LATE-ONSET VESTIBULOCEREBELLAR ATAXIA: CLINICAL AND GENETIC STUDIES IN A LONG FOLLOW-UP SERIES OF 50 PATIENTS.

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ABSTRACT

OBJECTIVE

To describe the clinical and genetic characteristics of a cohort of patients with late-onset vestibulo-cerebellar ataxia (LOVCA).

METHODS

We analyzed the clinical and genetic characteristics of a cohort of 50 patients with LOVCA (with an onset during their fifties or later).

RESULTS

Ten patients belonging to eight families had a positive family history while 40 patients presented sporadically. Forty-three patients had an episodic onset, with episodes of gait ataxia characterized by a sudden onset of instability associated with downbeat nystagmus, visual symptoms, dizziness, falls, and, less commonly, dysarthria and horizontal nystagmus. Progressive cerebellar ataxia developed on average 1.5 years after onset of episodic ataxia. Seven cases presented with a slowly progressive vestibulo-cerebellar syndrome without any episodes. In half of the cases the disease led to an inability to stand (FARS functional disability score of 5). Molecular genetic studies of EA1(*KCNA1*), EA2(*CACNA1*), EA5(*CACNB4*), EA6(*SLC1A3*) only identified two variants of uncertain significance (VUS) in *CACNA1A*. A post-mortem study revealed a case of polyglucosan body disease (APBD) with a VUS in the *GBE1* gene. An *FGF14* intronic GAA repeat expansion was found in 61% of patients. Treatment with 4-aminopyridine reduced the number and severity of episodes, although progression appeared not to be modified.

CONCLUSIONS

Late-onset vestibulo-cerebellar ataxia commonly presents as a sporadic slowly progressive episodic ataxia with downbeat nystagmus that leads to significant disability in a substantial

proportion of patients who are followed along their full course. The most common genetic cause in our cohort is a heterozygous GAA expansion in *FGF14* (SCA27B). Other rare variants can also lead to the same clinical picture. One third of our patients remain without an etiologic diagnosis.

INTRODUCTION

Vestibulo-cerebellar ataxia (VCA) results from the dysfunction of the vestibulo-cerebellar system, which connects vestibular nuclei with the flocculonodular lobe.[1] The first description of episodic and vestibulo-cerebellar ataxia (VCA) was reported in 1963 and, since then, there have been many reports of episodic ataxias although not all of these are vestibulocerebellar syndromes.[2] Until now, variants in genes have been proposed as causes of episodic ataxia: *KCNA1* (EA1), *CACNA1A* (EA2), *CACNB4* (EA5), *SLC1A3* (EA6), and *FGF14* (EA9/SCA27B), *UBR4, SCNA2, KCNA2, SCL1A3* .[3-12] However, there have been a few reports of hereditary EA in adults.[5,10-12,14,15] Far less attention has been paid to sporadic late-onset episodic ataxia: a single report describes four cases of a disease the authors named as "late-onset paroxysmal cerebellar ataxia," one of which could be attributed to a *CACNA1A* variant.[13,15,16] Other authors have reported cases in their series of familial late-onset ataxias that fulfilled the criteria for vestibulo-cerebellar ataxia[2,17-23] and so a definitive clinical profile of patients with sporadic late-onset vestibulo-cerebellar episodic ataxia, whether episodic or progressive, has yet to be firmly established.

In our geographical area in Spain, cases of sporadic late-onset vestibulo-cerebellar ataxia are found to be more common than familial ones. Here, we present the clinical and genetic characteristics of a cohort of 50 patients with vestibulo-cerebellar ataxia, whether episodic or progressive, that we have cared for over a period of up to two decades and in many cases following the full course of the disease from onset. Most of our patients began as episodic ataxias, which later became progressive.

Recently, a large study found an intronic GAA expansion in the *FGF14* gene to be often responsible for hereditary and sporadic late-onset episodic-progressive ataxia as well as non-

episodic progressive ataxias.[24] Another similar study reported the same expansion as being responsible for two different ataxia phenotypes: pure cerebellar ataxia and cerebellar ataxia with vestibulopathy.[25] Given that most of the patients carrying this expansion are affected by late-onset episodic ataxia, we decided to conduct a search for this expansion in our cohort of late-onset (episodic or progressive) hereditary and sporadic patients with vestibulo-cerebellar ataxia.

METHODS

Patients:

This retrospective study, approved by the Ethical Committee of the Dr. Josep Trueta University Hospital in Girona (2022.120), involves patients followed in the Ataxia Unit of the hospital over a period of two decades. This hospital has a catchment area of about 700,000 people and is the reference centre for six county hospitals around the province.

We included patients with isolated vestibulo-cerebellar syndrome whether presenting as late-onset episodic ataxia or as a slowly progressive form. Exclusion criteria included having a diagnosis of a vestibular disease, vertebro-basilar territory infarctions, or other pathologies of the posterior fossa. We also excluded patients with clinical criteria for the cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS) and, in order to be able to make the clinical evaluation with clarity, patients with previously diagnosed Parkinson's disease, Parkinson plus syndromes, chronic adult hydrocephalus, peripheral sensory neuropathy, and myelopathy causing deep sensory loss. Patient developing such disease after the diagnosis of VCA had been made were not excluded from the study.

For the evaluation of the functional status, we used the Functional Staging for Ataxia Subscore of the FARS scale (FARS-FS).[26] Here we refer to patients with a FARS-FS from 0 to 3

as not disabled or mildly disabled whereas subjects with FARS-FS 4 or 5 are referred to as moderately-to-severely disabled.

All patients meeting the inclusion criteria were from our catchment area (the province of Girona) except for three patients who were diagnosed and followed in a hospital in Barcelona.

MRI:

An MRI was performed in 48 out of 50 patients, while CT scan was obtained for the remaining two patients. Six subjects have been clinically assessed three times, 23 twice, and 19 only once. All the MRIs have been examined by two of the authors of this paper (BA and DG), who both have extensive experience in cerebellar ataxia. We classified the pattern of cerebellar atrophy into four types: no atrophy; isolated vermis atrophy; vermian-paravermian (VPV) with involvement of the vermis and adjacent areas that almost completely respects the hemispheres; and global atrophy, affecting vermian, paravermian and bilateral hemispheric atrophy in the anterior and posterior lobes. MRI was always performed shortly after the diagnosis and was repeated some years later during the disease.

Ataxia work-up:

Acquired causes of ataxia were excluded with a comprehensive blood work-up that included hematological, biochemical, thyroid, onconeuronal antibodies, antigliadin antibodies, B12, and vitamin E studies.

Eye movement recording:

Eye movement recording was performed by electro-oculogram (EOG) on nine patients by a neurologist with extensive experience in oculomotor disorders (CR).

vHIT:

Video head impulse test (vHIT) was conducted by specialized otologists in three patients.

Genetic molecular studies:

Screening of known frequent SCA genes: molecular studies of PolyQ expansions were performed in 35 subjects. Genomic DNA was isolated in these individuals of the series from whole peripheral blood leukocytes using automated DNA purification on the Chemagic Magnetic Separation Module I (Chemagen, Germany). These patients were tested for nucleotide repeat expansions at the SCA1, SCA2, SCA3, SCA6, SCA7, SCA10, SCA12, SCA17, and DRPLA genes following the published methodology.[27]

If a diagnosis of CANVAS was suspected, a molecular study for *RFC1* AAGGG repeat expansions was undertaken.[28]

Study of episodic ataxia gene variants:

In 28 patients, a molecular genetic study was undertaken searching for variants in the known episodic ataxia genes (*KCNA1, CACNA1A,* and *CACNB4, SLC1A3*), which were analyzed following the methodology described in earlier papers.[4-7,10,15]

Genetic screening for *FGF14* repeat expansions:

We also screened 36 patients with available DNA for *FGF14* GAA expansions using a standardized protocol, as described previously.[29]

Molecular genetic study of *GBE1* (adult polyglucosan body disease):

After a post-mortem study gave a diagnosis of a probable adult polyglucosan body disease (APBD), a search for variants in the *GBE1* gene was undertaken.

Exome sequencing (ES) was performed using the SureSelect Human All Exon v8 kit (Agilent Technologies, Santa Clara, CA, USA). Paired-end sequencing (2 × 150 bp) was carried out on a NextSeq 2000 sequencer (Illumina, San Diego, CA, USA).[30] Bioinformatic analysis was done as previously described. ES data was filtered by 28 genes previously associated with glycogenosis.[31]

Epidemiologic study:

We calculated the prevalence of the disease in our county basing ourselves exclusively on the direct catchment area of the two main hospitals (~200,000 inhabitants in the 2022 census), rather than taking into consideration the wider reference area in which it was less likely that these cases would be referred to us.

Treatment:

We initially used acetazolamide to treat patients with late-onset episodic ataxia but as the results were not satisfactory, patients were switched to 4-aminopyridine (4-AP). In some cases, alternative treatments with baclofen, gabapentin, or clonazepam were given to patients with more disabling evolutions.

Neuropathological work-up:

Neuropathological examination was performed following standardized protocols at the NTB. (REF: PMID 33576076). Briefly, half of the brain was dissected in the fresh state, frozen, and stored at −80°C, and the other half was fixed in a formaldehyde solution for three weeks. At least 25 representative brain areas were embedded in paraffin, cut at 5 µm, and stained with hematoxylin and eosin. Additionally, selected regions were stained with PAS, Gallyas, and Bielschowsky stainings. Immunohistochemistry was conducted in a BOND-MAX Automated Immunohistochemistry Stainer (Leica Biosystems Melbourne Pty Ltd, Melbourne, Australia) using a variety of antibodies, including anti-alpha B Crystallin (G2JF; Leica, Germany), anti-calbindin-D (CL-300, MilliporeSigma, USA), anti-neurofilament (RT97; Leica, Germany), anti-βA4 (6F/3D, Dako, Glostrup, Denmark), anti-tau (AT8; Thermo Scientific, USA), and anti-α-synuclein (5G4; Analytik Jena, Germany), anti-phosphoTAR DNA-binding protein 43 (11-9, Cosmo Bio. co, Japan). Disease assessment was performed in accordance with international consensus criteria.

Statistical study:

Categorical variables were expressed as absolute and relative frequencies and continuous variables as means and standard deviations, or as medians and interquartile range if the distribution is not normal. The normality of the distribution of the variables involved was checked by the Shapiro-Wilk test. Normally distributed data were analysed using either Student's t test or one-way ANOVA and non-parametric variables were compared using the Mann-Whitney U test or the Kruskal-Wallis test. Categorical variables were compared using the chi-square test or Fisher's exact test, where appropriate. Correlation between the size of the expansion and the age of onset was performed using the Spearman test.

The analyses were performed using statistical ware R version 4.2.1 with the compareGroups package. All p-values were 2-sided, and p less than 0.05 was considered statistically significant.

RESULTS

Clinical features of the cohort

There are no patients under the age of 50 in this cohort as no such case has come to our attention in over two decades paying close attention to this disease. The demographic data show a slight preponderance of males (58%) and a larger proportion of sporadic cases (80%) (Table 1). The median age of onset was 69.5 years and the progressive ataxic phase began on average at 71 years. The age of onset of sporadic cases was a decade later than that of familial cases 60 years vs 71.5 years (p=0.003) and this difference was longer in the case of the time of onset of the progressive phase (p=0.004) (Table 5).

Progression coincided with or followed episodes in 39 patients. Four additional patients, all of whom were male, had recurrent episodes without progressive cerebellar ataxia, and seven patients had progressive ataxia from onset without associated episodes.

The duration of episodes was known in 39 patients: in 26 patients (66.7%) this was less than one day, in 9 (23%) it was more than a day but less than a week, and in four (10%) the duration was a week or longer (Table 2). Regular daily morning worsening, which does not share the unpredictability of episodes, was reported in 11 patients (33.33%). This phenomenon was observed mainly in women ($p=0.009$) and it appears unrelated to the mutation causing the disease (Table 6). The number of episodes before and after progression showed no differences between males and females, disabled and non-disabled patients, familial and sporadic cases, and between cases with and without SCA27B. Emotion, physical exertion and fever were reported as triggering episodes in single subjects.

The clinical features during the episodes did not vary substantially: the central syndrome was a severe ataxia associated with downbeat nystagmus. The degree of disability was known in 37 patients, 20 (47.62%) were unable to walk and could not stand or needed aid to do so, whereas 22 (52.37%) could walk although with varying degrees of difficulties (FARS-FS 0 to 3). Downbeat nystagmus was present in 83% of patients and dizziness was also common. Many patients suffered falls. Oscillopsia, horizontal nystagmus, blurred vision, nausea, vomiting, diplopia, and dysarthria were less frequently observed. (Table 3)

In the interictal period, cerebellar signs were frequently observed, such as alterations in static balance and tandem walking, and difficulties with turning, but ataxia was frequent but not always present. Downbeat nystagmus was present in between episodes in 78% of patients but could be difficult to assess. The frequency of falls and positive Romberg's sign (56%) revealed the severity of the permanent damage to the vestibulo-cerebellar system.

Dysarthria was less common, being observed in only 14 patients (29%) and usually remained mild (Table 3).

Surprisingly, only three subjects with episodic ataxia were reported to have dysmetria during the episodes: this disappeared in one case and persisted in two. In five subjects, a slight dysmetria appeared during the progression: three were episodic whereas two had only a progressive picture without episodes.

Of the 32 out of the 50 patients whose disability status is known, it took a median of three years before a unilateral walking aid (FARS-FS: stage 3) was required (25 patients), five years for a walker (FARS-FS: stage 4) (19 patients), and seven years to finally require a wheelchair (FARS-FS: stage 5) (13 patients). Of the seven cases not recorded as having any level of disability, four have still had a relatively short period of evolution and three have been lost to follow-up. None of the 50 patients we have studied has ever reached FARS-FS: 6 (bedbound). Of the 26 patients who have died, we have been able to follow 16 patients throughout the whole process of their disease: 14 (87.5%) of these had become disabled (FARS-FS 4 or 5) whereas two died prematurely.

Fifty-eight percent of the disabled patients were women (FARS-FS: 4/5) whereas men made up 78% of the non-disabled group (p=0.061). Proportionally, it is observed that just 36% percent of males were disabled as opposed to 73% of women(Table 6). The age of onset, the duration of the disease, and the size of *FGF14* GAA expansion are similar in the two groups. Bias by sex is maintained in patients with SCA27B and in those without a known mutation (non-SCA27B). Many patients (89%) of those with a higher degree of disability continued to have episodes after the beginning of progression whereas this percentage was lower (58%) in patients without functional disability (p=0.023).

Of the 14 patients still being followed, none is wheelchair-bound but seven need to use either a cane or a walking frame. The same proportion was detected in the group lost to follow-up with 50% needing support at the time they were last seen.

Patients were followed for a median of 7 years (range 1-19), and 16 were followed for more than 10 years typically until death except when lost to follow-up. Ataxia progressed in 92% of the subjects, with an average onset of 2.1 years after the initial episode (ranging from 0 to 15 years). In 18 cases, progression coincided with the initial episode, while in 20 cases, the episodes began later. More than 70% of the patients who had episodes continued to have them after the beginning of progression.

The comparison of the evolution and the clinical findings between sporadic and familial cases only showed significant differences in the age of onset, the age at onset of progression, and the age at disability, which in all ten cases was earlier in the familial group. (Table 5)

Eleven patients were followed until death: nine became unable to stand over time, whereas two others used a cane when they died at 85 and 66, after 4 and 6 years of evolution, respectively.

The SCA27B group consisted of 22 patients (14 men). Seven were familial and 15 (68%) were sporadic cases. Seven of the sporadic cases (46.60%) were progressive from onset but without episodes. The median age of onset was 71 (range 50-87). Only two patients, both familial, have not had a progressive course. Six patients, three of whom were women, progressed to the point of moderate-to-severe disability (FARS 4 or 5) and 12 (eight men, 66.66%) had mild disability (FARS 0 to 3), and in four cases the degree of disability was unknown. The mean follow-up time was 7.75 years (range 1-19). The changes observed on MRI followed different patterns across the whole group with atrophy involving the vermian

(6) or paravermian areas (7), and only one patient had mild global atrophy. Eight did not show cerebellar atrophy.

Similar figures were found in the group without SCA27B (14 patients, six women) in whom three cases were familial and eleven sporadic. Progression was present in 13 and the age of onset was 66 (range 56-81). Seven reach FARS-FS 4-5, including five women. Four men were non-disabled, in three cases the evolution is unknown. MRI was normal in five, another five showed vermian-paravermian atrophy and in four there was global atrophy but no subjects with exclusive vermian atrophy unlike what was found in the SCA27B patients (Table 7).

APBD patient: his disease began in his sixties when he suffered several intense episodes during several days, progressing until reaching FARS-FS 5 in three years. Although the spinal cord atrophy was present from the onset of the picture, a pyramidal syndrome did not appear until some years of evolution. Dementia with altered behaviour developed more than 10 years after the onset. The basic clinical picture of ataxia and down-beat nystagmus worsening during the episodes remained over the course of the disease. Treatment with acetazolamide, 4-AP or clonazepam did not reduce the number of episodes or provide any other noticeable benefit.

MRI changes

Brain MRI showed cerebellar atrophy in 28 (58.3%) of patients. In patients with cerebellar atrophy, nine patients had isolated vermian atrophy, eleven had vermian/paravermian atrophy, and eight had global atrophy, which was mild in seven and more marked in one. There were no significant differences in the proportion of disabled subjects between those with and without cerebellar atrophy: 8 (66.7%) with FARS-FS 4/5 and 4 (33.3%) with FARS-FS 0 to 3 were in the non-atrophic group whereas 11 (39.29%) with FARS-FS 4/5 and 13 (54.17%) with FARS-FS 1/3 were in the group with cerebellar atrophy (p=0.068).

Vermian atrophy was in fact the most benign pattern as only 14% of these subjects were disabled, although we do not know the disability status in two. There are no significant differences between the disease duration in the different atrophy subtypes (Table 8). These patterns should not be considered as steps along the way from the absence of atrophy to global atrophy since the patterns seen in the images do not change over the course of the disease after years of evolution (Figures 1 to 3).

When we analyzed the association between MRI and genetic results, we found that the GAA *FGF14* expansion was detected in all the groups without significant differences between them, although it was present in all the subjects with vermian atrophy. In patients without cerebellar atrophy, 40% are carriers of this expansion. The degree of global atrophy was mild in all but one case. Therefore, the severity of the cerebellar atrophy did not correlate with the severity of the disease. There was no evidence of brainstem atrophy. Abnormalities such as vascular changes and brain atrophy were also detected in what is an elderly population.

MRI showed that the patient with APBD had a reduction in cerebellar volume, with medullar and spinal cord atrophy, and demyelination in the supra and infratentorial white matter, which are typical abnormalities seen in this condition (Figure 4).

Electro-oculogram (EOG)

The oculographic recordings detected downbeat nystagmus of different intensity in all the subjects examined. Upward optokinetic nystagmus was absent in six subjects and smooth pursuit mainly in the vertical plane was severely disturbed with pursuit gain between 20 and 60% of normal. Saccades were normal in all studied cases.

vHIT

Of the three patients studied with this technique, there was no associated vestibular dysfunction in two whereas in one case a hyperfunction of the right anterior and left posterior canals was detected.

Genetic studies

Polyglutamine gene expansions were not detected. A search for mutations in episodic ataxia genes, EA1(*KCNA1*) EA2(*CACNA1*), EA5(*CACBN4*), and EA6(*SLC1A3),* in 28 subjects found variants in two patients from the familial group: a woman who carried a c.3607_3609 del AAG (p.Lys 1203 del) variant in the 21st exon in the *CACNA1A* gene which is known to be benign (this variant results in the loss of the codon that codifies for lysine but is an in-frame deletion that does not affect the integrity of the rest of the protein). Another woman who reported having affected siblings presented variant c.3439G>A(pVal1147Ile) in the 20th exon in the *CACNA1A* gene, associated to a $GAA_{(200)}$ allele in the *FGF14* gene.[32, 33]

The study undertaken to detect intronic GAA expansions in the *FGF14* gene found pathogenic expansions in 22 patients. The size of the expansion ranged from $GAA_{(250)}$ to $GAA_{(516)}$. There was no clear correlation between the size of the expansion and the age of onset (Rs.0.1206). All familial cases carried this expansion except for the two patients with the benign *CACNA1A* variant. Three subjects have an intermediate allele with 200, 216 and 233 *FGF14* GAA repeats.

In the APBD patient, exome sequencing identified a novel homozygous missense variant c.1300C>G, p.(Arg434Gly) in the *GBE1* (NM_000158.4) gene. Although no CNVs were discarded, the variant is located in a run of homozygosity (ROH) of approximately 40Mb, supporting its zygosity. *In silico* tools suggest a deleterious effect of the variant on the protein. However, based on current evidence, this variant should be considered as a variant of unknown significance (VUS).

Neuropathological results

The brain weighed 1085 g. The macroscopic study revealed moderate to severe atrophy in the vermis and cerebellar hemispheres and spinal cord, moderate in the hippocampus and mammillary bodies, and mild in the remaining regions. In coronal sections, diffuse greyish discoloration was observed in the white matter with the preservation of U-shaped fibres.

The histological examination revealed widespread rarefaction and volume loss in both supra- and infratentorial white matter with chronic diffuse fibrillar gliosis and numerous intra-astrocytic polyglucosan bodies (PBs) as the main findings. The severity of these findings ranged from more to less in the occipital white matter of the parietal, temporal and frontal lobes. The internal capsule, brainstem, spinal cord, and cerebellar white matter were also affected. In contrast, the hemispheric cortical grey matter, basal ganglia, and the brainstem remained relatively intact, except for the lateral vestibular nuclei and the inferior olives. There was moderate gliosis and neuronal loss in these nuclei and the dentate nuclei of the cerebellum.

PBs were primarily concentrated in perivascular regions and along axons and fibres. The astrocytes in the white matter exhibited marked immunoreactivity for tau protein, both in the form of diffuse cytoplasmic staining and the characteristic thorn-shaped astrocytes (TSA), especially in perivascular and subpial regions, as described in "Aging-Related Tau Astrogliopathy" (ARTAG). However, it is noteworthy for both the abundance and the extension throughout the entire white matter in the same previously mentioned regions. This tauopathy was predominantly 4-repeat and showed weak positivity for Gallyas staining. In these regions, a co-deposition of TDP43 protein was also observed in the same astroglial cells, albeit in much smaller quantities, associated with abundant dystrophic neurites and occasional intraneuronal inclusions. This pathology was atypical and distinct from the TDP43

limbic proteinopathy associated with ageing (LATE), also present in this patient (LACTE-NC stage 2) associated with hippocampal sclerosis. (Figure 5)

Epidemiology

The calculated prevalence of LOVCA for the subjects in our area of maximal coverage is of $5.03/10^5$ inhabitants, $4.0/10^5$ for those affected by LOVCA due to SCA27B.

Treatment

Acetazolamide was used in 15 cases as the initial treatment for episodic ataxias. Unfortunately these patients did not report reduction in the number of episodes or in their balance sensation and walking capacities. Only one single case of unknown genetic mutation in which SCA27B was excluded reported an important reduction in episodes. 4-aminopyridine was prescribed to 18 patients and of the 16 in whom the response to the drug was known, 11 improved while five did not have any noticeable benefit. The improvement consisted in a noticeable reduction in the number and severity of the episodes without there being any clear influence in the progression of the ataxia and imbalance. Of the 11 SCA27B patients treated, seven improved (63.6%) and two did not respond (two are unknown). In the non-SCA27B group, five were treated, but only two improved. Where improvement was observed this consisted of a considerable reduction in the number and severity of episodes and in some cases with the cessation of falls. However, in this subgroup the progression of the disease did not appear to be modified. Of the four patients with episodes but without progression, two who had SCA27B were treated with 4-AP and remained without episodes or these became slight. Although other drugs were used in isolated cases (clonazepam, gabapentin, baclofen), none appeared to reduce the frequency or severity of the episodes or slow down the progression.

DISCUSSION

Hereditary vestibulo-cerebellar ataxia was first described in the 1960's and has been reported on several occasions.[2,17-23] Only a short early article reports sporadic cases, which they describe as late-onset paroxysmal ataxia,[16] although similar sporadic vestibulo-cerebellar patients are reported in a later study.[13] The main clinical difference with these authors is that in our series the picture appears as a vestibulo-cerebellar syndrome, indicated by ataxia almost always accompanied by downbeat nystagmus, which is the hallmark of vestibulo-cerebellar involvement.[34] In our series, the VCA syndrome presents in two forms: as a late-onset episodic ataxia followed by progression or not, and as a slowly progressive disease. Both of these forms were seen in two pairs of brothers, all of whom carried an *FGF14* GAA expansion. The disease begins with a mean age of onset at around seventy years of age. Only a few patients had a familial form. Statistical analysis reveals few differences between familial and sporadic cases except that in sporadic patients the age of onset and severe disability both occur a decade earlier. There are no other significant differences in the clinical course or in other parameters studied (Table 5).

The frequency, duration, and severity of the episodes are variable for every patient and even from one episode to another. Some patients have a few severe episodes whereas others have them weekly. The duration can be between just a few minutes to several weeks. In many cases, after one or more episodes the patient no longer returns to a state of having normal balance. The persistence of episodes during progression is variable: in some cases, they disappear completely whereas in others they continue throughout the evolution. The persistence of episodes after progression is a constant feature in disabled patients.

The clinical picture reaches its maximum level of severity during the episodes when the patient is frequently unable to achieve or maintain a standing position and has a downbeat

nystagmus of considerable amplitude. Visual symptoms such as oscillopsia are common. Vertigo, blurred vision, and diplopia can also form part of the symptoms. Autonomic manifestations, nausea, vomiting, and flushing are not uncommon. Dysarthria is not frequent and when present is mild (Table 3).

This phase usually wanes after hours or days and is followed by a reduction in the manifestations, although the residual symptoms increase as time goes by. In the early stages of the disease, the ataxia is often seen in the form of mild instability, with normal or unclear results when exploring the patient. However, after progression examination more clearly reveals the difficulties in turning, the impossibility of tandem walking and the difficulties in standing. Falls are frequent and many patients develop agoraphobia and dizziness as a result, increasing the sensation of instability. In this interictal phase the downbeat nystagmus can be severely reduced to the extent that close attention may be required for it to be detected. Horizontal nystagmus is sometimes present in both the acute and chronic phases. Constant dysarthria is present in one third of patients but is never severe. Slowly, over several years, standing becomes impossible in most patients and only in cases of premature death have we not found this to be the case.

Impossibility to stand seems to be the greatest degree of disability induced by the degeneration of the vestibulo-cerebellar system.

Some patients developed a spreading of the symptoms to other cerebellar areas, but a severe, generalized, cerebellar syndrome has not been observed in our series except in one patient. Another typical cerebellar syndrome, cognitive-affective cerebellar syndrome, has not been detected in any of our subjects. The four patients that have not progressed have only rare episodes and no residual symptoms.

An unexpected detail to emerge from the analysis of the data was that there seems to be a tendency for many women with VCA to have a worse prognosis than men (an observation that remained true in both the SCA27B and non-SCA27B patients), suggesting that the vestibulo-cerebellar system of women is more prone to damage than that of men. It also seems to indicate a greater sensitivity of the vestibulo-cerebellar system of women to the different mutations damaging this circuitry**.**

MRI also afforded some surprising findings, such as the absence of cerebellar atrophy being found to be quite common even in cases with advanced disease. Another unexpected finding was of the three different types of mild atrophy: vermian, vermian-paravermian, and global. Vermian atrophy appears more common in SCA27B males with a less disabling situation whereas global atrophy includes more disabled patients. Unfortunately, the main areas that are supposedly involved (flocculus-nodulus) could not be readily evaluated by MRI, and the mild atrophy reflected minor involvement of other cerebellar areas to a slight degree in concordance with the lack of clinical involvement of other cerebellar areas.

A GAA expansion in the *FGF14* gene was found in most of our patients. SCA27B was detected in 58% of sporadic subjects analyzed and in 78% of the familial cases that were studied. This newly reported expansion has allowed us to observe that despite subjects being classified as sporadic, they do in fact have a heterozygous variant. As reported recently,[24] 46% of patients carrying the *FGF14* GAA expansion presented as episodic ataxias. However, only a few patients in our cohort developed slight dysmetria or dysarthria in addition to the central picture of vestibulo-cerebellar syndrome. No correlation has been found between the repeat length and the age of onset, duration, and severity of disease.

In our cohort, only four patients with familial or sporadic episodic presentations did not progress, although we cannot be sure in the case of two of these who were lost to follow-up

after several years. As patients evolve and become more elderly, the picture can be complicated by other neurodegenerative diseases although no common pattern is observed. Familial forms of VCA have been detected and reported as rare syndromes[2,17-23] but only a single study reports sporadic cases as a separate syndrome.[16] The reasons for the previous low detection rate of this disease in general medical practice are diverse: for example, a finding of vertigo can lead to confusion, and permanent instability can be misattributed to the consequences of ageing or agoraphobia.

APBD is an ultrarare disease involving the central and peripheral nervous system and producing extensive demyelination in the CNS, leading to weakness, cognitive decline, and bladder dysfunction.[39] Cerebellar symptoms are rarely described in reports of APBD, although this may be partially explained by the pre-eminence of the pyramidal syndrome over the cerebellar syndrome. In some cases, patients with episodic ataxia have been reported.[40-42.] In our patient, the cardinal symptom was the episodic alteration of gait, thereby broadening the phenotypic spectrum of APBD.

With regard to the neuropathology, a severe diffuse degenerative leukoencephalopathy was observed with marked infra- and supratentorial involvement, especially in the occipital region. An extensive white matter ARTAG (astrocytic tau astrogliopathy) with prominent tau astrogliopathy was highlighted, featuring co-deposition of TDP43 protein and cerebellar degeneration.

The presence of abundant polyglucosan bodies in this patient mirrors the two cases described by Uemura et al. in which a family is reported with several individuals exhibiting a clinical phenotype of frontotemporal dementia (FTD) and underlying copathologies of adult polyglucosan body disease, FTLD-TDP, and ARTAG with a segregation of GBE1 loss-of-function. Therefore, we conducted a genetic study, detecting a variant of unknown

significance (VUS). We did not perform an expression study because it goes beyond the scope of this work. However, the presence and amount of these polyglucosan bodies is highly suggestive of a pathogenic mutation.

The prevalence of VCA 5.5/10^5 is similar to that of other dominant ataxias: 8.5/10^5 for all the dominant ataxias and 5.5 /10^5, which is exclusively due to the presence of two large families affected by SCA1,[44] and 1.75/10^5 for Friedreich's disease (unpublished data). The characteristics of vestibulo-cerebellar ataxia can lead to different diagnoses, and it is likely that many of the subjects with this picture are not referred for specialized neurological attention as they are diagnosed as having conditions such as vertigo, strokes, and the consequences of ageing. The SCA27B expansion is the predominant cause of sporadic and hereditary late-onset VCA but in our cohort one third of cases still do not have a definitive diagnosis and it seems likely that other variants will eventually be found to be responsible.

We also note that our cases of VCA do not have generalized hemispheric cerebellar or cognitive-affective cerebellar associated syndromes, although a role for cognition and behaviour had been attributed to *FGF14*.[45] Apart from mild dysarthria and rare cases with dysmetria, there was no clinical evidence of involvement beyond the vestibulocerebellar areas whether in the episodes or during the evolution of the disease. Therefore, the picture can be considered as remaining relatively pure over the years.

Treatment is still not standardized. Acetazolamide has been used for the treatment of episodic ataxias for many years and proved relatively successful in reducing the frequency of episodes in patients with episodic ataxia.[46,47] In a recent series of patients with SCA27B it has been reported as improving 44% of the treated patients, although this response was described as being mild or partial.[48] In our series, which only includes VCA patients, this

treatment has been found to be disappointing as only one patient responded to it satisfactorily.

4-AP has been reported as reducing the number of episodes in episodic ataxias and improving DBN.[49-50] In our cohort, 64% of those with SCA27B improved and many ceased to have episodes, but the drug does not appear to have an effect on progression. Treatment, therefore, can be considered as having been particularly useful in those patients without progression, given that they have remained asymptomatic or with minor symptoms as a result. We have not checked the effect of the drug on DBN. A similar situation has been reported in two recent series on the effect of 4-AP in patients with SCA27B and in patients with DBN in patients with intermediate FGF144-GAA expansions (less than 250 repeats). In all these series there has been a good response to 4-AP treatment.[51,52] However, we did not evaluate the parameters that would have been required to be able to measure whether or not the same effects were present in our patients.

As might be expected for a disease that mainly involves the vestibulo-cerebellar system connecting the vestibular nuclei to the flocculus and nodulus, cerebellar atrophy is not a common finding, and a significant proportion of cases have normal MRIs, even when disease have a long evolution or the patients are disabled. When atrophy was present, the vermian area was always affected, often together with the paravermian area and, less frequently, with the whole cerebellum. A surprising finding in our series is that of the high proportion of non-disabled patients in cases with only vermian atrophy, all of them bear the SCA27B mutation.

In these patients, atrophy was moderate or mild even in vermian areas. In any case, the presence or absence of atrophy was not found to be especially related to the severity of the evolution and it is surprising that, unlike in other ataxias, only slight and unchanged atrophy

is observed many years after the onset of the disease. However, the absence of abnormalities in many cases reduces the efficacy of this technique as a biomarker for the diagnosis of vestibulo-cerebellar ataxia and may reduce the rate of diagnosis of the disease.

Although previous authors[25] have reported common vestibular involvement in their patients, we did not observe this finding in our cohort, although only a few of our patients underwent vHIT. Further studies will be needed to evaluate the association of peripheral vestibular involvement in this syndrome and to determine whether or not there is an association with the different VCA-related mutations.

This syndrome has not been found to reduce patients' life expectancy as their mean age of death has been 81 years, which is only slightly lower than the 83.58 years recorded for Catalonia in 2019 for the general population. (ref.Idescat : https://www.idescat.cat/indicadors/?id=anuals&n=10380&t=201900&tema=SALUT).

Reported neuropathological studies have shown a severe loss of Purkinje cells in vermian areas, with few or no changes in dentate nuclei and the bulbar olive.[24] No abnormalities have been reported in the vestibular nuclei. No cytoplasmatic or intranuclear inclusions were found in the previously reported cases. The disappearance of Purkinje cells in vermian areas suggests that these cells are the target of the abnormalities.[24]

The high proportion of sporadic cases with the *FGF14* GAA expansion has been attributed to the high rate of expansion of this repeat in the general population and is associated with instability of intermediate alleles.[24]

Many questions still remain unresolved with regard to this newly described expansion as we do not yet understand why there is such a low rate of familial cases in a disease that is related to a heterozygous mutation. It is likely that the expansion causes loss of *FGF14*

function.[24] A signal-silencing epigenetic mechanism could contribute to this lack of function as happens with the intronic expansion in Friedreich's disease.[34]

The first molecular genetic studies of *FGF14* variants, now called SCA27A, detected missense, frameshift, and nonsense mutations. Some patients developed episodic ataxia.[36-38]

The axon initial segment is critical for the initiation of action potentials.[53] FGF14 is located mainly in proximal part of this area. It regulates the gating of sodium, potassium, and calcium channels and intervenes in the firing of Purkinje cells.[54-57]

SUMMARY

We present here a study of 50 subjects with late-onset VCA. Whereas most of the patients have late-onset episodic ataxia that presents as a pure VCA syndrome, others have a progressive picture without episodes. VCAs normally present as sporadic rather than hereditary forms. The clinical syndrome associates ataxia with downbeat nystagmus, altered vestibulo-cerebellar performance and, in some cases, dysarthria. About half of the subjects become unable to stand with the remaining subjects being disabled either to a lesser degree or not disabled at all. Women seem more prone to having a severe picture. MRI shows minor or slight abnormalities with vermian or paravermian atrophy, although normal MRI is also common. All of the few hereditary cases bore the *FGF14* GAA expansion as did two thirds of sporadic cases. In the remaining one third of sporadic cases, it has not been possible to detect a specific mutation so far, allowing us to conclude that this is an aetiologically heterogeneous syndrome. Treatment with 4-AP reduces the severity and frequency of episodes but does not influence the progression of disability.

REFERENCES

1. Voogd J, Gerrits NM, Ruigrok TJH. Organization of vestibulocerebellum. Ann NY Acad Sci 1996; 781:553-579.

2. Farmer TW, Mustian VM. Vestibulocerebellar ataxia. A newly defined hereditary syndrome with periodic manifestations. Arch Neurol 1963; 8:471-80.

3. Jen JC, Wan J. Handbook of Clinical Neurology. Episodic ataxias. 2018; 155: 205-215.

4. Browne DL, Gancher ST, Nutt JG, et al. Episodic ataxia/myokymia syndrome is associated with point mutations in the human potassium channel gene, KCNA1. Nat. Genet. 1994;8: 136-140.

5. Ophoff RA, Terwindt GM, Vergouwe MN, et al. Familial hemiplegic migraine and episodic ataxia type 2, are caused by mutations in the Ca^{2+} channel gene CACNL1A4. Cell 1996;87: 543-552.

6. Escayg A, De Waard M, Lee D, et al. Coding and noncoding variation of the human calcium-channel b 4-subunit gene CACNB4 in patients with idiopathic generalized epilepsy and episodic ataxia. Am. J. Hum. Genet. 2000;66: 1531-1539.

7. Jen J, Wan J, Palos TP et al. Mutation in the glutamate transporter EAAT1 causes episodic ataxia, hemiplegia, and seizures. Neurology 2005;65: 529-534.

8. Liao Y, Anttonen A, Liukkonen E, et al. SCN2A mutation associated with neonatal epilepsy, late-onset episodic ataxia, myoclonus, and pain. Neurology 2010;75: 1454–1458.

9. Conroy J, McGettigan P, Murphy R, et al. A novel locus for episodic ataxia: *UBR4* the likely candidate. European Journal of Human Genetics 2014; 22: 505–510.

10. Choi KD, Choi K,Jen J et al. Late-onset episodic ataxia associated with *SLC1A3* mutation. Journal of Human Genetics 2017; 62(3): 443-446.

11. Choquet K, La Piana R, Brais B. A novel frameshift mutation in FGF14 causes an autosomal dominant episodic ataxia. Neurogenetics 2015;16(3): 233-236.

12. Piarroux J, Riant F, Humbertclaude V, et al. FGF14-related episodic ataxia: delineating the phenotype of Episodic Ataxia type 9. Annals of Clinical and Translational Neurology 2020; 7 (4): 565–572.

13. Kerber K, Jen J, Lee H, et al. A new episodic ataxia syndrome with linkage to chromosome 19q13. Arch. Neurol. 2007; 64: 749-752.

14. Imbrici P, Eunson L, Graves T, et al. Late-onset episodic ataxia type 2 due to an-in-frame insertion in CACNA1A. Neurology 2005; 65: 944–946.

15. Cuenca-León E, Banchs I, Serra S, et al. Late-onset episodic ataxia type 2 associated with a novel loss-of-function mutation in the CACNA1A gene. Journal of the Neurological Sciences 2009; 280: 10–14.

16. Julien J, Denier C, Ferrer X, et al. Sporadic late onset paroxysmal cerebellar ataxia in four unrelated patients: a new disease? J Neurol 2001; 248: 209–221.

17. Kerber KA, Jen JC, Perlman S, Baloh RW**.** Late-onset pure cerebellar ataxia: differentiating those with and without identifiable mutations**.** J Neurol Sci 2005 Nov 15;238(1-2):41-5.

18. Theunissen EJ, Huygen PL, Verhagen WI. Familial vestibulocerebellar dysfunction: a new syndrome? J Neurol Sciences. 1989(89):149-155.

19. Small KW, Pollock SC, Vance JM et al. Ocular motility in North Carolina autosomal dominant ataxia. 1996 J Neuroophthalmol. **16** (2): 91–95.

20. Damji K, Rand Allingham R, Pollock S, et al. Periodic vestibulocerebellar ataxia, an autosomal dominant ataxia with defective smooth pursuit, is genetically distinct from other autosomal dominant ataxias. Arch Neurol 1996; 53: 338-344.

21. Steckley JL, Ebers GC, Cader MZ et al. An autosomal dominant disorder with episodic ataxia, vertigo, and tinnitus. Neurology. 2001;57(8):1499-1502.

22. Ragge NK, Hartley C, Dearlove AM et al. Familial vestibulocerebellar disorder maps to chromosome 13q31-q33: a new nystagmus locus. J Med Genet 2003; 40:37–41.

23. Gazulla J, Izquierdo-Alvarez S, Ruiz-Fernández E, et al. Episodic Vestibulocerebellar Ataxia Associated with a *CACNA1G* Missense Variant. Case Rep Neurol. 2021;13(2):347-354.

24. Pellerin D, Danzi MC, Wilke C, et al. Deep Intronic FGF14 GAA Repeat Expansion in Late-Onset Cerebellar Ataxia. N Engl J Med. 2023; 388(2): 128-141.

25. Rafehi H, Read J, Szmulewicz DJ et al. An intronic GAA repeat expansion in FGF14 causes the autosomal-dominant adult-onset ataxia SCA50/ATX-FGF14. Am J Hum Genet 2023; 110: 105-119.

26. Subramony SH, May W, Lynch D, et al. Measuring Friedreich ataxia: Interrater reliability of a neurologic rating scale. Neurology 2005; 64:1261–1262.

27. Pujana MA, Corral J, Gratacos M, et al. Spinocerebellar ataxias in Spanish patients: genetic analysis of familial and sporadic cases: The Ataxia Study Group. Hum Genet 1999; 104: 516–522.

28. 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

29. Bonnet C, Pellerin D, Roth V. et al. Optimized testing strategy for the diagnosis of GAA-FGF14 ataxia/spinocerebellar ataxia 27B. Scientific reports 2023; 13:9737.

30. Lossos A, Meiner Z, Barash V, et al. Adult polyglucosan body disease in Ashkenazi Jewish patients carrying the Tyr329Ser mutation in the glycogen-branching enzyme gene. Ann Neurol; 1998;44:867–872.

31. Alvarez-Mora MI; Rodríguez -Revenga L, Jodar M et al. Implementation of Exome Sequencing in Clinical Practice for Neurological Disorders. Genes (Basel) 2023 Mar 28;14(4):813.

32. National Center for Biotechnology Information. ClinVar; [VCV000195573.21], https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000195573.21

33. National Center for Biotechnology Information. ClinVar; [VCV000195470.13]. https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000195470.13.

34. Leigh RJ, Zee DS. The neurology of eye movements. Contemporary Neurology Series. Oxford University Press. Third Edition. 1999; 415-416.

35. Groh M, Lufino MM, Wade-Martins R, et al. R-loops associated with triplet repeat expansions promote gene silencing in Friedreich ataxia and fragile X syndrome. PLoS Genet 2014;10 (5): e1004318.

36. Van Swieten JC, Brusse E, de Graaf BM, et al. A mutation in the fibroblast growth factor 14 gene is associated with autosomal dominant cerebellar ataxia[corrected]. Am J Hum Genet 2003:72; 191-199.

37. Dalski A, Atici J, Kreuz FR, et al. Mutation analysis in the fibroblast growth factor 14 gene: frameshift mutation and polymorphisms in patients with inherited ataxias. Europ J Hum Genet 13: 118-120, 2005.

38. Miura S, Kosaka K, Fujioka R, et al. Spinocerebellar ataxia 27 with a novel nonsense variant (Lys177X) in FGF14. Europ J Med Genet 2019(62): 172-176.

39. Robitaille I, Carpenter S, Karpati G, et. Al. A distinct form of adult polyglucosan body disease with massive involvement of central and peripheral neuronal processes and astrocyte Brain 1980;103: 315-336.

40. Gray F, Gherardi R, Marshall A, et al. Adult polyglucosan body disease (APBD). Journal of Neuropathology and Experimental Neurology 1988; 44:459-474.

41. Paradas C, Akman HO, Ionete C, et al. Branching Enzyme Deficiency: Expanding the Clinical Spectrum. JAMA Neurol. 2014; 71(1): 41–47.

42. Sagnelli A, Savoiardo M, Marchesi M et al. Adult polyglucosan body disease in a patient originally diagnosed with Fabry's disease. Neuromuscular Disorders 2014; 24: 272–276.

43. Uemura MT , Suh ER, Robinson JL et al. Abundant copathologies of polyglucosan bodies, frontotemporal lobar degeneration with TDP-43 inclusions and ageing-related tau astrogliopathy in a family with a GBE1 mutation. Neuropathol Appl Neurobiol: 2023 Feb;49(1):e12865.

44. Genís D, Matilla T, Volpini V, et al. Clinical neuropathologic, and genetic studies of a large spinocerebellar ataxia type 1 (SCA1)kindred: (CAG), expansion and early premonitory signs and symptoms: Neurology 1995; 45:24-30.

45. Hoxha E, Marcinnò A, Montarolo F, et al. Emerging roles of Fgf14 in behavioral control. Behav Brain Res. 2019 Jan 1; 356: 257–265.

46. Zasorin NL, Baloh RW, Myers LB. Acetazolamide-responsive episodic ataxia síndrome. Neurology 1983; 33(9):1212-1214.

47. Gandini J, Manto M, Bremova-Ertl T, et al. The neurological update: therapies for cerebellar ataxias in 2020. J Neurol. 2020 Apr;267(4):1211-1220.

48. Ashton C, Indelicato E, Pellerin D, Spinocerebellar ataxia 27B: episodic symptoms and acetazolamide response in 34 patients Brain Commun. 2023 Sep 10;5(5):fcad239.

49. Strupp M, Kalla R, Dichgans M, et al. Treatment of episodic ataxia type 2 with the potassium channel blocker 4-aminopyridine. Neurology 2004; 62:1623–1625.

50. Strupp M, Kalla R, Glasauer S, et al. Aminopyridines for the treatment of cerebellar and ocular motor disorders. Prog Brain Res. 2008; 171: 535–54.

51. Wilke C, Pellerin D, Mengel D et al. GAA-*FGF14* ataxia (SCA27B): phenotypic profile, natural history progression and 4-aminopyridine treatment response. Brain. 2023 Oct 3;146(10):4144-4157.

52. Pellerin D, Heindl F, Wilke C, et al. Intronic *FGF14* GAA repeat expansions are a common cause of downbeat nystagmus syndromes: frequency, phenotypic profile, and 4-aminopyridine treatment response.

53. Xiao M, Bosch MK, Nerbonne JM. FGF14 localization and organization of the axon initial segment. Mol Cell Neurosci 2013; 56: 393-403.

54. Di Re J, Wadsworth PA, Laezza F.Intracellular fibroblast growth factor 14: emergin risk factor for brain disorders. Front Cell Neurosci 2017; 11: 103.

55. Pablo JL, Pitt GS. FGF14 is a regulator of KCNQ2/3 channels. Proc Natl Acad Sci USA 2017; 114: 154-159.

56. Yan H, Pablo JL, Pitt GS. FGF14 regulates presynaptic Ca2+ channels and synaptic transmission. Cell Rep 2013; 4: 66-75.

57. Bosch MK, Carrasquillo Y, Ransdell JL, et al. FGF14(iFGF14) is required for spontaneous and evoked firing in cerebellar Purkinje Neurons and for motor coordination and balance. J Neurosci 2015; 35:6752-6769.

Table 1. General findings.

Hereditary:	Median/IQR (Years)	N/%
Hereditary		10 (20%)
Sporadic		40 (80%)
Sex:		
Female		21 (42%)
Male		29 (58%)
Age of onset	69.50 [60.25;77]	50 (100%)
Age of first examination	72 [66;80]	50 (100%)
Age of last examination	81 [73;85]	50 (100%)
Progression:		
No		4 (8%)
Yes		46 (92%)
Episodes:		
No episodes		7 (14%)
Episodes		43 (86%)
Progression at first episode		18 (36%)
Age at progression:	71 [64.50;78]	
Disability chronology:		
Time to needing a crutch	3 [1;8]	25
Time to needing a walker	5 [2.50;6]	19
Time to needing a wheelchair	7 [5;9]	13
Disease duration:	8 [5;13.75]	50 (100%)
Time to FARS-FS: 4 or 5	5 [3;6]	19
Disability:		
Disabled (FARS-FS: 4 or 5)		19 (51.35%)
Time to FARS-FS: 4 or 5	5 [3;6]	19
Follow-up situation:		
Until death		12 (24%)
Lost		23 (46%)
Active		15 (30%)
Mean time of follow-up	7 [3.50;10]	47
Mortality:		
No		24 (48%)

Yes		26 (52%)
Age at death	81 [72.5;86.5]	

Table 2. Episodes.

Episode duration:	
Minutes to one hour	11 (28.21%)
Less than 24 hours	15 (38.46%)
More than a day, less than a week	9 (23.08%)
One week or more	4 (10.26%)
Episodes before progression:	
Five or less	16 (37.21%)
More than five	20 (46.51%)
No progression	4 (9.30%)
Unknown	3 (6.97%)
Episodes after progression:	
No	3 (6.97)
Yes	33 (76.74%)
No progression	4 (9.30%)
Unknown	3 (6.97%)
Daily worsening:	
No	22 (66.66%)
Yes	11 (33.33%)

Table 3. Clinical findings.

Clinical findings during episodes	**N (%)**	**N**
		43
Ataxia:	43 (100%)	43
Standing Impossible	9 (21.43%)	42
Severe but can stand with minimal support	11 (26.19%)	42
Can walk with aid	15 (35.71%)	42
Can walk unaided	7 (16.66%)	42
Down-beat nystagmus:	34 (82.93%)	41
Dizziness:	33 (78.57%)	42
Falls:	27 (62.79%)	43
Oscillopsia:	15 (36.59%)	41
Horizontal nystagmus:	14 (35.00%)	40
Blurred vision:	14 (33.33%)	42
Nausea/vomiting:	14 (33.33%)	42
Dysarthria:	13 (30.23%)	43
Diplopia:	9 (21.43%)	42
Dysmetria	3 (06.97%)	43
Interictal clinical findings		50
Altered static balance:	40 (88.88%)	45
Altered tandem walking:	44 (88.00%)	50
Ataxia:	45 (90.00%)	50
Altered turning:	37 (78.72%)	47
Down-beat nystagmus:	39 (78.00%)	50
Falls:	33 (66.00%)	50
Positive Romberg's sign:	28 (56.00%)	50
Dysarthria:	14 (28.57%)	49
Dysmetria:	5 (10.00%)	50

Table 4. Disability.

		Disabled	Less disabled		
		N=19 (%)	**N=18 (%)**	**p-value**	**N**
Heredity:				0.124	37
	Familial	2 (10.53%)	6 (33.33%)		
	Sporadic	17 (89.47%)	12 (66.67%)		
Sex:				0.061	37
	Female	11 (57.89%)	4 (22.22%)		
	Male	8 (42.11%)	14 (77.78%)		
Age of onset:		72.00 [63.00;79.50]	65.50 [59.25;76.25]	0.171	37
Age at first examination:		75.00 [69.00;81.00]	72.00 [66.00;79.75]	0.429	37
Age at last examination:		82.00 [76.50;88.00]	77.00 [73.00;82.75]	0.073	37
Progression:				0.046	37
	No	0 (0.00%)	4 (22.22%)		
	Yes	19 (100.00%)	14 (77.78%)		
Age at progression:		76.00 [67.00;80.00]	66.00 [62.25;72.75]	0.071	33
Follow-up situation:					37
	Complete (until death)	9 (47.37%)	2 (11.11%)		
	Lost to follow-up	7 (36.84%)	5 (27.78%)		
	Active follow-up	3 (15.79%)	11 (61.11%)		
Years of follow-up:		7.00 [5.50;11.00]	5.50 [3.00;9.75]	0.194	37
Episodes before progression:				0.673	26
	More than five	8 (44.44%)	5 (62.50%)		
	Less than five	10 (55.56%)	3 (37.5%)		
Episodes after progression:				1.000	25
	No	1 (5.88%)	1 (1.50%)		
	Yes	16 (94.12%)	7 (87.5%)		
Episode duration:				0.602	29
	Hour	5 (27.78%)	3 (27.27%)		

	Hours/day	5 (27.78%)	5 (45.45%)	
	Days	7 (38.89%)	2 (18.18%)	
	Week(s)	1 (5.56%)	1 (9.09%)	
Daily worsening:			1.000	25
	No	9 (69.23%)	8 (66.67%)	
	Yes	4 (30.77%)	4 (33.33%)	
***FGF14*GAA expansion:**			0.226	29
	Absent	6 (46.15%)	3 (18.75%)	
	Present	7 (53.85%)	13 (81.25%)	

Table 5. Differences between familial and sporadic groups.

	Familial (N=10)	**Sporadic** (N=40)	**p-value**
Sex:			1.000
Female	4 (40.00%)	17 (42.50%)	
Male	6 (60.00%)	23 (57.50%)	
Age of onset:	60.00 [57.25;64.50]	71.50 [65.00;78.25]	0.003
Age at first examination:	67.00 [60.00;75.00]	74.00 [68.75;80.25]	0.063
Progression:			0.174
No	2 (20.00%)	2 (5.00%)	
Yes	8 (80.00%)	38 (95.00%)	
Age at progression:	60.00 [58.50;64.00]	72.00 [66.00;78.00]	0.004
Age at disability:	65.00 [64.50;65.50]	81.00 [76.00;85.00]	0.033
Disease duration:	12.50 [7.75;18.75]	7.00 [5.00;11.25]	0.114
Time to reach disability:	5.50 [5.25;5.75]	5.00 [3.00;6.00]	0.639
Disability:			0.235
Disabled	2 (20.00%)	17 (42.50%)	
Less disabled	6 (60.00%)	12 (30.00%)	
Unknown	2 (20.00%)	11 (27.50%)	
Episodes after progression:			1.000 n=36
No	0 (0.00%)	3 (9.38%)	
Yes	4 (100%)	29 (90.62%)	
Episodes before progression:			0.574
More than Five	2 (66.66%)	14 (42.42%)	
Less than five	1 (33.33%)	19 (57.58%)	
SCA27B mutation:			0.246
No DNA available	1 (10.00%)	13 (32.50%)	
Performed	9 (90.00%)	27 (67.50%)	
SCA27B mutation type:			0.432
Positive	7 (77.78%)	15 (55.56%%)	

Negative	2 (22.22%)	12 (44.44%)

Table 6. Sex differences.

	Female N=21(%)	Male N=29(%)	p-value	N
Episodes before progression:			0.793	36
More than five	8 (50%)	8 (40%)		
Less than five	8 (50%)	12 (60%)		
Episodes after progression:				36
No	2 (12.50%)	1 (5%)	p=0.574	
Yes	14 (87.5%)	19 (95%)		
Disability:			0.061	37
Disabled	11 (73.33%)	8 (36.36%)		
Less disabled	4 (26.67%)	14 (63.64%)		
Daily worsening:			0.009	33
No	5 (38.46)	17 (85.00%)		
Yes	8 (61.54)	3 (15.00%)		

Table 7. SCA27B vs non-SCA27B.

		SCA27B	**Non-SCA27B**		
		(N=22)	(N=14)		
Familial:				0.432	36
	H	7 (31.82%)	2 (14.29%)		
	S	15 (68.18%)	12 (85.71%)		
Sex:				0.969	36
	Men	14 (63.3.%)	8 (57.14%)		
	Women	8 (36.36%)	6 (42.85%)		
Progression:				1.000	36
	Yes	20 (90.9%)	13 (92.85%)		
	No	2 (9.09%)	1 (7.14%)		
Age of onset:		71.00[59.25;78.00]	66.00[60.00;70.75]	0.426	36
Disability:				0.275	36
	FARS-FS: 4-5	6 (27.27%)	7 (50.0%)		
	FARS-FS: 0-3	12 (54,54%)	4 (28.57%)		
	Unknown	4 (18.18%)	3 (21.42%)		
Disability Sex:					
Women FARS-FS:4-5		3 (13.64%)	5 (35.71%)		
Women FARS-FS:0-3		4 (18.18%)	0 (0.00%)		
Men FARS-FS: 4-5		3 (13.64%)	2 (14.29%)		
Men FARS-FS: 0-3		8 (36.36%)	4 (28.57%)		
	Unknown	4 (18.18%)	3 (21.43%)		
Follow-up:				0.689	36
	Until death	7 (31.82%)	3 (21.43%)		
	Lost	6 (27.27%)	6 (42.86%)		
	Active	9 (40.91%)	5 (35.71%)		

Follow-up time:	7.00 [6.00;13.00]	7.50 [4.00;11.50]	0.410	35
MRI:			P=0.058	36
No cerebellar atrophy	8 (36.36%)	5 (35.71%)		
Vermian	6 (27.27%)	0 (0.00%)		
Vermian and paravermian	7 (31.82%)	5 (35.71%)		
Global	1 (4.55%)	4 (28.57%)		

Table 8. MRI features.

	MRI features (N=48/%)				
	No cerebellar atrophy 20 (41.6%)		**Cerebellar atrophy** 28 (58.3%)		
Disabled					p=0.409
FARS-FS 4/5	8 (66.67%)		11 (39.29%)		N=36
FARS-FS: 0-3	4 (33.33%)		13 (54.17%)		
Disease duration	**Years**		**Years**		p=0.502
	7.00 [5.00; 18.25]		10.00 [6.75; 13.25]		
MRI types:	**No atrophy**	**Vermian**	**Vermian and paravermian**	**Global**	
	20 (41.67%)	9 (18.75%)	11 (22.92%)	8 (16.67%)	N=48
Disability					p=0.153
FARS-FS: 0-3	4 (33.33%)	6 (85.71%)	4(44.44%)	3 (37.50%)	N=36
FARS-FS: 4-5	8 (66.67%)	1 (14.29%)	5 (55.56%)	5 (62.50%)	
Disease duration (y)	7.00 [5.00;18.25]	8.00 [4.00;12.00]	9.00 [7.00;12.00]	10.0 [9.00;15.75]	p=0.872