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Diagnostic Efficacy of Genetic Studies in a Series of Hereditary Cerebellar Ataxias in Eastern Spain

Raquel Baviera-Muñoz, MD, Lidón Carretero-Vilarroig, MSc, Juan Francisco Vázquez-Costa, MD, Carlos Morata-Martínez, MD, Marina Campins-Romeu, MD, Nuria Muelas, MD, Isabel Sastre-Bataller, MD, Irene Martínez-Torres, MD, Julia Pérez-García, BSN, Rafael Sivera, MD, Teresa Sevilla, MD, Juan J. Vilchez, MD, Teresa Jaijo, PhD, Carmen Espinós, PhD, Jose M. Millán, PhD, Luis Bataller, MD, and Elena Aller, PhD

Correspondence
Dr. Bataller
l_bataller@yahoo.com

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Abstract

Background and Objectives

To determine the diagnostic efficacy of clinical exome-targeted sequencing (CES) and spinocerebellar ataxia 36 (SCA36) screening in a real-life cohort of patients with cerebellar ataxia (CA) from Eastern Spain.

Methods

A total of 130 unrelated patients with CA, negative for common trinucleotide repeat expansions (SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA12, SCA17, dentatorubral pallidoluysian atrophy [DRPLA], and Friedreich ataxia), were studied with CES. Bioinformatic and genotype-phenotype analyses were performed to assess the pathogenicity of the variants encountered. Copy number variants were analyzed when appropriate. In undiagnosed dominant and sporadic cases, repeat primed PCR was used to screen for the presence of a repeat expansion in the *NOP56* gene.

Results

CES identified pathogenic or likely pathogenic variants in 50 families (39%), including 23 novel variants. Overall, there was a high genetic heterogeneity, and the most frequent genetic diagnosis was *SPG7* (n = 15), followed by *SETX* (n = 6), *CACNA1A* (n = 5), *POLR3A* (n = 4), and *SYNE1* (n = 3). In addition, 17 families displayed likely pathogenic/pathogenic variants in 14 different genes: *KCND3* (n = 2), *KIF1C* (n = 2), *CYP27A1A* (n = 2), *AFG3L2* (n = 1), *ANO10* (n = 1), *CAPN1* (n = 1), *CWF19L1* (n = 1), *ITPR1* (n = 1), *KCNA1* (n = 1), *OPA1* (n = 1), *PNPLA6* (n = 1), *SPG11* (n = 1), *SPTBN2* (n = 1), and *TPP1* (n = 1). Twenty-two novel variants were characterized. SCA36 was diagnosed in 11 families, all with autosomal dominant (AD) presentation. SCA36 screening increased the total diagnostic rate to 47% (n = 61/130). Ultimately, undiagnosed patients showed delayed age at onset ($p < 0.05$) and were more frequently sporadic.

Discussion

Our study provides insight into the genetic landscape of CA in Eastern Spain. Although CES was an effective approach to capture genetic heterogeneity, most patients remained undiagnosed. SCA36 was found to be a relatively frequent form and, therefore, should be tested prior to CES in familial AD presentations in particular geographical regions.

From the Neuromuscular Diseases Unit (R.B.-M., J.F.V.-C., C.M.-M., M.C.-R., N.M., I.S.-B., I.M.-T., J.P.-G., R.S., T.S., L.B.), Neurology Department, Hospital Universitari I Politècnic La Fe and Neuromuscular Reference Centre, ERN-EURO-NMD; Neuromuscular and Ataxias Research Group. Instituto de Investigación Sanitaria La Fe (R.B.-M., L.C.-V., J.F.V.-C., N.M., R.S., T.S., J.J.V., L.B.); Rare Diseases Joint Unit (R.B.-M., L.C.-V., J.F.V.-C., N.M., I.S.-B., I.M.-T., T.S., J.J.V., T.J., C.E., J.M.M., L.B., E.A.), CIPF-IIS La Fe; Cell Biology Department (L.C.-V.), University of Valencia; Department of Medicine (J.F.V.-C., N.M., T.S., L.B.), University of Valencia; Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER) (J.F.V.-C., N.M., R.S., T.S., J.J.V., T.J., J.M.M., L.B., E.A.); Department of Medicine (R.S.), University CEU Cardenal Herrera; Department of Genetics (T.J., E.A.), Hospital Universitari I Politècnic La Fe; Cellular (T.J., J.M.M., E.A.), Molecular and Genomics Biomedicine Group, Instituto de Investigación Sanitaria La Fe; and Laboratory of Rare Neurodegenerative Diseases (C.E.), Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain.

Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

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Glossary

aCGH = arrayCGH; **AD** = autosomal dominant; **AOA2** = ataxia with oculomotor apraxia type 2; **AR** = autosomal recessive; **CA** = cerebellar ataxia; **CES** = clinical exome-targeted sequencing; **CNV** = copy number variant; **MLPA** = multiple ligation-dependent probe amplification analysis; **NGS** = next-generation sequencing; **RP-PCR** = repeat primed PCR; **SCA** = spinocerebellar ataxia.

Hereditary cerebellar ataxias (CAs) are highly heterogeneous disorders frequently associated with additional neurologic and extraneurologic manifestations.[1,2] The genetic diagnosis in the clinical practice is challenging. Nearly 200 causal genes have been associated with ataxia,[3-5] and different technologies are needed to cover the full mutational spectrum: dynamic expansions, point mutations, and small and large duplications, insertions, or deletions. Sanger sequencing of candidate genes has been superseded in recent years by next-generation sequencing (NGS) techniques.[6,7] However, conventional NGS is not technically capable of detecting tandem repeat expansions, the most common sequence variant type in adult-onset CA.[1] Furthermore, ethnic and geographical background should be considered in diagnostic algorithms because some SCA subtypes are more common in certain populations.[8,9] Accordingly, spinocerebellar ataxia 36 (SCA36) was reported as the most frequent hereditary CA in the northwest regions of Spain.[10]

In this study, the authors aimed to analyze the diagnostic yield of clinical exome-targeted sequencing (CES) in our cohort of patients with CA. In addition, the authors screened for SCA36 in undiagnosed dominant and sporadic cases. This study may help clarify the most appropriate diagnostic algorithm in our population.

Methods

This is a retrospective descriptive study which includes clinical and genetic data collected from January 2012 to October 2021 at Hospital Universitari i Politècnic La Fe, Valencia (Spain), a national referral center for Hereditary Cerebellar Ataxia and Spastic Paraplegia. This work describes the experience of a Spanish Ataxia Unit and is influenced by local standards of care.

Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by the institutional ethics committee at Health Research Institute Hospital La Fe (PI18/00147; PI2022-388-1). Informed consent was obtained before the genetic analysis. Genetic counseling was offered prior to and after the genetic results.

Patient Recruitment

Between January 2012 and October 2021, a total of 234 index cases with familial or sporadic noncongenital CA were recruited from the Neurology and Genetics Department at Hospital Universitari I Politècnic La Fe. All included patients had familial or sporadic noncongenital CA,[11] with onset after 2 years. In all cases, acquired etiologies were fully excluded.

Common trinucleotide dynamic expansions in *ATXN1* (SCA1), *ATXN2* (SCA2), *ATXN3* (SCA3), *CACNA1A* (SCA6), *ATXN7* (SCA7), *ATXN8* (SCA8), *PPP2R2B* (SCA12), *TBP* (SCA17), *ATN1* (DRPLA), and *FXN* (Friedreich ataxia) were previously ruled out in all patients by PCR, followed by capillary electrophoresis. In addition, mutations in specific genes (*FMR1* and *RFC1*) were studied in patients with a highly suggestive phenotype. A total of 31 SCA3, 23 Friedreich ataxia, 14 SCA2, 14 SCA8, 7 SCA6, 3 SCA7, 2 SCA17, 1 DRPLA, 5 cerebellar ataxia neuropathy and vestibular areflexia syndrome, and 4 fragile X–associated tremor/ataxia syndrome families were identified and excluded from further analysis. A total of 172 patients from 130 unrelated families were ultimately selected to determine the diagnostic efficacy of CES and SCA36 screening in a real-life CA cohort.

Clinical Assessments

All patients were examined by consultant neurologists (J.J.V., L.B., N.M., and R.B.-M.) in clinical settings during their follow-up visits. Demographic, familial, age at onset, and phenotypic information was systematically collected in a prospective database. Dominant transmission was considered if vertical inheritance.[12] Recessive inheritance was assumed if consanguinity or family history of at least 2 affected siblings, with no cases in other generations. In all, a biological workup including albumin, immunoglobulin, cholesterol, triglycerides, α-fetoprotein, and vitamin E was routinely carried out. Additional metabolic testing (lactate, cholestanol, very long chain fatty acids, enzymatic activity assays, plasma amino acids, or urine organic acids) were performed based on clinical presumption. Brain MRI, nerve conduction studies, EMG, vestibular function testing, and muscle biopsy were performed and reviewed whenever needed.

Genetic Procedures

Genomic DNA was extracted from peripheral blood samples following standard procedures. A total of 130 probands from unrelated families were studied with a singleton Targeted-Exome Sequencing Panel (Agilent Technologies, Santa Clara, CA) for Illumina (San Diego, CA), in which 184 genes involved in CA and related forms were studied (additional data are provided in eAppendix 1, links.lww.com/NXG/A554). Family ATX-1 and ATX-27 were studied with the MovDisord-498 panel previously reported.[13] Genes related to episodic ataxia were initially studied in cases with prominent and recurrent spells of ataxia and mild CA at interictal examination. If negative, all genes involved in CA were analyzed.

The library preparation was carried out according to the Bravo NGS SureSelectQXT Automated Target Enrichment protocol

(Agilent Technologies) for Illumina Multiplexed Sequencing. The captured libraries were sequenced on NextSeq500 (Illumina) in a paired-end mode to generate a minimum median raw target coverage of 100×. The obtained sequences were aligned against the genome reference sequence (GRCh37/hg19) to perform the calling of variants with the Alissa Clinical Informatics Platform (Agilent Technologies). The annotated variants were initially filtered according to a minor allele frequency value ≤ 0.02, but only variants with a MAF ≤0.01 were considered. The frequency of the variants was explored in the Exome Aggregation Consortium database/gnomAD (gnomad.broadinstitute.org/) and 1,000 genomes (internationalgenome.org/). Filtered DNA variants were classified according to the American College of Medical Genetics guidelines.[14] To classify the variants, the authors consulted their annotation in the single nucleotide polymorphism database (dbSNP, ncbi.nlm.nih.gov/SNP/) and their description in ClinVar (ncbi.nlm.nih.gov/clinvar/), varsome (varsome.com/), Human Gene Mutation Database (hgmd.cf.ac.uk), and Leiden Open Variation Database (lovd.nl/). In addition, base conservation was assessed with Genomic Evolutionary Rate Profiling,[15] and in silico analysis were performed with the predictive tools: Protein Variation Effect Analyzer,[16] Sorting Intolerant from Tolerant,[17] Polyphen,[18] and MutationTaster.[19] All the likely pathogenic/pathogenic point mutations detected were confirmed by Sanger sequencing, and segregation of the variant in available family members was done when possible. Matching of phenotypic presentation to molecular diagnoses was evaluated in all cases. The presence of copy number variants (CNVs) was investigated by multiple ligation–dependent probe amplification analysis (MLPA; MRC Holland, Amsterdam, the Netherlands) and arrayCGH (aCGH) when appropriate. In all episodic ataxia cases undiagnosed after CES, *CACNA1A* CNVs were analyzed using SALSA P279. Furthermore, in the context of a compatible phenotype, CNVs in autosomal-recessive (AR) genes were analyzed if a single heterozygous pathogenic variant was detected by CES. Whenever MLPA commercial kit was not available for a gene, cytogenomic microarray (CytoScan XON array, Thermofisher, Waltham, MA) was performed. Coffalyser software and Chromosome Analysis Suite were used to analyze MLPA and aCGH results, respectively.

SCA36 Screening

NOP56 GGCCTG hexanucleotide repeat expansion was firstly studied by conventional PCR following standard procedures [sequence forward primer: TTTCGGCCTGCGTTCGGG (fluorescently labeled) and reverse: AGCCGACCGCGTGCTCAA; annealing temperature: 60°C]. Then, repeat-primed PCR (RP-PCR) was carried out as previously described.[20] PCR products were separated on an ABI Prism 3,130 Analyzer (Applied Biosystems, Vernon Hills, IL), and data were examined using GeneMapper software (Applied Biosystems, Vernon Hills, IL). A positive result was defined by the combination of a single peak in the conventional PCR electropherogram plus the typical sawtooth pattern in the RP-PCR.

Statistical Methods

Descriptive analysis of clinical and paraclinical data was performed. In addition, differences in clinical features between diagnosed and undiagnosed patients were studied. Statistical analysis was performed using IBM SPSS Statistics for Mac version 27.0.1. Distribution of continuous variables was assessed with the Kolmogorov-Smirnov test ($^*p < 0.05$), and subsequently, nonparametric tests were used for comparisons between groups. In all analysis, $p < 0.05$ was considered statistically significant.

Data Availability

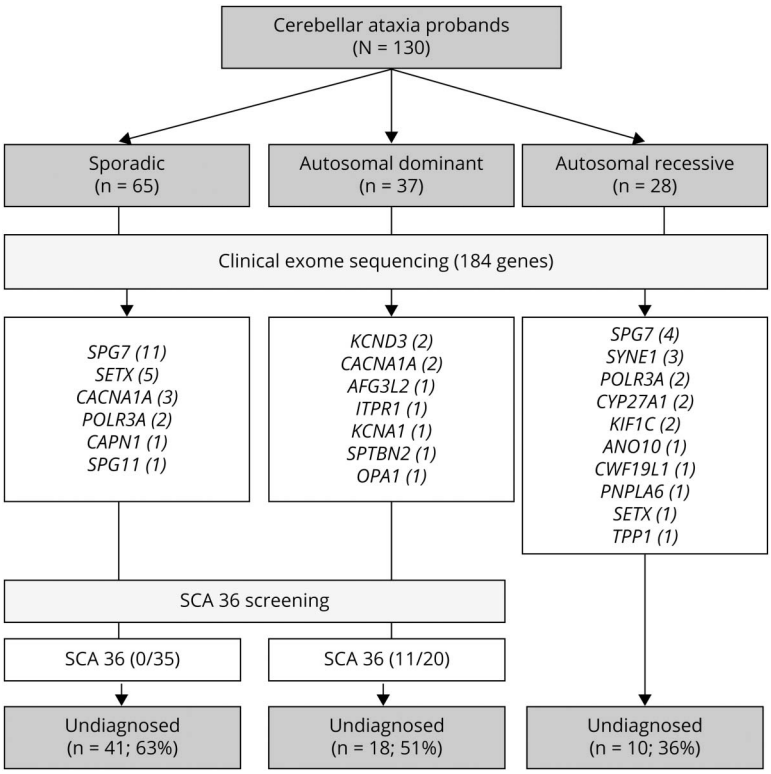
Anonymized data not published within this article will be made available by request from any qualified investigator.

Results

Overall, 172 individuals from 130 unrelated families were studied by CES. Screening of *NOP56* expansion was done in all undiagnosed families with a possible dominant disease and sporadic cases with a suggestive phenotype of hereditary CA (Figure 1 and eFigure 1, links.lww.com/NXG/A555). The mean age at onset was 39 years (range 2–83, SD ± 19.5). Ninety-four patients (55%) were male. Ethnicity was predominantly Caucasian (n = 125, 96%). Four families had Romani ancestry and one African. The pattern of disease presentation was autosomal dominant (AD) in 37 (29%), AR in 28 (21%), and sporadic (S) in 65 (50%). First-degree consanguinity was present in 16 families (12%), Table 1.

CES reached very probable or definite diagnoses in 50 families (39%). Clinical and genetic features of these patients are displayed in Table 2. Diagnosis yield was higher for AR presentation (18/28, 64%) than sporadic (23/65, 35%) or AD (9/37, 24%). Overall, the most frequently mutated gene was *SPG7* in 15 of 130 families (12%), followed by *SETX* in 6 (5%), *CACNA1A* in 5 (4%), *POLR3A* in 4 (3%), and *SYNE1* in 3 (2%). Sporadic cases harbored more frequently AR-associated genes (11 *SPG7*, 5 *SETX*, 2 *POLR3A*, 1 *CAPN*, and *1 SPG11*) than AD genes (3 *CACNA1A*). Our population showed a high degree of genetic heterogeneity. Only 2 recurrent variants were identified: the SPG7 change c.1529C > T; p.(Ala510Val)[21] was found in 19 alleles and the *POLR3A* variant c.1909 + 22G > A[22] in 4 alleles. Seventeen families displayed likely pathogenic/pathogenic variants in 14 different genes: *KCND3* (n = 2), *KIF1C* (n = 2), *CYP27A1* (n = 2), *AFG3L2* (n = 1), *ANO10* (n = 1), *CAPN1* (n = 1), *CWF19L1* (n = 1), *ITPR1* (n = 1), *KCNA1* (n = 1), *OPA1* (*n = 1*), *PNPLA6* (n = 1), *SPG11* (n = 1), *SPTBN2* (n = 1), and *TPP1* (n = 1). Twenty-two novel likely pathogenic/pathogenic variants were detected. Pathogenicity assessments are provided in Supplementary material, eTable 1 (links.lww.com/NXG/A556). Any gene with X-linked dominant or recessive transmission was identified. Copy number variations (CNVs) were studied by MLPA in *CACNA1A*, *SALSA P279* (n = 7); *SPG7*, *SALSA P213* (n = 5); *ATM*, SALSAs P041, and P042 (n = 1); *SACS*, *SALSA P441* (n = 1); and *SETX*, SALSA P316 (n = 1), but any CNVs were identified. aCGH was performed only in family ATX-5, which carried a heterozygous pathogenic variant in *POLR3A*.

Figure 1 Diagnostic Flowchart and Genetic Results of the Studied Patients



Clinical features were generally highly concordant with the genetic diagnosis. Spastic ataxia was the most frequent *SPG7* phenotype, but rarer clinical features such as progressive external ophthalmoplegia or dystonia were also identified.[23] Alpha-fetoprotein was elevated in 8 patients, and molecular analysis later confirmed that 7 of them harbored *SETX* (ataxia with oculomotor apraxia type 2, AOA2) biallelic pathogenic variants. AOA2 was a frequent cause of early-onset ataxia (mean age at onset 12.6 years, range 3–17). Patients displayed a phenotype consistent of ataxia, oculomotor apraxia, sensorimotor neuropathy, and elevated alpha-fetoprotein. All patients with *POLR3A*-related ataxia were compound heterozygotes carriers of the intronic variant c.1909 + 22G > A.[24] Three novel pathogenic/likely pathogenic *POLR3A* variants were identified: c.685C > T; p.(Arg229Ter), c.1628A > C; p.(Gln542Pro) and c.3688G > A; p.(Asp1230Asn). Phenotypes were rather homogeneous across different families. All 6 cases displayed short stature and central sensory tracts impairment with normal nerve conduction studies. Dystonia and dystonic tremor were prominent features in 4/6. Abnormal dentition (5/6) and mild MRI white matter hyperintensities (5/6) were also characteristic hallmarks. In 2 monozygotic twin siblings (ATX-5) with a highly suggestive phenotype, Figure 2, a cytogenic microarray (CytoScan XON array, Thermofisher, Waltham, MA) detected a formerly reported deletion (arr[GRCh37] 10q22.3(79781064_79782608) x 1) including exons 6, 7, and 8.[25] Recessive ataxia due to *SYNE1* was relatively common in our population (2%); the authors detected 2 novel pathogenic variants: c.368T > C; p.(Leu123Pro) and c.11253 + 2_11253+4dupTAG in 2 families a pure CA. Two siblings who carried a known pathogenic variant c.21148C > T; p.(Arg7050Ter)[26] presented with early-onset ataxia and upper motor neuron involvement. The most severely affected brother had a history of congenital cataracts.

Table 1 Demographic Characteristics of the Cohort

Total no. of CA patients/families	172/130
Male/female	55%/45%
Caucasian	96%
Mean age at onset (y)	39 (range 2–83)
Consanguinity	12%
AD inheritance pattern	29% (37/130)
AR inheritance pattern	21% (28/130)
Sporadic	50% (65/130)

Abbreviations: AD = autosomal dominant; AR = autosomal recessive; CA = cerebellar ataxia.

Channelopathies were the most frequent AD ataxia detected by NGS (9/13). *CACNA1A* was the channel gene most commonly mutated. Patients with episodic ataxia, belonging to the families ATX-39, ATX-41, ATX-42, and ATX-43, had loss of function variants while the family ATX-40's patients

Table 2 Clinical and Genetic Characteristics of the Cases With a Genetically Confirmed Diagnosis

Family	ID	Family history	Gene	Variants	Age onset, y	Age examination, y	SARA scale	UMN	Nerve§	Extrapyramidal	Others
ATX-1	1	AR	*KIF1C*	NM_006612.5: hom c.608+1G>A; p.(?)	18	55	30	+++	+	Cervical and UL dystonia	Eyelid opening and oculomotor apraxia
ATX-1	2	AR	*KIF1C*	NM_006612.5: hom c.608+1G>A; p.(?)	15	56	35	+++	+	Cervical and UL dystonia	Eyelid opening and oculomotor apraxia
ATX-2	3	AR (C)	*KIF1C*	NM_006612.5: hom c.455A>G; p.(Glu152Gly)	15	67	29	+++	+	Head tremor	–
ATX-3	4	AR, (C)	*TPP1*	NM_000391.3: hom c.887-10A>G; p.(?)[51]	29	53	29	+++	–	Rest tremor	Late-onset seizures
ATX-4	5	S	*POLR3A*	NM_007055.4: c.1909+22G>A[22], c.685C>T; p.(Arg229Ter)	11	61	23.5	+	–	Cervical and UL dystonia	Short stature, normal dentition.
											Spine atrophy, abnormal SEP
ATX-5	6	AR	*POLR3A*	NM_007055.4: c.1909+22G>A, arr[GRCh37] 10q22.3(79781064_79782608)x1[25]	13	34	20	+	–	Generalized dystonia	Short stature, abnormal dentition, brain WMH, spine atrophy, delayed SEP<!––Para Run–on––>
ATX-5	7	AR	*POLR3A*	NM_007055.4: c.1909+22G>A, arr[GRCh37] 10q22.3(79781064_79782608)x1	13	34	18	+	–	Cervical and UL dystonia	Short stature, abnormal dentition
											Brain WMH, absent SEP
ATX-6	8	AR	*POLR3A*	NM_007055.4: c.1909+22G>A, c.1628A>C; p.(Gln543Pro)	23	60	16.5	+	–	–	Short stature, abnormal dentition
											Eyelid myokymia; delayed SEP
ATX-6	9	AR	*POLR3A*	NM_007055.4: c.1909+22G>A, c.1628A>C; p.(Gln543Pro)	13	68	5	–	–	Right UL dystonic tremor	Short stature, abnormal dentition
ATX-7	10	S	*POLR3A*	NM_007055.4: c.1909+22G>A, c.3688G>A; p.(Asp1230Asn)	20	34	13	++	–	–	Scoliosis, normal dentition.
ATX-8	11	AR, (C)	*ANO10*	NM_001204831.1: hom c.1009T>G; p.(Phe337Val)[52]	23	70	24	–	–	–	Laryngeal stridor
ATX-9	12	AR, (C)	*SYNE1*	NM_182961.4: hom c.368T>C; p.(Leu123Pro)	25	46	15	–	–	–	–
ATX-9	13	AR, (C)	*SYNE1*	NM_182961.4: hom c.368T>C; p.(Leu123Pro)	23	37	NA	–	–	–	–
ATX-10	14	AR, (C)	*SYNE1*	NM_182961.4: hom c.11253+2_11253+4dupTAG	40	44	NA	–	–	–	–
ATX-11	15	AR	*SYNE1*	NM_182961.4: hom c.21148C>T; p.(Arg7050Ter)[26]	18	33	21	++	–	–	–
ATX-11	16	AR	*SYNE1*	NM_182961.4: hom c.21148C>T; p.(Arg7050Ter)	8	24	10.5	++	–	–	Congenital cataracts
ATX-12	17*	AR, (C)	*SPG7*	NM_003119.3: hom c.233T>A; p.(Leu78Ter)[53]	20	50	NA	++	–	–	–
ATX-12	18*	AR, (C)	*SPG7*	NM_003119.3: hom c.233T>A; p.(Leu78Ter)	20	52	NA	+	–	–	–

Continued

Table 2 Clinical and Genetic Characteristics of the Cases With a Genetically Confirmed Diagnosis *(continued)*

Family	ID	Family history	Gene	Variants	Age onset, y	Age examination, y	SARA scale	UMN	Nerve§	Extrapyramidal	Others
ATX-13	19*	S	*SPG7*	NM_003119.3:c.1529C>T; p.(Ala510Val),[21] c.1948G>A; p.(Asp650Asn)[30]	43	52	NA	+	–	–	–
ATX-14	20*	S	*SPG7*	NM_003119.3: hom c.1529C>T; p.(Ala510Val)	64	73	NA	+	–	–	PEO
ATX-15	21*	AR	*SPG7*	NM_003119.3: hom c.1529C>T; p.(Ala510Val)	43	59	NA	+	–	–	–
ATX-15	22*	AR	*SPG7*	NM_003119.3: hom c.1529C>T; p.(Ala510Val)	35	45	NA	+	–	–	–
ATX-16	23*	S	*SPG7*	NM_003119.3: hom c.1529C>T; p.(Ala510Val)	48	80	NA	+	–	–	PEO
ATX-17	24*	S	*SPG7*	NM_003119.3: c.1529C>T; p.(Ala510Val), c.2164delC; p.(Leu722TrpfsTer16)	34	48	NA	++	–	–	–
ATX-18	25*	S	*SPG7*	NM_003119.3: hom c.1529C>T; p.(Ala510Val)	43	51	NA	+	–	–	–
ATX-19	26	S	*SPG7*	NM_003119.3: c.1529C>T; p.(Ala510Val), c.758+2T>C; p.(?)	40	52	5.5	–	–	–	–
ATX-20	27*	AR	*SPG7*	NM_003119.3: c.1529C>T; p.(Ala510Val), c.1676delA; p.(Lys559ArgfsTer33)	15	42	NA	–	–	Left UL dystonia	Subclinical myopathy
ATX-20	28*	AR	*SPG7*	NM_003119.3: c.1529C>T, p.(Ala510Val), c.1676delA; p.(Lys559ArgfsTer33)	15	44	NA	–	–	–	Subclinical myopathy
ATX-21	29*	S	*SPG7*	NM_003119.3: c.1529C>T; p.(Ala510Val), c.1729G>C; p.(Gly577Arg) [rs72547552]	20	55	20	++	–	–	PEO
ATX-22	30*	S	*SPG7*	NM_003119.3: hom c.1529C>T; p.(Ala510Val)	32	41	NA	++	–	–	–
ATX-23	31	S	*SPG7*	NM_003119.3: c.1529C>T; p.(Ala510Val), c.1562T>C; p.(Ile521Thr)	64	70	NA	+	–	–	–
ATX-24	32	AR	*SPG7*	NM_003119.3: hom c.1450-1_1457del, p.(Arg485_Glu487del) [rs768823392]	55	66	NA	++	–	–	–
ATX-25	33	S	*SPG7*	NM_003119.3: c.1529C>T; p.(Ala510Val), c.1676delA; p.(Lys559ArgfsTer33)	35	53	NA	–	–	–	–
ATX-26	34	S	*SPG7*	NM_003119.3: hom c.1529C>T; p.(Ala510Val)	38	48	11.5	–	–	–	–
ATX-27	35	AR	*SETX*	NM_015046.7: c.4087C>T; p.(Arg1363Ter)9, c.7139G>A; p.(Arg2380Gln)[55]	17	33	25.5	-	+	-	Oculomotor apraxia, high AFP
ATX-27	36	AR	*SETX*	NM_015046.7: c.4087C>T; p.(Arg1363Ter), c.7139G>A; p.(Arg2380Gln)	17	30	21.5	-	+	-	Oculomotor apraxia, high AFP
ATX-28	37	S	*SETX*	NM_015046.7: hom c.2387_2390delAGAA; p.(Lys769SerfsTer16)[55]	10	59	NA	-	+	-	Oculomotor apraxia, high AFP
ATX-29	38	S	*SETX*	NM_015046.7: c.6843-2A>G; p.(?) c.7163C>T; p.(Thr2388Met) [rs765418950],	16	50	NA	-	+	Postural tremor	High AFP

Continued

Table 2 Clinical and Genetic Characteristics of the Cases With a Genetically Confirmed Diagnosis *(continued)*

Family	ID	Family history	Gene	Variants	Age onset, y	Age examination, y	SARA scale	UMN	Nerve§	Extrapyramidal	Others
ATX-30	39	S	*SETX*	NM_015046.7: c.7163C>T; p.(Thr2388Met) [rs765418950],	3	38	16.5	-	+	Cervical and upper limbs dystonia	Oculomotor apraxia, high AFP
				c.5895_5903delTGGAACAGG; p.(Gly1966_Gly1968del)							
ATX-31	40	S	*SETX*	NM_015046.7: c.6843-2A>G; p.(?), c.6133C>T; p.(Arg2045Ter)[56]	20	47	NA	-	+	Postural tremor	Oculomotor apraxia, high AFP
ATX-32	41	S	*SETX*	NM_015046.7: c.7385T>A; p.(Ile2462Asn), c.5224_5227delGATTinsAATC; p.(Asp1742_Tyr1743delinsAsnHis)[6]	5	29	NA	+	+	-	Oculomotor apraxia, high AFP
ATX-33	42	AR, (C)	*CWF19L1*	NM_018294.6: hom c.24-1G>C; p.(?)	25	51	NA	-	-	-	-
ATX-33	43	AR, (C)	*CWF19L1*	NM_018294.6: hom c.24-1G>C; p.(?)	35	54	NA	-	-	-	-
ATX-33	44	AR, (C)	*CWF19L1*	NM_018294.6: hom c.24-1G>C; p.(?)	30	59	NA	-	-	-	-
ATX-34	45	S	*SPG11*	NM_025137.3: c.6100C>T; p.(Arg2034Ter),[57] c.1440delG; p.(Cys481AlafsTer4)	10	41	NA	+++	+	Generalized dystonia	Oculomotor apraxia
ATX-35	46	AR, (C)	*PNPLA6*	NM_001,166,111.1: hom c.3373G>A; p.(Asp1125Asn) [rs372763461]	27	32	8.5	-	-	-	-
ATX-36	47	AR, (C)	*CYP27A1*	NM_000784.4: hom c.1016C>T; p.(Thr339Met)[58]	23	27	NA	+	-	-	Aquilles tendon xanthomas
ATX-37	48	AR, (C)	*CYP27A1*	NM_000784.4: hom c.1016C>T; p.(Thr339Met)	19	35	19	+	-	Right foot dystonia	Xanthomas in auricular pavilion, aquilles tendon, cataracts, cognitive impairment
ATX-38	49	S	*CAPN1*	NM_001198868.1: c.618_619delAG; p.(Gly208GlnfsTer7),[59] c.1605+5G>A[60]	44	65	-	++	-	-	Hand and feet interosseous amyotrophy
ATX-39	50	AD	*CACNA1A*	NM_023035.2: c.2042_2043delAG; p.(Gln681ArgfsTer103)[61]	10	42	5.5	-	-	-	EA
ATX-39	51	AD	*CACNA1A*	NM_023035.2: c.2042_2043delAG; p.(Gln681ArgfsTer103)	10	14	6	-	-	-	EA, attention deficit hyperactivity disorder
ATX-40	52	AD	*CACNA1A*	NM_023035.2: c.1748G>A; p.(Arg583Gln)[27]	2	63	19	+	-	Multifocal dystonia	Axial weakness, migraine with aura, dementia
ATX-40	53	AD	*CACNA1A*	NM_023035.2: c.1748G>A; p.(Arg583Gln)	40	56	9.5	-	-	-	Axial weakness, migraine with aura
ATX-40	54	AD	*CACNA1A*	NM_023035.2: c.1748G>A; p.(Arg583Gln)	26	29	2	-	-	-	-
ATX-41	55	S	*CACNA1A*	NM_023035.2: c.4045C>T; p.(Arg1349Ter)[62]	15	62	4	-	-	-	EA
ATX-42	56	S	*CACNA1A*	NM_023035.2: c.4600G>T; p.(Glu1534Ter)	30	50	NA	-	-	-	EA
ATX-43	57	S	*CACNA1A*	NM_023035.2: c.3704+1G>A; p.(?)[63]	12	71	NA	-	-	-	EA

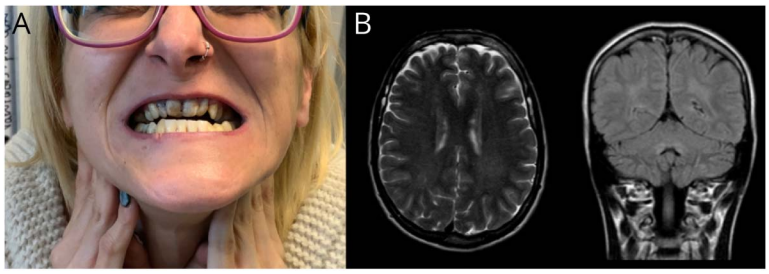
Continued

Table 2 Clinical and Genetic Characteristics of the Cases With a Genetically Confirmed Diagnosis *(continued)*

Family	ID	Family history	Gene	Variants	Age onset, y	Age examination, y	SARA scale	UMN	Nerve§	Extrapyramidal	Others
ATX-44	58	AD	*KCND3*	NM_004980.4: c.1117A>G; p.(Met373Val)	65	72	14	-	-	-	Paroxysmal exacerbations ACZ responsive
ATX-44	59	AD	*KCND3*	NM_004980.4:c.1117A>G; p.(Met373Val)	40	58	13	-	-	-	-
ATX-45	60	AD	*KCND3*	NM_004980.4: c.680_682delTCT, p.(Phe227del)[28]	29	40	10	-	-	-	Migraine, paroxysmal ataxic exacerbations
ATX-46	61	AD	*KCNA1*	NM_000217.2: c.904A>C; p.(Ile302Leu)	5	52	NA	-	-	-	EA, seizures
ATX-47	62	AD	*SPTBN2*	NM_006946.2: c.482A>G; p.(Gln161Arg)	8	8	15	+	-	-	Mild intellectual delay
ATX-47	63	AD	*SPTBN2*	NM_006946.2: c.482A>G; p.(Gln161Arg)	3	18	2.5	+	-	-	Mild psychomotor delay, oculomotor apraxia
ATX-48	64	AD	*OPA1*	NM_015560.2: c.1940G>T; p.(Gly647Val)	44	71	NA	-	-	-	-
ATX-48	65	AD	*OPA1*	NM_015560.2: c.1940G>T; p.(Gly647Val)	49	64	14.5	-	-	-	-
ATX-49	66	AD	*ITPR1*	NM_001168272.1: c.3601C>T; p.(Arg1201Trp)	62	66	NA	-	-	-	EA
ATX-49	67	AD	*ITPR1*	NM_001168272.1: c.3601C>T; p.(Arg1201Trp)	74	78	NA	-	-	-	-
ATX-50	68	AD	*AFG3L2*	NM_006796.2: c.2021_2023del; p.(Ser674del)	18	35	7.5	-	-	-	PEO, migraine with aura
ATX-50	69	AD	*AFG3L2*	NM_006796.2: c.2021_2023del; p.(Ser674del)	14	63	16.5	-	-	Dystonia	PEO, migraine with aura

Abbreviations: AD = autosomal dominant; AFP = alphafetoprotein; AR = autosomal recessive; ATX = cerebellar ataxia family identification code; C = consanguinity; hom = homozygous; ID = patient identification; S = sporadic; UL = upper limbs; UMN = upper motor neuron signs; WMH = white matter hyperintensity; +++ = severe; ++ = moderate; + = mild.
*EA = episodic ataxia; PEO = progressive external ophthalmoplegia.
These *SPG7* patients were included in a previously published work.[23]
§Neuropathy is assumed if clinical and/or electroneurographic findings are present. Novel variants are indicated in bold characters, and dbSNP references are indicated between brackets.

Figure 2 Family ATX-5, 2 Female Monozygotic Twins With Spastic Ataxia Had Compound Heterozygous Variants in *POLR3A* c.1909 + 22G > A and arr[GRCh37] 10q22.3(79781064_79782608)x1



(A) Both patients had typically abnormal dentition; (B) T2-weighted brain MRI (left) and fluid attenuated recovery (FLAIR) brain MRI (right) showing characteristic mild supratentorial and infratentorial white matter hyperintensities.

with the missense change c.1748G > A; p.(Arg583Gln)[27] displayed a complex phenotype. Both siblings in family ATX-40 showed marked axial weakness on examination, but EMG, including repetitive nerve stimulation and single fiber jitter, studies showed no abnormalities. Two novel changes in *KCNA1* c.904A > C; p.(Ile302Leu) and *KCND3* c.117A > G; p.(Met373Val) were identified. Patient 60, with the novel pathogenic variant *KCND3* c.117A > G; p.(Met373Val), had frequent paroxysmal ataxic exacerbations responsive to acetazolamide. Patient 50, with the previously reported KCND3 c.680_682delTCT; p.(Phe227del),[28] displayed occasional ataxic exacerbations triggered by emotional stress.

Very rare genetic etiologies were identified by CES. A novel homozygous pathogenic splicing variant in *CWF19L1* (c.24-1G > C) was identified in 3 affected siblings with late-onset pure CA. In addition, a homozygous likely pathogenic variants in *PNPLA6* c.3373G > A; p.(Asp1125Asn) was detected in a Romaní proband with adult-onset pure CA.

Spinocerebellar Ataxia 36 Screening

NOP56 hexanucleotide repeat expansion (GGCCTG) was screened in all undiagnosed families with an AD presentation (n = 20) and sporadic cases with a phenotype of late-onset (older than 20 years) progressive CA (n = 35). SCA36 was diagnosed in 31 patients of 11 families, all with AD presentation, and in none sporadic. Patients displayed a phenotype of pure or spastic CA. Sensorineural hearing loss was frequent (15/31, 48%), but facial and lingual myokymia were identified only in 3/31. Progressive external ophthalmoplegia and mild parkinsonism were present in 13/31 and 4/31 cases, respectively. *NOP56* hexanucleotide expansion represented the most common dominant variants in this study, which accounted for 30% (11/37) of cases with AD, increasing the overall diagnostic rate to 47% (61/130). Of interest most of the SCA36 apparently unrelated families lived in the geographical areas of *La Costera* and *La Vall d'Albaida*, pointing to a founder effect, as reported in other SCA36 series.[7]

Sixty-nine families (53%) were not genetically diagnosed despite CES and SCA36 screening. Undiagnosed patients showed a significantly delayed age at onset (n = 81, 47, SD ± 17.2 years) compared with diagnosed cases (n = 86, 32, SD ± 18.5 years), $p < 0.05$, but without differences in sex distribution. Negative cases were more frequently sporadic (n = 39/69, 49%), followed by AD (n = 18/69, 30%) and AR (n = 10/69, 21%).

Discussion

In this study, CES was performed in a series of 130 probands with CA, leading to an overall diagnostic yield of 39%. Although common classic repeat expansions were excluded, the *NOP56* hexanucleotide repeat expansion was further analyzed, which allowed us to diagnose 11 additional AD cases with SCA36. Several practical conclusions can be established from our study: (1) Diagnostic efficacy of CES applied to hereditary CA remains unsatisfactory because it missed the genetic diagnosis in more than 60% of cases in our series. This is concordant with other adult-onset CA cohorts, which reported a yield between 12.1% and 52%.[6,29-32] (2) There is an enormous genetic heterogeneity underlying CA phenotypes, and the routine clinical use of CES allows the diagnosis of very rare genetic etiologies. Most genes mutated were detected in individual families, and diverse novel pathogenic/likely pathogenic variants were identified. (3) Because SCA36 is a common form of AD CA in Eastern Spain,[10] the diagnostic workup of HCA in our geographical region should consider SCA36 testing in advance to NGS, especially if AD presentation. (4) The diagnostic efficacy was slightly higher in patients with a positive family history of dominant or, more frequently, recessive disease. The lowest diagnostic efficacy was found in sporadic and elderly cases. In this particular group, care should be taken to exclude neurodegenerative nonmendelian forms of CA such as multiple system atrophy. Future studies are also needed to ascertain if the so-called "idiopathic late-onset cerebellar ataxia" represents a distinct neurodegenerative disease entity.[33-36]

The correct interpretation of the countless number of detected DNA variants is the main challenge in NGS-based studies. Despite this, this study's CES allowed the identification of unexpected etiologies and expanded the clinical spectrum of known genes. A pathogenic variant in *CWF19L1* (c.24-1G > C)

was detected in 3 siblings with adult-onset pure CA. *CWF19L1* is a very rare gene associated with congenital or early-onset CA and mental retardation. Only 3 families have been described to date.[37-39] Thus, the clinical findings reported here contribute to broaden *CWF19L1* phenotypical spectrum. Similarly, *PNPLA6,* a gene traditionally related with complex congenital or childhood disorders, was recently associated with adult-onset CA presentations.[40] The novel *PNPLA6* c.3373G > A; p.(Asp1125Asn), substitution described here, reinforces previously reported observations.

The availability of updated genome variant databases (e.g., ExAc/gnomAD or ClinVar) is critical in the diagnostic workup of diseases with variable expressivity or incomplete penetrance and, also, in late-onset diseases where segregation studies are often difficult. Familial trio (proband and parents) whole-exome or genome sequencing approaches can further improve diagnostic efficacy by identifying new ataxia-related genes and mutations.

NGS studies across different populations have contributed to identify underdiagnosed mutations in commonly mutated genes. In keeping with other CA cohorts, few genes harbored most pathogenic variants in our series. *SPG7* (12%) and *SETX* (5%) were the most common AR genes detected,[6] and channelopathies were the most frequent AD ataxias.[41] Originally involved in 4H-leukodystrophy (hypomyelination and hypodontia), *POLR3A* is increasingly found in adult CA/hereditary spastic paraplegia cohorts (3.1%).[22,24] Concordantly, in this study, *POLR3A* represented 3% of the studied population. All cases carried the recurrent intronic variant c.1909 + 22G > A in compound heterozygosity and displayed a uniform phenotype of ataxia, spasticity, central sensory tract impairment, and dystonia. *SYNE1* mutations, initially described in Quebec, have also been reported as a frequent cause of ataxia outside French-Canadian population, with an estimated frequency of 5.3%.[26] Herein, homozygous *SYNE1* mutations were identified in 2% of cases. The authors reported 2 novel deleterious variants and described a family with ataxia, spasticity, and congenital cataracts.

The main limitations of this work are the retrospective nature of this study and the influence of local standards of care. Extensive metabolic testing was not systematically performed. Specific biochemical tests were carried out solely based on clinical presumption. Although CES is increasingly available and shall ultimately identify mutations in inherited metabolic disorders genes, metabolic screening should be done prior to molecular testing.[42] Many adult-onset forms of inherited metabolic disorders can present either with complex or isolated ataxia phenotypes[43,44] and some benefit from specific treatments that improve prognosis or prevent early death.[45] Therefore, our approach could have missed the diagnosis of some patients or led to a delay in the identification of treatable conditions.

Because CES does not detect dynamic expansions and SCA36 was a common cause of AD ataxia in Spain, the authors systematically studied *NOP56* hexanucleotide repeat expansion in AD and sporadic cases. SCA36 was a common cause of AD ataxia in this study, increasing the diagnostic yield from 39 to 47%. Studies in other populations showed that SCA36 has a worldwide distribution with reduced prevalence (France 1.9%, Japan 1.5%, and the United States 0.7%).[46,47] These findings support that SCA36 should be tested prior to CES in Spanish families with AD presentations. New AD repeat expansion disorders are continuously being discovered. For instance, SCA37, caused by the intronic ATTTC repeat expansion in *DAB1*, was recently identified in families from Portugal and Spain.[48-50] Screening of newly recognized repeat disorders should be carefully incorporated to genetic diagnostic algorithms.

In recent decades, advances in high-throughput sequencing technologies have enabled impressive achievements in the molecular diagnosis of mendelian diseases. However, an unsatisfactorily high proportion of patients remain undiagnosed among different CA series. Implementation of advanced sequencing strategies such as whole-genome sequencing together with collaborative international networks focused on ataxia are necessary steps toward reducing this diagnostic gap.

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Disclosure

The authors report no conflict of interest. Go to Neurology.org/NG for full disclosure.

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Appendix Authors

Name	Location	Contribution
Raquel Baviera-Muñoz, MD	Neuromuscular Diseases Unit, Neurology Department, Hospital Universitari I Politècnic La Fe; Neuromuscular Reference Centre, ERN-EURO-NMD; Neuromuscular and Ataxias Research Group. Instituto de Investigación Sanitaria La Fe; Rare Diseases Joint Unit, CIPF-IIS La Fe, Valencia, Spain	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data

Appendix *(continued)*

Name	Location	Contribution
Lidón Carretero-Vilarroig, MSc	Neuromuscular and Ataxias Research Group. Instituto de Investigación Sanitaria La Fe; Rare Diseases Joint Unit, CIPF-IIS La Fe; Cell Biology Department, University of Valencia, Spain	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Juan Francisco Vázquez-Costa, MD	Neuromuscular Diseases Unit, Neurology Department, Hospital Universitari I Politècnic La Fe; Neuromuscular Reference Centre, ERN-EURO-NMD; Neuromuscular and Ataxias Research Group. Instituto de Investigación Sanitaria La Fe; Rare Diseases Joint Unit, CIPF-IIS La Fe, Valencia, Spain; Department of Medicine, University of Valencia, Valencia; Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Spain	Drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data
Carlos Morata-Martínez, MD	Neuromuscular Diseases Unit, Neurology Department, Hospital Universitari I Politècnic La Fe, Valencia, Spain and Neuromuscular Reference Centre, ERN-EURO-NMD	Drafting/revision of the manuscript for content, including medical writing for content
Marina Campins-Romeu, MD	Neuromuscular Diseases Unit, Neurology Department, Hospital Universitari I Politècnic La Fe, Valencia, Spain and Neuromuscular Reference Centre, ERN-EURO-NMD	Drafting/revision of the manuscript for content, including medical writing for content
Nuria Muelas, MD	Neuromuscular Diseases Unit, Neurology Department, Hospital Universitari I Politècnic La Fe; Neuromuscular Reference Centre, ERN-EURO-NMD; Neuromuscular and Ataxias Research Group. Instituto de Investigación Sanitaria La Fe; Rare Diseases Joint Unit, CIPF-IIS La Fe, Valencia, Spain; Department of Medicine, University of Valencia, Valencia; Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Spain	Drafting/revision of the manuscript for content, including medical writing for content
Isabel Sastre-Bataller, MD	Neuromuscular Diseases Unit, Neurology Department, Hospital Universitari I Politècnic La Fe, Valencia, Spain and Neuromuscular Reference Centre, ERN-EURO-NMD; Rare Diseases Joint Unit, CIPF-IIS La Fe, Valencia, Spain	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data

Appendix *(continued)*

Name	Location	Contribution
Irene Martínez-Torres, MD	Neuromuscular Diseases Unit, Neurology Department, Hospital Universitari I Politècnic La Fe, Valencia, Spain and Neuromuscular Reference Centre, ERN-EURO-NMD; Rare Diseases Joint Unit, CIPF-IIS La Fe, Valencia, Spain	Drafting/revision of the manuscript for content, including medical writing for content Julia Pérez-García: Major role in the acquisition of data
Julia Pérez-García, BSN	Neuromuscular Diseases Unit, Neurology Department, Hospital Universitari I Politècnic La Fe, Valencia, Spain and Neuromuscular Reference Centre, ERN-EURO-NMD	Major role in the acquisition of data
Rafael Sivera, MD	Neuromuscular Diseases Unit, Neurology Department, Hospital Universitari I Politècnic La Fe; Neuromuscular Reference Centre, ERN-EURO-NMD; Neuromuscular and Ataxias Research Group. Instituto de Investigación Sanitaria La Fe, Valencia, Spain	Drafting/revision of the manuscript for content, including medical writing for content
Teresa Sevilla, MD	Neuromuscular Diseases Unit, Neurology Department, Hospital Universitari I Politècnic La Fe; Neuromuscular Reference Centre, ERN-EURO-NMD; Neuromuscular and Ataxias Research Group. Instituto de Investigación Sanitaria La Fe; Rare Diseases Joint Unit, CIPF-IIS La Fe, Valencia, Spain; Department of Medicine, University of Valencia, Valencia; Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Spain	Drafting/revision of the manuscript for content, including medical writing for content
Juan J. Vilchez, MD	Neuromuscular and Ataxias Research Group. Instituto de Investigación Sanitaria La Fe; Rare Diseases Joint Unit, CIPF-IIS La Fe, Valencia; Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Spain	Drafting/revision of the manuscript for content, including medical writing for content
Teresa Jaijo, PhD	Rare Diseases Joint Unit, CIPF-IIS La Fe, Valencia; Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER); Department of Genetics, Hospital Universitari I Politècnic La Fe; Cellular, molecular and genomics biomedicine Group. Instituto de Investigación Sanitaria La Fe, Valencia, Spain	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data

Continued

Appendix *(continued)*

Name	Location	Contribution
Carmen Espinós, PhD	Rare Diseases Joint Unit, CIPF-IIS La Fe; Laboratory of Rare Neurodegenerative Diseases, Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data
Jose M. Millán, PhD	Rare Diseases Joint Unit, CIPF-IIS La Fe, Valencia; Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER); Cellular, molecular and genomics biomedicine Group. Instituto de Investigación Sanitaria La Fe, Valencia, Spain	Drafting/revision of the manuscript for content, including medical writing for content
Luis Bataller, MD	Neuromuscular Diseases Unit, Neurology Department, Hospital Universitari I Politècnic La Fe; Neuromuscular Reference Centre, ERN-EURO-NMD; Neuromuscular and Ataxias Research Group. Instituto de Investigación Sanitaria La Fe; Rare Diseases Joint Unit, CIPF-IIS La Fe, Valencia, Spain; Department of Medicine, University of Valencia, Valencia; Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Spain	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
Elena Aller, PhD	Rare Diseases Joint Unit, CIPF-IIS La Fe, Valencia; Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER); Department of Genetics, Hospital Universitari I Politècnic La Fe; Cellular, molecular and genomics biomedicine Group. Instituto de Investigación Sanitaria La Fe, Valencia, Spain	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data

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