of them being French (69%). In all patients, the diagnosis of FA was previously excluded by molecular analysis. All patients underwent standardized clinical examinations and biological tests, including assays for AFP, albumin and cholesterol. Six of these families were considered to have possible AOA2, on the basis of CA associated with either oculomotor apraxia and/or an elevated level of AFP in at least one affected family member, after exclusion of AOA1 (Moreira *et al.*, 2001b).

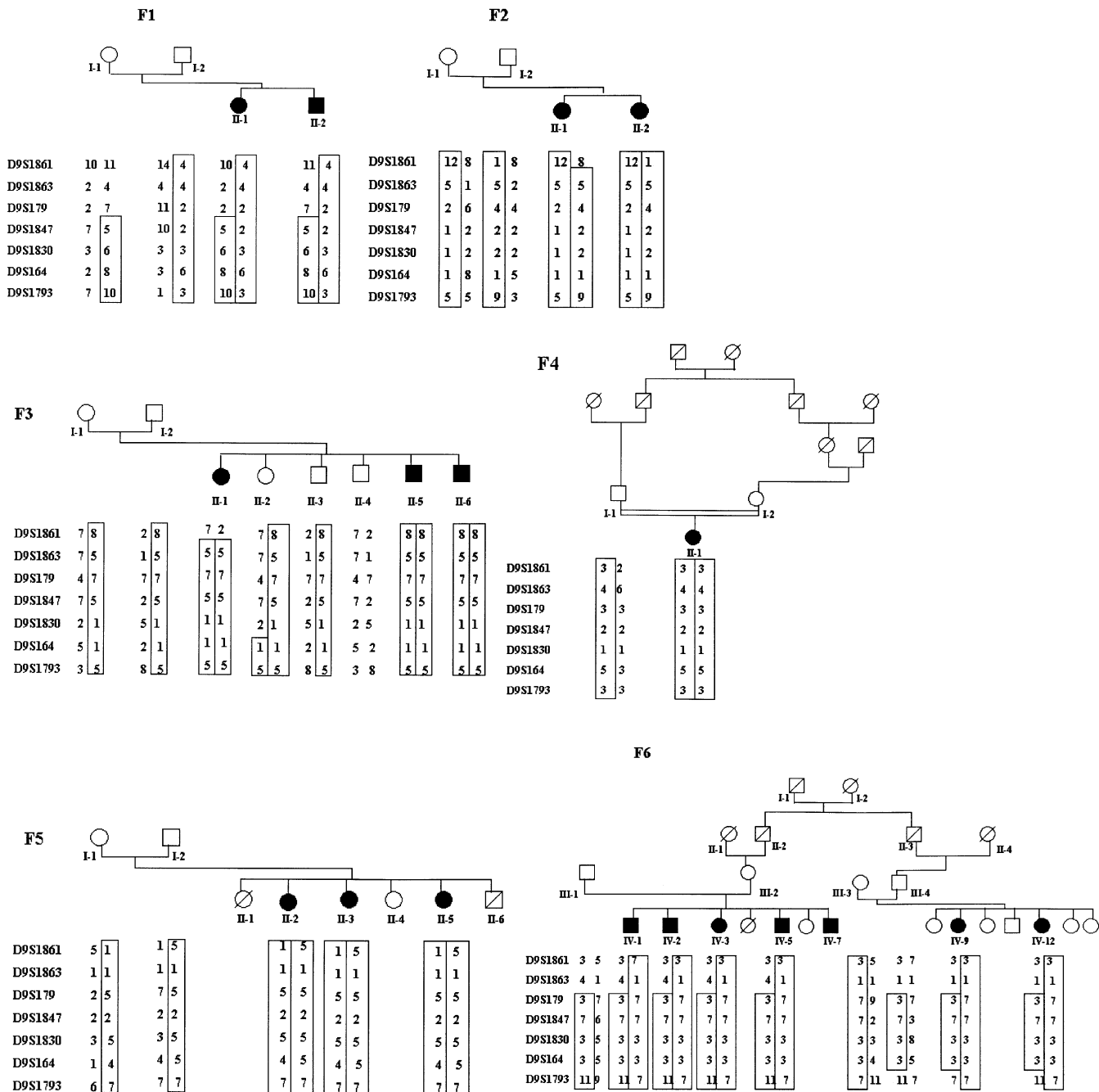
Molecular analysis

Molecular analyses were performed in the six families with possible AOA2. The patients and their relatives gave their informed consent. The study was approved by INSERM. DNA was extracted from peripheral leukocytes. Genotyping of the AOA2 locus (9q34) was performed in all patients and their relatives with seven microsatellite markers (D9S1863, D9S1861, D9S179, D9S1847, D9S1830, D9S164 and D9S1793) flanking the refined ~4 cM candidate region. DNA was polymerase chain reaction (PCR)-amplified using a 6 Fam-labelled forward primer in a final volume of 50 μ l containing 100 ng of genomic DNA, 0.2 μ M of dNTPs, 0.5 μ M of primers and 1 U of Taq DNA polymerase. After denaturation for 5 min at 95°C, the PCR consisted of 30 cycles of 15 s at 95°C, 20 s at 55°C and 20 s at 72°C, followed by a final extension for 5 min at 72°C. After electrophoresis for 3 h on an ABI-Prism 377 automated sequencer, data were analysed using the GenScan® and Genotyper® softwares (PE Applied Biosystems).

To exclude an 'A-T variant', we tested the *ATM* locus with three microsatellite markers (D11S1819, D11S2179 and D11S1778). To exclude an 'AT-like syndrome', the *hMRE11* locus was also tested with two closely flanking markers (D11S1311 and D11S4176) and an intragenic (CA)_n repeat marker located in intron 13 (forward primer, GGGTTTGCTAATTTATAGC; reverse primer, GGGTTTTGATTTTGAACACG). DNA was PCR-amplified and data analysed as indicated above. In addition, exons 5, 6 and 7 of the *APTX* gene, causing AOA1 when defective, were analysed by direct sequencing in all index cases, as described (Moreira *et al.*, 2001b).

Linkage analysis

For linkage analyses, two-point cumulative lod scores were calculated with the MLINK program of the LINKAGE package



(Lathrop *et al.*, 1985). We assumed a fully penetrant autosomal recessive mode of inheritance, with a disease allele frequency of 0.001 and no phenocopies. Equal frequencies were assumed for marker alleles.

All patients were interviewed with respect to their personal and familial histories of cancer and infection, and were examined. Six patients underwent detailed neuropsychological evaluations including the Mini-mental State Examination (MMSE; Folstein, 1975), the Mattis Dementia Rating Scale (MDRS; Mattis, 1988), the California

Other investigations

Table 1 Two-point lod scores at different recombination fractions for seven markers on chromosome 9q34

Markers	θ						
	0.0	0.01	0.05	0.1	0.2	0.3	0.4
D9S1861	-29.05	-2.30	0.57	1.28	1.25	0.75	0.25
D9S1863	-7.85	0.64	1.60	1.66	1.21	0.64	0.20
D9S179	-0.83	3.33	3.47	3.08	2.04	1.04	0.30
D9S1847	5.52	5.38	4.81	4.11	2.72	1.47	0.50
D9S1830	5.91	5.76	5.17	4.43	2.96	1.60	0.52
D9S164	4.54	4.85	4.73	4.17	2.81	1.50	0.47
D9S1793	-2.18	2.33	3.04	2.92	2.13	1.20	0.40

in addition to AFP, carcinoembryonic antigen, creatine phosphokinase (CK), electrophoresis and immunoelectrophoresis of serum proteins. All patients in families F1, F2, F3 and F4 underwent high resolution karyotyping.

Oculomotor recordings

Eye movements [fixation, gaze-holding, pursuit, vertical and horizontal saccades, presence and nature of nystagmus, evaluation of synergic eye-head movements in the head-free condition, vestibulo-ocular reflex (VOR) and VOR cancellation] were assessed clinically in all patients. Electro-oculographic recordings were made in seven patients, with a method previously detailed elsewhere (Le Ber *et al.*, 2003). We measured the latency, accuracy and velocity of horizontal saccades with amplitudes of $25 \pm 2^\circ$, in a gap (200 ms) and a no-gap task, when the subject's head was immobilized. Accuracy was expressed as a gain (amplitude of the first saccade over the eccentricity of the target). In addition, we calculated the percentage of errors of direction in an antisaccade task. The results were compared with the results of seven age-matched controls.

Results

We identified six families with progressive ARCA and oculomotor apraxia and/or elevated AFP, suggestive of AOA2. Consanguinity was known in one family and was probable in three others. None had telangiectasia, recurrent infections or cancer, immunodeficiency, elevated level of carcinoembryonic antigen or chromosomal rearrangements. A-T was therefore considered unlikely.

Molecular analyses

All families with clinical and biological profiles suggestive of AOA2 underwent genetic studies to confirm linkage to AOA2 and to exclude other loci for ARCA with oculomotor apraxia and/or elevated AFP. Possible linkage to the AOA2 locus was found by haplotype analysis in all six families, including five multiplex families and one isolated case with consanguineous parents (18 patients: seven men, 11 women) (Fig. 1). Two families originated from France (F2 and F3) and one each from the West Indies (F1), Turkey (F4), Morocco (F5) and Portugal (F6). In these families, linkage to the *ATM* and

hMRE11 loci was excluded by haplotype analysis, and no mutations were found in exons 5, 6 and 7 of the *APTX* gene (data not shown).

The two-point linkage analysis in this group of families generated significant positive lod score values with the markers D9S1847, D9S1830 and D9S164 at $\theta = 0$ (see Table 1). A maximal lod score of 5.91 was obtained for marker D9S1830. The patients from four families (F3, F4, F5 and F6), including three who were not known to be consanguineous, were homozygous for at least three consecutive markers at the AOA2 locus. Patients from the other two families (F1 and F2) showed haploidentity for all markers between D9S1847 and D9S164 (Fig. 1). Reconstruction of haplotypes revealed the existence of a recombination event between D9S179 and D9S1847 in family F1 and of a recombination event between D9S1830 and D9S164 in family F3. Analysis of the regions of homozygosity confirmed the centromeric boundary in family F6 and defined the telomeric boundary at D9S1793 in the same family. These results confirm that the AOA2 gene is located in the ~4 cM region between markers D9S179 (centromeric) and D9S164 (distal). No common haplotypes were found, excluding a founder effect for the AOA2 mutation in this group of families with different ethnic origins.

Clinical features

Clinical data on the 18 patients from the six AOA2 families linked to 9q34 are summarized in Table 2 and Fig. 2. The mean age at onset (15.1 ± 3.8 years, range 10–25) was higher than in both A-T (onset usually before age 5) and AOA1 (onset at 6.9 ± 4.7 years) (Le Ber *et al.*, 2003). The mean disease duration at examination was 26.0 ± 14.0 years. The initial symptom was gait ataxia in 78% of patients, which may have been preceded by strabismus in childhood in 11% and dystonia or postural tremor in 11% of patients. The disease progressed slowly. Only five patients of a single family (F6) were confined to a wheelchair at the time of examination, after a mean disease duration of 16.3 ± 3.8 years (range 11–22). In other families, disease progression was less rapid and all patients were still able to walk at the time of examination, after a mean disease duration of 19.4 ± 8.0 years.

Gait ataxia, the major clinical criterion for recruitment, was present in 100% of patients. All patients had predominantly axial cerebellar syndromes and consistent cerebellar atrophy on MRI (100% of patients) affecting predominantly the vermis (Fig. 3). Movement disorders were frequent (44% of patients), and consisted mainly of dystonic posture of the hands (28%), choreic movements (22%) and head or postural tremor (17%). The severity of movement disorders remained stable, in contrast to AOA1 where chorea tended to disappear over the course of the disease (Le Ber *et al.*, 2003). Clinical signs of neuropathy (abolished or diminished tendinous reflexes, sensorimotor deficit) were frequent but moderate, and severe disability was noted in only one family (F6), in which the patients

Table 2 Phenotypic characteristics of 18 patients with AOA2

Family	Patient	Age at onset	Disease duration	Initial symptom	Functional score	Disease duration until wheelchair	OMA	Pyramidal signs	Tendon reflexes	Motor deficit	Deep sensory loss	Chorea/dystonia	AFP level	CT level	Alb level (g/l)	Other signs
F1	II-1	15	11	Strabism	4	-	+	-	a	+	+	Dystonia	1-2N	N	44	-
				Dystonia								Chorea				
	II-2	15	9	Gait ataxia	2	-	+	-	a	+	+	Head tremor	1.5-3.5N	N	45	PC
F2	II-1	10	22	Strabism	4	-	+	-	d	-	-	L dystonia	1.5-2.5N	↑	44	-
												Chorea				
	II-2	22	12	Gait ataxia	3	-	+	-	a	-	+	Dystonia	2-3N	N	43	-
F3(*)	II-1	16	29	Dysarthria	4	-	+	-	a	+	+	Dystonia	na	na	na	PC
												Head tremor				
	II-5	25	14	Gait ataxia	3	-	+	+	d	-	-	LL	1-3N	↑	48	-
F4*	II-6	16	23	LL tremor								tremor				
				Dysarthria	4	-	+	+	d	-	-	Dystonia	1-1.5N	↑	40	Myoclonus
	II-1	11	9	Gait ataxia	2	-	+	-	d	-	+	Chorea	1.5-2.5N	N	37	-
F5(*)	II-2	15	18	Gait ataxia	5	-	+	-	a	+	+++	-	na	na	na	PC
	II-3	20	15	Gait ataxia	5	-	-	-	a	++	+++	-	na	na	na	PC
	II-5	12	22	Gait ataxia	5	-	+	-	a	+	+++	-	na	na	na	PC
F6(*)	IV-1	13	51	Gait ataxia	7	13	-	-	a	+++	+++	-	4N	N	34	PC, S
	IV-2	12	48	Gait ataxia	6	11	-	-	a	+++	+++	-	10N	N	32	PC, S
	IV-3	13	42	Gait ataxia	6	15	-	-	a	+++	+++	-	11N	N	34	PC, S, M
	IV-5	13	44	Gait ataxia	6	17	-	-	a	+++	+++	-	na	↑	na	PC, S
	IV-7	14	39	Gait ataxia	6	16	-	-	a	+++	+++	-	na	↑	na	PC, S
	IV-9	14	35	Gait ataxia	6	22	-	+	a	++	+++	-	na	na	na	PC, S, M
	IV-12	15	25	Gait ataxia	6	20	-	-	a	++	++	-	na	na	na	PC, S, M

+ = Present; ++ = moderate; +++ = severe; * = known consanguinity; (*) = probable consanguinity; a = abolished; Alb = albumin level (N < 38 g/l); d = diminished; L = laryngeal dystonia; LL = lower limbs; M = lower limbs; N = normal level; na = not available; OMA = oculomotor apraxia; PC = pes cavus; S = scoliosis. The disease duration until wheelchair is indicated in years. Motor disability was assessed by a seven-stage functional scale: 0 = normal; 1 = mild modifications at examination; 2, mild functional disability, able to walk and run; 3 = able to walk without help up to 500 m, unable to run; 4 = needs unilateral help to walk; 5 = needs bilateral help to walk; 6 = wheelchair-bound; 7, bedridden.

had the longest disease durations (mean disease duration in this family: 40.6 ± 8.7 years, see Table 2). Motor and/or sensory axonal neuropathies were detected in all seven patients who underwent EMG. Deep sensory loss was noted in 83% of the patients and extensor plantar reflex in three patients (17%) only. Six patients had difficulty swallowing and one had sphincter disturbances. In addition, it is noteworthy that all affected women in family F6 reached menopause between 21 and 24 years of age.

Neuropsychological results

Cognitive impairment was mild on the MMSE (mean score 26.5 ± 1.9 , range 25–30) and significantly milder on the MDRS (mean score 135.6 ± 2.9 , range 131–138), mostly due to a deficit in initiation and concept subtests. Nearly all patients showed impaired recognition (mean score 12.8 ± 2.2 , range 10–15; normal value 15.0 ± 1.3), perseverations (mean score 6.4 ± 3.8 ; normal value 3.6 ± 1.8) and intrusions (mean score 9.4 ± 7.7 ; normal value 4.9 ± 2.9). The frontal score was moderately disturbed (mean score 52.8 ± 5.0 range 45–58; normal value 60 ± 6), but more evident in two patients with disturbed WSCT (four criteria). In addition, phonemic fluency was mildly reduced (mean score 9.8 ± 2.0 ; normal value 15.5 ± 5.3). None had mental retardation or severe dementia, even after long disease duration. No obvious cognitive changes were observed in the 11 patients who did not undergo neuropsychological testing.

Oculomotor results

Oculomotor apraxia was noted in 56% of the patients at bedside examination. It was characterized by a dissociation of

eye–head movements in the head-free condition, in which the head reaches the lateral target before the eyes, rather than a true apraxia. In addition, saccadic pursuit (100%), gaze-evoked nystagmus (89%) and a limited abduction (61%) were noted. Only one patient had limited upward gaze. The VOR was normal in all patients, but VOR cancellation was lost.

Electro-oculographic recordings in seven patients showed normal mean latency in the gap task (210 ± 73 ms, versus 164 ± 27 ms in control subjects) and in the no-gap task (310 ± 90 ms, versus 310 ± 86 ms in controls) in the whole population. The mean velocity on 25° horizontal saccades was normal ($360 \pm 50^\circ/\text{s}$, versus $335 \pm 60^\circ/\text{s}$ in controls). However, in three out of the seven patients, the latencies were 2SD above the normal mean. In addition, visually guided saccades were hypometric (mean centrifugal gain in amplitude: 0.82 ± 0.18 , versus 0.94 ± 0.03 in controls). The percentage of errors in the antisaccade task (40%, range: 20–59, versus 4% in controls range: 0–14) was greatly increased.

Biological tests

AFP was mildly to moderately elevated (1.5–4 times the normal upper limit) in 75% of tested patients, when only one assay was done. This increased to 100% of the patients when measurements were repeated at least three times (Table 2). These values were variable over time and lower than usually observed in A-T, where they often reach 10 times the normal limit. A marked elevated level was noted only in one family (F6), where patients had the longest disease duration and the most severe phenotype. Moderately elevated levels should therefore be taken into account and measurements should be repeated. In family F4, CK levels were slightly elevated (1.5–2 times the normal upper limit). Elevated immunoglobulin levels (IgG and A) were found in three out of the six families

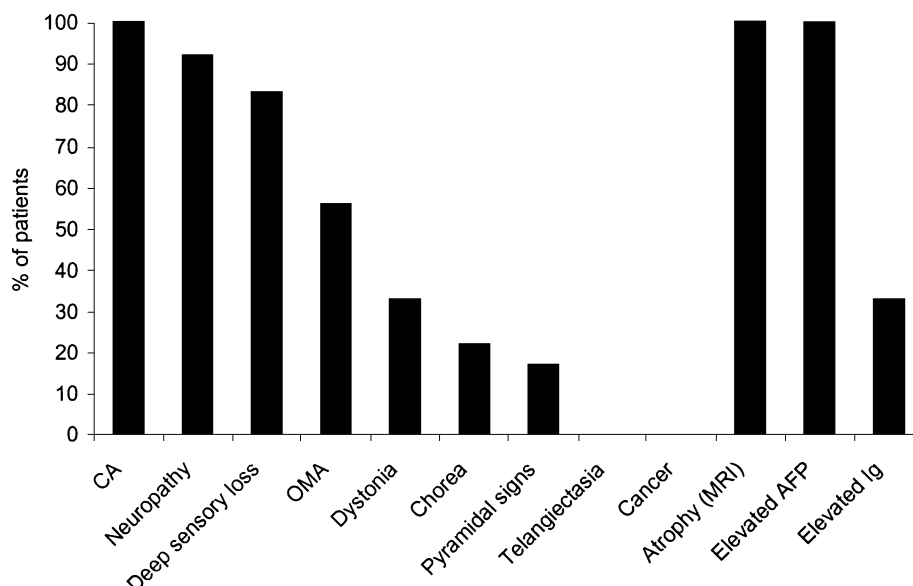


Fig. 2 Frequency of the main phenotypic and paraclinical characteristics in 18 patients with AOA2. CA = cerebellar ataxia; OMA = oculomotor apraxia. AFP = alphafoeto protein; Ig = immunoglobulin levels.

tested (F1, F3 and F4) and total cholesterol was increased in five patients from three out of the six families tested (F2, F3 and F6). Low albumin levels were noted in four patients from two families (F4 and F6), three of which had a disease duration longer than 40 years (Table 2). Carcinoembryonic antigen levels were normal in all patients, as were the karyotypes.

Discussion

This is the first report of a large series of AOA2 patients with detailed evaluations. The six families selected from a large cohort of families with non-FA ARCA have clinical and biological features suggestive of AOA2. They are also linked to the AOA2 locus and excluded from other loci for ARCA with oculomotor apraxia. AOA2 accounts for ~8% [95% confidence interval (CI) 2.91–16.29] of the 77 non-FA

families studied and 4% of the 53 families of French origin (95% CI 0.5–13.0). This is more frequent than A-T and AOA1 in our series, which represent only 1.9 and 5% of non-FA families, respectively (Le Ber *et al.*, 2003). The higher frequency of AOA2 than A-T is unexpected and may be explained in part by the fact that the series includes only adults and by the high early mortality rate in patients with A-T. We conclude that AOA2 is the most frequent cause of identified ARCA so far, after FA, in our series of adult patients. Therefore, this diagnosis should be considered systematically and AFP levels should be measured in all adults with CA beginning before the age of 35 years, after exclusion of FA. Our study also shows that AOA2 is not restricted to Japan and Pakistan where it was first described (Watanabe *et al.*, 1998; Bomont *et al.*, 2000; Németh *et al.*, 2000), but also exists in Europe, the West Indies and Mediterranean countries, but its relative frequency may vary according to ethnic origin.

In most families, the AOA2 phenotype associates CA with cerebellar atrophy on MRI, oculomotor apraxia, chorea or dystonia, and axonal sensory neuropathy with pes cavus, as in the three previously reported families (Watanabe *et al.*, 1998; Bomont *et al.*, 2000; Németh *et al.*, 2000). However, the number of patients studied here gives a more precise picture of the AOA2 phenotype and its severity. Although the AOA2 gene has not yet been identified, the wide phenotypic variability suggests that different mutations may be involved. Family F6 is characterized by severe neuropathy and a poor functional prognosis, in addition to the absence of oculomotor apraxia, chorea and dystonia. The levels of AFP were markedly higher than in other families. It is striking that all patients in this family became wheelchair-bound after 11–22 years of disease progression, whereas the nine patients from other families with a similar disease duration could still walk autonomously. Early menopause occurred in three affected women of family F6, but was not reported in the other



Fig. 3 Brain T2-weighted MRI sequences in sagittal sections (patient II-1, family F2). Severe cerebellar atrophy with vermian predominance.

Table 3 Phenotypic differences between FA, A-T, AOA1 and AOA2

Diseases	FA*	A-T**	AOA2	AOA1***
No. of patients	187	70	18	14
Age at onset, years (range)	15.5 ± 3.8 (2–51)	<5	15.1 ± 3.8 (9–25)	6.9 ± 4.7 (2–18)
Disease duration until wheelchair, years	10.8 ± 6	10	16.3	11.2
Gait ataxia	+	+	+	+
Neuropathy	S	SM	SM	SM
Oculomotor apraxia	–	+	+	+
Chorea and/or dystonia	–	+	+§	+
Pyramidal signs	+	–	±	–
Telangiectasia	–	+	–	–
Early cerebellar atrophy on MRI	–	+	+	+
Biological characteristics	–	↑AFP ↓Ig ↑CEA	↑AFP ±↑Ig ±↑CT ±↓Albumin	↑CT ↓Albumin

*From Dürr *et al.* (1996); **from Stankovic *et al.* (1998) and Woods and Taylor (1992); ***from Le Ber *et al.* (2003); + = present; – = absent; ± = present in a minority of patients; § = present at onset but disappearing with the course of the disease; AFP = α-fetoprotein; CEA = carcinoembryonic antigen; CT = cholesterol level; Ig = immunoglobulins; S = sensory; SM = sensory and/or motor.

families, and might be related to disease severity in this kindred or might be unrelated to the AOA2 phenotype.

Cognitive status was reportedly normal in the Pakistani and Japanese families. However, our study revealed subtle cognitive changes, consistent with executive dysfunction, in the patients who underwent neuropsychological testing.

Oculomotor apraxia, characterized by eye-head dissociation during voluntary lateral head movements, is found in only two-thirds of our patients. It should be emphasized that all cases without oculomotor apraxia, except one, occurred only in one family. Dysmetric horizontal saccades, saccadic pursuit and gaze-evoked nystagmus found in 89–100% of the patients indicate marked cerebellar dysfunction, mainly in the dorsal vermis and flocculus. The normal saccade velocities suggest, however, that the paramedian pontine reticular formation is intact. The oculographic characteristics in three out of the seven patients tested are similar to those described in A-T, with hypometria and increased horizontal saccade latency (Lewis *et al.*, 1999). Nevertheless, oculomotor abnormalities appear less severe than in AOA1, where apparent ‘slow saccades’ are due to the succession of multiple very hypometric saccades (Le Ber *et al.*, 2003). These oculomotor characteristics may therefore be helpful in distinguishing these two highly similar phenotypes.

Additional paraclinical investigations suggest the involvement of additional neural systems. Sensory and motor peripheral neuropathy was evident in 92% of the patients. A striking biological feature was the moderately elevated levels of AFP. This elevation is frequent in AOA2 and may vary with time in a given patient, indicating that assays should be performed repeatedly. High levels of AFP were noted in family F6 only, and could be related to the long disease duration or the peculiar severity of the disease in this family. Other biological abnormalities such as elevated CK, immunoglobulins, cholesterol and low albumin level are variably associated in a minority of patients. Albumin levels were very mildly decreased, compared with AOA1 (Le Ber *et al.*, 2003).

The group of ARCA with oculomotor apraxia is growing fast. Four distinct genetic entities are already known: A-T, A-T like, AOA1 and AOA2 (Table 3). The main clinical features distinguishing AOA2 from A-T are the later age at onset and the absence of extra-neurological signs, such as predisposition to cancer, infections and rare telangiectasia. Telangiectasias were described in the AOA2 family reported by Watanabe *et al.* (1998), but were not found in any of our patients. The clinical course in AOA2 is gradual and the functional deficit is less severe than in A-T (Table 3). The increase in AFP is also usually moderate in most cases (<5 times the normal upper limit) compared with in A-T (usually 10 times the normal upper limit) and karyotypes are normal. In ‘A-T variant’, oculomotor apraxia and movement disorders are often absent, whereas telangiectasia and cancer predisposition are more frequent than in AOA2. Moreover, the ‘A-T-like’ phenotype, described in two families with mutations

in the *hMRE11* gene, is associated with increased chromosomal rearrangements, but normal AFP and immunoglobulin levels (Stewart *et al.*, 1999).

In addition to their biological and oculographic characteristics, distinct phenotypic features may also help distinguish AOA2 from AOA1. Early onset, usually before the age of 10 years, and marked choreic movements are highly suggestive of AOA1 when present at onset, but their severity often decreases during the course of the disease (Le Ber *et al.*, 2003). In contrast, chorea is less frequent and less severe in AOA2 but, when present, abnormal movements persist throughout the course of the disease. Neuropathy in AOA1 and AOA2 is similar, but much more severe in AOA1, where it often leads to a disabling motor deficit, atrophy and deformity. The functional prognosis is therefore better in AOA2 than in AOA1, with a notably longer disease duration until the patients become wheelchair-bound (16.3 ± 3.8 years in AOA2, versus 11.2 years in AOA1).

AOA2 is easily distinguished from FA because of oculomotor apraxia, choreic and/or dystonic movement, and motor and sensory neuropathy, which is strictly sensory in FA (Dürr *et al.*, 1996). In addition, the biological markers (AFP, cholesterol and CK) and the severe cerebellar atrophy detected on MRI are not common in FA, whereas cardiomyopathy is absent and corticospinal tract involvement is rare in AOA2.

We conclude that AFP should be measured in all patients with CA beginning before the age of 35 years, and that variable and slightly elevated levels should be considered as suggestive of AOA2. This biological marker and the clinical features of AOA2 should help to distinguish it from the other three genetic entities with ARCA and oculomotor apraxia. Genetic analyses are underway to identify the defective gene. This will help understand the pathological mechanism of the disease and clarify its relationship to A-T and AOA1.

Acknowledgements

We wish to thank Dr Dominique Stoppa-Lyonnet who provided us with the intragenic primer in intron 13 of the *hMRE11* gene, the patients for their participation, the Centre d’Investigations Cliniques (CIC 9503), Hôpital de la Salpêtrière AP-HP, Paris, where many patients were examined, and Dr Merle Ruberg for her helpful suggestions on the manuscript. This study was supported by the SPATAX research network (INSERM-AFM grant 4MR12F-A0004DS), the VERUM foundation, CNRS, INSERM, AFM (Association Française contre les Myopathies) and the Strasbourg University Hospitals. I.L.B. had a fellowship from the Fondation pour la Recherche Médicale, the Association Française de l’Ataxie de Friedreich and the Société Française de Neurologie. M.C.M. had a graduate fellowship PRAXIS XXI/BD/18169/98 from Fundação para a Ciência e a Tecnologia, Portugal.

References

- Aicardi J, Barbosa C, Andermann E, Andermann F, Murcos R, Ghanem Q, et al. Ataxia-ocular motor apraxia: a syndrome mimicking ataxia-telangiectasia. *Ann Neurol* 1988; 24: 497–502.
- Barbot C, Coutinho P, Choro R, Ferreira C, Barros J, Fineza I, et al. Recessive ataxia with ocular apraxia: review of 22 Portuguese patients. *Arch Neurol* 2001; 58: 201–5.
- Bomont P, Watanabe M, Gershoni-Barush R, Shizuka M, Tanaka M, Sugano J, et al. Homozygosity mapping of spinocerebellar ataxia with cerebellar atrophy and peripheral neuropathy to 9q33–34, and with hearing impairment and optic atrophy to 6p21–23. *Eur J Hum Genet* 2000; 8: 986–90.
- Campbell C, Mitui M, Eng L, Coutinho G, Thorstenson Y, Gatti RA. ATM mutations on distinct SNP and STR haplotypes in ataxia-telangiectasia patients of differing ethnicities reveal ancestral founder effects. *Hum Mutat* 2003; 21: 80–5.
- Cogan DG. A type of congenital ocular motor apraxia presenting jerky head movements. *Am J Ophthalmol* 1953; 36: 433–41.
- Date H, Onodera O, Tanaka H, Iwabuchi K, Uekawa K, Igarashi S, et al. Early-onset ataxia with ocular motor apraxia and hypoalbuminemia is caused by mutations in a new HIT superfamily gene. *Nature Genet* 2001; 29: 184–8.
- Delis DC, Kramer JK, Kaplan E, Ober BA. The California Verbal Learning Test: research edition. San Antonio (TX): Psychological Corporation; 1987.
- Dürr A, Cossee M, Agid Y, Campuzano V, Mignard C, Penet C, et al. Clinical and genetic abnormalities in patients with Friedreich's ataxia. *N Engl J Med* 1996; 335: 1169–75.
- Folstein MF, Folstein SE, McHugh PR. 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; 12: 189–98.
- Gilad S, Khosravi R, Harnik R, Ziv Y, Shkedy D, Galanty Y, et al. Identification of ATM mutations using extended RT-PCR and restriction endonuclease fingerprinting, and elucidation of the repertoire of A-T mutations in Israel. *Hum Mutat* 1998; 11: 69–75.
- Harris CM, Shawkat F, Russell-Eggitt I, Wilson J, Taylor D. Intermittent horizontal saccade failure ('ocular motor apraxia') in children. *Br J Ophthalmol* 1996; 80: 151–8.
- Laake K, Jansen L, Hahnemann JM, Brondum-Nielsen K, Lonnqvist T, Kaariainen H, et al. Characterization of ATM mutations in 41 Nordic families with ataxia telangiectasia. *Hum Mutat* 2000; 16: 232–46.
- Lathrop GM, Lalouel JM, Julier C, Ott J. Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. *Am J Hum Genet* 1985; 37: 482–98.
- Le Ber I, Moreira MC, Rivaud-Pechoux S, Chamayou C, Ochsner F, Kuntzer T, et al. Cerebellar ataxia with oculomotor apraxia type 1: clinical and genetic studies. *Brain* 2003; 126: 2761–72.
- Lewis RF, Lederman HM, Crawford TO. Ocular motor abnormalities in ataxia telangiectasia. *Ann Neurol* 1999; 46: 287–95.
- Mattis S. Dementia Rating Scale. Odessa (FL): Psychological Assessment Resources; 1988.
- Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. *Br J Psychiatry* 1979; 134: 382–9.
- Moreira MC, Barbot C, Tachi N, Kozuka N, Mendonca P, Barros J, et al. Homozygosity mapping of Portuguese and Japanese forms of ataxia-oculomotor apraxia to 9p13, and evidence for genetic heterogeneity. *Am J Hum Genet* 2001a; 68: 501–8.
- Moreira MC, Barbot C, Tachi N, Kozuka N, Uchida E, Gibson T, et al. The gene mutated in ataxia-ocular apraxia 1 encodes the new HIT/Zn-finger protein aprataxin. *Nature Genet* 2001b; 29: 189–93.
- Moreira MC, Klur S, Barbot C, Tachi N, Bomont P, Watanabe M, et al. Autosomal recessive ataxia: a new gene—aprataxin—responsible for ataxia-ocular apraxia 1, and a new locus on 9q34. *Eur J Hum Genet* 2002; 10 Suppl 1: p. 272. PO936.
- Németh AH, Bochukova E, Dunne E, Huson SM, Elston J, Hannan MA, et al. Autosomal recessive cerebellar ataxia with oculomotor apraxia (ataxia-telangiectasia-like syndrome) is linked to chromosome 9q34. *Am J Hum Genet* 2000; 67: 1320–6.
- Pillon B, Gouider-Khouja N, Deweer B, Vidailhet M, Malapani C, Dubois B, et al. Neuropsychological pattern of striatonigral degeneration: comparison with Parkinson's disease and progressive supranuclear palsy. *J Neurol Neurosurg Psychiatry* 1995; 58: 174–9.
- Shawkat FS, Harris CM, Taylor DS, Kriss A. The role of ERG/VEP and eye movement recordings in children with ocular motor apraxia. *Eye* 1996; 10: 53–60.
- Shimazaki H, Takiyama Y, Sakoe K, Ikeguchi K, Nijima K, Kaneko J, et al. Early-onset ataxia with ocular motor apraxia and hypoalbuminemia: the aprataxin gene mutations. *Neurology* 2002; 59: 590–5.
- Stankovic T, Kidd AM, Sutcliffe A, McGuire GM, Robinson P, Weber P, et al. ATM mutations and phenotypes in ataxia-telangiectasia families in the British Isles: expression of mutant ATM and the risk of leukemia, lymphoma, and breast cancer. *Am J Hum Genet* 1998; 62: 334–45.
- Stavitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, et al. A single ataxia telangiectasia gene with a product similar to PI-3 kinase. *Science* 1995; 268: 1749–53.
- Stewart GS, Maser RS, Stankovic T, Bressan DA, Kaplan MI, Jaspers NG, et al. The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. *Cell* 1999; 99: 577–87.
- Tachi N, Kozuka N, Ohya K, Chiba S, Sasaki K. Hereditary cerebellar ataxia with peripheral neuropathy and mental retardation. *Eur Neurol* 2000; 43: 82–7.
- Thuillard F, Assal G. Données neuropsychologiques chez le sujet âgé normal. In: Habib M, Joannette Y, Puel M, editors. Démences et syndromes démentiels. Approche neuropsychologique. Paris: Masson; 1991. p. 125–33.
- Tranchant C, Fleury M, Moreira MC, Koenig M, Warter JM. Phenotypic variability of aprataxin gene mutations. *Neurology* 2003; 60: 868–70.
- Watanabe M, Sugai Y, Concannon P, Koenig M, Schmitt M, Sato M, et al. Familial spinocerebellar ataxia with cerebellar atrophy, peripheral neuropathy, and elevated level of serum creatine kinase, gamma-globulin, and alpha-fetoprotein. *Ann Neurol* 1998; 44: 265–9.
- Woods CG, Taylor AM. Ataxia telangiectasia in the British Isles: the clinical and laboratory features of 70 affected individuals. *Q J Med* 1992; 82: 169–79.