Background: The basis of heterogeneity in the clinical presentation and rate of progression of amyotrophic lateral sclerosis (ALS) is poorly understood.

Objectives: To use diffusion tensor imaging as a measure of axonal pathologic features in vivo in ALS and to compare a homogeneous form of familial ALS (homozygous D90A SOD1 [superoxide dismutase 1]) with sporadic ALS.

Design: Cross-sectional diffusion tensor imaging study.

Setting: Tertiary referral neurology clinic.

Patients: Twenty patients with sporadic ALS, 6 patients with homozygous D90A SOD1 ALS, and 21 healthy control subjects.

Main Outcome Measure: Fractional anisotropy in cerebral white matter.

Results: Patients with homozygous D90A SOD1 ALS showed less extensive pathologic white matter in motor and extramotor pathways compared with patients with sporadic ALS, despite similar disease severity assessed clinically using a standard functional rating scale. Fractional anisotropy correlated with clinical measures of severity and upper motor neuron involvement.

Conclusion: In vivo diffusion tensor imaging measures demonstrate differences in white matter degeneration between sporadic ALS and a unique familial form of the disease, indicating that genotype influences the distribution of cerebral pathologic features in ALS.


There is considerable variation in the clinical presentation and rate of progression of amyotrophic lateral sclerosis (ALS), but the basis of this heterogeneity is poorly understood. About 10% of ALS cases are inherited in a mendelian fashion, with mutations in the superoxide dismutase 1 (SOD1) gene (GenBank AY450286) being responsible for 20% of these.

The D90A SOD1 mutation is commonly inherited as a recessive trait with high penetrance in homozygotes.

Homozygous D90A SOD1 cases are genetically homogeneous, sharing a common founder about 60 generations ago.

The resulting clinical phenotype is stereotyped as a spastic paraparesis progressing to upper limb and bulbar involvement.

The disease is slowly progressive, with a much longer survival than with sporadic ALS (mean, 14 vs 3 years, respectively).

Groups of patients with genetic forms of ALS that are clinically homogeneous provide a unique opportunity to study the pathologic basis of the relationship between genotype and phenotype in ALS.

Neuroimaging techniques have been used in ALS in an attempt to improve diagnosis and to measure disease progression, as well as to provide a quantitative method of studying the pathophysiologic function of the disease in vivo. Diffusion tensor imaging (DTI) is a magnetic resonance (MR) imaging technique in which the signal is sensitized to the diffusion of water within brain tissue (which is restricted by barriers such as cell membranes and occurs preferentially along the direction of white matter tracts). Fractional anisotropy (FA) is a measure of the directionality of diffusion in a voxel [from 0 (complete lack of directionality of diffusion) to 1 (complete anisotropy)] and is reduced when white matter tracts are disrupted.

Previous studies using region-of-interest approaches to analyze DTI data in ALS have shown reduced FA in the corticospinal tract, consistent with the known pathologic features of the disease. Voxel-based approaches to analysis have demonstrated evidence of white matter damage in extramotor pathways.
Diffusion variables have been reported, but results have been inconsistent.12,13 Diffusion changes have been reported in asymptomatic patients with SOD1 mutations, suggesting that the technique is sensitive to early pathophysiologic changes.14 Diffusion tensor imaging has demonstrated distinct patterns of reduced FA in primary lateral sclerosis compared with ALS.15

We used whole-brain analysis of DTI data to test the hypothesis that FA changes in a clinically and genetically homogeneous form of ALS (homozygous D90A SOD1 variant) can be distinguished from those in sporadic ALS. Specifically, we hypothesized that extramotor involvement would be less marked in homozygous D90A SOD1 ALS cases, as recent evidence suggests that cognitive changes are lacking or less marked in ALS with SOD1 mutations10 and that immunoreactive inclusions of TAR DNA-binding protein of 43 kDa are absent or scarce in SOD1 compared with sporadic cases.17 In addition, we explored correlations between FA and clinical measures in ALS. We hypothesized that greater disease severity, more extensive clinical upper motor neuron (UMN) involvement, and longer disease duration would correlate with lower FA.

### METHODS

#### PATIENTS

The study included 20 patients with sporadic ALS, 6 patients with homozygous D90A SOD1 ALS, and 21 healthy control subjects. All subjects were right handed and had no history of hypertension, cerebrovascular disease, or diabetes mellitus. The study was approved by the Institute of Psychiatry, King's College London (London, England) local research ethics committee and was performed in accord with the ethical standards of the 1964 Declaration of Helsinki. All subjects gave written informed consent before their inclusion in the study.

All suitable patients with sporadic ALS attending the motor disorders clinic at King’s College Hospital were invited to participate in the study. Patients with ALS were diagnosed following clinical examination by a consultant neurologist. Other conditions were excluded by appropriate blood tests, neurophysiologic testing, and neuroimaging.18 Amyotrophic lateral sclerosis was categorized according to the revised El Escorial criteria:19 4 patients had definite ALS, 11 had probable ALS, and 5 had possible ALS. Patients with homozygous D90A SOD1 ALS were recruited from Umeå University Hospital (Umeå, Sweden) and traveled to London to take part in the study. Healthy controls without a history of neurologic disorder were recruited among the spouses and friends of patients and among members of a local volunteer organization.

All subjects underwent a clinical assessment, including full neurologic examination. Patients with ALS were assessed using the ALS Functional Rating Scale–Revised (ALSFRS-R).20 In addition, a UMN “burden” score was calculated to quantify the degree of UMN involvement in each patient.21 This score is the total number of pathologically brisk reflexes (including extensor plantar responses, brisk facial and jaw jerks, and biceps, supinator, triceps, finger, knee, and ankle reflexes), with a maximum possible score of 16.

#### DATA ACQUISITION

Diffusion tensor imaging data were acquired using a 1.5-T MR imaging scanner (GE Signa NV/i; General Electric, Milwaukee, Wisconsin) with actively shielded magnetic field gradients (maximum amplitude, 40 mT/m) and a standard quadrature transmit-and-receive birdcage head coil. All images were acquired from the whole brain, with sections parallel to the anterior commissure–posterior commissure line.

Using a multissection, peripherally gated echoplanar imaging pulse sequence, each DTI volume was acquired from 60 contiguous 2.5-mm-thick sections with a field of view of 240 × 240 mm and an acquisition matrix size of 96 × 96 pixels (zero filled to 128 × 128 pixels), giving an in-plane voxel size of 1.875 × 1.875 mm². Echo time was 107 milliseconds. Effective repetition time was 15 R-R intervals. At each location, 7 images were acquired without diffusion weighting, together with 64 images with a weighting of 1300 s/mm² applied along directions uniformly distributed in space.22 Data acquisition from patients and controls was interleaved to ensure that a shift in scanner performance would not lead to spurious results. The quality of echoplanar imaging data was assessed using an automated analysis technique.23

#### DATA ANALYSIS

Diffusion-weighted images were initially corrected for eddy-current distortion using in-house software that included a mutual information–based registration.24 Brain data were masked from the background using a semiautomated thresholding procedure. The diffusion tensor was then calculated for each brain voxel using multivariate linear regression analysis after logarithmic transformation of the signal intensities. The tensor matrix at each voxel was subsequently diagonalized to compute the eigenvalues and the corresponding eigenvectors. Fractional anisotropy maps were then constructed.

#### IMAGE PREPROCESSING

Preprocessing was performed using Statistical Parametric Mapping software (SPM2; Wellcome Department of Imaging Neurosciences, University College London, London). The FA maps for each subject were registered to a customized study-specific FA template. Images were then segmented, and a binary mask of white matter was created for each subject. The registered images were smoothed with a gaussian kernel of 4-mm full width at half maximum. Finally, the white matter masks were applied to the smoothed images.

#### STATISTICAL ANALYSIS

A nonparametric approach was used for the voxel-based statistical analysis. This allows a test statistic incorporating spatial information (3-dimensional cluster mass, which has no parametric distribution) to be used and reduces the need for extensive smoothing to ensure that the residuals of the model tested follow a gaussian distribution (which may not be true for DTI data). Between-group differences in diffusion variables were estimated by fitting an analysis of variance model at each intracerebral voxel. Permutation-based testing (http://www .brainmap.it) was used to assess statistical significance at voxel and cluster levels. Voxels of interest were initially selected using a lenient P value (P < .05) to reduce the subsequent search volume. We then searched for spatial clusters among these and tested the “mass” of each cluster (the sum of suprathreshold voxel statistics it comprises) for significance. The threshold level for statistical significance was set for each analysis at which less than 1 false-positive cluster would be expected per whole-brain map. In addition, a similar voxel-based analysis was performed to examine correlations between FA, disease duration, the UMN score, and the ALSFRS-R score within the sporadic ALS group.
IDENTIFICATION OF CLUSTERS

Significant clusters were identified. Identification was based on the knowledge of white matter anatomy by one of us (M.C.) in conjunction with the use of a published atlas.27

RESULTS

PATIENT CHARACTERISTICS

The demographic and clinical data for the 3 study groups are given in the Table. The 3 groups were similar in age, but the proportion of women was higher in the homozygous D90A SOD1 ALS group. The 2 patient groups were well matched for disease severity as assessed by the ALSFRS-R score ($t_{24}=0.475$, $P=.64$), and the degree of UMN involvement in the 2 groups was similar ($t_{24}=-0.770$, $P=.45$) (independent samples $t$ test for both). Disease duration was not significantly shorter among the sporadic ALS group ($t_{24}=-1.368$, $P=.18$), although the means were 28 and 46 months for the sporadic ALS and homozygous D90A SOD1 ALS groups, respectively.

Within the sporadic ALS group, disease duration, the UMN score, and the ALSFRS-R score were assessed. None of these variables significantly correlated with each other.

GROUP COMPARISON

Patients with sporadic ALS showed lower FA than controls in the body of the corpus callosum (particularly the region linking the precentral and postcentral gyri) and the corona radiata bilaterally ($P<.003$) (Figure 1). These significant clusters extended dorsally into the subcortical U-shaped short fibers and longitudinal association tracts. Patients with homozygous D90A SOD1 ALS showed higher FA than patients with sporadic ALS in these regions and in occipitotemporal and occipitoparietal white matter ($P<.003$) (Figure 2).

CORRELATION IN PATIENTS WITH SPORADIC ALS

ALSFRS-R Score

The ALSFRS-R score positively correlated with FA (ie, more disability equals lower FA) in motor and extramotor pathways ($P=.005$) (Figure 3). Significant correlations were found throughout the corticospinal tract, including the corona radiata, internal capsule, cerebral peduncles, and pons. In addition, the ALSFRS-R score positively correlated with FA in the middle and superior cerebellar peduncles, occipitotemporal fibers, occipitoparietal fibers, orbitofrontal fibers, and arcuate fasciculus.

Table. Demographic and Clinical Characteristics of Patients and Healthy Control Subjects

<table>
<thead>
<tr>
<th>Study Group</th>
<th>No.</th>
<th>Male to Female Ratio</th>
<th>Age, Mean (SD), y</th>
<th>Site of Disease Onset</th>
<th>Disease Duration, Mean (SD), mo</th>
<th>ALSFRS-R Score, Mean (SD)</th>
<th>UMN Score, Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control subjects</td>
<td>21</td>
<td>12:9</td>
<td>53 (17)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Patients with sporadic ALS</td>
<td>20</td>
<td>13:7</td>
<td>58 (10)</td>
<td>17 LO, 3 BO</td>
<td>28 (18)</td>
<td>38 (7)</td>
<td>7.9 (5)</td>
</tr>
<tr>
<td>Patients with homozygous D90A SOD1 ALS</td>
<td>6</td>
<td>1:5</td>
<td>50 (12)</td>
<td>6 LO</td>
<td>46 (50)</td>
<td>36 (6)</td>
<td>9.5 (2)</td>
</tr>
</tbody>
</table>

Abbreviations: ALS, amyotrophic lateral sclerosis; ALSFRS-R, ALS Functional Rating Scale–Revised; BO, bulbar onset; LO, limb onset; UMN, Upper Motor Neuron; ellipsis, not applicable.

Figure 1. Map of significant clusters of reduced fractional anisotropy in patients with sporadic amyotrophic lateral sclerosis compared with control subjects. Significant clusters are seen in the corona radiata (orange arrow), corpus callosum (green arrow), and subcortical white matter (yellow arrow).

Figure 3. Significant correlation of the ALSFRS-R score with fractional anisotropy (FA) in the middle and superior cerebellar peduncles, occipitotemporal fibers, occipitoparietal fibers, orbitofrontal fibers, and arcuate fasciculus.
UMN Score

The UMN score negatively correlated with FA in motor and extramotor pathways (\(P=.008\)) (Figure 4). Significant correlations were found throughout the corticospinal tract, including the subcortical white matter, corona radiata, internal capsule, cerebral peduncles, and pons. In addition, the UMN score positively correlated with FA in the body and splenium of the corpus callosum and in occipitotemporal fibers.

Disease Duration

Disease duration negatively correlated with FA in a few small clusters. These clusters involved the subcortical white matter, internal capsule, cerebral peduncles, and orbitofrontal fibers (\(P=.008\)).

COMMENT

We applied DTI in patients with homozygous D90A SOD1 ALS, patients with sporadic ALS, and healthy controls to explore the nature of the interaction of genotype and phenotype. Despite the few patients in the homozygous D90A SOD1 ALS group, the power to detect between-group differences remains good because of their homogeneity. Although the sex distribution of the patient groups was not balanced, FA did not differ significantly between men and women in the sporadic ALS group. We showed that patients with homozygous D90A SOD1 ALS have less extensive reductions in FA than patients with sporadic disease, despite similar UMN involvement and clinical scores. Extramotor pathways seem to be less involved in homozygous D90A SOD1 ALS cases. In addition, our results confirm the sensitivity of DTI to detect motor and extramotor pathologic white matter in ALS and demonstrate that diffusion variables correlate with clinical manifestations of the disease.

Our finding of less extensive cerebral pathologic white matter in the homozygous D90A SOD1 form of ALS is consistent with previously reported positron emission tomographic, volumetric MR imaging, and neurophysiologic data. Using flumazenil C 11 positron emission tomography as a marker of cortical neuronal loss or dysfunction, Turner et al28 noted a less extensive pattern of reduced binding among patients with homozygous D90A
SOD1 ALS compared with patients with sporadic ALS of similar disability. Subsequent investigation using voxel-based morphometric measurements supported these findings. Neurophysiologic evidence suggests that intracortical inhibition may be preserved in patients with homozygous D90A SOD1 ALS, in contrast to the increased cortical excitability seen in patients with sporadic ALS. Our findings are compatible with the notion that distinctive phenotypes, in this case determined by a unique and homogeneous genotype, may be identifiable using DTI.

It is now accepted that neurodegeneration in ALS extends outside of the motor system and that ALS overlaps with frontotemporal dementia. Whole-brain analysis of DTI data can characterize the distribution of pathologic white matter outside of motor pathways in ALS. Our data show involvement of the corpus callosum and more widespread degeneration of association tracts, in keeping with the findings of others showing reduced FA in several extramotor regions, particularly in the frontal lobes.

The correlations between FA and clinical measures highlight the potential of DTI as an objective way to assess ce-
Cerebral involvement in ALS in vivo and as a potential source of disease biomarkers for use in therapeutic monitoring. Previous studies\(^8\),\(^12\),\(^33\) have reported that FA correlates with disease severity, but results have been inconsistent.\(^13\) We find strong evidence that reduced FA correlates with increasing disease severity (ALSFRS-R score), greater UMN involvement as assessed clinically, and longer disease duration. Longitudinal studies are needed to confirm the potential of FA as a biomarker, although the use of longitudinal MR imaging may be limited by patients' ability to tolerate imaging as their disease progresses.\(^9\)

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Author Contributions: Dr Leigh had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Turner, V. C. Williams, S. C. R. Williams, Leigh, Andersen, and Simmons. Acquisition of data:

Figure 4. Map of significant clusters of voxels showing a negative correlation between fractional anisotropy and the Upper Motor Neuron score. Significant clusters are seen in the cerebellar peduncles (lime green arrow), corticospinal tracts in the brainstem (purple arrow), cerebral peduncles (red arrow), internal capsule (white arrow), occipitotemporal fibers (gray arrow), corpus callosum (green arrows), corona radiata (orange arrow), and subcortical white matter (yellow arrow).

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