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 carriers 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 a 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 leukoencephalopathy (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 electro- myographic (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 EIF2BS 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,
3 nM MgCl₂, 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 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 BaselmagIR v.4.0 software (Li-Cor, ON, Canada).

Multipoint linkage analyses were performed using GENEHUN-
TER 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 2.0
(Oxford, UK). For the candidate regions and the fine mapping
analyses, all markers were analysed, assuming equal allel frequen-
cies. 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 Pri-
mer3. Large exons were divided into overlapping fragments.
Oligonucleotide primers were synthesized by Invitrogen (Burlin-
ton, 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;
hpfrodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). For the
ALSIN screening, western blot analysis was done using protein
extracts 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 belong-
ing 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 cri-
terion. 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 genea-
logical relationship with the Portneuf County (a 4098 km²
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
with anticholinergic medications. Other clinical fea-
tures include ataxic and spastic dysthria (74%), dystonic
positioning (57%, including hemidystonia in one case), mild
horizontal nystagmus (44%), scoliosis (35%), optoc 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 cog-
nitive impairment (44%) are also present to a variable
degree.

Brain MRI of 21 participants were reviewed by an experi-
enced neuroradiologist (J.L.). One participant died before
gaining 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 cal-
losum atrophy was only observed in Family B (Fig. 2A and
C). Eleven patients (52.4%) show non-specific white matter
changes on T₂-weighted and FLAIR sequences on brain MRI
(Fig. 2). These changes range from diffuse periventricular T₂-
hyperintensities to mild punctiform T₂-hyperintensities loca-
lized in periventricular, deep white matter or juxtagocorti-
able 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 T₂ 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

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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.
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 T2 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.

Fig. 2 Sagittal and coronal T2-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).
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; SCNMI; 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 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 cosegregating 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](image-url) Multipoint LOD scores generated by the analysis of 14 ARSAL families for 11 chromosome 2q33–34 markers.
**Table 2**  Haplotype results of ARSL carrier chromosomes in 17 families for 2q33-34 markers

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- : 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 mutation; o: genotype unavailable.
of markers 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, NDUF51* 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 polynuropathy 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 vermal 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 T2-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 T2-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, NDUF51, 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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References