Clinical and genetic keys to cerebellar ataxia due to *FGF14* GAA expansions

Jean-Loup Méreaux,[a] Claire-Sophie Davoine,[a] David Pellerin,[b,c] Giulia Coarelli,[a,d] Marie Coutelier,[a] Claire Ewenczyk,[a,d] Marie-Lorraine Monin,[e] Mathieu Anheim,[f,ak] Isabelle Le Ber,[a] Stéphane Thobois,[g,ae,af] Florent Gobert,[h,am] Léna Guillot-Noël,[a] Sylvie Forlani,[a] Ludmila Jornea,[a] Anna Heinzmann,[a] Aude Sangare,[a,i] Bertrand Gaymard,[i] Lucie Guyant-Maréchal,[j,ag] Perrine Charles,[a,d] Cecilia Marelli,[k] Jérôme Honnorat,[l] Bertrand Degos,[m] François Tison,[n,ah] Sophie Sangla,[o] Marion Simonetta-Moreau,[p,ai,al] François Salachas,[a,q] Maya Tchikviladzé,[a] Giovanni Castelnovo,[r] Fanny Mochel,[a] Stephan Klebe,[s] Anna Castrioto,[t] Silvia Fenu,[u] Aurélie Méneret,[a,v] Frédéric Bourdain,[w] Marion Wandzel,[x] Virginie Roth,[x] Céline Bonnet,[x] Florence Riant,[y] Giovanni Stevanin,[a,z] Sandrine Noël,[aa] Anne-Laure Fauret-Amsellem,[aa] Melanie Bahlo,[ab] Paul J. Lockhart,[ac] Bernard Brais,[b] Mathilde Renaud,[ad,aj] Alexis Brice,[a] and Alexandra Durr[a,d,*]

[a]Sorbonne Université, Paris Brain Institute - ICM, Inserm, CNRS, AP-HP, Paris, France
[b]Department of Neurology and Neurosurgery, Montreal Neurological Hospital and Institute, McGill University, Montreal, QC, Canada
[c]Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology and the National Hospital for Neurology and Neurosurgery, University College London, London, United Kingdom
[d]Unité de Génétique Clinique, AP-HP, Hôpital Pitié-Salpêtrière, Paris, France
[e]Centre de Reference Maladies Rares « Neurogénétique », Service de Génétique Médicale, Bordeaux University Hospital (CHU Bordeaux), 33000, Bordeaux, France
[f]Department of Neurology, Strasbourg University Hospital, 67098, Strasbourg, France
[g]Department of Neurology C, Expert Parkinson Centre NS-Park/F-CRIN, Hospices Civils de Lyon, Pierre Wertheimer Neurological Hospital, 69677, Bron, France
[h]Neuro-Intensive Care Unit, Hospices Civils de Lyon, Neurological Hospital Pierre-Wertheimer, Lyon, France
[i]Department of Neurophysiology, University Hospital Group APHP-Sorbonne University, Pitié-Salpêtrière Site, Paris, France
[j]Neurophysiology Department, Rouen University Hospital, Rouen, France
[k]MMDN, University Montpellier, EPHE, INSERM and Expert Center for Neurogenetic Diseases, CHU, 34095, Montpellier, France
[l]Reference Center for Paraneoplastic Neurological Syndromes and Autoimmune Encephalitis, Hospices Civils de Lyon, MeLiS Institute UMR CNRS 5284 - INSERM U1314, Université Claude Bernard Lyon 1, Lyon, France
[m]Neurology Department, Avicenne Hospital, APHP, Hôpitaux Universitaires de Paris-Seine Saint Denis (HUPSSD), Sorbonne Paris Nord, Réseau NS-PARK/FCRIN, Bobigny, France
[n]Institut des Maladies Neurodégénératives-Clinique (IMNc), University Hospital Bordeaux, Bordeaux, France
[o]Neurology Department, Hôpital Fondation Adolphe de Rothschild, Paris, France
[p]Department of Neurology, University Hospital of Toulouse, 31300, Toulouse, France
[q]Département de Neurologie, Assistance Publique Hôpitaux de Paris (APHP), Centre de Référence SLA Ile de France, Hôpital de la Pitié-Salpêtrière, Paris, France
[r]Department of Neurology, Nîmes University Hospital, Hopital Caremeau, Nîmes, France
[s]Department of Neurology, University Hospital Essen, Essen, Germany
[t]Univ. Grenoble Alpes, Inserm, U1216, CHU Grenoble Alpes, Grenoble Institut Neurosciences, Neurology Department, 38000, Grenoble, France
[u]Unit of Rare Neurological Diseases, Department of Clinical Neurosciences, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
[v]Département de Neurologie, Hôpital de la Pitié-Salpêtrière, Assistance Publique Hôpitaux de Paris (APHP), Paris, France
[w]Service de Neurologie, Centre Hospitalier de la Côte Basque, Bayonne, France
[x]Laboratoire de Génétique Médicale, CHRU Nancy, Université de Lorraine, INSERM UMR_S1256, NGERE, Nancy, France
[y]Service de Génétique Moléculaire Neurovasculaire, AP-HP, Saint Louis Hospital, Paris, France
[z]Bordeaux University (Université de Bordeaux), Equipe « Neurogénétique Translationnelle - NRGEN », INCIA CNRS UMR5287, EPHE, 33000, Bordeaux, France
[aa]Unité de Neurogénétique Moléculaire et Cellulaire, AP-HP, Hôpital Pitié-Salpêtrière, Paris, France
[ab]Population Health and Immunity Division, Walter and Eliza Hall Institute of Medical Research, Department of Medical Biology, University of Melbourne, Parkville, VIC, 3052, Australia
[ac]Bruce Lefroy Centre, Murdoch Children's Research Institute and Department of Paediatrics, University of Melbourne, Parkville, VIC, 3052, Australia
[ad]Service de Génétique Clinique et de Neurologie, Hôpital Brabois, Nancy, France
[ae]Marc Jeannerod Cognitive Neuroscience Institute, CNRS, UMR 5229, Bron, France
[af]Faculté de Médecine Et de Maïeutique Lyon Sud Charles Mérieux, Université Claude Bernard Lyon 1, Université de Lyon, Lyon, France

*Corresponding author. ICM - 47 bd de l'Hôpital, CS21414, 75646, Paris Cedex 13, France.
E-mail address: alexandra.durr@icm-institute.org (A. Durr).

[ag]Medical Genetics Department, Rouen University Hospital, Rouen, France
[ah]Institut des Maladies Neurodégénératives, CNRS, UMR 5293, Bordeaux University, Bordeaux, France
[ai]Toulouse NeuroImaging Center (ToNIC), Inserm, UPS, Université de Toulouse, 31024, Toulouse, France
[aj]INSERM Unité 1256 N-GERE (Nutrition-Genetics and Environmental Risk Exposure), Université de Lorraine, Nancy, France
[ak]Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), INSERM-U964, CNRS-UMR7104, University of Strasbourg, 67400, Illkirch-Graffenstaden, France
[al]Clinical Investigation Center (CIC 1436), Toulouse University Hospital, INSERM, 31059, Toulouse, France
[am]University Lyon I, Villeurbanne, France

eBioMedicine
2024;99: 104931

Published Online 27 December 2023
https://doi.org/10.1016/j.ebiom.2023.104931

Summary

Background SCA27B caused by *FGF14* intronic heterozygous GAA expansions with at least 250 repeats accounts for 10–60% of cases with unresolved cerebellar ataxia. We aimed to assess the size and frequency of *FGF14* expanded alleles in individuals with cerebellar ataxia as compared with controls and to characterize genetic and clinical variability.

Methods We sized this repeat in 1876 individuals from France sampled for research purposes in this cross-sectional study: 845 index cases with cerebellar ataxia and 324 affected relatives, 475 controls, as well as 119 cases with spastic paraplegia, and 113 with familial essential tremor.

Findings A higher frequency of expanded allele carriers in index cases with ataxia was significant only above 300 GAA repeats (10.1%, $n = 85$) compared with controls (1.1%, $n = 5$) ($p < 0.0001$) whereas $GAA_{250–299}$ alleles were detected in 1.7% of both groups. Eight of 14 index cases with $GAA_{250-299}$ repeats had other causal pathogenic variants (4/14) and/or discordance of co-segregation (5/14), arguing against GAA causality. We compared the clinical signs in 127 $GAA_{\geq 300}$ carriers to cases with non-expanded GAA ataxia resulting in defining a key phenotype triad: onset after 45 years, downbeat nystagmus, episodic ataxic features including diplopia; and a frequent absence of dysarthria. All maternally transmitted alleles above 100 GAA were unstable with a median expansion of +18 repeats per generation ($r^2 = 0.44$; $p < 0.0001$). In comparison, paternally transmitted alleles above 100 GAA mostly decreased in size (–15 GAA ($r^2 = 0.63$; $p < 0.0001$)), resulting in the transmission bias observed in SCA27B pedigrees.

Interpretation SCA27B diagnosis must consider both the phenotype and GAA expansion size. In carriers of $GAA_{250-299}$ repeats, the absence of documented familial transmission and a presentation deviating from the key SCA27B phenotype, should prompt the search for an alternative cause. Affected fathers have a reduced risk of having affected children, which has potential implications for genetic counseling.

Funding This work was supported by the Fondation pour la Recherche Médicale, grant number 13338 to JLM, the Association Connaître les Syndrome Cérébelleux – France (to GS) and by the European Union's Horizon 2020 research and innovation program under grant agreement No 779257 ("SOLVE-RD" to GS). DP holds a Fellowship award from the Canadian Institutes of Health Research (CIHR). SK received a grant (01GM1905C) from the Federal Ministry of Education and Research, Germany, through the TreatHSP network. This work was supported by the Australian Government National Health and Medical Research Council grants (GNT2001513 and MRFF2007677) to MB and PJL.

Keywords: SCA27B; Hereditary cerebellar ataxia; GAA expansion; Diagnosis

Introduction

Spinocerebellar ataxias (SCA) are characterized by a lack of coordination, gait imbalance, clumsy voluntary limb movements, dysarthria, dysphagia, and oculomotor abnormalities. There are three major classes of autosomal dominant SCAs according to the underlying mechanisms[1]: polyglutamine SCAs, caused by the expansion of a translated CAG repeat, accounting for 60% of SCAs worldwide[2,3]; SCAs caused by single-nucleotide variants or genomic rearrangements such as micro-deletions, duplications, or insertions[4]; SCAs due to non-coding expansions, such as the recently described SCA27B. This SCA (OMIM: 620174)[5] is caused by one GAA expansion of more than 250 repeats in intron 1 of *FGF14* at chr13:102,161,575–102,161,726 (hg38).[6,7] This expansion has been reported to explain 10–60% of the yet unresolved cases of adult-onset cerebellar ataxia with diverse ancestry.[6–10] *FGF14* is highly expressed in the cerebellum and encodes fibroblast growth factor 14 (FGF14). FGF14 modulates the activity of voltage-

Research in context

Evidence before this study

Approximately 50% of individuals with cerebellar ataxia lack a genetic diagnosis. *FGF14* GAA expansions above 250 repeats have recently been identified as a cause of SCA27B in a significant proportion of late-onset autosomal dominant ataxias.

Added value of this study

This study tested 845 index cases and 324 affected relatives with cerebellar ataxia and revealed a frequency of 10% for the *FGF14* GAA expansion with a strict threshold of $GAA_{\geq 300}$. Maternal expansions during transmission result in a strong bias towards maternal inheritance. In several cases an additional variant explained the phenotype, therefore detailed clinical-genetic assessment is needed to establish the diagnosis of SCA27B, particularly with small expansions under 300 GAA.

Implications of all the available evidence

The *FGF14* GAA expansion is a frequent cause of late-onset ataxia and alleles ≥300 repeats are significantly more frequent among individuals with ataxias, but alleles in the range of $GAA_{250\text{-}299}$ repeats should be interpreted with caution. For individuals with $GAA_{250\text{-}299}$ alleles and a presentation which deviates from the key phenotype of SCA27B or when family segregation is absent, another cause should be looked for. Transmission bias results in a reduced risk for the disease in the offspring of affected fathers.

dependent channels and exerts an anti-apoptotic activity.[11] The SCA27B phenotype is comprised of a late-onset slowly progressive cerebellar ataxia frequently associated with cerebellar oculomotor signs, such as downbeat nystagmus, and episodic features. GAA expansion size is unstable during transmission with a trend towards maternal expansion and paternal contraction.[6–8,12] We sized the SCA27B GAA repeat in 1876 individuals to assess the frequency of expanded alleles and characterize genetic and clinical variability including instability upon transmission.

Methods

Participants

We included 845 index cases with cerebellar ataxia, 324 affected relatives and 197 unaffected blood-related individuals from these families, 119 index cases with spastic paraplegia, 113 with familial essential tremor, and 475 controls.

Index cases were selected with half of the cases having a negative result on a routine diagnostic molecular test for SCAs (consisting of targeted sizing of CAG repeats in *ATXN1, 2, 3, 7,* and *CACNA1A*). Some individuals had undergone video-oculography as previously described.[13] The cohort was from European (91%), American (6%), and African (2%) genetic origin.

Ethics

Blood samples were collected between 1990 and 2023 in accordance with local French legislation (approval from local ethics committees SPATAX RBM 01-29 and RBM 03-48 or BIOMOV NCT05034172) respecting the European General Data Protection Regulation (GDPR). All participants gave written informed consent to the research.

Exome sequencing

WES was done in 678 index individuals to detect single nucleotide variants as well as expansions with 100% sensitivity as described previously (paired-end read length of 150 bases and 100x median coverage of the Twist Exome 2.0 capture (Twist Bioscience, San Francisco, USA)).[14] The variants identified in hereditary neurological disorders genes labeled as pathogenic or likely pathogenic based on the American College of Medical Genetics (ACMG) classification were considered causative.[15] The index individuals without WES were clinically selected for SCA27B testing with the presence of downbeat nystagmus or episodic manifestations of cerebellar ataxia.

FGF14 targeted analysis

The *FGF14* GAA locus was amplified by long-range (LR) and repeat-primed (RP) polymerase chain reactions (PCR) as previously reported.[6,8] We pooled the LR-PCR with the two RP-PCR (3′ and 5′) to perform a capillary electrophoresis using the ABI3730XL machine (Thermo Fisher Scientific, Waltham, USA). The two RP-PCR were used to confirm the uninterrupted nature of the GAA sequence. The LR-PCR capillary electrophoresis provided very accurate sizing of alleles under 295 GAA. The Fragment Analyzer (Agilent Technologies, Santa Clara, USA) using a DNF-474 HS NGS Fragment Kit with 33 cm array was used to measure the size of larger alleles after purification with AMPure XP beads (Beckman Coulter, Brea, USA) with a 0.7X ratio. The amplicon size measured with both methods was adjusted with a linear and polynomial regression, respectively fitted with nine calibrators sized with targeted Oxford Nanopore long read sequencing, as described previously.[8]

Statistics

We used the R 4.2.3 and ggplot2 packages for statistics.[16,17] Groups were compared using Fisher's test for qualitative variables and Kruskal–Wallis test for quantitative variables, with a conventional two-tailed type I error of 0.05 and *p*-value adjustment for multiple testing with the Benjamini Hochberg procedure. Proportions

were related to the available data. For familial analyses, we inferred the parental transmitted allele by selecting the smallest parent-child size difference upon transmission. We used a linear model to estimate allele size differences, according to the size of the expansion above 100 GAA and the sex of the transmitting parent. Sex/gender was self-reported by study participants without considering it for statistical analyses except for intergenerational instability and parental transmission.

Role of funders

The funders had no role in study design, data collection, data analyses, interpretation, or writing of report.

Results

Defining a strict threshold for *FGF14* pathogenic expansions

We found 85/845 (10.1%) index cases with cerebellar ataxia carrying at least one *FGF14* GAA expansion ranging from 300 to 495 repeat units (flowchart of the distribution of carriers with $GAA_{\geq 300}$ in Supplementary Figure S1). We tested 232 additional index cases: 119 with spastic paraplegia because of overlapping causal genes, and 113 with familial essential tremor after having observed more frequent tremor in individuals with $GAA_{\geq 300}$. None carried a $GAA_{\geq 300}$ allele.

Previous reports have suggested that $GAA_{250\text{-}299}$ expansions are likely pathogenic albeit with incomplete penetrance, whereas larger expansions appear fully penetrant.[6,7] Indeed, we found the proportion of individuals carrying *FGF14* $GAA_{250\text{-}299}$ alleles to be similar in cases with ataxia and controls (1.7 versus 1.7%; $p = 1$ (Fisher's test); Fig. 1). In comparison, the proportion of individuals with $GAA_{\geq 300}$ was significantly greater in patients (10.1%) than in controls (1.1%) (odds ratio 10.5 with 95% confidence interval [4.3–33.4]; $p < 0.0001$ (Fisher's test)). Based on these findings, we proposed a stricter threshold of $GAA_{\geq 300}$ for further analyses.

Five of 475 controls had GAA-pure alleles ranging from 308 to 380 repeats. Their median age at examination was 37.9 years (IQR = 36.6–63.5) [min 27.6–max 69.5] not significantly different from the other controls examined at 59.7 (IQR = 47.4–68) [19.2–87.5] ($p = 0.16$ (Kruskal–Wallis test)) nor from individuals with ataxia (57 years (IQR = 45–66) [2–95]). One had compound heterozygous alleles above 250 GAA (250/308) and was unaffected at age 63.5 years.

In addition to GAA-pure expansions, we found four individuals with ataxia with an interrupted expanded allele above 250 repeats in length, ranging from 299 to 339 as well as two controls with 342 and 400 interrupted GAA repeats. They were considered nonpathogenic.[6,7]

Detailed phenotypic analysis for 127 individuals with $GAA_{\geq 300}$

In addition to the 85 index cases, 42 affected relatives also carried $GAA_{\geq 300}$. In total, 66 were women and 61 were men. Most exhibited late-onset ataxia with a median age at onset of 55 (IQR = 45–60) [6–79] years, starting with episodic symptoms in 47.9% (58/121) (Supplementary Tables S1 and S2). Only seven individuals had a disease onset prior to 30 years (5.6%), ranging from 6 to 27 with repeat sizes ranging from 305 to 496 GAA. Median SARA scores were 10.5 (IQR = 6.5–13.5)[2–28] despite a median disease duration of 10 (IQR = 5–18.8) [0–49] years. Median age at death was

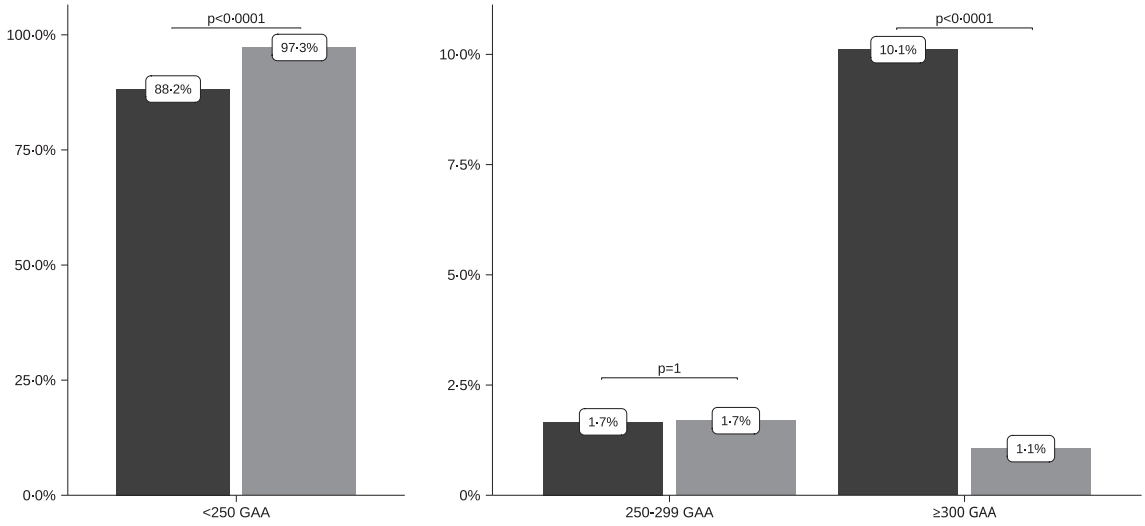

Fig. 1: Proportion of index patients with ataxia (black) (n = 845), and controls (gray) (n = 475) according to the size of their largest GAA repeat for *FGF14*. The proportion of carriers differed significantly above 300 GAA in patients (10.1%) compared to controls (1.1%) ($p < 0.001$; Fisher's test) as with <250 GAA whereas it was similar for GAA sizes between 250 and 299 GAA.

81.4 (IQR = 73.5–86.1) [48–104] years (n = 33). Survival was significantly longer compared to polyQ or other non-polyQ ataxias (p < 0.0001 (logrank test); Supplementary Figure S2).[18]

The absence of dysarthria in 50 of 99 individuals with $GAA_{\geq 300}$ (50.5%) was unusual compared with other genetic cerebellar ataxias (25.4%).[12,19] This absence of dysarthria was partially explained by a shorter disease duration (8 years (IQR = 5–12.5) versus 13 years (IQR = 9–20); p = 0.001 (Kruskal–Wallis test)), even though there were 31 patients with $GAA_{\geq 300}$ without dysarthria in the 61 with a disease duration above 10 years. Diplopia was frequent in 58.9% (53/90), as well as nystagmus in 83.3% (80/96), mostly downbeat (66.7% (56/84)). Decreased vibration sense was present in 57.5% (42/73). Reflexes in lower limbs were increased in 30.4% (21/69), extensor plantar reflexes were present in 14.7% (10/68), with overt spasticity in 20.7% (19/92). Postural tremor was present in 26.8% (26/97) and resting tremor in 14.3% (14/98). Extrapyramidal rigidity was found in 21% (13/62) and hypokinesia in 10.8% (7/65). No patient had dysautonomia defined as orthostatic hypotension (20 mmHg difference between supine and up wright position). Cognitive impairment was present in only 15.1% (13/86) despite advanced age at examination. We propose the following key feature triad: onset after 45 years, episodic ataxic features including intermittent diplopia and the presence of downbeat nystagmus, and an additional key: the absence of dysarthria.

Eye movement recordings were abnormal in 21/22 (95.5%, Supplementary Table S3). Main abnormalities were i) saccadic smooth pursuit in 20/22 (90.9%) mostly vertically; ii) nystagmus in 17/22 (77.3%); iii) downbeat nystagmus in 14/22 (63.6%) and iv) diplopia in 10/22 (45.5%), mainly caused by alternating skew deviation in 8/22 (36.4%). A moderate to important increase of square-wave jerks was present in only 3/22 (13.6%). No hypermetria was noted but horizontal hypometria was measured in 5/22 (22.7%) and vertical hypometria 11/22 (50%) (mainly downward). Antisaccade error rates were abnormal in 16/20 (80%). Saccade latencies were slightly increased in 6/22 (27.3%). A slight decrease of horizontal saccade velocities was noted in only 1/22 patient (4.5%).

Brain MRI showed cerebellar atrophy in 67/76 (88.2%) and sometimes more global cerebral atrophy in 11/27 (40.7%), but without pontine atrophy. All individuals with $GAA_{\geq 300}$ were European.

Phenotype comparison according to GAA size

We compared three groups defined by the size of the largest allele: <250 (n = 1016), 250–299 (n = 22) and ≥300 GAA (n = 127) (Fig. 2 and Supplementary Table S1). The $GAA_{250\text{-}299}$ group was phenotypically similar to $GAA_{\geq 300}$. Compared to the $GAA_{<250}$ group, the $GAA_{\geq 300}$ group had the latest median age at onset (55 versus 40 years in the $GAA_{<250}$ group; p < 0.0001 (Kruskal–Wallis test)) (Fig. 2a). Nystagmus was more frequent in the $GAA_{\geq 300}$ group (83.3% versus 55.5%; p = 0.002), especially downbeat nystagmus (66.7% versus 31.4%; p = 0.002 (Fisher's test)). Resting tremor also was more frequent in the $GAA_{\geq 300}$ group (14.3% versus 4.9%; p = 0.01 (Fisher's test)). Overt spasticity was less frequent in $GAA_{\geq 300}$ (20.7% versus 38.3%; p = 0.02 (Fisher's test)) as was dysarthria (49.5% versus 74.6%; p = 0.002 (Fisher's test)) (Fig. 2b).

Overall, 40% of patients with $GAA_{\geq 300}$ presented the key triad, more frequent than in those with $GAA_{<250}$ (4%; p = 0.002 (Fisher's test)) and similar to those with $GAA_{250\text{-}299}$ (13.3%; p = 0.13 (Fisher's test)) (Fig. 2c). Considering cases with the key triad, the proportion of SCA27B $GAA_{\geq 300}$ cases increased to 55.3% and to 75% when associated with the absence of dysarthria.

Neither GAA expansion size nor zygosity correlated with clinical severity

There was no correlation between the age at onset (Kendall tau –0.06, p = 0.3 (Kendall correlation test)), age at death (Kendall tau –0.15, p = 0.3 (Kendall correlation test)), SARA score (Kendall tau –0.12; p = 0.3 (Kendall correlation test)), and the expanded GAA allele size above 300 GAA. Eight cases were compound heterozygous with at least one allele expanded above 300 GAA and the second allele ranging from 265 to 289 GAA in 3/8 (Supplementary Table S2ter). There were no significant clinical differences with heterozygous carriers.

Intergenerational instability

Maternal transmission of $GAA_{\geq 300}$ was more frequent (76.9%, n = 60) than paternal transmission (23.1%, n = 18) in familial cases, p = 0.01 (Fisher's test). Indeed, including data from the three already described French-Canadian families,[6] mothers had a proportion of affected children of 48.6% (67/138) not significantly different (p = 0.9 (Fisher's test)) from the expected 50% for an autosomal dominant disease, whereas only 31.2% (24/77) of children of affected fathers were affected (p = 0.001 (Fisher's test)). We studied meiotic instability to see if a bias could account for the deviation from the expected 50% for both parental sexes. The instability of the GAA expansion during parental transmission depended on its size, repeats longer than 84 GAA were all unstable except one (n = 57/360, Fig. 3). All the 37 maternally transmitted alleles above 100 GAA were unstable and expanded by a median of +18 (IQR = 10–27) [–34 to +63] by generation (r^2 = 0.44; p < 0.0001 (Kendall correlation test)). The inverse occurred for the 16 paternally transmitted alleles above 100 GAA which contracted with a median difference of –15 (IQR = –23.5 to –5.75) [–59 to +16] (r^2 = 0.63; p < 0.0001 (Kendall correlation test)). Parental alleles above 300 GAA were larger when maternally than

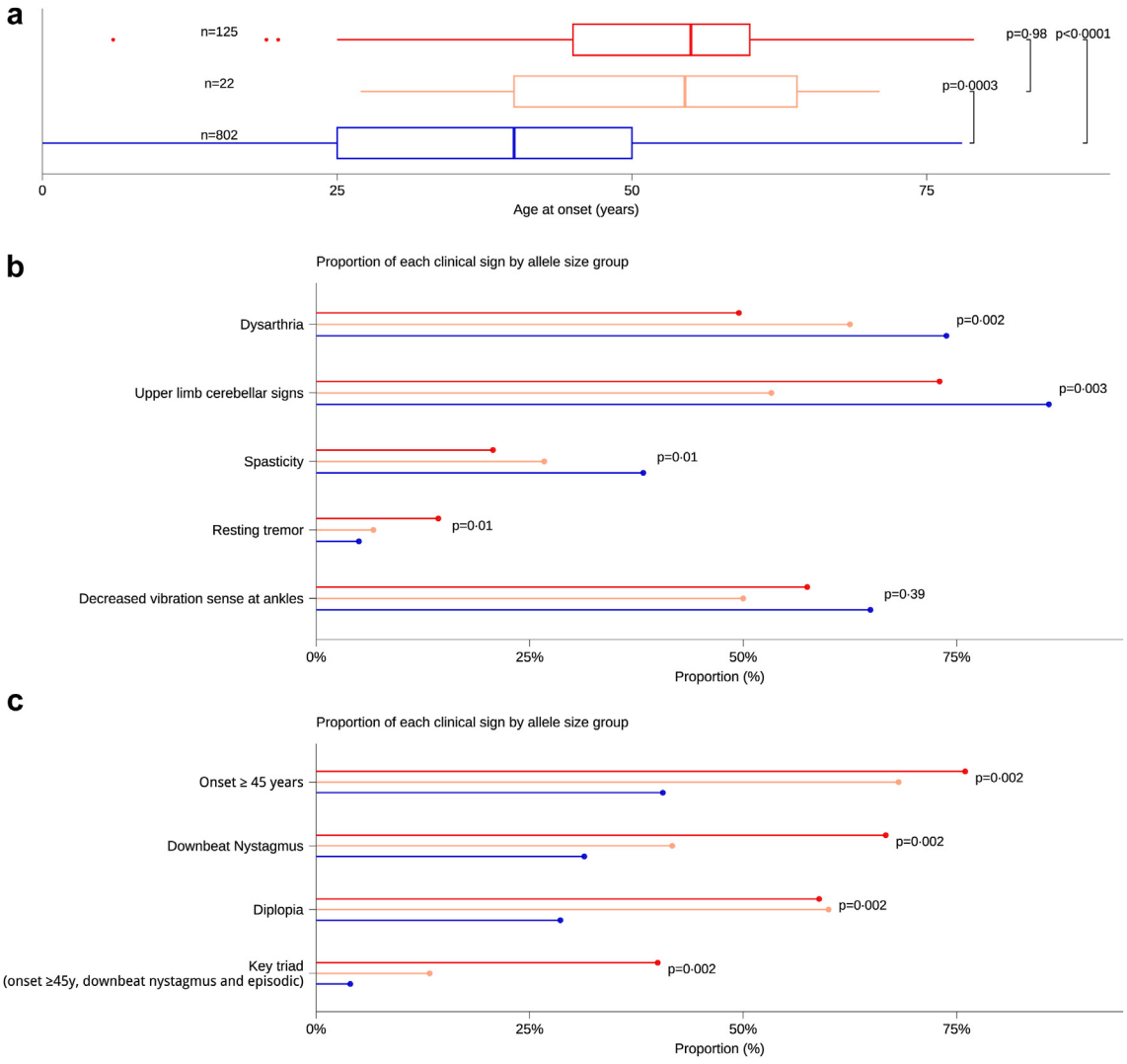

Fig. 2: Clinical features of patients with ataxia according to *FGF14* GAA size groups ($GAA_{\geq 300}$ in red, $GAA_{250\text{-}299}$ in light orange, and $GAA_{<250}$ in dark blue). a: boxplot of the age at onset (the ends of the boxes are 25th and 75th percentiles, the middle line is the median, and the whiskers are 1.5 IQR; adjusted *p*-values after Kruskal–Wallis test). b: clinical features of SCA27B individuals with $GAA_{\geq 300}$ were similar to those with GAA sizes between 250 and 299 GAA (Fisher's test). c: the three key features of SCA27B which were significantly different in individuals with less than 250 GAA repeats (Fisher's test).

paternally transmitted (395.5 (IQR = 345.3–454.5) [309–496] versus 334.5 (IQR = 318.5–375.8) [301–433] GAA; $p < 0.0001$ (Kruskal–Wallis test)).

The significance of pathogenic variants associated to *FGF14* GAA expansions

In 28.6% (4/14) families with $GAA_{250\text{-}299}$ we found another convincing pathogenic variant in a gene different from *FGF14*, versus only 4.7% (4/85) in those with $GAA_{\geq 300}$ ($p = 0.03$ (Fisher's test)) since 35.3% (298/845) of the index cases had a previously identified causal gene (Supplementary Table S4). Interestingly, 1.3% (4/298) of index cases with another causal gene carried $GAA_{\geq 300}$ with a frequency similar to that of carriers in controls ($p = 0.8$ (Fisher's test)), whereas 14.8% (81/547) without another identified causal gene had $GAA_{\geq 300}$.

In the four individuals with $GAA_{\geq 300}$, the additional pathogenic variant was in the following ataxia genes *ITPR1, NOP56, ATP1A2,* and *CACNA1A* (Table 1). The corresponding individuals presented usually with an earlier onset than in SCA27B and a complex phenotype. This suggested that the additional variant associated with genes is responsible for earlier onset and more severe phenotypes. The possible contribution of *FGF14* to the phenotype may be masked because of late onset and slow progression.

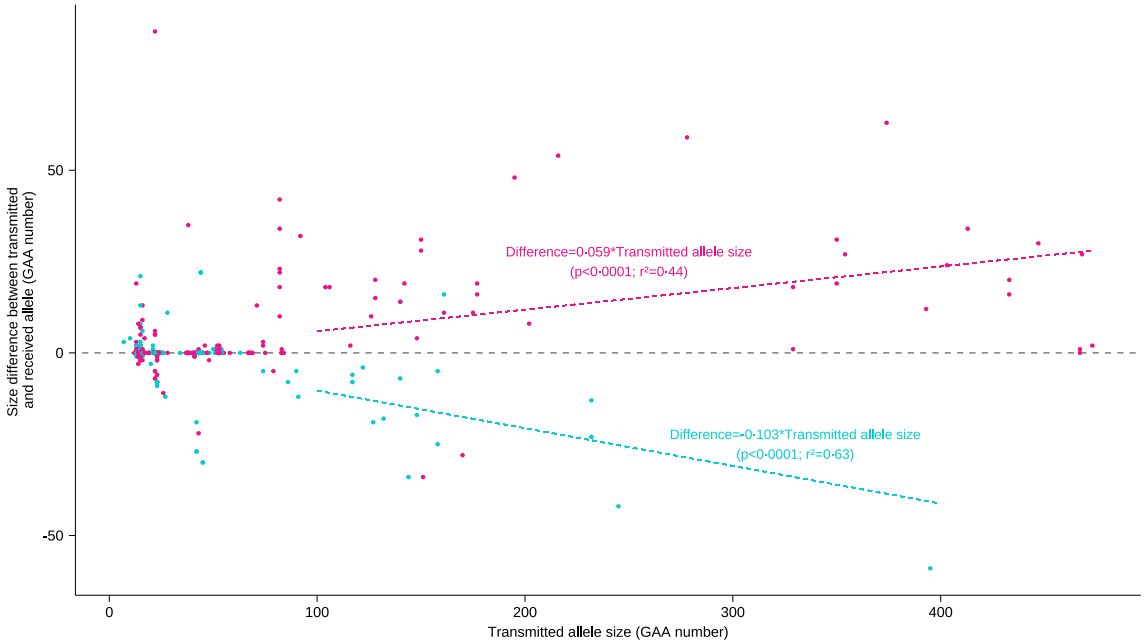

Fig. 3: Analysis of parent-offspring transmission of *FGF14* GAA alleles according to the size of the expansion and sex of the transmitting parent (375 maternal transmissions in magenta and 255 paternal transmissions in turquoise). Transmission of alleles above 100 GAA showed instability with a maternal propensity to expand ($p < 0.001$; Kendall correlation test; $r^2 = 0.42$) versus a paternal tendency to contract ($p < 0.001$; Kendall correlation test; $r^2 = 0.61$).

In individuals with $GAA_{250–299}$, disease-causing variants were found in four different genes: *ATXN3*, *SPTLC1*, *RFC1*, and *CACNA1A*. None of them only displayed the key phenotype of SCA27B but rather the phenotype expected given the other pathogenic variants identified (Supplementary Table S2bis).

Familial and Individual ID	*FGF14* GAA genotype	Other pathogenic gene	Transcript	Other pathogenic variant (c.)	Other pathogenic variant (p.)	ACMG criteria	Age at onset (years)	SCA27B key features
AAD-588-011	68/406	*ITPR1*	NM_001378452.1	c.(?_-350)_(708 + 1_709-1)del (exon 1_9 del)	p.?	P (Ib): PVS1, PM2, PP3, PP4, PP1	35	no
AAD-588-009	17/393	*ITPR1*	NM_001378452.1	c.(?_-350)_(708 + 1_709-1)del (exon 1_9 del)	p.?	P (Ib): PVS1, PM2, PP3, PP4, PP1	48	yes
AAD-1194-001	23/373	*NOP56*	NM_006392.4	c.3 + 71_4-62GGCCTG[1000+]	p.?	P (II): PS3, PS4, PM1, PM2, PP3, PP4	49	NA
AAD-1201-001	302/372	*ATP1A2*	NM_000702.4	c.2999A > G	p.Glu1000Gly	LP (IV): PP3, PM1, PM2, PM5	58	no
–399–498	265/326	*CACNA1A*	NM_001127222.1	c.889G > A	p.Gly297Arg	P (II): PS1, PM2, PS4, PP3, PP4, PP5	30	no
-SAL-382-069	15/283	*ATXN3*	NM_004993.6	c.874_915CAG[65]	p.292_305 [Gln65]	P (II): PS3, PS4, PM1, PM2, PP3, PP4	50	NA
-SAL-382-080	15/259	*ATXN3*	NM_004993.6	c.874_915CAG[72]	p.292_305[Gln72]	P (II): PS3, PS4, PM1, PM2, PP3, PP4	35	no
AAR-289-003	17/271	*SPTLC1*	NM_006415.4	c.115_117del	p.Leu39del	LP (V): PM4, PM2, PP5, PP4	27	no
–399–966	15/265	*RFC1*	NM_001204747.2	c.132 + 2864_132 + 2919TTCCC[1000+]/ c.132 + 2864_132 + 2919TTCCC[1000+]	p.?	P (II): PS3, PS4, PP3, PP4, PM1, PM3	56	no
AAR-173-001	15/263	*CACNA1A*	NM_001127222.1	c.4633C > T	p.Arg1545*	P (Ib): PVS1, PM2, PM5, PP4, PP5, PM6	36	no

NA: Not available; ACMG: American College of Medical Genetics; P: pathogenic; LP: likely pathogenic.

Table 1: **Cases with *FGF14* $GAA_{\geq 300}$ (top) or $GAA_{250-299}$ (bottom) and another pathogenic variant.**

Segregation in families

In two out 24 families with at least one individual with ataxia who had a $GAA_{\geq 300}$ expansion, three individuals with ataxia did not carry a $GAA_{\geq 250}$ expansion (AAD-431 and AAD-900; Supplementary Figure S3). In family AAD-431, the age at onset of three affected individuals was under 13 years, only one of the relatives carried an expansion above 300 GAA. This was earlier than usual in the individuals with $GAA_{\geq 300}$ and could represent an incidental finding. In family AAD-900, one affected individual with a phenotype compatible with SCA27B did not carry a large GAA allele, whereas five other affected cases had $GAA_{\geq 300}$ expansions. This suggests that the patient without the GAA expansion was a phenocopy.

A discordance of segregation of the $GAA_{\geq 250}$ expansion with cerebellar ataxia in affected individuals was present in five out ten families with at least one individual with $GAA_{250\text{-}299}$ expansion (SAL-382, -863, AAD-683, -907, -1328; Supplementary Figure S4). In family SAL-382, an *ATXN3* CAG repeat expansion accounted for the phenotype observed in 2/7 patients also carrying a $GAA_{250\text{–}299}$. In family SAL-863, an allele ranging from 176 to 270 GAA repeat units was found in three patients whereas the fourth had no expansion excluding a causal role of the GAA expansion. In the three remaining families, GAA expansions of 238 and 276 (AAD-683), 245 and 279 (AAD-907) and, 200 and 297 (AAD-1328) co-segregated with the phenotype (late-onset mild episodic ataxia) but they seemed not to be causative on their own alone due to their size. Exome analysis in two of these later families did not reveal additional disease-causing variants. Either these GAA expansions shorter than 250 are not responsible for the disease, or perhaps when associated with yet unknown modifying genetic variants they may become pathogenic.

In sum, more discordances in segregation with cerebellar ataxia were observed in families with $GAA_{250\text{–}299}$ expansions compared to those with $GAA_{\geq 300}$ (50% versus 8.3%; $p = 0.03$ (Fisher's test)).

Discussion

We screened a cohort of 1169 patients with ataxia and 475 controls for the recently reported *FGF14* GAA expansion responsible for SCA27B.[6,7] We showed, in contrast with what has previously been reported,[6,7,9] a higher frequency in cases with cerebellar ataxia versus controls was only observed in our cohort for *FGF14* $GAA_{\geq 300}$ expansions.[6,7] The proportion of *FGF14* $GAA_{250\text{-}299}$ alleles was similar in individuals with ataxia compared to controls (1.7 versus 1.7%; $p = 1$ (Fisher's test)). In controls, $GAA_{250\text{-}299}$ were as rare as in previous control cohorts ranging from 0.2%–2%.[6,7,20] We had fewer individuals with ataxia carrying $GAA_{250\text{-}299}$ than the first Australian and Canadian studies (5%–20%) but the percentage was comparable with that of the German cohort (1.7%).[6] Because alleles with $GAA_{\geq 300}$ were significantly more frequent in patients than in controls ($p < 0.0001$ (Fisher's test)), our data support the use of $GAA_{\geq 300}$ as the safe pathogenic threshold. The appropriateness of this more conservative threshold is reinforced by two additional findings observed in individuals with $GAA_{250\text{-}299}$ as compared with individuals with $GAA_{\geq 300}$: i) the presence of another genetic cause, and ii) the discordant segregation analysis. Indeed, another genetic causal variant was observed in 28.6% of index with $GAA_{250\text{-}299}$ ($n = 14$) but only in 4.7% of those with $GAA_{\geq 300}$ ($n = 85$) ($p = 0.03$ (Fisher's test)). Furthermore, the phenotype was compatible with the pathogenic variant observed in this other gene. Segregation analysis was discordant in 50% of families with $GAA_{250\text{-}299}$ versus 8.3% in those with $GAA_{\geq 300}$ ($p = 0.03$ (Fisher's test)). These data indicate that a significant proportion of cases with $GAA_{250\text{-}299}$ may have an ataxia of different origin and alleles in this range should be interpreted with care. Some cases with $GAA_{250\text{-}299}$, particularly those who present with the key phenotype, could still have true *FGF14* ataxia indicating reduced penetrance for these alleles, as previously suggested.[6,7] The size of the GAA could affect the degree of repression of transcription like in Friedreich ataxia GAA.[21] Additional genetic factors or digenism could be involved, as demonstrated for SCA48/*STUB1* where the full phenotype strongly depends on the presence of an intermediate allele at the SCA17/*TBP* locus.[22,23]

The key phenotype of SCA27B includes onset above 45 years, episodic cerebellar signs, and downbeat nystagmus, as previously reported.[6–9] An additional feature was the absence of dysarthria as previously described.[12,19] A recent study also highlighted the absence of dysautonomia, especially of orthostatic hypotension, which could be added as key for isolated cases with $GAA_{\geq 300}$.[9]

We recorded eye movement in 22 patients with $GAA_{\geq 300}$ in favour of involvement of the flocculus with downbeat nystagmus (68%) most frequently and vertical alteration of the pursuit (89%). This is not specific to SCA27B but allows us to distinguish the phenotype from polyQ SCAs.[24,25]

The fact that SCA27B is associated with late-onset and mild progression is well illustrated by the median age at death (81.4 years) close to that of the general French population (82.4 years).[26] The survival of SCA27B patients is preserved when compared with the decreased survival in SCAs with polyQ expansions and even in ataxias with conventional mutations in the most frequent genes.[18] This is supported by the disease-free survival which is longer in SCA27B than in sporadic late-onset ataxia including MSA, *RFC1*, and *SPG7*.[9]

There was no correlation between the size of the GAA repeat and clinical features (age at onset or death, SARA score) contrasting with discovery papers which found inverse correlation with age at onset and GAA

size.[6,7] Similarly, there is no evidence for a more severe phenotype in patients carrying SCA27B expansions on both alleles.

Although instability of large alleles was observed in both sexes upon transmission, we confirmed that the rate of contractions and expansions was different according to the sex of the transmitting parent.[6,20] The tendency for maternal expansion is reminiscent of that described for the intronic GAA repeat which causes Friedreich ataxia.[27] Predominantly maternal increased repeat alleles are also observed in *DMPK* CTG expansions or FRAXA CGG expansions.[28,29] In contrast, greater paternal instability with a tendency to expand is observed in translated CAG repeat expansions like most polyQ SCAs, particularly SCA1, 2 and 7.[30] The excess of contractions during paternal transmissions may explain why affected fathers had only 31% of affected offspring deviating from the expected 50%. This observation is important for genetic counselling as affected fathers have a reduced risk of having affected children compared to affected mothers. In addition, instability also depends on GAA purity and the presence of a stabilizing sequence before.[6,20]

Our study is limited by the fact that 20% of index cases had not been genetically explored by WES and therefore we may have underestimated the proportion of another genetic cause. In patients with decreased vibration sense at ankle (57.5%), we were not able to distinguish between peripheral neuropathy or isolated posterior cord syndrome due to only few ENMG data (n = 16 individuals with $GAA_{\geq 300}$) and absence of sensory evoked potentials measures.[9,12]

Our study confirms that the *FGF14* GAA expansion is a frequent cause of late-onset ataxia but that the threshold for strict diagnosis should be held at 300 repeats. For expansions within the $GAA_{250\text{-}299}$ range where penetrance is reduced, the results should be interpreted with caution and in consideration of supportive criteria such as: presence of the key triad, absence of dysarthria, familial co-segregation, and whole exome/genome sequencing excluding other genetic forms. Finally, the bias against paternal transmission is important to consider for genetic counselling.

Contributors

1) Conception and design of the study: J.L.M., C.S.D., D.P., M.B., P.J.L., B.B., M.R., A.B., A.D.
2) Acquisition and analysis of data: J.L.M., C.S.D., D.P., G.Co., M.C., C.E., M.L.M, M.A., I.L.B, S.T., F.G., L.G.N., S.Fo., L.J., A.H., A.S., B.G., L.G.M., P.C., C.M., J.H., B.D., F.T., S.S., M.S.M., F.S., M.T., G.Ca., F.M., S.K., A.C., S.Fe., A.M., F.B., C.B., M.W., V.R., F.R., G.S., M.B., P.J.L., S.N., A.L.F.A., B.B., M.R., A.B., A.D.
3) Drafting a significant portion of the manuscript or figures: J.L.M., C.S.D., D.P., M.A., G.Co., M.C., A.S., B.G., C.M., J.H., B.D., G.S., P.J.L., B.B., M.R., A.B., A.D.

All authors read and approved the final version of the manuscript. J.L.M. and C.S.D., A.B. and A.D. had full access and verified the data.

Data sharing statement

Detailed data was added in the Supplementary Materials.

Declaration of interests

MA received consulting fees from Reata pharmaceuticals, Merz, AbbVie, Orkyn, Ever Pharma, Ipsen; honoraria from Reata pharmaceuticals, Merz, AbbVie, Orkyn, Ever Pharma, Ipsen and participated on an advisory board for Reata Pharmaceuticals. ILB received grants from Pfizer, Fondation Plan Alzheimer, JPND/ANR, Alector; consulting fees from Prevail Therapeutics, Alector, JEITO and participated on an advisory board of Prevail Therapeutics. CM reveived financial support for attending meetings and travel from Nutricia and participated on an advisory board of Medesis Pharma society. BD had public funding contracts for Clinical Research: Contrat de Recherche Clinique (CRC) 2021 (APHP), CRC 2023 (APHP) and Agence Régionale de Santé (ARS); received honoraria from MERZ, IPSEN Pharma, LVL Medical; received support for attending meetings from MERZ Pharma, ADELIA; participated on an advisory board of MERZ, ORION Pharma and received equipment from MERZ. SF received support for participation in national and international meetings from Alnylam. MB received honoraria for thesis examinations; is member of Australian Academy of Health and Medical Sciences Australian Learned Academies Data Internetworking Network (ALADIN) Project Steering Committee; Australian Academy of Health and Medical Sciences Reports Committee; Clinical Genomics Advisory Committee, Kinghorn Sequencing Center; Gen V Scientific Advisory Committee, Murdoch Children's Research Institute; Viertel Foundation Medical Advisory Board; Australian Academy of Health and Medical Sciences Reports Committee; Present American Epilepsy Society Basic Sciences Committee; Gen V Bioresource Genetics Working Group. AD received grants from Biogen, WAVELIFE, ROCHE, TRIPLET Therapeutics, NIH RO1 (National Institute of Health), National Hospital Clinical Research Program; consulting fees from Wavelife science, ROCHE, TRIPLET Therapeutics, Pfizer, ASKBIO, Genome Quebec, VICO therapeutics; participated on an advisory board for REATA; is the president of the Société Francophone de Neurogénétique.

Acknowledgements

We thank the participants for their dedication to research and the SPATAX network. We thank Katja Lohmann and Mirja Thomsen from Institute of Neurogenetics at University of Lübeck and University Hospital of Schleswig–Holstein for sharing methodological insights. Special thanks to the DNA and cells bank of the Paris Brain Institute (ICM). We also thank Biomics Platform (C2RT, Institut Pasteur, Paris, France), supported by France Génomique (ANR-10-INBS-09) and IBISA with Marc Monot and Laurence Ma. This work benefited from equipment and services from the iGenSeq core facility, at Paris Brain Institute (ICM). This work was supported by the Fondation pour la Recherche Médicale, grant number 13338 to JLM and the Association Connaitre les Syndrome Cérébelleux—France (to GS) and by the European Union's Horizon 2020 research and innovation programme under grant agreement No 779257 ("SOLVE-RD" to GS). DP holds a Fellowship award from the Canadian Institutes of Health Research (CIHR). SK received a grant (01GM1905C) from the Federal Ministry of Education and Research, Germany, through the TreatHSP network. This work also was supported by the Australian Government National Health and Medical Research Council grants (GNT2001513 and MRFF2007677) to MB and PJL.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104931.

References

1 Coarelli G, Coutelier M, Durr A. Autosomal dominant cerebellar ataxias: new genes and progress towards treatments. *Lancet Neurol*. 2023;22:735–749.
2 Klockgether T, Mariotti C, Paulson HL. Spinocerebellar ataxia. *Nat Rev Dis Primer*. 2019;5:24.
3 Durr A. Autosomal dominant cerebellar ataxias: polyglutamine expansions and beyond. *Lancet Neurol*. 2010;9:885–894.
4 Cunha P, Petit E, Coutelier M, et al. Extreme phenotypic heterogeneity in non-expansion spinocerebellar ataxias. *Am J Hum Genet*. 2023;110:1098–1109.
5 *Online mendelian inheritance in man*; 2023. https://omim.org/. Accessed June 1, 2023.
6 Pellerin D, Danzi MC, Wilke C, et al. Deep intronic FGF14 GAA repeat expansion in late-onset cerebellar ataxia. *N Engl J Med*. 2023;388:128–141.
7 Rafehi H, Read J, Szmulewicz DJ, et al. An intronic GAA repeat expansion in FGF14 causes the autosomal-dominant adult-onset ataxia SCA50/ATX-FGF14. *Am J Hum Genet*. 2023;110:105–119.
8 Bonnet C, Pellerin D, Roth V, et al. Optimized testing strategy for the diagnosis of GAA-FGF14 ataxia/spinocerebellar ataxia 27B. *Sci Rep*. 2023;13:9737.
9 Wirth T, Clément G, Delvallée C, et al. Natural history and phenotypic spectrum of GAA-FGF14 sporadic late-onset cerebellar ataxia (SCA27B). *Mov Disord*. 2023. https://doi.org/10.1002/mds.29560.
10 Hengel H, Pellerin D, Wilke C, et al. As frequent as polyglutamine spinocerebellar ataxias: SCA27B in a large German autosomal dominant ataxia cohort. *Mov Disord*. 2023;38:1557–1558.
11 Biadun M, Sochacka M, Karelus R, et al. FGF homologous factors are secreted from cells to induce FGFR-mediated anti-apoptotic response. *FASEB J*. 2023;37:e23043.
12 Wilke C, Pellerin D, Mengel D, et al. GAA-FGF14 ataxia (SCA27B): phenotypic profile, natural history progression and 4-aminopyridine treatment response. *Brain J Neurol*. 2023:awad157.
13 Huin V, Coarelli G, Guemy C, et al. Motor neuron pathology in CANVAS due to RFC1 expansions. *Brain J Neurol*. 2022;145:2121–2132.
14 Méreaux J-L, Davoine C-S, Coutelier M, et al. Fast and reliable detection of repeat expansions in spinocerebellar ataxia using exomes. *J Med Genet*. 2023;jmg:2022–108924.
15 Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and genomics and the association for molecular pathology. *Genet Med*. 2015;17:405–424.
16 R Core Team. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing; 2023. https://www.R-project.org/.
17 Wickham H. *ggplot2: elegant graphics for data analysis*. Springer-Verlag New York; 2016. https://ggplot2.tidyverse.org.
18 Monin M, Montcel S, Marelli C, et al. Survival and severity in dominant cerebellar ataxias. *Ann Clin Transl Neurol*. 2015;2:202–207.
19 Pellerin D, Wilke C, Traschütz A, et al. Intronic FGF14 GAA repeat expansions are a common cause of ataxia syndromes with neuropathy and bilateral vestibulopathy. *J Neurol Neurosurg Psychiatry*. 2023;jnnp:2023–331490.
20 Pellerin D, Gobbo GD, Couse M, et al. A common flanking variant is associated with enhanced meiotic stability of the FGF14 -SCA27B locus. *bioRxiv*. 2023;2023.
21 Krasilnikova MM, Kireeva ML, Petrovic V, Knijnikova N, Kashlev M, Mirkin SM. Effects of Friedreich's ataxia (GAA)n*(TTC)n repeats on RNA synthesis and stability. *Nucleic Acids Res*. 2007;35:1075–1084.
22 Barbier M, Davoine C-S, Petit E, et al. Intermediate repeat expansions of TBP and STUB1: genetic modifier or pure digenic inheritance in spinocerebellar ataxias? *Genet Med*. 2023;25:100327.
23 Magri S, Nanetti L, Gellera C, et al. Digenic inheritance of STUB1 variants and TBP polyglutamine expansions explains the incomplete penetrance of SCA17 and SCA48. *Genet Med*. 2022;24:29–40.
24 Rivaud-Pechoux S, Dürr A, Gaymard B, et al. Eye movement abnormalities correlate with genotype in autosomal dominant cerebellar ataxia type I. *Ann Neurol*. 1998;43:297–302.
25 Alexandre MF, Rivaud-Péchoux S, Challe G, Durr A, Gaymard B. Functional consequences of oculomotor disorders in hereditary cerebellar ataxias. *Cerebellum*. 2013;12:396–405.
26 Chevreul K, Berg Brigham K, Durand-Zaleski I, Hernandez-Quevedo C. France: Health system review. *Health Syst Transit*. 2015;17:1–218.
27 Monrós E, Moltó MD, Martínez F, et al. Phenotype correlation and intergenerational dynamics of the Friedreich ataxia GAA trinucleotide repeat. *Am J Hum Genet*. 1997;61:101–110.
28 Joosten IBT, Hellebrekers DMEI, de Greef BTA, et al. Parental repeat length instability in myotonic dystrophy type 1 pre- and protomutations. *Eur J Hum Genet*. 2020;28:956–962.
29 Nolin SL, Glicksman A, Tortora N, et al. Expansions and contractions of the FMR1 CGG repeat in 5,508 transmissions of normal, intermediate, and premutation alleles. *Am J Med Genet*. 2019;179:1148–1156.
30 Stevanin G, Dürr A, Brice A. Clinical and molecular advances in autosomal dominant cerebellar ataxias: from genotype to phenotype and physiopathology. *Eur J Hum Genet*. 2000;8:4–18.