ATXN2 intermediate repeat expansions influence the clinical phenotype in frontotemporal dementia

ATXN2 intermediate repeat expansions influence the clinical phenotype in Frontotemporal Dementia

Elisa Rubino [a*], Cecilia Mancini [b*], Silvia Boschi[a, c]; Patrizia Ferrero[d], MD; Marina Ferrone[b], Stefano Bianca[a], Milena Zucca[a], Laura Orsi[c], Lorenzo Pinessi[d], Flora Govone[a]; Alessandro Vacca[a], Annalisa Gai[a], Maria Teresa Giordana[a,d], Alfredo Brusco[b,e], Innocenzo Rainero[a,d]

[a] Department of Neuroscience "Rita Levi Montalcini", University of Torino, Torino, Italy
[b] Department of Medical Sciences, University of Torino, Torino, Italy
[c] Department of Neuroscience, Psychology, Drug Research and Child Health (NEUROFARBA), University of Florence, Italy
[d] Neurology 1, AOU Città della Salute e della Scienza di Torino, Torino, Italy
[e] Medical Genetics Unit, AOU Città della Salute e della Scienza, Torino, Italy

* These authors equally contributed to the manuscript.

Corresponding author:
Elisa Rubino, MD, PhD
Department of Neuroscience "Rita Levi Montalcini", University of Torino
Via Cherasco 15, 10126 Torino, Italy.
Tel.: +39-011- 6634763
E-mail address: elisa.rubino@unito.it

ABSTRACT

Common genetic risk factors are associated with frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). Intermediate repeat expansions at the Ataxin-2 locus (*ATXN2*) are risk factor for ALS and influence the phenotype. We assessed whether *ATXN2* is a risk factor for FTD or modify clinical features in a dataset of Italian patients. Three hundred-68 unrelated FTD cases and 342 controls were enrolled. The frequency of intermediate CAG repeats in *ATXN2* gene was not different comparing patients and controls. CAG repeats were interrupted by CAA in all patients carrying intermediate repeats. Interestingly, patients with an increased number of CAG repeats had an earlier onset of the disease than those without expansions (p=0.011), and presented more frequently with parkinsonism (p=0.010), and psychotic symptoms (p=0.013) at disease onset. Our study does not support a major role of *ATXN2* intermediate CAG expansions in predisposising to FTD, but suggests that *ATXN2* may act as a phenotype modifier.

Keywords: Frontotemporal lobar degeneration, SCA2, *ATXN2*, polyQ repeats, intermediate expansions

Introduction

Several studies have showed that frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are part of the same clinical, genetic, and pathologic continuum (Van Langenhove et al., 2012). Indeed, these two disorders have a shared genetic background with genes causing either one or the other condition. Beyond Mendelian inherited forms, the great majority of cases underlay complex pattern of inheritance in both; the combination of multiple gene variants acts as predisposing factors, also behaving as genetic modifiers, able to modulate the clinical phenotype.

The boundary between causative and modifiers genes is often blurry, as exemplified by the *ATXN2* gene. A number of polyglutamine-encoding CAG triplet above 34 causes spinocerebellar ataxia type 2 (SCA2) (Pulst et al, 1998). However, intermediate repeat expansions (27-33 CAG repeats) in *ATXN2* gene have been reported as genetic risk factor or phenotype modifier in ALS (Elden et al., 2010; Lu et al., 2015). Inversely, the potential role of *ATXN2* gene repeats in FTD has been scarcely investigated (Ross et al., 2011; Lattante et al., 2014).

Interestingly, the CAG tract can be pure, or interrupted by the presence of CAA repeats. Both CAG and CAA encode for glutamine, but CAAs are known to stabilize the repeat and avoid expansions at somatic and germinal level. CAA interruptions have been identified in intermediate alleles both in healthy and ALS patients, but are lost in SCA2-associated alleles (Tsai et al., 2004; Yu et al., 2011).

Thus, the aim of this study was to evaluate the role of *ATXN2* CAG repeats in Italian patients with frontotemporal dementia.

Methods

We measured the number of CAG repeats in exon 1 of the *ATXN2* gene (NM_002973.3) by polymerase chain reaction amplification and fluorescent fragment analysis by capillary electrophoresis (ABI Prism 3730xl, Applied Biosystems), in 368 FTD patients (188 males, 180 females; mean age SD: 65.8 ± 9.3

years), attending the Department of Neuroscience, University of Torino. Three hundred forty-two healthy subjects were included as controls. Written informed consent was obtained from all participants, and the Internal Ethics review Board approved the study. CAG expansions and the interrupted sequences in intermediate alleles were verified by cloning the PCR product and Sanger sequencing (additional data in Supplemental 1).

Results

The distribution of the *ATXN2* CAG repeats in FTD patients and controls is shown in figure 1. The repeat length ranged from 14 to 32 repeats in FTD, and from 14 to 30 repeat units in controls, with 22 repeats being the most common allele. We did not find any fully expanded *ATXN2* allele. An intermediate repeat expansion was found in 18 out of 368 (4.9%) FTD patients, and in 8 out of 342 (2.3%) controls. No significant difference in the frequency of intermediate CAG expansions in *ATXN2* was found between cases and controls (p=0.07). Two patients with bvFTD were identified with a 32 allele, which was absent in our control population. When analyzing the CAG interruptions, CAG repeats in the *ATXN2* locus were interrupted by CAA in all of patients carrying intermediate repeats (additional data in Supplemental 1).

All patients carrying an intermediate expansion in *ATXN2* gene showed bvFTD phenotype at onset of disease, except one presenting with PNFA (additional data in Supplemental 1). Age at onset was lower in patients with intermediate number of CAG repeats (≥ 27) (p=0.011; mean age at onset 60.4± 3.3 years), than in those with repeats in a normal range (mean age at onset 65.7 ± 8.3 years). Interestingly, patients with intermediate repeats showed more frequently parkinsonism as the first symptom at onset when compared to patients with CAG repeats in normal range (p=0.01). Finally, FTD patients with intermediate expansions also showed more frequently psychosis at disease onset (p=0.013).

Discussion

Our study confirms previous reports suggesting that CAG intermediate repeats in the *ATXN2* gene are not likely to be associated with frontotemporal dementia. However, a detailed clinical evaluation allowed us uncovering that intermediate-length repeats may influence the clinical features of the disease. In our dataset,

carriers of intermediate repeats had a younger age at onset, and increased frequency of both parkinsonism and psychotic symptoms at the onset of the disease. This suggests that even if *ATXN2* expansions are not causative, they may act as a phenotype modifier of the FTD phenotype.

ATXN2 intermediate CAG repeats was previously reported in families with atypical parkinsonism with CAG repeats in the low expansion range and interrupted by CAA (Kim et al., 2007). Interestingly, a recent paper reported an FTD patient with TDP-43 proven pathology without pathogenic variants in known FTD genes; the patient carried an interrupted CAG expansions in *ATXN2* gene (Fournier et al., 2018). All these observations suggest that the configuration of the *ATXN2* repeat expansions might play a role in the phenotype variability, and that the presence of CAA interruptions might influence the clinical features; pathological uninterrupted *ATXN2* repeat may not have the same modifying effect as intermediate interrupted alleles.

In conclusion, we reported that *ATXN2* intermediate repeat expansions are not associated with FTD, but might influence the phenotype, being associated with an earlier age at onset, and parkinsonian, psychotic symptoms in the initial phase of the disease. Although a possible limitat of the study is its retrospective nature, we note that clinical data were recorded from neurologists without prior knowledge of patients' genetic background. Further clinical and experimental investigations are needed to analyze the role of *ATXN2* intermediate repeats in FTD.

Conflict of Interest

None of the authors has any conflict of interest to disclose.

Funding

This study was supported by Ministero dell'Istruzione, dell'Università e della Ricerca – MIUR project "Dipartimenti di Eccellenza 2018 – 2022" to Department of Neuroscience "Rita Levi Montalcini", University of Torino, and AIRAlzh Onlus-ANCC-COOP (SB).

REFERENCES

Elden, A.C., Kim, H.J., Hart, M.P., Chen-Plotkin, A.S., Johnson, B.S., Fang, X., Armakola, M., Geser, F., Greene, R., Lu, M.M., Padmanabhan, A., Clay-Falcone, D., McCluskey, L., Elman, L., Juhr, D., Gruber, P.J., Rüb, U., Auburger, G., Trojanowski, J.Q., Lee, V.M., Van Deerlin, V.M., Bonini, N.M., Gitler, A.D., 2010. Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. Nature 466, 1060-75.

Fournier, C., Anquetil, V., Camuzat, A., Stirati-Buron, S., Sazdovitch, V., Molina-Porcel, L., Turbant, S., Rinaldi, D., Sánchez-Valle, R., Barbier, M., Latouche, M., Neuro-CEB Neuropathology Network, Stevanin, G., Seilhean, D., Brice, A., Duyckaerts, C., Le Ber, I., 2018. Interrupted CAG expansions in ATXN2 gene expand the genetic spectrum of frontotemporal dementias. Acta Neuropathol Commun 6, 41.

Kim, J.M., Hong, S., Kim, G.P., Choi, Y.J., Kim, Y.K., Park, S.S., Kim, S.E, Jeon BS., 2007. Importance of low-range CAG expansion and CAA interruption in SCA2 Parkinsonism. Arch Neurol 64, 1510-8.

Lattante, S., Millecamps, S., Stevanin, G., Rivaud-Péchoux, S., Moigneu, C., Camuzat, A., Da Barroca, S., Mundwiller, E., Couarch, P., Salachas, F., Hannequin, D., Meininger, V., Pasquier, F., Seilhean, D., Couratier, P., Danel-Brunaud, V., Bonnet, A.M., Tranchant, C., LeGuern, E., Brice, A., Le Ber, I., Kabashi, E.; French Research Network on FTD and FTD-ALS, 2015. Contribution of ATXN2 intermediary polyQ expansions in a spectrum of neurodegenerative disorders. Neurology 83, 990-5.

Lu, H.P., Gan, S.R., Chen, S., et al., 2015. Intermediate-length polyglutamine in ATXN2 is a possible risk factor among Eastern Chinese patients with amyotrophic lateral sclerosis. Neurobiol Agin*g* 36, e11-1603.e4.

Pulst, S.M., Nechiporuk, A., Nechiporuk, T., at al. 1996. Moderate expansion of a normally biallelic trinucleotide repeat in spinocerebellar ataxia type 2. Nat Genet 14, 269-276.

Ross, O.A., Rutherford, N.J., Baker, M., Soto-Ortolaza, A.I., Carrasquillo, M.M., DeJesus-Hernandez, M., Adamson, J., Li, M., Volkening, K., Finger, E., Seeley, W.W., Hatanpaa, K.J., Lomen-Hoerth, C., Kertesz, A., Bigio, E.H., Lippa, C., Woodruff, B.K., Knopman, D.S., White, C.L. 3rd, Van Gerpen, J.A., Meschia, J.F., Mackenzie, I.R., Boylan, K., Boeve, B.F., Miller, B.L., Strong, M.J., Uitti, R.J., Younkin, S.G., Graff-Radford, N.R., Petersen, R.C., Wszolek, Z.K., Dickson, D.W., Rademakers, R., 2011. Ataxin-2 repeat-length variation and neurodegeneration. Hum Mol Genet 20, 3207-12.

Tsai, H.F., Liu, C.S., Leu, T.M., Wen, F.C., Lin, S.J., Liu, C.C., Yang, D.K., Li, C., Hsieh, M., 2004. Analysis of trinucleotide repeats in different SCA loci in spinocerebellar ataxia patients and in normal population of Taiwan. Acta Neurol Scand 109, 355-360.

Van Langenhove, T., van der Zee, J., Engelborghs, S., Vandenberghe, R., Santens, P., Van den Broeck, M., Mattheijssens, M., Peeters, K., Nuytten, D., Cras, P., De Deyn, PP., De Jonghe, P., Cruts, M., Van Broeckhoven, C., 2012. Ataxin-2 polyQ expansions in FTLD-ALS spectrum disorders in Flanders-Belgian cohorts. Neurobiol Aging 16, e17-20.

Yu, Z., Zhu, Y., Chen-Plotkin, A.S., Clay-Falcone, D., McCluskey, L., Elman, L., Kalb, R.G., Trojanowski, J.Q., Lee, V.M., Van Deerlin, V.M., Gitler, A.D., Bonini, N.M., et al. 2011. PolyQ repeat expansions in ATXN2 associated with ALS are CAA interrupted repeats. PLoS One 6, e17951.

Supplementary Material

Analysis of *ATXN2* intermediate repeat expansions in Italian patients with Frontotemporal Dementia

Rubino E., Mancini C., Ferrero P., Ferrone M., Boschi S., Bianca S., Zucca M., Orsi L., Gentile S., Pinessi L., Govone F., Vacca A., Gai A., Giordana M.T., Brusco A., Rainero I.

Introduction

Several studies have recently showed that Frontotemporal Dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS) are part of the same clinical, genetic, and pathologic continuum (Van Langenhove et al., 2012; Hardy and Rogaeva, 2014; Burrel et al., 2016, Nguyen et al, 2018). Indeed, these two disorders have a shared genetic background with genes causing either one or the other condition. In both, beyond Mendelian inherited forms, the great majority of cases underlay complex pattern of inheritance, where the combination of multiple gene variants acts as predisposing factors, also behaving as genetic modifiers, able to modulate the clinical phenotype.

The boundary between causative and modifiers genes is often blurry, as exemplified by the *ATXN2* gene. The ataxin-2 (*ATXN2*) gene is located on 12q24.12 and encodes ataxin, a multifunctional protein implicated in several cellular activities like mRNA processing, and endocytosis (Nonis et al., 2005; Blokhuis et al., 2016). A number of polyglutamine-encoding CAG triplet above 34 causes spinocerebellar ataxia type 2 (SCA2) (Pulst et al., 1996). However, intermediate repeat expansions (27-33 CAG repeats) in *ATXN2* gene have been reported as genetic risk factor or phenotype modifier in ALS (Elden et al., 2010; Lu et al., 2015). Contrariwise, the potential role of *ATXN2* gene repeats in FTD has been scarcely investigated (Ross OA et al., 2011; Van Langenhove et al., 2012; Lattante et al., 2015).

Interestingly, the CAG tract can be pure, or interrupted by the presence of CAA repeats. Both CAG and CAA encode for glutamine, but CAAs are known to stabilize the repeat and avoid expansions at somatic and

germinal level. CAA interruptions have been identified in intermediate alleles both in healthy and ALS patients, but are lost in SCA2-associated alleles (Chouldry et al., 2001; Tsai et al., 2004; Yu et al., 2011).

Therefore, the aim of this study was to evaluate the role of *ATXN2* CAG repeats in Italian patient with Frontotemporal Dementia.

Material and methods

Participants

We enrolled 368 unrelated patients with FTD (188 men, 180 women; mean age± SD= 65.8± 9.3 years), attending the Memory Clinic of the Department of Neuroscience "Rita Levi Montalcini" of the University of Torino (Italy). The diagnosis of FTD was made according to Neary et al. (1998) criteria with 249 patients fulfilled the diagnostic criteria for behavioural variant frontotemporal dementia (bvFTD), 62 for semantic dementia (SD), and 57 for progressive nonfluent aphasia (PNFA). Thirteen percent of FTD patients developed signs of motor neuron disease. Positive family history was found in 41% of the patients. Patients with corticobasal degeneration and progressive supranuclear palsy were not included in the study. Three hundred forty-two healthy subjects were included as controls. Patients and controls were of Caucasian origin and came from the same area of Northern Italy. Written informed consent was obtained from all participants, and the Internal Ethics review Board approved the study.

Genetic analysis of ATXN2

Genomic DNA was isolated from peripheral blood leukocytes using the Gene Eluate Blood Genomic DNA Kit (SIGMA-ALDRICH, USA) according to manufacturer's protocols. We measured the number of CAG repeats in exon 1 of the *ATXN2* gene (NM_002973.3) by polymerase chain reaction amplification and fluorescent-fragment analysis on an ABI PRISM 3130x1 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and GeneMapper version 3.9 software. In accordance with previous reports, the following

categorization for *ATXN2* polyQ was used: normal range (<27 repeats), intermediate (27-33 repeats), and expanded (≥34 repeats) alleles (Malandrini et al., 1998; Tsai HF et al., 2004).

To verify the CAG expansion and the interrupted sequences in alleles showing >27 CAG repeats, PCR amplicons were subcloned using a pGEM T-easy vector system (Promega, Madison, WI, USA) in JM109 Competent Cells (Promega). Blue/white screening was used to detect colonies in which PCR were correctly ligated. A total of ten colonies for each PCR were grown in liquid medium overnight and plasmid were extracted using PureYield™ Plasmid Miniprep System (Promega).

Four hundred nanograms of each plasmid were bidirectionally sequenced on an ABI PRISM 3130xl Genetic Analyzer, using the BigDye Terminator Cycle Sequencing Ready Reaction Kit version 3.1, following manufactors' instruction (Applied Biosystems). Sequences were analyzed using SeqScape version 2.5 software. All the patients were also genotyped for mutations in *MAPT*, *GRN* and for expansions in *C9orf72* genes.

Statistics

Data were analysed using SPSS 21 (Chicago, Il, USA). PolyQ repeats distributions between patients and controls were analysed using Fisher's exact test. Differences in clinical characteristics were measured using Student t test and correlation analysis. The statistical analysis was performed using as cut off for intermediate length expansions ≥ 27, ≥ 30, ≥ 32 alleles. A $p < 0.01$ value was considered as significant for all genetic comparisons, whereas $p<0.05$ was considered significant for clinical analyses.

Results

The distribution of the *ATXN2* CAG repeats in FTD patients and controls is shown in figure 1. The repeat length ranged from 14 to 32 repeats in FTD, and from 14 to 30 repeat units in controls, with 22 repeats being the most common allele. In our samples, we did not find fully expanded *ATXN2* allele. An intermediate repeat expansion was found in 18 out of 368 (4.9%) FTD patients, and in 8 out of 342 (2.3%) controls. No

significant difference in the frequency of intermediate CAG expansions in *ATXN2* was found between cases and controls (p=0.07). Two patients with bvFTD were identified with a 32 allele, which was absent in our control population. However, when considering intermediate polyQ expansions ≥ 32, the association remained negative. No patient with *C9orf72* expansion showed the occurrence of *ATXN2* intermediate expansion (all presented with 22/22 alleles). Of the other patients carrying a known genetic mutation, all were homozygous for 22 repeats allele, except for one patient heterozygous 22/23 repeats and carrier of the p.Pro301Leu variant in *MAPT* gene (Table S1). No neuropathologic data are available for FTD patients with intermediate expansions.

When analyzing the CAG interruptions, CAG repeats in the *ATXN2* locus were interrupted by CAA in all the patients carrying intermediate repeats.

All patients carrying an intermediate expansion in *ATXN2* gene showed bvFTD phenotype at onset of disease (n=17), except one presenting with PNFA (Table S2). Age at onset was lower in patients with intermediate number of CAG repeats (≥ 27) (p=0.011; mean age at onset: 60.4± 3.3 years), than in those with repeats in a normal range (mean age at onset: 65.7± 8.3 years). Interestingly, patients with intermediate repeats showed more frequently parkinsonism as the first symptom at onset in comparison with patients carrying CAG repeats in normal range (p=0.01). Finally, FTD patients with intermediate expansions showed more frequently psychosis at disease onset (p=0.013). In our population, no FTD patient who developed motoneuron disease carried an intermediate *ATXN2* expansion.

Discussion

Our study confirms previous reports suggesting that CAG intermediate repeats in the *ATXN2* gene are not likely to be associated with Frontotemporal Dementia. However, a detailed clinical evaluation allowed us uncovering that intermediate-length repeats may influence the clinical features of the disease. In our dataset, carriers of intermediate repeats had a younger age at onset, and increased frequency of both parkinsonism and psychotic symptoms at the onset of the disease. This suggests that even if *ATXN2* expansions are not causative, they may act as a modifier of the FTD phenotype.

Parkinsonian symptoms, such as bradikynesia, rigidity and gait dysfunctions, have been described up to 70% of FTD patients, with *in vivo* imaging showing significantly reduced dopamine transporter concentrations. FTD patients carrying *MAPT* and *PGRN* mutations frequently develop parkinsonism in respect to patients with *C9orf72* expansions (Baizabal-Carvallo et al., 2016). Here, we show that *ATXN2* intermediate expansions are associated with parkinsonism at the onset of the disease. Accordingly, intermediate *ATXN2* expansions are more frequent in patients with Parkinson's disease than in controls (Yamashita et al., 2014). Furthermore, *ATXN2* intermediate polyQ was reported in families with atypical parkinsonism with CAG repeats in the low expansion range and interrupted by CAA (Kim et al., 2007). Interestingly, a recent paper reported about a FTD patient with TDP-43 proven pathology without known FTD mutations, who carried interrupted CAG expansions in *ATXN2* gene (Fournier et al., 2018). All these findings suggest that the configuration of the *ATXN2* repeat expansions might play an important role in phenotype variability, and the presence of CAA interruptions could influence the clinical characteristics, whereas pathological uninterrupted *ATXN2* repeat may not have the same modifying effect as intermediate interrupted alleles.

The clinical presentation of FTD has a wide range of symptoms, including prominent psychotic symptoms that often mimic psychiatric disorders as late-onset mania and depression (Gossink et al., 2017). Hallucinations and delusions, especially of persecution, are present in about 20% of cases and may be sometimes the presenting symptoms of the disease. In literature, *C9orf72* patients were significantly more likely to exhibit psychotic symptoms, and they occurred to some degree in *GRN* mutated cases (Block et al., 2016). Our study shows that also FTD patients carrying intermediate expansions in the *ATXN2* gene may show a psychiatric presentation, with symptoms of psychosis at disease onset.

Neurobiological mechanisms underlying the effects of intermediate *ATXN2* expansions are, at present, unclear. In transgenic mice, decrease of ataxin-2 increase the aggregation of TDP-43, a protein involved in both ALS and FTD pathogenesis. In addition, intermediate polyglutamine repeats of ATXN2 combined with C9orf72 depletion impair the autophagic pathway in neuronal cell cultures. Additional studies are needed to better elucidate the interactions between *ATXN2* and different genes involved in neurodegeneration.

We recognize a few limitations to our study. The main limitation of the study is its retrospective nature. However, clinical data were recorded from neurologists without knowledge of patients' genetic background. Secondly, the number of patients and controls is relatively small, however, patients have been enrolled in the same Memory Clinic and have been involved in a standardized clinical protocol including neuroimaging as well as CSF examination; finally, a consensus on the definition of intermediate *ATXN2* repeat expansion is not univocal and different cutoffs have been used in published papers (Malandrini et al., 1998; Tsai et al., 2004). However, to overcome this latter issue, we performed statistical analyses with different cutoffs obtaining overlapping results.

In conclusion, we reported that *ATXN2* intermediate repeat expansions are not associated with FTD, but might influence the phenotype, being associated with an earlier age, parkinsonian, and psychotic symptoms at disease onset. Additional clinical investigations are needed to further analyze the role of ATXN2 intermediate repeats in the heterogeneity of FTD clinical phenotypes.

Acknowledgements

The authors thank the participants and their relatives, the staff and all the members of the Memory Clinic, Department of Neuroscience "Rita Levi Montalcini", University of Torino.

Funding

This study was supported by Ministero dell'Istruzione, dell'Università e della Ricerca – MIUR project "Dipartimenti di Eccellenza 2018 – 2022" to Department of Neuroscience "Rita Levi Montalcini", University of Torino, and AIRAlzh Onlus-ANCC-COOP (SB).

References

Baizabal-Carvallo JF, Jankovic J. Parkinsonism, movement disorders and genetics in frontotemporal dementia. Nat Rev Neurol 2016;12:175-85.

Block, N.R., Sha, S.J., Karydas, A.M., Fong, J.C., De May, M.G., Miller, B.L., Rosen, H.J., 2016. Frontotemporal Dementia and Psychiatric Illness: Emerging Clinical and Biological Links in Gene Carriers. Am J Geriatr Psychiatry 24:107–116.

Blokhuis, A.M., Koppers, M., Groen, E.J., van den Heuvel, D.M., Dini Modigliani, S., Anink, J.J., Fumoto, K., van Diggelen, F., Snelting, A., Sodaar, P., Verheijen, B.M., Demmers, J.A., Veldink, J.H., Aronica, E., Bozzoni, I., den Hertog, J., van den Berg, L.H., Pasterkamp, R.J., 2016. Comparative interactomics analysis of different ALS-associated proteins identifies converging molecular pathways. Acta Neuropathol 132, 175-96.

Burrell, J.R., Halliday, G.M., Kril, J.J., Ittner, L.M., Götz, J., Kiernan, M.C., Hodges, J.R., 2016. The frontotemporal dementia-motor neuron disease continuum. Lancet 27, 919-31.

Choudhry, S., Mukerji, M., Srivastava, A.K., Jain, S., Brahmachari, S.K., 2001. CAG repeat instability at SCA2 locus: anchoring CAA interruptions and linked single nucleotide polymorphisms. Hum Mol Genet 10, 2437–46.

Elden, A.C., Kim, H.J., Hart, M.P., Chen-Plotkin, A.S., Johnson, B.S., Fang, X., Armakola, M., Geser, F., Greene, R., Lu, M.M., Padmanabhan, A., Clay-Falcone, D., McCluskey, L., Elman, L., Juhr, D., Gruber, P.J., Rüb, U., Auburger, G., Trojanowski, J.Q., Lee, V.M., Van Deerlin, V.M., Bonini, N.M., Gitler, A.D., 2010. Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. Nature 466, 1060-75.

Fournier, C., Anquetil, V., Camuzat, A., Stirati-Buron, S., Sazdovitch, V., Molina-Porcel, L., Turbant, S., Rinaldi, D., Sánchez-Valle, R., Barbier, M., Latouche, M., Neuro-CEB Neuropathology Network, Stevanin, G., Seilhean, D., Brice, A., Duyckaerts, C., Le Ber, I., 2018. Interrupted CAG expansions in ATXN2 gene expand the genetic spectrum of frontotemporal dementias. Acta Neuropathol Commun 6, 41.

Gossink, F.T., Vijverberg, E., Krudop, W., Scheltens, P., Stek, M.L., Pijnenburg, Y.A., Dols, A., 2017. Psychosis in behavioral variant frontotemporal dementia. Neuropsychiatr Dis Treat 13, 1099–1106.

Hardy, J., Rogaeva, E., 2014. Motor neuron disease and frontotemporal dementia: sometimes related, sometimes not. Exp Neurol 262, 75-83.

Kim, J.M., Hong, S., Kim, G.P., Choi, Y.J., Kim, Y.K., Park, S.S., Kim, S.E., Jeon BS., 2007. Importance of low-range CAG expansion and CAA interruption in SCA2 Parkinsonism. Arch Neurol 64, 1510-8.

Lattante, S., Millecamps, S., Stevanin, G., Rivaud-Péchoux, S., Moigneu, C., Camuzat, A., Da Barroca, S., Mundwiller, E., Couarch, P., Salachas, F., Hannequin, D., Meininger, V., Pasquier, F., Seilhean, D., Couratier, P., Danel-Brunaud, V., Bonnet, A.M., Tranchant, C., LeGuern, E., Brice, A., Le Ber, I., Kabashi, E.; French Research Network on FTD and FTD-ALS, 2015. Contribution of ATXN2 intermediary polyQ expansions in a spectrum of neurodegenerative disorders. Neurology 83, 990-5.

Lu, H.P., Gan, S.R., Chen, S., et al., 2015. Intermediate-length polyglutamine in ATXN2 is a possible risk factor among Eastern Chinese patients with amyotrophic lateral sclerosis. Neurobiol Aging 36, e11-1603.e4.

Malandrini, A., Galli, L., Villanova, M., Palmeri, S., Parrotta, E., DeFalco, D., Cappelli, M., Grieco, G.S., Renieri, A., Guazzi, G., 1998. CAG repeat expansion in an Italian family with spinocerebellar ataxia type 2 (SCA2): a clinical and genetic study. Eur Neurol 40, 164-8.

Neary, D., Snowden, J.S., Gustafson, L., Passant, U., Stuss, D., Black, S., Freedman, M., Kertesz, A,, Robert, P.H., Albert, M., Boone, K., Miller, B.L., Cummings, J., Benson, D.F., 1998. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 51, 1546–54.

Nonis, D., Schmidt, M.H., van de Loo, S., 2008. Ataxin-2 associates with the endocytosis complex and affects EGF receptor trafficking. Cell Signal 20, 1725-39.

Nguyen, H.P., Van Broeckhoven, C., van der Zee, J., 2018. ALS Genes in the Genomic Era and their Implications for FTD. Trends Genet 34, 404-423.

Pulst, S.M., Nechiporuk, A., Nechiporuk, T., at al. 1996. Moderate expansion of a normally biallelic trinucleotide repeat in spinocerebellar ataxia type 2. Nat Genet 14, 269-276.

Ross, O.A., Rutherford, N.J., Baker, M., Soto-Ortolaza, A.I., Carrasquillo, M.M., DeJesus-Hernandez, M., Adamson, J., Li, M., Volkening, K., Finger, E., Seeley, W.W., Hatanpaa, K.J., Lomen-Hoerth, C., Kertesz, A., Bigio, E.H., Lippa, C., Woodruff, B.K., Knopman, D.S., White, C.L. 3rd, Van Gerpen, J.A., Meschia, J.F., Mackenzie, I.R., Boylan, K., Boeve, B.F., Miller, B.L., Strong, M.J., Uitti, R.J., Younkin, S.G., Graff-

Radford, N.R., Petersen, R.C., Wszolek, Z.K., Dickson, D.W., Rademakers, R., 2011. Ataxin-2 repeat-length variation and neurodegeneration. Hum Mol Genet 20, 3207-12.

Tsai, H.F., Liu, C.S., Leu, T.M., Wen, F.C., Lin, S.J., Liu, C.C., Yang, D.K., Li, C., Hsieh, M., 2004. Analysis of trinucleotide repeats in different SCA loci in spinocerebellar ataxia patients and in normal population of Taiwan. Acta Neurol Scand 109, 355-360.

Van Langenhove, T., van der Zee, J., Engelborghs, S., Vandenberghe, R., Santens, P., Van den Broeck, M., Mattheijssens, M., Peeters, K., Nuytten, D., Cras, P., De Deyn, PP., De Jonghe, P., Cruts, M., Van Broeckhoven, C., 2012. Ataxin-2 polyQ expansions in FTLD-ALS spectrum disorders in Flanders-Belgian cohorts. Neurobiol Aging 16, e17-20.

Yamashita, C., Tomiyama, H., Funayama, M., Inamizu, S., Ando, M., Li, Y., Yoshino, H., Araki, T., Ichikawa, T., Ehara, Y., Ishikawa, K., Mizusawa, H., Hattori, N., 2014. Evaluation of polyglutamine repeats in autosomal dominant Parkinson's disease. Neurobiol Aging 35:1779.e17-21.

Yu, Z., Zhu, Y., Chen-Plotkin, A.S., Clay-Falcone, D., McCluskey, L., Elman, L., Kalb, R.G., Trojanowski, J.Q., Lee, V.M., Van Deerlin, V.M., Gitler, A.D., Bonini, N.M., et al. 2011. PolyQ repeat expansions in ATXN2 associated with ALS are CAA interrupted repeats. PLoS One 6, e17951.

Supplementary Tables

Table S1. Number and percentage of FTD patients analysed for SCA2 CAG-repeat length

	Total n	<27 repeats (%)	≥ 27 repeats (%)
Familial FTD	151	145 (96.0)	6 (4.0)
Sporadic FTD	217	205 (94.5)	12 (5.5)
C9orf72 expansion	15	15 (100)	0
GRN mutations	5	5 (100)	0
MAPT mutations	6	6 (100)	0

Note: *ATXN2* alleleles were divided into two groups: <27 repeats or ≥ 27 repeats.
FTD patients were divided into familial and sporadic; the number of positive cases for *C9orf*, *GRN* and *MAPT* genes are indicated.

Table S2. *ATXN2* CAG-repeat length distribution according to clinical diagnosis.

Clinical diagnosis	Total n	< 24 repeats (%)	24-26 repeats (%)	27-29 repeats (%)	≥ 30 repeats (%)
bvFTD	249	232 (93.2)	1 (0.4)	11 (4.4)	5 (2.0)
SD	62	62 (100)	0	0	0
PNFA	57	56 (98.0)	0	0	1 (2.0)

Supplementary Figure

Figure S1. Distribution of *ATXN2* intermediate repeat lengths in FTD patients and controls.

In the upper right panel, histograms with ≥27 CAG repeats are magnified.