Original Investigation

TREM2 Variant p.R47H as a Risk Factor for Sporadic Amyotrophic Lateral Sclerosis

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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 disease. Whether the p.R47H variant is a risk factor for ALS is not known.

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

DESIGN, SETTING, AND PARTICIPANTS Samples of DNA from 923 individuals with sporadic ALS and 1854 healthy control individuals 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 were obtained on ALS participants for genotype/phenotype correlations. Expression of *TREM2* was measured by quantitative polymerase chain reaction and compared in spinal cord samples from 18 autopsied patients with ALS and 12 neurologically healthy controls, as well as from wild-type and transgenic $SOD1^{G93A}$ mice.

MAIN OUTCOMES AND MEASURES Minor allele frequency of rs75932628 and relative expression of *TREM2*.

RESULTS The *TREM2* variant p.R47H was more common in patients with ALS than in the controls and is therefore a significant risk factor for ALS (odds ratio, 2.40; 95% CI, 1.29-4.15; $P = 4.1\times10^{-3}$). Furthermore, *TREM2* expression was increased in spinal cord samples from ALS patients and $SOD1^{G93A}$ mice ($P = 2.8\times10^{-4}$ and $P = 2.8\times10^{-9}$, respectively), confirming dysregulated *TREM2* in disease. Expression of *TREM2* in the human spinal cord was negatively correlated with survival ($P = .04$) but not with other phenotypic aspects of disease.

CONCLUSIONS AND RELEVANCE This study demonstrates that the *TREM2* p.R47H variant is a potent risk factor for sporadic ALS. To our knowledge, these findings identify the first genetic influence on neuroinflammation in ALS and highlight the TREM2 signaling pathway as a therapeutic target in ALS and other neurodegenerative diseases.

JAMA Neurol. 2014;71(4):449-453. doi:10.1001/jamaneurol.2013.6237
Published online February 17, 2014.

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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 pathologic 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 (OMIM 605086) and TYROBP (OMIM 604142), also known as DAP12.[4] Simultaneous activation of TREM2/TYROBP results in a potentially neuroprotective microglial state, with improved phagocytosis of apoptotic cellular debris and downregulation 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*)(OMIM 221770), also known as Nasu-Hakola disease.[7,8] Furthermore, recent studies[9-13] have demonstrated that a rare nonsynonymous variant in *TREM2*, rs75932628 (encoding p.R47H), is a strong risk factor for Alzheimer disease, another neurodegenerative disease characterized by microglial activation. Other studies have implicated the same variant in frontotemporal dementia[14] and Parkinson disease.[15] It has been hypothesized[9,11] that this variant impairs TREM2/TRYOBP signaling, thereby blunting neuroprotective microglial activation and exacerbating the disease process.

In this study we demonstrated that p.R47H is a risk factor for sporadic ALS and observed upregulation of *TREM2* in human ALS spinal cord as well as in the spinal cord of the G93A mouse model of *SOD1* (OMIM 147450) ALS.[16]

Table 1. *TREM2* p.R47H Carriers in Published Cohorts and Present Study

Cohort	No. of Participants	No. of p.R47H Carriers	MAF, %
Controls			
ESP-EA[18]	4300	22	0.26
Spain[12]	550	0	0
Georgia[9]	402	1	0.12
Germany[9]	1891	7	0.19
The Netherlands[9]	4950	15	0.15
Norway[9]	2484	8	0.16
North America/ United Kingdom[11]	5166	20	0.19
Utah[13]	2540	12	0.24
France[10]	783	4	0.26
North America/Ireland/Poland[14]	1957	8	0.20
Present study (North America)	1848	3	0.08
Total	**26 871**	**100**	0.19
ALS			
Present study (North America)	920	10	0.54
North America[14]	765	5	0.33
Total	**1685**	**15**	0.45

Abbreviations: ALS, amyotrophic lateral sclerosis; ESP-EA, Exome Sequencing Project–European American; MAF, minor allele frequency.

Methods

Participants and *TREM2* p.R47H Genotyping

A total of 923 patients with sporadic ALS and 1854 healthy individuals serving as controls, all of self-reported non-Hispanic white background, were included. All participants provided written informed consent for genetic studies approved by the institutional review boards at Washington University School of Medicine and Virginia Mason Medical Center. There was no financial compensation. Diagnoses of probable or definite ALS were made by neuromuscular specialists according to El Escorial criteria[17] (Washington University, St Louis [n = 273]; Virginia Mason Medical Center [n = 143]; Methodist Neurological Institute [n = 47]; and Coriell Institute panels NDPT025, NDPT026, NDPT100, NDPT103, and NDPT106 [n = 460]). Control participants did not have ALS, Parkinson disease, or dementia and were drawn from ongoing studies (Washington University [n = 1390]) or Coriell Institute panels (NDPT020, NDPT079, NDPT082, NDPT095, and NDPT096 [n = 464]). The DNA was extracted from blood or saliva using standard methods and genotyped for rs75932628 (TREM2 p.R47H) using a custom assay (KASPar; KBioscience).[12] The genotype call rate was 99.7% in both cases and controls, resulting in 920 ALS and 1848 controls for comparison. The p.R47H carriers were validated by Sanger sequencing. Fifty-five percent of the sporadic ALS cases were men, and mean (SD) age at the time of DNA collection was 61.0 (11.6) years; the control cohort included 44% men aged 68 (13.6) years. 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 genotyped by whole-exome sequencing as part of the National Heart, Lung, and Blood Institute's Exome Sequencing Project.[18] Icelandic controls were not included given the isolation of the population and significantly higher minor allele frequency at rs75932628.[9]

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 patients with ALS (**Table 2**) and 12 controls without neurologic disease (miRNeasy kit; Qiagen). Extracted RNA was quantified, and 40 ng was used as input for the quantitative polymerase chain reaction testing (EXPRESS One-Step Superscript, universal, 11781-200; Invitrogen) with validated TaqMan assays for human *TREM2* and 3 endogenous controls: glyceraldehyde-3-phosphate dehydrogenase, peptidylprolyl isomerase, and ribosomal protein, large, P0 (Applied Biosystems 4331182,

Table 2. ALS Autopsy Subjects Studied for *TREM2* Expression in Lumbar Spinal Cord

Demographic Category	Mean (SD) [Range] or No. (%)	Correlation	*P* Value
Age at onset, y (n = 17)	61 (13) [29-75]	*r* = −0.03	.90[a]
Survival, mo (n = 18)[b]	31.3 (28.0) [4-108]	*r* = −0.49	.04[a]
Postmortem interval, h (n = 11)	12.4 (7.5) [2-28]	*r* = −0.45	.17[a]
Bulbar site of onset (n = 16)	5 (31.0)	...	.21[c]
Known genetic cause (n = 18)[d]	6 (33.3)	...	.21[c]

Abbreviations: ALS, amyotrophic lateral sclerosis; ellipses, correlation was not determined.

[a] Spearman correlation, unadjusted for multiple comparisons.

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

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

[d] Three individuals had *SOD1* mutations (A4V, G85R, and I133T) and 3 had *C9ORF72* repeat expansions.

4333764F, 4333763F, and 4333761F, respectively). Reactions were run in duplicate (ABI 7500 Fast thermocycler; Life Technologies). Expression of *TREM2* was normalized to the geometric mean of the 3 endogenous controls. Ten of the autopsied individuals had provided separate written 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 (B6.Cg-Tg[*SOD1**G93A]1Gur/J; Jackson Laboratory) and 6 negative littermate controls. Expression of *Trem2* was quantified using a mouse-specific *Trem2* TaqMan assay (4331182; Applied Biosystems) and normalized to the endogenous control *Ncor2* (primers and probe from Integrated DNA Technologies) (probe: AGACGTCTCACACAAGGAAGGACTCGCC, forward primer: GGGTATATTTTTGATACCTTCAATGAGTTA, and reverse primer: TCTGAAACAGTAGGTAGAGACCAAAGC). Reactions were run in duplicate (ABI 7500 Fast thermocycler; Life Technologies).

Statistical Analysis

All statistics were computed using R, version 3.0.1 (R Foundation for Statistical Computing; http://www.R-project.org/) except as noted otherwise. Fisher exact test was used to compare proportions of p.R47H carriers in cases and controls. Comparisons of *TREM2* expression were conducted using unpaired *t* tests, and correlations between *TREM2* expression and participants' characteristics were performed using Spearman correlations (continuous variables) or the Mann-Whitney (dichotomous variables) test. Logistic regression was performed in PLINK,[19] with age and sex as covariates, using cases and controls for whom these data were available (913 of 920 ALS patients and 1803 of 1848 controls). All tests were 2-tailed, with the significance level set at $P = .01$ to correct for multiple comparisons.

Results

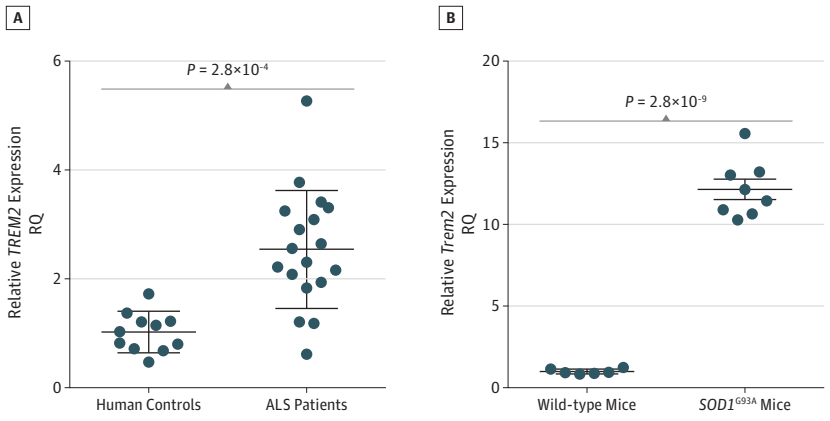
TREM2 p.R47H in Sporadic ALS

Heterozygous carriers of the p.R47H variant included 1.09% (10 of 920) of participants with sporadic ALS and 0.16% (3 of 1848) of healthy controls, showing a significant enrichment in ALS (odds ratio [OR], 6.77; 95% CI, 1.86-24.65; $P = .0016$). 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 the controls, we also analyzed our data by logistic regression with age and sex as covariates. This produced a similar risk estimate (OR, 7.38; 95% CI, 1.95-27.9; $P = .0032$). To provide a more conservative estimate of effect size, we also compared our sporadic ALS cohort with an aggregate control population of European ancestry gleaned from published studies[9-14] and databases[18] (n = 25 023) (Table 1). We again observed an enrichment in sporadic ALS, albeit with a lower effect size (OR, 2.81; 95% CI, 1.31-5.41; $P = 4.8\times10^{-3}$). A prior study[14] of a smaller cohort of North American ALS patients found a nonsignificant but increased frequency in cases vs controls (0.7% vs 0.45%). When data on ALS patients from this smaller cohort study were combined with ours and then compared with all available controls, the significant association persisted (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. The *TREM2* p.R47H carriers in our cohort showed no significant difference in age at symptom onset, site of first symptom, or survival compared with those who were not carriers. 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 characteristics.

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 patients with ALS and found a 2.8-fold upregulation compared with controls ($P = 2.8\times10^{-4}$) (**Figure**, A). Expression levels did not correlate with age at 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,[20] we evaluated *Trem2* expression in *SOD1*^{G93A} transgenic mice and found a 13-fold increase compared with expression in nontransgenic littermates ($P = 2.8\times10^{-9}$) (Figure, B).

Figure. Increased *TREM2* Expression in Human Amyotrophic Lateral Sclerosis (ALS) and *SOD1*^{G93R} Mouse Spinal Cord



A, *TREM2* expression was measured by quantitative polymerase chain reaction in lumbar spinal cord sections from 18 individuals with ALS and 12 control individuals and normalized to the geometric mean of 3 endogenous control genes. B, In mice, expression was measured in spinal cord samples from 8 *SOD1*^{G93R} mice and 6 wild-type littermates, with normalization to an endogenous control. *P* values were calculated using a 2-tailed *t* test.

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, to our knowledge, the first link between genetic variation and microglial activation in ALS pathogenesis. This is important in light of a recent study[21] demonstrating that higher degrees of microglial activation on pathological examination were correlated with both the degree of upper motor neuron symptoms and more rapid disease progression. 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 increased in spinal cords from *SOD1*^{G93A} mice is congruent with recent studies[2] of isolated microglia from this same model and suggests that studies of microglial activation in this model may provide insights relevant to human ALS.

The p.R47H variant was first shown[9-14] to increase the risk for Alzheimer disease, with subsequent associations with frontotemporal dementia and Parkinson disease. How the p.R47H variant affects TREM2 function and predisposes to neurodegeneration is unknown. Because TREM2 signaling mediates potentially neuroprotective microglial activities (including phagocytosis of apoptotic cells and secretion of anti-inflammatory cytokines), one model[9-11] hypothesizes that p.R47H is a loss-of-function allele. Inadequate cleanup of cellular debris and counterproductive 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 ligands or disrupt signaling through its receptor complex partner TYROBP. Because 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.

Conclusions

We have shown that rare variants in *TREM2* increase the risk for ALS and provide a genetic link to microglial activation in disease. This finding implicates the neuroinflammatory response as a target for further investigation in ALS pathogenesis and development of therapeutics.

ARTICLE INFORMATION

Accepted for Publication: December 18, 2013.

Published Online: February 17, 2014. doi:10.1001/jamaneurol.2013.6237.

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Statistical analysis: Cady, Koval, Zaidman, Cruchaga.
Obtained funding: Ravits, Goate, Miller, Cruchaga, Harms.
Administrative, technical, or material support: Allred, Appel, Pestronk, Miller.
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Conflict of Interest Disclosures: Dr Ravits receives research support from the ALS Association, Microsoft Research, and P2ALS; serves as an unpaid consultant to the Muscular Dystrophy Association; and serves as a consultant for Isis Pharmaceuticals, Inc. Dr Appel receives grant support from the Hamill foundation, not associated with the present work. Dr Pestronk receives revenue related to antibody patent licenses and speaker honoraria from Athena; owns stock in Johnson & Johnson; directs the Washington University Neuromuscular Clinical Laboratory, which performs antibody testing; and receives research support from the National Institutes of Health (NIH), Muscular Dystrophy Association, Neuromuscular Research Fund, Insmed, Knopp, Cytokinetics, Biogen Idec, ISIS, Genzyme, Glaxo SmithKline, sanofi-aventis, and Ultragenyx. Dr Goate provides consultation or expert testimony to Finnegan; receives grants from Genentech, Pfizer, and AstraZeneca for Alzheimer disease genetic research; has received honoraria from Genentech and Amgen for speaking on Alzheimer disease genetics; and receives royalties from Taconic for tau mutation patents. Dr Miller receives nonfinancial support from Regulus Therapeutics, not associated with the present work; receives grants and nonfinancial support from Isis Pharmaceuticals, not associated with the present work; and has a patent pending on miR-155 inhibition in ALS. Dr Harms has received honoraria from Genzyme for speaking on neuromuscular genetics; has provided expert testimony in medicolegal proceedings; has received grant support from Ultragenyx, not associated with the present work; and receives grant funding from the Barnes Jewish Foundation and NIH. No other disclosures were reported.

Funding/Support: This work was supported by NIH grants K08-NS075094 (Dr Harms), R01-AG044546 (Dr Cruchaga), R01-NS078398-02 (Dr Miller), and R01-NS069669 (Dr Baloh), as well as the Hope Center for Neurological Disorders. This research was conducted with Dr Cruchaga as the recipient of a New Investigator Award in Alzheimer's disease form the American Federation for Aging Research, and Ms Cady was funded by the Genetics Epidemiology Training grant 5 T32 HL 83822-5. Dr Baloh 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, a component of the NIH, and NIH Roadmap for Medical Research.

Role of the Sponsor: The sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Disclaimer: The contents of the article are solely the responsibility of the authors and do not necessarily represent the official view of the National Center for Research Resources or the NIH.

Additional Contributions: The authors thank the National Heart, Lung, and Blood Institute Grand Opportunity Exome Sequencing Project and its ongoing studies, which produced and provided exome variant calls for comparison: the Lung Grand Opportunity Sequencing Project (HL-102923), the Women's Health Initiative Sequencing Project (HL-102924), the Broad Grand Opportunity Sequencing Project (HL-102925), the Seattle Grand Opportunity Sequencing Project (HL-102926), and the Heart Grand Opportunity Sequencing Project (HL-103010). DNA panels and clinical data from the National Institute of Neurological Disorders and Stroke Human Genetics Resource Center DNA and Cell Line Repository (http://ccr.coriell.org/ninds) were used in this study. The individuals who 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. Tara Skorupa, BA (Department of Psychiatry, Washington University), and Paul Cooper, BA (Department of Neurology, Washington University), performed assays; Ryan Libby, BA, and Michael Baughn, BA (Department of Neurosciences, University of California, San Diego), contributed participant recruitment and sample processing; and Sharon Halton, MSW, LCSW, and Luis Lay Jr, MD (Department of Neurology, The Methodist Hospital), contributed participant recruitment and data collection.

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