



The *UNC13A* cryptic exon associates with cognitive impairment in Alzheimer's disease

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ABSTRACT

TAR DNA-binding protein of 43 kDa (TDP-43) is often found in the brains of patients with Alzheimer's disease (AD), where its co-occurrence with amyloid β plaques and tau neurofibrillary tangles associates with accelerated cognitive decline and brain atrophy. TDP-43's function of repressing the inclusion of cryptic exons (CEs) during RNA splicing is compromised in AD. A single-nucleotide polymorphism (SNP) located within the CE in the *UNC13A* gene [rs12973192 (C>G)] is associated with higher disease risk and reduced survival in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) by weakening TDP-43 binding promoting CE inclusion.

We evaluated the rs12973192 *UNC13A* CE SNP in a cohort of 1,672 AD, including 643 AD brains with available cognitive measurements and 73 AD cases for which we measured cryptic RNA levels in the amygdala, and investigated the influence of the *UNC13A* CE on TDP-43 pathology, survival and cognitive impairment in AD. We also evaluated a cohort of 466,517 from the UK Biobank to determine associations between the rs12973192 *UNC13A* CE SNP and dementia diagnosis..

Characteristics of the AD cohorts

Mayo Clinic Brain Bank

Variable	All AD (N = 1,672)	AD no TDP (N = 928)	AD/LATE (N = 744)
Sex (female)	906 (54.2%)	476 (51.3%)	430 (57.8%)
Age at onset (years)	72 (34, 101)	70 (34, 98)	74 (34, 101)
Age at death (years)	83 (38, 110)	80 (38, 110)	86 (54, 104)
Survival after onset (years)	9 (0.1, 36)	9 (1, 26)	10 (0.1, 36)
Race (White)	1,643 (98.4%)	908 (98.2%)	735 (98.8%)
Ethnicity (non-Hispanic or Latino)	1,627 (97.3%)	902 (97.2%)	725 (97.4%)
TDP-43 pathology	744 (44.5%)	NA	744 (100%)
Type α	327 (44.0%)	NA	327 (44.0%)
Type β	287 (38.6%)	NA	287 (38.6%)
Braak stage	6 (2, 6)	6 (2, 6)	6 (3, 6)
Thal phase	5 (2, 5)	5 (2, 5)	5 (2, 5)
Lewy body pathology	699 (63.1%)	377 (66.6%)	322 (59.5%)
Vascular dementia	709 (43.1%)	370 (40.3%)	339 (46.6%)

The sample median (minimum, maximum) is given for continuous variables; categorical variables were summarized with number and percentage of patients (calculated from total of patients with information in each category). Non-White individuals included 3 American Indian/Alaskan Native, 6 Asian, and 15 African Americans. A total of 5 individuals had undisclosed or unknown racial status, and 3 cases had unknown ethnic status..

UK Biobank

	All (N=466,517)	No dementia (N=465,012)	Dementia (N=1,505)
Sex (male)	212,732 (45.6%)	212,046 (45.6%)	837 (55.6%)
Average age (range) in years	57 (38-72)	57 (38-72)	65 (42-70)
BMI (kg/m ²)	27.4 (12.1-74.7)	27.5 (12.1-74.7)	27.5 (16.4-51.5)
Years of education (range)	14.8 (7-20)	14.8 (7-20)	12.5 (7-20)
Smoking (%)			
Never	266,905 (57.2%)	252,618 (54.3%)	703 (46.7%)
Previous	169,115 (36.3%)	163,000 (35.1%)	650 (43.2%)
Current	51,542 (11.1%)	49,394 (10.6%)	152 (10.1%)
rs12973192 genotype (C>G)			
C/C	200,794 (43%)	200,143 (43%)	651 (43%)
C/G	210,492 (45%)	209,843 (45%)	649 (43%)
G/G	55,231 (12%)	55,026 (12%)	205 (14%)
rs12973192 MAF	34.4%	34.3%	35.2%

The sample mean (range) is given for continuous variables; categorical variables were summarized with number and percentage of patients.

The *UNC13A* CE SNP is associated with cases that have received a diagnosis of dementia compared to those without (recessive model OR: 1.1732; 95% CI: 1.0074-1.3591; P value: 0.0364).

1. The rs12973192 *UNC13A* CE SNP does not predict TDP-43 pathology or survival in AD

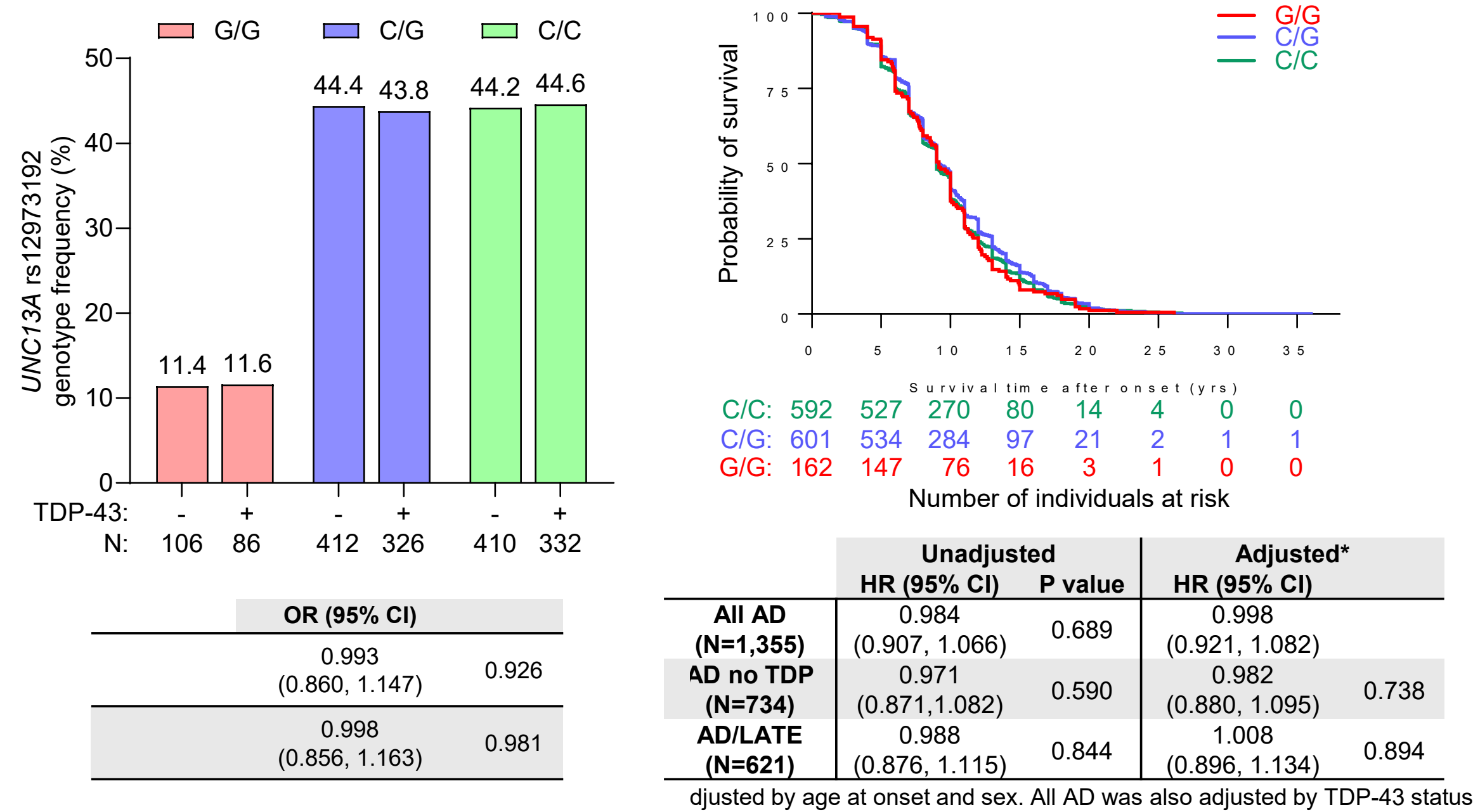


Fig. 1. **A**) Genotype frequencies of the rs12973192 *UNC13A* CE SNP in AD patients stratified by TDP-43 pathology status. Odds ratio (OR) and 95% confidence intervals (CI) were calculated using linear logistic regression analysis using the additive genetic model (CC=0 (reference), CG=1, GG=2) and allelic (C=0 (reference), G=1) adjusting of age of death, sex and APOE $\epsilon 4$. ORs can be interpreted as the odds of a patient exhibiting TDP-43 pathology vs. no TDP-43 pathology. **B**) Survival curves of all AD cases in this study stratified by rs12973192 *UNC13A* CE SNP. Hazard ratios (HR) and 95% CI were calculated using the additive model (CC=0 (reference), CG=1, GG=2), adjusting of age at symptom onset and sex for AD no TDP and AD/LATE groups, and age at symptom onset, sex and TDP-43 status for all AD cases.

2. The *UNC13A* CE SNP associates with worse cognitive function in AD

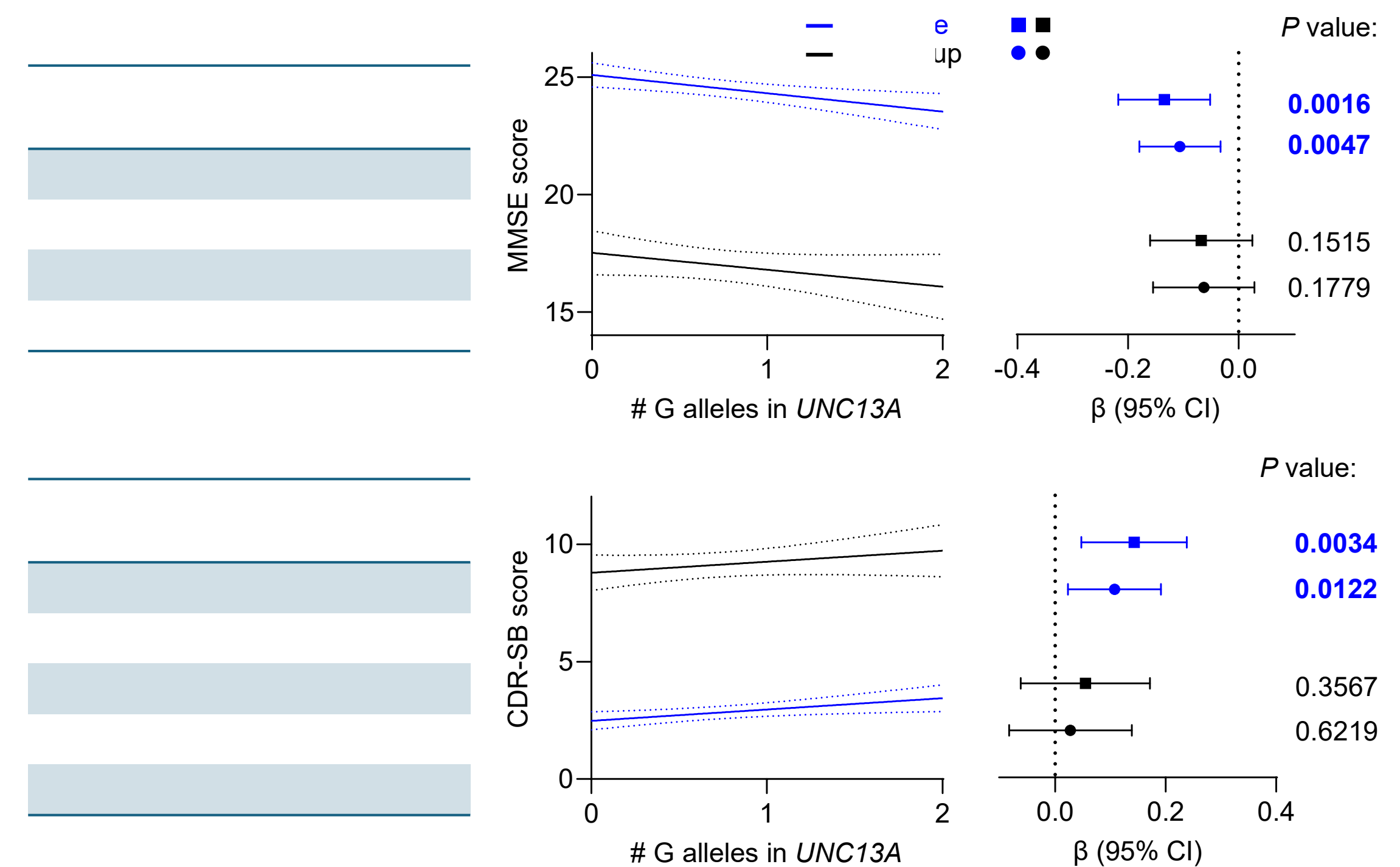


Fig. 2. Baseline (blue) and follow-up (black) scores for MMSE (**A**) and CDR-SB (**B**) stratified by presenting 0, 1 or 2 copies of the minor *UNC13A* CE SNP (G) allele (middle graphs). Linear regression models were used to determine the association between baseline (blue) and follow-up (black) scores and the *UNC13A* CE SNP using the additive (CC=0 (reference), CG=1, GG=2) genetic model, unadjusted (squares) adjusted (circles) for time from testing to death, sex and TDP-43 status (right graphs). After applying Bonferroni correction, adjusted p-values < 0.0125 are considered statistically significant.

3. The levels of *UNC13A*, but not of *STMN2*, cryptic RNA associate with worse cognitive function in AD

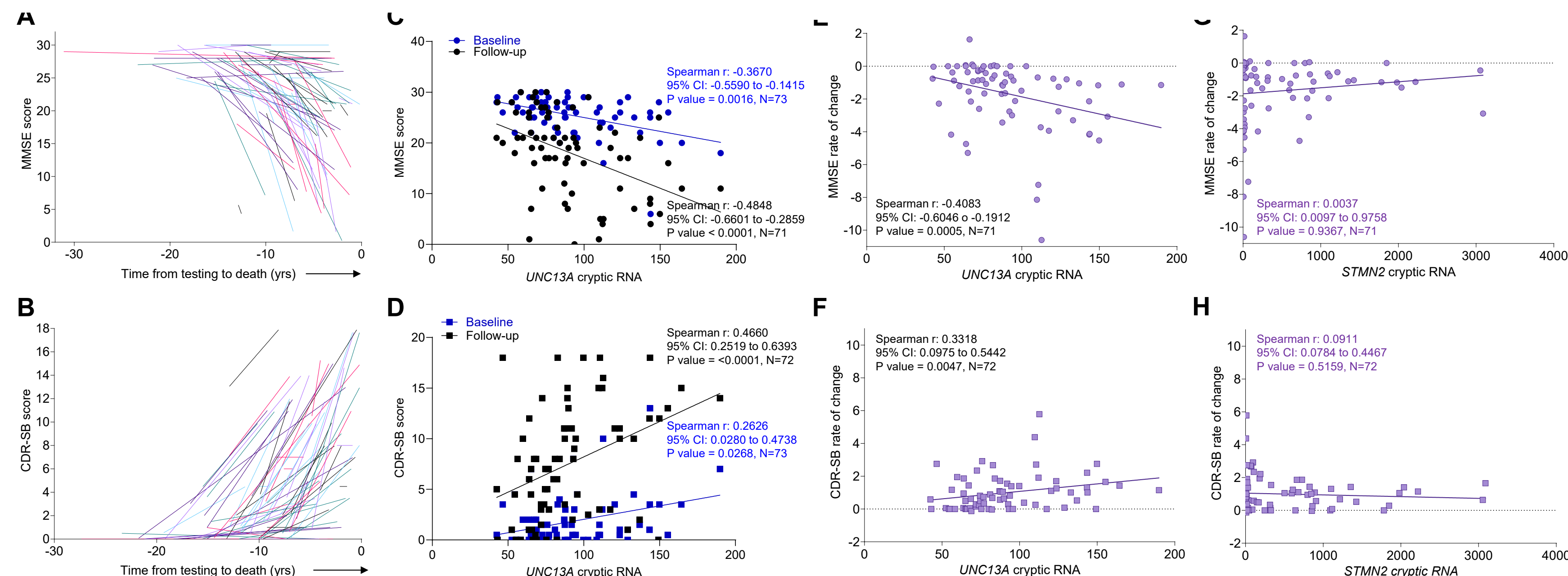


Fig. 3. **A**, **B**) Temporal trajectories, represented by each line, of cognitive scores for each AD/LATE case for which we had baseline and follow-up measurements of MMSE (**A**, N=71) and CDR-SB (**B**, N=72) and matching amygdala tissue. **C**, **D**) Spearman's partial correlation analysis between *UNC13A* cryptic RNA levels in the AD/LATE amygdala and corresponding baseline (blue) and follow-up (black) measurements for MMSE (**C**, circles) and CDR-SB (**D**, squares), and controlling for age at death and sex. **E-H**) Spearman's partial correlation analysis between *UNC13A* (**E**, **F**) or *STMN2* (**G**, **H**) cryptic RNA levels measured in the AD/LATE amygdala and corresponding rate of cognitive decline for MMSE (**E**, **G**) and CDR-SB (**F**, **H**) and controlling for sex.

CONCLUSIONS

- The *UNC13A* rs12973192 variant does not impact the presence of TDP-43 pathology or overall survival in AD patients.
- The risk *UNC13A* CE SNP is significantly associated with worse baseline cognitive and functional impairment in AD.
- Quantitative *UNC13A* cryptic RNA levels in the amygdala predict the rate of cognitive decline more accurately than the SNP alone.
- This association is specific to *UNC13A*, as *STMN2* cryptic RNA levels show no link with cognitive decline.

These findings underscore the potential role of genetic variants and TDP-43-regulated CEs in shaping AD phenotypes.

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