ARTICLE OPEN ACCESS

Prevalence of *RFC1*-mediated spinocerebellar ataxia in a North American ataxia cohort

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Abstract

Objective

We evaluated the prevalence of pathogenic repeat expansions in replication factor C subunit 1 (*RFC1*) and disabled adaptor protein 1 *(DAB1)* in an undiagnosed ataxia cohort from North America.

Methods

A cohort of 596 predominantly adult-onset patients with undiagnosed familial or sporadic cerebellar ataxia was evaluated at a tertiary referral ataxia center and excluded for common genetic causes of cerebellar ataxia. Patients were then screened for the presence of pathogenic repeat expansions in *RFC1* (AAGGG) and *DAB1* (ATTTC) using fluorescent repeat-primed PCR (RP-PCR). Two additional undiagnosed ataxia cohorts from different centers, totaling 302 and 13 patients, respectively, were subsequently screened for *RFC1*, resulting in a combined 911 subjects tested.

Results

In the initial cohort, 41 samples were identified with 1 expanded allele in the *RFC1* gene (6.9%), and 9 had 2 expanded alleles (1.5%). For the additional cohorts, we found 20 heterozygous samples (6.6%) and 17 biallelic samples (5.6%) in the larger cohort and 1 heterozygous sample (7.7%) and 3 biallelic samples (23%) in the second. In total, 29 patients were identified with biallelic repeat expansions in *RFC1* (3.2%). Of these 29 patients, 8 (28%) had a clinical diagnosis of cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS), 14 had cerebellar ataxia with neuropathy (48%), 4 had pure cerebellar ataxia (14%), and 3 had spinocerebellar ataxia (10%). No patients were identified with expansions in the *DAB1* gene (spinocerebellar ataxia type 37).

Conclusions

In a large undiagnosed ataxia cohort from North America, biallelic pathogenic repeat expansion in *RFC1* was observed in 3.2%. Testing should be strongly considered in patients with ataxia, especially those with CANVAS or neuropathy.

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Editorial

Intronic pentanucleotide expansion in the replication factor 1 gene (*RFC1*) is a major cause of adult-onset ataxia

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Glossary

CANVAS = cerebellar ataxia, neuropathy, and vestibular areflexia syndrome; ***DAB1*** = Disabled Adaptor Protein 1; ***RFC1*** = replication factor C subunit 1; **SCA37** = spinocerebellar ataxia type 37.

Cerebellar ataxia is a heterogeneous genetic disorder characterized by inability to control balance and coordination. Roughly 50% of patients remain undiagnosed despite advanced genomic testing.[1–4]The most common genetic ataxias, as well as several rarer forms, are caused by nucleotide repeat expansions, which typically require targeted non–sequence-based testing to identify.[5–8]

Recent studies identified a recessive intronic (AAGGG) repeat expansion in replication factor C subunit 1 (*RFC1*) related to cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS) in Australia and the United Kingdom.[9,10] In addition, this expansion may be responsible for up to 22% (33/150) of sporadic cerebellar ataxia and 63% (32/51) of ataxia associated with sensory neuropathy.[9] Similarly, a dominant pathologic pentanucleotide (ATTTC) repeat insertion was identified within a normal (ATTTT) tandem repeat element in the intronic 5′ untranslated region of the disabled adaptor protein 1 (*DAB1*) gene causing spinocerebellar ataxia type 37 (SCA37) in patients from the southern Iberian Peninsula.[11]

To address the frequency of these repeat expansion disorders in North America, we assessed a large cohort of 596 patients from the United States with unidentified cerebellar ataxia. We identified biallelic *RFC1* expansion in 1.5% (n = 9) and found no patients with a pathogenic *DAB1* expansion. We further tested 2 additional cohorts from different centers (the larger of which consisted of approximately one-third samples from patients in Canada, with the remainder from the United States) and identified *RFC1*-mediated ataxia cases in 5.6% (17/302) and 23% (3/13), respectively, for a total prevalence of 3.2% (29/911).

Methods

Standard protocol approvals, registrations, and patient consents

Patients were enrolled at the University of California, Los Angeles (UCLA) Ataxia Center, clinically assessed for acquired causes of ataxia, and then considered for genetic causes.[2] Only patients with negative testing for the common genetic ataxias (SCA1, SCA2, SCA3, SCA6, SCA7, and Friedreich ataxia) were included in this study. The majority (~ two-thirds) were adult and sporadic onset. All patients consented for DNA collection for genetic analysis. Peripheral blood was collected from patients, and DNA was then isolated and purified using the Gentra Puregene Blood Kit (Qiagen) for genetic testing. The study methods used were approved by the UCLA Institutional Review Board. Additional DNA samples from 302 patients enrolled at the University of Chicago and 13 patients enrolled at the Brigham and Women's Hospital under institutional review board-approved procedures with identical inclusion criteria were subsequently tested as well. For the purpose of assessing disease prevalence, only probands from affected families were included in this study, and all pathogenic repeat expansions were confirmed in additional affected family members when available.

Repeat expansion testing

RFC1 gene repeat expansion analysis

Fluorescent repeat-primed PCR (RP-PCR) was performed to detect *RFC1* pathogenic $(AAGGG)_n$ alleles using previously published primers[9,10] with an optimized touchdown PCR protocol and Qiagen HotStarTaq. One primer set included the forward 5′FAM-ACTGACAGTGTTTTTGCCTGT-3′ primer, the anchor 5′-CAGGAAACAGCTATGACC-3′ primer, and the repeat 5′-CAGGAAACAGCTATGACCAAGGGAAGGGA AGGGAAGGGAAGGG-3′ primer that identifies the (AAGGG) repeats. A second primer set included the forward 5′FAM-TCAAGTGATACTCCAGCTACACCGT-3′ primer, the anchor 5′-CAGGAAACAGCTATGACC-3′ primer, and 3 repeat primers that identify the (AAGGG) repeats 5′-CAGGAAA-CAGCTATGACCAACAGAGCAAGACTCTGTTTCA AAAAAGGGAAGGGAAGGGAAGGGAA-3′, 5′-CAGGAAA-CAGCTATGACCAACAGAGCAAGACTCTGTTTCA AAAAGGGAAGGGAAGGGAAGGGAA-3′, and 5′-CAG-GAAACAGCTATGACCAACAGAGCAAGACTCTGTT TCAAAAGGGAAGGGAAGGGAAGGGAA-3′. Fragment length analysis was performed using an Applied Biosystems 3730xl DNA Analyzer with Peak Scanner software (v. 2.0). To determine whether the genotype of samples with positive RP-PCR results was heterozygous or biallelic, standard PCR was performed using published primers,[9] forward 5′-TCAAGTGA-TACTCCAGCTACACCGTTGC-3′ primer and the reverse 5′ GTGGGAGACAGGCCAATCACTTCAG-3′ primer. Observation of a band at or near 348 bp (wild-type size) corresponding to repeat sizes of less than approximately 60 repeats (approximately 650 bp) identified patients as heterozygotes. As an internal control to prevent false positives, standard PCR was also performed simultaneously on the same sample with primers designed to amplify a 282-bp band from the *SPG11* gene (figure e-1, links.lww.com/NXG/A266).

For a proportion of samples at the University of Chicago, 3 separate reactions each using 100 ng of genomic DNA were performed to confirm the existence of a true biallelic (AAGGG) repeat expansion (figure e-2, links.lww.com/NXG/A267). First, a flanking PCR was performed using primers that surrounded the RFC1 region of interest. The flanking primers

Table 1 Patient demographics

	UCLA	University of Chicago	Harvard	Combined
A. Age and sex				
Total	596	302	13	911
Female[a]	300 (50.3%)	148 (49.0%)	8 (61.5%)	456 (50.1%)
Average age at onset[b] **(y)**	55 (±17)	48 (±22)	56 (±11)	53 (±19)
B. Clinical presentation				
CANVAS	3 (0.5%)	7 (2.3%)	1 (7.7%)	11 (1.2%)
Cerebellar ataxia neuropathy[b]	41 (6.9%)	18 (6.0%)	4 (30.8%)	63 (6.9%)
Episodic ataxia	19 (3.2%)	22 (7.3%)	0	41 (4.5%)
MSA	104 (17.4%)	6 (2.0%)	1 (7.7%)	111 (12.2%)
Pure cerebellar ataxia	129 (21.6%)	62 (20.5%)	2 (15.4%)	193 (21.2%)
Spastic ataxia	38 (6.4%)	24 (7.9%)	1 (7.7%)	63 (6.9%)
Spastic paraplegia	35 (5.9%)	4 (1.3%)	0	39 (4.3%)
Spinocerebellar ataxia	158 (26.5%)	144 (47.9%)	4 (30.8%)	306 (33.6%)
Other	69 (11.6%)	15 (5.0%)	0	84 (9.2%)
C. Race/ethnicity[c]				
Asian	54 (9.1%)	12 (4.0%)	0	66 (7.2%)
Native Hawaiian or Pacific Islander	1 (0.2%)	0	0	1 (0.1%)
Native America or Alaska Native	6 (1.0%)	0	0	6 (0.7%)
Black	15 (2.5%)	10 (3.3%)	0	25 (2.7%)
White, Hispanic, or Latino	58 (9.7%)	5 (1.7%)	0	63 (6.9%)
White, non-Hispanic	393 (65.9%)	192 (63.6%)	12 (92.3%)	597 (65.5%)
Unspecified	76 (12.8%)	84 (27.8%)	1 (7.7%)	161 (17.7%)

Abbreviations: CANVAS = cerebellar ataxia, neuropathy, and vestibular areflexia syndrome; MSA = multiple system atrophy.
A) Age and sex of the enrolled subjects are shown.
B) The major clinical presentations of the patients enrolled in this study are shown. The presence of peripheral neuropathy was determined either clinically or electrophysiologically. Spastic ataxia and spastic paraplegia are distinguished based on which symptom was clinically estimated to be predominant. Spinocerebellar ataxia is used to indicate patients with cerebellar ataxia as the primary symptom but with notable features other than spasticity or neuropathy (e.g., dementia, epilepsy, or extrapyramidal signs). Although all patients exhibited ataxia, patients who did not clearly fit the major diagnostic categories listed were labeled as other (e.g., primary extrapyramidal conditions such as parkinsonism). MSA includes both possible and probable cases as defined by current diagnostic criteria.
C) Race and ethnicity of the enrolled subjects are shown. Patients who did not choose to disclose this information are listed as unspecified.
[a] Ten subjects in the UCLA cohort did not report sex.
[b] Sixteen subjects in the University of Chicago cohort did not have a reported age at onset.
[c] Individuals identifying membership in more than 1 race were counted separately for each race.

included a labeled forward primer[9] (5′-/56-FAM/ACTGACAGTGTTTTTGCCTGT-3′) (10 μm) and a reverse primer[9] (5′-GGCTGAGGCAGGAGATTCAC-3′) (10 μm). Second, RP-PCR was performed to detect individual nonpathogenic (AAAAG) motifs. The primers for the (AAAAG) RP-PCR included the same forward primer as the flanking reaction, an M13 anchor primer (5′-CAGGAAACAGCTATGACC-3′) (10 μm) and an (AAAAG) specific primer (5′-CAGGAAACAGCTATGACC_AAAAGAAAAGAAAAGAAAAGAAAAG-3′) (1 μm). Both the flanking and (AAAAG) RP-PCR products were amplified using the Takara LA PCR kit in combination with a touchdown PCR. Third, to detect individual pathogenic (AAGGG) motifs, a PCR reaction was performed using the same forward primer as the flanking, the M13 anchor primer, and an (AAGGG) specific primer[9] (5′-CAGGAAACAGCTATGACC_AAGGGAAGGGAAGGGAAGGGAAGGG-3′). To obtain full amplification of the expanded (AAGGG) motif, the Qiagen HotStarTaq chemistry was used with 400 μm of deoxyribonucleotide triphosphates using a standard PCR. All 3 products were loaded on an ABI3730xl DNA analyzer after denaturing with a 5% GS500 Rox/formamide mixture and subsequently analyzed using GeneMarker v2.6.0 (SoftGenetics Inc.).

To validate the assays performed at the different centers, a proportion of samples (>50%) determined to have

heterozygous or biallelic AAGGG expansions were assessed at least 2 times by both expansion RP-PCR testing and standard PCR genotyping in 2 separate laboratories.

DAB1 gene repeat expansion analysis

RP-PCR was performed to detect expansion of the normal (ATTTT) *DAB1* repeat region and for detection of the pathogenic $(ATTTC)_n$ insertion using published primers.[11,12] Fragment length analysis was performed as described above.

Data availability

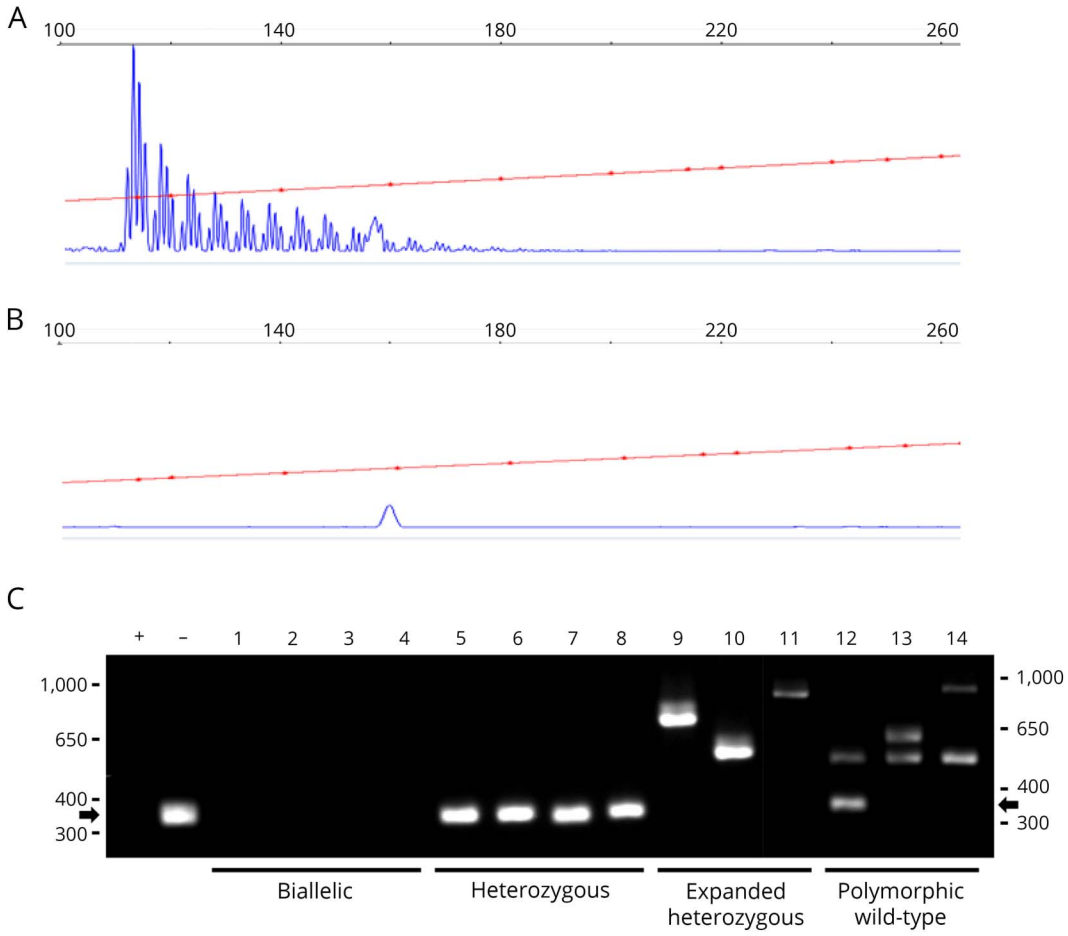
The data sets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Results

We examined the prevalence of repeat expansions in *RFC1* and *DAB1* in a large cohort from the tertiary referral Ataxia Center at UCLA. The demographics of this 596 subject cohort are described in table 1. Average age was 55 years, 50% of the patients were female, and 66% were white, non-Hispanic. The most common phenotypes were spinocerebellar ataxia (26.5%), pure cerebellar ataxia (21.6%), and multiple system atrophy (17.4%). To assess for the presence of pathogenic (AAGGG) repeat expansions in *RFC1*, fluorescent repeat-primed fragment analysis was performed and identified at least 1 expansion in 50 of 596 patients (8.4%, figure 1 and figure e-1, links.lww.com/NXG/A266). Standard PCR was used to genotype subjects for the presence of a heterozygous or a pathogenic biallelic expansion. Nine subjects (1.5%) were found with biallelic expansions. Of these patients, 3 subjects presented clinically with CANVAS (33%, 100% of phenotype), 5 had cerebellar ataxia with neuropathy (56%, 12% of phenotype), and 1 had spinocerebellar ataxia (11%, 0.6% of phenotype).

To validate these findings, we tested 2 additional cohorts from centers in different regions of the United States, the University of Chicago (UC) and Brigham and Women's Hospital affiliated

Figure 1 RFC1 expansion analysis



Representative RP-PCR results from a patient with disease due to (A) biallelic expanded (AAGGG) pathogenic alleles or a control individual (B) with wild-type alleles. Samples with RP-PCR evidence of an expanded *RFC1* allele were genotyped by standard PCR for biallelic expansion (C). Standard PCR allows categorization of individuals as biallelic with pathogenic expansions (no band, lanes 1-4), heterozygous wild-type (348 bp band, arrow, lanes 5-8), heterozygous with a non-pathogenic polymorphic expansion (variable sized bands, lanes 9-11), or wild-type with one or more non-pathogenic polymorphic expansion(s) (variable sized band(s), lanes 12-14). + = biallelic control; − = wild-type control; M = marker; *RFC1* = replication factor C subunit 1.

Table 2 Demographics of patients with heterozygous expansions in the *RFC1* gene

Sample	Phenotype	Sex	Age at onset (y)	Ethnicity	Inheritance
H1	CANVAS	Female	52	White, non-Hispanic	Familial
H2	Cerebellar ataxia + neuropathy	Male	72	White, Hispanic, or Latino	Sporadic
H3	Cerebellar ataxia + neuropathy	Male	20	White, non-Hispanic	Familial
H4	Cerebellar ataxia + neuropathy	Female	75	White, non-Hispanic	Sporadic
H5	Episodic ataxia	Male	36	White, Hispanic, or Latino	Sporadic
H6	Episodic ataxia	Male	30	White, non-Hispanic	Familial
H7	Episodic ataxia	Female	22	White, non-Hispanic	Familial
H8	Episodic ataxia	Male	10	White, non-Hispanic	Sporadic
H9	MSA	Female	66	White, non-Hispanic	Sporadic
H10	MSA	Male	63	White, non-Hispanic	Sporadic
H11	MSA	Female	56	Asian	Sporadic
H12	MSA	Male	Unknown	Unspecified	Unspecified
H13	MSA	Male	55	White, non-Hispanic	Sporadic
H14	MSA	Female	77	White, non-Hispanic	Sporadic
H15	MSA	Male	61	Unspecified	Sporadic
H16	MSA	Male	63	White, non-Hispanic	Sporadic
H17	MSA	Male	54	White, non-Hispanic	Sporadic
H18	Pure cerebellar ataxia	Male	72	White, non-Hispanic	Sporadic
H19	Pure cerebellar ataxia	Female	61	White, non-Hispanic	Sporadic
H20	Pure cerebellar ataxia	Female	66	Unspecified	Sporadic
H21	Pure cerebellar ataxia	Female	62	White, non-Hispanic	Sporadic
H22	Pure cerebellar ataxia	Female	76	Unspecified	Sporadic
H23	Pure cerebellar ataxia	Unknown	66	White, non-Hispanic, Native American, or Alaska Native	Sporadic
H24	Pure cerebellar ataxia	Female	37	White, non-Hispanic	Sporadic
H25	Pure cerebellar ataxia	Male	68	White, non-Hispanic	Sporadic
H26	Pure cerebellar ataxia	Female	70	White, non-Hispanic	Sporadic
H27	Pure cerebellar ataxia	Male	21	White, Hispanic, or Latino	Sporadic
H28	Pure cerebellar ataxia	Male	57	White, non-Hispanic	Familial
H29	Spastic ataxia	Male	54	White, non-Hispanic	Sporadic
H30	Spastic ataxia	Female	63	White, Hispanic, or Latino	Sporadic
H31	Spastic ataxia	Male	78	White, non-Hispanic	Sporadic
H32	Spastic ataxia	Female	57	White, non-Hispanic	Sporadic
H33	Spastic ataxia	Male	68	White, non-Hispanic	Familial
H34	Spastic ataxia	Female	38	Unspecified	Sporadic
H35	Spastic ataxia	Male	71	Unspecified	Familial
H36	Spastic paraplegia	Male	61	White, non-Hispanic	Unspecified
H37	Spastic paraplegia	Female	32	White, non-Hispanic	Sporadic
H38	Spinocerebellar ataxia	Male	84	Unspecified	Sporadic

Continued

Table 2 Demographics of patients with heterozygous expansions in the *RFC1* gene *(continued)*

Sample	Phenotype	Sex	Age at onset (y)	Ethnicity	Inheritance
H39	Spinocerebellar ataxia	Male	48	White, non-Hispanic	Sporadic
H40	Spinocerebellar ataxia	Female	66	White, non-Hispanic	Sporadic
H41	Spinocerebellar ataxia	Female	44	White, non-Hispanic	Sporadic
H42	Spinocerebellar ataxia	Male	Unknown	Unspecified	Unspecified
H43	Spinocerebellar ataxia	Male	Unknown	Unspecified	Unspecified
H44	Spinocerebellar ataxia	Female	69	White, Hispanic, or Latino	Sporadic
H45	Spinocerebellar ataxia	Female	76	White, Hispanic, or Latino	Familial
H46	Spinocerebellar ataxia	Female	62	White, non-Hispanic	Familial
H47	Spinocerebellar ataxia	Male	36	White, non-Hispanic	Sporadic
H48	Spinocerebellar ataxia	Female	35	White, non-Hispanic	Familial
H49	Spinocerebellar ataxia	Female		White, non-Hispanic	Familial
H50	Spinocerebellar ataxia	Female	42	White, non-Hispanic	Sporadic
H51	Spinocerebellar ataxia	Female	35	Asian	Sporadic
H52	Spinocerebellar ataxia	Female	47	Unspecified	Sporadic
H53	Spinocerebellar ataxia	Male	44	White, non-Hispanic	Sporadic
H54	Spinocerebellar ataxia	Male	41	White, non-Hispanic	Familial
H55	Other	Unknown	24	Unspecified	Unspecified
H56	Other	Female	69	White, non-Hispanic	Sporadic
H57	Other	Female	35	White, Hispanic, or Latino	Familial
H58	Other	Male	78	White, non-Hispanic	Sporadic
H59	Other	Unknown	65	Unspecified	Unspecified
H60	Other	Male	53	Unspecified	Sporadic
H61	Other	Female	55	White, non-Hispanic	Familial
H62	Other	Female	24	White, non-Hispanic	Sporadic

Abbreviations: CANVAS = cerebellar ataxia, neuropathy, and vestibular areflexia syndrome; MSA = multiple system atrophy; *RFC1* = replication factor C subunit 1.

with Harvard Medical School. Demographics were similar to the UCLA cohort (table 1). The larger UC cohort, consisting of both sporadic and familial cases with roughly one-third of subjects originating from Canada, showed heterozygous expansions in 20 individuals (6.6%) and pathogenic biallelic expansion in 17 patients (5.6%) (figure e-2, links.lww.com/NXG/A267). Four subjects had CANVAS (24%, 57% of phenotype), 7 had cerebellar ataxia with neuropathy (41%, 39% of phenotype), 4 had pure cerebellar ataxia (23.5%, 6.5% of phenotype), and 2 had spinocerebellar ataxia (12%, 1.4% of phenotype). In the smaller Harvard cohort, 1 individual showed heterozygous expansion (7.7%), and 3 of 13 patients (23%) had pathogenic biallelic expansions. Two of these had cerebellar ataxia with neuropathy (67%, 50% of phenotype), while 1 had CANVAS (33%, 100% of phenotype). Collectively, 62 individuals were found with heterozygous expansion (6.8%, 62/911, table 2), and 29 of 911 subjects showed pathogenic biallelic expansions across all cohorts for a total prevalence of 3.2% (29/911, table 3). Of the biallelic cases, the majority of the patients were white (24/29, 83%), 1 patient was Hispanic (1/29, 3.4%), and the race/ethnicity of 5 patients was not reported (17%). Twelve of the cases showed sporadic onset (41%). In total, 8 subjects had CANVAS (28%, 73% of phenotype), 14 had cerebellar ataxia with neuropathy (48%, 22% of phenotype), 4 had pure cerebellar ataxia (14%, 2.1% of phenotype), and 3 had spinocerebellar ataxia (10%, 1.0% of phenotype).

For *DAB1* repeat expansion analysis, 83/596 (13.9%) subjects showed an expanded ATTTT allele by RP-PCR analysis; however, none of these patients possessed the pathogenic ATTTC insertion, indicating that no patients within the cohort had SCA37.

Table 3 Demographics of patients with biallelic expansions in the *RFC1* gene

Sample	Phenotype	Sex	Age at onset (y)	Ethnicity	Inheritance
B1	CANVAS	Male	78	White, non-Hispanic	Sporadic
B2	CANVAS	Female	69	White, non-Hispanic	Familial
B3	CANVAS	Male	63	White, non-Hispanic	Familial
B4	CANVAS	Female	33	White, non-Hispanic	Familial
B5	CANVAS	Male	40	White, non-Hispanic	Sporadic
B6	CANVAS	Female	61	Unspecified	Unknown
B7	CANVAS	Female	56	White, non-Hispanic	Sporadic
B8	CANVAS	Male	60	White, non-Hispanic	Sporadic
B9	Cerebellar ataxia + neuropathy	Female	81	White, non-Hispanic	Familial
B10	Cerebellar ataxia + neuropathy	Female	66	White, Hispanic, or Latino	Familial
B11	Cerebellar ataxia + neuropathy	Female	71	White, non-Hispanic	Sporadic
B12	Cerebellar ataxia + neuropathy	Male	69	White, non-Hispanic	Sporadic
B13	Cerebellar ataxia + neuropathy	Female	71	White, non-Hispanic	Sporadic
B14	Cerebellar ataxia + neuropathy	Female	42	White, non-Hispanic	Familial
B15	Cerebellar ataxia + neuropathy	Female	39	White, non-Hispanic	Familial
B16	Cerebellar ataxia + neuropathy	Male	38	White, non-Hispanic	Familial
B17	Cerebellar ataxia + neuropathy	Female	64	Unspecified	Familial
B18	Cerebellar ataxia + neuropathy	Female	55	Unspecified	Familial
B19	Cerebellar ataxia + neuropathy	Female	45	White, non-Hispanic	Sporadic
B20	Cerebellar ataxia + neuropathy	Male	37	White, non-Hispanic	Familial
B21	Cerebellar ataxia + neuropathy	Female	55	White, non-Hispanic	Sporadic
B22	Cerebellar ataxia + neuropathy	Female	64	White, non-Hispanic	Sporadic
B23	Pure cerebellar ataxia	Male	45	Unspecified	Familial
B24	Pure cerebellar ataxia	Female	76	White, non-Hispanic	Familial
B25	Pure cerebellar ataxia	Female	62	White, non-Hispanic	Familial
B26	Pure cerebellar ataxia	Male	55	White, non-Hispanic	Familial
B27	Spinocerebellar ataxia	Female	Unknown	Unspecified	Unknown
B28	Spinocerebellar ataxia	Male	45	White, non-Hispanic	Sporadic
B29	Spinocerebellar ataxia	Female	38	White, non-Hispanic	Sporadic

Abbreviations: CANVAS = cerebellar ataxia, neuropathy, and vestibular areflexia syndrome; *RFC1* = replication factor C subunit 1.

Discussion

Overall, in a large undiagnosed ataxia cohort of 911 patients from 3 tertiary referral centers in the United States, biallelic pathogenic (AAGGG) repeat expansions in *RFC1* were observed in 3.2% (n = 29, 95% CI 2.0%–4.3%) of patients from the United States (25/29, 86%) and Canada (4/29, 14%). The observation that the majority of cases (24/24, 100%, table 3), where ethnicity was known, were white of European ancestry is consistent with previous reports.[9,10] Of the heterozygotes with race and ethnicity data available, 96% (47/49, table 2) were of white ancestry, and overall, single or biallelic expansions were detected in 7.1% and 3.6%, respectively, of the total white population surveyed in this study. The high rate of heterozygosity (6.8%) in our cohort is notable but is similar to 1 previous study, which calculated an allele frequency of 4% in control populations of 69 and 133 individuals based on haplotype in next-generation

sequencing data sets,[10] although another study found a frequency of 0.7% in a cohort of 304 healthy controls using RP-PCR.[9] Although our methods cannot accurately size larger repeats, standard PCR analysis indicated that under the conditions of our AAGGG RP-PCR assay, we were able to detect small expansions up to 60 repeats above wild type (~650 bp, figure 1 and figure e-1, links.lww.com/NXG/A266), and therefore, we suspect that our high rate of detection may be due, in part, to the detection of confounding mildly expanded alleles below the estimated 400 repeat margin of pathogenicity[9] (figure 1 and figure e-1, links.lww.com/NXG/A266). It is interesting to note that small AAGGG expansions have not previously been reported in patients or controls,[9,10] and because all subjects tested presented with some form of cerebellar ataxia, we cannot exclude the possibility that expanded AAGGG repeats may contribute to the development of cerebellar ataxia in some heterozygous individuals. We also cannot rule out a contribution of false-positive detection of other small polymorphic nonpathogenic non-AAGGG repeats[9] in some heterozygous individuals. We did confirm all biallelic subjects with RP-PCR and standard PCR in at least 2 experiments each across 2 separate laboratories and further determined that none of these individuals harbored the most common nonpathogenic expanded repeat, AAAAG[9] (data not shown).

In addition, the pathogenic (ATTTC) repeat in the *DAB1* gene, causative for SCA37, was not observed in our large undiagnosed ataxia cohort of 596 individuals of mostly white, non-Hispanic ancestry, consistent with the observation of a founder effect in the Iberian Peninsula[11,13] and suggesting that although this disorder should be considered in that population, it is likely extremely rare in the United States. Although no pathogenic ATTTC insertions were found, it is possible that extremely large repeats of ATTTT flanking a pathogenic ATTTC insertion might prevent amplification of products from the RP-PCR, so false negatives cannot be ruled out in this study, although this has not been commonly observed in published reports.[11,12]

Consistent with previous reports, our study identified biallelic *RFC1* expansions in a high percentage of patients with CANVAS (n = 8, 73%) and cerebellar ataxia with neuropathy[9] (n = 14, 22%). Although we do not have electrophysiologic data on all subjects, of the biallelic patients identified, all appeared to have a large fiber neuropathy (data not shown), which would be an important focus for further clinical investigation. In addition, we also observed biallelic expansion in a notable percentage of patients with pure cerebellar ataxia (n = 4, 2.1%) and generalized spinocerebellar ataxia (n = 3, 1.0%), a frequency on par with the majority of rare ataxic disorders identifiable by clinical sequencing.[2] Based on this study, the presence of neuropathy confers a 20.1% absolute benefit to testing (95% CI 9.7%–30.6%) over pure cerebellar ataxia alone, and the presence of both neuropathy and vestibulopathy further increases this to 70.6% (95% CI 44.2%–97.0%), with biallelic *RFC1* expansions anticipated in 1 of every 5.0 (95% CI 3.3–10.3) and 1 of every 1.4 (95% CI 1.0–2.3) patients tested, respectively. Taken together, these results suggest that *RFC1* expansion testing is high yield in cases of CANVAS and cerebellar ataxia with neuropathy but should also be considered in the genetic workup of patients with undiagnosed pure cerebellar and spinocerebellar ataxia.

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Disclosure

All authors declare that there is no conflict of interest. Go to Neurology.org/NG for full disclosures.

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Appendix Authors

Name	Location	Contribution
Dona Aboud Syriani	University of California, Los Angeles	Acquisition of data, analysis of data, and drafting of the manuscript
Darice Wong, PhD	University of California, Los Angeles	Analysis of data, study design, and drafting of the manuscript
Claudio M. De Gusmao, MD	Brigham and Women' Hospital and Harvard Medical School	Acquisition of data and revision of the manuscript for intellectual content
Sameer Andani, BS	University of Chicago	Acquisition of data, analysis of data, and revision of the manuscript for intellectual content
Yuanming Mao, BS	University of California, Los Angeles	Acquisition of data, analysis of data, and drafting of the manuscript for intellectual content
May Sanyoura, PhD	University of Chicago	Acquisition of data, analysis of data, and revision of the manuscript for intellectual content
Giacomo Glotzer	University of Chicago	Acquisition of data and revision of the manuscript for intellectual content

Appendix *(continued)*

Name	Location	Contribution
Paul J. Lockhart, PhD	Murdoch Children' Research Institute	Study design and revision of the manuscript for intellectual content
Sharon Hassin-Baer, MD	Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel	Acquisition of data and revision of the manuscript for intellectual content
Vikram Khurana, MD, PhD	Brigham and Women's Hospital and Harvard Medical School	Acquisition of data, study design, and revision of the manuscript for intellectual content
Christopher M. Gomez, MD, PhD	University of Chicago, Chicago	Acquisition of data, study design, analysis of data, and revision of the manuscript for intellectual content
Susan Perlman, MD	University of California, Los Angeles	Acquisition of data and revision of the manuscript for intellectual content
Soma Das, PhD	University of Chicago	Acquisition of data, data analysis, study design, and revision of the manuscript for intellectual content

Appendix *(continued)*

Name	Location	Contribution
Brent L. Fogel, MD, PhD	University of California, Los Angeles	Study design and supervision, acquisition of data, analysis of data, and drafting and revision of the manuscript for intellectual content

References

1. Sun M, Johnson AK, Nelakuditi V, et al. Targeted exome analysis identifies the genetic basis of disease in over 50% of patients with a wide range of ataxia-related phenotypes. Genet Med 2019;21:195–206.
2. Fogel BL, Lee H, Deignan JL, et al. Exome sequencing in the clinical diagnosis of sporadic or familial cerebellar ataxia. JAMA Neurol 2014;71:1237–1246.
3. Rexach J, Lee H, Martinez-Agosto JA, Nemeth AH, Fogel BL. Clinical application of next-generation sequencing to the practice of neurology. Lancet Neurol 2019;18:492–503.
4. Ngo KJ, Rexach JE, Lee H, et al. A diagnostic ceiling for exome sequencing in cerebellar ataxia and related neurological disorders. Hum Mutat 2020;41:487–501.
5. Paulson H. Repeat expansion diseases. Handb Clin Neurol 2018;147:105–123.
6. Mundwiler A, Shakkottai VG. Autosomal-dominant cerebellar ataxias. Handb Clin Neurol 2018;147:173–185.
7. Fogel BL. Autosomal-recessive cerebellar ataxias. Handb Clin Neurol 2018;147:187–209.
8. Wallace SE, Bird TD. Molecular genetic testing for hereditary ataxia: what every neurologist should know. Neurol Clin Pract 2018;8:27–32.
9. Cortese A, Simone R, Sullivan R, et al. Biallelic expansion of an intronic repeat in RFC1 is a common cause of late-onset ataxia. Nat Genet 2019;51:649–658.
10. Rafehi H, Szmulewicz DJ, Bennett MF, et al. Bioinformatics-based identification of expanded repeats: a non-reference intronic pentamer expansion in RFC1 causes CANVAS. Am J Hum Genet 2019;105:151–165.
11. Seixas AI, Loureiro JR, Costa C, et al. A pentanucleotide ATTTC repeat insertion in the non-coding region of DAB1, mapping to SCA37, causes spinocerebellar ataxia. Am J Hum Genet 2017;101:87–103.
12. Loureiro JR, Oliveira CL, Sequeiros J, Silveira I. A repeat-primed PCR assay for pentanucleotide repeat alleles in spinocerebellar ataxia type 37. J Hum Genet 2018;63:981–987.
13. Corral-Juan M, Serrano-Munuera C, Rabano A, et al. Clinical, genetic and neuropathological characterization of spinocerebellar ataxia type 37. Brain 2018;141:1981–1997.