Background: Twenty percent of familial amyotrophic lateral sclerosis (ALS) is caused by mutations in the superoxide dismutase 1 gene ($SOD1$). Few data exist on their clinicopathologic phenotypes.

Objectives: To determine the clinical and pathologic phenotype associated with the G93C mutation in $SOD1$ and to compare survival in familial ALS related to this mutation with survival in other ALS subgroups.

Design: Retrospective study.

Setting: Tertiary referral center for neuromuscular disorders.

Patients: Twenty patients with the G93C mutation for whom clinical data were available and 1 patient with pathologic data.

Main Outcome Measures: Characteristics and survival compared with other ALS subgroups, adjusting for known prognostic factors.

Results: The G93C mutation was associated with a purely lower motor neuron phenotype without bulbar involvement. Presence of the mutation independently predicted longer survival compared with other ALS subgroups. Pathologic examination showed degeneration of the anterior horn, spinocerebellar tracts, and posterior funiculi, with minimal involvement of corticospinal tracts and no degeneration of brainstem motor nuclei. Survival motor neuron gene copy number had no significant influence on age at onset or survival in patients with the G93C mutation.

Conclusions: These findings add to the knowledge of $SOD1$-related familial ALS and demonstrate further clinicopathologic variability between different $SOD1$ mutations. Finally, they demonstrate the independent prognostic value of the G93C mutation.
dicted forced vital capacity (FVC) soon after diagnosis. No patients received artificial ventilation.

NEUROPATHOLOGIC EXAMINATION

The brain and spinal cord of a 57-year-old man with SOD1(G93C) and a 67-month survival were examined by routine techniques. Briefly, representative tissue blocks from formalin-fixed brain and spinal cord were processed with paraffin, and 5-µm-thick sections (through motor and other neocortexes, basal ganglia, hippocampus, brainstem, and cervical, thoracic, and lumbar spinal cord) were stained with hematoxylin-eosin and with the Klüver-Barrera stain. Indirect immunoperoxidase stains were performed with the use of polyclonal antitubulin antibodies (DAKO, Glostrup, Denmark) and polyclonal antineurofilament antibodies (Sigma Chemical Co, St Louis, Mo).

GENETIC ANALYSIS

The SOD1 genotyping of patients with FALS with screening of all 5 exons was performed as described previously, after informed consent was obtained. The copy number of the survival motor neuron 1 (SMN1) and SMN2 genes in 15 of the patients with SOD1(G93C) was determined by means of the multiplex ligation-dependent probe amplification technology, with a diagnostic kit for spinal muscular atrophy (Salsa P021; MRC Holland BV, Amsterdam, the Netherlands).

STATISTICAL ANALYSIS

Age at onset and diagnostic delay in patients with FALS (SOD1 related [G93C, L38V, D90A] and non–SOD1 related) and SALS were compared by analysis of variance. Pairwise comparisons were performed by unpaired, 2-tailed t tests. Kaplan-Meier curves of survival in SOD1(G93C)–related FALS, SOD1(L38V)–related FALS, non–SOD1-related FALS, and SALS were generated and compared by the log-rank test. Because only 5 patients with SOD1(D90A) were available, their data were not included. A Cox proportional hazards regression was performed with SOD1(G93C), age at onset, diagnostic delay, bulbar vs spinal onset, and percentage of predicted FVC as covariates. Hazard ratios were calculated for presence of SOD1(G93C), per year of greater age at onset, per month of increased diagnostic delay, for spinal onset, and per 10% increase in percentage of predicted FVC.

Age at onset and survival were compared in patients with SOD1(G93C) with 1 vs 2 copies of the SMN1 or SMN2 gene with an unpaired, 2-tailed t test.

The Bonferroni method was used to correct for multiple comparisons. A 2-sided significance level of .05 was used. Values are represented as mean ± SD, unless indicated otherwise. All statistical analyses were performed with SPSS 10.0 (SPSS Inc, Chicago, Ill).

COMPARATIVE DATA

We reviewed the English-language literature to compare data for patients with SOD1 mutations for which at least 1 detailed illustrated neuropathologic description and detailed clinical information on at least 10 patients with FALS were available. Care was taken not to include the same patients repeatedly. Whenever possible, the same criteria for age at onset, survival, and upper motor neuron involvement as in our study population were used.

RESULTS

CLINICAL CHARACTERISTICS OF SOD1(G93C)

All 20 patients with SOD1(G93C) FALS had slowly progressive weakness and atrophy, starting distally in the
lower extremities. Symptoms gradually spread proximally and to the upper extremities. Throughout the course, distal involvement predominated and bulbar function was preserved. Death resulted from respiratory failure in all 11 patients who had died. Deep tendon reflexes were initially present but gradually disappeared. There were no brisk reflexes, spasticity, Babinski signs, jaw jerk, or other upper motor neuron signs. Several patients (not the patient who underwent autopsy) had mild sensory symptoms, and in 1 patient with advanced disease, mild distal loss of pinpoint sensation was found.

NEUROPATHOLOGIC FINDINGS

Brain and spinal cord of a 57-year-old man with SOD1(G93C) were obtained at autopsy, after a 67-month-long disease course.

The motor cortex appeared normal. No loss of brainstem motor neurons was observed, but in most brainstem motor nuclei, lipofuscin-loaded motor neurons were prominent. Lipofuscin was most prominent in hypoglossal motor neurons, which also showed hyaline inclusions. Loss of myelinated fibers was pronounced in the posterior column and spino-cerebellar tracts, but only mild in the lateral corticospinal tracts (Figure 1A). Severe loss of anterior horn cells was present, and many of the remaining neurons were atrophic and showed hyaline inclusions similar to the previously described Lewy body–like hyaline inclusions (Figure 1B) or ubiquitin-positive inclusions (Figure 1C). Most remaining neurons contained lipofuscin. The Clarke column was also degenerated. Neurofilament stains were unremarkable. Bunina bodies were absent.

DEMOGRAPHIC CHARACTERISTICS

Age at onset in SOD1(G93C)–related FALS was 45.9±10.6 years, significantly younger than in SALS (58.4±12.0 years; P<.001). The onset was earlier in SOD1(L38V)–related FALS than non–SOD1–related FALS (P=.004) and SALS (P<.001) (Table 1). Bulbar onset was noted in 105 (30.2%) of 348 patients with SALS but in none of the patients with SOD1–related FALS (P=.001).

Median disease durations were estimated by Kaplan-Meier curves (Table 1, Figure 2). Log-rank analysis showed a statistically significant difference among the groups (P<.001). Pairwise analysis showed that median disease duration in patients with SOD1(G93C) (153.0 months) was significantly longer than that of patients with SALS (30.0 months), SOD1(L38V) (24.0 months), and non–SOD1 FALS (43.0 months). There was no difference in survival between patients with SALS, non–SOD1 FALS, and SOD1(L38V).

In a multivariate analysis, the hazard ratios (and corresponding 95% confidence intervals) for age at onset, diagnostic delay, spinal site of onset, and percentage of predicted FVC were 1.03 (1.02–1.05; P<.001), 0.94 (0.91–0.96; P<.001), 0.64 (0.44–0.93; P=.02), and 0.84 (0.78–0.91; P<.001), respectively, all statistically significant. In addition, the presence of the G93C mutation was found to represent an independent, statistically significant, positive prognostic factor for survival (hazard ratio, 0.2; 95% confidence interval, 0.05–0.81; P=.02).

SMN GENE ANALYSIS

Two patients had 1 SMN1 gene copy but 3 SMN2 copies, and 9 patients had 1 SMN2 gene copy. There was no

Table 1. Demographic Characteristics of ALS Subgroups

<table>
<thead>
<tr>
<th></th>
<th>G93C</th>
<th>D90A</th>
<th>L38V</th>
<th>Non-SOD1 FALS</th>
<th>SALS</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset, mean ± SD, y</td>
<td>45.9 ± 10.6† (n = 20)</td>
<td>52.8 ± 17.4 (n = 5)</td>
<td>38.0 ± 6.6† (n = 10)</td>
<td>54.7 ± 10.8 (n = 18)</td>
<td>58.4 ± 12.0 (n = 359)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Diagnostic delay, mean ± SD, mo</td>
<td>15.3 ± 16.1 (n = 12)</td>
<td>38.0 ± 47.6‡ (n = 5)</td>
<td>NA</td>
<td>7.2 ± 4.3 (n = 16)</td>
<td>12.0 ± 11.3 (n = 269)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Survival, median ± SE (95% CI)§</td>
<td>153.0 ± 46.1 (n = 20)</td>
<td>62.7-243.3 (n = 12)</td>
<td>24.0 ± 6.0 (12-36) (n = 10)</td>
<td>43.0 ± 12.4 (18.9-67.2) (n = 16)</td>
<td>30.0 ± 1.6 (26.9-33.1) (n = 269)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: ALS, amyotrophic lateral sclerosis; CI, confidence interval; NA, not applicable; non–SOD1 FALS, familial ALS not related to the superoxide dismutase 1 gene; SALS, sporadic ALS.

*G93C FALS vs SALS, P<.001.
†L38V FALS vs SALS, P<.001; and vs non–SOD1 FALS, P=.004.
‡D90A vs G93C, non–SOD1 FALS, and SALS, P<.01.
§Estimated by Kaplan-Meier curves.
||P<.001 for difference vs SALS and for difference vs mutant SOD1(L38V) FALS; P=.001 for difference vs non–SOD1 FALS.
We present clinical data on the largest group of patients with SOD1 (G93C)–related FALS yet reported, and the first pathologic data of a patient with this mutation, to our knowledge.

In our population, SOD1 (G93C) was associated with a purely lower motor neuron clinical phenotype and absence of bulbar involvement. Aside from variability in age at onset and survival, this phenotype was quite constant in comparison with some other SOD1 mutations, especially SOD1 (I113T) (Table 2). The SMN gene copy number, previously proposed as a susceptibility or prognostic factor in SALS, did not explain this variability in our patients. This suggests that other modifiers exist.

Predominant lower motor neuron involvement, as found in our patients with SOD1 (G93C) FALS, is also a frequent finding in other SOD1 mutations, especially in SOD1 (A4V) and SOD1 (A4T) (Table 2). Although pathologic data on SOD1-related ALS are limited, findings similar to those in our patient, such as mild corticospinal tract involvement, prominent myelin loss in posterior columns and spinocerebellar tracts, and degeneration of Clarke column, seem to define the pathologic picture in SOD1 (A4V), SOD1 (A4T), and SOD1 (E100G). However, in these reports, brainstem involvement was more pronounced than in our patient, who showed only hyaline inclusions in the hypoglossal nucleus, without neuronal loss. Absence of Bunina bodies has been a consistent finding in SOD1–related ALS, except for 1 patient with a de novo H80A mutation and a patient with an insertion at codon 127.

Table 2. Clinicopathologic Comparison With Other SOD1 Mutations

<table>
<thead>
<tr>
<th>SOD1 Mutation*</th>
<th>A4V</th>
<th>H46R</th>
<th>I113T†</th>
<th>G93C</th>
<th>D101N*</th>
<th>A4T</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients‡</td>
<td>167</td>
<td>62</td>
<td>29</td>
<td>20</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>With clinical neurologic details</td>
<td>19</td>
<td>56</td>
<td>10</td>
<td>20</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>With pathologic details</td>
<td>9</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Age at onset, y</td>
<td>Mean ± SD</td>
<td>47 ± 14</td>
<td>45 ± 10</td>
<td>57 ± 13</td>
<td>46 ± 11</td>
<td>41 ± 10</td>
</tr>
<tr>
<td>Range</td>
<td>21-78</td>
<td>25-68</td>
<td>26-84</td>
<td>33-71</td>
<td>26-57</td>
<td>21-60</td>
</tr>
<tr>
<td>Survival, y</td>
<td>Mean ± SD</td>
<td>1.2 ± 0.7</td>
<td>1.7 ± 0.9</td>
<td>4.2 ± 4.2</td>
<td>13 ± 4</td>
<td>2.4 ± 0.9</td>
</tr>
<tr>
<td>Range</td>
<td>0.5-4.0</td>
<td>6-47</td>
<td>6.0-21</td>
<td>5-20</td>
<td>1.1-4.0</td>
<td>NA</td>
</tr>
</tbody>
</table>

Clinical Information||

<table>
<thead>
<tr>
<th>Onset site</th>
<th>Spinal/bulbar</th>
<th>Lower limb</th>
<th>Upper limb/ lower limb</th>
<th>Lower limb</th>
<th>Lower limb/ upper limb</th>
<th>Lower limb/ upper limb</th>
</tr>
</thead>
<tbody>
<tr>
<td>UMN signs</td>
<td>– (Sens) (EO)</td>
<td></td>
<td>–/ +/ +/ +</td>
<td>–</td>
<td>–/ (+)</td>
<td>–</td>
</tr>
<tr>
<td>Atypical signs</td>
<td>–/Sens</td>
<td>–/ (Sens)</td>
<td>–/ (Sens)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pathologic findings</td>
<td>BS motor nuclei</td>
<td>+/ +</td>
<td>XII</td>
<td>–/ +</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Motor cortex</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>–/+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CST degeneration</td>
<td>+/ +/ +/ +/</td>
<td>+/ +</td>
<td>–/ +/ +/</td>
<td>+/ +/</td>
<td>+/ +</td>
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<tr>
<td>PC degeneration</td>
<td>+/ +/ +/ +/</td>
<td>+/ +/ +/</td>
<td>+/ +/ +/</td>
<td>+/ +/ +/</td>
<td>+/ +/ +/</td>
<td>+/ +/ +/</td>
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<tr>
<td>SCT-Cl degeneration</td>
<td>+/ +/ +/</td>
<td>+/ +/ +/</td>
<td>+/ +/ +/</td>
<td>+/ +/ +/</td>
<td>+/ +/ +/</td>
<td>+/ +/ +/</td>
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<tr>
<td>Other neurodegeneration</td>
<td>–/ SN/dorsal vagal nucleus, gracile nucleus</td>
<td>–</td>
<td>OI/SN, LC, GP</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
| LBHI | + | – | +|+|+|+|+
| References | 2, 3, 10-14 | 13, 15-18 | 2, 13, 14, 19-26 | Current study | 27-29 | 30-32 |

Abbreviations: BS, brainstem; CST, corticospinal tract; EO, external ophthalmoparesis; GP, globus pallidus; LBHI, Lewy body–like hyaline inclusions; LC, locus ceruleus; NA, not available; OI, oliva inferior; PC, posterior column; SCT-Cl, spinocerebellar tract and Clarke nucleus; Sens, sensory signs; SN, substantia nigra; SOD1, superoxide dismutase 1 gene; UMN, upper motor neuron; –, absent; +, mild; ++, moderate to severe.

*Clinical signs and neuropathological findings of anterior horn degeneration were moderate to severe in all of the mutations.
††There were neurofibrillary tangles associated with the degeneration of SN, LC, and GP; in other cases, hyaline conglomerates and argyrophilic inclusions were observed.
\*\*Discordance between degeneration in Clarke nucleus and SCT.

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tations and FALS in general. Among SOD1 mutations, SOD1(L38V) and SOD1(G37R) have been shown to present earlier than other mutations, reflected in a mean age at onset of 38 years in our patients with SOD1(L38V). In our population, the comparison with patients with FALS carrying other mutations in SOD1 did not reach statistical significance, probably because of small numbers. Indications of a better prognosis associated with SOD1(G93C) (median survival, 153 months) were reported before, but these analyses were done only on patients with FALS and did not adjust for all the known prognostic variables. We demonstrated that SOD1(G93C)–related FALS has a significantly better prognosis than SALS, non–SOD1–related FALS, and SOD1(L38V)–related FALS, independent of age at onset, site of onset, percentage of predicted FVC, and diagnostic delay. The independent prognostic value of these latter factors was confirmed.

The reason for the better prognosis and particular phenotype of SOD1(G93C) remains elusive, as are the reasons why other mutations, like SOD1(A4V), have a worse prognosis. However, our data are useful to estimate prognosis in these rare families and may help to validate theories about the pathogenicity of SOD1 mutations. Indeed, the remarkable differences in clinical severity, age at onset, and type (lower-upper, spinal-bulbar) of motor neuron involvement between certain SOD1 mutations may be a reflection of their different biological properties. A hint to possible mechanisms in this diversity of phenotypes was provided in a recent report on the selective association of mutant SOD1 with mitochondria of affected tissues in transgenic mouse models of ALS. More research into differences in molecular mechanisms between SOD1 mutations with well-defined clinicopathologic phenotypes in humans is needed to solve these issues.

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Correspondence: Wim Robberecht, MD, PhD, Department of Neurology, University Hospital Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium (wim.robbrecht@u.z.kuleuven.ac.be).

Author Contributions: Study concept and design: Régal, Vanopdenbosch, Van Den Bosch, and Robberecht. Acquisition of data: Vanopdenbosch, Tilkin, Sciot, and Robberecht. Analysis and interpretation of data: Régal, Vanopdenbosch, Thijs, Sciot, and Robberecht. Drafting of the manuscript: Régal, Tilkin, and Robberecht. Critical revision of the manuscript for important intellectual content: Régal, Vanopdenbosch, Van Den Bosch, Thijs, Sciot, and Robberecht. Statistical analysis: Thijs and Robberecht. Obtained funding: Robberecht. Administrative, technical, and material support: Régal, Tilkin, Sciot, and Robberecht. Study supervision: Vanopdenbosch, Van Den Bosch, Sciot, and Robberecht.

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