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A new autosomal recessive spastic ataxia associated with frequent white matter changes maps to 2q33–34

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Recessive ataxias are a heterogeneous group of diseases. We identified a group of 23 French–Canadian cases belonging to 17 families affected by an autosomal recessive spastic ataxia associated with frequent white matter changes. The fact that 59% of these families have a genealogical relationship to the Portneuf County of Quebec suggests that this is a new form of ataxia with a regional founder effect. All cases present with cerebellar ataxia and spasticity. There is great intrafamilial and interfamilial variability, as illustrated by the spectrum of age of diagnosis (range: 2–59 years, mean: 15.0) and the presence of white matter changes on MRI in 52.4% of cases. The more severe cases have spasticity from birth, scoliosis, dystonia and cognitive impairment and were considered cases of cerebral palsy. Brain MRI constantly shows cerebellar atrophy, which in some cases may be associated with cortical atrophy, leucoencephalopathy and corpus callosum thinning. A genome wide scan uncovered linkage of three families to marker D2S2321 localized on chromosome 2q33–34. Linkage analysis confirmed that all families are linked to the same region [multipoint log of the odds (LOD) score of 5.95]. Haplotype analysis and allele sharing suggest that one common mutation may account for 97% of carrier chromosomes in Quebec. The uncovering of the mutated gene may point to a common pathway for pyramidal and cerebellar degeneration as both are often observed in recessive ataxias and complicated paraplegias.

Keywords: spastic ataxia; paraplegia; founder effect; linkage; genome-wide-scan

Abbreviations: ARSACS = spastic ataxia of Charlevoix–Saguenay; ARSAL = autosomal recessive spastic ataxia with frequent leucoencephalopathy; FRDA = Friedreich ataxia; IAHSP = infantile ascending hereditary spastic paralysis; LOD = log of the odds; PCR = polymerase chain reaction; GWS = genome wide scan

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Introduction

Recessive ataxias are a heterogeneous group of neurodegenerative diseases. To date, the mutated genes for 10 recessive ataxias have been uncovered and two others have been mapped (Van de Warrenburg *et al.*, 2005). The combination of pyramidal and cerebellar signs has been observed in a few recessive ataxias, complicated paraplegias, leucodystrophies and cerebral palsy (Leegwater *et al.*, 2001; Zhao *et al.*, 2001; Koenig, 2003). Spasticity has been observed in particular in the following well-characterized recessive ataxias: Friedreich ataxia (FRDA), spastic ataxia of Charlevoix–Saguenay (ARSACS) (Bouchard *et al.*, 1978) and to as lesser extent in ataxia with vitamin E deficiency (AVED) (Koenig, 2003). In the French–Canadian population, FRDA and ARSACS are the most common forms of spastic ataxias. FRDA in French–Canadians has been associated with a regional cluster in the Rimouski area (Bouchard *et al.*, 1979), though FRDA cases are

found in all regions of Quebec and in the Acadian populations of New Brunswick and Nova Scotia (Keats *et al.*, 1989; Richter *et al.*, 1996). On the other hand, cases of ARSACS, as its name implies, originate most often from the Charlevoix and Saguenay regions. This being said, both conditions are present in several other populations (Mrissa *et al.*, 2000; Gucuyener *et al.*, 2001; El Euch-Fayache *et al.*, 2003; Criscuolo *et al.*, 2005; Hara *et al.*, 2005). In our study of recessive ataxias in Quebec we initially focused on cases of spastic ataxia with a distinct phenotype from FRDA and ARSACS that frequently have a genealogical relationship with the Portneuf County of Quebec. The geographical clustering to this small region of Quebec, known for a genetic founder effect for Tay-Sachs (Laberge *et al.*, 2005) raised the possibility that at least one other form of recessive spastic ataxia is prevalent in the French–Canadian population. To test this hypothesis we collected additional families with Portneuf ancestry to gather a more homogeneous group to facilitate linkage analysis. We also recruited other autosomal recessive spastic ataxia families not affected by FRDA and ARSACS without a known relationship with Portneuf County. This paper describes the variable clinical features and the identification of a novel locus for an autosomal recessive spastic ataxia frequently associated with leucoencephalopathy (ARSAL) on chromosome 2q33–34.

Subjects and methods

Clinical evaluation

Relying on a network of ataxia and neuromuscular clinics that cater to >500 ataxic patients, we were able to recruit serially 17 Quebec kindreds with cases affected by a new form of spastic ataxia not associated with a polyneuropathy (Fig. 1). Neither the presence of white matter changes on MRI nor a known Portneuf County ancestry was used as selection criterion. All probands and family members underwent a detailed neurological examination by experienced neurologists. In addition to an extensive clinical evaluation, most probands underwent the following complementary biochemical screening tests: blood and urine amino acids, vitamin B and E levels, serum lactate–pyruvate ratio, long-chain fatty acids and electromyographic (EMG) studies. All medical records and imaging were reviewed. A total of 21 of the 23 affected individuals underwent brain MRI using standard methods in different radiology departments. All MRI were reviewed by a senior neuroradiologist (J.L.). This project was approved by institutional Ethics Committee of the Centre de recherche du CHUM. Informed consent was obtained from all patients and all participant living family members.

Exclusion of loci of diseases with overlapping phenotype by mutation and linkage analysis

Genomic DNA was extracted from peripheral blood lymphocytes using standard methods. Screening for the FRDA (GAA)n mutation and the two common mutations of ARSACS in Quebec were performed on all cases. Genetic analyses were also performed in the majority of ARSAL patients for dominant CAG expansions in SCA1, SCA3, SCA6, SCA7, SCA8, SCA12, SCA17 and DRPL. Candidate gene loci genotyping and linkage was performed using selected polymorphic STR markers. Seven loci for recessive spastic ataxia or other disorders with phenotype overlap with ARSAL were studied: FRDA locus (9q13.3, 9p23); spastic paraplegia 5B (8p12); ARSACS locus (13q12); spastic paraplegia 11 (15q13); Huntington disease-like 3 (*HDL3*) (4p15.3); sodium channel modifier (*SCNM1*) gene (1q21); spinocerebellar ataxia with blindness/deafness (SCABD) locus (6p23); and *EIF2B5* gene (3q27). Markers were selected using the UCSC genome browser (http://genome.ucsc.edu, May 2004 assembly).

Genome wide scan and linkage analyses

A genome wide scan (GWS) of 500 markers was conducted at deCODE Genetics (Reykjavik, Iceland) on 14 samples from families B, C and E. Fine mapping was performed using primer sequences of polymorphic markers obtained from deCODE and Marshfield genetic maps. Polymerase chain reactions (PCRs) were performed using 20 ng genomic DNA in 8 μl PCRs containing 1× PCR buffer,

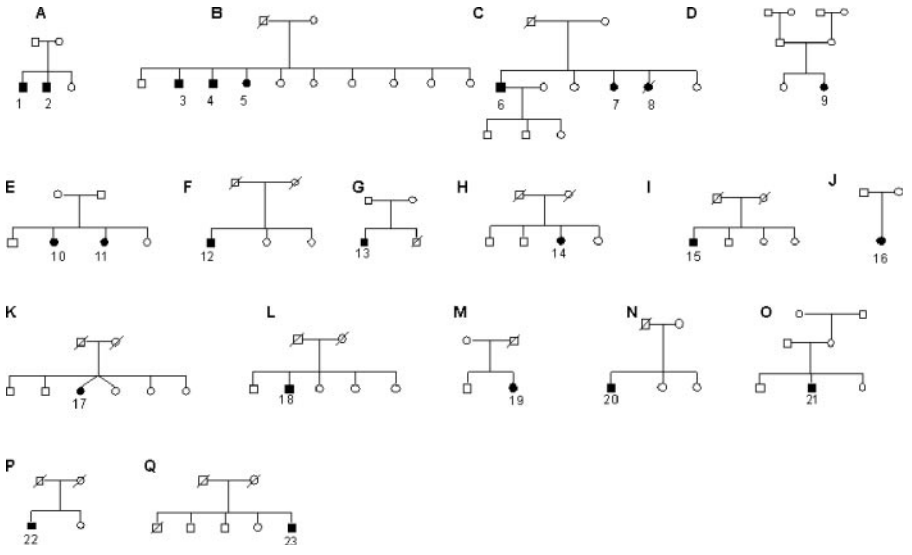

Fig. 1 Pedigrees of 17 French–Canadian ARSAL families. No consanguineous marriages have been reported. The symbols of family members affected by ARSAL are shaded black.

3 nM $MgCl_2$, 10 μM primer mix and 0.4 U Taq DNA polymerase (Invitrogen, Burlington, ON, Canada). Amplification conditions were obtained from the genome database (www.gdb.org). PCR products were amplified for allele-size analysis by adding 4 μl of STOP loading buffer to each sample, followed by a denaturing step of 5 min at 95°C, and final loading of 2 μl was onto a 64-lane 6% acrylamide gel containing 6 M urea. Data acquisition and analyses were performed using a Li-Cor 4100 automated DNA sequencer using BaseImagIR v.4.0 software (Li-Cor, ON, Canada).

Multipoint linkage analyses were performed using GENEHUNTER v.2.1. Marker order and genetic distances were based on the deCODE genetic map and UCSC (http://genome.ucsc.edu, May 2004 assembly) physical map. The haplotypes were reconstructed in a single section using the MAXPROB method of Genehunter v.2.1. The resulting haplotypes were imported into Cyrillic v.2.0 (Oxford, UK). For the candidate regions and the fine mapping analyses, all markers were analysed, assuming equal allele frequencies. The ARSAL phenotype was analysed as an autosomal recessive trait with complete penetrance of 100% on the basis of the observed pattern of affected individuals within the cohort, and with an estimated disease gene frequency of 0.001. No phenocopies were incorporated into the analysis.

Exclusion of candidate genes

A total of five candidate genes on chromosome 2q33–34 were studied for mutations: *ALSIN*, *EEF1B2*, *NRP2*, *NDUFS1* and *ALS2CR19* (entire coding and a minimum of 30 bp of intronic flanking sequences). Primers used to amplify exonic and flanking sequences were designed using ExonPrimer tool from UCSC website (http://ihg.gsf.de). *ALSIN*, *EEF1B2*, *NRP2* and *NDUFS1* genes were also investigated for mutations in alternative exons 5′- and 3′-UTR using Primer3 to design oligonucleotides (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). All designed primer sequences and PCR conditions were provided by Primer3. Large exons were divided into overlapping fragments. Oligonucleotide primers were synthesized by Invitrogen (Burlington, ON, Canada). The PCR products and primer pairs were sent to the McGill University and Genome Quebec Innovation Centre for forward and reverse sequencing. Sequences were aligned using SeqMan 4.03 (DNAStar, Wisconsin, USA) and analysed using Chromas 1.62 (Technelysium Pty Ltd, Australia; http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi. For the ALSIN screening, western blot analysis was done using protein extracted from lymphoblastoid cell lines generated from four unrelated affected patients. Each blot was probed with polyclonal antibodies specific for ALSIN protein provided by G. A. Rouleau.

Results

Clinical features of the French–Canadian ARSAL cohort

In Table 1 we summarize our findings on 23 patients belonging to 17 families that are linked to the ARSAL locus. All these French–Canadian cases were recruited serially because they were shown not to be affected by another known ataxia and presented a spastic ataxia without a polyneuropathy. Neither the presence of white matter changes nor a known Portneuf County ancestry was used as selection criterion. Phenotype segregation in pedigrees strongly suggests an autosomal recessive mode of inheritance (Fig. 1). None of the parents were known to be related or share family names. However, 10 of the 17 families (59%) have a known genealogical relationship with the Portneuf County (a 4098 km^2 region south of Quebec City). As shown on Table 1, all cases demonstrated ataxic gait, spasticity and hyperreflexia. The age of diagnosis is extremely variable (mean: 15.0 years, range: 2–59 years). The milder cases report that they were always less coordinated than their classmates and had leg stiffness in their youth. The majority of cases (57%) have urinary urgency. These symptoms respond well in all cases treated with anticholinergic medications. Other clinical features include ataxic and spastic dysarthria (74%), dystonic positioning (57%, including hemidystonia in one case), mild horizontal nystagmus (44%), scoliosis (35%), optic atrophy with cataract in two older patients (8.7%, Cases 3 and 4) and mild hearing impairment (13%, Cases 1, 14 and 20). Though formal neuropsychological evaluations were not performed, 10 cases seem to have mild cognitive impairment (44%) that limited their schooling.

One of the most remarkable features of ARSAL is the variability in the severity of the phenotype between siblings and cases from different families (Table 1). For instance, Case 4 from family B has been wheelchair-bound since the age of 19 while his 3-years younger brother (Case 3) has been using a wheelchair only since the age of 51. Their sister (Case 5), who has an intermediate presentation compared with her brothers, has been using a wheelchair since the age of 45. Therefore, not only is the age of diagnosis variable but the impact on walking is different, with 35% of participants not needing technical aid to walk. In the more severe cases, scoliosis (35%), dystonia (52%) and mild cognitive impairment (44%) are also present to a variable degree.

Brain MRI of 21 participants were reviewed by an experienced neuroradiologist (J.L.). One participant died before getting an MRI, while it was not requested for Case 11 because of her young age. Imaging demonstrated cerebellar atrophy in all patients (100%) (Table 1). The cerebellar atrophy involves proportionally the vermis and the cerebellar hemispheres and is mild to severe. Nine patients (42.9%) also show mild-to-moderate cerebral atrophy; in four patients the atrophy is more frontoparietal and in four patients the atrophy is more parieto-occipital. Corpus callosum atrophy was only observed in Family B (Fig. 2A and C). Eleven patients (52.4%) show non-specific white matter changes on T_2-weighted and FLAIR sequences on brain MRI (Fig. 2). These changes range from diffuse periventricular T_2-hyperintensities to mild punctiform T_2-hyperintensities localized in periventricular, deep white matter or juxtacortical white matter (Fig. 2B and D). Only two patients had white matter changes in the cerebellum and brainstem and one patient had white matter changes in the corpus callosum (Fig. 2B). The abnormal hyperintense T_2 white matter signals were observed mostly in the older patients with a mean age of 46.2 years (ranging from 13 to 59 years). These white

Table 1 Clinical and MRI findings on 23 ARSAL patients from 17 French-Canadian families

Family and patient	Portneuf ancestry	Age of diagnosis, year	Age at exam, year	Ataxia	Spasticity and hyperreflexia	Dysarthria	Neurogenic bladder	Dystonia	Nystagmus	Scoliosis	Wheel-chair use, year	Cognitive impairment	Cerebellar atrophy	Cerebral atrophy	White matter changes localization			
															PV	DWM	JC	PF
A-1	Yes	15	23	Yes	Yes	Yes	No	Yes	Yes	No	No	Mild	Moderate	Mild (AN)	–	–	–	–
A-2	Yes	3	19	Yes	Yes	Yes	No	Yes	No	No	No	Normal	Severe	None	–	–	–	–
B-3	Yes	3	55	Yes	Yes	Yes	Yes	Yes	Yes	Yes	51	Mild	Mild	Moderate (AN, PT)	+	+	+	–
B-4	Yes	6	52	Yes	Yes	Yes	Yes	Yes	Yes	Yes	19	Mild	Severe	Severe (PT, PF)	+	+	+	–
B-5	Yes	3	48	Yes	Yes	Yes	Yes	Yes	Yes	Yes	45	Mild	Mild	Moderate (PT, PF)	+	+	++	–
C-6	Yes	20	67	Yes	Yes	Yes	Yes	Yes	Yes	No	59	Normal	Moderate	Mild (PT)	++	+	+	–
C-7	Yes	17	59	Yes	Yes	Yes	No	No	Yes	No	No	Normal	Moderate	None	–	–	–	–
D-9	Yes	13	30	Yes	Yes	Yes	Yes	Yes	Yes	No	30	Normal	Mild	Mild (PT)	–	–	–	–
E-10	Yes	3	13	Yes	Yes	Yes	Yes	Yes	No	Yes	10	Normal	Moderate	None	+	–	–	–
E-11	Yes	3	7	Yes	Yes	Yes	Yes	No	No	No	No	Normal	n/a	n/a		n/a		
F-12	Yes	5	59	Yes	Yes	No	None	No	No	No	No	Mild	Mild	Moderate (AN, PT)	–	–	+	–
G-13	No	19	28	Yes	Yes	Yes	None	No	Yes	No	No	Normal	Severe	None	–	–	–	–
I-15	Yes	23	73	Yes	Yes	No	Yes	Yes	No	No	38	Mild	Mild	Mild (AN)	–	–	–	–
H-14	No	2	51	Yes	Yes	No	Yes	Yes	No	Yes	36	Mild	Mild*	None	–	–	–	–
J-16	Yes	59	51	Yes	Yes	No	None	No	No	Yes	72	Mild	Moderate	None	–	+	++	–
K-17	No	43	58	Yes	Yes	Yes	None	No	No	No	No	Normal	Moderate	None	–	+	++	–
L-18	No	26	40	Yes	Yes	No	None	Yes	No	No	38	Mild	Severe	None	–	–	–	–
M-19	Yes	4	52	Yes	Yes	Yes	Yes	No	No	No	No	Normal	Severe	None	–	–	–	–
N-20	No	2	16	Yes	Yes	Yes	No	No	No	Yes	26	Mild	Mild*	None	++	+	+	+++
O-21	No	9	38	Yes	Yes	Yes	Yes	No	Yes	No	16	Normal	Severe	None	–	–	+	–
P-22	No	32	51	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Normal	Moderate	Severe (PF)	–	–	–	++
Q-23	Yes	20	49	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Normal	n/a	n/a		n/a		
	59%	Mean 15 years	Mean 42.6 years	100%	100%	74%	57%	57%	44%	35%	52% Mean 36.6 years	44%	100%	42.9%		52.4%		

AN = anterior, PT = posterior, PF = posterior fossa, PV = periventricular, DWM = deep white matter, JC = juxtacortical, mild = +, moderate = ++, severe = +++, none = –
*Cerebellar atrophy was limited to the vermis, n/a = not available.

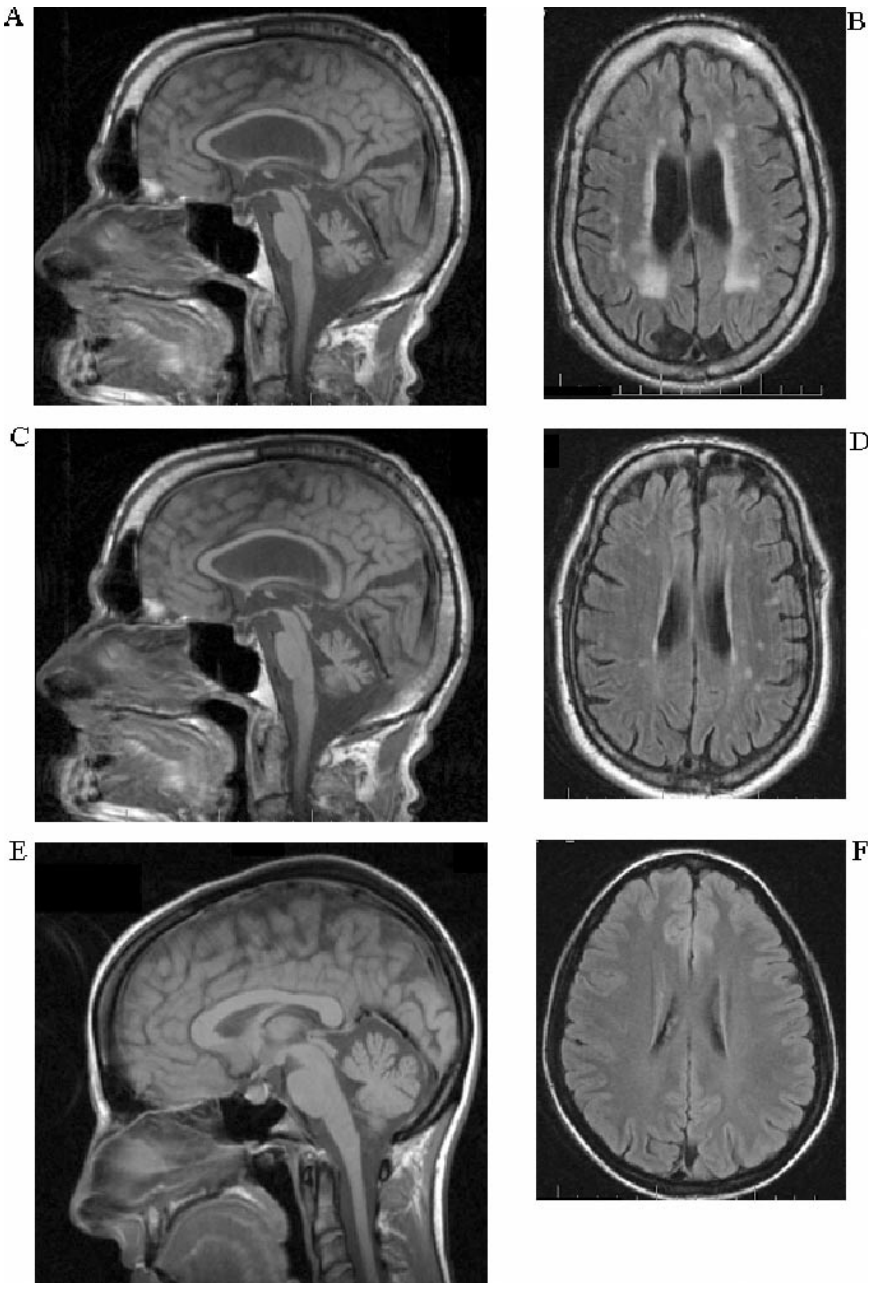

Fig. 2 Sagittal and coronal T_2-weighted MRI sequences demonstrating the variable cerebellar atrophy and white matter changes in three ARSAL cases. (**A** and **B**) Case 4 is the 52-year-old brother of Case 5 who has a more severe form of ARSAL. (**C** and **D**) Case 5 is the 46-year-old younger brother of Case 4 who has a less severe form of ARSAL. The degree of cortical and cerebellar atrophy and of white matter changes seem to correlate in this family (**B**) with the severity of the disease. (**E** and **F**) 30-year-old case with a mild-to-moderate ARSAL phenotype that is clearly less severe than that of Cases 4 and 5 (**A**, **B**, **C** and **D**). At age 30 she only has cerebellar atrophy and no white matter changes (Family E, Case 10).

matter changes being possibly later findings in this condition may explain why it is not universally present in our cohort. No correlation between the presence of white matter changes and the age of onset was noted, the mean age of onset of patients with leucoencephalopathy being 15.8 years compared with 15.0 for the entire cohort. The association of cognitive disabilities with white matter changes is not constant, but 6 out of 10 patients with such difficulties (60%) showed few to diffuse T_2 white matter hyperintense signals. The observation that the majority of our cases have a leucoencephalopathy led us to name this condition autosomal recessive spastic ataxia with frequent leucoencephalopathy (ARSAL). This name may help identify cases of ARSAL in other populations.

Exclusion of candidate loci and genome wide scan analysis

Exclusion of mutations in FRDA and ARSACS and linkage to disease loci with phenotype overlapping with ARSAL was undertaken using DNA extracted from peripheral blood lymphocytes. GAA triplet expansion causing FRDA and the two most common ARSACS mutations in French–Canadians were excluded in our cohort. The following eight candidate genes or disease loci did not demonstrate positive linkage: FRDA; spastic paraplegia 5B; ARSACS; spastic paraplegia 11; Huntington disease-like 3; *SCNM1*; *SCABD*; and *EIF2B5* (data not shown).

Fourteen DNA samples from affected and unaffected patients belonging to three unrelated ARSAL families (families B, C and E) with Portneuf County ancestry were sent to deCODE genetics (Reykjavik, Iceland) for a GWS. Multipoint log of the odds (LOD) scores > 1 were obtained for three loci centred on markers D2S2321, D14S1043 and D17S1832 (data not shown). The highest LOD score of 2.08 was observed for D2S2321. We genotyped a set of 25 French–Canadian families with spastic ataxia not associated with a polyneuropathy. We uncovered that out of the 25 families all the 17 families that were sufficiently large to be informative were linked to the ARSAL locus. Genotyping of these 17 families with 23 additional polymorphic markers spanning 49 cM confirmed linkage on chromosome 2q33–34. We obtained a maximum multipoint LOD score value of 5.95 (Fig. 3). The linkage analysis defines a 11.62 cM (13.89 Mb) candidate interval (D2S273-D2S2321, Fig. 3). Analysis of co-segregating haplotypes uncovered a three-marker (D2S2321-D2S2178-D2S2274) presumed 4-4-1 founder haplotype shared by 18 of 28 (64%) of phased carrier chromosomes (Table 2). The 4-4 haplotype for D2S2321 and D2S2178 is shared by 23 of the 28 (82%) phased chromosomes, while the four alleles for these two markers are also present on five or four of the unphased six carrier chromosomes (Table 2). As depicted by the shaded boxes in Table 2, the sharing of alleles between families suggests that up to 33 out of the 34 (97%) carrier chromosomes may be carrying the same historical ARSAL mutation. Five or more presumed historical recombination events between markers D2S1782 and D2S2321 suggest that D2S1782 should be considered the centromeric flanking marker of the haplotype-defined candidate interval. One such historical event has remodelled what appears to be a recombinant chromosome shared by families E, F and N (boxed area). Analysis of data for marker D2S2321 is more difficult because of its apparent higher mutation rate. However, a presumed historical recombination in family P would make D2S2321 the telomeric flanking marker. A more conservative estimate base on presumed historical recombinations in families C, H, L and Q would make D2S2274 the telomeric flanking marker. Together, these presumed historical recombinations delimit a 0.89 cM (1.25 Mb) to 2.51 cM (3.33 Mb) haplotype-defined candidate intervals. The typing

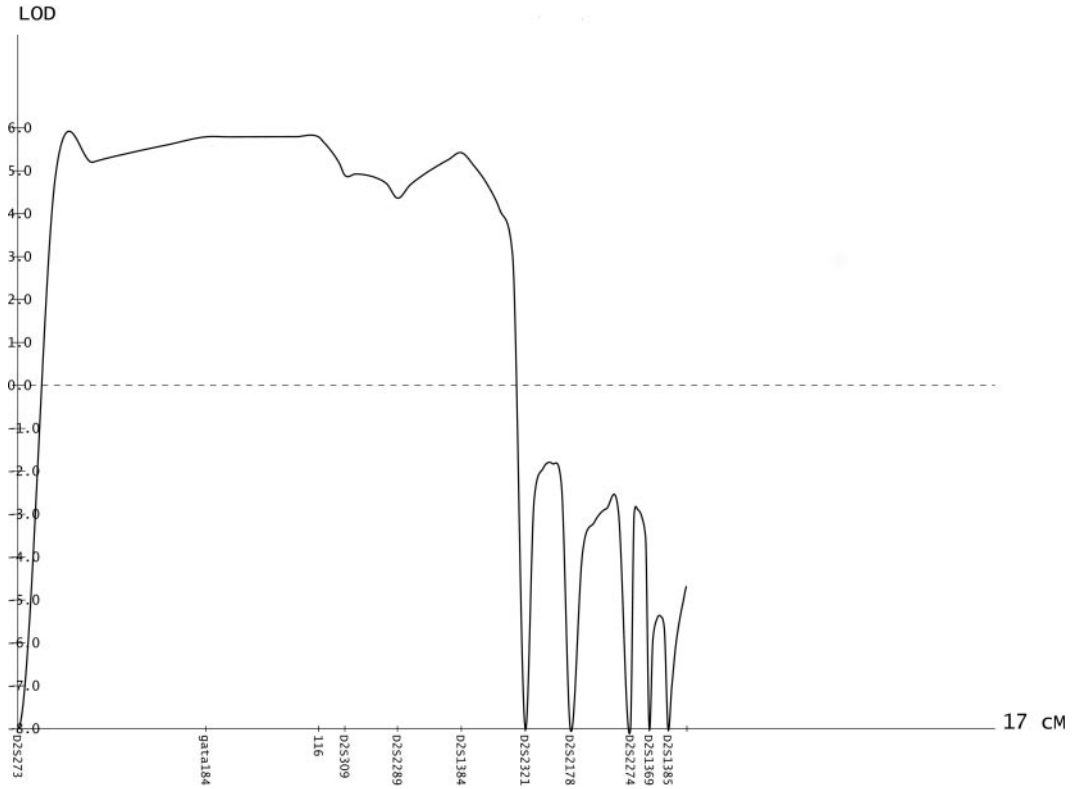

Fig. 3 Multipoint LOD scores generated by the analysis of 14 ARSAL families for 11 chromosome 2q33–34 markers.

Table 2 Haplotype results of ARSAL carrier chromosomes in 17 families for 2q33-34 markers

Family	Marker DeCODE UCSC (Mbp)	D2S318 193.26 192.4	D2S273 193.26 193.4	GATA149 199.5	D2S116 198.66 201.5	D2S309 198.66 201.79	D2S2289 200.48 203.39	D2S1384 201.34 205.05	D2S1782 203.99 206.84	D2S232 204.88 208.09	D2S2178 206.3 209.6	D2S2274 206.3 210.3	D2S1369 206.51 210.27	D2S1385 206.74 212.13	D2S371 207.58 213.17
A	1	4	3	2	3	5	3	3	4	4	4	1	5	3	5
	1	5	3	4	3	4	2	5	3*	4	4	1	2	2	4
B	3	4	1	4	6	4	7	3	4	4	4	1	4	3	4
	3	3	1	4	6	1	7	3	4	2m	4	1	5	3	5
C	6	0	5	2	6	6	2	3	4	4	4	1	5	3	5
	6	0	5	3	3	3	2	3	4	2m	4	4*	4	2	3
D	9	4	4	4	3	5	4	5	6*	4	4	1	1	1	3
	9	1	5	4	3	4	6	4	6	4	4	4	4	3	5
E	11	3	3	4	2	5	2	4	6*	4	4	1	5	3	5
	11	3	3	3	4	5	3	4	4	4	4	1	5	3	5
F	12	1	5	2	0	5	2	4	6*	4	4	1	5	3	5
	12	1	6	3	0	7	4	2	4	3	1	1	0	2	3
I	15	0	5	2	1	0	2	4	3*	4	4	1	5	3	5
	15	0	3	2	3	0	2	3	4	4	4	1	4m	3	5
H	14	2	4	2	3	6	3	1	4	4	4	2*	4	3	5
	14	3	4	2	2	5	7	3	4	4	4	1	4m	3	5
K	17	0	0	4	3	0	4	3	4	4	4	1	5	3	5
	17	0	0	4	2	0	2	3	4	4	4	1	5	3	5
L	18	2	7	4	3	5	7	7	6	6	4	4	1	3	5
	18	1	5	2	3	3	7	3	4	4	4	4*	1	1	5
N	20	1	5	3	3	0	7	3	4	3m	4	1	5	5	5
	20	1	5	2	6	0	2	4	6*	4	4	2m	5	3	5
O	21	3	5	3	4	0	4	3	1	2	2	2	2	3	5
	21	1	5	4	4	0	2	1	4	4	4	2m	5	3	4
P	22	1	4	4	3	2	3	3	6	4	4	0	4	3	3
	22	1	5	4	3	2	7	3	4	2**	5	0	5	3	5
Q	23	3	5	4	0	0	2	3	4	4	4	3*	4	2	5
	23	1	5	2	0	0	3	4	5*	4	4	1	4	3	5
Unphased															
G	13	2	3	0	1	1	1	3	4	4	4	2	0	3	0
	13	6	6	0	3	1	6	2	4	2	2	2	0	3	0
J	16	5	5	2	3	4	0	3	4	4	2	2	5	3	3
	16	6	6	3	6	5	0	3	4	4	4	1	5	3	5
M	19	0	2	2	3	4	3	3	4	4	4	2	5	1	5
	19	0	6	2	6	4	6	4	6	4	4	4	5	3	4

: Shared founder alleles or haplotype; ☐: shared presumed three-marker historical recombinant haplotype; *presumed historical recombination; **presumed historical recombination in family with limited allele sharing; m: presumed allele mutaion; o: genotype unavailable.

of makers D2S155 and D2S2237 in this region did not further narrow the interval because they were not found to be polymorphic in our families, while we failed to produce quality genotypes for markers D2S422 and D2S369 (data not shown).

Sequencing of candidate genes

Candidate gene mutation analysis was performed in parallel with the fine mapping. The 11.62 cM (13.89 Mb) linkage-based candidate interval contained numerous known neurological disease loci (Supplementary Table 1), some with phenotypes that overlap with ARSAL. The UCSC May 2004 freeze (http://genome.ucsc.edu/) predicts that 34 genes lie in the conservative 2.51 cM (3.33 Mb) haplotype-defined candidate region. The four best candidate genes based on their biological functions and expression patterns in the larger haplotype-defined candidate interval are *EEF1B2*, *NRP2*, *NDUFS1* and *ALS2CR19* genes. No mutations in these genes were uncovered by extensive sequencing. Though the *ALSIN* gene, ultimately, was excluded by further fine mapping from the candidate interval, it was extensively studied by western blot and sequencing and was not found to harbour any mutations (data not shown).

Conclusion

In this report, we describe a new autosomal recessive spastic ataxia with frequent leucoencephalopathy (ARSAL) that maps to chromosome 2q33–34. We chose to refer to this new complex disorder as a spastic ataxia rather than a complicated spastic paraplegia because ataxic features and cerebellar atrophy are constant features at the time of diagnosis. This will help in distinguishing it from the growing number of spastic paraplegias (Klebe *et al.*, 2006). Furthermore, we feel that including in the name the frequent presence of a leucoencephalopathy on MRI will help in the identification of other ARSAL cases worldwide, though its presence is not essential to the diagnosis and it may be a late manifestation in milder cases. This is even more important considering ARSAL's complicated and variable phenotype. Clinically, ARSAL can be distinguished from FRDA by the increased deep tendon reflexes, the absence of a peripheral neuropathy, the absence of a cardiomyopathy, the frequent presence of white matter changes on MRI and the usually less severe phenotype. The absence of a polyneuropathy and the limited ocular movement abnormalities also help in distinguishing ARSAL from ARSACS and spinocerebellar ataxia with axonal neuropathy (SCAN1) (Koenig, 2003).

The complexity of the ARSAL phenotype lies not only in its clinical spectrum but also in its involvement of various components of the CNS: cerebellum, pyramidal system, subcortical white matter, brainstem and corpus callosum. One of the challenges of contemporary neurogenetics is to uncover the genetic bases of diseases with variable phenotypes. The variable severity in the ARSAL phenotype and possible lower prevalence in other populations may have hampered its earlier definite description. The observed initial regional clustering of ARSAL cases in the Portneuf County and the early observation of significant intrafamilial variability allowed us to group cases with such different degrees of involvement. The mapping of all the informative families to the same locus and the high degree of haplotype sharing confirmed that indeed the same variable form of spastic ataxia segregates in these families. The identification of genes underlying such conditions is facilitated by the identification of cohorts originating from populations with well-established founder effects (Laberge *et al.*, 2005). It is clear that other non-French–Canadian families with overlapping complicated spastic phenotypes with cerebellar involvement map to this region (Eymard-Pierre *et al.*, 2002; Lesca *et al.*, 2003).

The mapping of the French–Canadian ARSAL families in the region of the ALS2 locus raised the possibility that mutation in the *ALSIN* gene could also be responsible for this new ataxia with upper motoneuron (UMN) involvement. Though slightly outside our final haplotype-defined 0.89 cM interval based on a presumed historical recombinations for marker D2S1782 (Table 2), the *ALSIN* gene was extensively studied because of its possible role in infantile ascending hereditary spastic paralysis (IAHSP) (Lesca *et al.* 2003). *ALSIN* has been most often found to be mutated in ALS2 (Yang *et al.* 2001; Eymard-Pierre *et al.*, 2002). No proven cases of ALS2 were reported to have cerebellar atrophy or white matter changes on MRI (Hadano *et al.*, 2001; Yang *et al.*, 2001; Gros-Louis *et al.*, 2003). Only one *ALSIN* mutation proven case with IAHSP was found to have mild vermian atrophy on MRI (Eymard-Pierre *et al.*, 2002). However, in this latter study, four families with *ALSIN* mutations were described as having mild periventricular white matter change on T_2-weighted sequences in the parieto-occipital regions, while two out of four also had similar lesions in their internal capsules (Lesca *et al.*, 2003). Interestingly, cases with similar IAHSP phenotype from six different Caucasian families that are probably linked to the same region were not found to harbour *ALSIN* mutations (Lesca *et al.*, 2003). This suggests either that mutations in the non-coding sequence of *ALSIN* need to be uncovered or alternatively that another gene in the region may be responsible for IAHSP and possibly also for ARSAL. Other cases of IAHSP with clear ataxia, cerebellar atrophy and T_2-weighted white matter changes have also been described, but *ALSIN* mutation has not been looked for in these cases (Brockmann *et al.*, 2005). Our extensive sequencing and western blot analysis suggests that *ALSIN* as presently characterized is unlikely to be the gene causing ARSAL.

In summary, we describe the clinical features of a large French–Canadian cohort affected by a novel ARSAL and define a 0.89–2.51 cM candidate region on chromosome 2q33–34. Thirty-four known or predicted genes (UCSC Human Genome Assembly, 2004) map to the candidate interval, including the already excluded *NRP2*, *NDUFS1*, *EEF1B2* and *ALS2CR19* genes. Further gene screening will be required to identify the causal mutations. Collecting additional families from Quebec and other countries with the same phenotype will

help better define the variability of the phenotype and reduce the candidate region. The identification of the ARSAL gene may lead to an understanding of the mechanisms responsible for the observed variable involvement of the CNS associated frequently with white matter changes.

Supplementary material

Supplementary data are available at *Brain* Online.

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