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Title: Aggregation of Neuropsychiatric Disease in Amyotrophic Lateral Sclerosis Kindreds:
Evidence of Clustering within Families. **Running head:** Clustering of neuropsychiatric disease
in ALS.

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27 **Key points**

28 Question: This study examines the incidence of neuropsychiatric conditions within ALS
29 kindreds.

30 Findings: This population-based, case–control family aggregation study confirms our
31 previous epidemiologic observations of an association between ALS and schizophrenia in
32 Irish kindreds. In addition, a significant family history of suicide, autism and alcohol overuse
33 was reported in ALS kindreds compared to controls.

34 Meaning: There are significant differences in the incidence of several neuropsychiatric
35 conditions in ALS kindreds compared to controls which is not accounted for by the *C9orf72*
36 expansion.

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38

39

40 **Abstract**

41 *Importance* ALS is a progressive neurodegenerative condition primarily involving the motor
42 system. There is increasing epidemiological evidence of an association between ALS and a
43 wider spectrum of neurodegenerative and neuropsychiatric disorders among family
44 members, including schizophrenia / psychotic illness and suicidal behaviour.

45 *Objective* To examine the frequency and range of neuropsychiatric conditions that occur
46 within individual ALS kindreds.

47 *Design and Setting* A population-based, case–control family aggregation study was
48 designed. All patients included in the Irish ALS Register between January 2012 and January
49 2014 with definite, probable, or possible ALS by El Escorial criteria were invited to
50 participate.

51 *Participants* All incident patients in the Irish ALS Register between January 2012 and January
52 2014 with definite, probable, or possible ALS by El Escorial criteria were invited to
53 participate (n=202). 75 patients were unable or refused to participate and were excluded.
54 127 incident ALS patients genotyped for the *C9orf72* repeat expansion, and 132 age and
55 gender matched controls were included in the study.

56 *Main Outcome and Measures* The prevalence of defined neuropsychiatric disease in first
57 and second degree relatives of ALS patients and matched controls was determined.

58 *Results* Mean age at diagnosis was 64.2, 58% of patients were female. Reported data from
59 2116 relatives of patients with ALS included 924 first-degree relatives and 1128 second-
60 degree relatives. Data from controls comprised 829 first- and 1310 second- degree relatives.
61 78 (61%) of ALS kindreds and 51 (39%) of control kindreds reported at least one first or
62 second degree relatives with a history of schizophrenia, psychosis, suicide, depression,
63 alcoholism or autism (RR1.5, 95% CI 1.08-2.17, P=0.017,). Cluster analysis suggested two
64 subgroups based on the number of family members with a neuropsychiatric condition:
65 Expected (0-2) and High (≥ 3). Within the high sub-group, ALS kindreds (71%) presented a
66 significantly higher than controls ((71% ALS; $X=4.29\pm 1.41$; $p=.001$). A strong family history of
67 schizophrenia (RR 3.4, 95% CI 1.27-9.3, P=0.015), suicide (RR 3.3, 95% CI 1.07-10.05,
68 $p=0.037$), autism (RR 10.1, 95% CI 1.3-78.8, $p=0.027$) and alcohol overuse (RR 1.48, 95% CI
69 1.01-2.17, $p=0.04$) was reported in ALS kindreds. 17% of probands with a strong family
70 history of neuropsychiatric conditions (>3 first or second degree relative) carried the
71 *C9orf72* repeat expansion.

72 *Conclusions and relevance* Neuropsychiatric symptoms in addition to schizophrenia,
73 including obsessive compulsive disorder, autism and alcoholism occur more frequently in

74 ALS kindreds than controls. The presence of the *C9orf72* expansion does not fully account
75 for this, suggesting the presence of additional pleiotropic genes associated with both ALS
76 and neuropsychiatric disease in the Irish population.

77 **Introduction**

78 Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative condition primarily
79 involving the motor system. Recent deep phenotyping studies have provided compelling
80 evidence of phenotypic heterogeneity, suggesting that ALS is a disease spectrum rather than
81 a single entity. Though originally considered a pure motor system degeneration, the
82 spectrum has expanded to include extra-motor involvement in sub-cohorts of ALS patients,
83 including the presence of behavioural variant frontotemporal dementia (bvFTD) in up to
84 13% at the time of diagnosis¹, with known executive dysfunction, behavioural and social
85 cognitive change in up to 70% of cases. The phenotypic heterogeneity in bvFTD and ALS can
86 be attributed in part to pathogenic mechanisms associated with the presence of a
87 hexanucleotide expansion in *C9orf72*, which accounts for up to 10% ALS and up to 30% of
88 FTD in European populations. We, and others, have shown that this variant can also be
89 associated with a range of neurological and neuropsychiatric phenotypes including bvFTD,
90 psychosis, Huntington disease phenocopies, obsessive-compulsive disorder and bipolar
91 affective disorder²⁻⁵.

92

93 Using a population-based case control cohort study, we previously demonstrated increased
94 aggregation of schizophrenia / psychotic illness and suicidal behaviour in ALS cohorts⁶.

95 While this was associated with the presence of the *C9orf72* variant in ALS probands, in some
96 cases, higher rates of psychosis and suicidal behaviour were also noted in relatives of
97 probands who did not carry the *C9orf72* repeat expansion. It remains unclear as to whether
98 this overlap with neuropsychiatric conditions is mediated by other pleiotropic genes of
99 major effect within individual kindreds, or occurs as a consequence of shared polygenic risk

100 across the entire ALS spectrum. In favour of the latter, we have recently reported 14%
101 shared polygenic risk between ALS and Schizophrenia using summary statistics from a
102 combined ALS/Schizophrenia GWAS analysis⁷. However, we, and others, have also
103 demonstrated that the genetic architecture of ALS seems to differ from schizophrenia, and
104 that rare variants and private mutations are likely to account for a higher proportion of ALS,
105 providing an explanation for the “missing heritability” in ALS⁸⁻¹⁰. An additional explanation
106 for missing heritability is the likely presence of genetic pleiotropy, in which a pathogenic
107 gene variant is associated with more than one phenotypic expression within a kindred. In
108 this instance, the presence of ALS within a kindred would be interpreted as either sporadic
109 or familial with incomplete penetrance, as the definition of familial disease purely based on
110 a recurrence of ALS within the pedigree would be excessively narrow.

111

112 The purpose of this study was to explore the possibility of genetic pleiotropy within ALS
113 kindreds using a case control design. We have expanded our previous familial aggregation
114 study of ALS kindreds⁶ by examining a second incident based cohort of patients with ALS to
115 determine the extent to which other DSM-IV axis I and II disorders might occur in individual
116 family members of patients with ALS. The objective was (1) to establish whether these traits
117 are distributed uniformly across all ALS kindreds and (2) whether they segregate within
118 individual kindreds suggesting the presence of single/oligogenic pleiotropic gene variants.

119

120 **Methods**

121 A population-based, case–control family aggregation study was designed. All patients
122 included in the Irish ALS Register between January 2012 and January 2014 with definite,
123 probable, or possible ALS by El Escorial criteria were invited to participate.

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

129 Written consent for the study was obtained from ALS patients and matched controls. The
130 study was approved by Beaumont Hospital Research Ethics Committee.

131

132 Data Collection

133 ALS probands and matched controls were asked to complete a family history questionnaire
134 in which details of neurological and neuropsychiatric conditions reported by all first and
135 second degree relatives were ascertained. This was followed by a semi-structured interview
136 with the proband or another family member, where possible. This semi-structured interview
137 ensured accuracy of the material recorded on the questionnaire, and addressed missing
138 data where possible. An identical methodology was used for patients and controls. Details
139 included questions about medical conditions in parents, siblings and children (first degree
140 relatives) and grandparents, uncles, and aunts (second degree relatives), aged over 18
141 years. All respondents were asked specifically about the occurrence among their immediate
142 relatives of psychiatric conditions (defined by DSM-IV at the time of this study's inception)
143 including major psychotic illness (schizophrenia, bipolar disorder), suicide, autism or autistic
144 spectrum disorder, obsessive compulsive disorder, addiction and alcohol dependence.

145

146 *C9orf72* Genotyping

147 DNA samples were screened using repeat-primed PCR for the presence of a GGGGCC

148 hexanucleotide repeat expansion in *C9orf72*. Representative DNA from positive and
149 negative controls was also analysed by Southern blot to confirm the sensitivity and
150 specificity of the analysis. PCR products were analyzed on an Applied Biosystems 3130xl
151 genetic analyzer and visualized using GeneMapper software (version 4.0). Patients with the
152 characteristic appearance of the expanded hexanucleotide repeat on repeat-primed PCR
153 consisting of a decaying series of 30 or more peaks in duplicate were regarded as having a
154 pathologic expansion as described previously¹¹.

155

156 Statistical Analysis

157 Baseline characteristics were tested for difference using the chi-square test for
158 independence. The relative risk (lambda), used in the majority of previously reported family
159 aggregation studies, was calculated by comparing the risk of developing a disease in the
160 relatives of ALS patients, compared to the risk in relatives of controls. K-means clustering
161 was employed as a non-hierarchical method to quantify the presence of psychiatric
162 diagnoses. Chi-square compared the distribution of ALS kindred to healthy control within
163 the k-means clusters. Statistical analysis was carried out using SPSS v24 (SPSS Inc, Chicago,
164 IL). All statistical testing was performed at the conventional 2-tailed a level of 0.05.

165

166

167 **Results**

168 127 incident ALS patients, diagnosed between 2012-2014, who agreed to complete the
169 questionnaire on family history, were included in the study. There were no significant
170 differences in age of diagnosis, gender, site of onset, or the proportion reporting a positive
171 family history of ALS between this cohort and our previously cohort of 172 ALS patients⁶. In

172 addition, there were no significant differences in gender, El Escorial criteria at first
173 assessment, site of onset, or the proportion reporting a positive family history between
174 those who agreed to inclusion in the study and were able to provide a comprehensive family
175 history compared to those who either declined inclusion or were unable to complete a
176 family history (Table 1). As in a previous study⁶ patients who declined to participate, or who
177 were included in the study were significantly older at symptom onset and diagnosis.

178

179 Reported data from 2116 relatives of patients with ALS included 924 first-degree relatives
180 and 1128 second-degree relatives. Data from controls comprised 829 first- and 1310
181 second- degree relatives. There was no statistical difference between the kindreds of ALS
182 probands and controls with respect to the number of first and second degree relatives.

183 Our previously observed increased risk of neuropsychiatric disorders including schizophrenia
184 / psychotic illness, and suicide observed in first and second degree relatives of patients with
185 ALS compared to relatives of controls was replicated (Table 2). Specifically, the relative risk
186 of developing schizophrenia or other psychotic illness among first and second degree
187 relatives of ALS probands compared with control was 3.4, $p= 0.015$, while the relative risk of
188 death by suicide in first- or second-degree relatives of ALS probands was 3.3, $p=0.037$.

189

190 The reported frequency of OCD, personality disorders, addiction and alcoholism and autistic
191 spectrum disorders) was also assessed in patients and controls. DSM criteria for each of
192 these conditions was applied by the interviewer, and only those cases reporting to fulfil the
193 criteria, or in whom a confirmed psychiatric diagnosis could be verified were included.

194 Higher rates of autism, alcohol dependence and other conditions associated with
195 personality rigidity (obsessive compulsive disorder, and personality disorder) were reported

196 in the kindreds of ALS probands compared with controls (Table 2).

197

198

199 *Clustering of neuropsychiatric disease in ALS within kindreds.*

200 Of the 127 ALS probands, 77 (61%) had at least one sibling, parent, uncle, aunt or adult child

201 with a history of schizophrenia / psychosis / suicide / depression / alcoholism / autism /

202 other neuropsychiatric condition, compared with 51 (39%) of controls. K-means clustering

203 identified two distinct subgroups within the data. These subgroups have been defined as an

204 *expected rate*: 0-2 affected family members (45% ALS; $X = 0.58 \pm 0.711$; Min= 0; Max =2) and a

205 *high rate* of psychiatric illness: ≥ 3 affected family members (71% ALS; $X = 4.29 \pm 1.41$; Min=3;

206 Max=7). Within the high rate of psychiatric illness group, ALS kindreds represented a

207 significantly higher rate when compared to health control kindreds ($p = .001$). This significant

208 clustering of neuropsychiatric disease within the kindreds of ALS probands was independent

209 of kindred size, and there was no significant difference between ALS probands and controls

210 in this regard. ALS probands with higher reported rates of neuropsychiatric disease reported

211 a mean number of 16 (range 6-32) first and second degree relatives, whereas controls

212 reported a mean of 21 (range 15-27) first and second degree relatives.

213

214 *Neuropsychiatric disease and C9orf72 status*

215 *C9orf72* genotyping was available in 111 of the 127 ALS patients (87%). Of these, 19% (21) of

216 probands carried the repeat expansion, while 81% (90) had a normal *C9orf72* repeat

217 expansion profile. A history of dementia affecting at least one other family member was

218 reported in 100% of kindreds of probands carrying the *C9orf72* expansion. This contrasted

219 with kindreds of ALS probands who were negative for the repeat expansion, in whom 27.5%

220 (33) reported a diagnosis of dementia in FDRs and SDRs and in controls, where 10%
221 reported the presence of dementia in FDRs and SDRs.

222

223 The majority of *C9orf72* positive ALS kindreds (19 of 21, 90%) also reported at least one
224 family member with a history of neuropsychiatric disease. The commonest neuropsychiatric
225 conditions associated with *C9orf72* included depression and alcoholism. However, of the 29
226 kindreds reporting more than 3 first or second degree relatives with a neuropsychiatric
227 condition, only 17% (6) of the ALS probands carried the expanded genotype. The remaining
228 23 (83%) of ALS probands did not carry any of the known pathogenic gene variants.

229

230 **Discussion**

231 This study confirms our previous epidemiologic observations of an association between ALS
232 and schizophrenia in Irish kindreds, and extends the finding to other neuropsychiatric
233 conditions characterized by impulse dyscontrol, addiction, alcoholism and rigid / autism
234 spectrum disorder, as defined by DSM-IV. Our data support the hypothesis that family
235 members of ALS probands are more likely to exhibit a neuropsychiatric endophenotype that
236 recapitulates in part the extra motor changes reported in ALS.

237

238 We have shown that 58% of ALS probands reported at least one relative with a history of
239 schizophrenia, psychosis, suicide, depression, alcohol dependence or autism, compared with
240 39% of control kindreds ($p=0.002$). While neuropsychiatric conditions are common within
241 the general population as demonstrated by their presence within our control cohort, our
242 data clearly suggest that kindreds of ALS probands are enriched for these disorders. Of the
243 participants who clustered in the high rate of familial psychiatric illness (≥ 3 family members

244 with psychiatric illness), over 70% were ALS patients ($p=.001$), with the same mean number
245 of relatives. Major psychiatric disorders that were specifically over-represented within these
246 ALS kindreds included schizophrenia / psychosis ($p=0.015$), suicide ($p=0.037$), autism
247 ($p=0.027$), rigid personality disorders ($p=0.02$) and alcohol overuse ($p=0.045$).

248

249 In patients with progressive neurodegenerative disease, the impact of psychological
250 stressors on the caregivers and wider family network is well recognised. A higher than
251 expected rate of neuropsychiatric disease in ALS kindreds may well be anticipated as simply
252 a function of disease-induced stress on the family. However, this study was specifically
253 designed to address this point as we identified those with neuropsychiatric symptoms that
254 had been present prior to any knowledge of ALS within kindreds, in which case the diagnosis
255 would be expected to have no impact on neuropsychiatric presentations within extended
256 kindreds. Moreover, the absence of evidence of increased rates of depression, which would
257 be anticipated were the presence of ALS a factor, suggests that the findings are not related
258 to the presence of ALS within kindreds.

259

260 Our findings suggest the presence of the *C9orf72* repeat expansions does not fully account
261 for the observed overlap between ALS and neuropsychiatric conditions. While 17% of
262 probands from kindreds reporting high rates of neuropsychiatric conditions (>3 affected
263 relatives) carried the *C9orf72* repeat expansion, the remaining 83% of probands carried the
264 normal variant.

265

266 Consistent with our previous study, there was no reported difference in the presence of risk

267 of underlying depression in the kindreds of the ALS probands compared with controls.
268 Given that depression is common within the general population and might be expected to
269 be over-represented in ALS kindreds due to a bias in reporting, this finding supports the
270 robustness of our finding of a relationship between specific neuropsychiatric conditions and
271 ALS.

272

273 Indeed, the absence of an association between depression and ALS, despite the presence of
274 higher rates of reported suicide among first and second degree relatives of ALS probands
275 may reflect an underlying dysregulation of impulse control, rather than a specific alteration
276 in mood. While further prospective family studies are required to confirm, the
277 dichotomization of depression and suicide was also noted in our previous study⁶. This
278 observation is congruent with the emerging concept of network disruption in ALS leading to
279 a range of behavioural changes including increasing disinhibition, impulse dyscontrol, and in
280 some cases increased personality rigidity¹³⁻¹⁶.

281

282 This study was unable to demonstrate a significant association between behavioural change
283 in ALS probands and the presence of a psychiatric endophenotype among first and second
284 degree relatives. This was most likely due to the low power of our study, and the relative
285 insensitivity of the employed behavioural screening tools.

286

287 Our study is limited by design. The neuropsychiatric signal among first and second degree
288 relatives was obtained by report rather than by direct examination of family members, and
289 verification was limited to a series of confirmatory questions by the interviewer. Verification

290 questions included clarification that the diagnosis had been made by a suitably qualified
291 medical practitioner. It is therefore possible that some of the diagnostic categorization is
292 incomplete. However, this limitation applied to ALS kindreds from probands with and
293 without the *C9orf72* repeat expansion and controls equally, and is therefore unlikely to have
294 substantially biased our findings. Secondly, it is possible that ALS probands over-reported
295 the presence of psychiatric disorders. We consider this to be unlikely, as a possible
296 association between ALS and neuropsychiatric conditions is not commonly known to the
297 majority of ALS probands. Moreover, the absence of a significant increase in depression
298 among family members supports the veracity of our findings. Our study cohort was slightly
299 enriched by kindreds of probands carrying the *C9orf72* repeat expansion – this is likely an
300 artifact of our collection method, as some families carrying the *C9orf72* expansion have
301 knowledge of ALS, and more likely to agree to participate in this type of study.

302 Notwithstanding, our study confirms our previous observation of higher rates of
303 neuropsychiatric conditions within ALS kindreds. We have shown that this aggregation is
304 driven primarily by kindreds enriched for particular neuropsychiatric conditions that
305 recapitulate the cognitive and behavioural subphenotypes described in ALS, and that this
306 effect is not primarily driven by the presence of the *C9orf72* expansion. Detailed
307 suphenotyping and genotyping of members of informative kindreds from ALS probands will
308 be required to further characterize this association which, if replicated, suggests the
309 presence of a distinct subphenotype of ALS that shares pleiotropic genetic risk with some
310 forms of neuropsychiatric illness.

311 K-means clustering was chosen as the process is a two-phase iterative heuristic, with data
312 assignment, and centroid updating staggered successively^{17,18}. K-means is a partitional

313 technique used to find clusters, whereby the clusters are represented by their centroids,
314 e.g., the arithmetic means of data points within the respective clusters. The statistical
315 convergence of these iterations, which are integral for clusters to be identified, further
316 increased the integrity and robustness of these analyses alongside the close matched case
317 and control cohorts.

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319

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323 Authorship

324 M.O.B. assisted with study design, collected family data, carried out the statistical analysis,
325 and wrote the manuscript. M.H. provided input with study design and collected family
326 history data. A.V. provided input with study design and collected family history data, R.M.
327 analysed genetic data, J.G. collected family data, S.B. designed the original study and
328 questionnaires used, M.P.G. performed neurocognitive testing and analysed cognitive data,
329 T.B. performed neurocognitive testing and analysed cognitive data, M.E. N.P. analyzed
330 cognitive data, and contributed to writing the manuscript. O.H. was the principal
331 investigator of this research, designed the study, wrote and edited the manuscript.

332

333 Potential Conflicts of Interest

334 The authors report no conflicts of interest.

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Characteristic	Not Included in Study (n=75)	Included in Study (n=127)	P value
Mean age onset, yr (SD)	67.2 (10.1)	62.8 (10.2)	0.004
Mean age diagnosis, yr (SD)	68.1 (10.1)	64.2 (10.7)	0.033
Sex, No. female (%)	30 (40%)	58 (45.6%)	0.47
Site onset, No. (%)	Bulbar, 30 (40); Limb 38 (50.7); other 7, (9.3)	Bulbar, 35 (27.5); Limb, 81 (63.7); other, 11 (9)	0.15
C9orf72 repeat status	Positive, 6 (8%); Negative, 35 (46.7%); Not available, 34 (45.3%).	Positive, 21 (16.5%); Negative, 90 (70.9%); Not available, 16 (12.6%).	0.64

389

390 Table 1. Comparison of the Demographic details of ALS patients included in the study and

391 those not included.

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Disease	Relatives of Cases, n=2116	Relatives of Controls, n= 2139	Risk Ratio	<i>P</i>
Suicide	13	4	3.3	0.037
Schizophrenia / psychotic illness	17	5	3.4	0.015
Autism	10	1	10.1	0.027
Depression	35	31	1.14	0.59
Alcoholism	63	43	1.48	0.045
OCD and Rigid Personality Disorders	11	2	5.6	0.02

395

396 Table 2. Prevalence and relative risk of neuropsychiatric conditions in first and second
397 degree relatives of ALS patients compared to controls relatives. ‘

398

Number of affected individuals per kindred	ALS Kindreds (n=127)	Control Kindreds (n=132)
0	50	81
1	32	31
2	16	14
3 or more	29	6

400 Table 3. Clustering of neuropsychiatric conditions in kindreds of patients with ALS compared
401 to those of controls.

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