Screening for SCA27B, CANVAS and other repeat expansion disorders in Greek patients with late-onset cerebellar ataxia suggests a need to update current diagnostic algorithms

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The authors report no conflict of interest.

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Abstract

Objective: Late-onset cerebellar ataxia (LOCA) is a term used to describe a slowly progressive cerebellar disorder with symptom onset ≥ 30 years of age. Intronic tandem repeat expansions (TREs) in *RFC1* and *FGF14* have recently emerged as common causes of LOCA. The relative contribution of classic vs. newly discovered TREs, has not been systematically investigated in LOCA cohorts. This study screened Greek LOCA patients for the commonest known causative TREs.

Methods: Over a 28-year period, 206 consecutive LOCA patients were referred for genetic testing. Based on clinical data and inheritance pattern, patients were screened for FRDA, SCA1,2,3,6,7 and FXTAS, followed by testing for CANVAS and SCA27B. PCR, RP-PCR, agarose gel electrophoresis and fragment analysis were performed, as appropriate, to detect the pathogenic TREs.

Results: A genetic diagnosis was reached in 60 of 206 cases (29.1%). Mean age was 60.1 ± 11.2 (35-87) years and mean age at onset (AAO) 52.5 ± 11.4 (30-80) years. SCA27B accounted for 9.7% of LOCA cases, CANVAS for 6.8% and FRDA for 4.4%. The overall frequency of SCA1, SCA2 and SCA7 was estimated at 6.8%. No cases of SCA3 and SCA6 were identified. FXTAS contributed another 1.5% of cases. In sporadic cases, the diagnostic yield was 22.8% (34 of 149), with SCA27B accounting for 8.7% of cases, CANVAS for 8.1%, FRDA for 2.7%, SCA2 for 1.3%, FXTAS for 1.3 % and SCA7 for 0.7%. In familial cases, the diagnostic yield was 45.6% (26 of 57). Patients with SCA27B, CANVAS and FXTAS had mean AAO > 50 years, whereas patients with FRDA, SCA1, SCA2 and SCA7 had mean AAO < 50 years. SCA27B had the broadest AAO range (34-80), overlapping substantially with both groups. Episodic onset was only seen in the SCA27B (35.5%) and undiagnosed cases (5.5%).

Conclusion: Our study provides data on the frequency of different TRE ataxias in LOCA patients, showing that recently-discovered TREs causing SCA27B and CANVAS, represent the commonest known genetic causes of LOCA. We, therefore, recommend prioritizing testing for *FGF14* and *RFC1* expansions in the diagnostic algorithm of LOCA.

Keywords: Ataxia; late-onset cerebellar ataxia; tandem repeat expansions; *FGF14*; spinocerebellar ataxia 27B (SCA27B); *RFC1*; CANVAS; Greek population

Introduction

Late-onset cerebellar ataxia (LOCA) is a term used to describe a slowly progressive cerebellar disorder presenting from the age of 30 years or older [van Gaalen and van de Warrenburg. Pract Neurol 2012]. It encompasses both sporadic patients with idiopathic LOCA (ILOCA), as well as familial cases with a presumed genetic cause [Harding, J Neurol Sci 1981]. Diagnostic molecular testing for patients with LOCA often combines a targeted approach for tandem repeat expansion (TRE) disorders with a non-targeted approach for conventional mutations using next-generation sequencing (NGS) [Coarelli et al. Lancet Neurol 2023].

Until recently, molecular testing yielded negative results in almost 75% of LOCA cases [Pellerin et al. NEJM 2023]. However, newly discovered intronic TREs in *RFC1* and *FGF14* have recently emerged as common causes of LOCA [Cortese et al. Nat Genet 2019; Rafehi et al. Am J Hum Genet 2019; Pellerin et al. NEJM 2023; Rafehi et al. Am J Hum Genet 2023]. To date, the relative contribution of classic TRE disorders, such as Friedreich's ataxia (FRDA), spinocerebellar ataxia (SCA) 1, 2, 3, 6, 7 and fragile-X tremor ataxia syndrome (FXTAS), vs. novel TRE disorders, such as cerebellar ataxia, neuropathy and vestibular areflexia syndrome (CANVAS) and SCA27B, has not been systematically investigated in LOCA cohorts. Such an investigation would be of substantial clinical importance, given that it may lead to the revision of established diagnostic algorithms in patients with adult-onset ataxia [van de Warrenburg et al. Eur J Neurol 2014].

The present study retrospectively assessed the contribution of the commonest causative TREs, including recently discovered TREs in *RFC1* and *FGF14*, in a large cohort of Greek LOCA patients.

Patients and methods

Patients

The Neurogenetics Unit, 1st Department of Neurology, National and Kapodistrian University of Athens (NKUA) at Eginitio Hospital is the only unit of its kind in Greece and serves as a referral center for patients with suspected hereditary ataxias from all regions of the country. From 1995 to 2023, 206 index cases with LOCA (age at onset >=30 years) and good DNA quality were referred to the Neurogenetics Unit for genetic testing. Sporadic cases had been investigated by referring neurologists to varying degrees for acquired causes of ataxia. Table 1 depicts basic demographic and clinical characteristics of the patient cohort. Based on clinical data and inheritance pattern, patients were screened for FRDA, SCA1,2,3,6,7 and FXTAS, followed by testing for CANVAS and SCA27B. Written informed consent was obtained from all patients. We presently analyzed retrospectively findings from this screening. Figure 1 depicts the flow diagram of this study. The study protocol was approved by the Eginitio Hospital Ethics Committee (Ethics ID: 345/21-05-2019).

Methods

Molecular analysis of LOCA patients was carried out in the Neurogenetics Unit, 1st Department of Neurology, NKUA, Eginition Hospital, Athens Greece (for all TREs), the Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, University College London, London, UK (for SCA27B), and the Department of Neurology and Neurosurgery, Montreal Neurological Hospital and Institute, McGill University, Montreal, QC, Canada (for SCA27B).

PCR, repeat primed-PCR (RP-PCR), agarose gel electrophoresis and fragment analysis were performed, as previously described and as appropriate, to detect the pathogenic TREs [Koutsis et al. J Neurol Sci 2014; Kontogeorgiou et al. Clin Genet 2021; Kartanou et al. Parkinsonism Relat Disord 2022; Kartanou et al. Clin Genet 2024]. For the diagnosis of FRDA, SCA1,2,3,6,7 and FXTAS, well-established laboratory guidelines were followed. For the diagnosis of CANVAS, cases with no band on sizing PCR, positive RP-PCR for the pathological AAGGG expansion, and negative RP-PCR for the non-pathological AAAGG and AAAAG expansions were considered positive for biallelic pathological AAGGG expansions [Kontogeorgiou et al. Clin Genet 2021]. For the diagnosis of SCA27B, cases with *FGF14* expansions longer than a threshold of 250 GAA repeat units were considered positive [Kartanou et al. Clin Genet 2024]. Capillary gel electrophoresis was performed on an ABI3500 genetic analyzer. Allele sizes were estimated using the GeneMarker® v2.2.0 software (SoftGenetics). All statistical analyses were performed on SPSS version 29 (Chicago, IL, USA).

Results

Frequency of different TRE ataxias in Greek patients with LOCA

Overall, a molecular genetic diagnosis was reached in 60 of 206 cases (29.1%). The diagnostic yield was highest in LOCA with presumed autosomal recessive inheritance (7 of 11, 63.6%), followed by LOCA with presumed autosomal dominant inheritance (18 of 45, 40.0%) and lowest in sporadic cases with ILOCA (34 of 149, 22.8%). Grouping all familial cases together gave a diagnostic yield of 45.6% (26 of 57).

In the entire LOCA cohort, SCA27B accounted for 9.7% of cases (n=20), CANVAS for 6.8% (n=14) and FRDA for 4.4% (n=9). The overall frequency of SCA1 (n=5), SCA2 (n=6) and SCA7 (n=3) was estimated at 6.8%. No cases of SCA3 and SCA6 were identified. FXTAS (n=3) contributed another 1.5% of cases (Figure 2).

In LOCA with presumed dominant inheritance, SCA27B accounted for 15.6% of cases (n=7), SCA1 for 11.1% (n=5), SCA2 for 8.9% (n=4) and SCA7 for 4.4% (n=2). In LOCA with presumed recessive inheritance, FRDA accounted for 45.5% of cases (n=5) and CANVAS for 18.2% (n=2). In sporadic cases with ILOCA, SCA27B accounted for 8.7% of cases (n=13), CANVAS for 8.1% (n=12), FRDA for 2.7% (n=4), SCA2 for 1.3% (n=2), FXTAS for 1.3 % (n=2) and SCA7 for 0.7% (n=1). A single case with presumed X-linked inheritance had FXTAS.

Clinical features of Greek patients with LOCA with and without identified TREs

Basic demographic and clinical characteristics of the entire LOCA cohort are shown in Table 1. Mean age was 60.1 ± 11.2 (35-87) years and mean age at onset (AAO) 52.5 ± 11.4 (30-80) years. The most frequent presenting symptom was gait ataxia, reported by 174 (84.5%) patients. An insidious, slowly progressive onset was reported by 191 (92.7%) patients.

Demographic and more detailed clinical features of patients with LOCA caused by different TREs are shown in table 2. Patients with SCA27B, CANVAS and FXTAS had mean AAO > 50 years, whereas patients with FRDA, SCA1, SCA2 and SCA7 had mean AAO < 50 years. SCA27B had the broadest AAO range (34-80), overlapping substantially with both groups. Episodic onset was only seen in the SCA27B (35.5%) and in the undiagnosed group (5.5%). Peripheral neuropathy was omnipresent in CANVAS patients, but only variably present in other TRE ataxias and rarely seen in SCA27B. Cerebellar atrophy on routine brain MRI was seen in around 75% of cases with SCA27B or CANVAS, but in all, albeit fewer, cases of other TRE

ataxias. In sporadic patients with ILOCA and no molecular diagnosis, 22 of 115 (19.1%) fulfilled diagnostic criteria for clinically probable MSA [Wenning et al. Mov Disord 2022].

Discussion

We herein screened a cohort of 206 Greek LOCA for the commonest causative TREs causing FRDA, SCA1,2,3,6,7 and FXTAS, as well as for the recently-discovered TREs in *RFC1* and *FGF14*, causing CANVAS and SCA27B respectively. We found that SCA27B is the commonest cause of LOCA, followed by CANVAS, and that the remaining classic TRE ataxias are, in fact, less common. This may have important implications for diagnostic algorithms.

The overall diagnostic yield in the present study was 29.1%. Previous studies investigating the diagnostic yield of classic TRE ataxias in LOCA have reported frequencies ranging from 9.9 to 36.5% [Wardle et al. J Neurol 2009; Moseley et al. Neurology 1998]. However, the overall frequency of molecular diagnosis in these studies largely depended on the percentage of familial cases included. The diagnostic yield for familial cases in our study was 45.6%, compared to 28.9% and 52.7% in these two previous reports [Wardle et al. J Neurol 2009; Moseley et al. Neurology]. It should be noted that previous studies included patients with a broader disease onset range (above 18 years), further limiting any direct comparison.

In the case of sporadic LOCA, also referred to as ILOCA, diagnostic yield was 22.8% in our cohort, comparing favorably to frequencies of 4.1% and 9.7% reported in the aforementioned studies [Wardle et al. J Neurol 2009; Moseley et al. Neurology 1998]. Diagnostic yield of classic TREs in ILOCA has been investigated more extensively, with a further study, specifically focusing on sporadic adult-onset ataxia, reporting a diagnostic yield of 14.2% [Abele et al. Brain 2002]. Adding the newly-discovered TREs in *RFC1* and *FGF14* appears, therefore, to substantially improve diagnostic yield in ILOCA.

Studies that have investigated the contribution of conventional mutations to sporadic LOCA cohorts using short-read NGS, have reported diagnostic yields of 4.9% and 6% [Bogdan et al. J Neurol 2022; Giordano et al. Neurology 2017]. The first of these studies also screened for classic TRE ataxias and CANVAS, reporting an overall frequency of 4.4%, of which *RFC1* expansions contributed 1.5% [Bogdan et al. J Neurol 2022]. However, the *FGF14* expansion, responsible for around 9% of ILOCA cases in our cohort, had not yet been discovered and was not included in the analysis.

A significant proportion of sporadic adult-onset cerebellar ataxia with AAO > 30 years can be due to MSA-C. Previous studies have reported frequencies of possible or probable MSA ranging from 29 to 43% [Abele et al. Brain 2002; Giordano et al. Neurology 2017; Bogdan et al. J Neurol 2022]. In 115 sporadic LOCA patients without a molecular diagnosis, we identified 22 cases (19.1%) that fulfilled recent diagnostic criteria for clinically probable MSA [Wenning et al. Mov Disord 2022]. This frequency appears lower, but cannot be directly compared to previous studies because we only included patients that had been referred for diagnostic genetic testing, whereas previous reports also recruited patients from movement disorder or ataxia clinics [Giordano et al. Neurology 2017; Bogdan et al. J Neurol 2022].

Current guidelines for genetic testing in adult-onset cerebellar ataxias recommend screening first for the classic TRE ataxias (SCA1,2,3,6,7 and 17, FRDA and FXTAS) [van de Warrenburg et al. Eur J Neurol 2014]. Even recent updates on this algorithm do not recommend changing this traditional approach, although testing for *FGF14* and *RFC1* TREs is advised, if available [Rudaks et al. Cerebellum 2024]. Findings from

the present study suggest that testing for SCA27B should be prioritized in all patients with LOCA and this should be combined with prioritized testing for either CANVAS or FRDA based on whether onset is after or before 50 years of age, in all cases lacking a dominant pedigree. In cases with dominant FH prioritized testing for SCA1,2,3,6,7 should accompany SCA27B analysis. When a dominant FH is clearly present, testing for recessive disorders can be safely omitted. However, an apparently recessive pedigree in everyday clinical practice does not always safely exclude dominant or X-linked inheritance, meaning that testing for SCA1,2,3,6,7 and FXTAS should also be performed in such cases. The above are illustrated in figure 3.

Regarding clinical features of LOCA that may be of particular diagnostic interest, the presence of an episodic onset should clearly raise suspicion of SCA27B [Pellerin et al. NEJM 2023]. No other TRE ataxia presently identified included cases with episodic onset. However, it should be noted that we did not identify any cases of SCA6, which has been rarely associated with episodic onset [Geschwind et al. Neurology 1997]. In our cohort, we observed a small percentage of cases with episodic onset (5.5%) in our undiagnosed group, which may represent cases with point mutations in ion channel genes, such as *CACNA1A* [Jaudon et al. Biomedicines 2020]. A further point to be raised is the relatively broader range of onset observed for SCA27B compared to CANVAS and FXTAS, despite similar mean AAO, as can be deduced from previous reports [Cortese et al. Brain 2020; Pellerin et al. NEJM 2023; Leehey et al. Mov Disord 2008]. This should be borne in mind by clinicians, to avoid *a priori* excluding this disorder in younger patients. Finally, cerebellar atrophy, at least on routine clinical imaging, is reported in only around 75% of cases with SCA27B or CANVAS, as has been previously noted. Thus, in the absence of cerebellar atrophy, physicians should not be necessarily directed away from considering a heredodegenerative cause in a sporadic patient with ataxia [Cortese et al. Brain 2020; Pellerin et al. NEJM 2023].

Our study has several limitations that need to be briefly outlined. Firstly, our genetic analysis was limited to TREs and did not include NGS to pick up conventional mutations. Given, however, the fact that NGS would probably be expected to identify no more than a further 10% of cases, we do not think that this shortcoming would change the main conclusions of our study [Coutelier et al. Brain 2017; Giordano et al. Neurology 2017]. Furthermore, we did not screen our LOCA cohort routinely for some of the rarer TRE-associated SCAs, such as SCA8,10,12,17 or 36. Nevertheless, previous studies from our group that have screened cohorts of Greek ataxia patients for these expansions, did not identify any positive cases, excepting a single family with SCA17 [Koutsis et al. J Neurol Sci 2014; Katsimpouris et al. J Neurol Sci 2019]. A further limitation relates to the varying spectrum of investigations performed by referring neurologists for the exclusion of acquired causes in sporadic LOCA cases. Nevertheless, these were all cases suspected of possibly harboring a genetic cause by experienced neurologists. This last point also relates to a final limitation regarding the origin of our LOCA cohort. As previously mentioned, we only included patients that had been referred for diagnostic genetic testing, rather than cases with undiagnosed LOCA from movement disorder or ataxia clinics.

In conclusion, our study provides comprehensive data on the frequency of different TRE ataxias in LOCA patients, showing that newly-discovered TREs causing SCA27B and CANVAS, appear to represent the commonest known genetic causes of LOCA. We, therefore, recommend prioritizing testing for *FGF14* and *RFC1* expansions in the diagnostic algorithm of LOCA.

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Table 1. Basic demographic and clinical characteristics of the Greek LOCA cohort

Variable	**LOCA cohort**
N	206
Gender (%)	
Male	99 (48.1)
Female	107 (51.9)
Age (years)	60.1 ± 11.2 (35-87)
Age at onset (years)	52.5 ± 11.4 (30-80)
Family History (%)	
Familial	57 (27.7)
Sporadic	149 (72.3)
Inheritance Pattern (%)	
Presumed autosomal dominant	45 (21.8)
Presumed autosomal recessive	11 (5.3)
Presumed X-linked	1 (0.4)
Apparently sporadic	149 (72.3)
Presenting symptom (%)	
Gait ataxia	174 (84.5)
Dysarthria	11 (5.3)
Postural tremor	8 (3.9)
Vertigo/dizziness	6 (2.9)
Appendicular ataxia	1 (0.5)
Other	6 (2.9)
Episodic presentation (%)	15 (7.3)

Data are mean ± SD (range); LOCA: late-onset cerebellar ataxia

Table 2. Demographic and clinical characteristics at presentation of patients with LOCA caused by different TREs and of as yet undiagnosed cases.

Variable	Undiagnosed	SCA27B	CANVAS	FRDA	SCA1	SCA2	SCA7	FXTAS
N (%)	146 (70.9)	20 (9.7)	14 (6.8)	9 (4.4)	5 (2.4)	6 (2.9)	3 (1.5)	3 (1.5)
Male sex (%)	65 (44.5)	10 (50)	8 (57.1)	8 (88.9)	3 (60.0)	2 (33.3)	1 (33.3)	2 (66.7)
Age (years)	59.7 ± 10.1	68.1 ± 11.3	71.3 ± 5.5	48.0 ± 8.4	48.6 ± 6.9	48.0 ± 3.7	43.7 ± 5.0	71.0 ± 5.3
Age at onset (years)	52.3 ± 10.0 (31-78)	60.9 ± 12.1 (34-80)	64.2 ± 6.9 (50-73)	36.9 ± 4.7 (31-45)	38.0 ± 6.2 (30-45)	44.0 ± 6.1 (34-50)	35.0 ± 0.0 (35)	58.3 ± 10.6 (47-68)
Familial cases (%)	31 (21.2)	7 (35.0)	2 (14.3)	5 (55.6)	5 (100.0)	4 (66.7)	2 (66.7)	1 (33.3)
Episodic onset (%)	8 (5.5)	7 (35.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gait ataxia (%)	145 (99.3)	20 (100.0)	14 (100.0)	9 (100.0)	5 (100.0)	6 (100.0)	3 (100.0)	3 (100.0)
Appendicular ataxia (%)	125 (85.6)	16 (80.0)	7 (50.0)	9 (100.0)	5 (100.0)	6 (100.0)	3 (100.0)	3 (100.0)
Nystagmus (%)	63 (43.1)	10 (50.0)	13 (92.9)	4 (44.5)	3 (60.0)	2 (33.3)	0 (0.0)	0 (0.0)
Downbeat nystagmus (%)	7 (4.8)	6 (30.0)	5 (35.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gaze-evoked nystagmus (%)	64 (43.8)	10 (50.0)	13 (92.9)	4 (44.5)	3 (60.0)	2 (33.3)	0 (0.0)	0 (0.0)
Dysarthria (%)	117 (80.1)	16 (80.0)	9 (64.3)	8 (88.9)	5 (100.0)	5 (83.3)	1 (33.3)	3 (100.0)
Vertigo (%)	3 (2.1)	5 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hyperreflexia (%)	56 (38.4)	9 (45.0)	1 (7.1)	6 (66.7)	4 (80.0)	0 (0.0)	1 (33.3)	1 (33.3)
Babinski sign (%)	30 (20.5)	4 (20.0)	0 (0.0)	9 (100.0)	4 (80.0)	0 (0.0)	1 (33.3)	1 (33.3)
Postural tremor (%)	13 (8.9)	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100.0)
Parkinsonism (%)	16 (11.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)
Dysautonomia (%)	11 (7.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Clinical signs of peripheral neuropathy (n=185) (%)	23/137 (16.8)	2/16 (12.5)	14/14 (100.0)	4/6 (66.7)	2/3 (66.7)	3/5 (60.0)	0/3 (0.0)	1/1 (100.0)
Cerebellar atrophy on MRI (n=185) (%)	92/132 (69.7)	13/17 (76.5)	8/11 (72.7)	2/2 (100.0)	5/5 (100.0)	5/5 (100.0)	3/3 (100.0)	3/3 (100.0)

Data are mean ± SD (range); LOCA: late-onset cerebellar ataxia; TRE: tandem repeat expansion; SCA: spinocerebellar ataxia: CANVAS: cerebellar ataxia, neuropathy, vestibular areflexia syndrome; FRDA: Friedreich ataxia; FXTAS: fragile X tremor ataxia syndrome

Figure 1. Patient flow diagram of the present study. A total of 206 patients with late-onset cerebellar ataxia underwent a two-tier screening, first for classic tandem repeat expansion ataxias and then for more recently-discovered expansions in *RFC1* and *FGF14*. Cases are divided bases on presumed inheritance pattern. The number of different molecular diagnoses is also depicted.

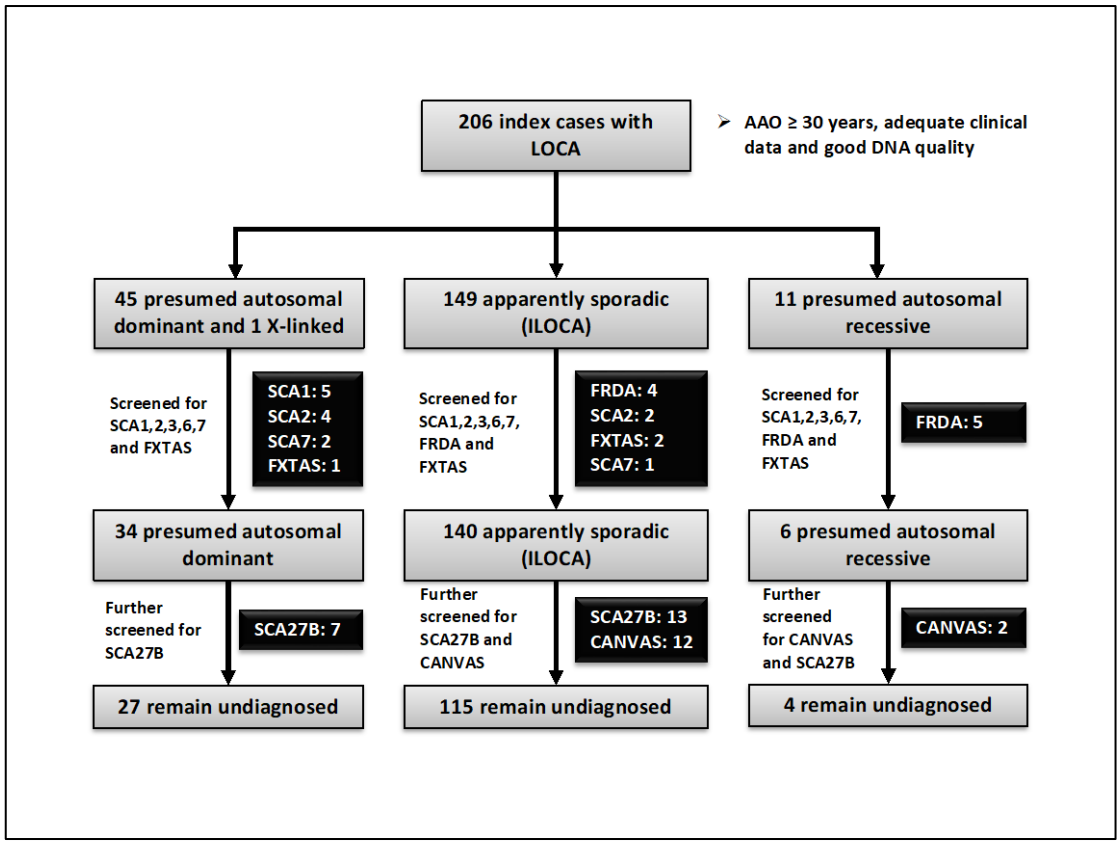

Figure 2. Pie diagram depicting the frequency of different tandem repeat expansion ataxias in Greek patients with late-onset cerebellar ataxia.

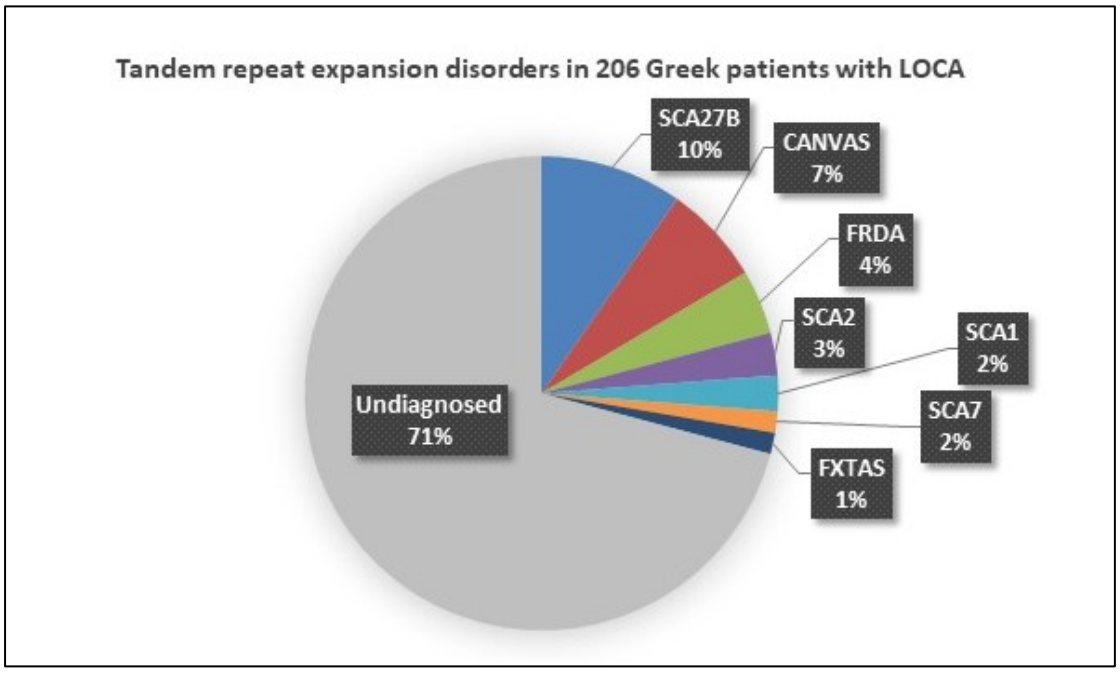

Figure 3. Suggested updated diagnostic algorithm for patients with late-onset cerebellar ataxia, based on findings from the present study.