doi:10.1093/brain/awp211 Brain 2009: 132; 2688–2698 | 2688

BRAIN
A JOURNAL OF NEUROLOGY

Ataxia with oculomotor apraxia type 2: clinical, biological and genotype/phenotype correlation study of a cohort of 90 patients

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Received March 6, 2009. Revised June 15, 2009. Accepted June 26, 2009. Advance Access publication August 20, 2009

Ataxia with oculomotor apraxia type 2 (AOA2) is an autosomal recessive disease due to mutations in the senataxin gene, causing progressive cerebellar ataxia with peripheral neuropathy, cerebellar atrophy, occasional oculomotor apraxia and elevated alpha-feto-protein (AFP) serum level. We compiled a series of 67 previously reported and 58 novel ataxic patients who underwent senataxin gene sequencing because of suspected AOA2. An AOA2 diagnosis was established for 90 patients, originating from 15 countries worldwide, and 25 new senataxin gene mutations were found. In patients with AOA2, median AFP serum level was 31.0 μg/l at diagnosis, which was higher than the median AFP level of AOA2 negative patients: 13.8 μg/l, $P = 0.0004$; itself higher than the normal level (3.4 μg/l, range from 0.5 to 17.2 μg/l) because elevated AFP was one of the possible selection criteria. Polyneuropathy was found in 97.5% of AOA2 patients, cerebellar atrophy in 96%, occasional oculomotor apraxia in 51%, pyramidal signs in 20.5%, head tremor in 14%, dystonia in 13.5%, strabismus in 12.3% and chorea in 9.5%. No patient was lacking both peripheral neuropathy and cerebellar atrophy. The age at onset and presence of occasional oculomotor apraxia were negatively correlated to the progression rate of the disease ($P = 0.03$ and $P = 0.009$, respectively), whereas strabismus was positively correlated to the progression rate ($P = 0.03$). An increased AFP level as well as cerebellar atrophy seem to be stable in the course of the disease and to occur mostly at or before the onset of the disease. One of the two patients with a normal AFP level at diagnosis had high AFP levels 4 years later, while the other had borderline levels. The probability of missing AOA2 diagnosis, in case of sequencing senataxin gene only in non-Friedreich ataxia non-ataxia-telangiectasia ataxic patients with AFP level $\geqslant 7$ μg/l, is 0.23% and the probability for a non-Friedreich ataxia non-ataxia-telangiectasia ataxic patient to be affected with AOA2 with AFP levels $\geqslant 7$ μg/l is 46%. Therefore, selection of patients with an AFP level above 7 μg/l for senataxin gene sequencing is a good strategy for AOA2 diagnosis. Pyramidal signs and dystonia were more frequent and disease was less severe with missense mutations in the helicase domain of senataxin gene than with missense mutations out of helicase domain and deletion and nonsense mutations ($P = 0.001$, $P = 0.008$ and $P = 0.01$, respectively). The lack of pyramidal signs in most patients may be explained by masking due to severe motor neuropathy.

Keywords: ataxia; oculomotor apraxia; polyneuropathy; alpha-feto-protein; cerebellar atrophy

Abbreviations: AFP = alpha-feto-protein; ALS4 = amyotrophic lateral sclerosis; AOA1 = ataxia with oculomotor apraxia type 1; AOA2 = ataxia with oculomotor apraxia type 2; ARCA = autosomal recessive cerebellar ataxia; AT = ataxia-telangiectasia; DD = disease duration; HD = helicase domain; OMA = occasional oculomotor apraxia; SDFS = spinocerebellar degeneration functional score corrected; *SETX* = Senataxin gene

Introduction

Ataxia with oculomotor apraxia type 2 (AOA2) belongs to the autosomal recessive cerebellar ataxias (ARCAs), which are rare and early-disabling neurodegenerative diseases, dominated by Friedreich ataxia (Campuzano *et al*., 1996; Durr *et al*., 1996). AOA2 is caused by mutations in the senataxin (*SETX*) gene (Moreira *et al*., 2004). The onset of the disease usually occurs between 12 and 20 years of age (Criscuolo *et al*., 2004, 2006; Le Ber *et al*., 2004; Duquette *et al*., 2005; Anheim *et al*., 2008; Tazir *et al*., 2009). The clinical phenotype is characterized by progressive cerebellar ataxia, sensorimotor peripheral neuropathy, occasional oculomotor apraxia (OMA), strabismus, chorea and/or dystonia. Laboratory examination of AOA2 reveals prominently elevated alpha-feto-protein (AFP) serum levels (Watanabe *et al*., 1998*b*; Izatt *et al*., 2004) and, less frequently, elevated creatine kinase (CK) serum level (Watanabe *et al*., 1998*b*). We have recently reported slightly elevated AFP levels in healthy subjects with *SETX* heterozygous mutation (Anheim *et al*., 2008). Brain magnetic resonance imaging (MRI) shows diffuse cerebellar atrophy and electroneuromyography (EMG) confirms the peripheral neuropathy. A post-mortem study revealed a marked loss of Purkinje cells as well as mild fibrous gliosis that was more severe in the vermis than in the hemispheres (Criscuolo *et al*., 2006).

AOA2 belongs to the group of ARCAs with OMA (Le Ber *et al*., 2005, 2006) which also includes ataxia-telangiectasia (AT) due to mutations in the AT mutated (*ATM)* gene (Savitsky *et al*., 1995; Chun and Gatti, 2004); ataxia with oculomotor apraxia type 1 (AOA1) related to mutations in the aprataxin (*APTX*) gene (Date *et al*., 2001; Tranchant *et al*., 2003; Le Ber *et al*., 2004; Moreira *et al*., 2004) with initial signs usually occurring earlier than AOA2; and AT-like disorder (ATLD) related to mutations in the *MRE11* gene (Stewart *et al*., 1999; Fernet *et al*., 2005). AOA2 and AT patients also share elevated AFP levels but in contrast to AT, there is no increased sensitivity to ionizing radiation (Nahas *et al*., 2007) and no susceptibility to cancer in AOA2.

Mutations in *SETX* have also been reported to cause dominantly inherited juvenile amyotrophic lateral sclerosis (ALS4) (Chen *et al*., 2004) and dominant tremor-ataxia (Bassuk *et al*., 2007). ALS4 occurs before 25 years of age and is defined by limb weakness, severe muscle wasting, pyramidal signs, normal sensation and a slow disease progression (Chance *et al*., 1998).

The predicted protein encoded by *SETX*, which comprises 24 exons, is 2677 amino acids long and contains at its C terminus a classical seven-motif domain found in the superfamily 1 of helicases. Senataxin is suspected to be a DNA/RNA helicase (Moreira *et al*., 2004; Ursic *et al*., 2004; Chen *et al*., 2006) and is considered to be involved in the defence against DNA damage (Moreira *et al*., 2004; Suraweera *et al*., 2007) and in processing

non-coding RNAs. It has been demonstrated that senataxin is located in the nucleus of cycling cells (Chen *et al*., 2006; Suraweera *et al*., 2007). *SETX* missense mutations are mostly located at either the N-terminal domain or the C-terminal helicase domain (HD), supporting the fact they are both key functional domains (Bassuk *et al*., 2007).

Several series of AOA2 patients have been reported but some clinical features, such as the frequency of OMA, remain a controversial issue (Le Ber *et al*., 2004; Duquette *et al*., 2005; Criscuolo *et al*., 2006; Anheim *et al*., 2008; Tazir *et al*., 2009) and no predictive prognosis factor has been identified. Moreover, *SETX* is a large gene that is not readily amenable to routine sequencing. Here, we investigated a series of 90 AOA2 patients in order to define an efficient selection strategy for sequencing and to assess genotype–phenotype correlations. As none of the parents had neurological complaints, they were not extensively examined.

Patients and Methods

We retrospectively analysed the clinical, laboratory, electrophysiological, imaging and molecular data of all patients who underwent *SETX* sequencing in our laboratory between 2004 and 2008, because of suspected AOA2. Sequencing of *SETX* was performed upon request in case of cerebellar ataxia with elevated AFP level and/or in case of cerebellar ataxia associated with clinical, electrophysiological and imaging features consistent with AOA2: progressive cerebellar ataxia occurring between 5 and 25 years of age combined with peripheral neuropathy and/or cerebellar atrophy on MRI.

Clinical analysis

Age at onset of the disease, disease duration (DD) and age at last examination were noted as well as gender and geographic origin. Spinocerebellar degeneration functional score (SDFS) was used to evaluate the disability stage from 1 to 7 (0: no functional handicap; 1: no functional handicap but signs at examination; 2: mild, able to run, walking unlimited; 3: moderate, unable to run, limited walking without help; 4: severe, walking with one stick; 5: walking with two sticks; 6: unable to walk, requiring wheelchair; 7: confined to the bed). Other assessed clinical findings included vibratory sense, deep tendon reflexes, plantar reflexes, OMA (defined as an intermittent saccade failure causing dissociation between the eyes and head movements during the rotation of the head which reaches the target before the eyes), slow and/or saccadic pursuit, hypometric saccades, strabismus, *pes cavus*, chorea, dystonia, tremor and parkinsonism. Peripheral neuropathy was defined by decreased or absent tendon reflexes, distal lower limbs vibratory sense loss and/or motor deficit, and/or electrophysiological evidence of nerve conduction abnormalities. Pyramidal tract involvement was retained in case of extension response of plantar reflexes and/or increased or diffused tendon reflexes. Cerebellar atrophy was considered on MRI sagittal and axial slides by both a neuroradiologist and a neurologist. The spinocerebellar degeneration functional score corrected for DD (SDFS/DD ratio), which is not yet validated, was used to evaluate the progression rate of the disease. Although not precise, this ratio is easily recordable and was the only available for the entire set of patients. AFP and CK serum level assessment as well as EMG/nerve conduction studies and brain MRI were performed. MRIs were performed for at least one patient per family and were reviewed by both neuroradiologists and neurologists. For some patients, immunoglobulin (Ig) serum level assessment, karyotype and/or *ATM* sequencing were performed.

AFP level assessment in controls

Serum AFP levels were determined for control non-ataxic patients, control non-AOA2 non-AT patients and ataxic patients with suspected AOA2. AFP level assessment in controls was performed using the immunoanalysis Kryptor Brahms method as established in the *Laboratoire de Biochimie Générale et Spécialisée* (LBGS) in the University Hospital of Strasbourg, France. The dosage of AFP level in 100 healthy subjects with this method provided a median value of 3.2 μg/l with a 97.5 percentile at 7 μg/l, which was considered as the upper limit in our study. All healthy subjects had an AFP level between 0.5 and 15.7 μg/l. We tested for AFP levels in 102 control patients who had no cerebellar ataxia but were affected with Parkinson's disease ($n = 37$), atypical parkinsonism ($n = 13$), Huntington's disease ($n = 3$), dystonia ($n = 1$), Alzheimer's disease ($n = 11$), multiple sclerosis ($n = 11$), amyotrophic lateral sclerosis ($n = 2$), peripheral neuropathy ($n = 16$) and myopathy ($n = 6$). These patients were not investigated for AOA2 mutations. Moreover, a series of 31 non-AOA2 non-AT ataxic patients (who were investigated in another study; Anheim *et al*., 2009) underwent AFP level analysis.

Genetic analysis and genotype/phenotype correlation studies

We sequenced all exons of *SETX* from both the forward and reverse strands after purification of the polymerase chain reaction (PCR) products, as reported earlier (Moreira *et al*., 2004) (flanking primer sequences and PCR conditions are available on request). Sequences were analysed using the Seqpilot software version 2.0 (JSI medisys, Kippenheim, Germany). Quantitative *SETX* exon copy number analysis was performed as described previously (Arning *et al*., 2008). The AOA2 patients were divided into three groups according to the type of mutation and/or to the location of the mutation in *SETX*: (i) at least one missense mutation into the HD; (ii) at least one missense mutation outside the HD; and (iii) no missense mutation (nonsense mutation or frameshift insertion or deletion or large in frame deletion). The three groups were compared regarding their clinical, electrophysiological, imaging and biochemical features. Finally, truncating mutations in the HD were compared to truncating mutations out of the HD.

Statistical analysis

Patients' data were collected in a computerized database and analysed using the statistical software package Statistical Analysis System (SAS) for Windows, release 9.1.3 (SAS Institute Inc., Cary, NC, USA). Categorical variables were analysed with the χ^2 test and Yates' correction or Fisher's exact test when necessary. Non-parametric statistical methods were used for analysis, as most analysed quantitative variables could not meet the assumption of normality. Such variables are presented as the median (Q1:25th–Q3:75th percentiles), while categorical variables are presented as frequencies (percentage of patients).

We used Wilcoxon rank-sum test (two independent groups) and Kruskal–Wallis test (more than two independent groups) for all comparisons of quantitative variables across groups. Pearson and Spearman correlation coefficients were computed in order to assess the strength of the linear relationship between the two quantitative variables.

AFP serum level distribution was assessed using the Kernel density estimate. For AOA2+ patients with repeated AFP assessments, Wilcoxon signed-rank test was applied in order to perform a comparison between AFP values at baseline, contemporary to the diagnosis of the disease and at the end-point at which the last assessment was performed.

Results

Between 2004 and 2008, 125 patients underwent *SETX* direct sequencing (Fig. 1). Sixty-seven were published previously (Watanabe *et al*., 1998; Bomont *et al*., 2000; Nemeth *et al*., 2000; Izatt *et al*., 2004; Le Ber *et al*., 2004; Moreira *et al*., 2004; Anheim *et al*., 2008; Gazulla *et al*., 2009; Tazir *et al*., 2009) and 25 new *SETX* mutations were identified among the remaining patients (Table 1). One hundred patients had elevated AFP levels and an AOA2 diagnosis was established for 90 patients (AOA2+), including two with normal AFP levels at diagnosis (Table 2). The second mutation of a compound heterozygous patient for which only one mutation was previously reported (Anheim *et al*., 2008) has now been identified by copy number determination of all *SETX* exons and consists of a deletion of exons 10 to 13 (online Supplementary material). This deletion results in an in-frame protein deletion (Table 1), explaining why this deletion does not result in *SETX* RNA decay (Anheim *et al*., 2008). The most typical AOA2− patients were also analysed by *SETX* exon copy number determination and no deletion or duplication was identified (for example, see online Supplementary material).

AOA2+ patients: 90 patients

The main features of the AOA2+ group are presented in Tables 2 and 3. Forty-nine AOA2+ patients were male. Patients originated from France ($n = 20$), Algeria ($n = 20$), Portugal ($n = 19$), Germany ($n = 6$), Japan ($n = 5$), Pakistan ($n = 5$), Saudi Arabia ($n = 2$), Tunisia ($n = 2$), Norway ($n = 2$), England ($n = 2$), Spain ($n = 2$), Sudan ($n = 1$), Ireland ($n = 1$), Belgium ($n = 1$), Switzerland ($n = 1$) and Canada ($n = 1$). Median age at onset was 14.0 years (Q1 = 12, Q3 = 17) and median current age was 32.0 years (Q1 = 25, Q3 = 40). Mean current SDFS was 4.68 ± 1.47 after a mean DD of 19 years, and mean SDFS/DD ratio was 0.39 ± 0.41. Most AOA2 patients needed unilateral or bilateral help for walking after 20 years of DD. The few patients who were wheelchair bound were so at a mean age of 29.9 years ± 3.84, after a mean DD of 15.3 years ± 3.52. Peripheral neuropathy, mostly axonal sensory motor neuropathy, was detected in 97.5% of patients. OMA was found in 51% of cases, saccadic pursuit without OMA in 4.5% and strabismus in 12.3%. Pyramidal signs were present in 20.5% of patients. Movement disorders were not rare, including head tremor (14%), dystonia (13.5%) and/or chorea (9.5%). *Pes cavus* was noted in all 21 patients for whom this item was documented. Cerebellar atrophy was found on MRI in 96% of patients. No AOA2 patient was lacking both peripheral neuropathy and cerebellar atrophy. Some AOA2 patients underwent several brain MRIs during the course of the disease, which revealed severe cerebellar atrophy early in the course of the disease (Fig. 2). Elevated AFP serum levels were found in 98% of patients at the first AFP level assessment and in 99% of patients during the course of the disease, with a median serum level of 31 μg/l. The single patient with normal AFP levels had levels at 6 μg/l (normal < 7 μg/l) after 27 years of DD and was part of a Pakistani sibship whose other affected members had moderately elevated AFP levels (Nemeth *et al*., 2000; Moreira *et al*., 2004). Another patient had normal AFP levels 12 years after the onset of the disease but AFP levels increased thereafter up to 31.5 μg/l and 29.3 μg/l, 4 and 7 years later, respectively. CK levels were moderately elevated in three patients, normal in 20, but not determined in 67 patients. Two patients presented with hypogonadotrophic hypogonadism: Patient 16 has previously been reported (Gazulla *et al*., 2009), and Patient 44 developed amenorrhoea at the age of 39 due to hypogonadotrophic hypogonadism with follicle-stimulating hormone (FSH) and lutenizing hormone (LH) at 69.6 IU/l and 41.4 IU/l, respectively.

In the AOA2+ group, age at onset was correlated with the progression rate of the disease that was evaluated using the SDFS/DD ratio ($r = 0.23$, $P = 0.04$). The progression rate of the disease was negatively associated with OMA (SDFS/DD ratio of 0.25 in patients with OMA and of 0.32 in patients without OMA; $P = 0.009$) and positively associated with strabismus [SDFS/DD median ratio of 0.38 (Q1 = 0.25, Q3 = 0.5) in case of strabismus and of 0.26 (Q1 = 0.20, Q3 = 0.35) in patients without strabismus; $P = 0.03$]. The earlier the onset, the higher was the frequency of strabismus ($P = 0.04$). The progression rate and the duration of the disease were not associated with incidence of peripheral neuropathy, pyramidal signs, cerebellar atrophy or AFP levels. AFP serum level was not correlated with age at onset of the disease. Surprisingly, AFP level was negatively associated with pyramidal signs (extensor plantar response; $P = 0.0008$): patients with pyramidal signs had a median AFP level of 16.6 μg/l (Q1 = 8.5, Q3 = 20.6) versus 37.7 μg/l (Q1 = 24, 53.4) for patients without pyramidal signs. AFP level was negatively associated with strabismus 21.5 μg/l (Q1 = 7, Q3 = 43.7) versus 35.5 μg/l (Q1 = 21, Q3 = 54) and with head tremor 20 μg/l (Q1 = 9, Q3 = 45) versus 35 μg/l (Q1 = 21, Q3 = 54) ($P = 0.03$ and 0.02, respectively) but was associated with neither progression rate nor OMA.

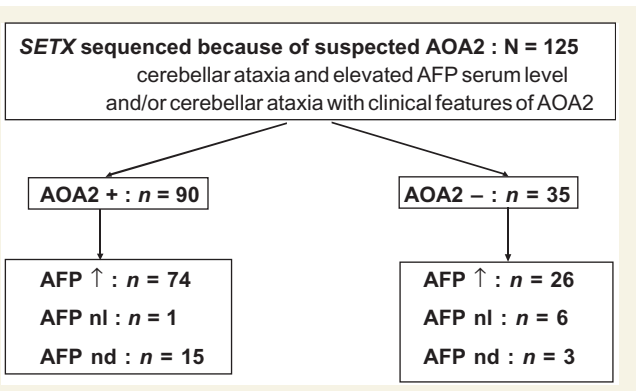

Figure 1 Classification of the 125 patients who underwent *SETX* sequencing showing the molecular diagnosis and serum AFP levels. *n* = number of patients; ↑ = elevated serum level; nl = normal serum level; nd = not documented.

Table 1 Description of the 25 new mutations

Patient number (geographic origin)	Nucleotide change (exon)	Amino acid change	Mutation status
3 (France)	5413C>T (10)	P1805S	Homozygous
6 (Ireland)	7139G>A (22)	R2380Q	Heterozygous[a]
10 (France)	2387_2390del AGAA (8)	fs after K796	Homozygous
15 (France)	2659C>T (8)	Q887X	Homozygous
26 (France)	*5264del C (8)*[b] del exons 10, 11, 12 and 13	*fs after N1784*[b] V1792_L2035del	Compound heterozygous
39 (Norway)	6792A>G (19)	I2264M	Homozygous
40 (Norway)	6792A>G (19) 4036C>T (8)	I2264M Q1346X	Compound heterozygous
41 (France)	6029A>G (13)	N2010S	Homozygous
44 (Saudi Arabia)	6340_6341del GA (16)	fs after D2113	Homozygous
46 (Saudi Arabia)	3604del G (8)	fs after I1201	Homozygous
47 (France)	7138C>G (22)	R2380G	Homozygous
48 (France)	2659C>T (8)	Q887X	Homozygous
49 (Algeria)	915G>T (6)	W305C	Homozygous
54 (France)	7240C>T (23)	R2414X	Homozygous
61 (Germany)	6590A>G (18)	H2197R	Homozygous
67 (France)	994C>T (6) 2966_2970del GGAAA	R332W fs after Q988	Compound heterozygous
69 (France)	5249ins T (8)	fs after L1750	Homozygous
89 (Belgium)	del exons 6, 7 and part of 8	fs after D279	Homozygous
90 (France)	*5929C > T (12)*[c] 7000_7012del 13ins T (21)	*L1977F*[b] R2234_S2338del ins C	Compound heterozygous
94 (Switzerland)	3070_3073del GATG (8)	fs after D1023	Homozygous
96 (England)	7157T>C (22)	I2386T	Heterozygous
117 (Portugal)	6017G>A (13) 6831_6836del AAAAAC (19)	C2006Y KT2278_2279del	Compound heterozygous
123 (Portugal)	*5308_5311del GAGA*[d]	*fs after R1769*	Homozygous
126 (Portugal)	7089C>G (exon 21)	F2363L	Homozygous

a For Patient 6, only one of the two mutations was identified.
b Previously reported mutation in a compound heterozygous patient (Anheim *et al*., 2008).
c Previously reported mutations but in distinct patients, (Fogel and Perlman, 2007).
d Previously reported mutations but in distinct patients (Nicolaou *et al*., 2008).
del. = deletion; ins. = insertion; fs = frameshift.

Table 2 Main quantitative variables of AOA2+ and AOA2− patients

	AOA2+ ($N^a = 90$)				AOA2− ($N^a = 35$)				*P**
	Min	Med	Mean (SD)	Max	Min	Med	Mean (SD)	Max	
Age at onset	7.0	14.0	14.6 (3.4)	25.0	1.0	12.0	15.9 (16.0)	71.0	0.03
Current age	10.0	32.0	33.5 (11.7)	68.0	4.0	31.0	34.4 (17.5)	77.0	NS
Disease dur.	1.0	17.0	18.9 (11.8)	55.0	3.0	17.0	18.5 (12.9)	46.0	NS
Current SDFS	1.0	5.0	4.7 (1.5)	6.0	2.0	5.0	4.35 (1.4)	6.0	NS
Progression rate[b]	0.11	0.26	0.39 (0.41)	3.0	0.07	0.27	0.39 (0.35)	1.67	NS
AFP[c]	5.0	31.0	41.8 (34.6)	185.2	0.7	13.8	27.6 (42.3)	236.0	0.0004

a Number of patients.
b Progression rate evaluated with SDFS/DD score.
c AFP serum level.
*Wilcoxon rank-sum test.
Min = Minimum; Med = Median; SD = Standard deviation; Max = Maximum; Dur = Duration; NS = not significant.

AOA2− patients: 35 patients

In the AOA2− group, median age at onset was 12.0 years of age (Q1 = 4, Q3 = 15) and median current age was 31.0 years (Q1 = 22, Q3 = 50; Table 2). Mean current SDFS was 4.35 ± 1.38 S.D after a mean DD of 18.5 years. Peripheral neuropathy was found in 64.5% of patients, OMA in 40.6%, saccadic pursuit without OMA in 11.8%, pyramidal signs in 25.8% and no strabismus was noted (Table 3). Movement disorders were observed, including head tremor (15%), dystonia (18.5%), chorea (21.4%) and parkinsonism (one patient). Cerebellar atrophy was found on MRI in 89% of patients.

Table 3 Main qualitative variables, in percent, of AOA2+ and AOA2− patients

	AOA2+ ($N^a = 90$) (%)	AOA2− ($N^a = 35$) (%)	P*
Elevated AFP	99	81	0.003
Peripheral neuropathy	97.5	64.5	<0.0001
Cerebellar atrophy	96	89	NS[b]
Oculomotor apraxia (OMA)	51	40.6	NS[b]
Pyramidal signs	20.5	25.8	NS[b]
Head tremor	14	15	NS[b]
Dystonia	13.5	18.5	NS[b]
Strabismus	12.3	0	0.03
Chorea	9.5	21.4	NS[b]
Saccadic pursuit without OMA	4.5	11.8	NS[b]

a Number of patients.
b Not statistically significant.
* χ^2 test.

Elevated AFP levels were found in 81% of patients, with a median serum level of 13.8 μg/l (Q1 = 9, Q3 = 40). CK levels were elevated in three patients, normal in 15 patients and not determined in 17 patients. There was no correlation between the current SDFS or AFP level and the DD. Age at onset was negatively associated with pyramidal signs ($P = 0.03$): patients with pyramidal signs had median onset of the disease at 24 (Q1 = 12, Q3 = 39.5) years versus 12 (Q1 = 3, Q3 = 14) for the patients without pyramidal signs. Friedreich ataxia was excluded in all AOA2− patients. Three AOA2− patients had clinical and biological presentations consistent with AT. AT was confirmed in one and is pending in the other two. AOA1 was diagnosed in two patients, who had AFP levels of 10 μg/l and 17.8 μg/l, respectively. AOA1 was excluded in six other AOA2− patients.

Comparison of AOA2+ with AOA2− patients

The main features of the AOA2 patients (AOA2+) and of the patients without *SETX* mutations (AOA2−) are presented in Tables 2 and 3. AFP levels differed significantly between the two groups ($P = 0.0004$) and were higher in AOA2+ (med = 31.0, Q1 = 20.0, Q3 = 50.7 μg/l) than in AOA2− (med = 13.8, Q1 = 9.0, Q3 = 40.0 μg/l) despite the fact that a few AOA2− patients had ataxia-telangiectasia and therefore very high AFP levels. AFP levels were more frequently elevated in AOA2+ than in AOA2− ($P = 0.003$). Age at onset of the disease was later in AOA2+ than in AOA2− ($P = 0.03$). There was no difference between the AOA2+ and AOA2− groups regarding the progression rate of the disease, the frequency of OMA nor the frequency of pyramidal signs, which are not rare in AOA2. Peripheral neuropathy was more frequent in AOA2+ than in AOA2− patients ($P < 0.0001$) but cerebellar atrophy on MRI was as frequent in both groups. Strabismus was more frequent in AOA2+ than in AOA2− ($P = 0.03$) as well as *pes cavus* ($P = 0.03$) but not head tremor, chorea or dystonia.

AFP serum level distribution

The AFP level distribution of the non-ataxic control patients, of the AOA2− patients, and of the AOA2+ patients is shown in Fig. 3A. The density is presented as a function of the logarithm base 10 of AFP serum level in order to obtain Gaussian distributions. The mean AFP level of the normal controls (3.4 ± 4 μg/l, range from 0.5 to 17.0 μg/l), non-AOA2 patients and non-AOA2 non-ataxia-telangiectasia patients from a distinct study (Anheim *et al*., 2009) were identical and lower than the mean level of AOA2− patients (27.6 ± 42.3 μg/l). The AOA2− group showed a broad AFP level distribution ranging from normal, slightly elevated (due to selection criteria) and markedly elevated corresponding to confirmed and potential ataxia-telangiectasia patients. The AOA2+ patients have a mean AFP level higher than that for AOA2− patients ($P = 0.0004$). The probability for the control patients to have an AFP <7 μg/l is 89.8%. The probability for an AOA2+ patient to have an AFP ⩾7 μg/l is 97.6%.

Time course of AFP serum level

By analysing the 12 AOA2+ patients with several AFP serum assessments (Fig. 3B), we found no difference with the Wilcoxon signed-rank test between AFP serum level measured at the diagnosis of the disease and the AFP level at the end-point, which corresponds to the last assessment ($P = 0.66$). The mean duration between the diagnosis of ataxia and the end-point was 55.7 months. Thus, the AFP level may be considered to be stable in the course of the symptomatic phase of AOA2. Figure 3B indicates that among the 12 AOA2 patients, six shared a moderate increase of AFP levels after the occurrence of the first signs of the disease and then a stability of AFP levels. However, some patients shared a slight decrease of the AFP level after the beginning of the disease.

Senataxin gene sequencing strategy according to AFP serum level

In our 102 non-ataxic control patients, the median AFP level was 3.4 μg/l with the 5th percentile at 1.6 μg/l and the 95th percentile at 9 μg/l. We considered the normal AFP level to be <7 μg/l based on the study of 100 healthy individuals assessed in the LBGS in Strasbourg, France. By using the Gaussian distribution of AFP level among AOA2 and control patients, we calculated the risk to miss an AOA2 diagnosis by sequencing only non-Friedreich ataxia non-ataxia-telangiectasia ataxic patients with AFP ⩾ 7 μg/l according to the following Bayesian probability:

$P(\text{AOA2/AFP} < 7) = [P(\text{AFP} < 7/\text{AOA2} \times P(\text{AOA2})]/\{[P(\text{AFP} < 7/\text{AOA2}) \times P(\text{AOA2})] + [P(\text{AFP} < 7/\text{non-AOA2}) \times P(\text{non-AOA2})]\}$,

non-AOA2 being the population of non-AOA2 ataxic patients. With $P(\text{AFP} < 7/\text{AOA2}) = 0.024$, $P(\text{AOA2}) = 0.08$ (AOA2 prevalence estimated at 8% for the population of non-Friedreich ataxia non-ataxia-telangiectasia ataxic patients (Le Ber *et al*., 2004), $P[(\text{AFP} < 7)/\text{non-AOA2}] = 0.90$ and $P(\text{non-AOA2}) = 0.92$,

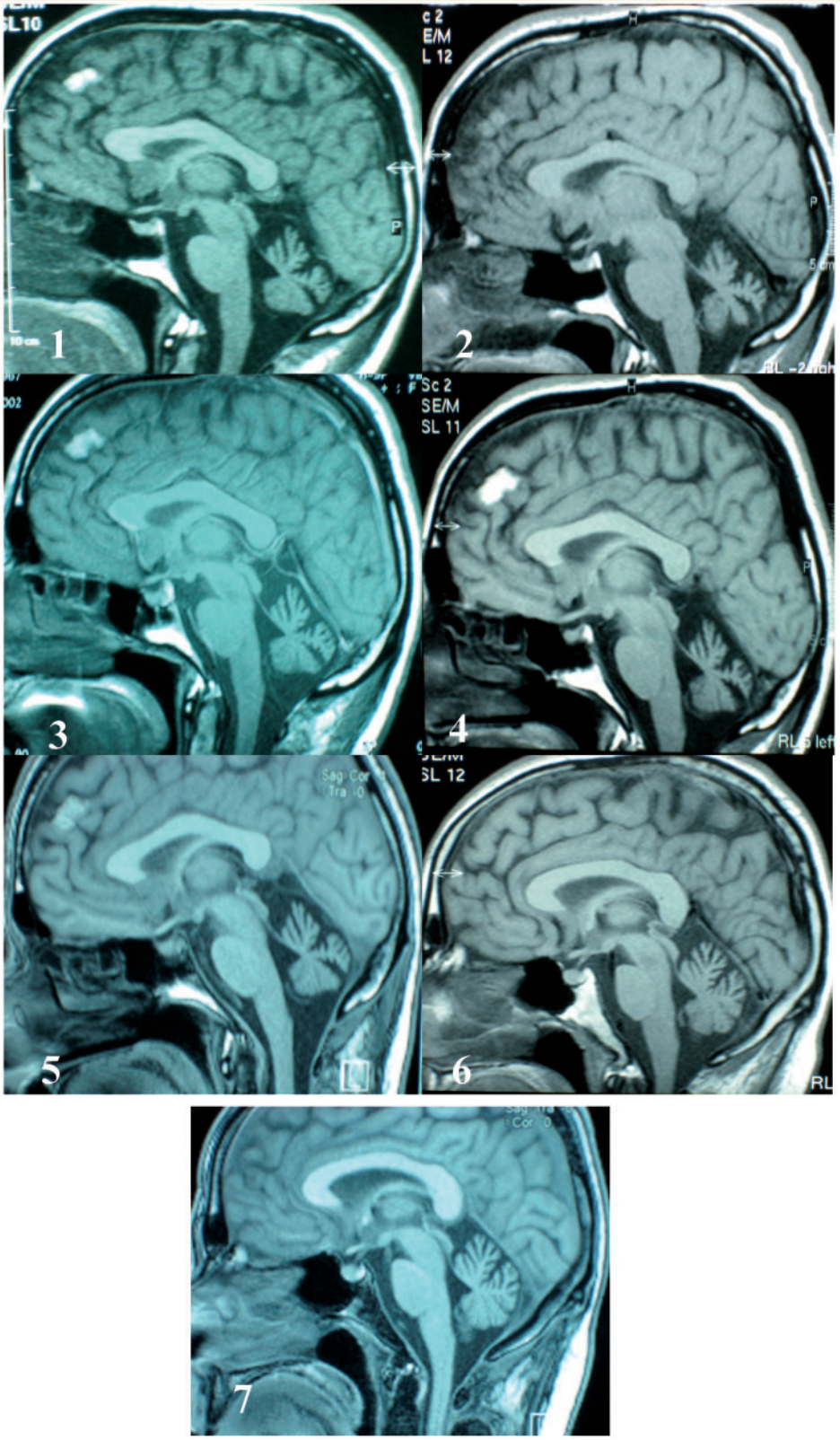

Figure 2 Sagittal T_1-weighted brain resonance magnetic imaging slices showing the stability over several years of the cerebellar atrophy in patients affected with AOA2. Marked cerebellar atrophy in Patient 27 after 15 years (**1**), 19 years (**2**), 21 years (**3**), 25 years (**4**) and 27 years (**5**) of DD (current age 39 years). Moderate cerebellar atrophy in Patient 22 after 15 years (**6**) and 17 years (**7**) of DD.

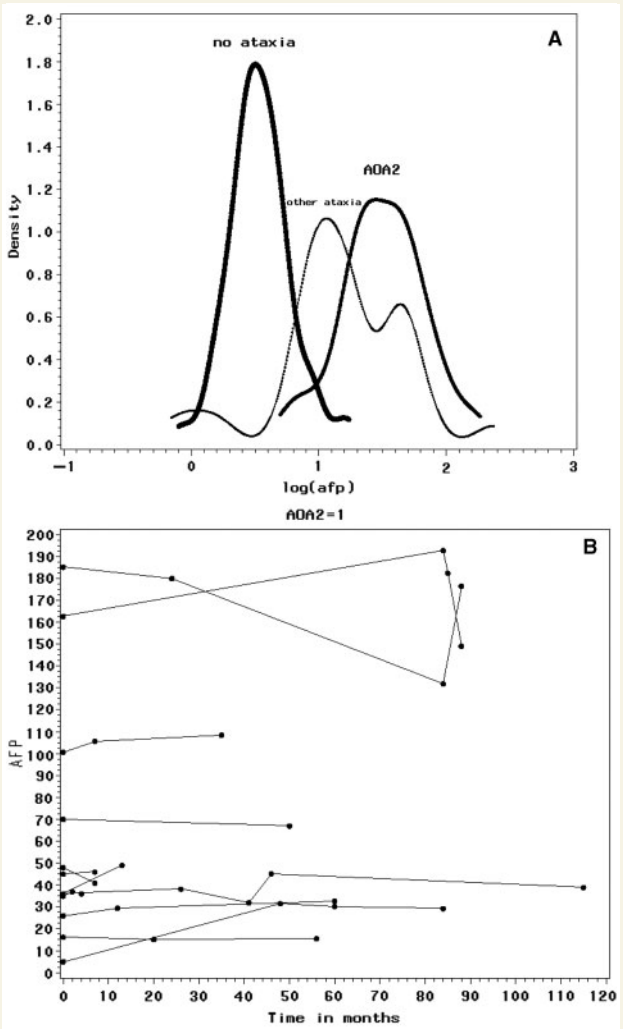

Figure 3 AFP studies in patients. (**A**) Distribution of AFP levels in 102 non-ataxic control patients (no ataxia), in the 35 AOA2− patients (other ataxias) and in the 90 AOA2+ patients (AOA2). The density of points is presented in function of the logarithm base 10 of the AFP serum level. (**B**) Time course of AFP serum levels in the 12 AOA2 patients for which we had several AFP level assessments.

the probability for a non-Friedreich ataxia non-ataxia-telangiectasia ataxic patient with an AFP below 7 μg/l to be affected with AOA2 was 0.23%.

We then calculated the probability for a non-Friedreich ataxia non-ataxia-telangiectasia ataxic patient to be affected with AOA2 in case of AFP ⩾ 7 μg/l using the following Bayesian ratio:

$P(\text{AOA2/AFP} \geqslant 7) = [P(\text{AFP} \geqslant 7/\text{AOA2}) \times P(\text{AOA2})]/\{[P(\text{AFP} \geqslant 7/\text{AOA2}) \times P(\text{AOA2})] + [P(\text{AFP} \geqslant 7/\text{non-AOA2}) \times P(\text{non-AOA2})]\}$.

With $P(\text{AFP} \geqslant 7/\text{AOA2}) = 0.976$, $P(\text{AFP} \geqslant 7/\text{non-AOA2}) = 0.1$ and $P(\text{non-AOA2}) = 0.92$ (Le Ber *et al*., 2004), this probability was 46%. Therefore, the selection of ataxic patients for *SETX* sequencing based on AFP serum levels appeared to be an efficient diagnosis strategy. A cut-off value of 7 μg/l or slightly less is a very reasonable threshold above which *SETX* sequencing should be recommended.

Genotype to phenotype correlation

We compared the phenotype of the AOA2+ patients according to the type and/or the location of the mutations in *SETX*. Eighteen AOA2+ patients had at least one missense mutation outside the HD, 15 had at least one missense mutations in the HD and 54 had only in-frame deletions or truncating mutations. Firstly, we compared truncating mutations and deletions with missense mutations. Only pyramidal signs were correlated with type of mutation, being more frequent in patients with missense mutations than in patients with deletion or truncating mutations ($P = 0.01$). There was no difference between the two groups regarding progression rate, dystonia, OMA, peripheral neuropathy or AFP level.

In order to further understand the increased rate of pyramidal signs when associated with missense mutations, we divided this group into patients with missense mutations in the HD and patients with missense mutations outside the HD (Fig. 4). The frequency of pyramidal signs was different in the three groups ($P = 0.001$) with a high frequency for missense in the HD (57%), compared to missense out of the HD (18.7%, $P < 0.05$) and to deletion and truncating mutations (11.5%, $P = 0.0002$). With this grouping of mutations, there were also statistically significant differences between the three groups considering dystonia ($P = 0.01$) and the progression rate of the disease measured with the SDFS/DD ratio ($P = 0.01$), with a clear difference between mutations in or out of the HD. The frequency of dystonia was 41.7% for missense mutations in the HD, 6.7% for missense mutations out of the HD and 8.7% for deletions or truncating mutations. The disease was more severe in cases of missense mutations out of the HD (SDFS/DD median ratio = 0.32, Q1 = 0.26, Q3 = 0.50) than in the HD (SDFS/DD median ratio = 0.21, Q1 = 0.16, Q3 = 0.27) and possibly also more severe than in the cases of deletions or truncating mutations (SDFS/DD median ratio = 0.26, Q1 = 0.21, Q3 = 0.40). No significant difference was found considering the age at onset of the disease or the AFP level. There was no difference between the progression rate of AOA2 patients with truncating mutations in the HD and out of the HD.

Discussion

Here, we report the features of 125 ataxic patients who underwent *SETX* sequencing because of suspected AOA2.

AOA2+

We present correlation studies of a large cohort of 90 patients affected with AOA2, originating from 15 different countries worldwide, including 65 previously reported patients (Watanabe *et al*., 1998*a*; Nemeth *et al*., 2000; Izatt *et al*., 2004; Le Ber *et al*., 2004; Moreira *et al*., 2004; Duquette *et al*., 2005; Asaka *et al*., 2006; Criscuolo *et al*., 2006; Anheim *et al*., 2008; Tazir *et al*., 2009) as well as 23 newly identified patients whose clinical, electrophysiological, imaging and molecular features are in accordance with previously reported smaller series (Watanabe *et al*., 1998*a*; Nemeth *et al*., 2000; Izatt *et al*., 2004;

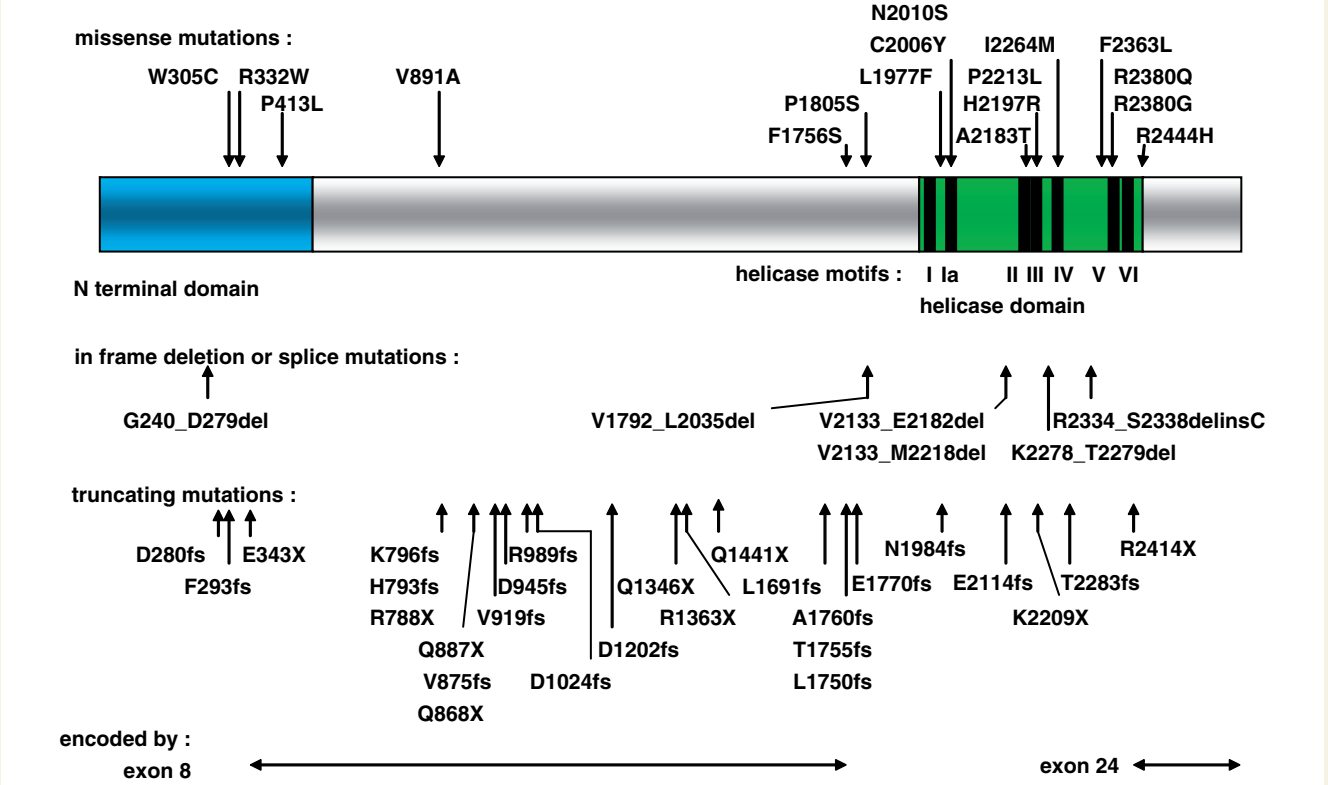

Figure 4 Distribution of the missense mutations, in frame deletion or splice mutations and truncating mutations along the *SETX* protein. Only six missense mutations were located outside of the helicase domain. Deletions and exons duplication (corresponding amino acid changes) are: Del exon 5 (G240_D279del), Del exons 6, 7 and part of 8 (D280fs), Dup exons 5, 6, 7 and 8 (A1760fs), Del exons 10 to 13 (V1792_L2035del), 6106G>A, exon 13 splice donor (N1984fs), 6546+5G>T, exon 17 splice acceptor (V2133_E2182del), Del exons 17 and 18 (V2133_M2218del). The portions of the protein encoded by the two large exons 8 and 24 are indicated at the bottom.

Le Ber *et al*., 2004; Moreira *et al*., 2004; Duquette *et al*., 2005; Asaka *et al*., 2006; Criscuolo *et al*., 2006; Anheim *et al*., 2008). AOA2 is a progressive, disabling cerebellar ataxia occurring within the second decade, usually around 15 years of age, associated mostly with axonal sensorimotor peripheral neuropathy (97.5%), cerebellar atrophy (96%) and elevated AFP levels (96%). Other signs may be encountered such as OMA (51%), pyramidal signs (20.5%) or, less frequently, strabismus, dystonia, chorea, head tremor and *pes cavus*. The mean age at onset in AOA2 is higher than in AOA1 and AT which are 7 years and 2–3 years, respectively (Savitsky *et al*., 1995; Moreira *et al*., 2001; Le Ber *et al*., 2003; Chun and Gatti, 2004) and is similar to the mean age at onset of Friedreich ataxia (Durr *et al*., 1996). However, the range of age at onset is broader in Friedreich ataxia (from 2 to 72 years of age) than in AOA2, which is from 7 to 25 years in our series. Interestingly, age at onset in AOA2 is correlated to the progression rate of the disease. The course of AOA2 appears to be less severe than in AT, AOA1 or Friedreich ataxia. Indeed, the majority of AOA2 patients are not wheelchair bound after a mean DD of 19 years, whereas most AT and AOA1 patients are wheelchair bound after a mean DD of 10 and 11 years, respectively (Woods and Taylor, 1992; Le Ber *et al*., 2003). In case of ataxia with elevated AFP levels, strabismus was very suggestive of AOA2 as it was not found at all in the group of AOA2− patients. Moreover, the lack of both peripheral neuropathy and cerebellar atrophy excludes the diagnosis of AOA2 since all patients in our series had one of the two signs. Cerebellar atrophy seems to be an early sign of AOA2, which stabilizes after several years of DD.

OMA was found in 50% of our patients and was negatively correlated with the progression rate of the disease. OMA is an optional finding in AOA2 and is less frequent than in AOA1 where it is found in 86% of patients (Le Ber *et al*., 2003). OMA in AOA2 is a controversial issue. OMA was initially defined as the inability to generate volitional horizontal saccades. Oculomotor recordings in AOA2 may reveal increased horizontal saccade latencies and hypometria (Le Ber *et al*., 2004). Other oculomotor signs may be encountered in AOA2 including strabismus which, contrary to OMA, is positively correlated with the progression rate of the disease in our series. Pyramidal involvement, described in 20.5% of our AOA2 patients, is not a rare feature although it was only found in four out of 60 previously reported AOA2 patients (Le Ber *et al*., 2004; Duquette *et al*., 2005; Criscuolo *et al*., 2006; Anheim *et al*., 2008). Interestingly, pyramidal signs were found to be negatively correlated with AFP levels. We describe one AOA2 case with hypogonadotrophic hypogonadism causing amenorrhoea. This contrasts the reported AOA2 case with ovarian failure related to hypergonadotrophic hypogonadism (Lynch *et al*., 2007),

therefore extending the spectrum of extraneurologic signs in AOA2.

AOA2− patients

In contrast to the AOA2+ group, the AOA2− group appeared to be heterogeneous. Indeed, there was no correlation in this group between current SDFS and DD. Two AOA2− patients were subsequently diagnosed with AOA1, demonstrating that elevated AFP levels may also be found in AOA1.

AFP serum level

In the case of sequencing *SETX* only in ataxic patients with AFP levels >7 μg/l, the probability to miss an AOA2 diagnosis has been found to be 0.23%. On the other hand, the probability for a non-Friedreich ataxia non-AT ataxic patient with AFP levels >7 μg/l to have AOA2 is 46%. Thus, the AFP values at or ~7 μg/l appear to be a good cut-off for selecting which patient should undergo sequencing of *SETX*, given the hurdles to sequence this large gene. In our series, AOA2+ had AFP levels higher than AOA2− patients, despite the fact that patients were partly selected because of elevated AFP levels. In AOA2+ patients, there was no correlation between AFP levels and progression rate or DD. No correlation was found between AFP levels and DD despite a mean period of nearly 5 years between the first and last measurements. However, one patient presented with delayed AFP level increase after a normal AFP level period at the beginning of the disease. We may hypothesize that AFP levels increases in the pre-symptomatic stage of AOA2 and then stabilize, in contrast to AT where patients present a progressive increase of AFP levels (Stray-Pedersen *et al*., 2007) and higher values, mostly >70 μg/l. In case of ataxia with normal AFP levels (<7 μg/l) in the early stage of the disease, the diagnosis of AOA2 is unlikely (0.23%) but a second assessment of AFP levels, 1 year later for instance, is highly recommended. AFP appears to be a more useful biomarker for AOA2 diagnosis than hypoalbuminaemia for AOA1 diagnosis since the latter occurs late in the course of the disease (Le Ber *et al*., 2003). The pathophysiology of elevated AFP levels in AOA2 remains unclear but a hepatic origin is suspected in AT patients. The defect of polynucleotide unwinding in the AOA2 liver may be the cause of aberrant AFP gene transcription. Whether AFP has a pathogenic role in AOA2 or is only a biochemical marker remains unclear.

Genotype to phenotype correlation

Little is known about genotype/phenotype correlation in AOA2. It has been suggested that homozygous *SETX* missense mutations in the N-terminal domain or in the HD were causing similar phenotypes to those due to deletions, nonsense and/or frameshift mutations (Chen *et al*., 2006; Bassuk *et al*., 2007). However, we found that missense mutations in the HD caused less severe AOA2 phenotypes than those due to missense mutations out of the HD or those due to deletions and truncating mutations of *SETX* and are more frequently associated with pyramidal signs and dystonia. The present study highlights the fact that pyramidal signs and dystonia are important features of AOA2. The lower frequency of pyramidal signs in patients with truncating or missense mutation out of the HD could be due to the masking of the pyramidal signs by severe motor neuropathy. The fact that missense mutations out of the HD are causing more severe phenotypes than missense mutations located in the HD could be consistent with the existence of one or more additional functional domains in senataxin. This may include the N-terminal domain and a conserved domain located just before the HD. However, further studies are needed to confirm this hypothesis.

Acknowledgements

We wish to thank A. Nemeth, M. Pandolfo, J. Schulz, P. Calvas, A. Munnich, P. Aubourg, E. Roze, C. Tallaksen, D. Devos, L. Perrin, C. Verny, M.T. Calvo, I. Benavente, P. Modrego, I. Pérez, S. Elmalik, M. Kabiraj, A. Ahmed, M. Manto, T. Kuntzer and M-C. Moreira for up-dates on previously reported patients and for referring AOA2− patients.

Funding

The Ministère de la santé, de la famille et des personnes handicapées (réseau neuromusculaire DHOS-OPRC No. 2003-227); the Agence Nationale pour la Recherche (grant ANR-05-MRAR*013-01); the Institut National de la santé et de la recherche médicale and the Centre National de la Recherche Scientifique. B; the GIS-Maladies Rares of the French Agence Nationale pour la Recherche (to B.M.); and the CRCM (Project No. 05-495 to S.M.), College of Medicine, King Saud University, Riyadh, Saudi Arabia.

Supplementary material

Supplementary material is available at *Brain* online.

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