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Ataxin-2 intermediate-length polyglutamine expansions in European ALS patients

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Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset neurodegenerative disease primarily affecting motor neurons. We recently identified intermediate-length polyglutamine (polyQ) expansions (27–33 Qs) in ataxin 2 as a genetic risk factor for sporadic ALS in North American ALS patients. To extend these findings, we assessed the ataxin 2 polyQ repeat length in 1294 European ALS patients and 679 matched healthy controls. We observed a significant association between polyQ expansions and ALS (>30 Qs; $P = 6.2 \times 10^{-3}$). Thus, intermediate-length ataxin 2 polyQ repeat expansions are associated with increased risk for ALS also in the European cohort. The specific polyQ length cutoff, however, appears to vary between different populations, with longer repeat lengths showing a clear association. Our findings support the hypothesis that ataxin 2 plays an important role in predisposing to ALS and that polyQ expansions in ataxin 2 are a significant risk factor for the disease.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a devastating adult-onset neurodegenerative disease caused primarily by a loss of motor neurons in the motor cortex, brainstem and spinal cord, leading to generalized paralysis and death within 2–5 years of disease onset. The disease is mostly sporadic but ~10% of patients have a self-reported family history of ALS (FALS). Since the familial and sporadic forms of the disease are clinically indistinguishable, it is hoped that identifying the genetic contributors to FALS will provide insight into sporadic forms of the disease as well.

The first gene associated with FALS was *SOD1*, which encodes superoxide dismutase 1 (1). SOD1 mutations account for 12–23% of FALS and 1–7% of sporadic ALS (SALS) cases, for an overall incidence of ~5% (2). For many of the remaining cases, the RNA-binding protein TDP-43 (TAR DNA-binding protein) has been found to accumulate abnormally in ubiquitinated pathological lesions of brain and spinal cord neurons (3) and pathogenic mutations in the TDP-43 gene, *TARDBP*, have been identified in rare familial and SALS patients (4). Following the identification of TDP-43, mutations in a second gene encoding a related RNA-binding protein, FUS/TLS, was linked to ALS (5,6), underscoring a role for RNA-binding proteins and RNA-

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processing pathways as critical for ALS pathogenesis (7). In addition to TDP-43 and FUS, mutations in additional genes, including *VAPB, OPTN* and *VCP*, have also been linked to ALS (8–10). As more genetic contributors to ALS are discovered, they will aid our understanding of disease mechanisms and will suggest novel avenues for therapeutic intervention.

Spinocerebellar ataxia type 2 (SCA2) is one of a heterogeneous group of 28 autosomal dominant hereditary ataxias (11) and is caused by polyglutamine (polyQ) tract expansions in ataxin 2 (12–15). These polyQ expansions are encoded at the DNA level in the ataxin 2 gene (*ATXN2*) by trinucleotide repeats of CAG. The ataxin 2 polyQ tract length, though variable, is most frequently 22–23, with expansions of >34 causing SCA2 (12–15). Like other spinocerebellar ataxias, the hallmark pathology in SCA2 is the atrophy and loss of Purkinje neurons from the cerebellar cortex. This manifests clinically as deficits in motor coordination that affect gaze, speech, gait and balance (16). In SCA2, motor neurons are also known to degenerate, as in ALS, although these features typically occur later than the cerebellar degeneration. However, in selected cases, the motor neuron features of SCA2 are prominent enough to mimic an ALS presentation (17,18), indicating the potential for clinicopathological overlap between SCA2 and ALS.

Recently, using a combination of yeast and fly genetics, biochemistry, cell biology and human genetics, we identified intermediate-length polyQ repeat expansions in ataxin 2 as a genetic risk factor for ALS (19,20). We analyzed 915 North American ALS patients and 980 healthy control individuals and found that ataxin 2 polyQ repeat tracts of 27–33 Qs were significantly associated with increased risk for ALS (19).

To extend these findings, we sought to test whether ataxin 2 intermediate-length polyQ expansions increase risk of ALS in additional patient populations. In the present study, we compared the ataxin 2 polyQ lengths of ALS and healthy controls from European populations.

RESULTS

We compared the ataxin 2 polyQ repeat length in genomic DNA from 1294 ALS patients and 679 neurologically normal controls (Fig. 1, Table 1). We found that 20 of 679 control individuals (2.9%) harbored an ataxin 2 intermediate-length polyQ expansion (range 27–30) compared with 45 of 1294 ALS patients (3.5%, range 27–35). This difference was not statistically significant. However, we observed that, within the intermediate-length range of repeats (27–33Q), longer repeats were more common in ALS patients than the controls (Fig. 1). Out of 679 controls, no individuals harbored a polyQ repeat >30, whereas 14 of 1294 ALS patients (1%) had a polyQ repeat length >30 (range 31–35). This difference was statistically significant ($P = 6.2 \times 10^{-3}$). Thus, intermediate-length ataxin 2 polyQ repeat expansions are associated with increased risk for ALS. The specific polyQ length cutoff likely varies between different ethnic populations, with longer repeat lengths showing a stronger association.

Six of the 13 patients with an ataxin 2 polyQ >30 were FALS cases and 7 were SALS cases, but unfortunately DNA was not available from any relatives for comparison. Clinically, none of the patients showed signs or symptoms, including age of onset or disease duration, different from patients with polyQ <30. In particular, ataxia, dementia, or any atypical features have not been observed. Unfortunately, postmortem tissue is not available from any of the 13 patients. The longest ataxin 2 polyQ repeat length among the controls was 30Q, which was found in two cases; a 72-year-old male and a 57-year old male control subject, both without signs of ALS or ataxia.

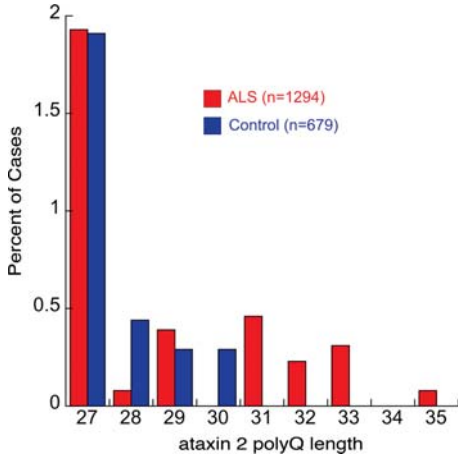

Figure 1. Distribution of ataxin 2 polyQ repeat lengths in ALS patients and healthy controls. Compared with controls, there was a greater number of ALS cases with polyQ repeats >30 (Fisher's exact test, $P = 6.2 \times 10^{-3}$).

Table 1. Intermediate-length ataxin-2 polyQ repeat expansions in ALS

	Total	≤30Q Repeats	27-33Q Repeats	>30Q Repeats	*P*-value (Fisher's)
ALS	1294	1281	45	13	6.2×10^{-3}
Neurologically normal	679	679	20	0	

DISCUSSION

We recently identified intermediate-length ataxin 2 polyQ repeat expansions as a genetic risk factor for ALS (19). In the present study, we have assessed the role of ataxin 2 polyQ expansions in European ALS patients. These findings indicate a significant association of polyQ repeats of >30Q with increased risk for ALS. In the previous analysis of North American ALS patients and controls (19), we found that repeat lengths 27–33Q were significantly associated with ALS, whereas in this study of European ALS patients and controls, only repeats >30Q were significantly different between ALS cases and controls ($P = 6.2 \times 10^{-3}$).

It is likely that the specific cutoff for ataxin 2 polyQ repeat length and risk for ALS will vary from population to population, and future studies aimed at replicating these findings in additional ALS patient and control populations will help define the clinically relevant range further. However, it is perhaps unsurprising that the association of polyQ expansions

on ALS risk is more robust with longer polyQ lengths, given the biology of polyQ disease proteins and the effects of polyQ expansions on their properties: longer polyQ lengths have a more severe effect in model organisms ranging from yeast to fly and worm to mouse (22–26). Furthermore, our findings also suggest the possibility that SCA2 and ALS might lie on different ends of a clinical spectrum, with intermediate-length repeat expansions presenting with more prominent motor neuron degeneration, indicative of ALS, and much longer polyQ expansions resulting in cerebellar degeneration and ataxia. This would predict that some SCA2 patients might occasionally present with clinical symptoms indicative of ALS (17,18) and vice versa—namely that ALS patients would present with ataxia (27, see Clinical Anecdote in Supplementary Information to 19).

In addition to ataxin 2, there are also emerging hints that other ataxins and ataxin-interacting proteins could be involved in ALS. Copy number variants (CNVs) in *ataxin 1* (*ATXN1*) and *ataxin 3-like protein* (*ATXN3L*) were identified in a genomewide search for CNVs associated with ALS (28) and an intronic SNP in *ataxin-2-binding protein 1* (*A2BP1*) was identified in analysis of Dutch and Irish SALS cases (29,30). Since ataxin 1 and ataxin 3 have both been shown to interact genetically with ataxin 2 in *Drosophila* (31,32) and A2BP1 was discovered by virtue of its ability to physically interact with ataxin 2 (33), a testable hypothesis is that intermediate-length polyQ expansions in ataxin 2 disrupt, alter or enhance its normal interactions with one or more of these proteins. Apart from these ataxin genes, our initial studies identified genetic and physical interactions between ataxin 2 and TDP-43 (19), so it will be important to define further the functional and/or pathological interaction between TDP-43 and ataxin 2 and how intermediate-length polyQ repeats affect this interaction.

In summary, we confirm an association between ataxin 2 polyQ repeat expansions and increased risk for ALS by analyzing a large population of European ALS patients and controls. In contrast to our initial study for ALS patients and controls from North America (19), where the statistically significant cutoff is polyQ length $\geq$ 27Q, the cutoff for this population was $>$30Q. Our studies support the hypothesis that ataxin 2 contributes to ALS. Future studies will be aimed at defining the mechanisms by which intermediate-length ataxin 2 polyQ expansions contribute to ALS pathogenesis.

MATERIALS AND METHODS

ALS patients and control samples

With written informed consent, blood samples were obtained from 400 patients diagnosed with FALS [Sweden (184 patients), Germany (57), Switzerland (49), Finland (37), Norway (20), Denmark (28), Portugal (23) and Iceland (2)] and 894 patients diagnosed with SALS [Sweden (658), Switzerland (236); 389 females and 505 males]. The mean age of onset was 56.7 years for the FALS cases and 59.9 years for the SALS cases. All patients were diagnosed according to standard criteria at specialized ALS clinics in Europe (21). The patient cohort was compared with 679 controls matched for age and gender. These were either spouses of patients or healthy blood donors. The study was approved by the institutional ethical review boards in each country.

PolyQ repeat size determination in ALS patients and controls

We amplified ataxin 2 CAG repeats from individual samples by polymerase chain reaction (PCR). PCR primers used for amplification were designed to amplify the CAG repeat region of human Ataxin-2 (bp 442–598). The 5′ primer was SCA2-A6FAMnew: 5′-CCC CGC CCG GCG TGC GAG CCG GTG TAT G-3′ (modified with the 6FAM fluorophor into PCR). The 3′ primer was SCA2-B: 5′-CGG GCT TGC GGA CAT TGG-3′. PCR cycles were as follows: 2 min at 94°C, 35 cycles (1 min at 94°C, 1 min at 60°C, 1 min at 72°C), and 5 min at 72°C. PCR products were mixed with Liz-500 size standard (Applied Biosystems) and were processed for size determination on an ABI3730 sequencer. The size of the repeats was determined with GeneMapper™ 4.0 software (Applied Biosystems).

Statistical analysis

Two-tailed Fisher's exact tests were used to evaluate genetic association between intermediate-length Ataxin-2 repeats and ALS.

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Conflict of Interest statement. None declared.

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REFERENCES

1. Rosen, D., Siddique, T., Patterson, D., Figlewicz, D., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O'Regan, J., Deng, H. *et al.* (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature*, **362**, 59–62.
2. Eisen, A., Mezei, M.M., Stewart, H.G., Fabros, M., Gibson, G. and Andersen, P.M. (2008) SOD1 gene mutations in ALS patients from British Columbia, Canada: clinical features, neurophysiology and ethical issues in management. *Amyotroph. Lateral. Scler.*, **9**, 108–119.

3. Neumann, M., Sampathu, D.M., Kwong, L.K., Truax, A.C., Micsenyi, M.C., Chou, T.T., Bruce, J., Schuck, T., Grossman, M., Clark, C.M. *et al.* (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science*, **314**, 130–133.
4. Pesiridis, G.S., Lee, V.M. and Trojanowski, J.Q. (2009) Mutations in TDP-43 link glycine-rich domain functions to amyotrophic lateral sclerosis. *Hum. Mol. Genet.*, **18**, R156–162.
5. Kwiatkowski, T.J. Jr, Bosco, D.A., Leclerc, A.L., Tamrazian, E., Vanderburg, C.R., Russ, C., Davis, A., Gilchrist, J., Kasarskis, E.J., Munsat, T. *et al.* (2009) Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science*, **323**, 1205–1208.
6. Vance, C., Rogelj, B., Hortobagyi, T., De Vos, K.J., Nishimura, A.L., Sreedharan, J., Hu, X., Smith, B., Ruddy, D., Wright, P. *et al.* (2009) Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science*, **323**, 1208–1211.
7. Lagier-Tourenne, C., Polymenidou, M. and Cleveland, D.W. (2010) TDP-43 and FUS/TLS: emerging roles in RNA processing and neurodegeneration. *Hum. Mol. Genet*., **15**, R46–64.
8. Nishimura, A.L., Mitne-Neto, M., Silva, H.C., Richieri-Costa, A., Middleton, S., Cascio, D., Kok, F., Oliveira, J.R., Gillingwater, T., Webb, J. *et al.* (2004) A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. *Am. J. Hum. Genet.*, **75**, 822–831.
9. Maruyama, H., Morino, H., Ito, H., Izumi, Y., Kato, H., Watanabe, Y., Kinoshita, Y., Kamada, M., Nodera, H., Suzuki, H. *et al.* (2010) Mutations of optineurin in amyotrophic lateral sclerosis. *Nature*, **465**, 223–226.
10. Johnson, J.O., Mandrioli, J., Benatar, M., Abramzon, Y., Van Deerlin, V.M., Trojanowski, J.Q., Gibbs, J.R., Brunetti, M., Gronka, S., Wuu, J. *et al.* (2010) Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron*, **68**, 857–864.
11. Orr, H.T. and Zoghbi, H.Y. (2007) Trinucleotide repeat disorders. *Annu. Rev. Neurosci.*, **30**, 575–621.
12. Imbert, G., Saudou, F., Yvert, G., Devys, D., Trottier, Y., Garnier, J.M., Weber, C., Mandel, J.L., Cancel, G., Abbas, N. *et al.* (1996) Cloning of the gene for spinocerebellar ataxia 2 reveals a locus with high sensitivity to expanded CAG/glutamine repeats. *Nat. Genet.*, **14**, 285–291.
13. Lorenzetti, D., Bohlega, S. and Zoghbi, H.Y. (1997) The expansion of the CAG repeat in ataxin-2 is a frequent cause of autosomal dominant spinocerebellar ataxia. *Neurology*, **49**, 1009–1013.
14. Pulst, S.M., Nechiporuk, A., Nechiporuk, T., Gispert, S., Chen, X.N., Lopes-Cendes, I., Pearlman, S., Starkman, S., Orozco-Diaz, G., Lunkes, A. *et al.* (1996) Moderate expansion of a normally biallelic trinucleotide repeat in spinocerebellar ataxia type 2. *Nat. Genet.*, **14**, 269–276.
15. Sanpei, K., Takano, H., Igarashi, S., Sato, T., Oyake, M., Sasaki, H., Wakisaka, A., Tashiro, K., Ishida, Y., Ikeuchi, T. *et al.* (1996) Identification of the spinocerebellar ataxia type 2 gene using a direct identification of repeat expansion and cloning technique, DIRECT. *Nat. Genet.*, **14**, 277–284.
16. Lastres-Becker, I., Rub, U. and Auburger, G. (2008) Spinocerebellar ataxia 2 (SCA2). *Cerebellum*, **7**, 115–124.
17. Infante, J., Berciano, J., Volpini, V., Corral, J., Polo, J.M., Pascual, J. and Combarros, O. (2004) Spinocerebellar ataxia type 2 with Levodopa-responsive parkinsonism culminating in motor neuron disease. *Mov. Disord.*, **19**, 848–852.
18. Nanetti, L., Fancellu, R., Tomasello, C., Gellera, C., Pareyson, D. and Mariotti, C. (2009) Rare association of motor neuron disease and spinocerebellar ataxia type 2 (SCA2): a new case and review of the literature. *J. Neurol*., **256**, 1926–1928.
19. 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. *et al.* (2010) Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. *Nature*, **466**, 1069–1075.
20. Lagier-Tourenne, C. and Cleveland, D.W. (2010) Neurodegeneration: an expansion in ALS genetics. *Nature*, **466**, 1052–1053.
21. Andersen, P.M., Borasio, G.D., Dengler, R., Hardiman, O., Kollewe, K., Leigh, P.N., Pradat, P.F., Silani, V. and Tomik, B. (2007) Good practice in the management of amyotrophic lateral sclerosis: clinical guidelines. An evidence-based review with good practice points. EALSC Working Group. *Amyotroph. Lateral. Scler.*, **8**, 195–213.
22. Gidalevitz, T., Ben-Zvi, A., Ho, K.H., Brignull, H.R. and Morimoto, R.I. (2006) Progressive disruption of cellular protein folding in models of polyglutamine diseases. *Science*, **311**, 1471–1474.
23. Krobitsch, S. and Lindquist, S. (2000) Aggregation of huntingtin in yeast varies with the length of the polyglutamine expansion and the expression of chaperone proteins. *Proc. Natl Acad. Sci. USA*, **97**, 1589–1594.
24. Lim, J., Crespo-Barreto, J., Jafar-Nejad, P., Bowman, A.B., Richman, R., Hill, D.E., Orr, H.T. and Zoghbi, H.Y. (2008) Opposing effects of polyglutamine expansion on native protein complexes contribute to SCA1. *Nature*, **452**, 713–718.
25. Warrick, J.M., Paulson, H.L., Gray-Board, G.L., Bui, Q.T., Fischbeck, K.H., Pittman, R.N. and Bonini, N.M. (1998) Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneration in *Drosophila*. *Cell*, **93**, 939–949.
26. Zoghbi, H.Y. and Orr, H.T. (2009) Pathogenic mechanisms of a polyglutamine-mediated neurodegenerative disease, spinocerebellar ataxia type 1. *J. Biol. Chem.*, **284**, 7425–7429.
27. Andersen, P.M., Forsgren, L., Binzer, M., Nilsson, P., Ala-Hurula, V., Keranen, M.L., Bergmark, L., Saarinen, A., Haltia, T., Tarvainen, I. *et al.* (1996) Autosomal recessive adult-onset amyotrophic lateral sclerosis associated with homozygosity for Asp90Ala CuZn-superoxide dismutase mutation. A clinical and genealogical study of 36 patients. *Brain*, **119** (Pt 4), 1153–1172.
28. Cronin, S., Blauw, H.M., Veldink, J.H., van Es, M.A., Ophoff, R.A., Bradley, D.G., van den Berg, L.H. and Hardiman, O. (2008) Analysis of genome-wide copy number variation in Irish and Dutch ALS populations. *Hum. Mol. Genet.*, **17**, 3392–3398.
29. Cronin, S., Berger, S., Ding, J., Schymick, J.C., Washecka, N., Hernandez, D.G., Greenway, M.J., Bradley, D.G., Traynor, B.J. and Hardiman, O. (2008) A genome-wide association study of sporadic ALS in a homogenous Irish population. *Hum. Mol. Genet.*, **17**, 768–774.
30. van Es, M.A., Veldink, J.H., Saris, C.G., Blauw, H.M., van Vught, P.W., Birve, A., Lemmens, R., Schelhaas, H.J., Groen, E.J., Huisman, M.H. *et al.* (2009) Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. *Nat. Genet*., **41**, 1083–1087.
31. Al-Ramahi, I., Perez, A.M., Lim, J., Zhang, M., Sorensen, R., de Haro, M., Branco, J., Pulst, S.M., Zoghbi, H.Y. and Botas, J. (2007) dAtaxin-2 mediates expanded Ataxin-1-induced neurodegeneration in a *Drosophila* model of SCA1. *PLoS Genet*, **3**, e234.
32. Lessing, D. and Bonini, N.M. (2008) Polyglutamine genes interact to modulate the severity and progression of neurodegeneration in *Drosophila*. *PLoS Biol*, **6**, e29.
33. Shibata, H., Huynh, D.P. and Pulst, S.M. (2000) A novel protein with RNA-binding motifs interacts with ataxin-2. *Hum. Mol. Genet.*, **9**, 1303–1313.