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 SOD1G93A 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; \( P = 4.1 \times 10^{-3} \)). Furthermore, TREM2 expression was increased in spinal cord samples from ALS patients and SOD1G93A mice (\( P = 2.8 \times 10^{-4} \) and \( P = 2.8 \times 10^{-9} \), 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.
A myotrophic 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, but whether such activation is a beneficial response or an 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 and may transition from a neuroprotective phenotype at symptom onset to become more neurotoxic later in the disease course.

There are many signaling pathways governing microglial phenotype, including a complex formed by TREM2 (OMIM 605086) and TYROBP (OMIM 604142), also known as DAP12. Simultaneous activation of TREM2/TYROBP results in a potentially neuroprotective microglial state, with improved phagocytosis of apoptotic cellular debris and downregulation of inflammatory cytokines. The importance of signaling through TREM2/TYROBP is made clear by the fact that recessive mutations in either gene cause early-onset frontotemporal-dementia, either in isolation or as part of the recessive human disease poly cystic lipomembranous osteodysplasia with sclerosis leukoencephalopathy (PLOSL) (OMIM 221770), also known as Nasu-Hakola disease. Furthermore, recent studies 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 and Parkinson disease. It has been hypothesized that this variant impairs TREM2/TYROBP 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.

### 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 (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 NDPT016 [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).

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 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 Human, Lung, and Blood Institute’s Exome Sequencing Project. Icelandic controls were not included given the isolation of the population and significantly higher minor allele frequency at rs75932628.

#### 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 (miR-Neasy kit; Qiagen). Extracted RNA was quantified, and 40 ng was used as input for the quantitative polymerase chain reaction testing (EXPRESSION 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, 4321513, 4312880). The expression of TREM2 was determined relative to the expression of its endogenous controls and normalized to the controls.

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

<table>
<thead>
<tr>
<th>Cohort</th>
<th>No. of Participants</th>
<th>No. of p.R47H Carriers</th>
<th>MAF, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESP-EA18</td>
<td>4300</td>
<td>22</td>
<td>0.26</td>
</tr>
<tr>
<td>Spain12</td>
<td>550</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Georgia9</td>
<td>402</td>
<td>1</td>
<td>0.12</td>
</tr>
<tr>
<td>Germany9</td>
<td>1891</td>
<td>7</td>
<td>0.19</td>
</tr>
<tr>
<td>The Netherlands9</td>
<td>4950</td>
<td>15</td>
<td>0.15</td>
</tr>
<tr>
<td>Norway9</td>
<td>2484</td>
<td>8</td>
<td>0.16</td>
</tr>
<tr>
<td>North America/United Kingdom11</td>
<td>5166</td>
<td>20</td>
<td>0.19</td>
</tr>
<tr>
<td>Utah13</td>
<td>2540</td>
<td>12</td>
<td>0.24</td>
</tr>
<tr>
<td>France10</td>
<td>783</td>
<td>4</td>
<td>0.26</td>
</tr>
<tr>
<td>North America/Ireland/Poland14</td>
<td>1957</td>
<td>8</td>
<td>0.20</td>
</tr>
<tr>
<td>Present study (Total)</td>
<td>1848</td>
<td>3</td>
<td>0.08</td>
</tr>
<tr>
<td>ALS</td>
<td>26 871</td>
<td>100</td>
<td>0.19</td>
</tr>
</tbody>
</table>

**Abbreviations:** ALS, amyotrophic lateral sclerosis; ESP-EA, Exome Sequencing Project—European American; MAF, minor allele frequency.
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: AGAGCCTCACAAGGAAGGACTCGCC, forward primer: GGGTATATTTTTGATACCTTCAATGAGTTA, and reverse primer: TCTGAAACAGTAGTTAGAGCCAAAGG). Reactions were run in duplicate (ABI7500 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, 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, and controls for whom these data were available (913 of 920). Expression of Trem2 in ALS 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 Spinal Cord 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 = 2.8×10⁻⁴ (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,26 we evaluated Trem2 expression in SOD1*G93A transgenic mice and found a 13-fold increase compared with expression in nontransgenic littermates (P = 2.8×10⁻⁶) (Figure, B).

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

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

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

a Spearman correlation, unadjusted for multiple comparisons.

b Mann-Whitney rank test, unadjusted for multiple comparisons.

c Three individuals had SOD1 mutations (A4V, G85R, and I135T) and 3 had C9ORF72 repeat expansions.
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 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 SOD1G93A mice is congruent with recent studies 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 to increase the risk of 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 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.
TREM2 Expression in Amyotrophic Lateral Sclerosis

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 Neuroumuscular Clinical Laboratory, which performs antibody testing; and receives research support from the National Institutes of Health (NIH), Muscular Dystrophy Association, Neuroumuscular Research Fund, Insmed, Knopp, Cytokinetiks, Biogen Idec, ISIS, Genzyme, Glaxo SmithKline, sanofi-aventis, and Ultragrenex. 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 Aagen 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 Ultragrenex, not associated with the present work; and receives grant funding from the Barnes Jewish Foundation and NIH. No other disclosures were reported.

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REFERENCES