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Serologic Anti-GD3 IgM-positive CANVAS Syndrome with RFC1 rare mutation type: A Case Report

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Abstract

Cerebellar ataxia, neuropathy, and vestibular reflex syndrome (CANVAS) is a late-onset, slowly progressive neurological disorder. Double allelic intron (AAGGG)n repeat amplification in the replication factor C1 (RFC1) gene is a common cause of CANVAS. This paper presents a unique case of CANVAS in a patient with a heterozygous mutation in exon 11 of the RFC1 gene, along with multimodal mutations of (AAAAGG)exp and (AAAGG)exp in the bi-allelic introns, and serological positivity for anti-GD3 IgM. This case highlights the diagnostic challenges of CANVAS, especially when symptoms overlap with other conditions. The patient exhibited abnormal limb sensations, diminished tendon reflexes, and positive anti-GD3 IgM findings, which facilitated the diagnosis of Guillain-Barre syndrome (GBS). Although treatment improved some symptoms, the patient continued to experience an unsteady gait and prolonged sensory abnormalities. Genetic testing ruled out other ataxic disorders and confirmed CANVAS syndrome. The identification of a rare mutation in the RFC1 gene expands our understanding of genetic heterogeneity in CANVAS. This case underscores the importance of comprehensive clinical evaluation and genetic testing in diagnosing complex neurological disorders.

Introduction

CANVAS is an adult-onset, slowly progressive neurological disorder characterized by cerebellar ataxia, neuropathy, and bilateral vestibular failure. Some patients may also experience chronic cough and autonomic dysfunction. The median age of onset for CANVAS is reported to be 52 years, with a range of 19 to 76 years. The median age at death is 77 years, ranging from 59 to 87 years [1-2]. The double allele intronic sequence (AAGGG) exp in the RFC1 gene is a common cause of delayed-onset ataxia, particularly in patients with CANVAS syndrome. The prevalence of this recessive trait at birth is estimated to be approximately 1/20,000, with allele frequencies ranging from 0.7% to 4% [3]. We report a case of a patient with CANVAS syndrome who has a rare RFC1 mutation and is serologically positive for anti-GD3 IgM. The diversity and complexity of the patient's symptoms can lead to misdiagnosis and underdiagnosis. This article presents a multifaceted examination of the patient to enhance understanding of this disorder.

Case Presentation

A 56-year-old male patient presented with symmetrical limb numbness for 5 years, with no obvious triggers. The numbness began in the palms and soles and gradually progressed to the proximal regions. The patient did not report limb weakness, pain, double vision, dysphagia, or bowel issues. Over the past year, he began to experience unsteady gait and slurred speech, which progressively worsened. He struggled with fine motor tasks such as writing and experienced occasional dizziness, particularly when standing or walking. He reported no headaches, choking while drinking, or limb stiffness. The patient consulted the

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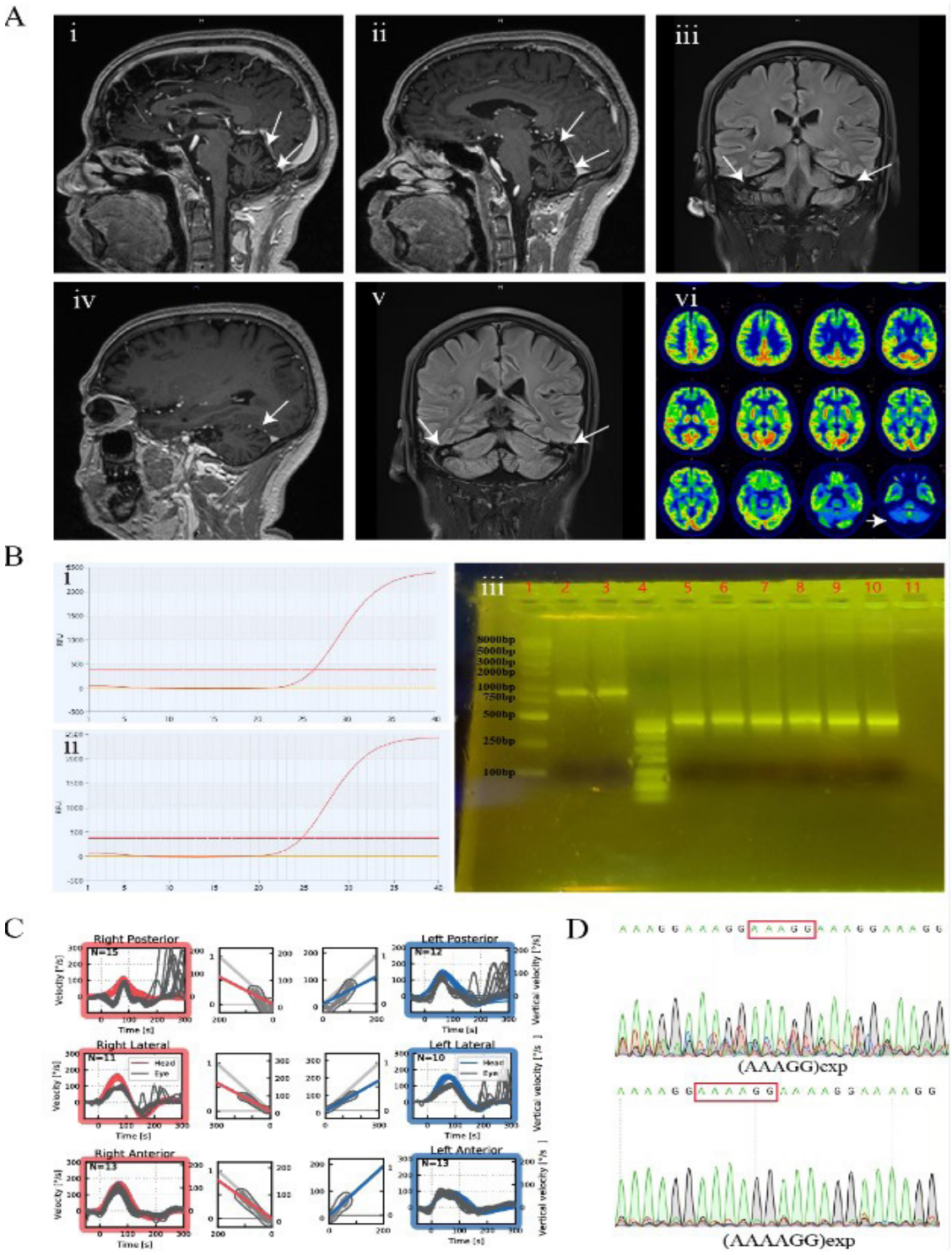

Figure 1: *Imaging, vestibular function tests, and RFC1-related genetic information for this patient. (A) The arrows indicate cerebellar vermis, anterior dorsal, and superior hemispheric lobular atrophy in the patient. (i), (ii), and (iv) are cranial magnetic resonance imaging (MRI) enhanced T1 sagittal views of the patient; (iii) and (v) are T2-FLAIR coronal MRI images; (vi) shows PET-CT imaging of the patient's brain. (B) Internal reference gene amplification effect and RCF1 gene (AAAAG)n or (AAGGG)n repeat sequence amplification results. Amplification results of the internal reference gene in the patient (i) and in the control (ii). The amplification results of the actin internal reference gene are normal, indicating satisfactory sample quality. (iii) Testing for short tandem repeat sequence mutations in the RFC1 gene in the patients. Wells: 1: DNA Marker (8K); 2: Patient Blood Sample 1; 3: Patient Blood Sample 2; 4: DNA Marker (500); 5: Control Blood Sample 1; 6: Control Blood Sample 2; 7: Control Blood Sample 3; 8: Control Blood Sample 4; 9: Control Blood Sample 5; 10: Control Blood Sample 6; 11: Negative Control. (C) The head impulse test report indicates high-frequency hypoplasia of the left and right lateral semicircles, as well as the left and right posterior semicircles in the patient. (D) Sequencing of the 3' end of the Alu element in the patient's blood RFC1 gene identified multiple mutation types: (AAAAGG)exp and (AAAGG)exp.*

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neurology department, where examination revealed slurred speech with poor control of speed and volume, along with occasional explosive speech. He exhibited spontaneous downbeat nystagmus and a positive bilateral bedside head-shaking test. Limb muscle tone and strength were normal, but the bilateral finger-nose and heel-knee-tibia tests were poorly performed. Romberg's sign indicated unsteadiness with eyes open and closed, and he demonstrated a broad-based gait. There was a suspected decrease in vibratory sensation in the right lower limb, and bilateral tendon reflexes were diminished.

The patient's coagulation profile, anemia triple test, blood sedimentation rate, liver and kidney function tests, thyroid function tests, glycosylated hemoglobin, vitamin D levels, hypertension quintuple test, and urinary and stool analyses, along with male tumor markers, showed no abnormalities. The cerebrospinal fluid pressure, routine analysis, biochemistry, and protein content were within normal limits. Blood tests for peripheral nerve antibodies returned positive for anti-GD3 IgM. The paraneoplastic syndrome antibody test was negative. Electromyography showed: 1. Peripheral nerve involvement (motor and sensory) in both upper and lower limbs. 2. Suspicious neurogenic changes in the muscles of both lower limbs, with F-wave abnormalities also noted. Video nystagmography findings included spontaneous downbeat nystagmus, smooth tracking type IV, and bilateral gain reduction in optokinetic response. The head shake and positional tests were positive, suggesting a possible central conditioning abnormality associated with the observed nystagmus. The head impulse test report indicates high-frequency hypoplasia of the left and right lateral semicircles, as well as the left and right posterior semicircles in the patient. (Fig. 1C). Cranial MRI revealed atrophy of the brainstem and cerebellum, specifically in the anterior and dorsal regions of the vermis (Fig. 1A i-v). Brain PET-CT suggested cerebellar atrophy with diffuse hypometabolism of glucose (Fig. 1A vi).

Genetic testing for ataxia types SCA1/2/3/6/7/8/10/12/17/36/DRPLA and FRDA revealed no significant abnormalities. Full-exon sequencing identified a variant potentially associated with the patient's clinical phenotype: chr4:39314514, NM_001204747.2:c.1240del, located in exon 11 of RFC1 (total of 25 exons). We designed amplification primers targeting the repeated sequences (AAAAG)n and (AAGGG)n, employing PCR and DNA electrophoresis to test for short tandem repeat mutations in the RFC1 gene. Simultaneously, six blood samples from healthy subjects were randomly selected for amplification using the same PCR system as a control. The results indicated that the patient's blood sample displayed a single amplified band near 1000 bp, suggesting a pure synonymous mutation. This finding suggests a mutation in the repeat sequence of the RFC1 gene (Fig. 1B). Subsequent sequencing identified multiple mutation types at the (AAAAG)n position of the Alu element in the patient's RFC1 gene, specifically (AAAAGG) exp and (AAAGG)exp (Fig. 1D).

After hospital admission, the patient received hormone and gammaglobulin shock therapy, along with nutritional nerve and gastric protective treatment. Although the symptoms of limb numbness and unsteady walking improved, they were not completely alleviated.

Discussion

In 1998, Rinne et al. reported cerebellar degeneration in 7 out of 51 patients (13%) with bilateral vestibular failure, which included 4 patients with mild peripheral neuropathy [4]. Vera C. Zingler et al. analyzed the etiology and epidemiology of 255 patients with bilateral vestibular lesions, finding that a significant subgroup exhibited cerebellar dysfunction and peripheral polyneuropathy [5]. Szmulewicz et al. retrospectively analyzed 18 subjects meeting the criteria for bilateral vestibulopathy with cerebellar ataxia, finding that all had sensory neuropathy. MRI scans revealed cerebellar atrophy in 16 patients. This led to the proposal of a new syndrome termed cerebellar ataxia with neuropathy and bilateral vestibular reflex syndrome (CANVAS) [6]. MRI in patients with CANVAS demonstrated a pattern of anterior and dorsal cerebellar atrophy, specifically in lobules VI, VIIa, and VIIb, while lateral atrophy predominantly affected the cerebellar hemispheres, corresponding to lobule VII [7]. Microscopically, diffuse loss of Purkinje cells is evident, primarily affecting the vermis region but also significantly impacting the lateral cerebellum [8]. Recently, Cortese et al. identified a double allelic intron (AAGGG)n repeat amplification in the poly-A tail at the 3' end of the Alu element of replication factor C1 (RFC1) as the causative mutation in CANVAS [9]. Notably, no correlation was observed between the age of onset of neuropathy in CANVAS syndrome and the size of the RFC1 repeat expansion [3].

In this paper, we report a unique case of CANVAS in a patient with a heterozygous mutation in exon 11 of the RFC1 gene, as well as multimodal mutations of (AAAAGG) exp and (AAAGG)exp in the bi-allelic introns, combined with serological positivity for anti-GD3 IgM. This finding is relatively rare and offers valuable clinical insights. In clinical practice, CANVAS syndrome is often misdiagnosed or underdiagnosed, particularly when it overlaps with other conditions. Upon reviewing this case, we noted that the patient exhibited abnormal limb sensations, weakened tendon reflexes upon examination, and positive findings for anti-GD3 IgM. These indicators facilitated the diagnosis of Guillain-Barre syndrome (GBS). Following treatment with hormone and gammaglobulin shock therapy, the patient's symptoms improved, although he continued to experience unsteady gait. Concurrently, the patient exhibited prolonged

sensory abnormalities in the extremities, a positive bilateral bedside head-shaking test, signs of cerebellar atrophy on imaging, and vestibular function tests suggesting bilateral vestibular hypoplasia, which could not be solely attributed to GBS. Consequently, we suspected that the patient might be comorbid with other ataxic disorders. To investigate this, we conducted genetic testing for various ataxic types (SCA1/2/3/6/7/8/10/12/17/36/DRPLA and FRDA), ruling out SCA-related disorders and Friedreich's ataxia, ultimately diagnosing CANVAS syndrome [10]. Additionally, we identified a rare mutation locus in the RFC1 gene in this patient with CANVAS syndrome. Whole-exon sequencing revealed a heterozygous deletion mutation at the locus of exon 11 of the RFC1 gene. Most literature indicates that the causative mutation in RFC1 for CANVAS patients is the amplification of the AAGGG sequence in intron 2 [3]. Consequently, we designed amplification primers targeting the repeated sequences of the RFC1 gene in the poly-A tail, specifically (AAAAG)n or (AAGGG)n within the Alu element, to amplify blood nucleic acids from patients. Concurrently, six randomly selected blood samples from healthy individuals were amplified using the same PCR system as controls. The amplification results for the patient samples revealed a single amplified band close to 1000 bp, indicating a homozygous mutation. Comparisons with healthy controls suggest that the patient harbors a mutation in the (AAAAG)n or (AAAGG)n repeats of the RFC1 gene. Subsequent sequencing identified multiple mutation types at the (AAAAG)n position of the Alu element in the patient's RFC1 gene, specifically (AAAAGG)exp and (AAAGG)exp. The former represents a 6-nucleotide repeat sequence amplification, which is less commonly reported. Given the unclear pathogenesis of CANVAS, we speculate that this mutation may represent a rarer mutation type associated with the disease.

In recent years, there have been several reports concerning the RFC1 non-AAGGG mutations in CANVAS syndrome. Natalia Dominik et al. expanded the genetic heterogeneity of RFC1-associated CANVAS disorders, identifying three additional pathogenic repeat motifs: AAGGC, AGGGC, and AGAGG [11]. Carolin K. Scriba et al. screened an Asia-Pacific CANVAS cohort and identified a novel RFC1 repeat motif, (ACAGG), amplified in three affected individuals. This motif was associated with additional clinical features, including muscle fasciculations and elevated serum creatine kinase levels [12]. Sacha Weber et al. reported genetic and clinical data from two families with heterozygous mutations in the RFC1 gene, specifically in a splice variant located in exon 19 and another in exon 20, respectively [13]. RFC1 encodes the large subunit of replication factor C, a five-subunit DNA polymerase accessory protein. It facilitates the loading of proliferating cell nuclear antigen onto DNA, activates DNA polymerases δ and ε, and promotes the coordinated synthesis of both DNA strands during DNA replication or DNA damage. The 3' end of the Alu element in RFC1 contains a long A-rich region that is crucial for its amplification mechanism [14]. The cerebellum and peripheral nerves are particularly vulnerable to reduced DNA repair function [15]. Therefore, when the 3' end of the Alu element is mutated, it may undergo abnormal over-amplification, leading to pathogenic mRNA accumulation and resulting in CANVAS [3].

In summary, we present a case of CANVAS syndrome involving mutations in both the intron and exon of RFC1 in a patient with a recent history of GBS. This case provides valuable clinical insights for diagnosing similar combined disorders and expands the genetic heterogeneity of CANVAS syndrome. The specific role of this RFC1 mutation warrants further investigation.

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Data Availability: Anonymized data can be requested from any qualified researcher.

Declarations

Conflicts of Interest: The authors declare that they have no conflicts of interest.

Ethical Approval: This study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

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