The Distinct Traits of the *UNC13A* Polymorphism in Amyotrophic Lateral Sclerosis

Harold H. G. Tan, MD,[1] Henk-Jan Westeneng, MD,[1] Hannelore K. van der Burgh, PhD,[1] Michael A. van Es, MD, PhD,[1] Leonhard A. Bakker, MSc,[1,2] Kevin van Veenhuijzen, MD,[1] Kristel R. van Eijk, PhD,[1] Ruben P. A. van Eijk, MD, PhD,[1,3] Jan H. Veldink, MD, PhD,[1] and Leonard H. van den Berg, MD, PhD[1]

Objective: The rs12608932 single nucleotide polymorphism in *UNC13A* is associated with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) susceptibility, and may underlie differences in treatment response. We aimed to characterize the clinical, cognitive, behavioral, and neuroimaging phenotype of *UNC13A* in patients with ALS.
Methods: We included 2,216 patients with ALS without a *C9orf72* mutation to identify clinical characteristics associated with the *UNC13A* polymorphism. A subcohort of 428 patients with ALS was used to study cognitive and behavioral profiles, and 375 patients to study neuroimaging characteristics. Associations were analyzed under an additive genetic model.
Results: Genotyping rs12608932 resulted in 854 A/A, 988 A/C, and 374 C/C genotypes. The C allele was associated with a higher age at symptom onset (median years A/A 63.5, A/C 65.6, and C/C 65.5; $p < 0.001$), more frequent bulbar onset (A/A 29.6%, A/C 31.8%, and C/C 43.1%; $p < 0.001$), higher incidences of ALS-FTD (A/A 4.3%, A/C 5.2%, and C/C 9.5%; $p = 0.003$), lower forced vital capacity at diagnosis (median percentage A/A 92.0, A/C 90.0, and C/C 86.5; $p < 0.001$), and a shorter survival (median in months A/A 33.3, A.C 30.7, and C/C 26.6; $p < 0.001$). *UNC13A* was associated with lower scores on ALS-specific cognition tests (means A/A 79.5, A/C 78.1, and C/C 76.6; $p = 0.037$), and more frequent behavioral disturbances (A/A 16.7%, A/C 24.4%, and C/C 27.7%; $p = 0.045$). Thinner left inferior temporal and right fusiform cortex were associated with the *UNC13A* single nucleotide polymorphism (SNP; $p = 0.045$ and $p = 0.036$).
Interpretation: Phenotypical distinctions associated with *UNC13A* make it an important factor to take into account in clinical trial design, studies on cognition and behavior, and prognostic counseling.

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that is highly heterogenic in terms of clinical manifestations, genetic profiles, and pathophysiological mechanisms involved.[1] In search of new strategies to find more effective therapies, it has been proposed that ALS be treated as a disease that comprises multiple subgroups, rather than a single disease entity.[1–4] Different genetic mutations may affect different pathways but subsequently result in a similar phenotype. Recognition of these subtypes of ALS could eventually lead to development of pathway-specific therapies and might pave the way toward precision medicine. Furthermore, identification of subgroups allows for more targeted clinical trial designs

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Address correspondence to Leonard H. van den Berg, Department of Neurology, G03.228, University Medical Center Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands. E-mail: l.h.vandenberg@umcutrecht.nl

From the [1]Department of Neurology, UMC Utrecht Brain Center, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands; [2]Center of Excellence for Rehabilitation Medicine, UMC Utrecht Brain Center, University Medical Center Utrecht, Utrecht University and De Hoogstraat Rehabilitation, Utrecht, The Netherlands; and [3]Biostatistics and Research Support, Julius Centre for Health Sciences and Primary Care, University Medical Centre Utrecht, Utrecht University, Utrecht, The Netherlands

and genomewide association studies, which can increase statistical power.

Patients with ALS carrying a hexanucleotide repeat expansion in the *C9orf72* gene show a distinct clinical phenotype compared to sporadic patients with ALS: they have a shorter survival,[5–7] more cognitive deficits,[6,7] and more widespread brain involvement as shown by neuroimaging studies.[8,9] Another gene that could serve for subgroup stratification is *UNC13A*. The C allele of the rs12608932 single nucleotide polymorphism (SNP) within this gene has been identified as a risk locus for both ALS and frontotemporal dementia (FTD).[10,11] A recent meta-analysis of lithium trials in ALS suggests that *UNC13A* may underlie differences in treatment effects on survival.[12] Furthermore, the risk allele is associated with a shorter survival in ALS,[13–15] and more extensive cerebral involvement of frontal and temporal regions, as shown in neuroimaging and neuropathological TDP-43 burden.[16] Although these studies reveal that there are distinct traits associated with *UNC13A*, extensive phenotyping of its genotypes, also independent from *C9orf72*, has not yet been performed.

In this study, we perform this comprehensive phenotyping in ALS by studying clinical characteristics, cognition, behavior, brain morphology, and cerebral white matter integrity in a large set of patients with ALS from a population-based cohort.

Subjects/Materials and Methods

Patients

As part of an ongoing population-based study of ALS in The Netherlands, 2,397 patients diagnosed with ALS were included between January 2006 and June 2018. To avoid the known phenotype modifying effects of *C9orf72*, we excluded 181 patients (7.6%) with a *C9orf72* hexanucleotide repeat expansion. The *UNC13A* SNP (rs12608932) was determined in the remaining 2,216 patients using multiple methods: 1,395 genotypes were obtained through whole genome sequencing, 562 through genomewide SNP arrays, 259 through polymerase chain reaction (PCR)-amplified capillary sequencing described in previous work.[13] In addition, whole genome sequencing data were analyzed for genetic variants in ALS-FTD-related genes, that is *FUS*, *TARDBP*, *TBK1*, *SQSTM1*, *VCP*, and *UBQLN2*, which had a minor allele frequency smaller than 1%. Frameshift mutations, in-frame deletions and insertions, splice acceptor mutations, splice donor mutations, stop-gain mutations, stop-loss mutations, and start-loss mutations were considered high-risk mutations. Missense mutations and splice region mutations were considered to be of moderate risk. This study was approved by the Medical Ethical Committee of the University Medical Center (UMC) Utrecht and written informed consent was obtained from all participants.

Clinical Characteristics

Clinical characteristics were collected at the time of diagnosis or thereafter. We determined the site of symptom onset, age at symptom onset, and the presence of FTD. Disease progression rates were calculated using the slope of the revised ALS functional rating scale (ALSFRS-R),[17] defined as (48 ALSFRS-R score)/months since onset.[18] Respiratory function was measured using the forced vital capacity (FVC), expressed as a percent of the value predicted based on age, sex, and height. Survival status was obtained from municipal records.

Cognition and Behavior

A subset of 428 patients with ALS was screened for cognitive impairment using the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) within 3 months of diagnosis.[19] ECAS cognition scores were classified as normal or abnormal based on normative data of the Dutch version of the ECAS.[20] Behavioral deficits were assessed using the ECAS behavior screen and the ALS-FTD Questionnaire (ALS-FTD-Q).[21] Presence of behavioral impairment (ALS-bi) and FTD were assessed from the ECAS using the revised diagnostic criteria reported by Strong et al.[22] In addition, using previously reported cutoff values of the ALS-FTD-Q, patients were categorized as having mild behavioral impairment (score ≥ 22), or severe behavioral impairment (score ≥ 29).[21]

Neuroimaging

Another subset of 375 patients with ALS underwent 3 Tesla magnetic resonance imaging (MRI) scans of the brain. All participants with a history of epilepsy, stroke, psychiatric disorders, or any overt structural brain abnormalities were excluded from this subpopulation.

Images were acquired using a 3 Tesla Achieva Medical Scanner from Philips. A high-resolution T1-weighted image was acquired using the following parameters: three-dimensional fast field echo using parallel imaging; repetition time/echo time (TR/TE) = 10/4.6 ms, flip angel 8°, slice orientation sagittal, 0.80 × 0.75 × 0.75 mm voxel size, field of view = 176 × 240 × 240 mm, and reconstruction matrix = 220 × 320 × 320 covering the entire brain.

Diffusion-weighted imaging was performed for reconstruction of the complete white matter brain network (ie, connectome). Two sets of 30 weighted diffusion scans and 2 unweighted B0 scans were acquired with the following parameters: diffusion-weighted imaging using parallel imaging SENSE, p-reduction 3, high angular gradient set of 30 different weighted directions, TR/TE = 7,035/68 ms, 2 × 2 × 2 mm voxel size, 75 slices, b = 1,000 s/mm^2, second set with reversed k-space readout to correct for susceptibility-induced distortions.

T1-weighted images were preprocessed at high-resolution using Freesurfer version 6.0.[23] The cerebral cortex was parcellated into 68 regions according to the Desikan-Killiany atlas and segmented into 18 subcortical structures (deep gray matter and cerebellum).[24] From these regions, either cortical thickness or subcortical volumes were calculated.

Diffusion-weighted imaging data was used to analyze the connectome. White matter connections were reconstructed using Fiber Assignment for Continuous Tracking.[25] For each subject, an individual brain network was reconstructed, which contained a set of nodes (83 segmented brain regions from the Desikan-Killiany atlas) and a set of connections linking these nodes (white matter tracts interlinking these regions). The white matter connectivity strength was measured in terms of fractional anisotropy. A detailed description of the structural network reconstruction can be found in previous work.[26]

Statistical Analysis

In this study, we analyzed the additive genetic effects of rs12608932 C allele. SNP genotype A/A, A/C, and C/C were recoded as 0, 1, and 2, respectively. Statistical analyses were carried out using R (version 3.4.3). Analyses were performed using linear and logistic regression. Survival analyses were carried out using a robust Cox proportional hazard model, which provides accurate standard errors for Cox regression when the proportional hazards assumption is violated.[27] Survival analyses are corrected for the linear predictor of the ENCALS survival prediction model, which was calculated for every subject and represents a weighted sumscore of a previously established set of independent predictors for survival.[28] Because of their non-normal distributions, ECAS cognition scores were analyzed by first reversing the scores (ie, maximum ECAS score per domain – ECAS domain score), subsequently using a negative binomial model with age, sex, education, and bulbar onset of symptoms as covariates. The Wald-test was applied for p value calculations, using a threshold of significance at $p < 0.05$. Pairwise deletions were used when missing data was present. The package "effects" in R was used to report marginal effects of multivariable regression models.[29]

To account for the possible genotype-modifying effects of ALS-FTD-related genes (*FUS*, *TARDBP*, *TBK1*, *SQSTM1*, *VCP*, and *UBQLN2*), two types of sensitivity analyses were performed for all analyses of cognition and behavior. The first excluded only patients with a high-risk mutation in these genes. The second excluded patients with either a high-risk mutation or moderate-risk mutation in these genes.

MRI analyses were carried out using linear regression, adjusted for age and sex. Volumetric measures were additionally corrected for estimated total intracranial volume. For the gray matter analyses, a stringent permutation-based correction for multiple testing was performed using 10,000 random group assignment permutations. For each permutation, the lowest T-statistic over all cortical and subcortical gray matter regions was retained to create for multiple testing corrected null distributions over which p values were calculated. This means that only associations for gray matter thinning or volume loss were analyzed. This permutation-based correction controls the family-wise error rate. For the connectome-based white matter analyses, significance was assessed by means of the Network-Based Statistic.[30] Edges in the brain network were first labeled as affected if the linear model resulted in a p value < 0.05. Next, the size of the largest connected component of these affected connections was tested for significance by comparing it to a null distribution of 10,000 random group assignment permutations. The p values < 0.05 after these corrections for multiple testing were considered significant.

Results

UNC13A *Genotypes*

In the nonmutated *C9orf72* patients of the population-based cohort, rs12608932 showed a minor allele frequency of 0.39, with 854 A/A, 988 A/C, and 374 C/C genotypes. Subcohorts for cognition and behavior, and for neuroimaging showed similar minor allele frequencies at 0.39 and 0.38, respectively. An overview of the demographics for each (sub)cohort can be found in Table 1.

Clinical Characteristics

Clinical characteristics, stratified for genotype, are shown in Table 2. The risk allele of *UNC13A* is associated with a higher age at onset (ß = 1.17, 95% confidence interval [CI] = 0.52–1.81, $p < 0.001$), higher frequencies of bulbar symptom onset (odds ratio [OR] = 1.30, CI = 1.15–1.47, $p < 0.001$), FTD at diagnosis (OR = 1.51, CI = 1.15–1.99, $p = 0.003$), and lower FVC at diagnosis (ß = −2.50, CI = −3.95 to −1.05, $p < 0.001$). Multivariable analyses showed that the association with age at onset was independent of bulbar symptom onset (ß = 1.05, CI = 0.30–1.70, $p = 0.001$), and the association with bulbar symptom onset was independent of age at onset (OR = 1.28, CI = 1.29–1.45, $p < 0.001$). FTD was still associated with *UNC13A* in a multivariable analysis with age at onset and bulbar symptom onset as covariates (OR = 1.45, CI = 1.09–1.92, $p = 0.010$). These results did not change in our sensitivity analyses in which we (1) excluded patients with a high-risk mutation in ALS-FTD-related genes, or (2) excluded patients with either a high or moderate risk mutation. Furthermore, the multivariable analysis of FVC showed that it was still associated to *UNC13A* when correcting for bulbar symptom onset (ß = −2.22, CI = −3.68 to −0.77, $p = 0.003$). Last, multivariable survival analysis demonstrated that *UNC13A* was associated with survival (hazard ratio = 1.16, CI = 1.06–1.26, $p < 0.001$) independent from the linear predictor of the ENCALS survival model (Fig 1).

Cognition and Behavior

The results for the cognitive analyses are described in Table 3. The C-allele of *UNC13A* was associated with a lower ALS-specific score on the ECAS (incident rate ratio [IRR] = 1.070, CI = 1.005–1.140, $p = 0.037$), which seem to be mainly driven by a lower score on the language domain (IRR = 1.134, CI = 1.000–1.287, $p = 0.049$), and the executive domain (IRR = 1.070, CI = 0.996–1.150,

TABLE 1. Demographics Per Subcohort

	Subcohort:			
	Population-based	**Cognition and behavior**	**Neuroimaging**	**Missing data (%)**
n	2216	428	375	
UNC13A genotype (%)				0.0
A/A	854 (38.5)	164 (38.3)	148 (39.5)	
A/C	988 (44.6)	192 (44.9)	171 (45.6)	
C/C	374 (16.9)	72 (16.8)	56 (14.9)	
Sex, Female (%)	892 (40.3)	170 (39.7)	126 (33.6)	0.0
Age at onset, years, median (IQR)	64.7 (57.8–71.2)	66.5 (58.1–71.2)	61.4 (53.4–67.2)	0.5
Diagnostic delay, months, median (IQR)	9.9 (6.2–15.9)	11.1 (6.2–17.9)	8.8 (5.8–13.1)	0.5
Bulbar onset of symptoms (%)	722 (32.8)	114 (26.7)	95 (25.4)	0.6
Survival since onset,[a] months, median (CI)	31.3 (30.1–32.4)	32.9 (29.4–36.0)	40.4 (36.1–45.4)	0.5
FTD at diagnosis (%)	101 (5.6)	30 (7.6)	7 (2.1)	16.2
FVC% at diagnosis, median (IQR)	90.5 (75.0–104.0)	93.0 (78.0–106.0)	98.5 (88.0–108.0)	17.7
ALSFRS-R, median (IQR)	39.0 (34.0–43.0)	40.0 (37.0–43.0)	42.0 (39.0–45.0)	24.9
ALSFRS-R slope,[b] median (IQR)	0.6 (0.4–1.1)	0.7 (0.4–1.3)	0.5 (0.3–0.9)	25.3
Disease duration at assessment, months, median (IQR)		10.6 (6.0–17.6)	13.4 (9.4–21.9)	0.0
ECAS behavioral screen present (%)		387 (90.4)		
ALS-FTD-related genes[c]				33.3
High-risk mutation (%)	27 (1.2)	0 (0.0)	3 (0.8)	
Moderate-risk mutation (%)	169 (7.6)	6 (1.4)	25 (6.7)	

Overview of demographics and clinical characteristics for each (sub)cohort.
ALS = amyotrophic lateral sclerosis; ALSFRS-R = revised amyotrophic lateral sclerosis functional rating scale; CI = 95% confidence interval; ECAS = Edinburgh Cognitive and Behavioural ALS Screen; FTD = frontotemporal dementia; FVC% = forced vital capacity as a percentage of the value predicted based on age, sex, and height; IQR = interquartile range.
[a]The *p* value is calculated using a Cox proportional hazard model corrected for the linear predictor of the ENCALS model.
[b]ALSFRS-R slope is displayed in points decrease per month.
[c]ALS-FTD-related genes are *FUS*, *TARDBP*, *TBK1*, *SQSTM1*, *VCP*, and *UBQLN2*. Frameshift mutations, in-frame deletions and insertions, splice acceptor mutations, splice donor mutations, stop-gain mutations, stop-loss mutations, and start-loss mutations were considered high-risk mutations. Missense mutations and splice region mutations were considered to be of moderate risk.

$p = 0.066$). The proportion of patients scoring below cut-off did not differ significantly between the genotypes.

The results for the behavioral analyses are described in Table 4. The *UNC13A* risk allele was associated with higher frequencies of patients who fulfilled the revised Strong criteria for ALS-bi (OR = 1.40, CI = 1.01–1.97, $p = 0.045$) and ALS-FTD (OR = 1.89, CI = 1.18–3.06, $p = 0.008$), as derived from ECAS cognitive and behavioral scores. On the behavioral subdomains, disinhibition was associated with *UNC13A* (OR = 1.93, CI = 1.17–3.22, $p = 0.011$). In addition, *UNC13A* was associated with higher ALS-FTD-Q scores (ß = 1.78, CI = 0.13–3.42, $p = 0.034$), but no significant differences were found regarding the presence of mild or severe behavioral impairment ($p = 0.068$ and $p = 0.050$, respectively). Sensitivity analyses, which excluded patients carrying ALS-FTD-related gene mutations, showed similar results for all analyses on cognition and behavior (Table 5).

TABLE 2. Clinical Characteristics

	UNC13A genotype: A/A	A/C	C/C	*p* value	Missing data (%)
N	854	988	374		
Sex, F (%)	347 (40.6)	387 (39.2)	158 (42.2)	0.80	0.0
Age at onset, yr, median (IQR)	63.5 (55.6–70.3)	65.6 (59.0–71.8)	65.5 (58.9–71.4)	<0.001	0.7
Diagnostic delay, mo, median (IQR)	10.1 (6.4–16.7)	9.9 (6.1–15.6)	9.6 (6.0–13.9)	0.097	0.7
Bulbar onset of symptoms (%)	251 (29.6)	312 (31.8)	159 (43.1)	<0.001	0.8
Survival since onset,[a] mo, median (CI)	33.4 (32.3–35.6)	30.7 (29.4–32.8)	26.6 (24.9–29.5)	<0.001	0.7
Family history of ALS or FTD (%)	29 (3.6)	35 (3.7)	12 (3.4)	0.92	4.2
FTD at diagnosis (%)	30 (4.3)	42 (5.2)	29 (9.5)	0.003	18.4
FVC% at diagnosis, median (IQR)	92 (78–106)	90 (75–104)	86.5 (69–101)	<0.001	21.2
ALSFRS-R, median (IQR)	39 (33–43)	39 (35–43)	39 (34–43)	0.98	32.1
ALSFRS-R slope,[b] median (IQR)	0.7 (0.4–1.1)	0.6 (0.3–1.1)	0.6 (0.4–1.2)	0.42	32.6

Overview of clinical characteristics stratified for genotype. The *p* values are derived from univariable linear or logistic models.
ALS = amyotrophic lateral sclerosis; ALSFRS-R = revised amyotrophic lateral sclerosis functional rating scale; CI = 95% confidence interval; FTD = frontotemporal dementia; IQR = interquartile range; FVC% = forced vital capacity as a percentage of the predicted value.
[a]The *p* value is calculated using a Cox proportional hazard model corrected for the linear predictor of the ENCALS model.
[b]ALSFRS-R slope is displayed in points decrease per month.

Neuroimaging

Outcomes of gray and white matter analyses are visualized in Figure 2. Cortical gray matter analyses revealed 2 regions that showed significant cortical thinning after corrections for multiple testing: the left inferior temporal cortex (ß = −0.027, CI = −0.043 to −0.010, $p = 0.045$)

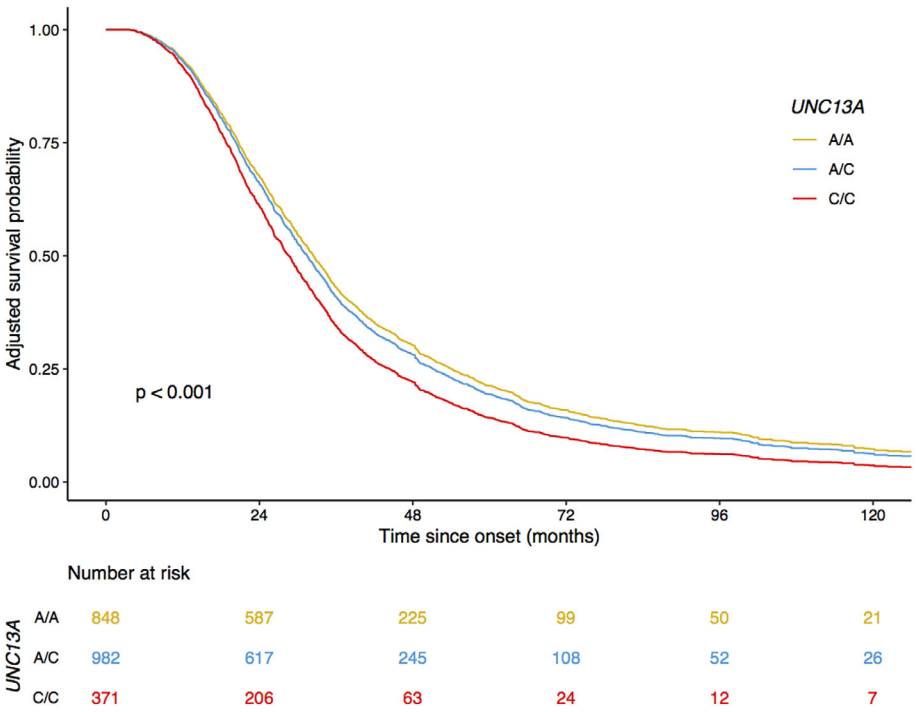

FIGURE 1: Adjusted survival curves corrected for the linear predictor of the ENCALS survival prediction model. The *p* values display the additive effect of the C allele of the *UNC13A* single nucleotide polymorphism (SNP).

TABLE 3. Cognitive Profile According to the ECAS

	UNC13A genotype: A/A	A/C	C/C	*p* value	Missing data (%)
N	164	192	72		
Age at ECAS, yr, median (IQR)	66.3 (56.7–71.5)	68.5 (60.4–72.6)	68.1 (62.4–72.7)	0.057	0.0
Time since onset, mo, median (IQR)	10.3 (5.4–16.6)	11.5 (6.3–19.1)	9.2 (5.9–12.4)	0.62	0.0
Education (%)				0.33	0.2
ISCED 0–4	117 (71.3)	152 (79.2)	53 (74.6)		
ISCED 5–6	47 (28.7)	40 (20.8)	18 (25.4)		
ECAS cognitive screen					
Abnormal total score[a] (%)	29 (18.0)	36 (19.9)	10 (15.2)	0.78	4.7
Abnormal ALS specific score[a] (%)	18 (11.1)	27 (14.8)	13 (19.7)	0.088	4.0
Abnormal ALS nonspecific score[a] (%)	23 (14.3)	40 (21.4)	9 (13.2)	0.70	2.8
Total score[b]	106.6 (104.7–108.3)	105.1 (103.7–106.4)	103.5 (100.7–106.1)	0.10	4.7
ALS specific score[b]	79.5 (78.1–80.9)	78.1 (77.0–79.1)	76.6 (74.4–78.6)	0.037	4.0
ALS nonspecific score[b]	26.9 (26.2–27.6)	26.8 (26.3–27.3)	26.7 (25.7–27.7)	0.81	2.8
Language score[b]	25.9 (25.6–26.2)	25.6 (25.4–25.9)	25.3 (24.8–25.8)	0.049	2.6
Fluency score[b]	18.8 (18.3–19.2)	18.5 (18.1–18.8)	18.1 (17.4–18.7)	0.13	2.3
Executive score[b]	34.7 (33.7–35.7)	33.8 (33.0–34.5)	32.8 (31.1–34.3)	0.066	2.8
Memory score[b]	15.5 (14.8–16.1)	15.4 (14.8–15.8)	15.2 (14.2–16.1)	0.71	2.3
Visuospatial score[b]	11.5 (11.4–11.6)	11.5 (11.4–11.6)	11.5 (11.3–11.6)	0.76	2.1

Patient characteristics are reported at time of assessment. Analyses were reported for both normative data and raw ECAS scores.
ALS = amyotrophic lateral sclerosis; CI = 95% confidence interval; ECAS = Edinburgh ALS cognitive and behavioural screen; IQR = interquartile range; ISCED = International Standard Classification of Education.
[a]Cutoff is set at the 5th percentile of normative data after correction for age, sex, and education levels as previously reported.[20] The *p* values are derived from logistic models.
[b]Estimated means (95% confidence interval) corrected for age, sex, and education. The *p* values are calculated using a negative binomial model corrected for age, sex, and education.

and the right fusiform cortex (ß = −0.027, CI = −0.043 to −0.011, $p = 0.036$). To assess whether this effect was driven by patients with FTD, we performed a sensitivity analysis in which 7 patients with FTD were excluded. The results of this analysis were similar, with the 2 regions most strongly associated to *UNC13A* being: the left inferior temporal cortex (ß = −0.027, CI = −0.043 to −0.010, $p = 0.049$) and the right fusiform cortex (ß = −0.024, CI = −0.041 to −0.008, $p = 0.083$). However, in this sensitivity analysis, the right fusiform cortex is not significantly associated to *UNC13A*. Connectome analyses did not show statistically significant differences regarding white matter connectivity (Fig 3).

Discussion

In this population-based study, we found distinct patient characteristics in ALS associated with the minor allele of the rs12608932 polymorphism in *UNC13A*. The risk allele is independently associated with a higher age at symptom onset, higher frequency of bulbar onset, lower respiratory function, and reduced survival. In a relatively

TABLE 4. Behavioral Profile

	UNC13A genotype: A/A	A/C	C/C	*p* value	Missing data (%)
N	150	172	65		
ECAS behavioral screen					
Disinhibition (%)	6 (4.0)	17 (9.9)	9 (13.8)	0.011	0.0
Apathy (%)	24 (16.0)	34 (19.8)	15 (23.1)	0.20	0.0
Loss of sympathy (%)	13 (8.7)	27 (15.7)	11 (16.9)	0.054	0.0
Perseverative behavior (%)	15 (10.0)	14 (8.1)	9 (13.8)	0.57	0.0
Hyperorality (%)	7 (4.7)	19 (11.0)	5 (7.7)	0.20	0.0
Any behavioral changes (%)	39 (26.0)	57 (33.1)	22 (33.8)	0.17	0.0
Presence of psychiatric symptoms (%)	1 (0.7)	10 (5.8)	1 (1.5)	0.28	0.0
Presence of ALS-bi[a] (%)	25 (16.7)	42 (24.4)	18 (27.7)	0.045	0.0
Presence of ALS-FTD[a] (%)	8 (5.3)	18 (10.5)	11 (16.9)	0.008	0.3
ALS-FTD-Q					
Total score, median (IQR)	6.5 (3–12.2)	9 (3–19)	10 (4.5–21)	0.034	10.1
Behavioral disturbances (%)					
Mild to severe, ≥ 22	19 (14.4)	30 (19.1)	15 (25.4)	0.068	
Severe, ≥ 29	7 (5.3)	16 (10.2)	8 (13.6)	0.050	

Behavioral changes as observed from the ECAS and ALS-FTD-Q behavioral screens. The *p* values are derived from a univariable logistic model.
ALS-bi = amyotrophic lateral sclerosis with behavioral impairment; FTD = frontotemporal dementia; ALS-FTD-Q = amyotrophic lateral sclerosis with frontotemporal dementia questionnaire; ECAS = Edinburgh ALS cognitive and behavioural screen; IQR = interquartile range.
[a]Classification according to the revised criteria by Strong et al (2017).[22]

large cognition dataset, we further showed that *UNC13A* was associated with poorer cognitive functioning, higher rates of behavioral impairment, and higher rates of FTD. Our neuroimaging analyses reveal *UNC13A*-associated cortical thinning in bilateral temporal regions, which provide a potential anatomic basis for these cognitive and behavioral deficits.[31] The results of this study demonstrate the importance of comprehensive phenotyping in multiple domains of the disease for the discovery of ALS subgroups.

In this study, we establish that the polymorphism in *UNC13A* contributes to the clinical heterogeneity found in ALS. In previous smaller studies, we and others have shown that *UNC13A* is associated with a reduced survival,[13–15] and in our current study of 2,216 patients, we show that this effect is independent of the linear predictor of the ENCALS survival prediction model; an internationally validated prognostic model consisting of 8 survival predictors.[28] In addition to this, we discovered previously undescribed associations of the *UNC13A* minor allele to 4 clinical characteristics, namely: a higher age at symptom onset, a higher frequency of bulbar onset, lower FVC at diagnosis, and a higher percentage of FTD at diagnosis. Despite the fact that a higher age at onset has been associated with higher proportions of bulbar symptom onset,[32] we show that each of these clinical characteristics is independently correlated to the *UNC13A* risk allele. Likewise, the findings of a lower FVC is not confounded by bulbar symptoms. The minor allele frequency of 0.39 is within the range of ALS populations in previous studies, in which a minor allele frequency between 0.32 and 0.41 is found for patients with ALS and between 0.29 and 0.36 for controls.[10,13–15,33,34] Combined with the distinct set of *UNC13A*-associated clinical characteristics, there is a possibility for *UNC13A* to substantially impact the heterogeneity of study samples.

This study builds upon our previous work, which showed that *UNC13A* is a risk factor for both ALS and

TABLE 5. Analysis of Subcortical Structures

	UNC13A genotype: A/A	A/C	C/C	Uncorrected *p* value	Corrected *p* value
N	148	171	56		
Deep gray matter					
Left					
Thalamus	6.95 (6.85–7.05)	6.90 (6.83–6.97)	6.85 (6.72–6.99)	0.34	0.97
Putamen	4.76 (4.69–4.82)	4.71 (4.67–4.76)	4.67 (4.58–4.77)	0.22	0.92
Globus pallidus	1.85 (1.82–1.88)	1.85 (1.83–1.87)	1.85 (1.81–1.90)	0.84	1.00
Caudate nucleus	3.52 (3.46–3.59)	3.48 (3.43–3.53)	3.44 (3.34–3.53)	0.20	0.90
Nucleus accumbens	0.532 (0.519–0.545)	0.516 (0.506–0.525)	0.499 (0.481–0.517)	0.010	0.20
Hippocampus	3.81 (3.75–3.88)	3.80 (3.76–3.85)	3.79 (3.70–3.88)	0.79	1.00
Amygdala	1.58 (1.55–1.62)	1.58 (1.56–1.61)	1.58 (1.53–1.63)	0.94	1.00
Right					
Thalamus	6.76 (6.67–6.85)	6.72 (6.65–6.78)	6.68 (6.55–6.80)	0.35	0.97
Putamen	4.76 (4.69–4.82)	4.70 (4.65–4.75)	4.64 (4.55–4.74)	0.089	0.72
Globus pallidus	1.81 (1.78–1.84)	1.82 (1.80–1.84)	1.84 (1.79–1.88)	0.35	1.00
Caudate nucleus	3.65 (3.58–3.72)	3.58 (3.53–3.63)	3.51 (3.41–3.60)	0.036	0.47
Nucleus accumbens	0.557 (0.544–0.569)	0.541 (0.532–0.55)	0.525 (0.508–0.542)	0.010	0.20
Hippocampus	3.95 (3.88–4.01)	3.93 (3.88–3.97)	3.91 (3.82–4.00)	0.56	0.99
Amygdala	1.81 (1.77–1.84)	1.79 (1.76–1.81)	1.77 (1.72–1.81)	0.23	0.93
Cerebellum					
Left cortex	51.6 (51.0–52.3)	51.3 (50.9–51.8)	51.1 (50.2–52.0)	0.41	0.98
Left white matter	13.9 (13.7–14.1)	13.8 (13.7–14.0)	13.8 (13.5–14.1)	0.73	1.00
Right cortex	51.8 (51.2–52.4)	51.4 (51.0–51.9)	51.1 (50.2–51.9)	0.22	0.91
Right white matter	13.4 (13.1–13.6])	13.2 (13.0–13.3)	13.0 (12.7–13.3)	0.15	0.85

Estimated mean volumes of deep gray matter and cerebellum after corrections for age, sex, and total intracranial volume. Uncorrected *p* values are calculated using the same linear model. The *p* values were corrected for multiple testing using 10,000 random permutations. Values are in mean (95% confidence interval). Volumes are reported in cm^3.

FTD separately.[11] The association of *UNC13A* and the ALS-FTD phenotype has been further explored in another study,[16] which analyzed cognitive function, neuroimaging (restricted to the frontal and temporal cortices), and TDP-43 pathology on autopsy. In a cohort of 109 patients, they found a worse performance on a reverse digit span task in homozygous *UNC13A* carriers, indicating frontal lobe-mediated cognitive defects. Furthermore, their neuroimaging analyses revealed small regions of cortical thinning in prefrontal and temporal regions, as well as an increased TDP-43 pathologic burden in the frontal and temporal cortices. In our study, we investigated the association of *UNC13A* and the ALS-FTD phenotype by administering the ECAS—a validated cognitive screen specifically designed for patients with ALS—to 428 patients in order to detect cognitive and behavioral changes in an

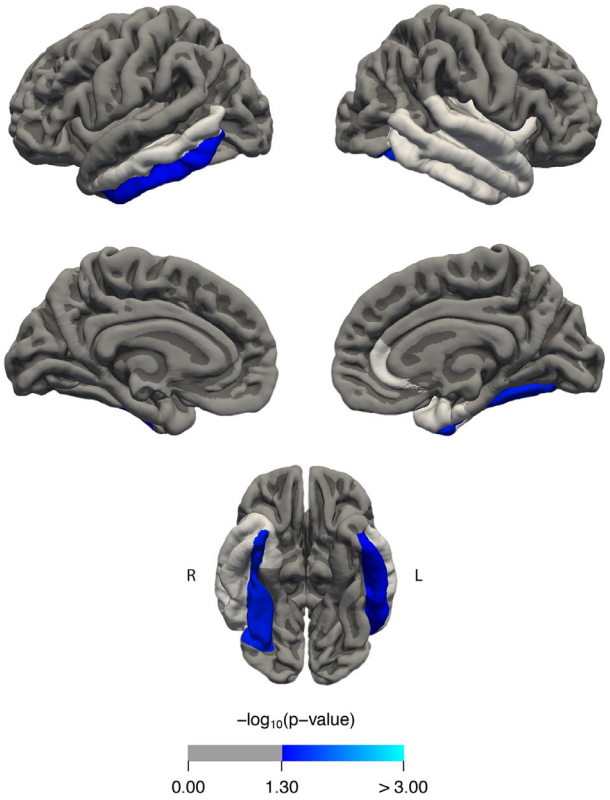

FIGURE 2: Regions showing reduced cortical thickness associated with *UNC13A*. Blue areas indicate the level of significance after correction for multiple testing. White areas indicate significant cortical thinning without corrections for multiple testing.

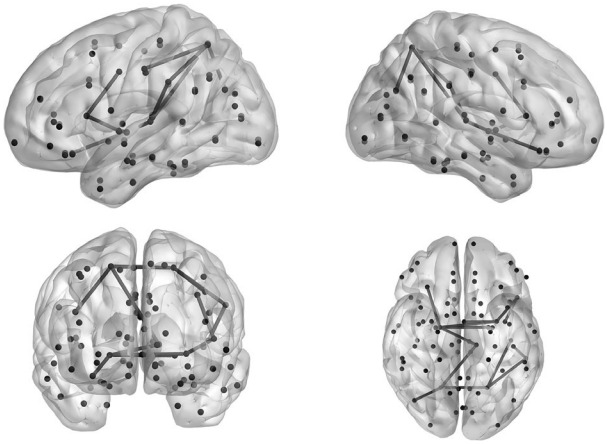

FIGURE 3: Largest connected component showing decreased fractional anisotropy associated with *UNC13A*. Network based statistics did not reveal a significant largest connected component.

additive model. Used in conjunction with the revised Strong criteria,[22] we found that carriers of the risk allele not only showed higher rates of ALS-FTD, but also more frequent cognitive and behavioral impairment, which predominantly manifested as language impairment, executive dysfunction, and behavioral disinhibition. Moreover, sensitivity analyses rule out the possibility that these cognitive and behavioral changes are an effect of other ALS-FTD-related genetic mutations. To explore the anatomic basis for these findings, we performed a whole-brain neuroimaging analysis involving 375 patients, in which we observed temporal cortical thinning associated with *UNC13A*, but did not find associated thinning in prefrontal regions (even without correction for multiple testing). Additional analyses show that these gray matter changes may indeed be partially explained by patients with FTD, but for a large part could still be driven by patients with milder cognitive or behavioral symptoms. Overall, we provided comprehensive evidence showing *UNC13A* is not only a risk factor for ALS or FTD separately, but also for the ALS-FTD phenotype.

Identification of disease modifiers, such as common genetic variants, can help to tailor medicine to individual patients. Common variant disease modifiers also present a challenge, as evidence show they easily create an imbalance between study arms in clinical trials of smaller sample size, subsequently influencing outcome measures of clinical trials.[35] This effect of *UNC13A* in clinical trials has already been shown in a previous meta-analysis of lithium trials: the treatment group show a prolonged survival but only in homozygous carriers of the *UNC13A* risk allele.[12] As we show that *UNC13A* is related to cognitive and behavioral deficits, we suggest that these domains could be relevant outcome measures in *UNC13A* carrier specific trials. Such a trial design could also be extrapolated to patients with *UNC13A* minor allele-carrying FTD, where cognitive and behavioral changes form the hallmark symptoms of the disease. The findings of this study strengthen the argument that the *UNC13A* SNP could provide another dimension for stratification in trials, as its associations are not limited to survival time, but encompass a variety of clinically relevant outcome measures.

The UNC13A protein is known to have a variety of functions that facilitate presynaptic vesicle release.[36] Yet, it is uncertain how this protein actually plays a role in ALS pathophysiology, or if the rs12608932 SNP increases disease susceptibility through other genetic variants that are in linkage disequilibrium with this SNP. Counterintuitively, the risk allele in *UNC13A* is associated with both higher susceptibility and higher age at onset, which, in a multistep etiology, conflicts with the idea that a higher genetic burden will reduce the number of steps needed to develop ALS.[37] Moreover, it is still unclear how the phenotypical distinctions result from the *UNC13A* genotype. Seeing that the *UNC13A* risk allele is associated with a shorter survival, but not with a faster progression of motor symptoms (ALSFRS-R slope), it is possible that *UNC13A* affects survival through other factors than ALSFRS-R

slope. A previous study has identified multiple independent predictors of survival besides motor symptom progression (ie, age at symptom onset, a bulbar onset of symptoms, and the presence of FTD).[28] As *UNC13A* is also correlated to these predictors, it is possible that the effect of survival results from disease mechanisms associated with these clinical characteristics. Furthermore, the increased frequency of both bulbar symptom onset and extra-motor impairment gives rise to the idea that *UNC13A* selectively increases vulnerability of neurons in associated regions of the brain. This could be related to earlier studies, which found correlations between bulbar onset and cognitive impairment,[38,39] although these findings are debated.[40] Alternatively, one could speculate that *UNC13A* is involved in the process of TDP43 propagation from primary affected motor regions to extra-motor regions, a mechanism that has been proposed by Brettschneider et al[41] and Braak et al.[42] However, the association of *UNC13A* with the pure form of FTD suggests that primary affected regions could also lie in extra-motor regions.[11]

One of the limitations of the study is that we used only the additive genotypic model to analyze the effect of *UNC13A*. As the exact pathophysiological mechanism model is unknown, we do not know in which genotypic model *UNC13A* affects ALS. Earlier studies mainly found associations with recessive and additive models,[13,14] but there were also dominant and overdominant associations.[15] Hence, in this study, we aimed primarily to describe the characteristics of each genotype and then to analyze structures in the data using the additive model as a versatile genotypic model. Another limitation is the missing data in our analyses. When using pairwise deletions to cope with missing data, higher proportions of missing data could lead to biased estimates requiring careful interpretation. In our study, this is especially the case for the ALSFRS-R and its slope. Yet, for the majority of the analyses, the proportions of missing data are relatively low, and we were able to test other proxies of disease progression, such as diagnostic delay and survival, with a completeness of 99.3%. Last, we have to deal with the fact that we could not include the entire ALS population in our cognition and neuroimaging subcohort, because the Dutch version of the ECAS has only been available since 2015, and because of exclusion criteria in our neuroimaging study concerning patient safety. We did aim to minimize inclusion bias by offering the ECAS to all newly included patients in our population-based study and also offered home visits when needed. Furthermore, we tried to include patients in our neuroimaging study shortly after diagnosis to minimize the exclusion of those who had progressed to severe bulbar and respiratory symptoms, which would hinder the acquisition of MRI scans.

Finally, we conclude that the rs12608932 minor allele in *UNC13A* is a highly frequent genetic variant in ALS that shows a distinct phenotype over a wide range of disease aspects, covering clinical, cognitive, behavioral, and neuroimaging domains. Comprehensively phenotyping a large number of patients enabled us to detect distinct characteristics for both motor symptoms (age at onset, bulbar onset, early respiratory decline, and survival), as well as extra-motor symptoms in the form of ALS-FTD (cognitive, behavioral, and temporal neuroimaging findings). We stress the importance of comprehensive phenotyping in search of phenotypical subgroups in ALS, as we demonstrate that heterogeneity can be found in multiple aspects of the disease. Although the role of *UNC13A* in ALS pathophysiology is still unknown, its phenotypical distinctions make it an important factor to take into account in clinical trial design, studies on cognition and behavior, and prognostic counseling.

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Author Contributions

H.H.G.T., H.J.W., H.K.v.d.B., M.A.v.E., R.P.A.v.E., J.H.V., and L.H.v.d.B. contributed to the conception and design of the study. H.H.G.T., H.J.W., H.K.v.d.B., L.A.B., K.v.V., and K.R.v.E. contributed to the acquisition and analysis of data. H.H.G.T., H.J.W., H.K.v.d.B., and L.H.v.d.B. contributed to drafting the text and preparing the figures.

Potential Conflicts of Interest

The authors report no conflicts of interest.

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