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CASE REPORT

New Nonsense Variant c.2983G>T; p.Glu995* in the *CACNA1A* Gene Causes Progressive Autosomal Dominant Ataxia

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ABSTRACT

The genetic testing of hereditary ataxias includes screening for CAG-repeat expansions as well as pathogenic variants and nontranslated oligonucleotide expansion, which can cause spinocerebellar ataxia (SCA). Genotype-phenotype correlations of several SCA subtypes are difficult to establish, and the underlying mechanisms remain unclear. Here, we report a 58-year-old male patient who presented with severe generalized ataxia, horizontal gaze-evoked nystagmus, cognitive impairment and a positive family history of gait difficulties. Genetic panel diagnostics revealed a new nonsense pathogenic variant in the *CACNA1A* gene (c.2983G>T; p. Glu995*) that segregated with the phenotype in three clinically affected family members. This gene is related to SCA type 6 (SCA6), episodic ataxia type 2, familial hemiplegic migraine type 1, among others. When it is supported by the clinical findings and family history, additional DNA sequencing beyond fragment length analysis should be performed.

Key Words *CACNA1A*; Channelopathies; Spinocerebellar ataxia type 6.

Spinocerebellar ataxias (SCAs) are a heterogeneous group of neurodegenerative diseases causing progressive autosomal dominant ataxia. SCA 1, 2, 3, and 6 are the most common causes of hereditary ataxia in Western and Asian countries. SCA screening typically involves routine diagnostics for polyglutamine disease. We report a family with generalized ataxia and cognitive impairment due to a newly described nonsense variant in the *CACNA1A* gene.

CASE REPORT

Between 2018 and 2019, five family members were examined in our clinic (Department of Neurology, DRK-Kliniken Nordhessen, Kassel, Germany). After written informed consent was obtained, genetic analyses were performed in four clinically affected patients as well as in one unaffected family member. DNA was extracted from peripheral blood according to standard protocols. We initiated molecular genetic panel analyses of the index patient by investigating CAG repeats of the responsible genes for SCA1, SCA2, SCA3, SCA6, SCA7, and SCA17, with negative results. Afterwards, initial sequence analysis was performed by using a panel-based next-generation sequencing (NGS) approach. The following genes were analyzed: *ATM* (NM_000051.3), *APTX* (NM_175073.2), *SETX* (NM_015046.6), *ANO10* (NM_ 018075.4), *PRKCG* (NM_002739.4), and *CACNA1A* (NM_001127 221.1). After a new pathogenic nonsense variant c.2983G>T; p.Glu995* was identified in *CACNA1A* in the index patient, all other family members were tested for the

identified variant according to a Sanger sequencing protocol. Informed consent for collecting and publishing the data was obtained from all of the examined patients. The study was approved by the Institutional Review Board at the University Medical Center Göttingen (approval no: 20/11/19).

Patient 1 (index patient; patient II.3 in Figure 1)

In 2018, a 58-year-old male patient presented with persistent pain in both knees in the Department of Pain Medicine of our clinic. He had lumbar spinal disk herniations that were treated by hemilaminectomy earlier that year. He required two additional surgeries afterwards due to discitis. He also complained of the progressive inability to lift both of his arms and has only been able to walk with a walker since 2015. The patient had a history of chronic cervicobrachialgia with weakness in both arms and underwent fusion as well as cervical spinal canal decompression the previous year. The surgery relieved the pain without affecting motor function. His medical history included mild hypertension and mild alcohol and non-steroidal anti-inflammatory drug abuse. The neurological exam revealed proximal weakness in both upper extremities, which he could not lift by more than 90 degrees; shoulder girdle atrophy including the pectoral and trapezoid muscles, saccades and horizontal gaze-evoked nystagmus on both sides; unsteady gait without assistance; and straddle-legged ataxia. The Babinski sign was positive bilaterally. In the detailed history taking, the patient reported progressive difficulties with walking for at least 10 years. He had been working as a furniture mover until 2001. His two children were reported to be healthy; however, his 54-year-old sister also needed a walker. Both parents died of an unknown cause long ago. A history of migraine was not reported.

The diagnostic tests included extensive laboratory blood and cerebrospinal fluid tests as well as MRI scans of the head and cervical spine, evoked potential examinations (sensory and motor) and electroneurography. Ultimately, the tests showed moderate creatine kinase elevation, slight generalized brain atrophy, vascular leukoencephalopathy, cervical myelopathy C3/C4 and the beginning stage of spinal canal stenosis at the level of C5/C6 on the MRI examinations (Figure 2). After excluding the common causes of ataxia, we initiated genetic panel testing. However, the results did not show polyglutamine expansions in the common SCA-associated genes for SCA1, SCA2, SCA3, SCA6, SCA7, or SCA17. Then, NGS revealed a nonsense variant c.2983G>T; p.Glu995* in the *CACNA1A* gene.

Patients 2, 3, and 4 (patients II.4, III.2, and III.3 in Figure 1)

Patient 2

The 57-year-old sister of the propositus presented with mild generalized ataxia, cognitive impairment, gait difficulties with an unsteady broad-legged gait and undirected falling tilt. Additionally, she showed pes cavus and claw toes on both sides. She required a walker for a couple of years due to the progressive
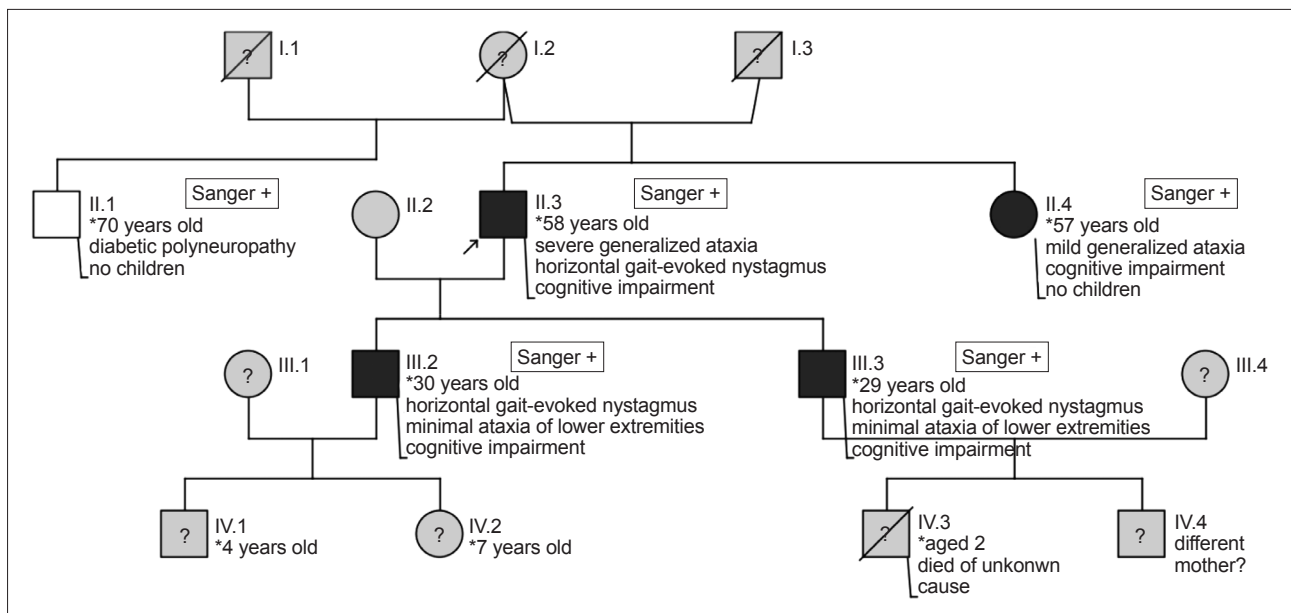

Figure 1. Pedigree showing the four affected individuals. Sanger sequencing ('Sanger +' in figure) was also performed in patient II.1, who was clinically unaffected and did not show the c.2983G>T; p.Glu995* variant in *CACNA1A*. The black color corresponds to the examined patients who were clinically affected and had confirmed variants in the *CACNA1A* gene, the white color corresponds to the healthy subject who did not have the pathogenic variant found in the propositus, and the light gray color and question mark correspond to the phenotype and unclear genotype. Circle: female; square: male; arrow: propositus; cross: deceased; asterisk: age.

worsening of her gait and had chronic back pain and osteoporosis. She had undergone multiple spine surgeries. Unfortunately, MRI scans could not be taken.

Patient 3

The 30-year-old son of the propositus did not report any difficulties with walking or coordination or additional diseases, and he had two healthy children. The neurological exam revealed inexhaustible horizontal gaze-evoked nystagmus on both sides, a slight ataxic heel-knee test result and cognitive impairment. The MRI brain scan was unremarkable.

Patient 4

The 29-year-old son of the propositus reported always being clumsy during sports in elementary school and had a legal guardian due to cognitive impairment. The clinical examination showed an exhaustible horizontal bilateral gaze-evoked nystagmus, a slight ataxic heel-knee test and cognitive impairment. No MRI scans were performed.

Patients 2, 3, and 4 did not have a history of migraine or recurrent hemiparesis. Segregation analyses by Sanger sequencing confirmed the presence of the new nonsense variant c.2983G>T; p.Glu995* in *CACNA1A* in patients 2, 3, and 4.

Patient 5 (patient II.1 in Figure 1)

The half-brother of the propositus was 70 years old and had the same mother as the propositus. He had a history of hyper-
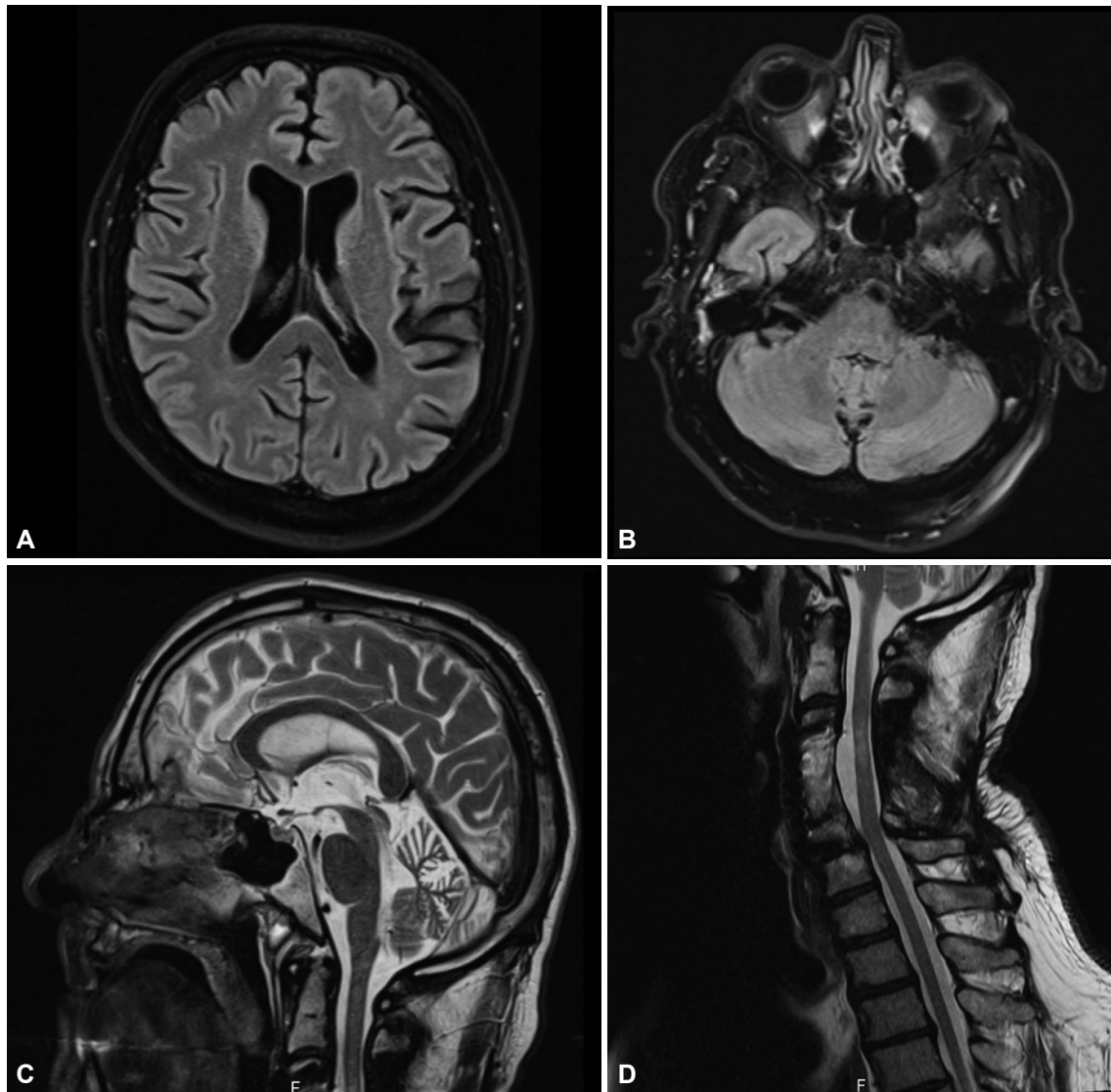

Figure 2. MRI scans of the propositus (pedigree patient II.3 in Figure 1). FLAIR (A and B) and T2-weighted (C) images show generalized brain atrophy, affecting the cerebrum and cerebellum. At level C3/C4, a myelopathy measuring 1.7 cm in length was evident without spinal canal stenosis or compression of the spinal cord. At level C5/C6, disc herniation was evident without a myelopathy signal (D).

tension and a known polyneuropathy due to insulin-dependent diabetes mellitus. The clinical exam showed mild clinical signs of polyneuropathy but no signs of ataxia or oculomotor disturbances. Sanger sequencing did not show the pathogenic variant in *CACNA1A*.

DISCUSSION

We identified a new nonsense variant c.2983G>T; p.Glu995* in the *CACNA1A* gene to be a cause of progressive ataxia and cognitive impairment. To the best of our knowledge, p.Glu995* in *CACNA1A* has not been described in the literature to date. The family showed an autosomal dominant pattern of inheritance. The nonsense variant most likely leads to a truncated protein or nonsense-mediated mRNA decay. Furthermore, all four family members with a confirmed variant showed signs of cerebellar syndrome. According to the American College of Medical Genetics and Genomics (ACMG) guidelines, there is strong evidence for the pathogenicity of this new nonsense variant.[1] The pathogenicity of the variant was confirmed by an online genetic variant interpretation tool based on the ACMG guidelines (using PVS1, PS4, and PP1).[2]

Interestingly, variants in the *CACNA1A* gene are associated with familial hemiplegic migraine type 1 (FHM1), episodic ataxia type 2 (EA2), and SCA6. The *CACNA1A* gene codes for the α1A subunit of the CaV2.1 or P/Q-type voltage-gated calcium channel.[3] A polyglutamine expansion in *CACNA1A* is the cause of SCA6, while in FMH1 pathogenic missense variants and in EA2 missense variants, deletions, insertions and splice site variants in *CACNA1A* have been found to be causative factors.[4,5] The mechanism that causes SCA6 is not fully understood. Different mechanisms have been proposed, but some of the reported results are conflicting.[3,6] There might also be a clinical overlap between SCA6, EA2, and FHM1.[4] However, in the family in our study, none of the patients showed clinical signs of EA2 or FHM1.

SCA6 is caused by CAG-repeat expansion in exon 47 of the *CACNA1A* gene. In SCA6, the number of CAG repeats does not have an effect on disease progression but instead affects the age of onset.[7] In 1997, Yue et al.[8] described a type of dominantly inherited ataxia caused by a variant in the *CACNA1A* gene. Recently, more variants in addition to the polyglutamine expansion in the *CACNA1A* gene have been detected: Coutelier et al.[9] defined channelopathies as point mutations in channel genes such as the *CACNA1A, KCND3,* and *KCNC3,* genes rather than CAG-repeat expansions in ataxia-linked genes. Here, we describe another point mutation, the pathogenic nonsense variant c.2983G>T; p.Glu995* in the *CACNA1A* gene that causes progressive ataxia.

A previous study established a correlation between the type of variant and the age of onset of the disease as well as some of the clinical features.[9] The authors found that in patients with point mutations, the onset of the disease occurs at an earlier age, the disease progression is slower and the phenotype is slightly different (pure cerebellar syndrome, often combined with intellectual deficiency).

However, in the family included in our study, patients 1 and 2 did not show an early onset of the disease, while the severity of cerebellar syndrome in patients 3 and 4 was mild. While the latter two patients did not complain about any disabilities, signs of ataxia were detected in our neurological examinations. Patient 1 and 3 also did not show prominent cerebellar atrophy, which Coutelier et al.[9] found to be especially present in patients with *CACNA1A* point-mutated ataxias. Cognitive impairment was indeed present in all four affected patients. Even when there is a difference between the genotype-phenotype correlations, it is still important to distinguish between polyglutamine expansion ataxias and non-polyQ repeat ataxias since patients with the former ataxias have a reported shorter survival.[10]

In summary, we found a previously undescribed nonsense variant in the *CACNA1A* gene that causes progressive autosomal dominant ataxia. When classic fragment length analyses do not show CAG repeat expansions, additional genetic testing such as NGS should be performed to screen for additional pathogenic variants, especially in cases of hereditary ataxias. The clinical differentiation between channelopathies and polyQ ataxias is difficult. The patient history needs to be taken carefully to screen for other possible phenotypes of a variant in *CACNA1A*, such as episodic ataxia or familial hemiplegic migraine.

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