ALS in Finland

Major Genetic Variants and Clinical Characteristics of Patients With and Without the *C9orf72* Hexanucleotide Repeat Expansion

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Abstract

Background and Objectives
To analyze the frequencies of major genetic variants and the clinical features in Finnish patients with amyotrophic lateral sclerosis (ALS) with or without the *C9orf72* hexanucleotide repeat expansion.

Methods
A cohort of patients with motor neuron disease was recruited between 1993 and 2020 at the Helsinki University Hospital and 2 second-degree outpatient clinics in Helsinki. Finnish ancestry patients with ALS fulfilled the diagnosis according to the revised El Escorial criteria and the Awaji-criteria. Two categories of familial ALS (FALS) were used. A patient was defined FALS-A if at least 1 first- or second-degree family member had ALS, and FALS-NP, if family members had additional neurologic or psychiatric endophenotypes.

Results
Of the 815 patients, 25% had FALS-A and 45% FALS-NP. *C9orf72* expansion (*C9pos*) was found in 256 (31%) of all patients, in 58% of FALS-A category, in 48% of FALS-NP category, and in 23 or 17% of sporadic cases using the FALS-A or FALS-NP definition. *C9pos* or *SOD1* p.D91A homozygosity was found in 328 (40%) of the 815 patients. We compared demographic and clinical characteristics between *C9pos* and patients with unknown cause of ALS (*Unk*). We found that the age at onset was significantly earlier and survival markedly shorter in the *C9pos* vs *Unk* patients with ALS. The shortest survival was found in bulbar-onset male *C9pos* patients, whereas the longest survival was found in *Unk* limb-onset males. Older age at onset associated consistently with shorter survival in *C9pos* and *Unk* patients in both limb-onset and bulbar-onset groups. There were no significant differences in the frequencies of bulbar-onset and limb-onset patients in *C9pos* and *Unk* groups. ALS-frontotemporal dementia (FTD) was more common in *C9pos* (17%) than in *Unk* (4%) patients, and of all patients with ALS-FTD, 70% were *C9pos*.

Discussion
These results provide further evidence for the short survival of *C9orf72*-associated ALS. A prominent role of the *C9orf72* and *SOD1* variants was found in the Finnish population. An unusually high frequency of *C9pos* was also found among patients with sporadic ALS. The enrichment of these 2 variants likely contributes to the high incidence of ALS in Finland.

Glossary

ALS = amyotrophic lateral sclerosis; **AUC** = area under the curve; **FALS** = familial ALS; **FTD** = frontotemporal dementia; **GWAS** = genome-wide association study; **MST** = mean survival time; **OR** = odds ratio; **SALS** = sporadic ALS.

Introduction

The most common genetic cause of amyotrophic lateral sclerosis (ALS) in European populations is the hexanucleotide repeat expansion in the *C9orf72* gene.[1,2] In these populations, up to 50% of patients with familial ALS (FALS) and about 5% of patients with sporadic ALS (SALS) carry this expansion (*C9pos*).[3] The variant is much less common among Asian populations.[4]

Frontotemporal dementia (FTD) is another common phenotype associated with the *C9orf72* expansion; it accounts for about 25% of familial FTD in Europeans[3] and up to 86% of familial patients having both FTD and ALS. The *C9pos* patients may rarely have other clinical presentations than ALS or FTD such as movement disorders, psychiatric symptoms, and idiopathic normal pressure hydrocephalus.[5,6] A younger than average age at onset and shorter survival have been relatively uniformly reported among the *C9pos* patients with ALS, although the penetrance of this expansion is variable.[7] A higher proportion of bulbar onset has been inconsistently reported.[8–16]

Finland is among the countries with the highest incidence of ALS in the world.[17–19] We aimed to study the frequencies of the major genetic variants and compare the clinical features of ALS in Finnish patients with the *C9orf72* expansion (*C9pos*) and in patients with unknown cause of ALS (*Unk*).

Methods

Study Design and Participants

Between 1993 and 2020, we recruited 836 patients with motor neuron disease attending the Neurology Department at the Helsinki University Hospital or 2 private neurology outpatient clinics in Helsinki. The clinics serve the population of the Helsinki and Uusimaa health care district (population size 1,634,319 on December 31, 2016). These units also received referrals from other parts of the country. We excluded patients where both parents were of non-Finnish descent (n = 15), patients with spinal and bulbar muscular atrophy variant in androgen receptor gene[20] (n = 2), and patients with Jokela-type spinal muscular atrophy variant in the *CHCHD10* gene[21] (n = 4). Of the remaining 815 patients, 74% lived in the province of Uusimaa in southern Finland. eFigure 1 (links. lww.com/NXG/A517) illustrates the geographic distribution of the patients and the frequencies of the *C9orf72* and *SOD1* variants in Uusimaa and other regions. Four hundred five patients were included in the discovery study of the *C9orf72* expansion[1] and a subsequent population frequency analysis of the expansion.[3] We excluded homozygotes for *SOD1* p.D91

and heterozygotes for *SOD1* p.A90V from the clinical data analysis of the present study because patients with these variants differ from classical ALS.[22,23] The details of the patients with the *SOD1* p.A90V variant have been previously reported.[23] We also excluded patients with respiratory onset and patients with incomplete clinical data. The final statistical analysis for phenotypic comparisons included 707 patients with ALS (Figure 1).

Diagnosis

Patients were assessed by H.L. and at least 1 other neurologist. The patients fulfilled the criteria of definite or probable ALS according to the El Escorial criteria,[24] and from 2001 onward, according to the revised version. From 2008, the Awaji-criteria (electroneuromyography, ENMG) were combined to the clinical criteria. The last day of follow-up was June 21, 2021.

Clinical Variables and Familiality

Data on the age at onset, age at death, duration of disease, site of onset, sex, family history, and comorbid neurodegenerative and psychiatric diseases were gathered by interview and from the patients' charts and death certificates. Death certificates were obtained from Official Statistics of Finland.[25] The date of death was confirmed from the Digital and Population Data Services Agency of Finland using a unique personal id-code available at reference 26, ensuring that no patients were lost to follow-up. The age at symptom onset was defined as the age at which the patient first experienced motor dysfunction that led to the ALS diagnosis. The disease duration was defined as the number of years starting from the year of onset to the year of death or mechanical ventilation required for more than 12 hours per day.

In *C9pos* ALS families, an increased frequency of neurologic and psychiatric disorders has been reported.[27,28] We used 2 definitions of FALS. A patient was defined as FALS-A if at least 1 first- or second-degree family member had ALS and FALS-NP if 1 or more first- or second-degree family member had neurologic or psychiatric entities as confirmed from the index patient's chart, interview, or from relatives' hospital records, or death certificate in equivocal cases. The family history interview was conducted by H.L. and the information came from the patient and family members. Systematic questionnaires were not used. Our FALS-NP category (described in Figure 1) had slightly broader criteria than the definite and probable categories proposed by Byrne,[29] but for FALS-A, we did not count in family members with pure FTD without evidence of motor symptoms. ALS patients' comorbid disorders were coded as present or absent if they had been observed during the course of ALS. FTD was coded separately from other dementias and cognitive deficits. Comorbid cognitive deficit was a widely defined variable, which includes

Figure 1 Flowchart of the Study
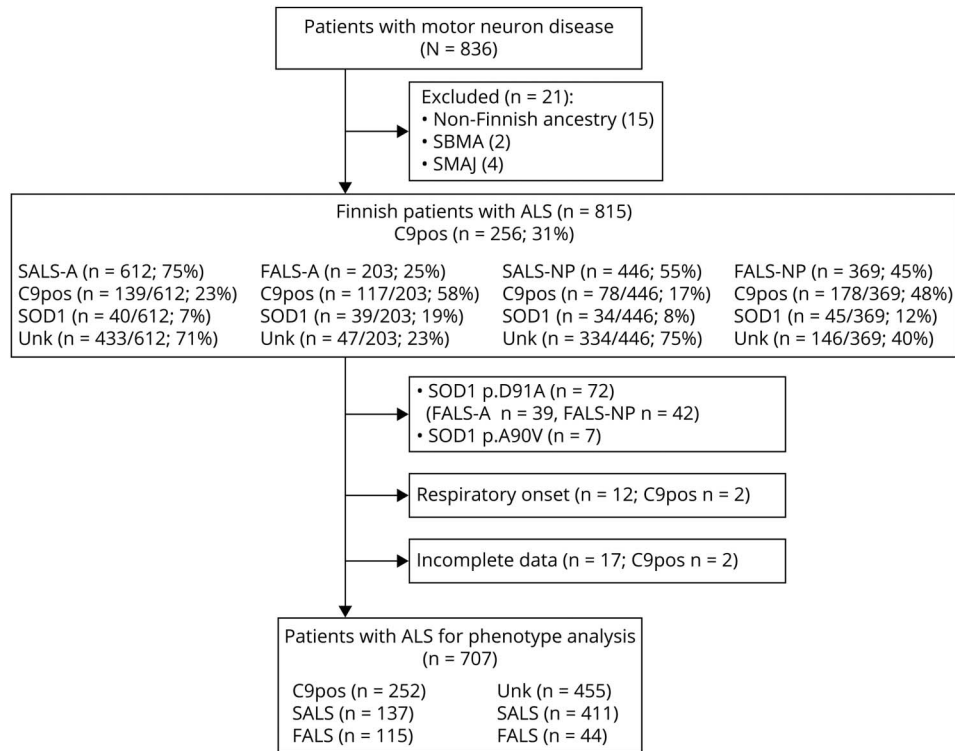
ALS = amyotrophic lateral sclerosis; *C9pos* = carrier of the hexanucleotide repeat expansion in the *C9orf72* gene; FALS-A = familial ALS, only first- and second-degree relatives with ALS (A) were considered in the definition of FALS; FTD = frontotemporal dementia; SALS-A = sporadic ALS; SBMA = spinobulbar muscular atrophy; SMAJ = spinal muscular atrophy Jokela type; *Unk* = noncarrier. SALS-NP/FALS-NP = broad definition, first- and second-degree relatives with various neurologic and psychiatric (NP) disorders were included in FALS-NP. FALS-A was classified in 158 families, *C9pos* in 91 (58%), *Unk* in 41 (26%), and *SOD1* p.D91A in 26 (16%) families. FALS-NP was classified in in 326 families, of which 167 (51%) families were *C9pos*, 126 (39%) *Unk*, and 33 (10%) *SOD1* p.D91A. Respiratory-onset cases were considered outliers in survival and were excluded from the phenotype analysis. FALS-NP categorization included the following neurologic disorders: ALS, FTD, Alzheimer disease, Lewy body disease, other dementia or cognitive deficit not linked to vascular or traumatic etiology, Parkinson disease, and Huntington disease. Neurodegenerative conditions such as corticobasal degeneration and multiple system atrophy as well as disorders causing muscular weakness or stiffness such as polyneuropathies, myasthenia gravis, myasthenic syndromes, and Stiff-person syndrome were grouped. We did not include mild cognitive deficits, unspecified memory problems, tremors, ataxias, seizures, epilepsies, stroke, severe trauma, post-polio syndrome, multiple sclerosis, migraine, congenital disorders, metastases, primary tumors of the nervous system, anecdotal recollections of autism-type disorders, and very late-onset dementias, i.e., when the relative was known or suspected to develop dementia at age 80 years or over. FALS-NP categorization included the following psychiatric conditions: schizophrenia, psychosis, delusions, bipolar disorder, significant alcohol or narcotics abuse/dependence, and suicide. Mood disorders were grouped (anxiety and depression when severe and diagnosed before the index patient was diagnosed with ALS). We did not include anecdotal mental problems, character disorders, obsessive-compulsive disorder, unspecified apathy, and recital faux pas.

Alzheimer disease and other dementias or cognitive deficits not linked to vascular or traumatic etiology. Riluzole, lithium, and gabapentin were used by some patients during the study period, but because the dose and length of use varied, we did not include these treatments in survival analysis.

Standard Protocol Approvals, Registrations, and Patient Consents

The study protocol was approved by the Institutional Review Boards at the Haaga Neuro Clinic (December 10, 1993) and the Helsinki University Hospital (Drno 299/E9/2001, Drno 401/13/03/01/2009, HUS/1720/2019). Written informed consent was obtained from each patient or a close relative, depending on whether the patient was physically able to give a written consent.

Genotyping

DNA was extracted using standard methods from peripheral blood leukocytes. The *C9orf72* repeat expansion was newly genotyped in 2019–2020 in all samples with DNA available (796 of the 815). We assessed the *C9orf72* hexanucleotide repeat expansion as previously described; samples with more than 45 repeats and the typical sawtooth pattern in repeat-primed PCR, which only produced the smaller amplicon in over-the-repeat PCR, were categorized as expansions.[30] In 2 patients, the expansion status was based on the discovery study,[1] and in 17, it was based on clinical genetic diagnostics at the University of Turku or University of Tampere, Finland. Seven patients had 2 copies of the *C9orf72* intermediate-length alleles and a genotype (7–45/17–45 repeats), which is a risk factor for ALS.[31] Six of these patients were included in the *Unk* group. One was *SOD1* p.D91A homozygote and was excluded from phenotype analysis. The *SOD1* p.D91A variant was verified as a diagnostic service in 72 samples at the University of Turku, Finland, and 799 samples were also screened for this variant using genome-wide association study (GWAS) data that allowed reliable calling or imputation of the *SOD1*

p.D91A variant. Of these, 772 samples were genotyped by FinnGen ThermoFisher Axiom custom array v2 (finngen.fi/fi/node/59) and 27 samples as described.[32] The *SOD1* p.D91A genotype was missing in 16 patients, but none of these had a clinical course compatible with this subtype of ALS. *CHCHD10* p.G66V variant rs730880031 was screened in 772 samples using the FinnGen array, but none of the patients had this variant. The *SOD1* p.A90V was discovered in 1 patient along with neuropathologic and exome-wide analysis and in 6 patients by the imputation of this variant in the 772 genotyped patients (rs1280042397, IMPUTE2 INFO score 1.0 indicating reliable imputation) and confirmed by sequencing. We assessed possible cryptic relatedness in the patients with ALS using the GWAS data using a threshold of >0.185 proportion identity by descent between 2 patients to define relatedness.

Statistical Analysis

Survival, age at onset, and age at end point were expressed in years. Univariate comparisons were made with the Mann-Whitney *U* test. We used binary logistic regression to analyze which phenotypes were associated with *C9pos* or *Unk* status. Cox proportional hazards models were built to analyze the hazard ratios associated with clinical variables. The Efron likelihood was used for estimation, coding of factors was sigma restricted, and models included all effects. The proportionality assumption was tested for all factors using Schoenfeld residual plots. We added an interaction term familiality × genotype on the assumption that familial *C9pos* patients might have a disproportionately severe presentation. Both complete and censored cases were included in the mean survival time (MST) analysis[33,34] and were expressed in years and calculated as the area under the curve (AUC) of a Kaplan-Meier curve. The AUC can be viewed as the definite integral of a curve that describes variation with time. For this calculation, we smoothed the survival data by adding a 2-decimal random number between −0.5 and 0.5. The same jittered data are used in figures for clarity. For MST, data from living (66/707) patients were excluded from analysis.[33,34] Statistical analysis was performed using Statistica version 13.3 software (StatSoft, Inc., 2013). AUC was calculated in Microsoft Excel using the trapezoidal rule.

Data Availability

Anonymized data not published within this article will be made available by request to a qualified investigator with institutional review and approval.

Results

Frequencies of the *C9orf72* and SOD1 Variant Carriers

We identified 815 Finnish patients with ALS over the 27-year study period. Of these, 256 patients (31%) carried the *C9orf72* repeat expansion (*C9pos*), 79 (9.7%) had a *SOD1* variant, and 480 (59%) had an unknown cause of ALS (*Unk*)

(Figure 1). Overall, the 2 most common variants *C9orf72* or *SOD1* p.D91A homozygosity were found in 328 (40%) of the patients.

Familiality

We used 2 definitions of FALS (see Methods: Clinical variables and familiality). Using the FALS-A definition, 203 (25%) of all patients were familial and were distributed in 158 unique families: 91 families were *C9pos* (58%), 41 families *Unk* (26%), and 26 families had *SOD1* p.D91A (16%). Using the broader FALS-NP definition, 369 (45%) of all patients were familial. These were distributed in 326 unique families, of which 167 (51%) were *C9pos*, 126 (39%) were *Unk*, and 33 (10%) had *SOD1* p.D91A. The distribution of the neurologic and psychiatric endophenotypes among relatives of *C9pos* and *Unk* FALS-NP cases is shown in eFigure 2A and eFigure 2B (links.lww.com/NXG/A517).

Demographics and Clinical Features of Patients With ALS With and Without the *C9orf72* Expansion

Here, we focus on the characteristics of the *C9pos* and *Unk* patients with ALS. Patients with *SOD1* variants, respiratory-onset disease (outliers in survival), or incomplete clinical data were removed from the subsequent analysis, as shown in Figure 1. Familiality was categorized only as FALS-A in this analysis.

Table 1 shows the demographic and clinical characteristics of the *C9pos* and *Unk* patients with ALS. The median age at onset in the *C9pos* and *Unk* patients with ALS was 58.0 and 61.0 years, respectively (Mann-Whitney U $Z = 3.6025$, $p = 0.0003$). The observed age at onset varied according to the site of onset and sex (eTable 1, links.lww.com/NXG/A517). The most pronounced effect was found in ALS *Unk* limb-onset vs bulbar-onset patients; the median age at onsets were 57 vs 66 years ($p = 4.6 × 10^{-12}$). Limb-onset patients had slightly, but significantly, earlier age at onset also in the *C9pos* group ($p = 0.048$), and males had earlier age at onset in the *Unk* group ($p = 0.018$). The median survival was 2.0 years (0.5–13.0) in the *C9pos* vs 3.0 years (0.5–26.0) in the *Unk* group (Mann-Whitney U -3.6021, $p = 0.0003$). We did not find any significant differences in the demographic or clinical features in comparisons between *C9pos* FALS-A and SALS-A. However, among the *Unk* group, the patients with FALS-A had a significantly lower age at onset than SALS-A (Table 1). ALS-FTD was significantly more common in the *C9pos* (17%) than in the *Unk* (4%) group (Fisher exact test $p = 2.27 × 10^{-8}$). ALS-FTD with bulbar onset was slightly more common than bulbar onset in pure ALS (46% vs 36%), but the difference was not statistically significant ($p = 0.13$).

The distribution of the age at onset and survival in the *C9pos* and *Unk* patients is shown in Figure 2. These data demonstrate that the age at onset modulates survival more in the *Unk* than in the *C9pos* patients. As expected, the *Unk* group is more heterogeneous in survival and age at onset distributions. The *C9pos* group

Table 1 Demographic and Clinical Characteristics of the Patients According to the Carriership of the *C9orf72* Hexanucleotide Expansion (*C9pos/Unk*)

		C9pos			*Unk*		
	All patients (n = 707)	Total (n = 252)	FALS (n = 115)	SALS (n = 137)	Total (n = 455)	FALS (n = 44)	SALS (n = 411)
Female, n (%)	366 (52)	138 (55)	64 (56)	74 (54)	228 (50)	19 (43)	209 (51)
Median AOO, y (range)	59.0 (27–88)	58.0 (35–79)	59.0 (41–76)	57.0 (35–79)	61.0 (27–88)	57 (31–87)	62.0 (27–88)
Median survival, y (range)	3.0 (1–26)	2.0 (0.5–13)	3.0 (0.5–13)	2.0 (0.5–7)	3.0 (0.5–26)	3.0 (1–23)	3.0 (0.5–26)
N alive (June 2021)	66	14	6	8	52	1	51
Bulbar onset, n (%)	265 (37)	89 (35)	40 (35)	49 (36)	176 (39)	19 (43)	157 (38)
Limb onset, n (%)	442 (63)	163 (65)	75 (65)	88 (64)	279 (61)	25 (57)	254 (62)
FTD, n (%)	60 (8)	42 (17)	27 (23)	15 (11)	18 (4)	4 (10)	14 (3)
Cognitive deficit, n (%)	51 (7)	28 (11)	10 (9)	18 (13)	23 (5)	3 (7)	20 (5)

Abbreviations: ALS = amyotrophic lateral sclerosis; AOO = age at onset; FALS = familial ALS; FTD = frontotemporal dementia; SALS = sporadic ALS.
*SOD1*p.D91A homozygotes and *SOD1*p.A90V heterozygotes were excluded.

was relatively homogeneous and did not include many outliers in either survival or age at onset; there were only 5 patients (2%) with survival longer than 8 years, and only 16 (6%) patients had an age at onset outside the age band 40–70 years (Figure 2). The cumulative incidence by age of *C9pos* ALS in males/females, bulbar-/limb-onset, and SALS-A/FALS-A dichotomies is shown in eFigure 3 (links.lww.com/NXG/A517).

Association of Clinical Variables With C9pos ALS

We analyzed features that differentiate *C9pos* from *Unk* ALS using binary logistic regression. We found a significant association of *C9pos* with FALS-A, earlier age at onset, and shorter survival (Table 2). The effect size[33] was large in survival (odds ratio [OR] 10.7–14.4) and FALS-A (OR 8.9) and medium for the age at onset (OR 5.3–5.4). The site of onset was not associated with *C9* status.

Effects of the Expansion, Age at Onset, Site of Onset, Sex, and MST

Next, we analyzed features modulating survival. Figure 3 shows the MST of *C9pos* and *Unk* patients grouped by age at onset, site of onset, and sex. First, the MST of the *C9pos* patients was shorter than the cohort's average, regardless of whether the onset site was bulbar or in the limbs. Second, bulbar-onset *Unk* patients had almost equally short survival as *C9pos* patients, whereas limb-onset *Unk* patients survived longer than the cohort on average. Third, the MST was slightly longer in females than males with bulbar onset, whereas the opposite was found in limb-onset patients. Fourth, the age at onset had a clear impact on the patient's survival. In all groups, there was a progressive shortening in the MST with later age at onset.

Cox Regression Hazard Ratios for Death

Using a Cox proportional hazard model of the whole cohort, we found that the genotype *C9pos*, bulbar onset, and late age

at onset statistically significantly increased the risk of death by a factor of 1.5–2.0 (Table 3). FALS-A and sex did not have a significant effect on survival.

C9orf72 Intermediate-Length Alleles in C9pos Patients

We have previously provided evidence that the carriership of 2 copies of the intermediate-length alleles (7–45 repeats) increases the risk of ALS when the longer allele is ≥17 repeats.[31] Therefore, we tested whether the carriership of an intermediate-length allele in *C9pos* patients would modulate the age at onset or survival. Five *C9pos* patients had ≥17 repeat alleles (3 were FALS-A, and 5 were FALS-NP). Forty-two *C9pos* patients had 7–16 repeat alleles, 22 (52%) were FALS-A, and 31 (74%) FALS-NP. We did not find a statistically significant association between survival or age at onset and carriership of an intermediate-length allele. The median survival was 3.0 years in carriers of ≥17 repeat allele, 3.0 years in carriers of 7–16 repeat allele, and 2.0 years in carriers of 2–6 repeat allele (Kruskal-Wallis test: $H = 0.657$, $p = 0.719$). The mean age at onset was 50.8 years in carriers of ≥17 repeat allele, 56.5 years in carriers of 7–16 repeat allele, and 57.0 years in carriers of 2–6 repeat allele (1-way analysis of variance $F = 1.39$; $p = 0.25$).

Discussion

We describe the major genetic variant frequencies, familiality, and clinical features of a large cohort of patients with ALS with the *C9orf72* hexanucleotide repeat expansion collected within a single country. The frequency of the *C9orf72* repeat expansion was 31% in the whole cohort, 48%–58% among FALS families, and 17%–23% in SALS depending on the FALS criteria used. Survival was shortest in bulbar *C9pos* and longest in limb-onset *Unk* patients.

Figure 2 Distribution of Finnish Patients With the *C9orf72* Expansion (A) *C9pos* and Patients With Unknown Cause of ALS (B) *Unk* Plotted Against Age at Onset and Survival
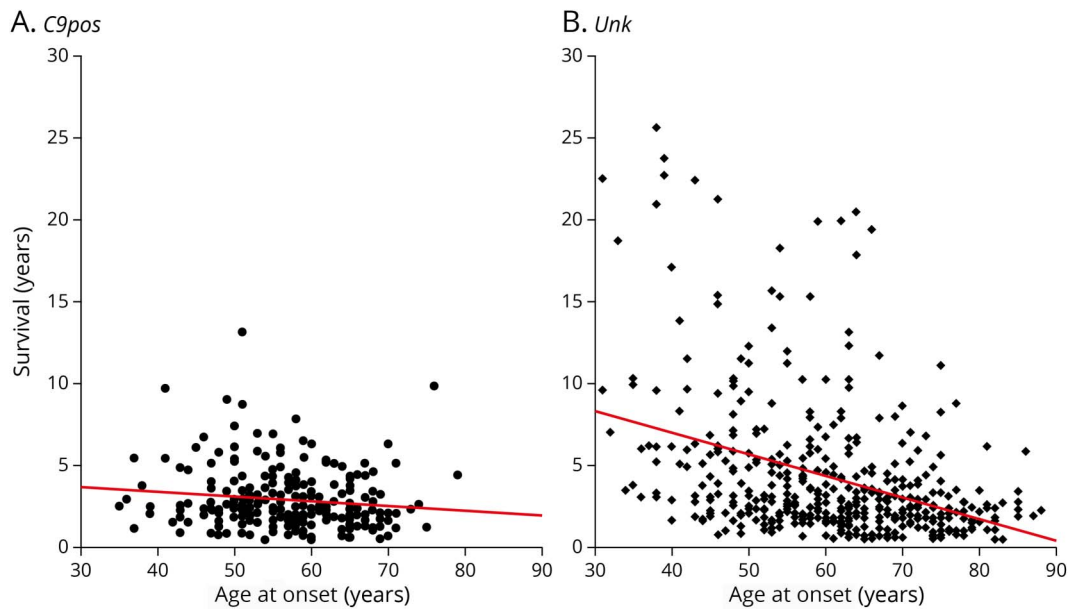
The regression line between age at onset and survival is given in red.

The 2 most common variants (*C9orf72* and *SOD1* p.D91A) were found in 40% of the 815 patients; 31% were *C9pos*, and 9% were *SOD1* p.D91A homozygous. These data suggest an exceptional enrichment of these 2 variants in Finland. However, it should be noted that these percentages do not represent the true population rate because of possible referral bias, which usually increases the proportion of FALS. It has been reported that hospital-based studies have more *SOD1* but fewer *C9orf72* variants in FALS than population-based studies.[4] Also, referral bias of unusually prolonged clinical course is possible, which may increase the proportion of *SOD1* p.D91A. Although the percentages probably have an upward bias compared with the genuine population-based frequencies, these referral biases cannot be the sole cause for the high variant frequencies. When we excluded patients from outside referrals (which may have most bias), the frequencies of *C9pos* and *SOD1* p.D91A patients were 30.3% and 5.5%, respectively. A significant increase of the *SOD1* p.D91A (from 5.5% to 18.5%, eFigure 1, links.lww.com/NXG/A517) but not of *C9pos* patients was observed in outside referrals. This is expected given the high carrier frequency of *SOD1* p.D91A in Northern Finland. In systematic comparisons of worldwide studies (excluding isolates in the Western Pacific), Finland has had the highest[17] or second highest[18] age-adjusted incidence of ALS in the world. We conclude that the high incidence of ALS is contributed by the enrichment of the *C9orf72* expansion and, to a lesser extent, the *SOD1* p.D91A.

Table 2 Association of Variables With *C9pos* ALS Using Binary Logistic Regression

	Effect	Compared with effect	Odds ratio	95% CI	*p* Value
Sex	Female	Male	1.30	0.90–1.87	0.170
Familial	FALS	SALS	8.91	5.74–13.85	<0.0001
Site of onset	Limb	Bulbar	1.23	0.83–1.82	0.315
Age at onset, y	27–52	68–88	5.26	2.90–9.56	0.003
	53–59		5.41	3.00–9.76	0.002
	60–67		3.98	2.23–7.09	0.205
Survival, y	1–2	10–32	14.37	4.05–51.0	<0.0001
	3–4		10.69	3.00–38.09	0.001
	5–9		5.59	1.53–20.49	0.891

Abbreviations: ALS = amyotrophic lateral sclerosis; bulbar = bulbar onset; CI = confidence interval; FALS = familial ALS; limb = limb onset; SALS = sporadic ALS.

The dotted vertical line indicates the mean value of all groups.
ALS = amyotrophic lateral sclerosis.
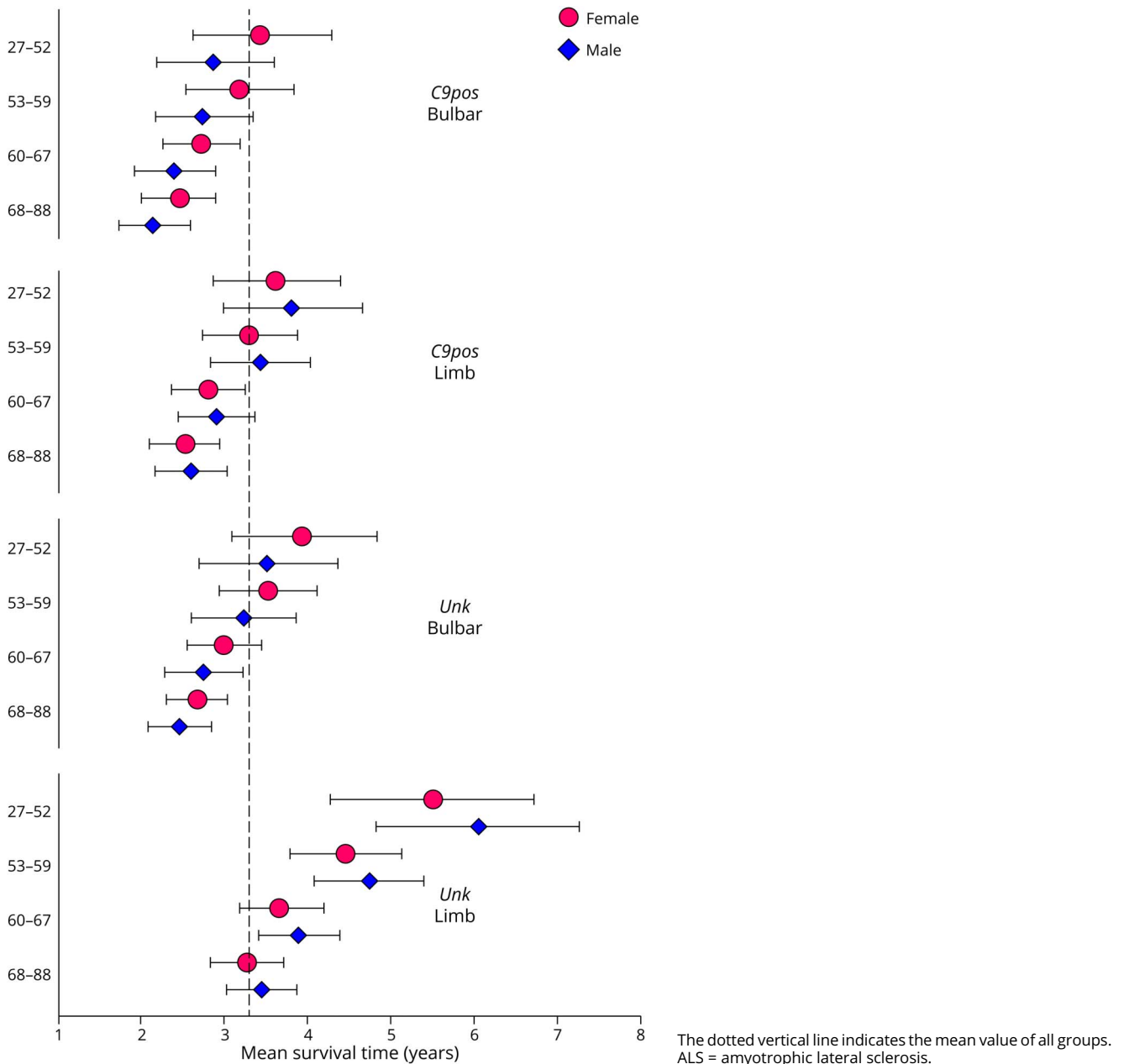
An explanation for the high frequency of the *C9orf72* and *SOD1* variants as well as the high incidence of ALS in Finland could be the Finnish population structure, which is featured with genetic isolation and genetic bottlenecks resulting in geographically clustered founder populations.[35] This distinctive history has led to an enrichment of rare, mainly recessive Mendelian diseases among the population, collectively known as the Finnish disease heritage.[36] The enrichment of certain variants can also be seen in adult-onset autosomal dominant neurologic disorders. For example, adult-onset spinal muscular atrophies are caused mainly by 2 founder variants in Finland: the X-linked Kennedy disease and SMA Jokela type by *CHCHD10* variant.[20,21] Enrichment of certain ALS variants has also been reported in Sardinia, another population with genetic founder effect, where 41% of the 375 studied patients with ALS had variants (in decreasing order) in *TARDBP, C9orf72, SOD1,* or *MATR3* genes.[37]

Our FALS-A definition follows the traditional dichotomy defining a case FALS if there are 2 or more ALS cases in first- or second-degree relatives. At the beginning of our study (1993), there was some evidence about the link between ALS and FTD, but knowledge of other endophenotypes was limited. The information obtained in this study comprised of interviews and document reviews rather than direct examinations of relatives. We conducted the whole study uniformly and used the same

Table 3 Hazard Ratios for Death in Cox Proportional Hazards Regression Analysis

	Level of effect	Compared with effect	Hazard ratio	95% lower CL	95% upper CL	*p* Value
Case	*C9pos*	*Unk*	1.50	1.03	2.18	0.03
Sex	Female	Male	1.01	0.86	1.19	0.88
Familial	FALS	SALS	0.98	0.79	1.22	0.88
Site of onset	Bulbar	Limb	1.55	1.30	1.85	<0.0001
Age at onset, y	53–59	23–52	0.89	0.68	1.15	0.24
Age at onset, y	60–67		1.46	1.17	1.83	0.51
Age at onset, y	68–88		2.02	1.59	2.56	<0.0001

Abbreviations: ALS = amyotrophic lateral sclerosis; bulbar = bulbar onset; CL = confidence limit; FALS = familial ALS; limb = limb onset; SALS = sporadic ALS.
All patients with ALS.

information-gathering methods throughout. Today the somewhat anachronistic definition of FALS can be questioned in many ways, and the knowledge of the various endophenotypes has changed the picture. Our broader FALS-NP category included neuropsychiatric variables, and this resulted as a significant change from FALS-A (25%) to FALS-NP (45%). An

Table 4 *C9pos* Patients With ALS in Different Cohorts of FALS and SALS

	FALS		SALS		
Population	**n**	***C9pos* (%)**	**n**	***C9pos* (%)**	**Reference**
Finnish (FALS-A)	203	58	612	23	This study
Finnish (FALS-NP)	369	48	446	17	This study
Israel (AJ)	10	80	339	11	38
Belgian	62	52	461	9.6	12
United States (Hispanic)	—	—	72	8.3	3
Sardinian	100	39	275	7.3	35
English	98	46	916	6.8	3
Dutch	78	37	1422	6.1	13
United States (White)	163	36	890	5.4	3
Australian	—	—	263	5.3	3
German	69	22	421	5.2	3
Irish	49	41	386	5.0	9
Italian	90	38	465	4.1	3
United States (Black)	—	—	49	4.1	3
Spanish	155	27	781	3.2	10
Turkish	116	18	361	3.1	39
Indian	28	11	565	2.8	40
Russian	20	15	238	2.5	41
Taiwanese	22	18	102	2.0	42
Japanese	11	0	552	0.4	43
Chinese	—	—	1092	0.3	15

Abbreviations: AJ = Ashkenazi Jews; FALS-A and FALS-NP definitions presented in Figure 1 footnote.

Irish population-based study[38] found that inclusion of the presence of neuropsychiatric endophenotypes within kindreds increased the FALS incidence rate to 30%. Our study also resulted in a slight change in the proportions of SALS/FALS in C9pos patients. The sensitivity to capture all these endophenotypes by interview is much more difficult than an established ALS diagnosis in a relative. Hence, we may have underestimated the amount of patients with FALS-NP.

A recent review[39] on the prevalence of SOD1 and C9orf72 in 22 countries (Finland not included) suggested that the majority of patients with ALS with these variants may be found within the SALS group. Our results parallel this, and the high rate of C9pos among patients with SALS in Finland is interesting. Table 4 shows C9pos frequencies in patients with FALS and SALS from different countries and suggests that the Finnish population is an outlier in the frequency of the C9pos SALS. Several interpretations for this finding should be considered. First, the studies differ in FALS definition, case ascertainment (population based vs second or tertiary clinic based), and by including or excluding other variants. Not all studies report the version of El Escorial criteria used. Also, life expectancy and different family sizes among the countries may have influenced the recognition of FALS. Second, we might have misclassified some cases as SALS due to missed recognition of endophenotypes among relatives. On the other hand, we were able to monitor cryptic relatedness between patients using the GWAS data, which increased the sensitivity to detect patients with FALS-A. Third, the penetrance of the C9orf72 repeat expansion could be reduced in Finland, which would imply unknown protective factors in family members of the C9pos patients with SALS. This view is supported by the observed expansion carrier frequency 0.19% in the older Finnish population without ALS or FTD diagnosis; the expansion carriers were aged 68–79 years.[33] Fourth, frequent de novo mutations could be 1 contributing factor. The frequency of the C9orf72 hexanucleotide repeat intermediate-length alleles of ≥20 repeats is about 2 times higher in Finland than in other European countries.[30] Some of the ≥20 repeat alleles may represent unstable premutation alleles that could generate expansions. A larger pool of premutation alleles could partially explain the more significant proportion of C9pos SALS in Finland. Large intermediate-length alleles as the source of expansion have been previously shown in the Huntington disease CAG repeats.[46,47]

Several factors modified the age at onset of ALS in our data. Limb onset was the most decisive factor associated with earlier onset in the Unk group; the site of onset had a much smaller effect in the C9pos group (eTable 1, links.lww.com/NXG/A517). The C9pos patients overall had 3 years earlier onset than the Unk patients, which is in line with most previous reports.[9–11,13] Our results also indicate that the survival of C9pos was significantly shorter than in Unk, and corresponding results have been reported in previous studies but occasionally with a broader age range.[12–14] We did not observe an excess of bulbar onset in our C9pos group. This agrees with many studies,[10,11,13,14] but bulbar-onset

phenotype has dominated in others.[8,9,16] Logistic regression analysis revealed that it is likely for C9pos patients to have relatives with ALS and an earlier age at onset and shorter survival than Unk patients. These findings confirm previous results reported in a large European population-based cohort.[13]

Although concomitant FTD indicates the existence of the C9orf72 expansion, this is not absolute. In our study, 70% of the patients with ALS-FTD were C9pos, divided into 64% familial and 36% sporadic patients (Table 1). This partly corresponds to the results of a study that combined 5 European populations[15] and reported C9pos frequency in 72% C9pos patients with familial ALS-FTD and 17% in C9pos patients with sporadic ALS-FTD.

Risk factors for shorter survival in a Cox regression model including the total cohort indicated that the C9pos genotype, bulbar onset, and higher age at onset shortened survival, which is in agreement with previous data.[48] The hazard ratios were in the range of 1.5–2, which represent relatively small effect sizes. A small effect size of a hazard ratio has been proposed to be in the range of >1.3, a medium effect size of >1.9, and a high effect size >2.8.[49] Most studies report hazard ratios that are small to medium, which may explain why risk profiles have sometimes been inconsistent in different studies.

The MST is often used as a point estimate in comparing survival. The AUC of a survival curve is an intuitive measure of survival and allows direct comparison between groups. The analysis of MST revealed a clear pattern of how the age at onset affects the survival in both C9pos and Unk groups, with earlier age providing a more favorable survival, and this is consistent with a recent systematic meta-analysis of C9orf72-linked disease survival and prognosis.[48] Another finding is the monotonic survival pattern in the bulbar-onset groups of both C9pos and Unk. The limb-onset C9pos group had an almost equally poor prognosis. In contrast, limb-onset Unk patients lived substantially longer (Figure 3). It has been suggested[50] that the reduced survival in the C9orf72 expansion carriers is accountable on male sex, and especially on the limb-onset male patients, but this intriguing observation was not confirmed in a recent meta-analysis,[48] in a population-based study[51] or in our study. In our study, males tended to survive longer if they had limb onset and shorter if they had bulbar onset. There are many reasons for divergent results which are probably due to complex interactions between genes, environment, and lifestyle. Modifiable causal relationships like alcohol use and smoking have been shown to link to genetically determined ALS, primarily to C9orf72 noncarriers.[52]

Our study has several strengths. The cohort is large and originates from a relatively homogeneous population. As a result, we described a relatively uniform clinical picture of C9pos ALS in a Nordic population. The patients were traced, and their survival and course of the disease could be followed reliably due to the unique individual id-code used in Finland.

In addition, GWAS data helped to detect cryptic relatedness between patients. The main limitation is that this is not a population-based study. Another limitation associates with the context of FALS. Our FALS-A definition likewise represents the traditional clinical situation, where ALS family history is considered either positive or negative, but the expanded FALS-NP is far from complete as well.[53]

The confirmation of the uniform clinical outcome of *C9orf72* expansion carriers facilitates the design and result interpretations of trials using symptomatic and neuroprotective therapies. The better perception of *C9orf72* characteristics also helps the clinician to estimate the prognosis and treatment challenges of a single patient. The connection with neuropsychiatric endophenotypes expands the consequences of the expansion. Accordingly, there is a need to rethink the concepts of sporadic and familial ALS.

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Disclosure
H. Laaksovirta, J. Launes, and L. Jansson report no disclosures relevant to this manuscript. B.J. Traynor holds patents on the diagnostic and therapeutic implication of the *C9orf72* repeat expansion. K. Kaivola reports no disclosures relevant to this manuscript. P.J. Tienari holds patents on the diagnostic and therapeutic implication of the *C9orf72* repeat expansion. Go to Neurology.org/NG for full disclosures.

Appendix Authors

Name	Location	Contribution
Hannu Laaksovirta, MD	Department of Neurology, Helsinki University Hospital, Finland; Translational Immunology, Research Programs Unit, University of Helsinki, Finland	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data

Appendix (continued)

Name	Location	Contribution
Jyrki Launes, MD, PhD	Department of Psychology and Logopedics, University of Helsinki, Finland	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data
Lilja Jansson	Translational Immunology, Research Programs Unit, University of Helsinki, Finland	Major role in the acquisition of data and analysis or interpretation of data
Bryan J. Traynor, MD, PhD	Neuromuscular Diseases Research Section, Laboratory of Neurogenetics, National Institute on Aging, Bethesda, MD	Drafting/revision of the manuscript for content, including medical writing for content
Karri Kaivola, MD, PhD	Department of Neurology, Helsinki University Hospital, Finland; Translational Immunology, Research Programs Unit, University of Helsinki, Finland	Drafting/revision of the manuscript for content, including medical writing for content, and analysis or interpretation of data
Pentti J. Tienari, MD, PhD	Department of Neurology, Helsinki University Hospital, Finland; Translational Immunology, Research Programs Unit, University of Helsinki, Finland	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data

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