ORIGINAL ARTICLE

Genetic and Epidemiological Study of Adult Ataxia and Spastic Paraplegia in Eastern Quebec

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ABSTRACT: ***Objective:*** To estimate the minimum prevalence of adult hereditary ataxias (HA) and spastic paraplegias (HSP) in Eastern Quebec and to evaluate the proportion of associated mutations in identified genes. ***Methods:*** We conducted a descriptive cross-sectional study of patients who met clinical criteria for the diagnosis of HA ($n = 241$) and HSP ($n = 115$) in the East of the Quebec province between January 2007 and July 2019. The primary outcome was the prevalence per 100,000 persons with a 95% confidence interval (CI). The secondary outcome was the frequency of mutations identified by targeted next-generation sequencing (NGS) approach. Minimum carrier frequency for identified variants was calculated based on allele frequency values and the Hardy–Weinberg (HW) equation. ***Results:*** The minimum prevalence of HA in Eastern Quebec was estimated at 6.47/100 000 [95% CI; 6.44–6.51]; divided into 3.73/100 000 for autosomal recessive (AR) ataxias and 2.67/100 000 for autosomal dominant (AD) ataxias. The minimum prevalence of HSP was 4.17/100 000 [95% CI; 4.14–4.2]; with 2.05/100 000 for AD-HSP and 2.12/100 000 for AR-HSP. In total, 52.4% of patients had a confirmed genetic diagnosis. AR cerebellar ataxia type 1 (2.67/100 000) and AD spastic paraplegia SPG4 (1.18/100 000) were the most prevalent disorders identified. Mutations were identified in 23 genes and molecular alterations in 7 trinucleotides repeats expansion; the most common mutations were c.15705–12 A > G in *SYNE1* and c.1529C > T (p.A510V) in *SPG7*. ***Conclusions:*** We described the minimum prevalence of genetically defined adult HA and HSP in Eastern Quebec. This study provides a framework for international comparisons and service planning.

RÉSUMÉ : Étude sur la forme génétique et la fréquence épidémiologique de l'ataxie et de la paraplégie spastique dans l'Est du Québec. ***Objectifs :*** Évaluer la prévalence minimale de l'ataxie héréditaire (AH) et de la paraplégie spastique héréditaire (PSH) dans une population adulte de l'Est du Québec, ainsi que la proportion des mutations associées dans les gènes identifiés. ***Méthodes :*** Il s'agit d'une étude descriptive et transversale, réalisée chez des patients de l'Est du Québec, ayant présenté les critères cliniques compatibles avec les diagnostics de l'AH (N=241) ou de la PSH (N=115), et couvrant la période entre janvier 2007 et Juillet 2019. Le critère d'évaluation principal était la prévalence de chaque maladie pour 100 000 personnes, avec un intervalle de confiance à 95% (IC à 95%); le critère d'évaluation secondaire était la fréquence des mutations identifiées par la méthode de séquençage de nouvelle génération. La fréquence minimale des porteurs des différents variants génétiques repérés dans la population de l'Est du Québec a été calculée sur la base des fréquences alléliques de chaque variant et de la loi de Hardy-Weinberg. ***Résultats :*** La prévalence minimale de l'AH dans l'Est du Québec a été évaluée à 6,47/100 000 personnes [IC à 95 % : 6,44-6,51] et était ainsi répartie : 3,73/100 000 pour l'ataxie autosomique récessive et 2,67/100 000 pour l'ataxie autosomique dominante. La prévalence minimale de la PSH a été évaluée à 4,17/100 000 [IC à 95 % : 4,14-4,2] et était ainsi répartie : 2,05/100 000 pour la PSH autosomique dominante et par 2,12/100 000 pour la PSH autosomique récessive. Dans l'ensemble, un diagnostic génétique d'AH ou de PSH avait été posé chez 52,4% des sujets. L'ataxie cérébelleuse autosomique récessive de type 1 (2,67/100 000) et la paraplégie spastique autosomique dominante SPG4 (1,18/100 000) étaient les deux formes de maladie les plus fréquentes. Des mutations dans 23 gènes et 7 expansions pathologiques de triplets répétés ont été détectées; les mutations les plus fréquentes étaient la c.15705-12A>G dans le gène *SYNE1* et la c.1529C>T (p.A510V) dans le gène *SPG7*. ***Conclusion :*** L'étude a permis de calculer la prévalence minimale des cas adultes atteints de l'AH ou de la PSH et confirmés génétiquement à l'Est du Québec. Elle établit aussi une base pour les prochaines analyses comparatives et la planification des services de la santé.

Keywords: Hereditary ataxia, Hereditary spastic paraplegia, Minimum prevalence, Mutation spectrum

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Table 1: Prevalence of hereditary ataxia and spastic paraplegia in previous regional studies

Region (country)	Study population	Recruitment sources	Included diagnoses	Number of subjects	Prevalence per 100,000 people			References
					All included	HA	HSP	
Benghazi province (Libya)	519,000	Regional health institutions	HA, AD-HSP, AR-HSP	25	4.8	2.7	2.1	3
Turin province (Italy)	2,327,996	Regional hospitals	HA, AD-HSP, AR-HSP, isolated HSP	142	6.1	4.8	1.3	4
Cantabria region (Spain)	510 000	Regional hospitals	HA (AR + AD + Isolated), HSP (pure)	103	20.2	7.2 + 1.2 + 2.2	9.6	5
Molise region (Italy)	335,211	Mail and phone survey	HA, AD-HSP, AR-HSP	25	7.5	4.8	2.7	6
Valle d'Aosta region (Italy)	115,270	Regional health institutions	HA, AD-HSP, AR-HSP, isolated HSP	17	14.8	10.5	4.3	7
Padua province (Italy)	845,203	Multiple search strategies	HA	79	9.33	-	-	8
Southeast Wales (UK)	742,000	Multiple search strategies	AR-late-onset HA, sporadic late-onset ataxia	76	10.2	-	-	9
Cantabria region (Spain)	527,000	Diagnostic centers	HA	8	1.6	-	-	10
Southeast region (Norway)	2,633,893	Multiple search strategies	HA, AD-HSP, AR-HSP, isolated HSP	365	13.9	6.5	7.4	11
Sfax district (Tunisia)	869,700	Regional hospitals	AD-HSP, AR-HSP	50	-	-	5.8	12
Alsace region (France)	1,800,000	Regional hospitals	HA	95	-	5.3	-	13
Al-Kharga district (Egypt)	62,583	Door-to-door survey	HA	7	-	11.2	-	14
North East region (UK)	2,610,000	Regional hospitals	HSP	74	-	-	2.8	15
Eastern Quebec province (Canada)	1,606,463	Regional health institutions	HA, AD-HSP, AR-HSP, isolated HSP	356	10.64	6.47	4.17	Present study

Introduction

Hereditary ataxias (HA) and hereditary spastic paraplegias (HSP) are heterogeneous neurodegenerative disorders that present with progressive gait impairment and lead to significant disability. Although these diseases are diverse in their pathophysiology – with HA involving mainly the cerebellar system and HSP affecting primarily the corticospinal tracts, there is some clinical and genetic overlap between them. Autosomal dominant (AD), autosomal recessive (AR), X-linked, and mitochondrial inheritance are reported.[1,2]

Over the last two decades, the advent of next-generation sequencing (NGS) has led to improved molecular diagnosis and a better understanding of the genetic heterogeneity and phenotypic pleiotropy of HA and HSP. Updated epidemiological studies are required to evaluate the prevalence of these disorders and their associated mutations in the light of these developments. Most recent studies were performed in European countries, and some originate from Asia, North Africa, and South America (Table 1).[3–15] These studies have found the existence of a west to east prevalence gradient in Europe with highest levels in the east of France, north of Spain, and Norway, and lowest levels in England. Many factors may influence the prevalence proportions reported, including geography, ethnicity, consanguinity, and the ascertainment method. The reported prevalences of HA and HSP range between 1–5 per 100,000 and 1–10 per 100,000, respectively.[16,17] A large population study was performed in southeast Norway, between 2002 and 2008, and reported a regional prevalence of 6.5/100 000 and 7.4/100 000 for HA and HSP, respectively.[11] Another prospective multicentric study of a large cohort of autosomal recessive cerebellar ataxia (ARCA) from the Alsace region, Eastern France showed an overall prevalence of 5.3/100 000 without genetic features and mutation distribution.[13] In Canada, there were no reports specifically studying the prevalence of HA and HSP, apart from one cross-sectional study of HSP patients in the provinces of Alberta, Ontario, and Quebec, which showed SPG4 as the most common HSP form (48%).[18] However, epidemiological data regarding the prevalence of HA and HSP, as well as the frequency of associated mutations in North America is lacking.

Quebec is a province located in east-central Canada (Figure 1A) where more than 82% of the population are of French-Canadian descent. Friedreich ataxia, AR spastic ataxia of Charlevoix-Saguenay, and ARCA 1 account for the majority of ARCA cases in Quebec.[19] The prevalence and genetic characterization of the other HA and HSP subtypes remain poorly studied. In addition, a large number of HA and HSP cases from Eastern Quebec remain without a molecular diagnosis, especially in late-onset presentations. Diagnosis is challenging due to their individual low prevalence, poor medical awareness, and heterogeneous clinical presentations with many overlapping features.[20,21]

The aim of this study was to calculate the minimum prevalence of HA and HSP in the Eastern Quebec population and to evaluate the minimum carrier frequency of the mutations associated with these disorders.

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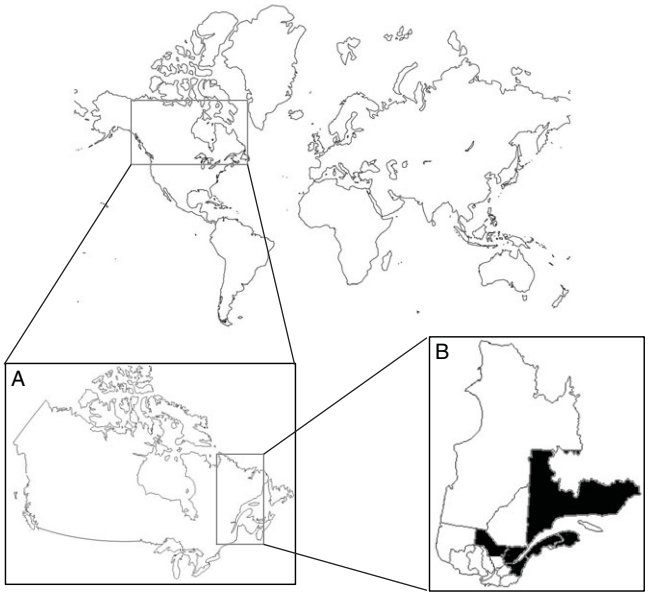

Figure 1: *Map of the world showing Canada (A) and the Quebec province (B). Eastern regions are shown in black. Eight-two percent of Eastern Quebec population are descendants of French-Canadian ancestry.*

Methods

Study Design and Patient Selection

This study is a case identification covering adults with suspected HA and HSP referred to our reference center (Neurology Department of the CHU de Québec/Centre Hospitalier Affilié Universitaire de Québec) for specialist clinical or genetic assessments. In total, 356 patients were enrolled in this study and referred by their treating physician in several family medicine units of Eastern Quebec between 2007 and 2019. Patients were included if alive and affected on the prevalence day according to the following criteria: (1) an adult phenotype with a clinical diagnosis being detected at ≥ 18 years old; (2) for HA, patients had to present with progressive cerebellar ataxia for which acquired etiologies had been excluded; (3) for HSP, patients had to present pure progressive lower extremity weakness associated with upper motor neuron signs for which acquired cases were excluded or deemed unlikely. All patients provided written informed consent for genetic testing.

Data Collection

Data about demographic information, family history, parental consanguinity, age at disease onset, age at diagnosis, and genetic diagnoses were collected from an anonymous clinical database elaborated by a neurological clinic in CHU de Quebec in collaboration with the biochemical department for continuous quality control of genetic testing. According to the standardized clinical assessment in the neurology department, the referred patients underwent laboratory, imaging, and electrophysiological investigations at the first evaluation. Patients were regularly followed to characterize clinical symptoms and disease progression.

Genetic Analysis and Mutation Screening

Blood samples were obtained from consenting participants. According to clinical suspicion for specific HA or HSP subtypes, single-gene testing by Sanger sequencing was used. All undiagnosed patients with a clinical suspicion of ataxia were tested for SCA 1, 2, 3, 6, 7, 8, 17, FRDA repeat expansion analysis, and Fragile X syndrome by PCR or had an NGS ataxia gene panel done (NGS420 Ataxia/Episodic Ataxia disorders; 362 genes and mitochondrial DNA). For suspected HSP, all patients had an NGS gene panel testing for 58 genes known to cause HSP (NGS337 Spastic Paraplegia NextGen DNA Sequencing Panel).

Sanger validation was performed according to standard protocols using BigDye terminator v1.1 (Life Technologies, Carlsbad, CA, USA). Variants nomenclature is given according to Locus Reference Genomics number LRG_269 tl. All identified variants were classified according to international guidelines.[22] Reported variants are those classified as pathogenic (class 1) and likely pathogenic (class 2). Identified likely pathogenic variants not reported previously are under submission to the ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar).

Data Analysis

Eastern Quebec was selected as the geographical area for the prevalence analysis, with a total population of 1,606,463 according to the most recent census of July 2019 for Quebec province (http://www.bdso.gouv.qc.ca). July 1, 2019 was chosen as the prevalence day. The study population was defined according to the socio-sanitary regions of the province of Quebec. Eastern Quebec includes the following regions: Capitale-Nationale, Côte-Nord, Gaspésie-Îles-de-la-Madeleine (except Madeleine Islands), Chaudières-Appalaches, Bas-Saint-Laurent, and half of Mauricie and Centre-du-Québec (Figure 1B). Minimum point prevalence was calculated as the number of affected patients in the study area on the prevalence day divided by the total number of at-risk individuals (population of Eastern Quebec). Prevalence proportions were expressed as cases/100 000% and 95% confidence intervals (CI) were calculated using the following equation: $CI = p \pm 1.96 \times ((p \times (1-p)/n)^{1/2}$ where p was the prevalence proportion and n the sample size.

In addition, using the Hardy–Weinberg (HW) equation, we calculated carrier frequency values based on the number of affected individuals with AR inheritance in our cohort of patients. Carrier frequency was calculated as $2pq$ where $p = 1 - q$; p represents the frequency of wild type allele, whereas q the frequency of mutant allele. q was calculated as the root square of the number of homozygous patients plus half the number of compound heterozygous patients divided by the population size, as shown in the following equation: $q = \sqrt{}$ (NHo+0.5 × NHe)/NI in studied population where NHo: Number of homozygous individuals; NHe: Number of heterozygous individuals, and NI: Number of individuals.

Results

Minimum Prevalence Estimates

A total of 356 subjects (174 women and 182 men) from 324 families were included in the present study. By July 2019, 241 HA and 115 HSP patients were identified in the study population. The mean age at HA diagnosis was 44.2 years ± 13.4 SD and the mean age at last examination was 57.4 years ± 13.2 SD. Female patients represented 56.3% of the total (Table 2).

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Table 2: Demographic characteristics and minimum prevalence of HA in Eastern Quebec 2007–2019

Disease group	Patients (Pedigree)	Male/Female	Age, years Mean ± SD*[a]	Age at diagnosis, years Mean ± SD[†]	Minimum prevalence per 100,000, [95% CI]
AR-ATX					
ARCA1	43(30)	16/27	48.7 ± 12.5	25 ± 8.6	2.67 [2.65-2.7]
FRDA	3(3)	2/1	45.7 ± 20	37.7 ± 17	0.18[0.18-0.19]
AOA2	4(4)	2/2	42.2 ± 14.7	38.5 ± 15.1	0.25[0.24-0.26]
ARSACS	7(7)	0/7	39.4 ± 11.5	32.7 ± 11.6	0.43[0.41-0.45]
POLG-MIRAS	2(2)	1/1	30.5 ± 16.2	21.5 ± 5	0.12[0.12-0.13]
SPG7	1(1)	1/0	65	55	0.06[0.06-0.07]
Total	60 (47)	22/38	45.2 ± 11.5	35.1 ± 11.9	3.73 [3.71-3.76]
AD-ATX					
SCA1	1(1)	0/1	72	70	0.06 [0.06-0.07]
SCA2	17(17)	13/4	54.5 ± 16	43.3 ± 14	1.06 [1.04-1.07]
SCA6	6(6)	2/4	61.6 ± 19.4	33.5 ± 24	0.37 [0.36-0.38]
SCA8	3(3)	½	73 ± 4.6	38.1 ± 31.2	0.18 [0.17-0.19]
SCA15	1(1)	0/1	57	47	0.06 [0.06-0.07]
SCA17	1(1)	0/1	70	63	0.06 [0.06-0.07]
SCA34	7(4)	2/5	70.1 ± 14.3	47.3 ± 8.2	0.43 [0.41-0.45]
EA2	7(7)	4/3	54.6 ± 22.5	44.8 ± 24	0.43 [0.41-0.45]
Total	43 (40)	22/21	64.3 ± 8.3	47.2 ± 10.2	2.67[2.65-2.7]
X-linked-ATX					
FXTAS	1 (1)	0/1	74	61	0.06 [0.06-0.07]
Total[‡]	104(88)	44/60	57.2 ± 13.7	43.8 ± 13.8	6.47 [6.44-6.51]
Unidentified gene	137 (136)	70/67	60.5 ± 13.8	49.8 ± 15.6	8.52 [8.49-8.57]
Total	241 (224)	114/127	57.4 ± 13.2	44.2 ± 13.4	15 [14.9-15.06]

AR = Autosomal recessive; AD = Autosomal dominant; ATX = Ataxia; CI = confidence interval;
ARCA = Autosomal recessive cerebellar ataxia; FRDA = Friedreich ataxia; AOA = Ataxia with oculomotor apraxia; ARSACS = Autosomal recessive spastic ataxia of Charlevoix-Saguenay; MIRAS = Mitochondrial recessive ataxia syndrome; SCA = Spinocerebellar ataxia; FXTAS = Fragile X-associated tremor/ataxia syndrome.
*At the last examination.
[†]Age-at-being clinically detected.
[‡]Total number of patients with positif genetic diagnosis.

The mean age at HSP diagnosis was 38.9 years ± 2.7 SD and the mean age at last examination was 53.1 years ± 3.4 SD. Women represented 40.9% of the total (Table 3). The minimum prevalence of HA and HSP in Eastern Quebec was estimated at 10.64/100 000.

Hereditary Ataxia

A total of 241 HA patients from 224 families (127 women and 114 men) were identified on prevalence day. Based on a population of 1,606,463 in Eastern Quebec at the prevalence day, an overall regional prevalence is estimated at 15/100 000 [95% CI; 14.9–15.06]. Among them, 104 patients (60 women and 44 men) had a confirmed pathogenic mutation in a known ataxia gene, which gave a minimum regional prevalence of HA of 6.47/100 000 [95% CI; 6.44–6.51] (Table 2) covering AD-HA at 2.67/100 000, AR-HA at 3.73/100 000 and X-linked ataxia at 0.06/100 000 (Table 2). Among the AR forms, pathogenic mutations in *SYNE1* were identified in 43 patients belonging to 30 non-related families making ARCA1 the most prevalent form with a minimum prevalence of 2.67/100 000 [95% CI; 2.65–2.7]. Pathogenic mutations in *SACS* were found in seven non-related patients with a late clinical onset, which yielded a prevalence estimate of 0.43/100 000 [95% CI; 0.41–0.45]. Four non-related patients had pathogenic mutations in *SETX*, whereas two others had *POLG* mutations (Table 2). Among the AD ataxia forms, abnormal *ATXN2* (CAG)n repeat expansions were identified in 17 patients making SCA2 the most prevalent AD form in our cohort with a minimum prevalence of 1.06/100 000 [95% CI; 1.04–1.07] followed by mutations in *ELOVL4* and *CACNA1A* found each in seven patients (0.43/100 000; [95% CI; 0.41–0.45]). Abnormal *CACNA1A* (CAG)n and *ATXN8OS* (CTG)n repeat expansions were also identified in 6 (0.37/100 000) and 3 (0.18/100 000) patients, respectively. The remaining pathogenic mutations were identified in *ATXN1*, *IPTR1*, *TBP*, and *SPG7* genes in our cohort (Figure 2 A).

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Table 3: Demographic characteristics and minimum prevalence of HSP in Eastern Quebec 2007–2019

Disease group	Patients (Pedigree)	Male/Female	Age, years Mean ± SD*	Age at onset, years Mean ± SD†	Minimum prevalence per 100,000, [95% CI]
Identified genetic mutations					
AR-HSP					
SPG7 (AR)	14 (14)	6/8	59.4 ± 8.7	42.9 ± 11.9	0.87 [0.86-0.89]
SPG11	9 (8)	6/3	37.7 ± 6.4	25 ± 5.7	0.56 [0.55-0.57]
SPG15	2 (2)	1/1	48 ± 4.2	44.5 ± 6.3	0.12 [0.12-0.13]
SPG18	2 (1)	2/0	28.5 ± 12	20.5 ± 0.7	0.06 [0.06-0.07]
SPG5A	3 (3)	0/3	54 ± 13	30 ± 6	0.18 [0.18-0.19]
SPG46	2 (1)	2/0	25.5 ± 0.7	20.7 ± 3.9	0.06 [0.06-0.07]
SPG48	1 (1)	0/1	50	44	0.06 [0.06-0.07]
SPG75	1 (1)	0/1	63	54	0.06 [0.06-0.07]
Total	34(31)	17/17	45.8 ± 13	35.2 ± 12.7	2.12[2.09-2.14]
AD-HSP					
SPG4	19 (14)	12/7	56.4 ± 13.7	33.7 ± 12.7	1.18 [1.17-1.20]
SPG3A	8 (2)	5/3	32 ± 21	24 ± 16.9	0.49 [0.48-0.51]
SPG9A	2 (1)	2/0	63.5 ± 3.5	34.5 ± 3.5	0.12 [0.12-0.13]
SPG7 (AD)	2 (2)	1/1	71.5 ± 13.4	49.2 ± 16.6	0.12 [0.12-0.13]
SPG17	1 (1)	0/1	60	46	0.06 [0.06-0.07]
SPG31	1(1)	1/0	50	45	0.06 [0.06-0.07]
To-tal	33 (21)	21/12	55.5 ± 13.6	38.7 ± 9.6	2.05[2.03-2.08]
Total AR + AD	67 (52)	38/29	50.7 ± 6.9	36.9 ± 2.5	4[4.14-4.2]
Unidentified gene					
	48 (48)	30/18	55.5 ± 16	40.8 ± 17.8	2.98 [2.84-3.13]
Total	115 (100)	68/47	53.1 ± 3.4	38.9 ± 2.7	7.15[7.12-7.20]

AR = autosomal recessive; AD = autosomal dominant; SPG = Spastic gait gene.
*At the last examination.
†Age-at-being clinically detected.

Hereditary Spastic Paraplegia

A total of 115 subjects from 100 families (47 women and 68 men) were identified on the prevalence day. The HSP prevalence for all patients was estimated at 7.15/100 000 [95% CI; 7.12–7.2] and at 4.17/100 000 [95% CI; 4.14–4.2] for those genetically confirmed (67 out of 115 patients) equally distributed between AD-HSP and AR-HSP (prevalence at 2.12/100 000 [95% CI; 2.09–2.14]) (Table 3). Among AD-HSP, pathogenic mutations in *SPAST* were found in 19 patients making SPG4 the most prevalent AD form in Eastern Quebec (1.18/100 000 [95% CI; 1.17–1.2]) followed by mutations in *ATL1* linked to SPG3A form (0.49/100 000 [95% CI; 0.49–0.51]. For AR-HSP, pathogenic mutations in *SPG7* were identified in 14 patients belonging to 14 non-related families making SPG7 the most prevalent form with a minimum prevalence estimated at 0.87/100 000 [95% CI; 0.86–0.89] followed by mutations in *SPG11* (0.56/100 000 [95% CI; 0.55–0.57]. The remaining identified HSP forms ranged between 0.06/100 000 and 0.18/100 000 with pathogenic mutations identified in *CYP7B1*, *ALDH18A1*, *ZFYVE26*, *BSCL2*, *ERLIN2*, *REEP1*, *GBA2*, *AP5Z1*, and *MAG* (Table 3; Figure 2B).

Genetic Diagnoses

Hereditary Ataxia

We report 36 different pathogenic variants in 15 genes previously associated with HA (Table 4). In AR-HA, the most common mutation was the c.15705-12 A > G in *SYNE1 (ARCA1)* with a minimum carrier frequency of 1/134 in Eastern Quebec. In total, 11 unrelated patients were homozygous carriers of the c.15705-12 A > G mutation and 23 patients from 15 families were compound heterozygous carriers of this mutation and a different *SYNE1* mutation, which included c.8716 A > T (p.R2906X), c.22918C > T (p.Q7640X), c.16177-2 A > G, c.17603_17607delATTTG (p.D5868AfsX13) and c.21721C > T (p.Q7241X). Furthermore, 2 patients were homozygous and 16 were compound heterozygous carriers of the c.8716 A > T nonsense mutation, making it the second most frequent mutation causing AR-HA with a minimum carrier frequency of 1/200. Seven different mutations were found in *SACS* (ARSACS); the c.8844delT (p.I2949Ffs) mutation was the most common variant (three in homozygous state and two in compound heterozygous state). In addition,

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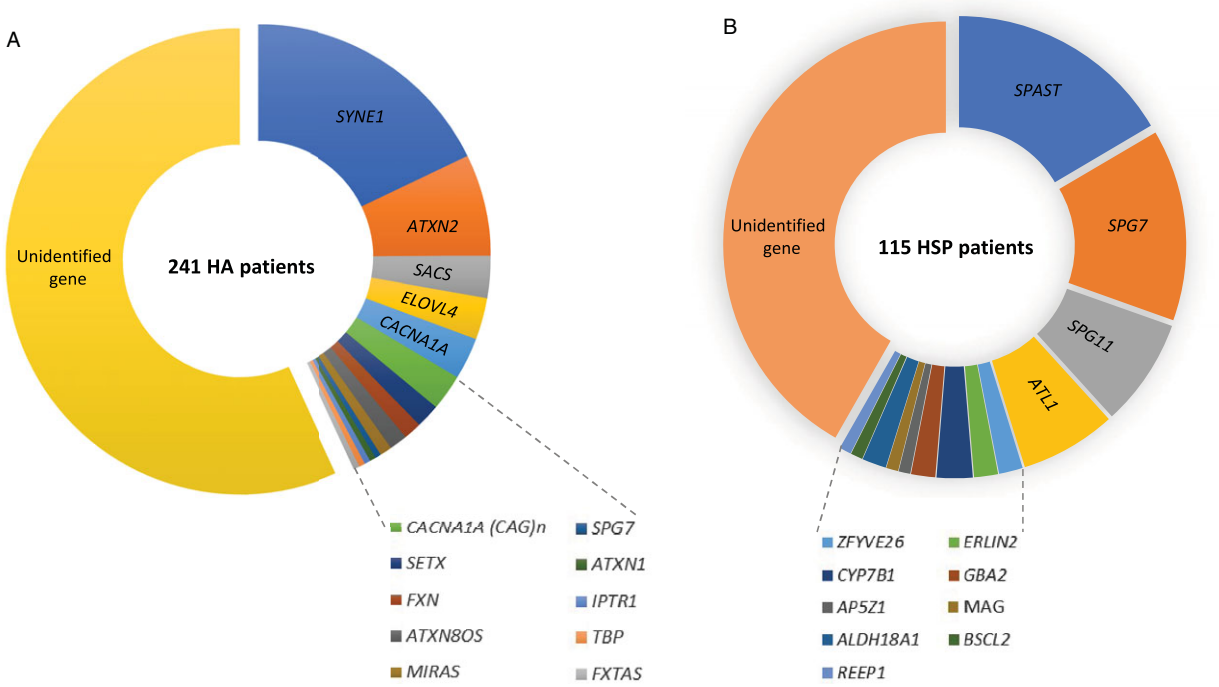

Figure 2: *Molecular diagnosis underlying HA (A) and HSP (B) in the Eastern Quebec population. Molecular diagnosis has been established in 43% and 58 % of patients for HA and HSP, respectively.*

we found pathogenic variants in *POLG1* associated with mitochondrial recessive ataxia syndrome (MIRAS) in three cases.

In AD-HA, the most common mutation was the *ATXN2* (CAG)n repeat expansion (SCA2) (range 35–49 in our patients, whereas reference range is < 31 CAG trinucleotide repeats). Seven patients from four families were heterozygous for the same c.504 G > C (p.L166F) mutation in the *ELOVL4* gene responsible for spinocerebellar ataxia 34, and seven other patients harbored a heterozygous mutation in *CACNA1A* (episodic ataxia type 2). We also report patients with mutations in the following genes: *SETX, ITPR1*, and *SPG7* and alteration in (GAA)n, (CGG)n, (CAG)n, (CTG)n, (CAG)n, and (CAG/CAA)n trinucleotide repeat expansions in *FXN*, *FXTAS*, *ATXN1*, *CACNA1A*, *ATXN8OS*, and *TBP* (Table 4).

Hereditary Spastic Paraplegia

Thirty-three different mutations were detected in 13 genes responsible for HSP (Table 5), among which 52 mutations (71%) represent the heterozygous state (21 in homozygous state). In our cohort, eight variants in the *SPAST* gene related to SPG4 form were responsible for the majority of AD-HSP. The most common mutation was the c.1209C > A (p.F403L) missense mutation found in six patients representing three families. For AR-HSP, five different variants were found in *SPG7* with c.1529C > T (p.A510V) being the most common with a minimum carrier frequency of 1/200, whereas seven different mutations in *KIAA1840* were found to cause SPG11 form showing a minimum carrier frequency ranged between 1/328 and 1/900 (Table 5). Nine patients belonging to two non-related families were found to be heterozygous for c.1306_1308 delAAT in ALT1 responsible for the SPG3A form. We also report patients with mutations in the following genes: *CYP7B1*, *ALDH18S1*, *ZFYVE26*, *BSCL2*, *ERLIN2*, *REEP1*, *GAB2*, *AP5Z1*, and *MAG* (Table 5, Figure 2B).

Discussion

To the best of our knowledge, this study is the first to estimate the minimum regional prevalence of hereditary ataxia and spastic paraplegia adult-onset forms in Eastern Québec. We report also for the first time information on carrier frequency of mutations associated with these disorders. The overall pooled prevalence of HA and HSP was 10.64 per 100,000 which is similar to previously reported prevalence estimates with a genetic distribution comparable with other countries.[7,11,12] Previous epidemiological studies for these conditions have been conducted separately in different age population where clusters of the disease have previously been found and yielded variable results.[11,13] The type and size of populations, inclusion criteria, and diagnoses included vary considerably, which makes it difficult to compare reports.

Prevalence Study

Hereditary Ataxia

In the present study, the minimum regional prevalence of hereditary ataxia in Eastern Quebec was 6.47/100 000 made up of the following: AR inheritance in 3.73/100 000 and AD pattern of inheritance in 2.67/100 000. A previous study of HA prevalence from southeast Norway showed a comparable prevalence of 6.5/100 000 with respect to patient inclusion strategies and source population size.[11] However, a higher prevalence of AR-HA was reported in Alsace, Eastern France (5.3/100 000)[13] and in Cantabria, Spain (7.2/100 000),[5] which may reflect a real difference between these regions or may be due in part to the fact that some

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Table 4: Minimum carrier frequency of common mutations in patients with HA in Eastern Quebec

Subtype	Locus/-gene	Variant (Protein)	ACGM	Zygosity*	Minimum carrier frequency¥	Reported in
ARCA1	6q25.2/*SYNE1*	c.15705–12 A > G	1	11/23	1/134	19,32
		c.8716 A > T (R2906X)	1	2/16	1/200	19,32
		c.17603_17607delATTTG (D5868AfsX13)	1	0/7	1/333	32
		c.21721C > T (Q7241X)	1	0/5	1/400	Undocumented
		c.4393_4394delCT (L1465VfsX6)	1	0/2	1/666	Undocumented
		c.22918C > T (Q7640X)	1	0/2	1/666	32
		c.21250C > T (R7084X)	1	0/1	1/900	41
		c.16177–2 A > G	1	0/3	1/500	Undocumented
AOA2	9q34.13/*SETX*	c.5927 T > G (L1976R)	1	1/2	1/454	42
		c.195 G > A (Q65K)	2	1/1	1/200	Undocumented
		Exon 19 Deletion	1	0/1	1/900	43
ARSACS	13q12.12/*SACS*	c.8844delT (I2949Ffs)	1	3/2	1/332	44
		c.237dupA (S80Ifs)	1	0/1	1/900	45
		c.5836 A > G (D1946N)	1	0/1	1/900	46
		c.4205 A > T (D1402V)	1	0/1	1/900	45
		c.6663delA (K2221AsnfsX33)	2	0/1	1/900	Undocumented
		c.5838 T > C (W1946R)	2	0/1	1/900	Undocumented
		SACS gene deletion	1	0/1	-	47
SCA34	6q14.1/*ELOVL4*	c.504 G > C (L166F)	1	0/7	-	48
MIRAS	15q26.1/*POLG1*	c.2542 G > A (G848S)	1	0/2	1/870	49
		c.428C > T (A143V)	2	0/1	1/900	49
		c.248 T > C (L83P)	1	0/1	1/900	50
EA2	19p13.13/CACNA1A	c.835C > T (R279C)	1	0/2	-	51
		c.6840 G > A (R2280P)	2	0/2	-	Undocumented
		c.6205C > T (R2069G)	1	0/1	-	52
		c.6672_6674dup	2	0/1	-	53
		c.1818_1821del (L607SfsX4)	2	0/1	-	Undocumented
SPG7	16q24.3/*SPG7*	c.1529C > T (A510V)	1	1/0	1/870	37
SCA1	6p22.3/*ATXN1*	(CAG)n expansion				
SCA2	12q24.12/*ATXN2*	(CAG)n expansion				
SCA6	19p13.13/*CACNA1A*	(CAG)n expansion				
SCA8	13q21.33/*ATXN8OS*	(CTG)n expansion				
SCA15	3p26.1/*IPTR1*	*IPTR1* gene deletion				
SCA17	6q27/*TBP*	(CAG/CAA)n expansion				
FRDA	9q21.11/*FXN*	(GAA)n expansion				
X-Fragile	Xq27.3 /*FXTAS*	(CGG)n expansion				

ARCA = autosomal recessive cerebellar ataxia; AOA2 = ataxia with oculomotor apraxia; ARSACS = autosomal recessive spastic ataxia of Charlevoix-Saguenay; SCA = spinocerebellar ataxia; MIRAS = mitochondrial recessive ataxia syndrome; EA = Episodic ataxia; SPG7 = spastic paraplegia type 7; FRDA = Freidreich ataxia; SYNE1 = Spectrin Repeat Containing Nuclear Envelope Protein 1; *SETX* = senataxin gene; SACS = sacsin gene; ELOVL4 = Elongation of Very Long-Chain Fatty Acids-Like 4; POLG1 = DNA Polymerase Gamma 1; CACNA1A = Calcium channel, voltage-dependent, L type, α1S subunit gene; ATXN2 = ataxin 2; ATXN8OS = ataxin 8 opposite strand; FXN = frataxin; FXTAS = fragile X tremor/ataxia syndrome. ACMG categories: Class 1: Pathogenic variant; Class 2: Likely pathogenic variant; Class 3: Variant of uncertain clinical significance; Class 4: Likely benign variant; Class 5: Benign variant.

*The number of homozygous patients/the number of heterozygous patients,

¥Minimum carrier frequency was calculated only for autosomal recessive inheritance subtypes.

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Table 5: Minimum carrier frequency of common mutations in patients with HSP in Eastern Quebec

Subtype	Locus/gene	Variant (Protein)	ACGM	Zygosity*	Minimum carrier frequency¥	Reported in
SPG4	2p22.3/*SPAST*	c.1209C > A (F403L)	1	0/6		54
		c.1245 + 1 G > A	1	0/3		55
		c.1345 G > T (E449X)	2	0/2		Undocumented
		c.1801 G > A (G559D)	1	0/3		56
		c.562delG (A188PfsX8)	1	0/2		57
		c.1775_1778delTAAA (I592NfsX9)	2	0/1		Undocumented
		c.1413 + 3_1413 + 6del	2	0/1		Undocumented
		Deletion exons 10–16	1	0/1		Undocumented
SPG7	16q24.3/*SPG7*	c.1529C > T (A510V)	1	9/3	1/200	37
		c.998–1 G > A	1	0/2	1/666	37
		c.1715C > T (A572V)	1	1/2	1/454	37
		c.233 T > A (L78X)	1	0/2	1/666	58
		c.25C > T (R9C)	2	0/1	1/900	Undocumented
SPG11	15q21/*SPG11*	c.6598 A > T (K2200X)	1	5/0	1/328	59
		c.1478_1482del	1	1/0	1/666	Undocumented
		c.2466delT (A834X)	1	0/1	1/900	Undocumented
		c.6882_6884delCAC	1	0/1	1/900	Undocumented
		c.1108 G > A (E370K)	1	0/1	1/900	Undocumented
		c.2083 G > A (A695T)	1	0/1	1/900	Undocumented
		Exon 11 and 27 deletion	1	1/0	1/666	Undocumented
SPG5A	8q12.3/ *CYP7B1*	c.1250 G > A (R417H)	1	1/1	1/500	60
		c.1456C > T (R486C)	1	1/1	1/500	60
SPG3A	14q22.1/*ATL1*	c.1306_1308delAAT (del436N)	1	0/9		36
SPG9A	10q24/*ALDH18A1*	c.359 T > C (V120A)	2	0/1		61
SPG15	14q24/*ZFYVE26*	c.363 + 1 G > A	1	0/1		Undocumented
		*ZFYVE26*gene deletion	1	0/1		Undocumented
SPG17	11q12.3/*BSCL2*	c.263 A > G (N88S)	1	0/1		Undocumented
SPG18	8p11.23/*ERLIN2*	c.899 A > T (D300V)	3	2/0	1/666	Undocumented
SPG31	2p11.2/*REEP1*	c.478del (R160GfsX63)	1	0/1		62
SPG46	9p13.3/*GBA2*	c.2202delC (Y735IfsX26)	1	2/0	1/666	Undocumented
SPG48	7p22.1/*AP5Z1*	c.1560C > A (Y529X)	1	0/1		Undocumented
SPG75	19q13.12/*MAG*	c.308 T > A (L103Q)	2	0/1	1/900	Undocumented
		c.313 A > C (S105R)	2	0/1	1/900	Undocumented

ACMG categories: Class 1: Pathogenic variant; Class 2: Likely pathogenic variant; Class 3: Variant of uncertain clinical significance; Class 4: Likely benign variant; Class 5: Benign variant.

*The number of homozygous patients/the number of heterozygous patients.

¥Minimum carrier frequency was calculated only for autosomal recessive inheritance subtypes.

presumed ARCA patients without molecular diagnosis do not have an inherited form of ataxia.

Fifteen separate HA are represented in our cohort, which underlines the genotypic heterogeneity of this group of diseases (Figure 2 A). As suspected, ARCA1, which was initially identified in a large cluster of French-Canadian families who originated from Beauce in southeastern Quebec, Canada[19,23], is the most prevalent AR-HA in our cohort. For AD-HA, SCA2 was the most prevalent diagnosis followed by SCA34 and SCA6, which is in line with previous results in other populations.[10,11] Although SCA3 is the most commonly encountered SCA worldwide[24], no cases were found in our cohort. A previous single-center retrospective chart review with a total of 21 SCA families was published 15 years ago and has identified SCA3 as occurring at

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the highest frequency (24%) in Canada followed by SCA2 and SCA6.[25] However, the small population size and the evolution of NGS panels, among other factors, may play an important role in the wide range of epidemiological and phenotypic diversity emerging from studies showing population-specific and region-specific aspects of SCAs. For example, a systematic population-based survey of general practitioners and other health care providers in Portugal showed a HA prevalence of 8.9/100,000 inhabitants with a major contribution of SCA3 (3.1/100,000), possibly due to a founder effect which markedly increases prevalence.[26]

Hereditary Spastic Paraplegia

In the present study, a comparable HSP prevalence (4/100 000) and genetic distribution with previous reports were found[7,12] with equal distributions with respect to the inheritance pattern. Based on a systematic review of prevalence studies, the range of prevalence proportions for HSP was estimated to be 0.5–5.3 per 100,000 with the highest value for AD-HSP reported in southeast Norway.[11] The results from southeast Norway (2009) showed a higher prevalence of AD-HSP (5.5/100 000) compared to our study. The authors included patients from birth to 80 years of age, and used stringent methods that probably yielded a high participation rate, for example; patients could be evaluated close to their residence if they could not travel. Moreover, HSP is often misdiagnosed initially and most patients experience the onset of symptoms between the second and fourth decades. Hence, including patients with childhood-onset diseases may have caused an overestimation as patients with other etiologies may have been erroneously included. The most common AD-HSP form in our estimated figure was SPG4 followed by SPG3A which appears to be comparable to previous studies.[27,28] Although AD-HSP is the most common type of HSP, found in 75%–80% of affected individuals, equal prevalence was found for AR-HSP to AD forms with SPG7 and SPG11 found to be the most prevalent in our cohort. The AR pattern represents the major growing group of newly described HSP, and is more frequent in consanguineous families and non-European populations, such as SPG11 in Tunisia with a prevalence of 5.75/100.000.[12]

Great care was taken to avoid including false-positive subjects. The differential diagnosis between hereditary and neurodegenerative disease is challenging. By using molecular testing and including only genetically confirmed patients in our estimation, we estimate that we minimized the risk of contaminating the results with misdiagnosed patients showing neurodegenerative diseases of other etiologies. The size of the study population is also important in prevalence studies, and many previous studies have been conducted on small populations, which can bias the results.[6,10]

Our study has a number of limitations. Using monocentric study takes away a potential source of bias in the recruitment, notably for the rare forms of HA and HSP. Neurology department of the CHU de Québec has long been the only one to offer investigation for screening panels of adult ataxia and spastic paraplegia genes in eastern regions of the province of Quebec. One finding that could be attributable to such a bias in this study is an underestimation of the true prevalence, and sporadic subjects, mild forms, and asymptomatic carriers may have been missed. All patients were followed biannually to annually once acquired causes have been ruled out and a genetic etiology was considered, which avoid loss of patients during the study. We estimate, however, that the number of missed subjects is small and that obtaining an exact number is impossible.

In addition, as patients were included only when a clinical diagnosis of HA or HSP was detected, the estimates obtained may represent an underestimation of the true prevalence since these hereditary disorders have an early onset. Moreover, our estimation was based on index subjects referred to our tertiary care clinic. About 42% of patients had a positive family history, but unfortunately, we could not reach all family members, which may underestimate the true prevalence of these conditions. The use of multiple search strategies might ensure the inclusion of more subjects which was the case in previous population-based studies with multi-source ascertainment.[11]

Genetic Aspects

Our study describes for the first time the genetic spectrum of HA and HSP in Eastern Quebec. The frequency of gene mutations in these disorders varies considerably across populations, and a precise picture may help clinicians guide molecular testing and interpret results. A positive molecular diagnosis was established in 43.1% of HA patients and 58.2% of HSP patients, which is similar to the diagnostic yield in recent NGS studies in cohorts of HA[29] and HSP[30,31] (Figure 2). The distribution of identified genes was different from those described in studies from different populations.[11,17] The *SYNE1* c.15705–12 A > G variant is the most frequent pathogenic mutation in our cohort. It is most prevalent in the French-Canadian descent due to a founder effect.[23,32] Moreover, the c.8716 A > T variant is the second most frequent variant in our cohort. Despite these two more common variants in our cohort, the identification of nine other distinct *SYNE1* variants supports the presence of allelic heterogeneity in the Eastern Quebec population, even for rare diseases.[33] In European countries, Ataxia-telangiectasia and Friedreich's ataxia were the most common recessive HA forms.[5,34] The prevalence of Friedreich's ataxia in our study was low compared to European studies, but similar to southeast Norway[11] or Finland.[35]In addition to the inclusion criteria of an adult study, the difference may be due to the fact that Friedreich's ataxia was a clinical diagnosis and revision of the diagnoses in line with new insights in molecular genetics and reduced consanguinity may explain our results.

We show that point mutations in *SPAST* are the most common cause of AD-HSP in our cohort, which is consistent with other studies.[18,28] The c.1306_1308 delAAT *ATL1* variant was the second most common cause of AD-HSP, suggesting a founder effect in the Eastern Quebec population. This deletion was first labeled as a variation of unknown significance, but its pathogenicity was demonstrated in a recent study[36], and its presence in our AD-HSP cohort supports this observation. SPG7 was the commonest cause of AR-HSP in accordance with the previous findings[18]; the c.1529C > T mutation was the most frequent mutation identified, which provides additional evidence of the high frequency of this variant in the Quebec population.[37]

Pathogenic or potentially pathogenic variants were not detected in 185 patients after NGS panel investigation although about 11% had a positive family history. According to

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population-based studies, the proportion of families without genetic diagnosis ranged from 45% to 67% in AD-HSP, from 71% to 82% in the AR-HSP groups.[31] This suggests that more genes involved in HA or HSP remain to be discovered. To overcome the diagnostic challenges caused by genetic heterogeneity, different NGS diagnostic techniques have been applied.[38,39] Our work shows that an NGS gene panel approach can be an efficient way to make a genetic diagnosis in a heterogeneous population native to genetic testing. Whole-exome and whole-genome sequencing are alternative diagnostic methods that have shown promising results in undiagnosed patients with genetic conditions.[40]

Conclusion

HA and HSP are rare disorders with complex clinical presentations and significant genetic heterogeneity. This study is the first to estimate their minimum prevalences in Eastern Quebec. The results enable comparative analyses across different regions in other countries, as a necessary framework for service planning, and as a platform for the development of disease-specific registries. Both HA and HSP cause significant morbidity, and accurate genetic diagnosis is essential to optimize the management and genetic counseling for patients with these disorders and their families.

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Dr. Gan-Or reports personal fees from Lysosomal Therapeutics, personal fees from Denali, personal fees from Handl Therapeutics, personal fees from Idorsai, personal fees from Inception Sciences, personal fees from Lighthouse, personal fees from Deerfield, personal fees from Neuron23, personal fees from Prevail Therapeutics, personal fees from Sanofi, outside the submitted work. The remaining authors have no conflicts of interest to declare.

Statement of Authorship

IHS: data collection, analysis, and interpretation of data, writing and revising the manuscript. MB: writing and critical revision of the manuscript. POC and MS: participated in data collection and revised the manuscript. MAE: provided genetic results and revised the manuscript. FB: data collection and critical revision of the manuscript. PLG: performed statistical analysis. GAR: revised the manuscript for important intellectual content. KMA: coordinated clinical molecular diagnostic testing; contributed clinical laboratory data; and critically reviewed the manuscript. ZGO: revised the manuscript for important intellectual content. ND: study concept and design, critical revision of the manuscript, study supervision, and coordination.

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