Clustering of Neuropsychiatric Disease in First-Degree and Second-Degree Relatives of Patients With Amyotrophic Lateral Sclerosis

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**IMPORTANCE** Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative condition primarily involving the motor system. There is increasing epidemiologic evidence of an association between ALS and a wider spectrum of neurodegenerative and neuropsychiatric disorders among family members, including schizophrenia and psychotic illness and suicidal behavior.

**OBJECTIVE** To examine the frequency and range of neuropsychiatric conditions that occur within individual first-degree and second-degree relatives of patients with ALS.

**DESIGN, SETTING, AND PARTICIPANTS** In this population-based, case-control family aggregation study, all 202 patients included in the Irish ALS Register between January 1, 2012, and January 31, 2014, with definite, probable, or possible ALS as defined by El Escorial criteria were invited to participate. A total of 75 patients were unable or refused to participate and were excluded; the remaining 127 patients with incident ALS were genotyped for the C9orf72 repeat expansion and 132 age- and sex-matched controls were included in the study.

**MAIN OUTCOME AND MEASURES** The prevalence of defined neuropsychiatric disease in first-degree and second-degree relatives of patients with ALS and matched controls was determined.

**RESULTS** Mean (SD) age at diagnosis of the 127 patients in the study (58 women and 69 men) was 64.2 (10.7) years. Data from 2116 relatives of patients with ALS were reported, including 924 first-degree relatives, 1128 second-degree relatives, and 64 third-degree relatives. Data from controls were reported from 829 first-degree and 1310 second-degree relatives. A total of 77 patients with ALS (61.4%) and 51 control participants (38.6%) reported at least 1 first-degree or second-degree relative with a history of schizophrenia, psychosis, suicide, depression, alcoholism, or autism (relative risk [RR], 1.50; 95% CI, 1.08-2.17; \( P = .02 \)). Cluster analysis suggested the following 2 subgroups based on the number of family members with a neuropsychiatric condition: expected (0-2) and high (\( \geq 3 \)). Within the high subgroup, ALS kindreds presented a significantly higher rate of psychiatric illness than did controls (28 of 39 [71.8%]; mean [SD] number of siblings, 4.29 [1.41]; \( P = .001 \)). A strong family history of schizophrenia (RR, 3.40; 95% CI, 1.27-9.30; \( P = .02 \)), suicide (RR, 3.30; 95% CI, 1.07-10.05; \( P = .04 \)), autism (RR, 10.10; 95% CI, 1.30-78.80; \( P = .03 \)), and alcoholism (RR, 1.48; 95% CI, 1.01-2.17; \( P = .045 \)) was reported in ALS kindreds. A total of 5 of 29 probands (17.2%) with a strong family history of neuropsychiatric conditions (\( \geq 3 \) first-degree or second-degree relatives) carried the C9orf72 repeat expansion.

**CONCLUSIONS AND RELEVANCE** Neuropsychiatric symptoms in addition to schizophrenia, including obsessive-compulsive disorder, autism, and alcoholism, occur more frequently in ALS kindreds than in controls. The presence of the C9orf72 repeat expansion does not fully account for this finding, suggesting the presence of additional pleiotropic genes associated with both ALS and neuropsychiatric disease in the Irish population.

Published online October 16, 2017.

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative condition primarily involving the motor system. Deep phenotyping studies have provided compelling evidence of phenotypic heterogeneity, suggesting that ALS is a disease spectrum rather than a single entity. Although it was originally considered a pure motor system degeneration, the spectrum has expanded to include extramotor involvement in subcohorts of patients with ALS, including the presence of behavioral variant frontotemporal dementia in up to 13% of patients at the time of diagnosis, with known executive dysfunction and behavioral and social cognitive change in up to 70% of cases. The phenotypic heterogeneity in behavioral variant frontotemporal dementia and ALS can be attributed in part to pathogenic mechanisms associated with the presence of a hexanucleotide expansion in C9orf72 (NCBI reference sequence NC_000009.12), which accounts for up to 10% of cases of ALS and up to 30% of cases of frontotemporal dementia in European populations. Several studies have shown that this variant can also be associated with a range of neuropsychologic and neuropsychiatric phenotypes including behavioral variant frontotemporal dementia, psychosis, Huntington disease phenocopies, obsessive-compulsive disorder, and bipolar affective disorder.

A previous population-based case-control cohort study demonstrated increased aggregation of schizophrenia and psychotic illness and suicidal behavior in ALS cohorts. Although this aggregation was associated with the presence of the C9orf72 variant in probands with ALS, in some cases, higher rates of psychosis and suicidal behavior were also noted in relatives of probands who did not carry the C9orf72 repeat expansion. It remains unclear as to whether this overlap with neuropsychiatric conditions is mediated by other pleiotropic genes of major effect within individual kindreds, or occurs as a consequence of shared polygenic risk across the entire ALS spectrum. In favor of the latter, a recent report described a 14% shared polygenic risk between ALS and schizophrenia using summary statistics from a combined ALS and schizophrenia genome-wide association study. However, several studies have also demonstrated that the genetic architecture of ALS seems to differ from that of schizophrenia, and that rare variants and private mutations are likely to account for a higher proportion of cases of ALS, providing an explanation for the missing heritability in ALS. An additional explanation for missing heritability is the likely presence of genetic pleiotropy, in which a pathogenic gene variant is associated with more than 1 phenotypic expression within a kindred. In this instance, the presence of ALS within a kindred would be interpreted as either sporadic or familial with incomplete penetrance, as the definition of familial disease based purely on a recurrence of ALS within the pedigree would be excessively narrow.

The purpose of this study was to explore the possibility of genetic pleiotropy within ALS kindreds using a case-control design. We have expanded the previous familial aggregation study of ALS kindreds by examining a second incident-based cohort of patients with ALS to determine the extent to which other Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), axis I and II disorders might occur in individual family members of patients with ALS. The objectives were to establish whether these traits are distributed uniformly across all ALS kindreds and whether these traits segregate within individual kindreds, suggesting the presence of single or oligogenic pleiotropic gene variants.

Methods

We designed a population-based, case-control family aggregation study. All patients included in the Irish ALS Register between January 1, 2012, and January 31, 2014, with definite, probable, or possible ALS by El Escorial criteria were invited to participate. Informed written consent for the study was obtained from patients with ALS and matched controls. The study was approved by the Beaumont Hospital Research Ethics Committee.

For each patient, an age- and sex-matched control was recruited at random from the records of the patient’s general practitioner, or, if this proved impossible, from the records of a general practitioner in the same area. The presence or absence of neuropsychiatric disease did not form part of the recruitment criteria. The family history questionnaire was administered only after recruitment of probands.

Data Collection

Probands with ALS and matched controls were asked to complete a family history questionnaire in which details of neurologic and neuropsychiatric conditions reported by all first-degree and second-degree relatives were ascertained, followed by a semistructured interview with the proband or another family member, where possible. This semistructured interview ensured accuracy of the material recorded on the questionnaire, and addressed missing data where possible. An identical method was used for patients and controls. Details included questions about medical conditions in parents, siblings, and children (first-degree relatives) and grandparents, uncles, and aunts (second-degree relatives), who were older than 18 years of age. All respondents were asked specifically about the occurrence among their immediate relatives of psychiatric conditions (defined by DSM-IV at the time of this study’s inception), including major psychotic illness (schizophrenia or bipolar disorder), suicide, autism or autism...
Cluster of Neuropsychiatric Disease in Relatives of Patients With ALS

Table 1. Demographics of Patients With ALS Included or Not Included in the Study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients, No. (n = 127)</th>
<th>Patients, No. (n = 75)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>66.2 (10.1)</td>
<td>62.8 (10.2)</td>
<td>.004</td>
</tr>
<tr>
<td>At onset</td>
<td>68.1 (10.1)</td>
<td>64.2 (10.7)</td>
<td>.03</td>
</tr>
<tr>
<td>Female sex</td>
<td>30 (40.0)</td>
<td>58 (45.7)</td>
<td>.47</td>
</tr>
<tr>
<td>Site of onset</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulbar</td>
<td>30 (40.0)</td>
<td>35 (27.6)</td>
<td>.15</td>
</tr>
<tr>
<td>Limb</td>
<td>38 (50.7)</td>
<td>81 (63.8)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>7 (9.3)</td>
<td>11 (8.7)</td>
<td></td>
</tr>
<tr>
<td>C9orf72 repeat status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>6 (8.0)</td>
<td>21 (16.5)</td>
<td>.64</td>
</tr>
<tr>
<td>Negative</td>
<td>35 (46.7)</td>
<td>90 (70.9)</td>
<td></td>
</tr>
<tr>
<td>Not available</td>
<td>34 (45.3)</td>
<td>16 (12.6)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: ALS, amyotrophic lateral sclerosis.

Table 2. Prevalence and Relative Risk of Neuropsychiatric Conditions in First- and Second-Degree Relatives of Patients With ALS Compared With Controls

<table>
<thead>
<tr>
<th>Condition</th>
<th>Relatives, No. (n = 2116)</th>
<th>Relatives, No. (n = 2139)</th>
<th>RR</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suicide</td>
<td>13</td>
<td>4</td>
<td>3.30</td>
<td>.04</td>
</tr>
<tr>
<td>Schizophrenia and psychotic illness</td>
<td>17</td>
<td>5</td>
<td>3.40</td>
<td>.02</td>
</tr>
<tr>
<td>Autism</td>
<td>10</td>
<td>1</td>
<td>10.10</td>
<td>.03</td>
</tr>
<tr>
<td>Depression</td>
<td>35</td>
<td>31</td>
<td>1.14</td>
<td>.59</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>63</td>
<td>43</td>
<td>1.48</td>
<td>.045</td>
</tr>
<tr>
<td>Obsessive-compulsive disorder and rigid personality disorders</td>
<td>11</td>
<td>2</td>
<td>5.60</td>
<td>.02</td>
</tr>
</tbody>
</table>

Abbreviations: ALS, amyotrophic lateral sclerosis; RR, relative risk.

Results

A total of 127 patients with incident ALS, diagnosed between January 1, 2012, and January 31, 2014, who agreed to complete the questionnaire on family history, were included in the study. There were no significant differences in age of diagnosis, sex, site of onset, or the proportion of patients reporting a positive family history of ALS between this cohort and a previously reported cohort of 172 patients with ALS.8 In addition, there were no significant differences in sex, El Escorial criteria at first assessment, site of onset, or the proportion reporting a positive family history between those who agreed to inclusion in the study and were able to provide a comprehensive family history and those who either declined inclusion or were unable to complete a family history (Table 1). As in a previous study,8 patients who declined to participate were significantly older at symptom onset and diagnosis than those who were included in the study.

Data from 2116 relatives of patients with ALS were reported, including 924 first-degree relatives, 1128 second-degree relatives, and 64 third-degree relatives. Data from controls were reported from 829 first-degree and 1310 second-degree relatives. There was no statistically significant difference between the kindreds of probands with ALS and controls with respect to the number of first-degree and second-degree relatives.

The previously observed8 increased risk of neuropsychiatric disorders including schizophrenia and psychotic illness and suicide in first-degree and second-degree relatives of patients with ALS compared with relatives of controls was replicated (Table 2). Specifically, the relative risk of developing schizophrenia or other psychotic illness among first-degree and second-degree relatives of probands with ALS compared with controls was 3.40 (95% CI, 1.27-9.30; P = .02), while the relative risk of death by suicide in first-degree or second-degree relatives of probands with ALS was 3.30 (95% CI, 1.07-10.05; P = .04).

The reported frequency of obsessive-compulsive disorder, personality disorders, addiction and alcoholism, and autistic spectrum disorders was also assessed in patients and controls. The DSM-IV criteria for each of these conditions were applied by the interviewer, and only patients who fulfilled the criteria, or for whom a confirmed psychiatric diagnosis could be verified, were included. Higher rates of autism (relative risk, 10.10; 95% CI, 1.30-78.80; P = .03),

C9orf72 Genotyping

Samples of DNA were screened using repeat-primed polymerase chain reaction for the presence of a GGGGCC hexanucleotide repeat expansion in C9orf72. Representative DNA from controls who were positive and negative for the presence of a GGGGCC hexanucleotide repeat expansion in C9orf72 was also analyzed by Southern blot to confirm the sensitivity and specificity of the analysis. Polymerase chain reaction products were analyzed on an Applied Biosystems 3130xl genetic analyzer and visualized using GeneMarker software, version 4.0 (Thermo Fisher Scientific). Patients with the characteristic appearance of the expanded hexanucleotide repeat on repeat-primed polymerase chain reaction consisting of a decaying series of 30 or more peaks in duplicate were regarded as having a pathologic expansion, as described previously.14

Statistical Analysis

Baseline characteristics were tested for difference using the χ² test for independence. The relative risk (λ), used in most previously reported family aggregation studies, was calculated by comparing the risk of relatives of patients with ALS patients developing a disease with the risk in relatives of controls.

k-Means clustering was used as a nonhierarchical method to quantify the presence of psychiatric diagnoses and χ² tests compared the distribution of ALS kindred with healthy controls within the k-means clusters. Statistical analysis was carried out using SPSS, version 24 (SPSS Inc). P < .05 (2-tailed) was considered significant.

Spectrum disorder, obsessive-compulsive disorder, addiction, and alcohol dependence.

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alcoholism (relative risk, 1.48; 95% CI, 1.01-2.17; \( P = .045 \)), and other conditions associated with personality rigidity (obsessive-compulsive disorder and rigid personality disorders) (relative risk, 5.60; 95% CI, 1.23-25.05; \( P = .02 \)) were reported in the kindreds of probands with ALS compared with controls (Table 2).

**Clustering of Neuropsychiatric Disease in ALS Within Kindreds**

Of the 127 probands with ALS, 77 (60.6%) had at least 1 sibling, parent, uncle, aunt, or adult child with a history of schizophrenia, psychosis, suicide, depression, alcoholism, autism, or other neuropsychiatric condition, compared with 51 of the 132 controls (38.6%) (Table 3). \( \chi^2 \)-Means clustering identified the following 2 distinct subgroups within the data: expected rate of psychiatric illness, defined as 0 to 2 affected family members (99 of 220 patients with ALS [45.0%]; mean [SD] number of siblings, 0.580 [0.711]; minimum = 0; maximum = 2), and high rate of psychiatric illness, defined as 3 or more affected family members (28 of 39 patients with ALS [71.8%]; mean [SD] number of siblings, 4.29 [1.41]; minimum = 3; maximum = 7). Within the high rate of psychiatric illness group, ALS kindreds represented a significantly higher rate when compared with control kindreds (\( P = .001 \)). This significant clustering of neuropsychiatric disease within the kindreds of probands with ALS was independent of kindred size, and there was no significant difference between probands with ALS and controls in this regard. Probands with ALS with higher reported rates of neuropsychiatric disease reported a mean number of 16 (range, 6-32) first-degree and second-degree relatives, whereas controls reported a mean of 21 (range, 15-27) first-degree and second-degree relatives.

**Neuropsychiatric Disease and C9orf72 Status**

C9orf72 genotyping was available in 111 of the 127 patients with ALS (87.4%). Of these, 21 of the probands (18.9%) carried the repeat expansion, while 90 (81.1%) had a normal C9orf72 repeat expansion profile. A history of dementia affecting at least 1 other family member was reported in all kindreds of probands carrying the C9orf72 repeat expansion. This finding contrasted with kindreds of probands with ALS who were negative for the repeat expansion, in whom 33 (36.7%) reported a diagnosis of dementia in first-degree and second-degree relatives and in controls, in whom 13 of 132 (9.8%) reported the presence of dementia in first-degree and second-degree relatives.

Most C9orf72-positive ALS kindreds (19 of 21 [90.5%]) also reported at least 1 family member with a history of neuropsychiatric disease. The most common neuropsychiatric conditions associated with C9orf72 included depression and alcoholism. However, of the 29 kindreds reporting 3 or more first-degree or second-degree relatives with a neuropsychiatric condition, only 6 of the probands with ALS (20.7%) carried the expanded genotype. The remaining 23 probands with ALS (79.3%) did not carry any of the known pathogenic gene variants.

**Discussion**

This study confirms previous epidemiologic observations of an association between ALS and schizophrenia in Irish kindreds, and extends the finding to other neuropsychiatric conditions characterized by impulse control disorder, addiction, alcoholism, personality rigidity, and autism spectrum disorder, as defined by DSM-IV criteria. Our data support the hypothesis that family members of probands with ALS are more likely to exhibit a neuropsychiatric endophenotype that recapitulates in part the extra motor changes reported in ALS.

We have shown that 60.6% of probands with ALS reported at least 1 relative with a history of schizophrenia, psychosis, suicide, depression, alcoholism, or autism, compared with 38.6% of control kindreds (\( P = .002 \)). Although neuropsychiatric conditions are common within the general population, as demonstrated by their presence within our control cohort, our data clearly suggest that kindreds of probands with ALS are at increased risk for these disorders. Of the participants who clustered in the high rate of familial psychiatric illness (≥3 family members with psychiatric illness), 71% were patients with ALS (\( P = .001 \)), with the same mean number of relatives. Major psychiatric disorders that were specifically overrepresented within these ALS kindreds included schizophrenia and psychosis, suicide, autism, rigid personality disorders, and alcoholism.

In patients with progressive neurodegenerative disease, the effect of psychological stressors on the caregivers and wider family network is well recognized. A higher than expected rate of neuropsychiatric disease in relatives of patients with ALS may be anticipated as a function of disease-induced stress on the family. However, this study was specifically designed to address this point, as we identified those with neuropsychiatric symptoms that had been present prior to any knowledge of ALS within kindreds, in which case the diagnosis would be expected to have no effect on neuropsychiatric presentations within first-degree or second-degree relatives. Moreover, the absence of evidence of increased rates of depression, which would be anticipated if the presence of ALS were a factor, suggests that the findings are not associated with the presence of ALS within kindreds.

Our findings suggest that the presence of the C9orf72 repeat expansions does not fully account for the observed overlap between ALS and neuropsychiatric conditions. Although 5 of 29 probands (17.2%) from kindreds reporting high rates...
of neuropsychiatric conditions (≥3 affected relatives) carried the C9orf72 repeat expansion, the remaining 24 of 29 probands (82.8%) carried the normal variant.

Consistent with results of a previous study, there was no reported difference in the presence of risk of underlying depression in the kindreds of the probands with ALS compared with controls. Given that depression is common within the general population and might be expected to be overrepresented in ALS kindreds owing to a bias in reporting, this finding supports the robustness of our finding of an association between specific neuropsychiatric conditions and ALS.

The absence of an association between depression and ALS, despite the presence of higher rates of reported suicide among first-degree and second-degree relatives of probands with ALS, may reflect an underlying dysregulation of impulse control, rather than a specific alteration in mood. Although further prospective family studies are required to confirm this finding, the dichotomization of depression and suicide was also noted in a previous study. This observation is congruent with the emerging concept of network disruption in ALS leading to a range of behavioral changes, including increasing disinhibition, impulse dyscontrol, and in some cases increased personality rigidity.

This study was unable to demonstrate a significant association between behavioral change in probands with ALS and the presence of a psychiatric endophenotype among first-degree and second-degree relatives. This finding was most likely owing to the low power of our study and the relative insensitivity of the behavioral screening tools that were used.

Limitations
Our study is limited by design. The neuropsychiatric signal among first-degree and second-degree relatives was obtained by report rather than by direct examination of family members, and verification was limited to a series of confirmatory questions by the interviewer. Verification questions included clarification that the diagnosis had been made by a suitably qualified medical practitioner. It is therefore possible that some of the diagnostic categorization is incomplete. However, this limitation applied equally to ALS kindreds from probands with or without the C9orf72 repeat expansion and controls groups, and is therefore unlikely to have substantially biased our findings. Second, it is possible that probands with ALS overreported the presence of psychiatric disorders. We consider this possibility to be unlikely, however, as a possible association between ALS and neuropsychiatric conditions is not commonly known to most patients with ALS. Moreover, the absence of a significant increase in depression among family members supports the veracity of our findings. Our study cohort was slightly enriched by kindreds of probands carrying the C9orf72 repeat expansion, which is likely an artifact of our collection method, as some families carrying the C9orf72 repeat expansion have knowledge of ALS, and are more likely to agree to participate in this type of study.

Conclusions
Notwithstanding, our study confirms a previous observation of higher rates of neuropsychiatric conditions within ALS kindreds. We have shown that this aggregation is driven primarily by kindreds at risk for particular neuropsychiatric conditions that recapitulate the cognitive and behavioral subphenotypes described in ALS, and that this effect is not driven primarily by the presence of the C9orf72 repeat expansion. Detailed subphenotyping and genotyping of members of informative kindreds from probands with ALS will be required to further characterize this association that, if replicated, suggests the presence of a distinct subphenotype of ALS that shares pleiotropic genetic risk with some forms of neuropsychiatric illness.

k-Means clustering was chosen, as the process is a 2-phase iterative heuristic, with data assignment and centroid updating staggered successively. k-Means clustering is a partitional technique used to find clusters, whereby the clusters are represented by their centroids (eg, the arithmetic means of data points within the respective clusters). The statistical convergence of these iterations, which are integral for clusters to be identified, further increased the integrity and robustness of these analyses alongside the closely matched case and control cohorts.

ARTICLE INFORMATION
Accepted for Publication: August 6, 2017.
Published Online: October 16, 2017.
Author Contributions: Dr Hardiman had full access to all 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: O’Brien, Heverin, Byrne, Pender, Hardiman. Acquisition, analysis, or interpretation of data: All authors. Drafting of the manuscript: O’Brien, Heverin, Gibbons, Elamin, Hardiman. Critical revision of the manuscript for important intellectual content: O’Brien, Heverin, Byrne, Vajda, McLaughlin, Byrne, Pinto-Grau, Elamin, Pender, Hardiman.

Statistical analysis: O’Brien, Burke, Gibbons, Elamin, Pender.
Obtained funding: O’Brien, Hardiman.
Administrative, technical, or material support: O’Brien, Heverin, Pinto-Grau, Hardiman.
Supervision: O’Brien, Heverin, Pender, Hardiman.
Conflict of Interest Disclosures: None reported.

Additional Contributions: We thank the ALS Association, the Irish Motor Neurone Disease Association, and the Motor Neurone Disease Association of Great Britain and Northern Ireland, for support in maintenance and access to the ALS registry.

REFERENCES


