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CANVAS: A New Genetic Entity in the Otorhinolaryngologist's Differential Diagnosis

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Abstract

Objective. The biallelic inheritance of an expanded intronic pentamer $(AAGGG)_{exp}$ in the gene encoding replication factor C subunit 1 (*RFC1*) has been found to be a cause of cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS). This study describes clinical and genetic features of our patients with clinical suspicion of the syndrome.

Study Design. A retrospective descriptive study from an ataxia database comprising 500 patients.

Setting. The study was performed at the Otorhinolaryngology Department of a hospital in the north of Spain.

Methods. Specific genetic testing for CANVAS was performed in 13 patients with clinical suspicion of complete or incomplete syndrome. The clinical diagnosis was supported by quantitative vestibular hypofunction, cerebellar atrophy, and abnormal sensory nerve conduction testing.

Results. Nine of 13 (69%) patients met clinical diagnostic criteria for definite CANVAS disease. The first manifestation of the syndrome was lower limb dysesthesia in 8 of 13 patients and gait imbalance in 5 of 13. Eleven of 13 (85%) patients were carriers of the biallelic $(AAGGG)_{exp}$ in *RFC1*.

Conclusion. A genetic cause of CANVAS has recently been discovered. We propose genetic screening for biallelic expansions of the AAGGG pentamer of *RFC1* in all patients with clinical suspicion of CANVAS, since accurate early diagnosis could improve the quality of life of these patients.

Keywords

CANVAS, bilateral vestibulopathy, DNA repeat expansion

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Cerebellar ataxia, neuropathy, and vestibular areflexia syndrome (CANVAS) is a recently described neurodegenerative multisystem disease characterized by the progressive impairment of 3 of the 4 cardinal props of balance: cerebellar, vestibular, and sensory (only vision remains unaffected).[1] Most CANVAS phenotypes have been described in individuals of European ancestry, and its real prevalence remains unknown.[2-4] The prevalence in Asians might be lower than in Europeans.[5]

Typical symptoms associated with CANVAS diagnosis are gait imbalance, lower limb dysesthesia, oscillopsia, dizziness, and intrinsic falls. An unexplained spasmodic dry cough was reported in over 60% of patients.[6] A high prevalence of autonomic dysfunction has been found in patients with CANVAS.[7-9]

Bilateral vestibular areflexia has been attributed to a sensory neuropathy that affects Scarpa's ganglion of the VIII nerve.[2,10] Bilateral vestibulopathy (BVP) is a very disabling situation. The origin of BVP often remains unknown, and CANVAS is one of the few known etiologies of this condition.[11] Sensory impairment in CANVAS is caused by a marked dorsal root ganglionopathy that leads to reduced proprioception. The cerebellar symptoms are likely associated with a loss of Purkinje cells.[12] Hearing loss and pyramidal signs are not components of CANVAS.[10]

CANVAS diagnosis has been based on clinical findings.[6,13] The onset of the 3 cardinal CANVAS features (cerebellar impairment, BVP, and a somatic sensory deficit) does not follow a consistent sequence, and patients may manifest only 1 or 2 of these 3 features many years before fulfilling the minimal diagnostic clinical requirements.[10] However, recently,

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Cortese et al[14] and Rafehi et al[15] found that CANVAS is caused by the biallelic inheritance of a previously undescribed expanded short tandem repeat (STR) in intron 2 of the gene encoding replication factor C subunit 1 (*RFC1*).[16,17]

STRs are stretches of DNA containing multiple iterations of the same 1- to 7-bp units, typically forming series from half a dozen to several dozens of the unit. Expansions of certain STRs (increases in the number of their units beyond the upper limit found in the healthy population) are associated with more than 20 human diseases, including fragile X syndrome, Huntington's disease, myotonic dystrophy, or certain types of spinocerebellar ataxia.[18] Although many of these STRs are found in exons, and thus their expansions change the structure of the proteins encoded by the affected genes, thereby causing disease, others are outside genes, although normally close enough to disrupt their functions. In the case of the RFC1-associated CANVAS, a pentamer (AAGGG) placed in intron 2 and repeated 11 times in the reference genome was recently found to be expanded to 400 to 2000 repeats in both alleles of affected individuals.[14,15] Patient cells from the Cortese et al[14] study showed expression levels of *RFC1* messenger RNA (mRNA) and protein comparable to controls, and brain tissue from 1 patient with CANVAS had normal levels of RFC1. Retention of intron 2 in *RFC1* pre-mRNA was observed in patient fibroblasts, but these cells did not show increased susceptibility to DNA damage. Thus, no evidence of a loss-of-function effect or of an alternative mechanism for disease has been described to date.[14] So far, no phenotypic alterations have been found in heterozygote carriers of the expansion.

The aim of this study was to test the $(AAGGG)_{exp}$ in the *RFC1* gene in our patients with clinical suspicion of CANVAS, based on their phenotypes, and describe them from a clinical and a genetic point of view.

Patients and Methods

From a database with 500 ataxia patients from our hospital, we reviewed clinical and genetic data from 13 patients who at least partially met the Szmulewicz et al[13] criteria for CANVAS. Medical records were retrospectively evaluated. Written informed consent was obtained from each patient. A regional ethics committee approved this study (Comité de Ética de la Investigación del Principado de Asturias, study 2020.239).

The proposed diagnostic criteria for clinically definite CANVAS are abnormal visually enhanced vestibulo-ocular reflex (VVOR); cerebellar atrophy on magnetic resonance imaging (MRI) mainly involving vermian lobes VI, VII, and VIIa; neurophysiological evidence of a somatosensory neuronopathy; and exclusion of genetic ataxias susceptible of genetic testing.[13] When all features were fulfilled, we classified the patients as complete CANVAS cases. Incomplete CANVAS was diagnosed when 1 of the diagnostic criteria was either not found at all or incompletely present. We also considered cough and autonomic dysfunction as complementary symptoms that increased the suspicion of the disorder.

A progressive disease course and an absence of a compelling alternative differential diagnoses were mandatory.

Vestibular Assessment

A complete vestibular assessment was conducted in all 13 patients, including pure-tone and speech audiometry, video-nystagmography to visualize spontaneous nystagmus, video head impulse test (v-HIT), and computerized dynamic posturography (CDP).

The gain of the horizontal vestibulo-ocular reflex was quantified by the ICS Impulse of Optometrics video-oculography system, together with an accelerometer. According to the diagnostic criteria of the Barany Society, using vHIT, we set a value of 0.6 in the horizontal angular vestibulo-ocular reflex as the threshold for considering vestibular areflexia.

Balance was assessed with the CDP by the Neurocom Smart Equitest Posturographic Platform, NATUS balance and mobility, version 9.0. The main posturography resource analyzed was the sensory organization test (SOT), which quantifies the patient's displacements from the gravity center in 6 different sensory information conditions.

Neurological Study

A complete neurological study was carried out in all 13 patients, including neurological examination, a nerve conduction study, and MRI. Regarding the nerve conduction test, it was examined with 4-limb nerve conduction studies, performing a neurographic study of the sensory and motor dimensions of the peroneal, posterior tibial, sural, and ulnar nerves.

Genetic Study

All patients had been genetically tested to discard possible mutations in loci related to Friedreich's ataxia and the most common spinocerebellar ataxias (SCA): *ATXN1* (SCA1), *ATXN2* (SCA2), *ATXN3* (SCA3), *CACNA1A* (SCA6), *ATXN7* (SCA7), *PPP2R2B* (SCA12), *TBP* (SCA17), *ATN1/DRPLA*, and the CTG triplet expansion in *ATXN8OS* (SCA8).

Genetic testing was performed as previously described by Cortese et al.[14] Briefly, patients were screened by a short-range polymerase chain reaction (PCR) with oligonucleotides flanking the *RFC1* intron 2 region where the expansion occurs. In those patients without a short-range PCR product, the presence of a biallelic expansion, the identity of the expanded pentanucleotide, and the estimated size of the expanded region were confirmed by repeat-primed PCR, long-range PCR, and Sanger sequencing.

Results

Clinical Findings

Thirteen patients were included in the study. The clinical characteristics of the patients are shown in **Table 1**.

Nine of 13 patients (69%) met the clinical diagnostic criteria for definite CANVAS disease (complete syndrome),[13] manifesting ataxia, gait imbalance, and lower limb dysesthesia. They were 6 males and 3 females, and their onset ages ranged from 46 to 74 years (median, 67 years). The remaining 4 patients (4/13, 31%) only showed some of the features, so they were classified as having incomplete syndromes. They were patients 8, 9, 12, and 13. Cases 8 and 9 showed

Table 1. Summary of Clinical Data.

Case No.	Sex	Age, y/onset	Initial symptoms	Autonomic dysfunction	Cough	Cerebellar ataxia	Peripheral neuropathy	Vestibular areflexia	Clinical diagnosis[a]	Genetic diagnosis	Familiar history
1	M	65/61	LLD	No	No	+	+	+	+	–	No
2	M	74/62	LLD	No	Yes	+	+	+	+	+	No
3	M	46/40	Gait imbalance	UI	No	+	+	+	+	–	No
4	F	67/61	LLD	No	Yes	+	+	+	+	+	No
5	M	62/56	Gait imbalance	Dry mouth, cold feet	Yes	+	+	+	+	+	No
6	F	70/65	Gait imbalance	Persistent constipation	Yes	+	+	+	+	+	Yes (sibling of case 7)
7	M	60/59	LLD	Syncope	Yes	+	+	+	+	+	Yes (sibling of case 6)
8	F	70/69	LLD	Hypotension	Yes	–	+	+	–	+	No
9	F	70/69	LLD	UI	No	–	+	+	–	+	No
10	M	70/68	Gait imbalance	Cold feet	Yes	+	+	+	+	+	Yes (sibling of case 11)
11	F	73/70	LLD	No	Yes	+	+	+	+	+	Yes (sibling of case 10)
12	F	71/67	Gait imbalance	Hypotension	Yes	+	+	–	–	+	No
13	F	68/62	LLD	UI	Yes	–	+	–	–	+	No

Abbreviations: LLD, lower limb dysesthesia; UI, urinary incontinence.
[a]Met the diagnostic criteria for definitive cerebellar ataxia, neuropathy, and vestibular areflexia syndrome disease by Szmulewicz et al.[13]

peripheral neuropathy and vestibular areflexia but did not show cerebellar ataxia. Case 12 showed cerebellar ataxia and peripheral neuropathy but did not show vestibular areflexia. This patient was sent to our department because he had instability. Case 13 showed peripheral neuropathy but did not show cerebellar atrophy on the MRI, nor did she show vestibular areflexia. She had instability, autonomic dysfunction (urinary incontinence [UI]), and cough.

Vestibular symptoms, including oscillopsia or other visual disturbances while walking or during head movements, were reported in 5 of 13 (38%). These symptoms worsened in the dark in all 5 patients. Gait imbalance was the main cerebellovestibular symptom and was present in all patients. It was the first symptom in 5 of 13 (38%) patients.

Lower limb dysesthesia was the first manifestation in 8 of 13 (62%) patients, and it was present in all patients as a decrease in sensory conduction velocity with well-shaped potentials of low amplitude.

Chronic dry cough was present in 9 of 13 (69%) cases and was the initial symptom in 3 of 9 (23%) patients (cases 5, 7, and 10), preceding gait imbalance by more than 5 years.

Different degrees of autonomic dysfunction (specified in **Table 1**) were observed in 9 of 13 (69%) patients.

Four affected adults from 2 unrelated consanguineous families have been identified: case 6 and case 7 and cases 10 and 11 were siblings. The age of onset of the symptoms was similar within each family. Patients did not refer any other relatives with similar phenotypes.

Regarding clinical evolution, 2 of 13 (15%) patients required gait aid (cases 1 and 6), and 1 of 13 (8%) needed a wheelchair to move safely (case 3).

Vestibular Findings

Abnormal findings on videonystagmography (VNG) were detected in 8 of 13 (62%) patients, the most common type being vertical downbeat nystagmus, which was observed in 6 of 8 (75%). Spontaneous nystagmus was observed on all gaze positions in the 8 patients.

Eleven of 13 (85%) patients exhibited abnormal bilateral v-HIT with vestibulo-ocular reflex (VOR) gains lower than 0.6, while 2 of 13 (15%) had normal vestibular function (cases 12 and 13). The 5 cases without any kind of nystagmus showed high values of VOR gain (cases 8, 9, 11, 12, and 13).

It was possible to perform CDP on 9 of 13 (69%). Two of 13 cases were unable to stand without support (cases 2 and 3), and 2 of 13 cases had normal vestibular function (cases 12 and 13). The average balance score was reduced in all patients evaluated. Four of 9 cases (45%) presented with an isolated vestibular deficiency pattern, 4 of 9 (45%) with a vestibular and proprioceptive deficiency pattern, and 1 of 9 (10%) with a visual and vestibular deficiency pattern.

Vestibular data are summarized in **Table 2**.

Neurological Findings

The MRI studies of our cohort showed mild to severe atrophy in the anterior and dorsal vermis in all 13 cases (100%). All had a decrease in sensory conduction velocity with well-shaped potentials of low amplitude, compatible with sensitive polyneuropathy.

Genetic Study

Eleven of 13 patients (85%) carried the biallelic AAGGG repeat expansion in *RFC1*. According to long-range PCR

Table 2. Summary of Vestibular Data.

Case No.	VA	VOR RE	VOR LE	VNG	CDP	Audiology
1	Yes	0.44	0.57	Downbeat	Vestibular deficit pattern	Normal for age
2	Yes	0	0	Downbeat	Not possible	Normal for age
3	Yes	0.1	0.15	Multidirectional	Not possible	Normal for age
4	Yes	0.33	0.43	Right horizontal	Vestibular deficit pattern	Normal for age
5	Yes	0	0	Downbeat	Vestibular and proprioceptive deficit pattern	Normal for age
6	Yes	0.02	0.1	Downbeat	Vestibular and visual deficit pattern	Normal for age
7	Yes	0.3	0.35	Downbeat	Vestibular and proprioceptive deficit pattern	Normal for age
8	Yes	0.64	0.55	Normal	Vestibular deficit pattern	Normal for age
9	Yes	0.56	0.49	Normal	Vestibular and proprioceptive deficit pattern	Normal for age
10	Yes	0.24	0.12	Downbeat	Vestibular deficit pattern	Normal for age
11	Yes	0.52	0.47	Normal	Vestibular and proprioceptive deficit pattern	Normal for age
12	No	1.10	0.94	Normal	Not possible	Normal for age
13	No	0.83	0.81	Normal	Not indicated	Normal for age

Abbreviations: CDP, computerized dynamic posturography; LE, left ear; RE, right ear; VA, vestibular areflexia; VNG, videonystagmography; VOR, vestibulo-ocular reflex, determined by video head impulse test.

results, expansion sizes were over 400 repeat units in all cases. Seven of 11 patients showed complete CANVAS, and 4 of 11 (cases 8, 9, 12, and 13) did not show all features of the diagnostic triad (incomplete CANVAS).

Two of 13 (15%) (cases 1 and 3) patients were negative for biallelic expansions. Both of them showed the complete clinical features (complete CANVAS).

Discussion

This article shows the importance of making a precise phenotypic clinical diagnosis to obtain high rates of success in genetic testing and highlights the possibility of ordering the genetic study in patients with incomplete CANVAS syndrome as a way of obtaining an early diagnosis. Recent studies focus on the variability of the clinical spectrum of CANVAS and recommend pursuing a genetic diagnosis as soon as possible in the family member with the clinical findings most suggestive of the disease. In fact, we had 4 of 11 patients with an incomplete syndrome and a genetic confirmation of the disorder, emphasizing the usefulness of requesting a genetic test in those patients with features characteristic of CANVAS. Moreover, in 2 patients fulfilling the criteria for a complete syndrome, a genetic diagnosis could not be confirmed, prompting alternative diagnoses such as Wernicke's encephalopathy (case 1) and Niemann-Pick dementia (case 3).

Pathology findings[1-3,6,9] suggest that the vestibular areflexia of CANVAS is due to vestibular ganglionopathy.[10,19] All of our patients showed progressive imbalance, which was the presenting symptom in 5 of 13 (38%). The median age in our patients at diagnosis was 67 years, which is similar to other studies.[19] However, symptoms can be present as early as in the third decade.

CANVAS is also frequently associated with dry cough and with different degrees of dysphagia.[16] Cough can be reported as early as in the second decade of life, many years before neurologic symptoms develop, and it is considered one of the main features of the condition.[20] In our series, 3 patients with a clinical diagnosis of CANVAS (cases 1, 3, and 9) had no cough. Two of them (cases 1 and 3) did not have a genetic confirmation. Moreover, just 1 patient (case 9) with a genetic diagnosis, belonging to the incomplete syndrome group, did not show cough.

This disease is part of the differential diagnosis of BVP. Known causes of BVP include ototoxicity, vestibular neuritis, Ménière's disease, genetic mutations, and bilateral schwannoma, but the etiology of BVP often remains unclear.[11,21] For the diagnosis of BVP, the horizontal angular VOR gain on both sides should be <0.6 (angular velocity 150-300 degrees/s).[11] We assessed the vestibular function using v-HIT and the stability in sensorial impaired conditions using CDP. In our series, there were only 2 of 13 patients without vestibular areflexia (cases 12 and 13), but they had gait imbalance and lower limb dysesthesia, and molecular genetic testing detected in both cases the intronic $(AAGGG)_{exp}$ in *RFC1*. The phenotypic spectrum associated with biallelic intronic $(AAGGG)_{exp}$ in *RFC1* includes idiopathic instability.[20] We suggest performing genetic testing in those patients with idiopathic BVP, as it causes great instability.

We do not perform vestibular evoked myogenic potential (VEMP) in our patients. There are some references indicating that otolith organs are not affected in CANVAS. There is only 1 pathologic postmortem study including otopathologic examinations. Despite severe atrophy of the vestibular nerves bilaterally, the cristae and maculae showed a normal population of hair and supporting cells. VEMPs showed no abnormalities in previous works[22] and inconsistent results in another study.[23] Further VEMP evaluations are needed to clarify the frequency and implications of otolith dysfunction in CANVAS.

Recent efforts to identify causative mutations led to the discovery of a biallelic pentanucleotide repeat expansion in intron 2 of the *RFC1* gene.[15,16] The recessive intronic $(AAGGG)_{exp}$ in the *RFC1* gene differs in both size and

nucleotide sequence from the reference $(AAAAG)_{11}$ allele.[17] *RFC1* encodes the largest subunit of replication factor C.[24]

After failing to identify mutations by whole-exome sequencing (WES), Cortese et al[14] and Rafehi et al[15] performed whole-genome sequencing (WGS) to examine both coding and noncoding regions, including STRs. Pathogenic alterations in STRs are involved in a growing list of human diseases primarily affecting the nervous system.[15,18,25,26] Although the expansion size varied across different families, ranging from around 400 to 2000 repeats, in most cases, approximately 1000 repeats were observed.[15] We found more than 400 repeats in 11 of 13 (85%) patients in whom the above-described genetic alteration was found.

We had 2 patients without a genetic diagnosis. One of them (case 1) has an alcoholic background, and Wernicke's encephalopathy should be considered in the differential diagnosis, since it is associated with altered mental status, cerebellar ataxia, and ocular dysfunction as well as vestibular areflexia and chronic neuropathy. The other one (case 3) is a 47-year-old man. He is below the median age of onset (over 60 years), has symptoms of dementia, and was being studied for a suspicion of a Niemann-Pick dementia. The genetic studies were negative in these patients even though they clinically resembled CANVAS. Three possibilities could underlie these results: (1) a false-negative genetic test; (2) another, still undescribed, genetic alteration; or (3) a different entity with similar clinical features.

As the monoallelic carrier's frequency of *RFC1* $(AAGGG)_{exp}$ ranges from ~1% to 5% of the healthy population, the estimated prevalence at birth of biallelic carriers ranges from 1:400 to 1:10,000 individuals, suggesting that the disease is likely to be largely underdiagnosed.[14-16] This prevalence, together with the fact that subclinical vestibular involvement was detected in an additional 30% of cases,[14] indicates that otoneurologists must pay special attention to this possibly hidden syndrome. Besides this fact, Cortese et al[16] found that the percentage of positive cases increased to 92% in patients with full-blown CANVAS syndrome, arguing for considerable genetic homogeneity and, thus, a high diagnostic accuracy and positive predictive value in a preselected patient sample. They also found that 22% of patients with sporadic late-onset ataxia of unknown etiology carried the biallelic recessive $(AAGGG)_{exp}$. Moreover, a recent study found that 62% of patients with a combination of cerebellar ataxia and sensory neuropathy were carriers of the biallelic mutation.[12]

According to recent studies,[12,20] we suggest performing genetic screening for biallelic inheritance of the $(AAGGG)_{exp}$ of *RFC1* in patients with clinical suspicion of CANVAS, including those with an incomplete syndrome.

The later appearance of vestibular and cerebellar dysfunction, compared to peripheral neuropathy,[16] prompts us to look for the earliest signs of the syndrome, with the aim of having the diagnosis as soon as possible. In accordance, we believe that we should pay attention to any downbeat nystagmus as a possible early predictor. We observe this kind of nystagmus as the most prevalent (6/8; 75%) among our patients.

An additional advantage of getting an accurate genetic diagnosis is the possibility of offering genetic counseling to unaffected relatives. A patient not included in our series, a sibling of cases 6 and 7, is currently an asymptomatic carrier of a biallelic expansion (he does not have any features of the triad of CANVAS at this moment). He is 50 years old, and he only shows intermittent coughing. Having the diagnosis before the appearance of the clinical features gives us the opportunity of understanding how the development of the disease takes place in a prospective way.

The current management of this disease is not very encouraging, especially considering that most patients have both ataxia and sensorial impairment. No medical treatment to restore lost vestibular function exists, and physical therapy is only mildly effective in patients with BVP.[27] Efforts should be aimed at referring patients for an individualized combination of neurological and vestibular rehabilitation.[9]

In conclusion, CANVAS is a recently described disease with a genetic origin in which vestibular disorder is one of the main pillars of the diagnosis. Thus, otorhinolaryngologists should keep up to date with the genetic diagnosis and the advances in the treatment of this new entity.

Author Contributions

María Costales, conceptualization and writing (original draft preparation), read and agreed to the published version of the manuscript; **Rodrigo Casanueva**, conceptualization and writing (original draft preparation), read and agreed to the published version of the manuscript; **Vanessa Suárez**, writing (review and editing), read and agreed to the published version of the manuscript; **José María Asensi**, writing (review and editing), read and agreed to the published version of the manuscript; **Guadalupe A. Cifuentes**, methodology, read and agreed to the published version of the manuscript; **Marta Diñeiro**, methodology and writing (review and editing), read and agreed to the published version of the manuscript; **Juan Cadiñanos**, methodology, writing (review and editing) and supervision, read and agreed to the published version of the manuscript; **Fernando López**, writing (review and editing), read and agreed to the published version of the manuscript; **César Álvarez-Marcos**, writing (review and editing), read and agreed to the published version of the manuscript; **Andrea Otero**, methodology, read and agreed to the published version of the manuscript; **Justo Gómez**, writing (review and editing), read and agreed to the published version of the manuscript; **José Luis Llorente**, writing (review and editing), read and agreed to the published version of the manuscript; **Rubén Cabanillas**, methodology, writing (review and editing) and supervision, read and agreed to the published version of the manuscript.

Disclosures

Competing interests: The following authors are currently employed by IMOMA, which is the institution that performed the genetic testing: Marta Diñeiro (clinical molecular geneticist), Guadalupe A. Cifuentes (biotechnologist), Andrea Otero (genetic counsellor), and Juan Cadiñanos (scientific director). The authors declare no other conflicts of interests.

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