Published in final edited form as:
JAMA Neurol. 2014 April ; 71(4): 449–453. doi:10.1001/jamaneurol.2013.6237.

The *TREM2* variant p.R47H is a risk factor for sporadic amyotrophic lateral sclerosis

Janet Cady, BS[1], **Erica D. Koval, BA**[1,†], **Bruno A. Benitez, MD**[2,†], **Craig Zaidman, MD**[1], **Jennifer Jockel-Balsarotti, BS**[1], **Peggy Allred, DPT**[3], **Robert H. Baloh, MD/PhD**[3,4], **John Ravits, MD**[5], **Ericka Simpson, MD**[6], **Stanley H. Appel, MD**[6], **Alan Pestronk, MD**[1], **Alison M. Goate, PhD**[1,2,7], **Timothy M. Miller, MD/PhD**[1,7], **Carlos Cruchaga, PhD**[2,7], and **Matthew B. Harms, MD**[1,7]

[1]Department of Neurology; Washington University School of Medicine. St. Louis, MO, USA

[2]Department of Psychiatry; Washington University School of Medicine. St. Louis, MO, USA

[3]Department of Neurology; Cedars Sinai Medical Center. Los Angeles, CA, USA

[4]Department of Neurology; University of California, Los Angeles. Los Angeles, CA, USA

[5]Department of Neurosciences; University of California, San Diego. La Jolla, CA, USA

[6]Department of Neurology, Methodist Neurological Institute, Methodist Research Institute, The Methodist Hospital. Houston, TX, USA

[7]Hope Center for Neurological Disorders; Washington University School of Medicine. St. Louis, MO, USA

Abstract

Importance—Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease in which microglia play a significant and active role. Recently, a rare missense variant (p.R47H) in the microglial activating gene *TREM2* was found to increase the risk of several neurodegenerative diseases, including Alzheimer's disease. Whether the p.R47H variant is a risk factor for ALS is not currently known.

Objective—To determine if p.R47H (rs75932628) in *TREM2* is a risk factor for ALS and assess whether *TREM2* expression is dysregulated in disease.

Design, setting, and participants—923 sporadic ALS subjects and 1854 normal controls self-reported as non-Hispanic white were collected from ALS clinics in the United States and genotyped for the p.R47H variant in *TREM2*. Clinical data was obtained on ALS subjects for genotype/phenotype correlations. Expression of *TREM2* was measured by quantitative PCR and compared in spinal cord from 18 ALS subjects, 12 neurologically normal controls, as well as from wildtype and transgenic $SOD1^{G93A}$ mice.

Corresponding Author: Matthew Harms, MD, Campus Box 8111, Department of Neurology, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, Missouri, 63110, harmsm@neuro.wustl.edu, 314-362-2155 (office), 314-362-3752 (fax).
†Authors contributed equally to this work.

Main outcome measures—Minor allele frequency of rs75932628 and relative expression of *TREM2*.

Results—The *TREM2* variant p. R47H was more common in subject with ALS than in controls and is therefore a significant risk factor for ALS (OR=2.40; 95%CI=1.29-4.15; $p=4.1\times10^{-3}$). Furthermore, *TREM2* expression was increased in spinal cords from ALS patients and $SOD1^{G93A}$ mice ($p=2.8\times10^{-4}$, $p=2.8\times10^{-9}$ respectively), confirming dysregulated TREM2 in disease. *TREM2* expression in human spinal cord was negatively correlated with survival (p=0.04), but not other phenotypic aspects of disease.

Conclusion and relevance—This study demonstrates that the *TREM2* p.R47H variant is a potent risk factor for sporadic amyotrophic lateral sclerosis. These findings identify the first genetic influence on neuro-inflammation in ALS and highlight the TREM2 signaling pathway as a therapeutic target in ALS and other neurodegenerative diseases.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal disease caused by the progressive degeneration of upper and lower motor neurons. Activated microglia in the vicinity of degenerating neurons are a long-recognized pathological feature of ALS[1], but whether such activation is a beneficial response or injurious contributor to the disease process remains unclear. In fact the answer may be both- mouse model data show that microglia express both neuroprotective and neurotoxic factors simultaneously[2] and may transition from a neuroprotective phenotype at symptom onset to become more neurotoxic later in the disease course[3].

There are many signaling pathways governing microglial phenotype, including a complex formed by TREM2 (MIM 605086) and TYROBP (also known as DAP12, MIM 604142)[4]. Activation of TREM2/TYROBP results in a potentially neuroprotective microglial state, with improved phagocytosis of apoptotic cellular debris and down-regulation of inflammatory cytokines[5]. The importance of signaling through TREM2/TYROBP is made clear by the fact that recessive mutations in either gene cause early onset frontotemporal-like dementia, either in isolation[6] or as part of the recessive human disease polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL or Nasu-Hakola Disease, MIM 221770)[7,8]. Furthermore, recent studies have demonstrated that a rare non-synonymous variant in *TREM2*, rs75932628 (encoding p.R47H) is strong risk-factor for Alzheimer's disease (AD), another neurodegenerative disease characterized by microglial activation[9-13]. Other studies have implicated the same variant in frontotemporal dementia and Parkinson's disease[14,15]. It has been hypothesized that this variant impairs TREM2/TRYOBP signaling, thereby blunting neuroprotective microglial activation and exacerbating the disease process[9,11].

In this study we demonstrate that p.R47H is a risk factor for sporadic ALS, and demonstrate upregulation of TREM2 in human ALS spinal cord and in spinal cord from the G93A mouse model of *SOD1* ALS[16].

METHODS

Study Subjects and TREM2 p.R47H genotyping

923 sporadic ALS (SALS) subjects and 1854 normal controls, all of self-reported non-Hispanic white background, were included and provided written informed consent at their contributing institution. Diagnoses of probable or definite ALS were made by neuromuscular specialists according to El Escorial criteria (Washington University in St. Louis, n= 273; Virginia Mason Medical Center, n=143; Methodist Neurological Institute, n= 47; Coriell plates NDPT025, NDPT026, NDPT100, NDPT103, NDPT106, n=460). Control subjects were without ALS, Parkinson's disease, or dementia and were collected from ongoing studies (Washington University, n=1390) or Coriell panels (NDPT020, NDPT079, NDPT082, NDPT095, NDPT096, n=464). 55% of SALS cases were male and age at DNA collection was 61.0±11.6 years old (mean ± stdev), while the control cohort was 44% male and 68±13.6 years old (mean ± stdev). An additional control group of 25,023 individuals of European or European American descent was collated from published studies (Table 1) and from the unrelated European Americans (EA) genotyped by whole-exome sequencing as part of the NHLBI's Exome Sequencing Project (ESP)[17]. Icelandic controls were not included given the isolation of the population and significantly higher MAF at rs75932628[9].

DNA was extracted from blood or saliva using standard methods and genotyped for rs75932628 (*TREM2* p.R47H) using a custom KASPar (KBioscience) assay[12]. Genotype call rate was 99.7% in both cases and controls. p.R47H carriers were validated by sequencing.

TREM2 Expression Analysis

Expression in human lumbar spinal cord—Total RNA was extracted from snap-frozen transverse sections of lumbar spinal cord of 18 autopsied subjects with ALS (Table 2) and 12 controls without neurological disease using the miRNeasy kit (Qiagen). Extracted RNA was quantified and 40ng was used as input for the Express One-Step Superscript qRT-PCR Universal (Invitrogen: 11781-200) with validated Taqman assays for human TREM2 and three endogenous controls, GAPDH, PPIA, and RPLPO (Applied Biosystems 4331182, 4333764F, 4333763F, 4333761F respectively). Reactions were run in duplicate on an ABI 7500 fast thermocycler. *TREM2* expression was normalized to the geometric mean of the three endogenous controls. 10 subjects had provided separate informed consent for genetic analysis, but none were found to carry the p.R47H variant.

Expression in mouse spinal cord—Total RNA was extracted from saline-perfused and snap-frozen spinal cords of 8 end-stage $SOD1^{G93A}$ transgenic mice (Jackson Lab B6.Cg-Tg(SOD1*G93A)1Gur/J) and 6 negative littermate controls. *TREM2* expression was quantified using a mouse-specific *TREM2* Taqman assay (Applied Biosystems 4331182) and normalized to the endogenous control SMRT using primers and probe from IDT DNA (Probe: AGACGTCTCACACAAGGAAGGACTCGCC, Forward primer: GGGTATATTTTTGATACCTTCAATGAGTTA, Reverse primer TCTGAAACAGTAGGTAGAGACCAAAGC). Reactions were run in duplicate on an ABI 7500 fast thermocycler.

Statistics

All statistics were computed using R version 3.0.1 except as noted. Fisher's exact test was used to compare proportions of p.R47H carriers in cases and controls. Comparisons of TREM2 expression utilized student t-tests, while correlations between TREM2 expression and subject characteristics utilized Spearman correlations (continuous variables) or Mann-Whitney U (dichotomous variables). Logistic regression was performed in PLINK with age and gender as covariates using cases and controls for whom this data was available (913/920 of ALS subjects and 1803/1848 of controls). All tests were two-tailed, with the significance level set at p=0.01 to correct for multiple comparisons.

RESULTS

TREM2 p.R47H in sporadic ALS

1.09% (10/920) of sporadic ALS subjects and 0.162% (3/1848) of normal controls were heterozygous carriers of the p.R47H variant, showing a significant enrichment in ALS (OR=6.77; 95% CI 1.86-24.65; p=0.0016). No cases or controls were homozygous for this allele. Because the proportion of p.R47H carriers in the population declines with age[7] and our cases were younger than controls, we also analyzed our data by logistic regression with age and gender as covariates. This produced a similar risk estimate (OR=7.38; 95%CI = 1.95-27.9, p= 0.0032). To provide a more conservative estimate of effect size, we also compared our sporadic ALS cohort to an aggregate control population of European ancestry gleaned from published studies and databases (n=25,023, Table 1). We again observed an enrichment in sporadic ALS, albeit with a lower effect size (OR=2.81; CI 1.31-5.41; $p=4.8\times10^{-3}$). A prior study of a smaller cohort of North American ALS patients found a non-significant but increased frequency in cases versus controls (0.7% vs. 0.45%)[14]. A combined analysis of this study with ours compared to all available controls also showed a significant association (OR=2.40; 95%CI=1.29-4.15; $p=4.1\times10^{-3}$), confirming that *TREM2* p.R47H is a risk factor for ALS. *TREM2* p.R47H carriers in our cohort showed no difference in age of symptom onset, site of first symptom, or in survival compared to those without. However, the rarity of the variant limited our power to detect such a difference. A larger cohort of p.R47H carriers with ALS will be required to definitively determine effects on disease parameters.

TREM2 expression in spinal cords from humans with ALS and SOD1^{G93A} mice

We examined spinal cord expression of TREM2 in lumbar spinal cord sections from 18 subjects with ALS and found a 2.8-fold upregulation compared to controls ($p=2.8\times10^{-4}$; Figure panel A). Expression levels did not correlate with age of onset, site of symptom onset, or presence of a known disease-causing mutation (Table 2). However, the degree of upregulation showed a modest inverse correlation with disease survival that was not statistically significant after correction for multiple comparisons. Because markers of microglial activation are also upregulated in models of SOD1 ALS[18], we evaluated *TREM2* expression in SOD1^{G93A} transgenic mice and found a 13-fold increase compared to non-transgenic littermates ($p=2.8\times10^{-9}$; Figure panel B).

DISCUSSION

Our study demonstrates that a rare variant in *TREM2* (p.R47H), more than doubles the risk of ALS. In addition to identifying a novel risk factor for ALS, this finding provides the first link between genetic variation and microglial activation in ALS pathogenesis. This is important in light of a recent study demonstrating that higher degrees of microglial activation on pathological examination were correlated with both the degree of upper motor neuron symptoms and a more rapid disease progression[19]. Interestingly, our evaluation of TREM2 expression in ALS spinal cord showed a similar trend, with higher levels of TREM2 correlating with shorter survival. Furthermore, our finding that TREM2 expression is also increased in spinal cords from $SOD1^{G93A}$ mice is congruent with recent studies of isolated microglia from this same model[2] and suggests that studies of microglial activation in this model may provide insights relevant to human ALS.

p.R47H was first shown to increase risk for Alzheimer's disease with subsequent associations with frontotemporal dementia and Parkinson's disease[9-14]. How the p.R47H variant affects TREM2 function and predisposes to neurodegeneration is currently unknown. Because TREM2 signaling mediates potentially neuroprotective microglial activities (including phagocytosis of apoptotic cells and secretion of anti-inflammatory cytokines), one model hypothesizes that p.R47H is a loss-of-function allele. Inadequate clean-up of cellular debris and counter-productive inflammation would predispose to symptomatic disease. The p.R47H variant is located in the extracellular domain of TREM2 where it could interfere with binding to unidentified ligand(s) or disrupt signaling through its receptor complex partner TYROBP. As dysregulated TREM2 signaling confers risk for several neurodegenerative disorders, insights gleaned from the study of TREM2 in ALS are likely to be applicable to other diseases and vice versa. This includes the important possibility that manipulation of TREM2 signaling or microglial activation would be a worthwhile therapeutic strategy.

Acknowledgments

This work was supported by National Institutes of Health (NIH) grants K08-NS075094 (M.B.H.), R01-AG044546 (C.C.), R01-NS078398-02 (T.M.M.), R01-NS069669 (R.H.B.), as well as the Hope Center for Neurological Disorders. This research was conducted with C.C. was a recipient of a New Investigator Award in Alzheimer's disease form the American Federation for Aging Research, and while J.C. was funded by the Genetics Epidemiology Training Grant (5 T32 HL 83822-5). R.H.B. holds a career Award for Medical Scientists from the Burroughs Wellcome Fund. This publication was made possible by grant UL1 RR024992 from the National Center for Research Resources (NCRR), a component of the NIH, and NIH Roadmap for Medical Research. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH. The authors thank the NHLBI GO Exome Sequencing Project and its ongoing studies which produced and provided exome variant calls for comparison: the Lung GO Sequencing Project (HL-102923), the WHI Sequencing Project (HL-102924), the Broad GO Sequencing Project (HL-102925), the Seattle GO Sequencing Project (HL-102926) and the Heart GO Sequencing Project (HL-103010). DNA panels and clinical data from the NINDS Human Genetics Resource Center DNA and Cell Line Repository (http://ccr.coriell.org/ninds) were used in this study. The submitters that contributed samples are acknowledged in detailed descriptions of each panel: NDPT020, NDPT025, NDPT026, NDPT079, NDPT82, NDPT095, NDPT096, NDPT100, NDPT103, NDPT106, and NDPT116. DNA samples from the Washington University Neuromuscular Genetics Project and autopsy tissue from the Washington University ALS Tissue Donation Program were used in this study. The authors also thank Tara Skorupa, Paul Cooper, Ryan Libby, Michael Baughn, Sharon Halton, and Luis Lay for research coordination or technical assistance.

Preliminary Disclosures (to be updated by JAMA's form):

Janet Cady:	has nothing to disclose
Erica D. Koval;	has nothing to disclose
Bruno A. Benitez;	has nothing to disclose
Jennifer Jockel-Balsarotti:	has nothing to disclose
Peggy Allred:	has nothing to disclose
Robert H. Baloh:	has nothing to disclose
John Ravits:	
receives research support from the ALS Association, Microsoft Research, P2ALS.	
Serves as an unpaid consultant to the Muscular Dystrophy Association	
Consults for Isis Pharmaceuticals, Inc.	
Ericka Simpson:	has nothing to disclose
Stanley H. Appel:	receives grant support from the Hamill foundation outside the submitted work
Alan Pestronk:	
Receives revenue related to antibody patent licenses & speaker honoraria from Athena;	
Owns stock in Johnson & Johnson;	
Directs the Washington University Neuromuscular Clinical Laboratory which performs antibody testing;	
Receives research support from the NIH, Muscular Dystrophy Association, Neuromuscular Research	
Fund; Insmed, Knopp, Cytokinetics, Biogen Idec, ISIS, Genzyme, GSK, Sanofi & Ultragenyx.	
Alison M. Goate:	
Provides consultation or expert testimony to Finnegan	
Receives grants from Genetech, Pfizer, and Astrazeneca for Alzheimer's genetic research	
Has received honoraria from Genentech and Amgen for speaking on Alzheimer's genetics	
Receives royalties from Taconic for tau mutation patents	
Timothy M. Miller:	
Non-financial support from Regulus Therapeutics, outside the submitted work	
Grants and non-financial support from Isis Pharmaceuticals, outside the submitted work Has a patent inhibiting miR-155 in ALS pending.	
Carlos Cruchaga:	has nothing to disclose
Matthew B. Harms:	
Has received honoraria from Genzyme for speaking on neuromuscular genetics	
Has provided expert testimony in medicolegal proceedings	
Has received grant support from Ultragenyx, outside the submitted work	
Receives grant funding from the Barnes Jewish Foundation and NIH	

References

1. Engelhardt JI, Appel SH. IgG reactivity in the spinal cord and motor cortex in amyotrophic lateral sclerosis. Arch Neurol. Nov; 1990 47(11):1210–1216. [PubMed: 2122877]

2. Chiu IM, Morimoto ET, Goodarzi H, et al. A neurodegeneration-specific gene-expression signature of acutely isolated microglia from an amyotrophic lateral sclerosis mouse model. Cell Rep. Jul 25; 2013 4(2):385–401. [PubMed: 23850290]
3. Liao B, Zhao W, Beers DR, Henkel JS, Appel SH. Transformation from a neuroprotective to a neurotoxic microglial phenotype in a mouse model of ALS. Exp Neurol. Sep; 2012 237(1):147–152. [PubMed: 22735487]
4. Kierdorf K, Prinz M. Factors regulating microglia activation. Front Cell Neurosci. 2013; 7:44. [PubMed: 23630462]
5. Takahashi K, Rochford CD, Neumann H. Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. J Exp Med. Feb 21; 2005 201(4):647–657. [PubMed: 15728241]
6. Guerreiro RJ, Lohmann E, Bras JM, et al. Using exome sequencing to reveal mutations in TREM2 presenting as a frontotemporal dementia-like syndrome without bone involvement. JAMA Neurol. Jan; 2013 70(1):78–84. [PubMed: 23318515]
7. Paloneva J, Kestila M, Wu J, et al. Loss-of-function mutations in TYROBP (DAP12) result in a presenile dementia with bone cysts. Nat Genet. Jul; 2000 25(3):357–361. [PubMed: 10888890]
8. Paloneva J, Manninen T, Christman G, et al. Mutations in two genes encoding different subunits of a receptor signaling complex result in an identical disease phenotype. Am J Hum Genet. Sep; 2002 71(3):656–662. [PubMed: 12080485]
9. Jonsson T, Stefansson H, Steinberg S, et al. Variant of TREM2 associated with the risk of Alzheimer's disease. N Engl J Med. Jan 10; 2013 368(2):107–116. [PubMed: 23150908]
10. Pottier C, Wallon D, Rousseau S, et al. TREM2 R47H variant as a risk factor for early-onset Alzheimer's disease. J Alzheimers Dis. Jan 1; 2013 35(1):45–49. [PubMed: 23380991]
11. Guerreiro R, Wojtas A, Bras J, et al. TREM2 variants in Alzheimer's disease. N Engl J Med. Jan 10; 2013 368(2):117–127. [PubMed: 23150934]
12. Benitez BA, Cooper B, Pastor P, et al. TREM2 is associated with the risk of Alzheimer's disease in Spanish population. Neurobiol Aging. Jun; 2013 34(6):1711 e1715–1717. [PubMed: 23391427]
13. Gonzalez Murcia JD, Schmutz C, Munger C, et al. Assessment of TREM2 rs75932628 association with Alzheimer's disease in a population-based sample: the Cache County Study. Neurobiol Aging. Jul 12.2013
14. Rayaprolu S, Mullen B, Baker M, et al. TREM2 in neurodegeneration: evidence for association of the p.R47H variant with frontotemporal dementia and Parkinson's disease. Mol Neurodegener. 2013; 8:19. [PubMed: 23800361]
15. Benitez, Ba; C, C. TREM2 and Parkinson's disease. NEJM. 2013 In press.
16. Gurney ME, Pu H, Chiu AY, et al. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. Science. Jun 17; 1994 264(5166):1772–1775. [PubMed: 8209258]
17. Exome Variant Server. NHLBI GO Exome Sequencing Project (ESP). Seattle, WA: Oct. 2012 URL: http://evs.gs.washington.edu/EVS/
18. Hall ED, Oostveen JA, Gurney ME. Relationship of microglial and astrocytic activation to disease onset and progression in a transgenic model of familial ALS. Glia. Jul; 1998 23(3):249–256. [PubMed: 9633809]
19. Brettschneider J, Toledo JB, Van Deerlin VM, et al. Microglial activation correlates with disease progression and upper motor neuron clinical symptoms in amyotrophic lateral sclerosis. PLoS ONE. 2012; 7(6):e39216. [PubMed: 22720079]

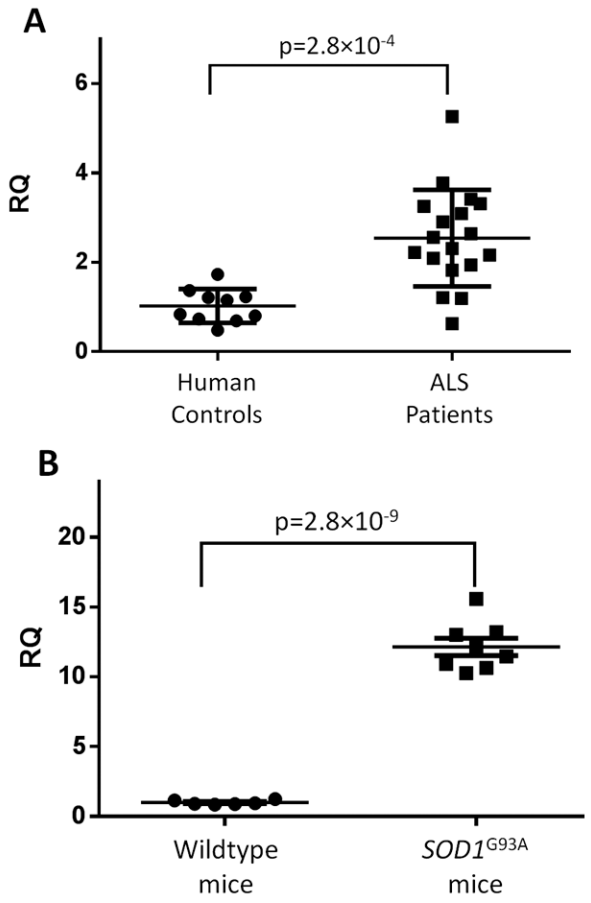

Figure 1. *TREM2* expression is increased in human ALS and SOD1^{G93R} mouse spinal cord

TREM2 expression was measured by qPCR in **A)** lumbar spinal cord sections from 18 ALS subjects and 12 controls and normalized to the geometric mean of three endogenous control genes.

B) In mice, expression was measured in spinal cords from 8 SOD^{G93R} mice and 6 wild-type littermates with normalization to an endogenous control. p-values were calculated using two-tailed student's t-test.

Table 1

TREM2 p.R47H carriers in published cohorts and this study

Cohort		No. Subjects	p.R47H carriers	MAF (%)
Controls	ESP-EA[17]	4,300	22	0.26
	Spain[12]	550	0	0.00
	Georgia, USA[9]	402	1	0.12
	Germany[9]	1,891	7	0.19
	Netherlands[9]	4,950	15	0.15
	Norway[9]	2,484	8	0.16
	N. America/UK[11]	5,166	20	0.19
	Utah, USA[13]	2,540	12	0.24
	France[10]	783	4	0.26
	N. America/Ireland/Poland[14]	1,957	8	0.20
	This Study (N. America)	1,848	3	0.08
	Total Controls	26,871	100	0.19
ALS	This Study (N. America)	920	10	0.54
	N. America[14]	765	5	0.33
	Total ALS Subjects	1685	15	0.45

Abbreviations: MAF= minor allele frequency; ESP-EA= Exome Sequencing Project European American; N. America=North American

Table 2

ALS autopsy subjects studied for TREM2 expression in lumbar spinal cord

Demographic Category	Metric		Correlation	P-value
Age at onset (years, n=17)	Mean ± stdev (range)	61±13 (29-75)	r=-0.03	0.9[c]
Survival[a] (months, n=18)	Mean ± stdev (range)	31.3±28.0 (4-108)	r=-0.49	0.04[c]
Postmortem interval (hours, n=11)	Mean ± stdev (range)	12.4±7.5 (2-28)	r=-0.45	0.17[c]
Site of onset (n=16)	% Bulbar (n)	31 (5)		0.21[d]
Genetic cause[b] (n=18)	% With known gene (n)	33 (6)		0.21[d]

[a] Survival defined as symptom onset to death or full-time ventilation.

[b] 3 subjects had *SOD1* mutations (A4V, G85R, I133T) and 3 had *C9ORF72* repeat expansions.

[c] Spearman correlation, unadjusted for multiple comparisons.

[d] Mann-Whitney U rank test, unadjusted for multiple comparisons.