Development of an UNC13A cryptic exon skipping antisense oligonucleotide as a treatment for ALS and FTD

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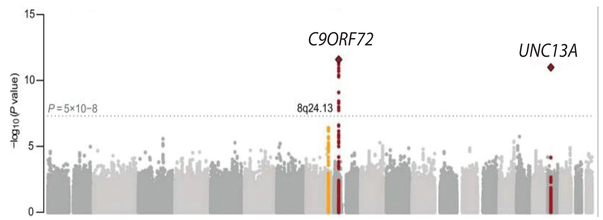
Abstract

Single nucleotide polymorphisms (SNPs) in UNC13A are among the strongest hits associated with FTD and ALS in human genome-wide association studies. Recent analyses by researchers from the labs of Aaron Gitler and Pietro Fratta identified a cryptic exon (CE) in *UNC13A* mRNA in postmortem tissues and patient-induced pluripotent stem cells (iPSC) derived neurons depleted of TDP-43(1,2). UNC13A is a particularly compelling target for ALS/FTD therapeutics because natural history studies show that patients carrying the normal transcript of *UNC13A* SNPs survive 3-4 years longer after disease onset than patients carrying two copies of the risk alleles(1). We sought to develop and identify a novel development candidate antisense oligonucleotides(ASOs) treatment that could potently and safely suppress the inclusion of the cryptic exon in *UNC13A* transcript using patient-derived models and animal models.

References:
1. Ma R et al. Nature. 2022 Mar;603(7899):124-130
2. Brown A et al. Nature. 2022 Mar;603(7899):131-137

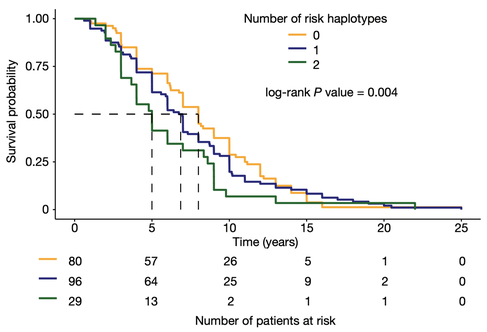
Background

UNC13A is a top GWAS hit in a meta-analysis of >19k FTD+ALS patients and controls



Adapted from Diekstra et al *Ann Neurol* 2014. Joint meta analysis conducted by Diekstra FP and colleagues using published GWAS data of 4,377 ALS patients and 13,017 controls and 435 pathology-proven FTD-TDP cases and 1,414 controls for genotype imputation.

SNPs in UNC13A are predictive of disease severity in FTD(shown) and ALS patients



Adapted from Ma et al 2022 *Nature*. Top, survival curves of FTLD-TDP patients stratified on the basis of the number of risk haplotypes. Heterozygous (1) and homozygous (2) patients had shorter survival time after disease onset (n = 205, Mayo Clinic Brain Bank; score (log-rank) test, P = 0.004). Dashed lines mark median survival for each genotype. Risk haplotype effect is modelled additively using Cox multivariable analysis adjusted for genetic mutations, sex and age at onset. Bottom, risk table.

UNC13A could be a core disease mechanism of TDP-43 proteinopathy

The copy number of SNPs in *UNC13A* is correlated with the disease severity of the FTD and ALS patients. Recently, it has been shown that the SNPs are located at TDP-43 protein binding sites which directly affect the splicing mechanism mediated by TDP-43, which leads to cryptic exon inclusion. This strongly suggests that altered UNC13A function is a core disease mechanism of TDP-43 proteinopathy.

UNC13A cryptic exon inclusion in ALS/FTD

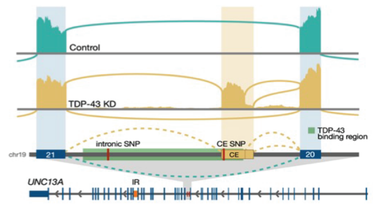
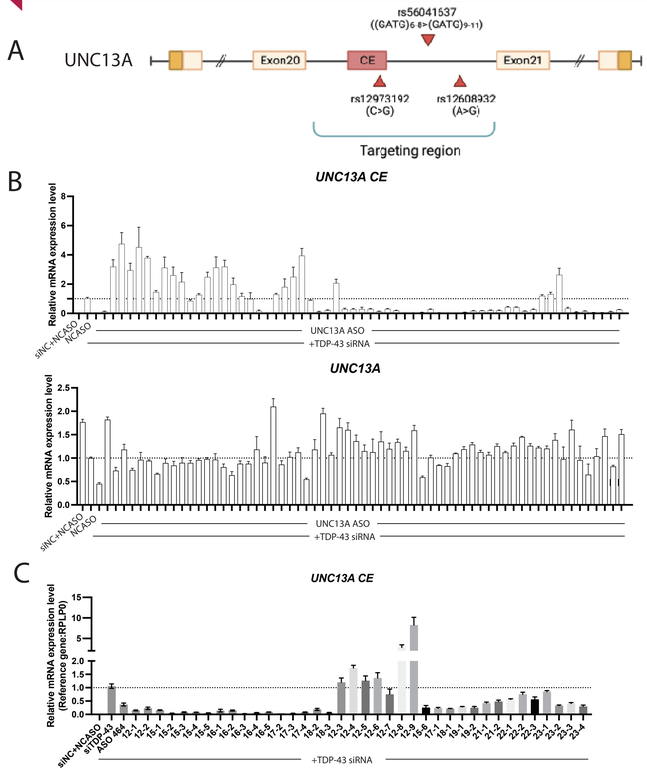


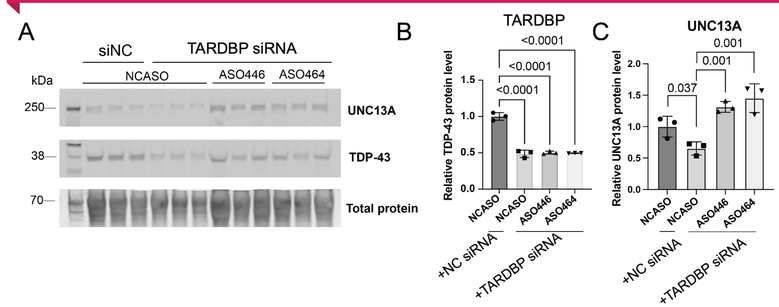
Illustration adapted from Brown et al 2022 Nature. Representative sashimi plots showing cryptic exon(CE) inclusions between exon 20 and exon 21 upong TDP-43 knockdown. TDP-43 binding region is marked in green. The location of the two SNPs(rs 12973192 and rs12608932) are marked in red.

Identification of UNC13A CE skipping ASOs



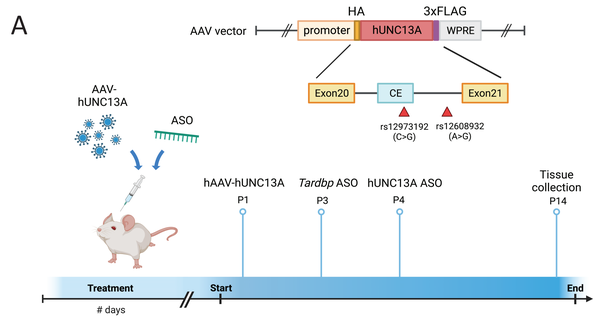
(A)Schematic showing the ASO targeting regions in respect to the location of the cryptic exon and ALS/FTD associated SNPs in UNC13A. (B)Hundreds of 20-mer ASOs were designed for the initial screen to identify the regions of interest by examining the CE including transcript and the normal transript by qPCR. Part of the screening results are shown here as representative data. fALS/FTD patient iPSC-derived cortical neuron were transfected with TDP-43 siRNA and UNC13A ASO. (C)The top candidate sequence was optimized more thouroughly by testing the variations of various length.

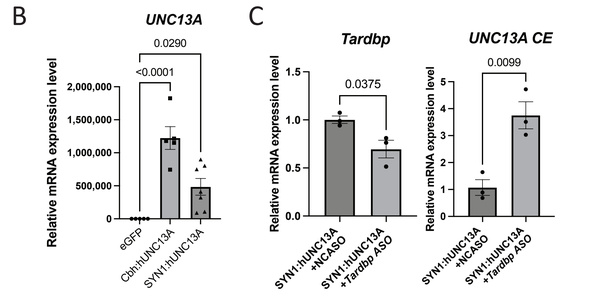
Lead candidates rescue UNC13A levels



(A-C) The protein expression of UNC13A was reduced by TDP-43 siRNA compared to negative control siRNA and the effect can be rescued by lead UNC13A ASOs (B) The level of TDP-43 protein was reduced significantly by TDP-43 siRNA (siTDP-43). (C) The UNC13A protein level was significantly reduced by TDP-43 KD and was rescued by lead ASO treatment. One-way ANOVA was performed for statistical significance.

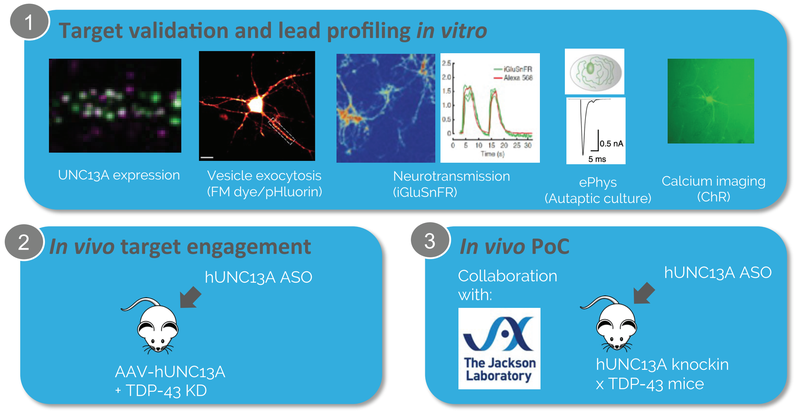
In vivo target engagement model





(A)Illustration of AAV vector design and experiment timeline for model generation (B) AAVs with Cbh or SYN1 promoter driven hUNC13A expressed significantly higher expression of hUNC13A than the eGFP control. (C) Tardbp knockdown and subsequent cryptic exon inclusion was detected in the model. Brain tissue was used for qPCR analysis. One-way ANOVA.

In vivo and in vitro functional readouts



Summary

We identified *UNC13A* cryptic exon suppressing antisense oligonucleotides using human iPSC-derived neurons with TDP-43 suppression. The top candidates can lower the cryptic exon inclusion rate and restore the expression of UNC13A at both the RNA and protein level. We also establish an *in vivo* target engagement model that can be used to assess in vivo potency of the lead candidates. The development candidate will be selected by using the functional and target engagement assays. The efficacy of the therapeutics will be test in a fully humanized mouse developed in collaboration with the Jackson Lab. This project will, for the first time, determine the impact of *UNC13A* CE inclusion on synaptic function and neurotransmission. Suppressing the *UNC13A* CE inclusion is a novel therapeutic approach that has a high probability of modifying disease progression for diverse forms of ALS.