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Mutations In Rare Ataxia Genes Are Uncommon Causes of Sporadic Cerebellar Ataxia

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Abstract

BACKGROUND—Sporadic-onset ataxia is common in a tertiary care setting but a significant percentage remains unidentified despite extensive evaluation. Rare genetic ataxias, reported only in specific populations or families, may contribute to a percentage of sporadic ataxia.

METHODS—Patients with adult-onset sporadic ataxia, who tested negative for common genetic ataxias (SCA1, SCA2, SCA3, SCA6, SCA7, and/or Friedreich ataxia), were evaluated using a stratified screening approach for variants in seven rare ataxia genes.

RESULTS—We screened patients for published mutations in *SYNE1* (n=80) and *TGM6* (n=118), copy number variations in *LMNB1* (n=40) and *SETX* (n=11), sequence variants in *SACS* (n=39) and *PDYN* (n=119), and the pentanucleotide insertion of spinocerebellar ataxia type 31 (n=101). Overall, we identified one patient with a *LMNB1* duplication, one patient with a *PDYN* variant, and one compound *SACS* heterozygote, including a novel variant.

CONCLUSIONS—The rare genetic ataxias examined here do not significantly contribute to sporadic cerebellar ataxia in our tertiary care population.

Keywords

Cerebellar Ataxia; Spinocerebellar Ataxia; Dominant Genetic Conditions; Recessive Genetic Conditions; Copy Number Variation

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Introduction

Sporadic cases of ataxia are common in a tertiary care setting and a significant percentage remain etiologically obscure despite extensive evaluation [1, 2]. Genetic ataxias comprise a large heterogeneous class of diseases difficult to distinguish by phenotype alone [3, 4]. Many rare genetic ataxias have thus far been found only in specific populations or in a few families and little is known regarding their contributions to sporadic ataxia worldwide. Screening sporadic-onset patients with large genetic panels can be exceedingly costly and there is minimal evidence to support this as a routine practice [4]. To address this issue, we conducted a stratified genetic screen of seven rare genes to assess their potential contribution to sporadic ataxia.

We selected rare ataxia genes for screening based on their potential to appear in a sporadic-onset clinical population. Phenotypic or genotypic characteristics which could lend themselves to such a presentation include a late age of onset (defined as age 50 or greater) [1], autosomal recessive inheritance [3], or copy number variations, whose frequency of mutation rate exceeds that of single point mutations by several orders of magnitude [5]. Based on these criteria, we selected the following seven genes for analysis in our clinical population. *PDYN*, which has been shown to be causative for spinocerebellar ataxia (SCA), type 23 (SCA23, MIM #610245), an autosomal dominant disorder reported in the Netherlands and characterized clinically by a late-onset, slowly progressive, pure cerebellar ataxia [6]. *TGM6*, which causes SCA35 (MIM #613908), another autosomal dominant ataxia identified in China, with onset in the 4th decade and clinical features of cerebellar ataxia associated with upper motor neuron features [7]. *SACS*, responsible for Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS, MIM #270550), a disease typically characterized by early-onset (age less than 5 years) spastic ataxia and sensorimotor peripheral neuropathy, originally reported in Canada but more recently seen worldwide including adult ages of onset [8, 9]. *SYNE1*, the causative gene for Autosomal Recessive Cerebellar Ataxia, Type 1 (ARCA1, MIM #610743), an ataxia seen in French-Canadians featuring a pure cerebellar ataxia with onset in the 3rd decade [10, 11]. *LMNB1*, where a gene duplication event gives rise to Autosomal Dominant Leukodystrophy (ADLD, MIM #169500), an adult-onset condition presenting clinically in the 4th decade with cerebellar ataxia, upper motor neuron signs, and autonomic dysfunction [12]. We also examined copy number variations in the *SETX* gene responsible for Ataxia with Oculomotor Apraxia Type 2 (AOA2, MIM #606002) , an adolescent-onset cerebellar ataxia with sensorimotor peripheral neuropathy [13]. Although this disease is known to be caused by a diverse variety of mutations including missense, nonsense, frameshifts, or those affecting RNA splicing [14–16], as well as copy number variations [17], heterozygous variants are often seen in ataxia referral clinics due to the gene's high degree of polymorphism, so we therefore specifically examined copy number variations in such individuals. Lastly, we further examined our population for the intergenic insertion and pentanucleotide repeats associated with SCA31 (MIM #117210), a late-onset pure cerebellar ataxia commonly found in Japan [18].

Overall, using this strategy, we molecularly identified less than 3% of our screened population with one of these seven disorders suggesting that they are not significant contributors to sporadic ataxia and should not be routinely screened in patients without the appropriate clinical context.

Patients and Methods

Patients initially presented to our tertiary referral ataxia center with primarily adult-onset sporadic cerebellar ataxia. For enrollment in this study, patients were required to have negative testing for the most common genetic ataxias worldwide [3, 4], specifically SCA1,

SCA2, SCA3, SCA6, SCA7, and/or Friedreich ataxia, although some participants may have had additional testing prior to referral or guided by phenotype as appropriate. No patient met criteria for probable multiple system atrophy (MSA) as defined by Gilman et al[19]. Consent was obtained to extract DNA for genetic analysis and all patients were provided genetic counseling. Patients were then stratified by phenotype for further genetic screening of either the *SACS*, *PDYN*, *TGM6*, *SYNE1*, *LMNB1*, and/or *SETX* genes as described in the text. Ethnicity was not considered in the stratification. Sequencing of all expressed exons was performed for *SACS*[8] and for *PDYN*[6], whereas targeted sequencing, directed to the exons containing previously reported mutations, was performed for *TGM6*[7], and *SYNE1*[11]. All sequencing was performed using the Sanger method. Copy number analysis was directed to gene regions previously reported to be variable in patients for *LMNB1*[12], and *SETX*[17] using RT-PCR (TaqMan, Applied Biosystems) with probes targets to exon 4 (*LMNB1*, NM_005573) and exons 8 and 10 (*SETX*, NM_015046) . PCR analysis of genomic DNA was performed to assess for the intergenic insertions and pentanucleotide repeats found in SCA31 according to previously published methods[18]. Upon study completion, patients were provided general non-identifiable study information in aggregate form and/or additional genetic counseling as appropriate. All study methods were approved by the Institutional Review Board of the University of California, Los Angeles (UCLA).

Results

Patients were stratified for screening of rare ataxia genes based on a generalized clinical strategy to maximize the chances of obtaining a positive result based on previous reports of the phenotype associated with mutations in the various genes (see Table 1). For *LMNB1*, patients were selected if they presented with spastic paraplegia, spastic ataxia, or if they had ataxia with white matter hyperintensities on brain MRI. The same population was screened for *SACS* variants except that patients with white matter hyperintensities were excluded. Patients with pure cerebellar ataxia or a spinocerebellar ataxia phenotype[4] were selected for screening *PDYN*, *SYNE1*, *TGM6*, and SCA31. Patients with a spinocerebellar phenotype known to have one or more variants of unknown significance on a single allele of the *SETX* gene were screened for copy number variations. In some cases, patients with overlapping phenotypes were screened in multiple categories (see Table 1).

A summary of the sequencing analysis is shown in Table 2. In our population, we did not identify any previously reported causal mutations in *SYNE1* or *TGM6* nor any of the reported structural variations in *SETX* or SCA31. We did identify one patient with the reported causal duplication of the *LMNB1* gene[12] and another patient with a reported mutation in the *PDYN* gene, (p.R138S)[6]. Another patient was found to be a compound heterozygote in *SACS* for both a previously reported mutation (p.N4549D) [20] as well as a novel nonsense variant (p.E174X). All three patients presented with the documented phenotypes for their respective diseases, without notable clinical deviations. The remaining genetic variation (see Table 2) either represented known or suspected polymorphisms, based on detection in normal populations, or was of uncertain clinical significance (e.g. a single variant in a gene not known to cause a dominant phenotype). Unfortunately, unaffected parental DNA samples were not available in the majority of cases to determine whether these variants were inherited or arose *de novo*.

Discussion

In this report we screened subsets of a tertiary care sporadic-onset ataxia population for seven rare genetic ataxias and found that none of these provided a notable contribution to diagnosis in this population. In the case of the *SYNE1* and TGM6 genes, this may be related to patients possessing novel mutations outside of the region sequenced, or with regard to

LMNB1 and *SETX*, structural variants involving regions outside those tested, as our study was designed to detect only those variants previously reported in the literature as disease-causing. One further caveat to this result arises from our use of stratification as opposed to indiscriminate screening. Methodologically, a phenotype-based approach to patient testing was chosen because, given the large number of ataxia genes available to the clinician for testing [3, 4], it is generally impractical to engage in non-directed screening as this can quickly become quite costly and time-consuming for the patient and clinician. The obvious disadvantage to this strategy is that novel genetic variants in unanticipated genes may result in unexpected phenotypes which would consequently be missed. Ultimately, newer technologies, such as next-generation sequencing [21], will dramatically reduce the cost and throughput of more widespread testing, enabling more detailed screening of patients for multiple rare ataxia genes to identify such novel phenotypes, if they exist. This may include novel dominant phenotypes associated with genes currently established as causing recessive disease. An alternate hypothesis is that a majority of sporadic-onset ataxia may be idiopathic in nature, including such entities as idiopathic late-onset cerebellar ataxia and others, but as these are typically diagnoses of exclusion, more detailed analysis of populations of these patients must be done to fully rule out complex genetic contributions.

At best, stratified genetic screening for these seven rare ataxias yielded a diagnosis in less than 3% of patients tested in our sporadic-onset cerebellar ataxia tertiary clinical population. As testing was driven by the predicted phenotype, barring unforeseen clinical variability, we predict generalized screening for these rare ataxia genes is unlikely to be helpful diagnostically. We therefore recommend their testing only in the appropriate clinical context.

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Table 1

Patient characteristics and phenotypic stratification

A) The demographics of the patient population screened for the seven genes in this study is shown. F, female; SD, standard deviation; y, year. B) The phenotypic categorization of the patient population screened is shown for each individual gene. Features guiding patient selection for a particular genetic test were selected to maximize discovery based on published clinical presentations (see text). Note that patients are listed only by a single phenotypic descriptor although individuals with overlapping features were included in multiple test categories.

A.				
Gene	**N**	**Age (y)**	**SD (y)**	**Sex (%F)**
LMNB1	40	46.1	15.8	55.0
PDYN	119	52.8	15.4	51.7
SACS	39	46.5	16.0	56.4
SCA31	101	56.0	14.2	53.5
SETX	11	43.1	18.0	66.7
SYNE1	80	53.3	15.2	58.8
TGM6	118	52.6	15.4	51.3

B.	**Gene**						
Phenotype	***LMNB1***	***PDYN***	***SACS***	**SCA31**	***SETX***	***SYNE1***	***TGM6***
Pure cerebellar	2	12	2	48	0	51	12
Spastic ataxia	29	30	28	0	0	17	30
Spastic paraplegia	6	8	7	0	0	3	8
Spinocerebellar ataxia	1	69	1	53	11	4	68
Episodic ataxia	0	0	0	0	0	5	0
Leukodystrophy	2	0	1	0	0	0	0
Total	40	119	39	101	11	80	118

Table 2

Genetic variants identified in this study

Variants are shown using genomic coordinates from the hg19 human genome build (http://genome.ucsc.edu). Nomenclature is based on the NCBI reference sequences for *PDYN* (NM_024411), *SACS* (NM_014363), *SYNE1* (NM_182961), and *TGM6* (NM_198994). SNP reference numbers (RefSNP) are from dbSNP (NCBI version dbSNP132). Frequency is based on the presence of the variant in the 1000 Genomes population database (November 2010 release) and variants reported there are presumed here to be polymorphisms. Causal variants identified in this study are highlighted. A compound heterozygote for *SACS* variants is indicated (*). Variants of unknown clinical significance are designated as such.

Gene	Variant	N	HZs	HMs	Location	Transcript	Protein	dbSNP ID	Frequency	Pathogenic
PDYN	chr20:1960876 G>A	1	1	0	UTR3	n/a	n/a	-	0.002	-
	chr20:1961134 A>G	28	25	3	exonic	c.T600C	p.H200H	rs6045819	0.125	-
	chr20:1961298 T>G	2	2	0	exonic	c.A436C	p.M146L	rs77155664	0.005	-
	chr20:1961320 C>A	1	1	0	exonic	c.G414T	p.R138S	-	-	Yes
	chr20:1963610 T>C	1	1	0	exonic	c.A121G	p.N41D	rs59191035	0.005	-
SACS	chr13:23904298 T>G	1	1	0	exonic	c.A13717C	p.N4573H	rs34382952	0.002	-
	chr13:23904370 T>C	1	1*	0	exonic	c.A13645G	p.N4549D	-	-	Yes
	chr13:23904897 T>C	1	1	0	exonic	c.A13118G	p.D4373G	-	-	Unknown
	chr13:23906235 G>A	1	1	0	exonic	c.C11780T	p.A3927V	-	-	Unknown
	chr13:23906983 G>C	5	5	0	exonic	c.C11032G	p.P3678A	rs17078601	0.03	-
	chr13:23907909 A>G	13	7	6	exonic	c.T10106C	p.V3369A	rs17078605	0.22	-
	chr13:23913939 A>G	1	1	0	exonic	c.T4076C	p.M1359T	-	-	Unknown
	chr13:23928671 C>T	1	1	0	exonic	c.G2080A	p.A694T	rs17325713	0.01	-
	chr13:23930055 A>T	10	10	0	exonic	c.T696A	p.N232K	rs2031640	0.07	-
	chr13:23932558 C>A	1	1*	0	exonic	c.G520T	p.E174X	-	-	This study
	chr13:23939319 A>G	1	1	0	exonic	c.T443C	p.M148T	-	-	Unknown
SYNE1	chr6:152639184 C>G	1	1	0	intronic	n/a	n/a	rs17082448	0.083	-
	chr6:152522926 G>A	20	18	2	intronic	n/a	n/a	rs2253512	0.13	-
	chr6:152540031 T>C	4	3	1	intronic	n/a	n/a	rs78900103	0.07	-
	chr6:152540187 T>G	1	1	0	exonic	c.A21995C	p.H7332P	-	-	Unknown
	chr6:152540278 A>C	6	6	0	exonic	c.T21904G	p.F7302V	rs2147377	1.0	-
	chr6:152615042 G>T	10	8	2	intronic	n/a	n/a	rs73007780	0.04	-
	chr6:152615200 G>A	14	14	0	exonic	c.C17745T	p.H5915H	rs12664753	0.14	-

Gene	Variant	N	HZs	HMs	Location	Transcript	Protein	dbSNP ID	Frequency	Pathogenic
	chr6:152638934 C>G	1	1	0	intronic	n/a	n/a	-	-	Unknown
	chr6:152640110 G>A	2	2	0	exonic	c.C16277T	p.T5426M	rs2306914	0.05	-
	chr6:152668211 A>G	1	1	0	exonic	c.T12061C	p.C4021R	rs111449472	0.007	-
	chr6:152668272 C>T	1	1	0	exonic	c.G12000A	p.A4000A	-	0.005	-
	chr6:152671975 A>G	1	1	0	intronic	n/a	n/a	rs1387549	0.56	-
TGM6	chr20:2380933 G>A	1	1	0	intronic	n/a	n/a	rs79778733	0.01	-
	chr20:2397883 C>T	3	3	0	exonic	c.C1342T	p.R448W	-	0.01	-
	chr20:2398017 G>A	46	38	8	exonic	c.G1476A	p.K494K	rs2295077	0.312	-
	chr20:2398064 G>A	1	1	0	exonic	c.G1523A	p.G508D	-	0.002	-
	chr20:2398121 T>A	1	1	0	exonic	c.T1580A	p.V527E	rs61729226	0.003	-