Movement Disorders
CLINICAL PRACTICE

BRIEF REPORT

Spinocerebellar Ataxia 36 is a Frequent Cause of Hereditary Ataxia in Eastern Spain

Raquel Baviera-Muñoz, MD,[1,2,3,4] Lidón Carretero-Vilarroig, MSc,[2,3,5] Nuria Muelas, MD, PhD,[1,2,3,6,7] Rafael Sivera, MD, PhD,[1,2,7,8] Pablo Sopena-Novales, MD, PhD,[9] Begoña Martínez-Sanchis, MD,[9] Isabel Sastre-Bataller, MD,[1,3] Marina Campins-Romeu, MD,[1] Irene Martínez-Torres, MD, PhD,[1,3] Jose Manuel García-Verdugo, MSc, PhD,[5] Jose M. Millán, MSc, PhD,[3,4,7] Teresa Jaijo, MSc, PhD,[3,4,7,10] Elena Aller, MSc, PhD,[3,4,7,10] and Luis Bataller, MD, PhD[1,2,3,6,7,*]

Abstract: Background: Autosomal dominant spinocerebellar ataxia 36 (SCA36) is caused by hexanucleotide repeat expansion in the *NOP56* gene.

Objectives: To assess frequency, clinical and genetic features of SCA36 in Eastern Spain.

Methods: *NOP56* expansion was tested in a cohort of undiagnosed cerebellar ataxia families (n = 84). Clinical characterization and haplotype studies were performed.

Results: SCA36 was identified in 37 individuals from 16 unrelated families. It represented 5.4% of hereditary ataxia patients. The majority were originally from the same region and displayed a shared haplotype. Mean age at onset was 52.5 years. Non-ataxic features included: hypoacusis (67.9%), pyramidal signs (46.4%), lingual fasciculations/atrophy (25%), dystonia (17.8%), and parkinsonism with evidence of dopaminergic denervation (10.7%).

Conclusions: SCA36 is a frequent cause of hereditary ataxia in Eastern Spain, and is associated with a strong founder effect. SCA36 analysis should be considered prior to other studies, especially in AD presentations. Parkinsonism reported here broadens SCA36 clinical spectrum.

SCA36 is caused by a large GGCCTG hexanucleotide non-coding repeat in the nucleolar protein 56 (*NOP56*) gene. It was initially described in Western Japan (3.6% of all SCA)[1] and in the Costa da Morte region of Spain (6.3%).[2] Patients usually exhibit late-onset progressive cerebellar ataxia, characteristically associated with upper and lower motor neuron involvement in the form of lingual and/or skeletal muscle atrophy and fasciculations, and sensorineural hearing loss.[2,3] In recent years, additional features were added to SCA36 phenotypes such as dystonia[4–6] and cognitive-affective cerebellar syndrome (CCAS).[7]

In this study, we investigated the frequency and clinical characteristics of SCA36 in Eastern Spain. Haplotype analysis was conducted to ascertain a founder effect in our population.

Methods

Patients were enrolled from the Ataxia Clinic at Hospital Universitari i Politècnic La Fe, a national referral center for Hereditary Cerebellar Ataxia and Spastic Paraplegia. The inclusion criteria were: (1) patients with a progressive cerebellar ataxia with negative studies for acquired etiologies; (2) sporadic or AD presentation; and (3) negative genetic studies including analysis of trinucleotide repeat expansion ataxias (SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA12, SCA17, DRPLA and Friedreich ataxia) and/or clinical exome sequencing.[8] Ultimately, SCA36 was screened in 84 index patients from 297 pedigrees. Written informed consent was obtained before the genetic analysis.

[1]*Neurology Department, Hospital Universitari I Politècnic La Fe, Valencia, Spain;* [2]*Neuromuscular and Ataxias Research Group, Instituto de Investigación Sanitaria La Fe, Valencia, Spain;* [3]*Rare Diseases Joint Unit, CIPF-IIS La Fe, Valencia, Spain;* [4]*Cellular, Molecular and Genomics Biomedicine Group, Instituto de Investigación Sanitaria La Fe, Valencia, Spain;* [5]*Cavanilles Institute of Biodiversity and Evolutionary, University of Valencia, Valencia, Spain;* [6]*Department of Medicine, University of Valencia, Valencia, Spain;* [7]*Centro de Investigación Biomédica en Red de Enfermedades Raras U755, U763, (CIBERER), Madrid, Spain;* [8]*Department of Medicine, University CEU Cardenal Herrera, Valencia, Spain;* [9]*Nuclear Medicine Department, Hospital Universitari I Politècnic La Fe, Valencia, Spain;* [10]*Department of Genetics, Hospital Universitari I Politècnic La Fe, Valencia, Spain*

***Correspondence to:** Dr Luis Bataller, Department of Neurology, Hospital Universitari i Politècnic La Fe. Av. Fernando Abril Martorell 106, 46026 Valencia, Spain; E-mail: luis.bataller@uv.es

Keywords: spinocerebellar ataxia, genetics, haplotype.

Raquel Baviera-Muñoz and Lidón Carretero-Vilarroig contributed equally to the manuscript.

Received 28 December 2022; revised 9 March 2023; accepted 29 March 2023.
Published online 5 May 2023 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mdc3.13740

Clinical Assessments

Symptomatic subjects and family members at risk were clinically assessed. Demographic, familial and clinical data, including the Scale for Assessment and Rating of Ataxia (SARA) were collected. Preclinical stage was considered if the SARA score was <3 while carriers free of symptoms and signs were considered asymptomatic.[9] Cognitive assessment was based on patient, caregiver and clinician impression. Disability was assessed at last visit with the ataxia disability scale.[10] I-123-Iofluopane presynaptic nigrostriatal imaging (DaTSCAN®) was performed if parkinsonian signs (bradykinesia, rigidity and/or tremor) were identified. The DaTQUANT version 2.000 Software application (GE Healthcare, Little Chalfont, UK) was used to perform semiquantitative analysis. The volumes of interest were semiautomatically placed over the right and left putamen and caudate nucleus in the transaxial slice showing most intense tracer uptake. To calculate the striatum to background ratios (SBR), tracer uptake was compared with a reference region placed in the occipital cortex. In addition, SBR scores were compared to an age-matched healthy controls database.

Genetic Analysis

Screening of the hexanucleotide repeat was ruled out with both conventional PCR and Repeat-Primed PCR (RP-PCR) as previously described.[1,8] The number of repeats in non-expanded alleles was calculated according to an amplicon of 174pb with a static region of 132 pb.

Haplotype analysis: D20S113 (chr20:2,035,488) and D20S842 (chr20:2,686,204) microsatellite markers flanking the *NOP56* gene were studied through PCR-fragment analysis. D20S113 was amplified using primers FOR:5′-TAACAGTGGTTGACTCTCAGAGG-3′ and REV:5′-AAGAGGTGCTGTCACATATTTATTC-3′, while D20S842 using FOR:5′-ACAGCCTTCATCGACTTCGT-3′ and REV:5′-GTCCACCCCTTCTCCTAACC-3′. Forward primers were labeled with 6-FAM at the 5′-end. The annealing temperature was 60°C. Capillary electrophoresis was conducted on ABI PRISM 3130XL. Fragment analysis was performed using Gene Mapper software (Thermo Fisher).

Statistical Methods

Chi-squared or Fisher's test were used to compare categorical variables. Distribution of continuous variables was assessed with Kolmogorov–Smirnov test and subsequently non-parametric tests were used. In all analysis $P < 0.05$ was considered statistically significant.

Results

NOP56 expansion was identified in 37 individuals from 16 apparently unrelated families. Of these, 28 were clinically affected, three were in the preataxic stage (SARA <3) and six were asymptomatic. All were Caucasian and most of them were originally from the same geographical area of *La Costera*, except pedigrees F4 and F13 that were originally from the city of Valencia and Murcia respectively. All kindreds showed AD transmission, except F6 that was sporadic, Figure 1.

Clinical Characteristics

The main clinical characteristics of the 28 affected subjects are summarized in Table S1.. Mean age at onset was 52.5 (range: 44–67) and mean age at exam was 63.7 (range: 49–77). All cases displayed a phenotype of progressive cerebellar ataxia. At onset, most patients presented with unsteady gait (n = 23) and rarely with other symptoms such as hearing loss (n = 2), loss of hand dexterity (n = 1), dysarthria (n = 1) or muscle cramps (n = 1). The average SARA score was 19 ± 8.3 (mean ± SD). Gait ataxia was the most prominent feature followed by lower limb ataxia and dysarthria. Mild eye movement saccadic abnormalities were common but only 11 (39.3%) patients showed horizontal nystagmus. A majority of patients (64%) exhibited ptosis and/or gaze limitation, Figure 2, with no significant association with longer disease duration or SARA scores.

Hypoacusia was the most distinguishable non-cerebellar feature (67.9%). Audiometry studies were available in four cases, and showed bilateral sensorineural hearing loss with a drop of approximately 40 dB at high frequencies. The presence of hearing loss was not correlated with SARA scores. Mild pyramidal signs were present in 46.4%. Lingual fasciculations and atrophy were seen in five cases and orbicularis oculi myokymia in two. Lower limb fasciculations were only identified in a patient with axonal sensory-motor neuropathy. Few cases showed extrapyramidal features. Cervical dystonia was detected in three and asymmetric upper limb dystonic posturing and tremor in two, Figure 2. In three patients signs of parkinsonism (bradykinesia, rest tremor) were identified. Rigidity was not observed. DaTSCAN® and DaTQUANT analysis confirmed dopaminergic denervation in these three cases compared to age-matching healthy controls, Figure 2. Levodopa was administered only to one patient without clinical improvement. The majority of patients did not report cognitive impairment. Six patients referred mild cognitive difficulties and three developed severe dementia. In some cases, CCAS features such as disinhibition or pseudobulbar affect were identified in usual clinical interviews.

Asymptomatic carriers were younger than preataxic cases (mean age at exam 38 vs. 47.7 years). Age at exam of preataxic carriers was 40, 35 and 54. Two referred mild gait imbalance and one had been diagnosed with bilateral sensorineural hypoacusis at 32 years and had orbicularis oculi myokymia.

Brain MRI was available for review in 15 ataxic patients, 2 asymptomatic and 1 preataxic subject. Cerebellar atrophy was evident in all symptomatic patients. Mild cerebellar atrophy was noted in the preataxic case and in one asymptomatic 50 year-old subject. After a mean disease duration of 11.3 ± 6.6 years (mean ± SD; range: 2–27) independent gait was preserved in 9 (33.3%), permanent gait support was required in 8 (29.6%) and 9 were wheelchair-bound (33.3%). Mean disease duration was

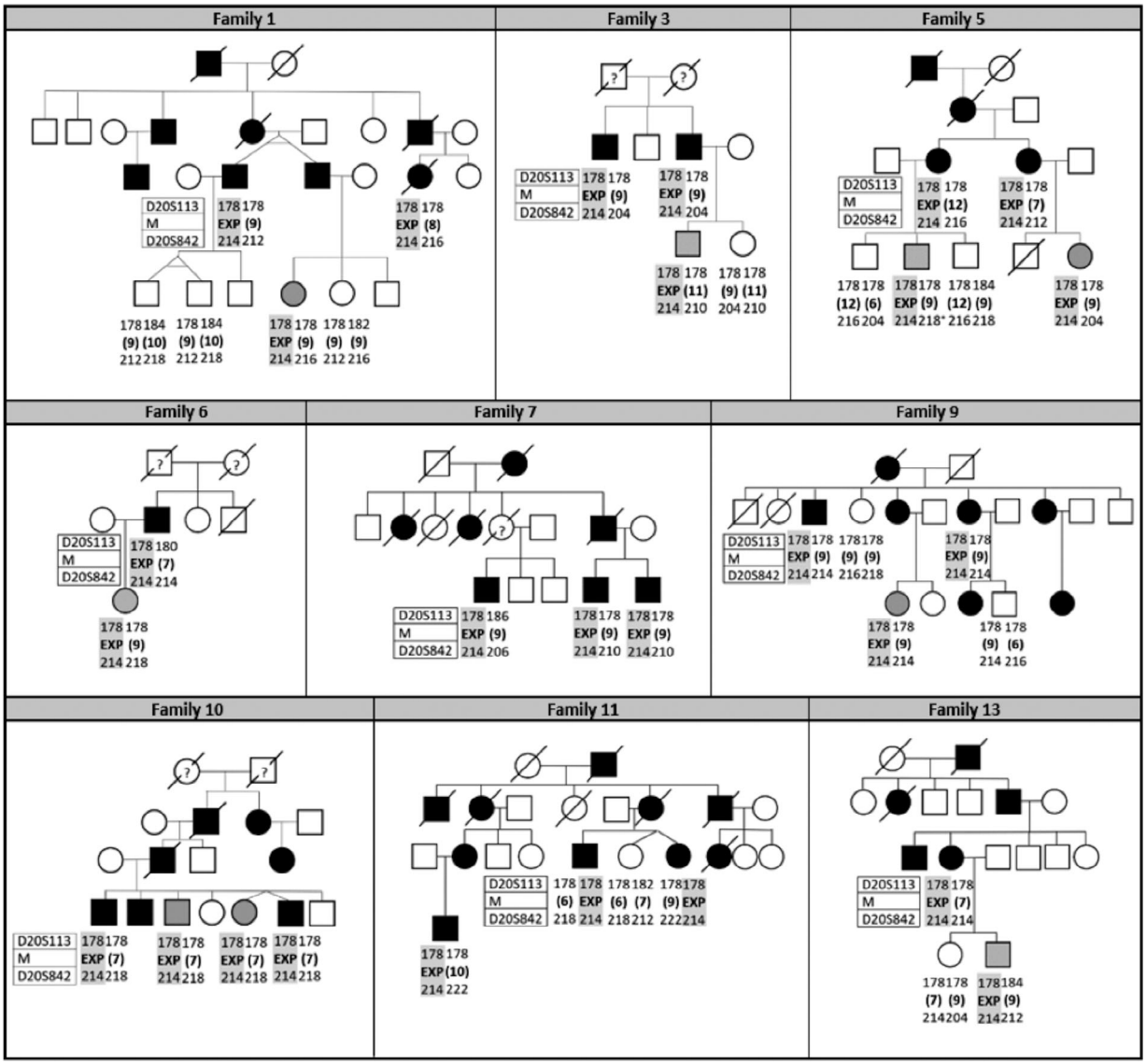

FIG 1. Pedigrees and haplotype analyses of nine Spanish SCA36 families. The common haplotype shared by all families is shaded in gray. Squares represent males and circles females. A slash indicates a deceased individual. Black-filled symbol: symptomatic individual; gray-filled symbol: asymptomatic carriers and preataxic individuals. (?) indicates no reliable phenotypic data. Asterisk on family 5 indicates a probable recombination event on paternal chromosome.

higher in wheelchair-bound patients than in ambulant cases (mean disease duration 17.6 ± 4.5 vs 8,3 ± 4.9 years, $P < 0.05$).

Genetic Analysis

A total of 15 autosomal dominant SCA36 families and one sporadic case were identified. The majority of non-expanded alleles carried between six and nine repeats, with the nine repeats-allele being the most common (43%). The study of Short Tandem Repeats (STRs) markers was performed in 37 individuals from nine families (25 affected and 12 unaffected), Figure 1. Haplotype construction revealed a common haplotype composed of an amplicon of 178pb for the D20S113 marker and 214pb for the D20S842, thus spanning 0.65 Mb. The STR marker D20S113 showed a low heterozigosity rate. This common allele was present in all individuals tested. Contrarily, the D20S842 marker was more polymorphic, with the 214pb allele being tightly linked to the *NOP56* expansion. STRs were also studied in isolated affected patients from F4, F8, F12, F15 and F16 pedigrees. Subjects from F8, F12, F15 and F16 harbored the common alleles, although their phase could not be inferred due to the lack of DNA sample from relatives. The individual from F4, originally from a different geographical area, did not carry the common allele for D20S842.

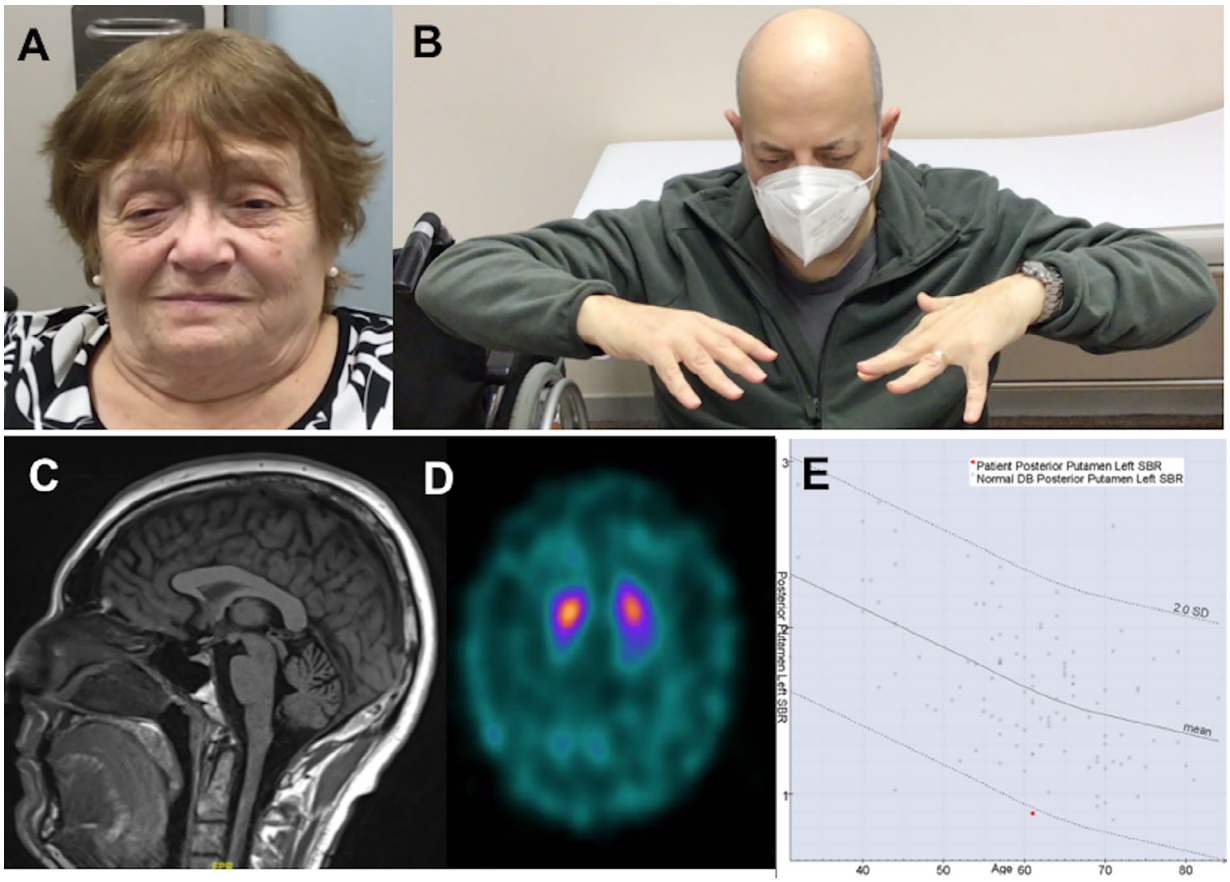

FIG 2. Clinical and radiological findings in SCA36. **(A)** Bilateral ptosis in a patient that also displayed ophthalmoplegia; **(B)** Left-hand dystonic postures in a SCA36 patient with dystonic tremor. **(C)** Sagital T-1 weighted MRI imaging showing cerebellar atrophy. **(D)** 123-Ioflupane reveals bilateral asymmetrical denervation with right striate reduced uptake. **(E)** Semiquantitative analysis with DaTQUANT™ for left posterior putamen confirms a decline (red spot) in tracer uptake compared to mean tracer uptake for age-matching healthy controls.

Discussion

This is the second SCA36 Spanish series reported demonstrating a high frequency of SCA36 in the Spanish population. *NOP56* expansion was found in 37 individuals from 16 different families. In a retrospective analysis of our total cohort of 297 index cases, SCA36 represented 5.4% (n = 16), (Figure S1.). It was the second most frequent AD ataxia after SCA3. SCA36 frequency was similar to that observed in northwestern regions of Spain.[2] Therefore, SCA36 testing should be implemented and performed in our regional population prior to other genetic tests. Similar to other studies, we found a strong founder effect.[2,6,11]

Comparable to other series, all affected patients presented with a late-onset cerebellar syndrome with slow progression.[2,3] Unlike previous reports, isolated facial or lingual fasciculations and tongue atrophy were scarce in our series (25%).[1,2,4,5] Hearing loss was present in two-thirds of our patients. Although hypoacusis is a distinctive clinical feature of SCA36,[12] it might be encountered in diverse types of hereditary ataxia,[13,14] especially mitochondrial disorders.[14,15] Ptosis and gaze limitation were also common. Therefore, it should be noted that in a given patient with ataxia, ophthalmoparesis, and hearing impairment that might suggest a mitochondrial disease phenotype, SCA36 testing should be considered.

Extrapyramidal features such as oromandibular or cervical dystonia[4,5] have occasionally been reported in SCA36. In our cohort, two siblings developed laterocaput and anterocaput respectively, and one patient displayed asymmetric dystonic posturing and tremor in the upper limbs. Dysfunction of the cerebello-thalamo-cortical pathway is believed to be involved in the pathophysiology of dystonia-ataxia syndromes.[16,17] Bradykinesia and resting tremor with evidence of dopaminergic denervation was encountered in three patients (10.7%). An additional case reported REM sleep behavior disorder, a marker of dopaminergic deficiency.[18] Postural tremor was identified in SCA36 series (7–29%),[2,6,11] but parkinsonism has not been reported to date.

We tested two STR markers included in previous SCA36 haplotype studies. A common haplotype spanning 0.65 Mb was identified in all the SCA36 families except in one subject originally from a different geographical area. This may be due to a different founder effect or to the occurrence of recombination events. Similarly to Japanese[1] and northwestern Spanish families,[2] D20S842 showed a conserved allele in our cohort. A common allele for the D20S113 marker has also been reported,[2,3] but it also showed a high frequency in the control group.[3] Nevertheless, the lack of a universal nomenclature makes it difficult to perform comparisons with previously reported data.

In conclusion, SCA36 was the second most frequent AD ataxia in our series. Thus, in our geographical area, *NOP56* expansion analysis should be prioritized to other genetic testing in probands whether with an AD or sporadic presentation. Although most patients display a predominant progressive cerebellar ataxia phenotype, less frequent clinical features such as hearing loss, ophthalmoparesis, or extrapyramidal features might assist the clinician to focus the genetic diagnosis.

Author Roles

(1) Research project: A. Conception, B. Organization, C. Execution; (2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique; (3) Manuscript Preparation: A. Writing of the first draft, B. Review and Critique.

R.B.M.: 1A, 1B, 1C, 2A, 2B, 3A
L.C.V.: 1A, 1B, 1C, 2A, 2B, 3A
N.M.: 1C, 2C, 3B
R.S.: 1C, 2C, 3B.
P.S.N.: 1C, 2C, 3B
B.M.S.: 1C, 2C, 3B
I.S.B.: 2C, 3B
M.C.R.: 2C, 3B
I.M.T.: 2C, 3B
J.M.G.V.: 2C, 3B
J.M.M.:1C, 2C, 3B
T.J.: 1C, 2C, 3B
E.A.: 1B, 1C, 2C, 3B
L.B.: 1B, 1C, 2C, 3B

Disclosures

Ethical Compliance Statement: This study was approved by the Research Ethics Committee from Hospital Universitari I Politècnic La Fe (2021–435-1). The authors affirm that additional written informed consent was obtained for the publication of patient identifiable images in Fig. 2. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

Funding Sources and Conflicts of Interest: This work was supported by the Instituto de Salud Carlos III (ISCIII)-Subdirección General de Evaluación y Fomento de la Investigación [PI18/00147; PI21/00103] and by the Generalitat Valenciana [PROMETEO/2018/135; PROMETEO/2019/075], within the framework of the National R + D + I Plan co-funded with ERDF funds. Part of the equipment employed in this work has been funded by Generalitat Valenciana and co-financed with ERDF funds (OP ERDF of Comunitat Valenciana 2014–2020). The authors have no competing interests to declare that are relevant to the content of this article.

Financial Disclosures for the Previous 12 Months: The authors declare that there are no additional disclosures to report. ■

References

1. Kobayashi H, Abe K, Matsuura T, et al. Expansion of intronic GGCCTG hexanucleotide repeat in NOP56 causes SCA36, a type of spinocerebellar ataxia accompanied by motor neuron involvement. *Am J Hum Genet* 2011;89(1):121–130. https://doi.org/10.1016/j.ajhg.2011.05.015.
2. García-Murias M, Quintáns B, Arias M, et al. "Costa da Morte" ataxia is spinocerebellar ataxia 36: clinical and genetic characterization. *Brain* 2012;135(5):1423–1435. https://doi.org/10.1093/brain/aws069.
3. Ikeda Y, Ohta Y, Kobayashi H, et al. Clinical features of sca36: a novel spinocerebellar ataxia with motor neuron involvement (asidan). *Neurology* 2012;79(4):333–341. https://doi.org/10.1212/WNL.0b013e318260436f.
4. Nakazato Y, Mochizuki H, Ishii N, et al. Spinocerebellar ataxia 36 accompanied by cervical dystonia. *J Neurol Sci* 2015;357(1–2):304–306. https://doi.org/10.1016/j.jns.2015.07.012.
5. Miyashiro A, Sugihara K, Kawarai T, et al. Oromandibular dystonia associated with SCA36. *Mov Disord* 2013;28(4):558–559. https://doi.org/10.1002/mds.25304.
6. Obayashi M, Stevanin G, Synofzik M, et al. Spinocerebellar ataxia type 36 exists in diverse populations and can be caused by a short hexanucleotide GGCCTG repeat expansion. *J Neurol Neurosurg Psychiatry* 2015;86(9):986–995. https://doi.org/10.1136/jnnp-2014-309153.
7. Martínez-Regueiro R, Arias M, Cruz R, et al. Cerebellar cognitive affective syndrome in Costa da Morte ataxia (SCA36). *Cerebellum* 2020;19(4):501–509. https://doi.org/10.1007/S12311-020-01110-0.
8. Baviera-Muñoz R, Carretero-Vilarroig L, Francisco Vázquez-Costa J, et al. Diagnostic efficacy of genetic studies in a series of hereditary cerebellar ataxias in eastern Spain. Published online. 2022;8:e200038. https://doi.org/10.1212/NXG.0000000000200038.
9. Maas RPPWM, van Gaalen J, Klockgether T, van de Warrenburg BPC. The preclinical stage of spinocerebellar ataxias. *Neurology* 2015;85(1):96–103. https://doi.org/10.1212/WNL.0000000000001711.
10. Abele M, Bürk K, Schöls L, et al. The aetiology of sporadic adult-onset ataxia. *Brain* 2002;125(5):961–968. https://doi.org/10.1093/brain/awf107.
11. Lee YC, Tsai PC, Guo YC, Hsiao CT, Liu GT, Liao YC, Soong BW. Spinocerebellar ataxia type 36 in the Han Chinese. *Neurol Genet* 2016;2(3):1–10. https://doi.org/10.1212/NXG.0000000000000068.
12. Ikeda Y, Ohta Y, Kurata T, Shiro Y, Takao Y, Abe K. Acoustic impairment is a distinguishable clinical feature of Asidan/SCA36. *J Neurol Sci* 2013;324(1–2):109–112, 112. https://doi.org/10.1016/j.jns.2012.10.013.
13. Guissart C, Drouot N, Oncel I, et al. Genes for spinocerebellar ataxia with blindness and deafness (SCABD/SCAR3, MIM# 271250 and SCABD2). *Eur J Hum Genet* 2015;24(8):1154–1159. https://doi.org/10.1038/ejhg.2015.259.
14. Winkelmann J, Lin L, Schormair B, et al. Mutations in DNMT1 cause autosomal dominant cerebellar ataxia, deafness and narcolepsy. *Hum Mol Genet* 2012;21(10):2205–2210. https://doi.org/10.1093/HMG/DDS035.
15. Chinnery PF, Elliott C, Green GR, Rees A, Coulthard A, Turnbull DM, Griffiths TD. The spectrum of hearing loss due to mitochondrial DNA defects. *Brain* 2000;123(1):82–92. https://doi.org/10.1093/BRAIN/123.1.82.
16. Bologna M, Berardelli A. Cerebellum: an explanation for dystonia? *Cerebellum Ataxias* 2017;4(1):1–9. https://doi.org/10.1186/s40673-017-0064-8.

17. Kaji R, Bhatia K, Graybiel AM. Pathogenesis of dystonia: is it of cerebellar or basal ganglia origin? *J Neurol Neurosurg Psychiatry* 2018;89(5):488–492. https://doi.org/10.1136/jnnp-2017-316250.

18. Wasserman D, Bindman D, Nesbitt AD, et al. Striatal dopaminergic deficit and sleep in idiopathic rapid eye movement behaviour disorder: an explorative study. *Nat Sci Sleep* 2021;13:1–9. https://doi.org/10.2147/NSS.S267037.

Supporting Information

Supporting information may be found in the online version of this article.

Table S1. Clinical characteristics of SCA36 symptomatic patients. M, male; F, female; y, years; −, absent; +, present; Pyramidalism, + mild, ++ moderate, +++ severe; GI, Gait instability; H, Hypoacusia; ScP, saccadic pursuit; ↓Sc, slow saccades; HpS, hypo/hypermetric saccades; LVG, limitation of vertical gaze; LHG, limitation of horizontal gaze; P, ptosis; Fasc., fasciculations; mk, myokymias; Tr, tremor; Br, bradykinesia; NA, not available; MCI, mild cognitive impairment; Disability scale, 0 = no gait difficulties; 1 = disease onset as defined by onset of gait difficulties; 2 = loss of independent gait, as defined by permanent use of a walking aid or reliance on a supporting arm; 3 = confinement to wheelchair; 4 = death; S-M, sensory-motor neuropathy.

Figure S1. Overall frequencies of different Genetic subtypes of ataxia in our cohort of index cases. CANVAS: Cerebellar Ataxia with Neuropathy and Vestibular Areflexia Syndrome; FXTAS, Fragile-X Tremor Ataxia Syndrome.