Overexpressing a protective variant while simultaneously lowering toxic APOE as potential treatment for Alzheimer Disease

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BACKGROUND

Alzheimer's Disease (AD) is a multifactorial neurodegenerative disorder and the most common form of dementia in people over 65 years (~50 million), manifesting as progressive loss of memory and cognitive function. The strongest genetic risk factor for late onset AD (LOAD) is a variant of the gene encoding Apolipoprotein E (APOE).

The E4 variant is present in 45-60% of all AD cases[1] and is associated with increased risk and decreased age of onset of AD, whereas E2 is associated with reduced risk and increased age of onset[2]. The potential of protective variants was shown in preclinical studies in humanized (h) APOE-AD animal models in which a reduction of hAPOE4 as well as expression of hAPOE2 could prevent neurodegeneration at various stages of AD pathology.

OBJECTIVES

uniQure is developing an AAV gene therapy product to treat patients with LOAD. Our approach is to silence toxic APOE variants using miQURE® and simultaneously overexpress a protective APOE variant.

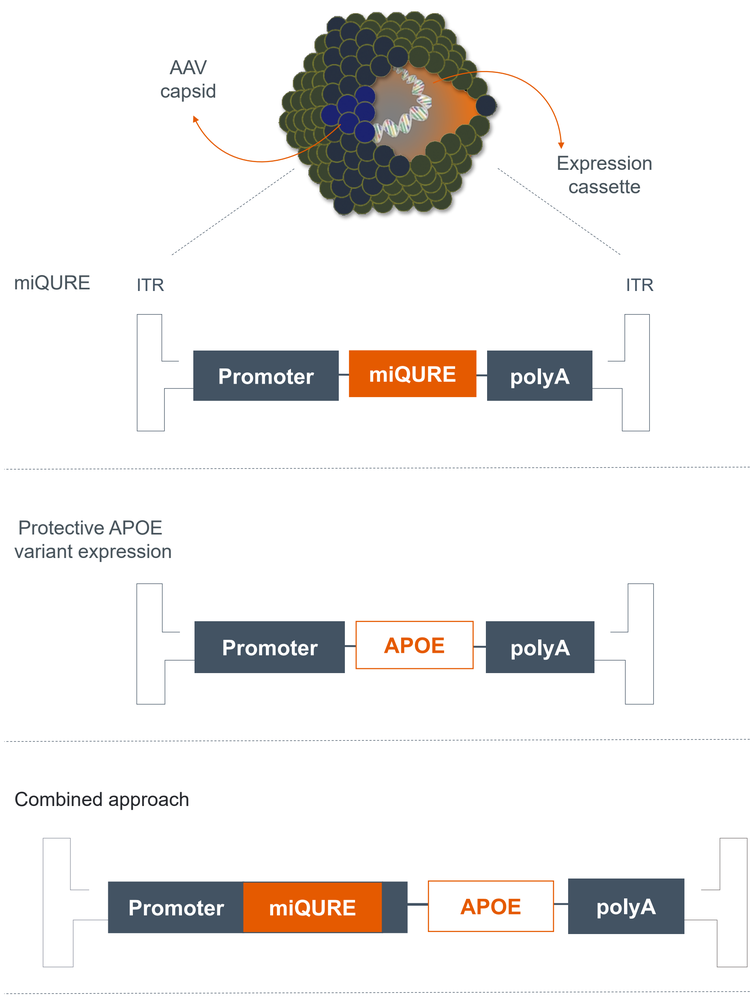

Fig 1 Visualisation of the miQURE® strategy (upper panel), protective APOE variant expression strategy (middle panel), and the combined approach (bottom panel). miQUREs® are processed by endogenous cellular machinery to mature guide miRNAs (miAPOE), which selectively binds and degrades hAPOE mRNA.

METHODS

hAPOE4-TR mouse study

AAV vectors were tested in hAPOE4-TR mice expressing human APOE4 under the control of the murine Apoe regulatory sequences.

- Mice received bilateral intrastriatal injections at three months of age at a mid and/or high dose. At 2 months post-injection, animals were sacrificed.
- Vector genome copies, APOE4 mRNA expression, mature miAPOE copies were determined by (RT)-qPCR, APOE4 protein was quantified by MSD.

Designs and *in vitro* screenings

- Various protective APOE variant constructs were tested for transgene expression and secretion in HEK293T and U-118 MG cells upon transfection.
- Selected APOE variants and miAPOE candidates were combined and tested for APOE overexpression and their knockdown efficiency on luciferase reporter.

RESULTS

Efficient APOE4 lowering *in vivo* upon AAV5-miAPOE delivery in the brain

Vector genome copies, miAPOE, APOE4 mRNA copies and protein levels were analyzed in the brain of hAPOE4-TR mice two months post-treatment (**Figure 2A**).

- Efficient transduction in striatum (**2B**)
- Substantial levels of mature miRNA in striatum (**2C**)
- Up to 85% silencing of APOE4 mRNA *in vivo* in striatum (**2D**)
- Up to 60% APOE4 protein reduction in the cortex (**2E**)

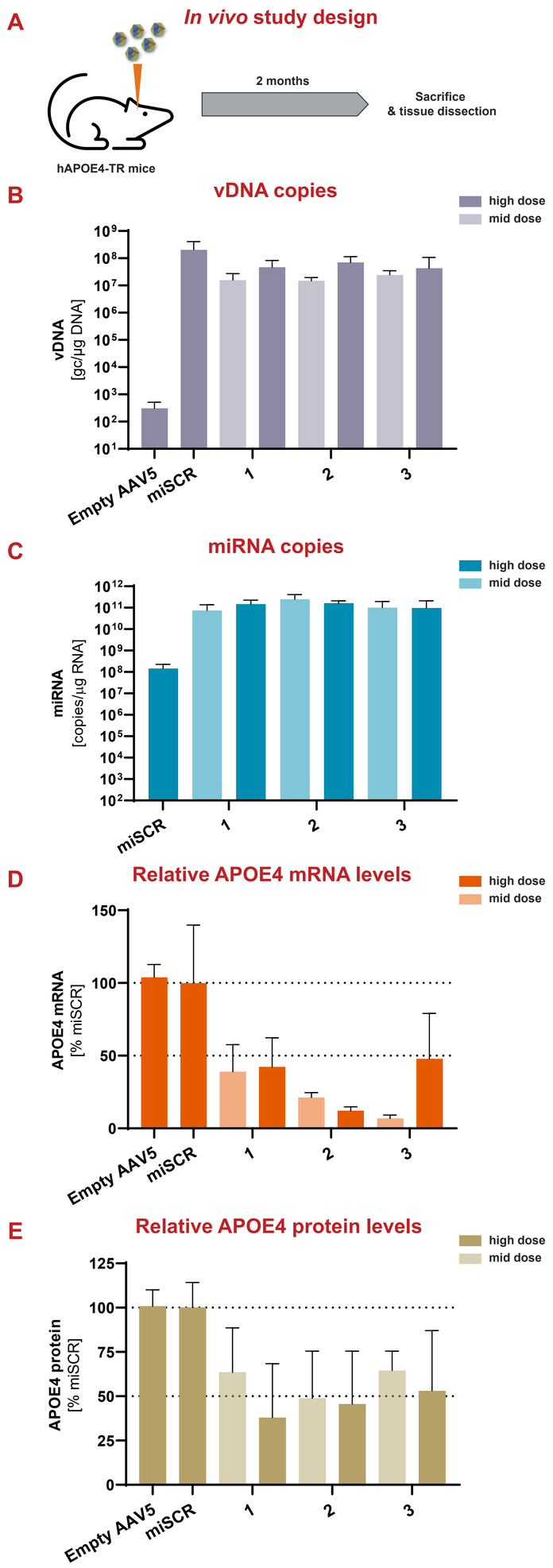

Fig 2 Empty AAV5, AAV5-miSCR and AAV5-miAPOE 1-3 were injected in hAPOE4-TR mice. *(A) Schematic overview of the in vivo study setup. (B) vDNA copies and (C) miAPOE copy numbers in striatum. (D) APOE4 mRNA in the striatum and (E) APOE4 protein in the cortex relative to miSCR.*

APOE variants are expressed *in vitro*

Several protective (wild-type or codon-optimized) APOE variants were tested for protein level expression *in vitro*.

- APOE protein expression could be detected in the supernatant of transfected HEK293T (**Figure 3A**) and U-118 MG astrocytoma cells (**3B**).

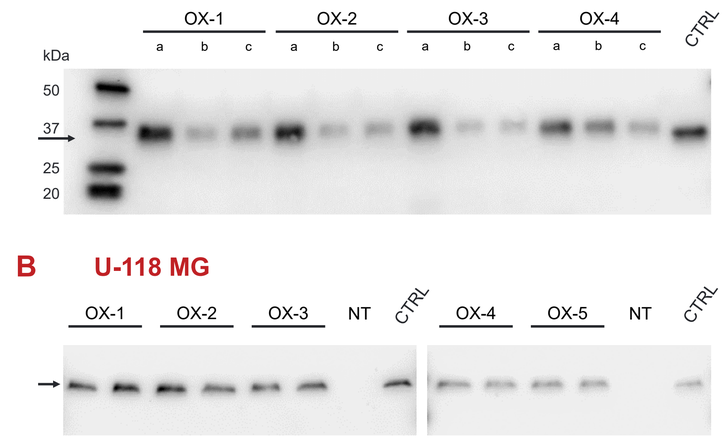

Fig 3 APOE variant expression. *APOE expression in the supernatant of transfected cells was detected by Western blot. OX1-5; encoded different APOE variants. a-c; codon adapted APOE variant expression constructs. CTRL; positive control (recombinant human APOE). NT; non-transfected.*

Combined approach constructs silence APOE4 reporter and express APOE protein *in vitro* .

A selection of combined constructs were tested in HEK293T cells for (i) knockdown efficiency in reporter assays and (ii) ability to overexpress and secrete APOE.

- APOE4 luciferase reporter silencing is comparable between single miAPOE and combined approach (**Figure 4A**).
- APOE expression was detected in cells transfected with all combined constructs (**4B**).

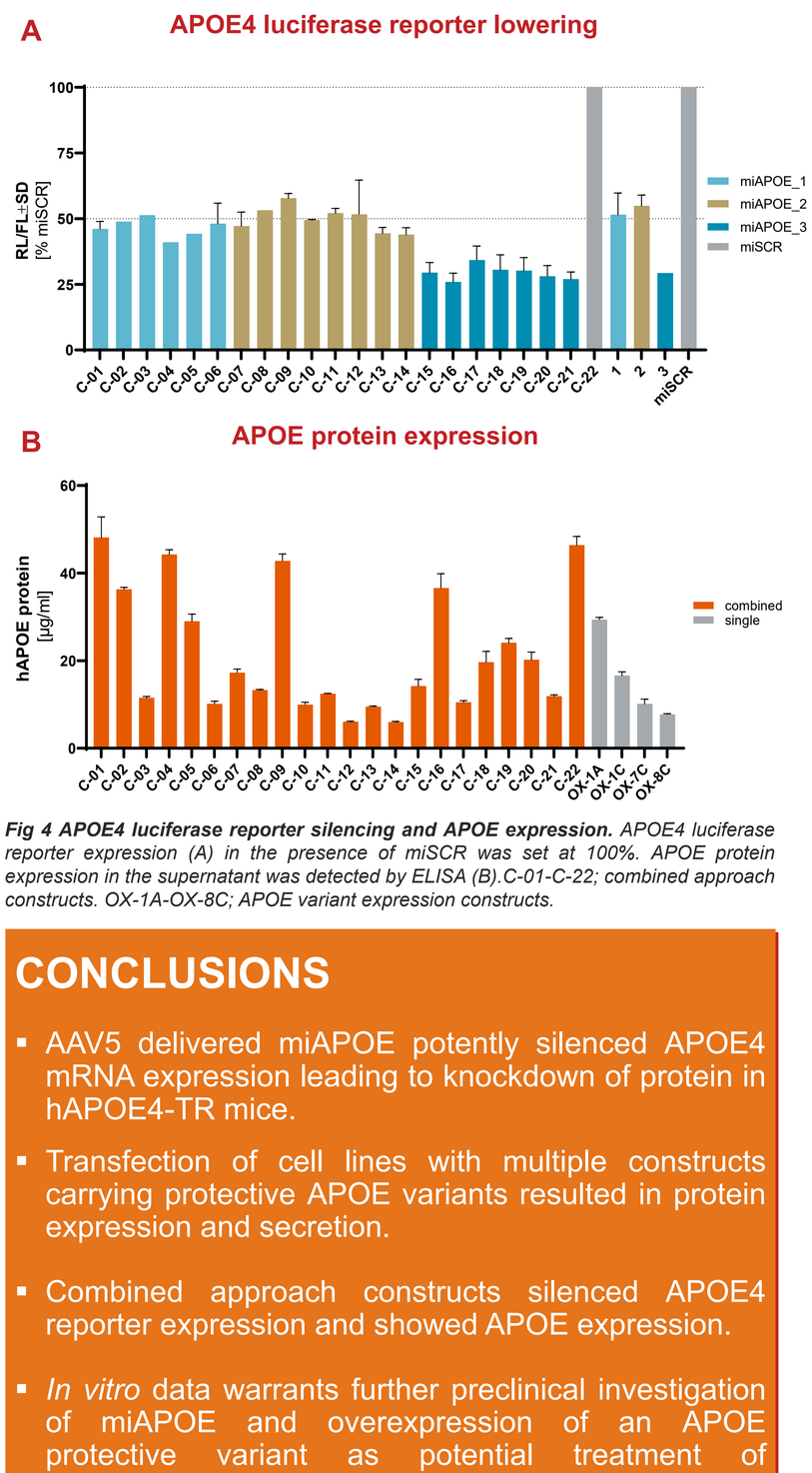

Fig 4 APOE4 luciferase reporter silencing and APOE expression. *APOE4 luciferase reporter expression (A) in the presence of miSCR was set at 100%. APOE protein expression in the supernatant was detected by ELISA (B).C-01-C-22; combined approach constructs. OX-1A-OX-8C; APOE variant expression constructs.*

CONCLUSIONS

- AAV5 delivered miAPOE potently silenced APOE4 mRNA expression leading to knockdown of protein in hAPOE4-TR mice.
- Transfection of cell lines with multiple constructs carrying protective APOE variants resulted in protein expression and secretion.
- Combined approach constructs silenced APOE4 reporter expression and showed APOE expression.
- *In vitro* data warrants further preclinical investigation of miAPOE and overexpression of an APOE protective variant as potential treatment of Alzheimer's disease.

ACKNOWLEDGMENTS

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REFERENCES

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