Movement Disorders
CLINICAL PRACTICE
RESEARCH ARTICLE

RFC1 and *FGF14* Repeat Expansions in Serbian Patients with Cerebellar Ataxia

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Abstract: Background: The newly discovered intronic repeat expansions in the genes encoding replication factor C subunit 1 (*RFC1*) and fibroblast growth factor 14 (*FGF14*) frequently cause late-onset cerebellar ataxia.
Objectives: To investigate the presence of *RFC1* and *FGF14* pathogenic repeat expansions in Serbian patients with adult-onset cerebellar ataxia.
Methods: The study included 167 unrelated patients with sporadic or familial cerebellar ataxia. The *RFC1* repeat expansion analysis was performed by duplex PCR and Sanger sequencing, while the *FGF14* repeat expansion was tested for by long-range PCR, repeat-primed PCR, and Sanger sequencing.
Results: We identified pathogenic repeat expansions in *RFC1* in seven patients (7/167; 4.2%) with late-onset sporadic ataxia with neuropathy and chronic cough. Two patients also had bilateral vestibulopathy. Repeat expansions in *FGF14* were found in nine unrelated patients (9/167; 5.4%) with ataxia, less than half of whom presented with neuropathy and two-thirds with global brain atrophy. Tremor and episodic features were the most frequent additional characteristics in carriers of uninterrupted *FGF14* repeat expansions. Among the 122 sporadic cases, 12 (9.8%) carried an expansion in either *RFC1* or *FGF14*, comparable to 4/45 (8.9%) among the patients with a positive family history.
Conclusions: Pathogenic repeat expansions in *RFC1* and *FGF14* are relatively frequent causes of adult-onset cerebellar ataxia, especially among sporadic patients, indicating that family history should not be considered when prioritizing ataxia patients for testing of *RFC1* or *FGF14* repeat expansions.

Until recently, there has been an unsatisfactory gap in the genetic diagnosis of patients with late-onset (age at onset [AAO] >30 years), sporadic, presumably degenerative ataxia.[1] A new milestone in the field of late-onset cerebellar ataxias (LOCA) was reached in 2019 when biallelic repeat expansions (mainly involving an AAGGG repeat, but also AAAGG, AGGGC, AAGGC, or AGAGG) in the second intron of the *RFC1* gene, encoding the Replication Factor C subunit, were found to cause cerebellar ataxia-neuropathy-vestibular areflexia syndrome (CANVAS, *RFC1* repeat expansion).[2–4] A second exciting step forward was recently achieved when two independent research teams discovered GAA repeat expansions in the first intron of the gene encoding the Fibroblast Growth Factor 14 (*FGF14*) as a cause of another LOCA subtype (SCA27B, GAA-FGF14-related ataxia, *FGF14* repeat expansion).[5,6]

Here, we report the results of genetic screening for these two novel pathogenic repeat expansions in *RFC1* and *FGF14* in a large group of Serbian patients with cerebellar ataxia of unknown cause.

Methods

Patients

Patients with progressive ataxia, referred to the Department for Neurodegenerative Diseases and Movement Disorders at the

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Neurology Clinic at the University Clinical Center of Serbia, were recruited for this study. All patients were examined by movement disorder specialists (M.S, N.D.M, V.K), and acquired causes of ataxia were excluded. Likewise, we excluded patients suggestive of multiple system atrophy and those positive for other well-established hereditary ataxias (spinocerebellar ataxias 1, 2, 3, 6, 7, Friedreich's ataxia, and fragile X-associated tremor ataxia syndrome (FXTAS) when appropriate). In total, we included 167 unrelated (index) patients in this study. There were 45 patients (45/167; 26.9%) with a positive family history. Brain magnetic resonance imaging (MRI) was performed to evaluate cerebellar and cerebral atrophy in 154 patients, while the remainder underwent computed tomography. Nerve conduction studies (NCS) were carried out in all but 10 patients. Available patients ($n = 4$) with newly identified repeat-expansions in *RFC1* or *FGF14* were reexamined for the presence of vestibulopathy with the head impulse test (HIT) or the video-HIT (vHIT), and the presence of cough was documented by the patient or family member reports in all genetically confirmed patients.

To better understand the frequency of pathogenic expansions in *RFC1* and *FGF14* in certain subgroups of ataxia patients, we divided the entire patient group (n = 167) into an early-onset group (AAO < 30 years, $n = 52$) and a late-onset group ($n = 115$) and subdivided them further by the presence of neuropathy based on NCS (Group A and C) and absence (Group B or D) (Fig. 1).[1] AAO was defined by the first occurrence of cerebellar symptoms (ie, gait unsteadiness, difficulties with speaking). The 10 patients in whom NCS could not be performed were assigned to the group without defined neuropathy, according to their AAO.

All patients signed an informed consent to participate in the study. The Ethics Committee of the Medical Faculty, University of Belgrade approved the study. The work was carried out in accordance with the ethical standards of the Declaration of Helsinki.

Genetic Analysis of Repeat Expansions in the *RFC1* and *FGF14* Genes

Genetic analysis was performed at the Institute of Neurogenetics, University of Lübeck, Lübeck, Germany. For *RFC1*, we applied genetic analyses for all patients as described,[6] including duplex PCR and Sanger sequencing. For *FGF14*, we performed long-range PCR followed by fragment length analysis as described.[6] In addition, repeat-primed PCR was used to test for interruptions of the GAA repeat in *FGF14* using the following primers (F: CACGACGTTGTAAAAC GACCTTCTTCTTCTTCTTCTTCTT, R: AGCAATCGTCAG TCAGTGTAAGC) and confirmed with Sanger sequencing of a repeat-spanning PCR product. *FGF14* repeat expansions were considered disease-causing with a pure GAA repeat number > 250.[7]

Statistical Analysis

Most of the data is presented on a descriptive level. For statistical analysis, we used SPSS version 23. We explored the association between the repeat number and AAO by Spearman correlation. *P* values <0.05 were considered significant.

Results

Clinical Description and Genetic Analysis of *RFC1* and *FGF14* Repeat Expansions

In total, 167 patients (women: 90, men: 77) underwent genetic testing for repeat expansions in *RFC1* and *FGF14*. The average

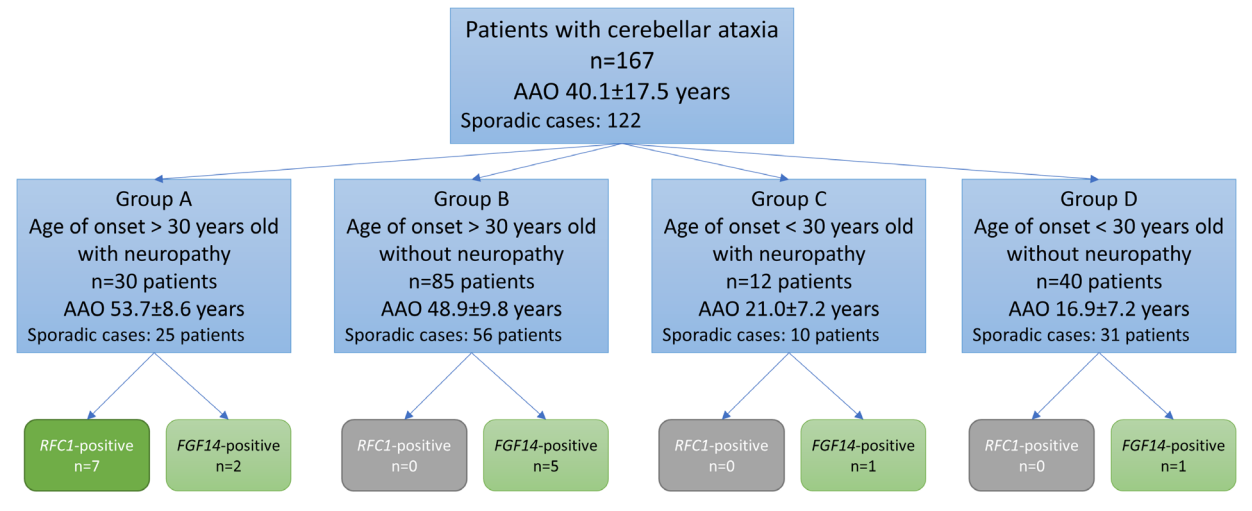


Figure 1. Patients included in the study were divided into four groups based on AAO and the presence of neuropathy. Genetic results are indicated. AAO, age at onset.

AAO (± standard deviation, STD) was 40.1 (±17.5) years, and the mean disease duration (±STD) was 10.0 (±8.2) years. Signs of neuropathy were found in 25.1% (42/167) of the patients, including 12 patients with early disease onset. Family history was positive in 26.9% (45/167), while the other patients, 73.1% (122/167), had sporadic onset of the disease.

We found pathogenic biallelic AAGGG repeat expansions in the *RFC1* gene in seven (7/167, 4.2%) patients. All seven patients belonged to subgroup A and had onset of the disease after 45 years of age, a negative family history, and the presence of neuropathy. Thus, among the 25 sporadic patients from Group A (Fig. 1), pathogenic expansions in *RFC1* were found in 28% (7/25) of the patients. We did not find carriers of other expanded repeat motifs.

Heterozygous repeat expansions (>250 repeats) in the *FGF14* gene were found in 11 patients (11/167, 6.6%). Repeat-primed PCR indicated pure GAA repeats in nine patients (9/167, 5.4%) and interrupted repeats in the remaining two individuals. Only uninterrupted repeats were considered disease-causing. Among the carriers of uninterrupted repeats, four patients had a positive family history compatible with an autosomal dominant pattern of inheritance. Thus, pathogenic repeat expansions in *FGF14* accounted for 8.8% (4/45) of the patients with a positive family history. Among the sporadic patients, 4.1% (5/122) were found to have uninterrupted repeat expansions in *FGF14*. With respect to AAO, two patients had an AAO before 30 years of age, which represent 3.8% of the tested patients with early onset ataxia (2/52, Groups C + D in Fig. 1). Among the carriers of pathogenic *FGF14* repeat expansions, 22.2% (2/7) showed an early onset. Neuropathy was present in three patients, accounting for 7.1% of all included patients with neuropathy (3/42, Groups A + C in Fig. 1) and for 33.3% (3/9) of *FGF14*-positive patients. The two early-onset patients, with AAO at 18 and 27 years, had ∼280 and ∼450 GAA repeats, respectively. Three patients had repeat numbers between 250 and 300, while six patients had >300 repeats. We did not find any significant correlation between AAO and the number of GAA repeats among these nine carriers ($r = -0.290$, $P = 0.449$).

Clinical Findings in Patients Harboring Pathogenic Repeat Expansions in the *RFC1* and *FGF14* Genes

The range and mean (±STD) AAO of the disease were 46 to 69 and 54.1 (±7.6) years in *RFC1* and 18 to 66 and 49.7 (±13.9) years in *FGF14* repeat expansion-positive patients. The mean disease duration (±STD) was 10.7 ± 6.0 and 7.8 ± 4.5 years in *RFC1* and *FGF14* repeat expansion-positive patients, respectively. Clinical features and genetic findings are presented in detail in Tables 1 and 2, as well as in Video 1.

All patients had gait ataxia. Further, 85.7% and 100% had limb ataxia among the *RFC1*- and *FGF14*-patients, respectively. Dysarthria was present in 57.1% of the *RFC1* and in 77.8% of the *FGF14* pathogenic expansion carriers.

Among the seven *RFC1* repeat expansion-positive patients, three patients had impaired smooth pursuit, two gaze-evoked nystagmus, and one downbeat nystagmus, while three patients had normal findings on oculomotor examination. As for the nine *FGF14* expansion carriers, seven patients had oculomotor disturbances, including four with gaze-evoked nystagmus, three with downbeat nystagmus, three with impaired smooth pursuit, and two patients also had skew deviation and slow saccades in all directions. In the *RFC1* repeat expansion-positive group, two patients had vestibular system dysfunction on vHIT. We found head tremor in two patients from both groups and prominent hand tremor in four *FGF14* repeat expansion carriers. MRI of the brain in *RFC1* repeat expansion-positive patients showed cerebellar atrophy in five and normal findings in two patients. In the *FGF14* group, 66.6% of patients had global brain atrophy (Fig. 2), from mild to marked; one patient had combined frontal and cerebellar atrophy, and two had normal findings. The atrophy was more pronounced than it would be expected for the respective age group. Signs of sensory neuropathy were present in all *RFC1*-positive patients and in 33.3% of the *FGF14*-positive patients. All but one *RFC1* repeat expansion-positive patient reported chronic cough without known etiology, while no one reported this feature among the *FGF14* repeat expansion-positive patients.

As for the two patients with interrupted repeat expansions in *FGF14*, they both have progressive cerebellar ataxia with a disease duration of more than 15 years, but without any episodic features, a negative family history, downbeat nystagmus and absent neuropathy. Both had cerebellar atrophy on MRI of the brain.

Discussion

Here, we investigated the frequency of newly reported repeat expansion disorders, ie, *RFC1* and *FGF14*, in a large group of Serbian patients with cerebellar ataxia. In this group of patients with previously unknown disease etiology, we were able to provide 9.6% (16/167) of the patients with a genetic diagnosis of either an *FGF14* repeat expansion (5.4%, $n = 9$) or an *RFC1* repeat expansion (4.2%, $n = 7$). Among the sporadic patients, the diagnostic yield was 9.8% (12/122).

Notably, all our *RFC1* repeat expansion-positive patients had an AAO after the age of 45 years and signs of neuropathy. Thus, the diagnostic yield with respect to *RFC1* repeat expansions was 23.3% (7/30) within the group of patients with LOCA and the presence of neuropathy. The proportion was even higher among sporadic patients with these two features, ie, 28.0% (7/25). This confirms previous clinical descriptions suggesting that *RFC1* repeat expansion-positive patients frequently have LOCA and neuropathy (and often also additional vestibulopathy [CANVAS]),[2,8] but AAOs as early as 19 years have also been reported.[9]

TABLE 1 *Clinical and diagnostic features of the* RFC1 *repeat expansion carriers*

Patient ID	SCN 329	SCN 345	SCN 366	SCN 34	SCN 204	HNPP 398	SCN 179
Family history	No	No	No	No	No	No	No
Sex	F	F	M	F	F	F	M
AAO	50	50	58	51	46	69	55
AAE	60	70	63	68	56	72	65
DD	10	20	5	17	10	3	10
First symptoms	Instability	Instability	Instability (increased with closed eyes)	Instability	Instability (increased with closed eyes)	Tingling in hands and feet, instability	Instability (increased with closed eyes)
Cough	Yes	Yes	Yes	Yes	Yes	Yes	No
Gait ataxia	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Walk assistance	No	Yes (walker)	No	No	No	No	Yes
Limb ataxia	Yes	Yes	No	Yes	Yes	Yes	Yes
Dysarthria	Yes	Yes	No	No	Yes	No	Yes
Oculomotor findings	ISP, downbeat nystagmus	ISP, GEN	ISP	GEN	Normal	Normal	Normal
Vestibulopathy	Yes	Yes	N/A	N/A	N/A	N/A	N/A
Spasticity	No	No	No	No	No	No	No
DTR	Absent on the LL	Absent on the LL	Normal	Absent on the LL	Absent on the LL	Absent on the LL	Decreased on the LL
Extensor plantar response	No	No	No	No	No	No	No
Other	Distal hypo-trophy, episodic diplopia	Head tremor, paraparesis, impaired vibration sense	N/A	Headache, impaired vibration sense	Dysphagia, impaired vibration sense	Head tremor, distal hypo-trophia, impaired vibration sense, pes cavus, global muscle weakness	Dysphagia, incontinence, dystal hypo-trophy and muscle weakness
MRI findings	Cerebellar atrophy	Cerebellar atrophy	Cerebellar atrophy	Cerebellar atrophy	Normal	Normal	Cerebellar atrophy
Neuropathy (NCS)	Yes	Yes	Yes	Yes	Yes	Yes	Yes

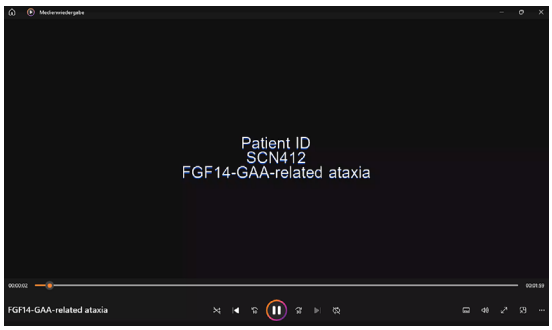
Abbreviation: AAO, age at onset (in years); AAE, age at examination (in years); DD, disease duration (in years); ISP, impaired smooth pursuit; DTR, deep tendon reflexes; GEN, gaze-evoked nystagmus; LL, lower limbs; NA, not assessed; NCS, nerve conductions studies; MRI, magnetic resonance imaging; UL, upper limbs.

TABLE 2 *Clinical and diagnostic features of the* FGF14 *repeat expansion carriers*

Patient ID	SCN 412	SCN 352	SCN 88	HSP 185	SCN 486	SCN 46	SCN 400	FAN 46	SCN 305
Family history	No	No	Yes	Yes	Yes	No	No	Yes	No
Sex	F	M	F	F	F	M	M	F	M
AAO	53	56	54	35	36	54	66	27	18
AAE	69	57	59	43	46	66	74	33	35
DD	16	1	5	8	10	12	8	6	17
First symptoms	Episodic vertigo and nausea	Instability	Instability	Incoordination with hands, episodic vertigo	Instability	Instability	Instability	Instability	Episodic vertigo and nausea
Cough	No	No	No	No	No	No	No	No	No
Gait ataxia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Walk assistance	Yes	No	No	No	No	Yes (walker)	Yes (walker)	No	No
Limb ataxia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Dysarthria	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes
Oculomotor findings	ISP, DBN	GEN	DBN	Normal	GEN, skew deviation, ISP	DBN	GEN, slow saccades	GEN, ISP	Normal
Vestibulo-pathy	N/a	N/a	N/a	No	No	N/a	N/a	N/a	N/a
Spasticity	No	No	No	No	No	No	No	No	No
DTR	Absent on the LL	Normal	Normal	Increased on UL and LL	Increased	Decreased on the LL	Normal	Increased patellar reflex, absent achillis reflex	Normal
Extensor plantar response	No	No	No	No	No	No	No	Yes	No
Other	Head tremor, distal hypo-trophy, tremor of hands and legs, inconti-nence	Tremor of the right hand, distal hypo-trophy	Inter-mittent tremor of the head and hands, urinary urgency	Muscle weakness of LL decreased vibrational sense, dysphagia, inconti-nence, headache	Tremor of the hands	Diplopia, hypo-trophy of the LL and weakness	Positional vertigo	N/a	N/a
MRI findings	Marked global atrophy	Marked global atrophy (more severe on the left side)	Marked global atrophy	Mild global atrophy	Normal	Frontal and cerebellar atrophy	Marked global atrophy	Moderate global atrophy	Normal
Neuropathy (NCS)	No	Yes	No	No	No	Yes	No	Yes	No
Number of repeats	480	330	280	300	400	350	270	450	280

Abbreviations: AAO, age at onset (in years); AAE, age at examination (in years); DD, disease duration (in years); ISP, impaired smooth pursuit; DBN, downbeat nystagmus; DTR, deep tendon reflexes; GEN, gaze-evoked nystagmus; LL, lower limbs; NA, not assessed; NCS, nerve conductions studies; MRI, magnetic resonance imaging; UL, upper limbs.

Although the previous studies mainly reported that *FGF14* repeat expansion carriers had an onset later than 30 years of age,[5,6,10–12] we found expansions in one patient in whom symptoms started when she was 27 years old and another one who had episodic features from the age of 18 years. These patients harbored 450 and 280 GAA repeats in the *FGF14* gene, respectively. On the contrary, another patient, carrying 480 repeats in the *FGF14* gene, had a late onset of the disease (after the age of 50 years). However, this individual presented with a complex clinical manifestation in the form of a severe gait ataxia and a high-amplitude hand and leg tremor combined with head tremor, leading to pronounced disability in daily activities, while previous studies mainly reported slow progression in *FGF14*-repeat expansion carriers, ie, slow increase of the score on the Scale for the Assessment and Rating of Ataxia (SARA) and rare dependence on a wheelchair.[11,12] One explanation for the faster and atypical disease progression and increased severity of *FGF14* repeat expansion disorder in the above-mentioned patient could be the presence of another neurological disorder with a different etiology.[11] We did not find any correlation between the repeat number and AAO in the *FGF14* repeat expansion carriers, as previously suggested,[5,6] maybe due to the small sample size.[11]



Video 1. The clinical findings (downbeat nystagmus, dysmetria, and gait ataxia, tremor of the extremities with static and action component, titubation) in one patient carrying *FGF14* repeat expansion (Patient ID SCN412).
Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/mdc3.14020

While neuropathy was present in 100% of the *RFC1* repeat expansion-positive patients, more than half of the *FGF14* repeat expansion-positive patients had normal findings on NCS. Thus, we suggest that all patients with LOCA associated with neuropathy should be first tested for *RFC1* repeat expansions (Fig. 1). Based on this and other studies, the highest predictive value would be achieved with additional vestibulopathy and cough.[13–15] In previous studies, the frequency of biallelic *RFC1* repeat expansion-positive patients was reported to range from 1.8% in Japanese patients[16] to approximately 30% of patients with adult-onset ataxia at European neurological centers.[9] The frequency of *RFC1* repeat expansion-positive patients in our entire cohort lies in between these numbers, reflecting heterogeneity in terms of AAO, presence of neuropathy, and bilateral vestibulopathy.[9] The phenotype of *RFC1* repeat expansion carriers has been expanded and is referred to as incomplete CANVAS when one feature of the previously mentioned triad is not present.[8] Since

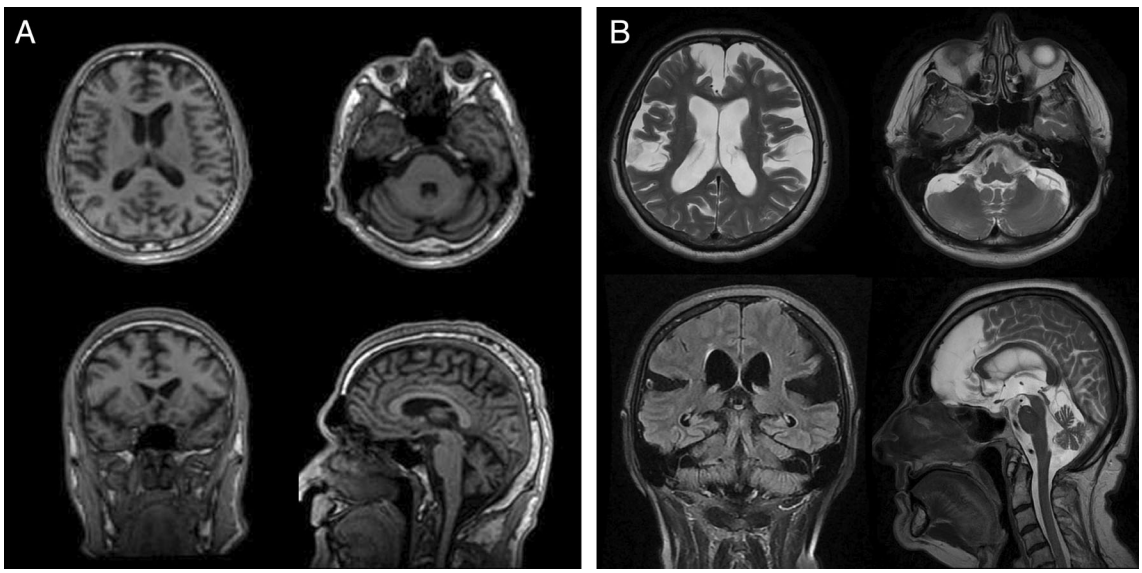


Figure 2. MRI of the brain of two patients with pathogenic repeat expansions in *FGF14*. (A) Patient SCN46, who is 10 years after disease onset and shows frontal and cerebellar atrophy. (B) Patient SCN412, who is 15 years after disease onset and shows marked global brain and cerebellar atrophy.

the first papers, it was noted that the most prominent sign is the presence of neuropathy, and subsequent studies described *RFC1* repeat expansion carriers with only isolated neuropathy,[9,17,18] even though neuropathological studies revealed loss of Purkinje and other cells in the cerebellum.[2,19,20] Additional features may include chronic cough, autonomic dysfunction, and parkinsonism.[21–24] A few studies reported a high percentage of *RFC1* repeat expansion carriers (~65%) in groups of patients with at least two of the three CANVAS features or in combination with cough.[18,21] In our cohort, two patients had full-blown CANVAS, and five had incomplete CANVAS. Nevertheless, according to new findings, in patients with full-blown or incomplete CANVAS, *FGF14* repeat expansion should also be considered in the differential diagnosis.[25]

Certain point and frameshift variants in the coding region of the *FGF14* gene lead to SCA27A, an autosomal dominant form of spinocerebellar ataxia, while the more recently found intronic repeat expansion in this gene can cause LOCA, often referred to as SCA27B.[5,26] This novel pathogenic repeat expansion was found in 5.4% (9/167) of all tested Serbian patients, involving 4.8% (5/122) of the tested patients with sporadic ataxia. Three of our patients had an expansion between 250 and 300 repeats in the *FGF14* gene. These repeat sizes are considered to be pathogenic with reduced penetrance in the initial studies, given that they were also found in a small percentage among controls.[5,7] On the other hand, the purity of GAA repeats is an important factor, as only uninterrupted repeats seem to be pathogenic.[7,27,28] [7,27,28] Of our 11 initially identified patients with expansions, only nine carried uninterrupted, ie, uninterrupted, disease-causing expansions. Among the two carriers of interrupted *FGF14* repeat expansions, no specific clinical features were found.

Notably, 75% of patients with GAA expansions in the *FGF14* gene had early episodic ataxia and downbeat nystagmus in previous reports.[5] Among our patients, one-third manifested paroxysmal symptoms early on in the disease. Oculomotor abnormalities were present in a higher percentage of *FGF14* repeat expansion-positive patients than in those with biallelic *RFC1* repeat expansions, with gaze-evoked nystagmus being the most frequent presentation.[17,18,29]

Nearly half of our patients with a GAA expansion in *FGF14* had either limb or head tremor, which is consistent with the phenotype of SCA27A,[30] although it was less frequent in previously reported cohorts with *FGF14* repeat expansions.[5] Some authors found isolated cerebellar atrophy,[5,11] others reported mostly global brain atrophy in *FGF14* repeat expansion carriers,[6] which is similar to our findings of mild to marked global atrophy.[11]

Today's genetic and bioinformatic analyses have made it possible to discover new disease-causing repeat expansions, especially in non-coding regions.[31] Since its identification in 2019, repeat expansions in the *RFC1* gene have been reported in populations worldwide.[16,19,24,32] Similarly, *FGF14* repeat expansions were found in ataxia patients in French-Canadian, Australian, Indian, German, French, Spanish, and Brazilian populations.[5,6,10,12,33] Notably, our study expands the findings of both repeats to Serbian ataxia patients, revealing that they represent a relevant disease cause among individuals with LOCA also in the Southeastern European population, especially in sporadic patients. This discovery is of great prognostic and treatment value for newly diagnosed patients, particularly considering potential therapeutic trials and the suggested beneficial effect of amantadine, acetazolamide in SCA27A,[30,34] and 4-aminopyridine in *FGF14* repeat expansion carriers.[5,11]

In summary, our data confirm the known clinical features of *RFC1* repeat expansion carriers regarding AAO, neuropathy combined with cerebellar signs, and chronic cough in most patients. As for the novel GAA repeat expansion in *FGF14*, we broaden the spectrum of the clinical manifestation in the context of a more frequent presence of tremor and significant global brain atrophy on MRI scans, which can be a distinguishing factor in the group of LOCA, as well as earlier manifestation of the disease.

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Author Roles

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

AM: 1B, 1C, 1C, 2A, 2B, 3A.
ND-M: 1B, 1C, 1C, 2A, 2B, 3A.
MT: 1C, 3B.
MB: 1A, 3B.
FH: 1C, 3B.
AW: 1B, 3B.
CK: 1B, 3B.
NB: 1A, 3B.
MS: 1C, 3B.
MB: 1C, 3A,B.
AM: 1C, 3B.
VSK: 1A, 1C, 3B.
KL: 1A, 1B, 2C, 3A, 3B.

Disclosures

Ethical Compliance Statement: The study was approved by the Ethics Committee of the Medical Faculty, University of Belgrade. All patients signed a written informed consent prior to inclusion in the study. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines.

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