Movement Disorders
CLINICAL PRACTICE

BRIEF REPORT

Detection of *ATXN2* Expansions in an Exome Dataset: An Underdiagnosed Cause of Parkinsonism

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Abstract: Background: CAG-repeat expansions in *Ataxin 2* (*ATXN2*) are known to cause spinocerebellar ataxia type 2 (SCA2), but CAA interrupted expansions may also result in autosomal dominant Parkinson's disease (AD PD). However, because of technical limitations, such expansions are not explored in whole exome sequencing (WES) data.

Objectives: To identify *ATXN2* expansions using WES data from PD cases.

Methods: We explored WES data from a cohort of 477 index cases with PD using ExpansionHunter (Illumina DRAGEN Bio-IT Platform, San Diego, CA). Putative expansions were confirmed by combining polymerase chain reaction and fragment length analysis followed by sub-cloning and sequencing methods.

Results: Using ExpansionHunter, we identified three patients from two families with AD PD carrying either *ATXN2* 22/39 or 22/37 repeats, both interrupted by four CAA repeats.

Conclusion: These findings demonstrate the usefulness of WES to detect pathogenic CAG repeat expansions, which were found in 1.7% of AD PD in the *ATXN2* gene in our exome dataset.

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a triad of symptoms: akinesia, rigidity, and rest tremor, and is associated with a good response to levodopa therapy. These symptoms are because of the degeneration of dopaminergic neurons of the substantia nigra secondary to the accumulation of aggregated α-synuclein.[1–3]

Although the cause of PD is commonly sporadic, Mendelian forms account for 5% to 10% of cases.[4] Disease causing variants, mostly in *SNCA, LRRK2*, and *VPS35* have been identified in patients with autosomal dominant (AD) PD. In addition, we and others have previously described families with heterozygous expansions in *Ataxin 2* (*ATXN2*) presenting with predominant parkinsonian symptoms and in some cases with typical AD PD.[5–11] As in ataxic forms of spinocerebellar ataxia type 2 (SCA2), the expanded allele contained >33 repeats, but in PD, they were often interrupted by one or more CAA codons.[12]

Next-generation sequencing (NGS) has proven to be of great diagnostic value in clinical practice,[13] but until recently, was thought to have a limited ability to assess for loci containing repeat expansions. Over the last few years, several bioinformatic tools for genome-wide genotyping of short tandem repeats (STRs) in short read sequencing data, mainly from whole genome sequencing (WGS) data have been developed.[14–17] However, despite the extensive application of whole exome

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sequencing (WES) in routine diagnostic genetic testing and the publication of several STR detection studies, STRs have not been systematically looked for in these data. Furthermore, large-scale assessments of the diagnostic potential of STR detection from WES have not been undertaken yet in the context of PD. In this study, we assessed the clinical use of detecting the most common PD-related *ATXN2* expansions using the ExpansionHunter software,[15,18] which can estimate the repeat size in the target loci in WES data from a large undiagnosed cohort enriched in familial and early-onset PD patients.

Patients and Methods

Patients

We selected a total of 477 PD index cases (192 females, 285 males), recruited between 1990 and 2021 for whom WES data were available. In addition, WES data were also available for 98 affected relatives for co-segregation analyses.

Clinical assessment was performed by specialists in movement disorders, mainly through the French Parkinson Disease Genetics Network[19,20] (the PDG group). Diagnosis of parkinsonism was established according to the United Kingdom Parkinson's Disease Society Brain Bank (PDSBB) criteria.[21] We excluded all patients with mutations in known genes causing or related to PD/parkinsonism.[19,20] Written informed consent was obtained from all participants, and genetic studies were approved by local ethics committees.

Methods

Exons were captured using different enrichment kits: the Roche Seqcap Ez MedExome (Roche Diagnostics Corporation, Indianapolis, IN) (n = 139) or the Human Twist exome refseq 40 Mb (Twist Biosciences, San Francisco, CA) (n = 338) kits followed by 150-bp paired-end sequencing performed on Ilumina NovaSeq 6000 instrument (Illumina Inc, San Diego, CA). Mean coverage was 104.6× (range, 51.1–216.4×) and 25, 30, 50-fold average sequencing depth was achieved across 73.1% (range, 55.6–102.8%), 93% (range, 51.1–205.1%) and 111.1% (range, 56.8–216.4%) of targeted regions, respectively. Read alignment and variant calling were made using an in-house pipeline. Briefly, FastQC was used to check the quality of the reads and low-quality reads were removed using Trimmomatic. Sequencing data were then aligned to the human reference genome hg19 using the bwa[22,23] suite and variant calling was performed using the HaplotypeCaller function of GATK suite or using Dragen (Illumina). Mutations in known PD and related parkinsonism genes (Table S1) were excluded using VarAFT software[24] (version 2.17–1) and Illumina DRAGEN BioIT Platform v3.9. Exomes alignment files were then analyzed with ExpansionHunter v.5.0.0 Illumina DRAGEN Bio-IT Platform (Illumina, San Diego, CA) for targeted screening of CAG-repeat in *ATXN2*.

Molecular Analysis

All likely expanded *ATXN2* alleles (>33 repeats) were confirmed using polymerase chain reaction (PCR) and fragment length analysis. To further verify the number of CAG repeats and assess the presence of CAA interruptions, the amplified fragments were cloned into a pJET1,2/blunt x-plasmid (ThermoFisher Scientific, Waltham, MA) and sequenced bi-directionally, using primer pairs: forward (5′-CGTGCGAGCCGGTGTATGGG-3′) and reverse (5′-GGCGACGCTAGAAGGCCGCT-3′).

Violin plots representing the distribution of CAG-repeat size according to the different exome enrichment kits were plotted in R v.4.1.1 using ggplot2.[25,26]

Results

Patients' Characteristics

The cohort consisted of 203 familial PD index cases 121 with at least two cases in two generations compatible with AD inheritance, 82 with at least two affected siblings compatible with autosomal recessive (AR) inheritance and 274 isolated cases. Of them, 223 were from Europe, 31 from North Africa and 223 of unknown origin. Mean age at onset of motor signs was 41 ± 12 years (range, 3–83 years) (Table S2).

Exonic Expansion Detected in WES Data

Using different exome enrichment kits for library preparation, we found that the mean coverage of the *ATXN2* region depended on the enrichment kit used. As a result, the mean coverage for the Roche Seqcap Ez MedExome was 60.67 ± 27.86× (range, 28–181×) and of 85.13 ± 48.75× (range, 5–367×) for the Human Twist exome refseq 40 Mb.

CAG-repeat lengths in index cases were estimated using ExpansionHunter. The mean estimated CAG-repeat allele length in WES data obtained with the Roche Seqcap Ez MedExome kit was 22.27 ± 2.22 (range, 15–52), and of 22.2 ± 1.24 (range, 17–39) with the Human Twist exome refseq 40 Mb enrichment capture kit (Fig. 1). Of the 477 PD index cases, expanded CAG-repeat alleles were detected in two, both from AD PD families for whom the *ATXN2* repeat size was within the pathogenic range (≥33 CAG-repeat) (Fig. 1). No instances of *ATXN2* expansions were detected among the 274 isolated cases or the 82 AR families. Estimated confidence intervals of the *ATXN2* CAG-repeat allele sizes were 22-22/39-39 for index case A-1 and 22-22/41-61 for index case B-2, accounting for 1.7% of our series of 121 AD PD families. Co-segregation analysis of additional affected relatives in family B for whom WES data was available showed an expanded allele length of 22–22/40–52 in the affected proband's uncle (individual 1), but normal CAG-repeat alleles (22/22) in the proband's aunt (individual 4) (Fig. 2A).

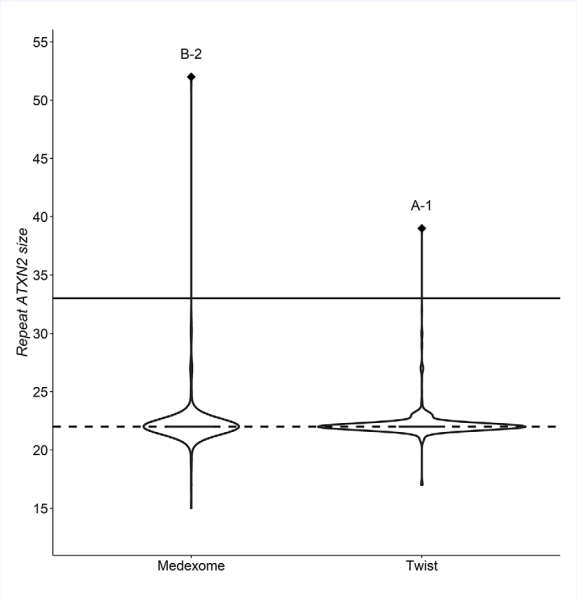


FIG. 1. Estimated ATXN2 repeat size using ExpansionHunter according to the enrichment kits used. The solid line delineates the mean ATXN2 CAG-repeat size to 22, estimated from Caucasian populations; the dashed line indicates the pathological threshold up to 32.

PCR and fragment analysis confirmed the pathogenic CAG-repeat allele size in all three patients, although it was overestimated by ExpansionHunter in family B (Fig. 2A). Subcloning and sequencing analysis revealed the presence of four CAA interruptions within the pathogenic CAG-repeat expanded fragment for all three patients: 39 repeats, $(CAG)_8$-CAA-$(CAG)_4$-CAA-$(CAG)_4$-CAA-$(CAG)_9$-CAA-$(CAG)_{10}$ for patient A-1 and 37 repeats, $(CAG)_8$-CAA-$(CAG)_9$-CAA-$(CAG)_4$-CAA-$(CAG)_4$-CAA-$(CAG)_8$ for patients B-1 and B-2 (Fig. 2B).

Clinical Features

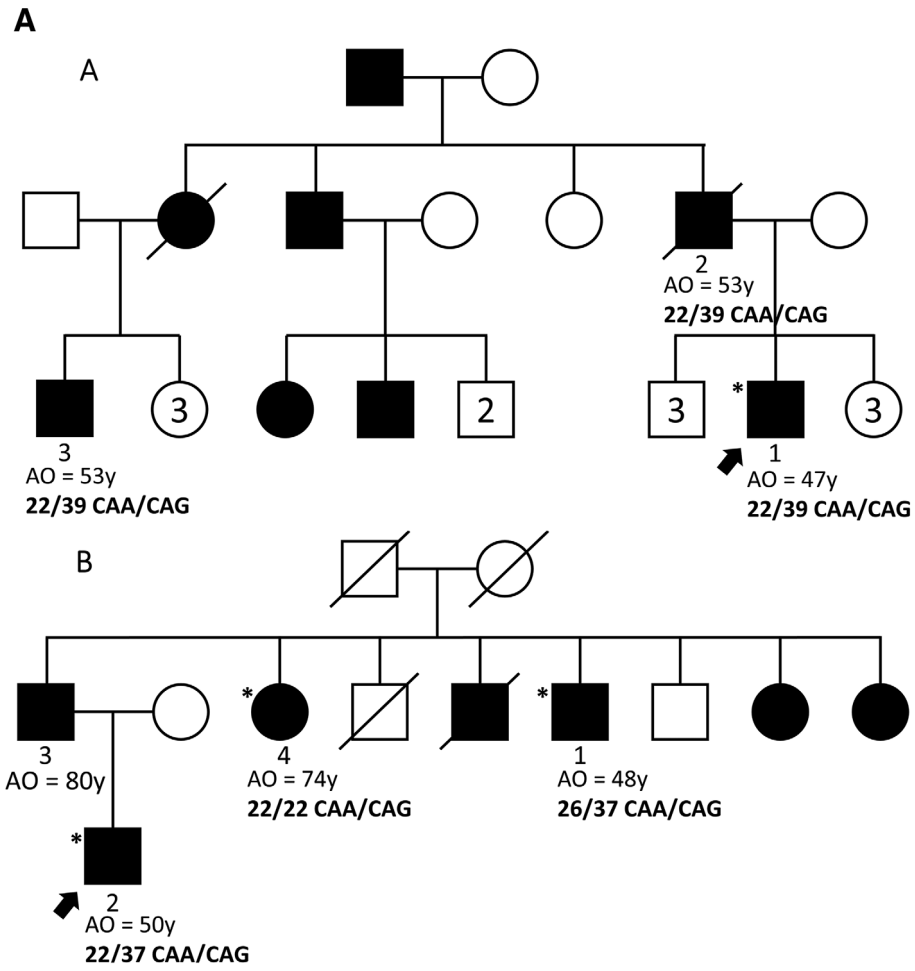
Patient A-1 had a diagnosis of PD, but with an atypical initial presentation with a slowly progressive feeling of discomfort of the lower limbs, predominantly on the right side, at age 47. At age 57, he had asymmetric brisk reflexes of the four limbs and discrete bradykinesia predominantly in the right lower limb. No rest tremor was observed. The dopamine transporter (DAT) scan performed at age 56 showed asymmetrical decreased uptake in the caudate nucleus and putamen. The magnetic resonance imaging (MRI) was normal, with no cerebellar atrophy reported. L-dopa treatment only partially relieved his symptoms. He had spasticity with preserved strength, other than just mild weakness in the right lower limb, and became wheelchair bound at the age of 58. At age 58, there was an increase in upper limb rigidity and the development of mild dysarthria, which was not L-dopa-responsive. He had increased body mass index (BMI) and presented an obstructive sleep apnea syndrome that required positive airway pressure therapy. His father (A-2) had a L-dopa-responsive akineto-rigid syndrome with a marked asymmetric resting tremor and was diagnosed with typical PD. His cousin (A-3) developed an asymmetric akineto-rigid syndrome at age 53, which responded well to L-dopa therapy and was diagnosed with PD. The presence of an *ATXN2* expanded allele was confirmed by genetic testing in the clinical setting in his father and his cousin.

The two patients from family B (patients 1 and 2) with *ATXN2* expanded alleles presented with typical L-dopa-responsive parkinsonism, diagnosed at ages 48 and 50, respectively. A DAT scan performed in patient B-2 confirmed dopaminergic denervation. In patient B-1, the positive response to treatment was quantified with an Unified Parkinson's Disease Rating Scale III score of 36 on "*off*" state, which decreased to 14 on medication. Interestingly, the third affected relative was also diagnosed with PD at an older age of onset (74 years), but with normal *ATXN2* repeat alleles, suggesting that she had idiopathic PD unrelated to the familial *ATXN2*-related PD. No signs of ataxia or oculomotor abnormality were reported in any of these three patients.

Discussion

WES has been used in multiple settings as a first-line diagnostic test for several neurological disorders, but was previously thought to have a low ability to detect repeat expansions. Several tools have been recently developed to detect repeat expansions from whole exome/genome sequencing in the research setting. We used ExpansionHunter here, which was previously reported as the most sensitive and specific bioinformatics tool,[27] to genotype *ATXN2* CAG-repeats in our WES data from a large cohort enriched of undiagnosed familial and early-onset PD patients. Using the same setting, Méreaux et al,[28] compared the results of ExpansionHunter with the sizing of the *ATXN2* repeats by PCR followed by capillary electrophoresis in 247 ataxic patients and found that both specificity and sensitivity reached 100%. Our data showed that WES combined with ExpansionHunter can reliably distinguish between non-expanded and expanded alleles at the *ATXN2* locus. However, a good coverage (>10×) is required, which might depend on the enrichment kit used.

In this study, we identified two AD PD families with *ATXN2* expanded alleles, accounting for 1.7% (2/121) of AD PD in our cohort, consistent with our previous study[5] (2%) and that of another Caucasian population[10] (1.5%). In these studies, PD patients with *ATXN2* expansions were only observed in AD PD cases. Indeed, *ATXN2* expansion frequency varies depending on ethnicity and family history of parkinsonism: up to 2.5% in Caucasians and up to 8.7% in Asians in familial PD, but is much lower in sporadic cases (ranging from 0% to 2.2% in all populations).[29] SCA2-parkinsonism is associated with relatively short *ATXN2* CAG-repeat expansion ranging from 33 to 43 in exon 1 of *ATXN2*, but with one to four CAA interruptions. In comparison, patients presenting with pure SCA2-ataxia harbor a wider range of expansion sizes from 32 to over 200, usually



B

Patient	Repeat length	Repeat structure
A-1 A-2 A-3	39	○○○○○○○○●○○○○●○○○○●○○○○○○○○○●○○○○○○○○○○
B-1 B-2	37	○○○○○○○○●○○○○○○○○○●○○○○●○○○○●○○○○○○○○

FIG. 2. Pedigrees of families A and B with autosomal dominant Parkinson's disease (AD PD) and expanded CAG-repeat alleles in *ATXN2*. **A**: Pedigrees of families A and B. Square = male; circles = female; black symbol = affected; white symbol = unaffected; slashed symbol = deceased. Asterisks indicate individuals with genetic testing and arrow, the proband. AO, age at onset. **B**: Length and structure of the expanded CAG/CAA repeats in *ATXN2* for the three carriers. Open circles = CAG; filled circles = CAA interruptions.

without CAA interruptions.[5] Interestingly, in the last years, several studies have shown that intermediate repeat size (29–32) in *ATXN2* could act as genetic risk factors or modifiers in other neurodegenerative diseases, such as amyotrophic lateral sclerosis, frontotemporal dementia, progressive supranuclear palsy, Alzheimer's disease, and multisystem atrophy.[30–35]

In conclusion, our findings provide guidance for the implementation of *ATXN2* CAG-repeat analysis in routine diagnostic clinical exome sequencing. Moreover, our results show that systematic STR evaluation may increase diagnostic yield of exome sequencing by identifying SCA2 Parkinsonism cases. This could contribute to a significant percentage of AD PD families, who might have been previously undiagnosed because of the lack of systematic testing.

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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; (4) Clinical investigation.

F.C.: 1A, 1C, 3A, 3B
T.C.: 1A
C.T.:1A, 1C, 3B
M.F.: 1C
S.N.: 1C
A.L.F.A.: 1C
T.G.: 1A
J.G.: 1A
M.A.: 4
L.L.M.: 3B, 4.
N.L.F.: 4
C.T.: 4
J.C.C.: 1B, 3B, 4
S.L.: 1A, 1B, 3B
A.B.: 1A, 1B, 3B, 4

Study Group: The French clinicians' network for Parkinson's disease genetics (the PDG group) members: 4. All authors have read and agreed to the published version of the manuscript.

Disclosures

Ethical Compliance Statement: Informed consent was obtained from all participants, and genetic studies were approved by local ethics committees (INSERM, CCPPRB du Groupe Hospitalier Pitié-Salpêtrière, Paris, France, No. 44814). 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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References

1. de Lau LM, Breteler MM. Epidemiology of Parkinson's disease. *Lancet Neurol* 2006;5(6):525–535. https://doi.org/10.1016/S1474-4422(06)70471-9.
2. Spillantini MG, Schmidt ML, Lee VMY, Trojanowski JQ, Jakes R, Goedert M. α-Synuclein in Lewy bodies. *Nature* 1997;388(6645):839–840. https://doi.org/10.1038/42166.
3. Braak H, Del Tredici K, Rüb U, de Vos RAI, Jansen Steur ENH, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* 2003;24(2):197–211. https://doi.org/10.1016/s0197-4580(02)00065-9.
4. Lunati A, Lesage S, Brice A. The genetic landscape of Parkinson's disease. *Rev Neurol* 2018;174(9):628–643. https://doi.org/10.1016/j.neurol.2018.08.004.
5. Charles P, Camuzat A, Benammar N, et al. Are interrupted SCA2 CAG repeat expansions responsible for parkinsonism? *Neurology* 2007;69(21):1970–1975. https://doi.org/10.1212/01.wnl.0000269323.21969.db.
6. Kim JM, Hong S, Kim GP, et al. Importance of low-range CAG expansion and CAA interruption in SCA2 Parkinsonism. *Arch Neurol* 2007;64(10):1510–1518. https://doi.org/10.1001/archneur.64.10.1510.
7. Lu CS, Wu Chou YH, Kuo PC, Chang HC, Weng YH. The parkinsonian phenotype of spinocerebellar ataxia type 2. *Arch Neurol* 2004;61(1):35–38. https://doi.org/10.1001/archneur.61.1.35.
8. Payami H, Nutt J, Gancher S, et al. SCA2 may present as levodopa-responsive parkinsonism. *Mov Disord* 2003;18(4):425–429. https://doi.org/10.1002/mds.10375.
9. Shan DE, Liu RS, Sun CM, Lee SJ, Liao KK, Soong BW. Presence of spinocerebellar ataxia type 2 gene mutation in a patient with apparently sporadic Parkinson's disease: clinical implications. *Mov Disord* 2004;19(11):1357–1360. https://doi.org/10.1002/mds.20212.
10. Simon-Sanchez J, Hanson M, Singleton A, et al. Analysis of SCA-2 and SCA-3 repeats in Parkinsonism: evidence of SCA-2 expansion in a family with autosomal dominant Parkinson's disease. *Neurosci Lett* 2005;382(1–2):191–194. https://doi.org/10.1016/j.neulet.2005.03.015.
11. Wang JL, Xiao B, Cui XX, et al. Analysis of SCA2 and SCA3/MJD repeats in Parkinson's disease in mainland China: Genetic, clinical, and positron emission tomography findings. *Mov Disord* 2009;24(13):2007–2011. https://doi.org/10.1002/mds.22727.
12. Coarelli G, Brice A, Durr A. Recent advances in understanding dominant spinocerebellar ataxias from clinical and genetic points of view. *F1000Research* 2018;7:F1000 Faculty Rev-1781. https://doi.org/10.12688/f1000research.15788.1.
13. Vissers LELM, van Nimwegen KJM, Schieving JH, et al. A clinical utility study of exome sequencing versus conventional genetic testing in pediatric neurology. *Genet Med* 2017;19(9):1055–1063. https://doi.org/10.1038/gim.2017.1.
14. Dashnow H, Lek M, Phipson B, et al. STRetch: detecting and discovering pathogenic short tandem repeat expansions. *Genome Biol* 2018;19(1):121. https://doi.org/10.1186/s13059-018-1505-2.
15. Dolzhenko E, van Vugt JJFA, Shaw RJ, et al. Detection of long repeat expansions from PCR-free whole-genome sequence data. *Genome Res* 2017;27(11):1895–1903. https://doi.org/10.1101/gr.225672.117.

16. Halman A, Oshlack A. Accuracy of short tandem repeats genotyping tools in whole exome sequencing data. *F1000Research* 2020;9:200. https://doi.org/10.12688/f1000research.22639.1.

17. Tankard RM, Bennett MF, Degorski P, Delatycki MB, Lockhart PJ, Bahlo M. Detecting expansions of tandem repeats in cohorts sequenced with short-read sequencing data. *Am J Hum Genet* 2018;103(6):858–873. https://doi.org/10.1016/j.ajhg.2018.10.015.

18. Dolzhenko E, Deshpande V, Schlesinger F, et al. ExpansionHunter: a sequence-graph-based tool to analyze variation in short tandem repeat regions. *Bioinformatics* 2019;35(22):4754–4756. https://doi.org/10.1093/bioinformatics/btz431.

19. Lesage S, Houot M, Mangone G, et al. Genetic and phenotypic basis of autosomal dominant Parkinson's disease in a large multi-center cohort. *Front Neurol* 2020;11:11. Accessed September 6, 2022. https://www.frontiersin.org/articles/10.3389/fneur.2020.00682.

20. Lesage S, Lunati A, Houot M, et al. Characterization of recessive Parkinson disease in a large multicenter study. *Ann Neurol* 2020;88(4):843–850. https://doi.org/10.1002/ana.25787.

21. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry* 1992;55(3):181–184. https://doi.org/10.1136/jnnp.55.3.181.

22. Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* 2009;25(14):1754–1760. https://doi.org/10.1093/bioinformatics/btp324.

23. Li H, Durbin R. Fast and accurate long-read alignment with Burrows–Wheeler transform. *Bioinformatics* 2010;26(5):589–595. https://doi.org/10.1093/bioinformatics/btp698.

24. Desvignes JP, Bartoli M, Delague V, Krahn M, Miltgen M, Béroud C, Salgado D. VarAFT: a variant annotation and filtration system for human next generation sequencing data. *Nucleic Acids Res* 2018;46(W1):W545–W553. https://doi.org/10.1093/nar/gky471.

25. R Core Team. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing; 2022. https://www.R-project.org/.

26. Wickham H. *Ggplot2: elegant graphics for data analysis*. New York: Springer-Verlag; 2016. https://ggplot2.tidyverse.org.

27. van der Sanden BPGH, Corominas J, de Groot M, et al. Systematic analysis of short tandem repeats in 38,095 exomes provides an additional diagnostic yield. *Genet Med* 2021;23(8):1569–1573. https://doi.org/10.1038/s41436-021-01174-1.

28. Méreaux JL, Davoine CS, Coutelier M, et al. Fast and reliable detection of repeat expansions in spinocerebellar ataxia using exomes. *J Med Genet* 2023;jmg-2022-108924. Online ahead of print. https://doi.org/10.1136/jmg-2022-108924.

29. Park H, Kim HJ, Jeon BS. Parkinsonism in spinocerebellar ataxia. *Biomed Res Int* 2015;2015:125273. https://doi.org/10.1155/2015/125273.

30. Corrado L, Carlomagno Y, Falasco L, et al. A novel peripherin gene (PRPH) mutation identified in one sporadic amyotrophic lateral sclerosis patient. *Neurobiol Aging* 2011;32(3):552.e1–552.e6. https://doi.org/10.1016/j.neurobiolaging.2010.02.011.

31. Lattante S, Millecamps S, Stevanin G, et al. Contribution of ATXN2 intermediary polyQ expansions in a spectrum of neurodegenerative disorders. *Neurology* 2014;83(11):990–995. https://doi.org/10.1212/WNL.0000000000000778.

32. Fournier C, Anquetil V, Camuzat A, et al. Interrupted CAG expansions in ATXN2 gene expand the genetic spectrum of frontotemporal dementias. *Acta Neuropathol Commun* 2018;6(1):41. https://doi.org/10.1186/s40478-018-0547-8.

33. Mongelli A, Sarro L, Rizzo E, et al. Multiple system atrophy and CAG repeat length: a genetic screening of polyglutamine disease genes in Italian patients. *Neurosci Lett* 2018;678:37–42. https://doi.org/10.1016/j.neulet.2018.04.044.

34. Rosas I, Martínez C, Clarimón J, et al. Role for ATXN1, ATXN2, and HTT intermediate repeats in frontotemporal dementia and Alzheimer's disease. *Neurobiol Aging* 2020;87:139.e1–139.e7. https://doi.org/10.1016/j.neurobiolaging.2019.10.017.

35. Glass JD, Dewan R, Ding J, et al. ATXN2 intermediate expansions in amyotrophic lateral sclerosis. *Brain* 2022;145(8):2671–2676. https://doi.org/10.1093/brain/awac167.

Supporting Information

Supporting information may be found in the online version of this article.

Table S1. List of Parkinson's disease-associated genes excluded from our cohort of patients

Table S2. Characteristics of the study population according to the different exome enrichment kits used