A CAG repeat-targeting artificial miRNA lowers the mutant huntingtin level in the YAC128 model of Huntington's disease

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Background: Among many proposed therapeutic strategies for Huntington's disease (HD), allele-selective therapies are the most desirable and the most challenging at the same time. We have previously demonstrated that RNA interference (RNAi) tools that target CAG repeats selectively reduced the mutant huntingtin level in cellular models of HD.

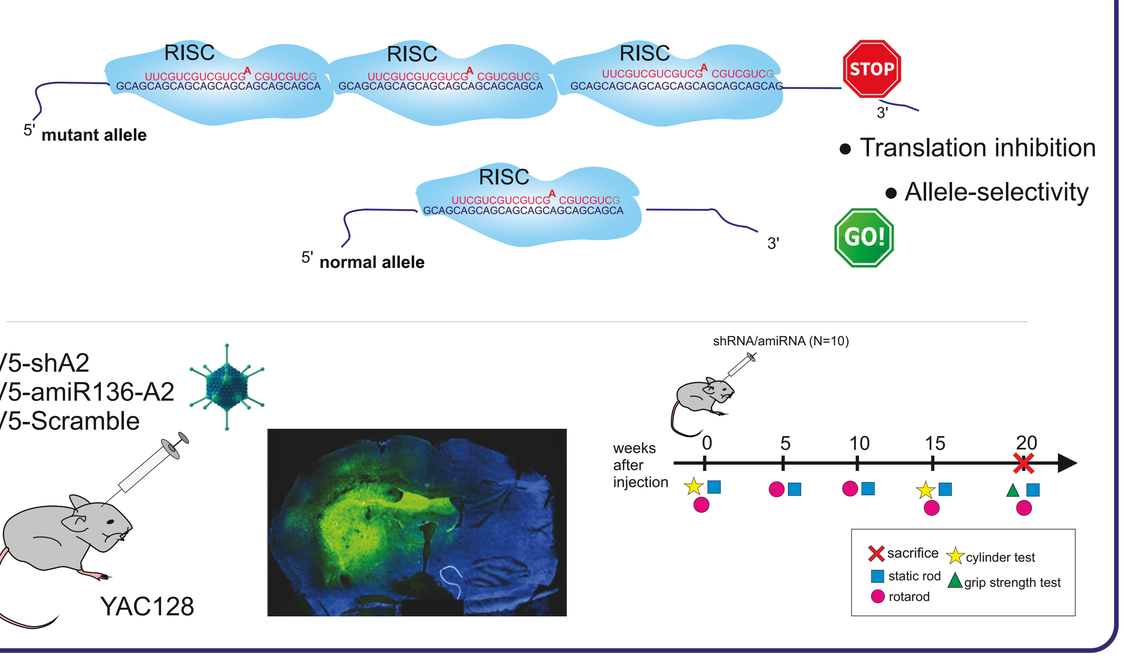
Aim: The purpose of this study was to test efficacy, selectivity and safety of two vector-based RNAi triggers in an animal model of HD.

Methods: CAG repeat-targeting short hairpin RNA (shRNA) and artificial miRNA (amiRNA) were delivered to brains of YAC128 mouse model in two doses via intrastriatal injections of AAV5 vectors. Vector genome copies, protein and transcript levels in the striatum, hippocampus and cortex were analyzed four months post injection. Behavioral tests were performed every five weeks post injection. Activation of toxicity markers and protein aggregates were analyzed by immunohistochemical staining of brain tissues.

Results: Molecular tests demonstrated that both shRNA and amiRNA reduced the level of mutant huntingtin to 50% without an influence on endogenous mouse huntingtin. We observed concentration-dependent reduction of HTT aggregates in the striatum and an improvement of motor performance using a static rod test. Expression of mutant huntingtin has previously been shown to increase organ weight. Interestingly, a treatment with amiRNA reduced the spleen weight to values characteristic of healthy mice (WT). In contrast to shRNA, amiRNA was well tolerated and did not reveal any signs of toxicity during the course of the experiment.

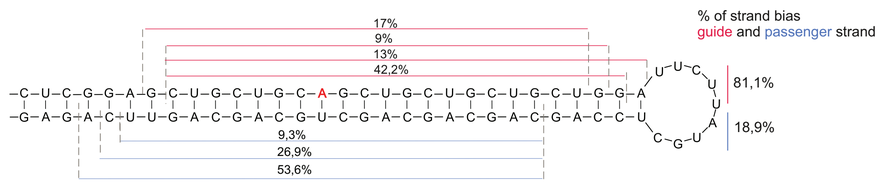
Conclusions: We confirmed that vector-based RNAi molecules targeting CAG tracts can be used to lower mutant huntingtin levels in vivo in an allele-selective manner. The amiRNA molecule has been shown to be effective, selective and safe. Therefore, this strategy could make an original and valuable contribution to currently used therapeutic approaches for HD.

CAG repeat-targeting strategy

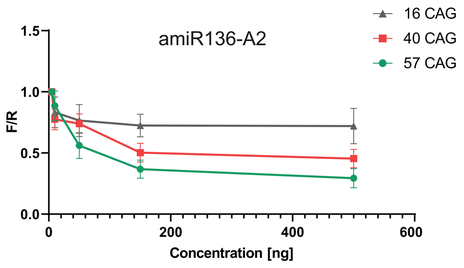


Design and characteristics of CAG repeat-targeting artificial miRNA

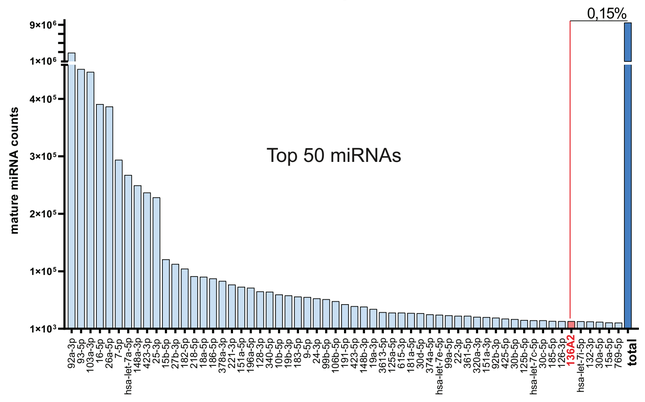
amiR136-A2 processing



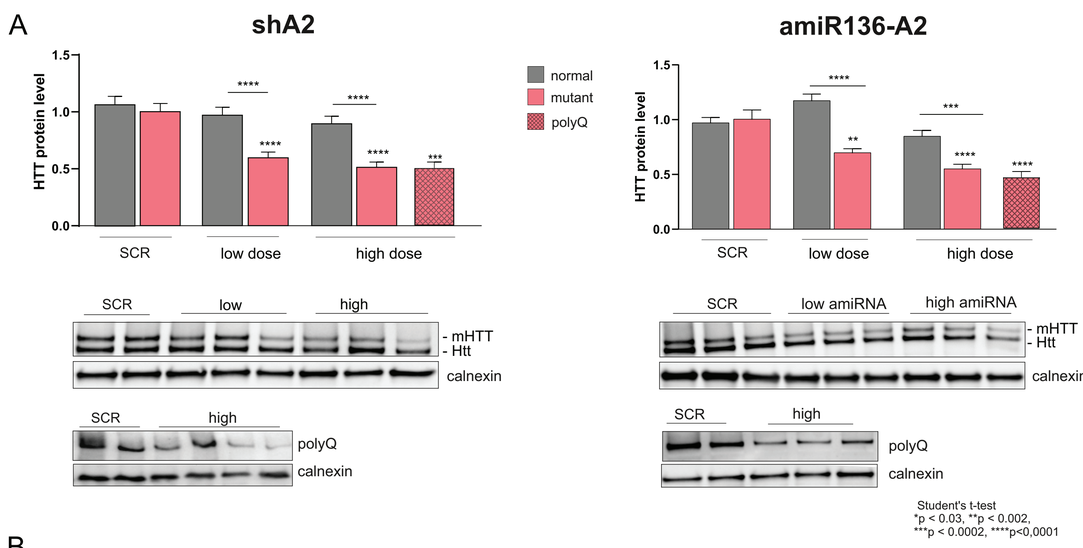
CAG length-dependent reduction of HTT level (Luc reporter system)



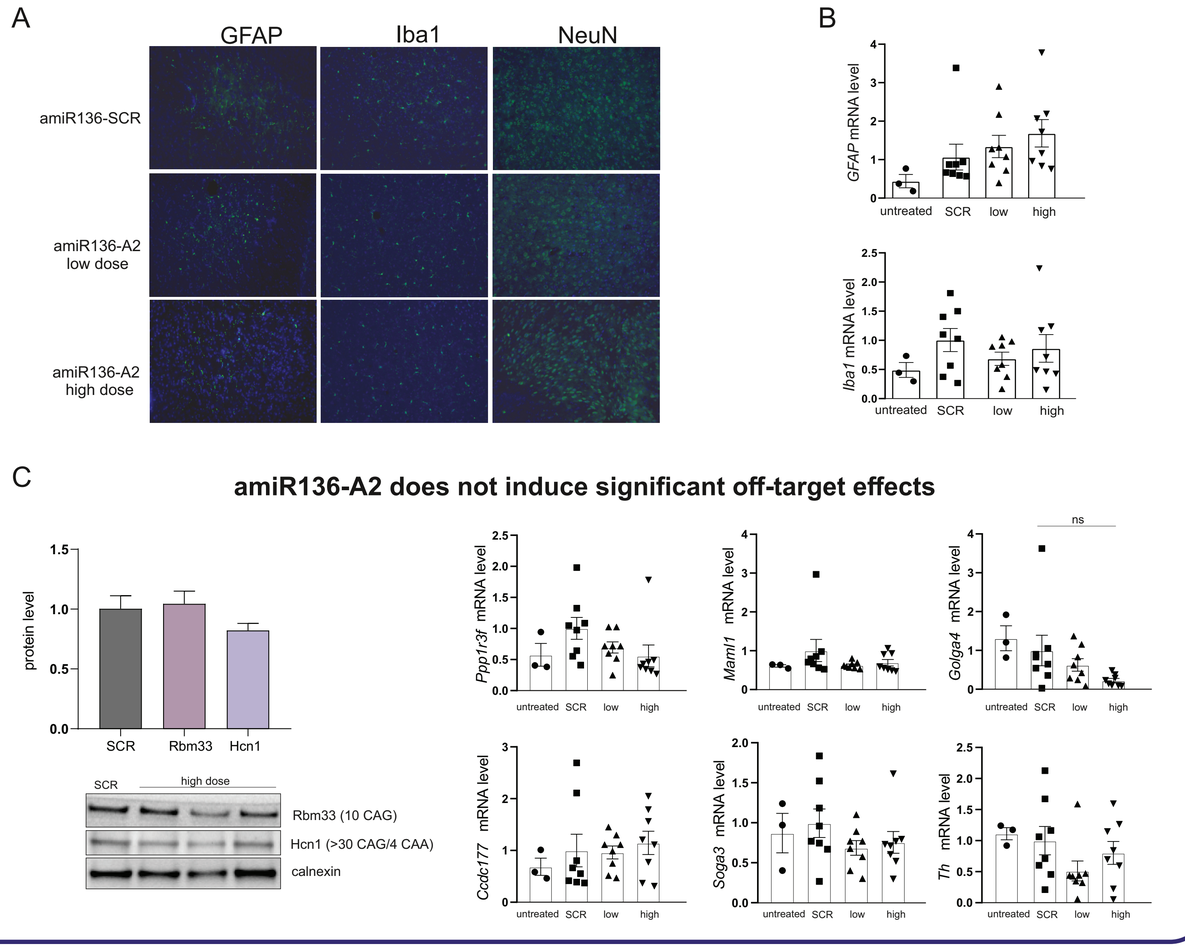
amiR136-A2 expression level



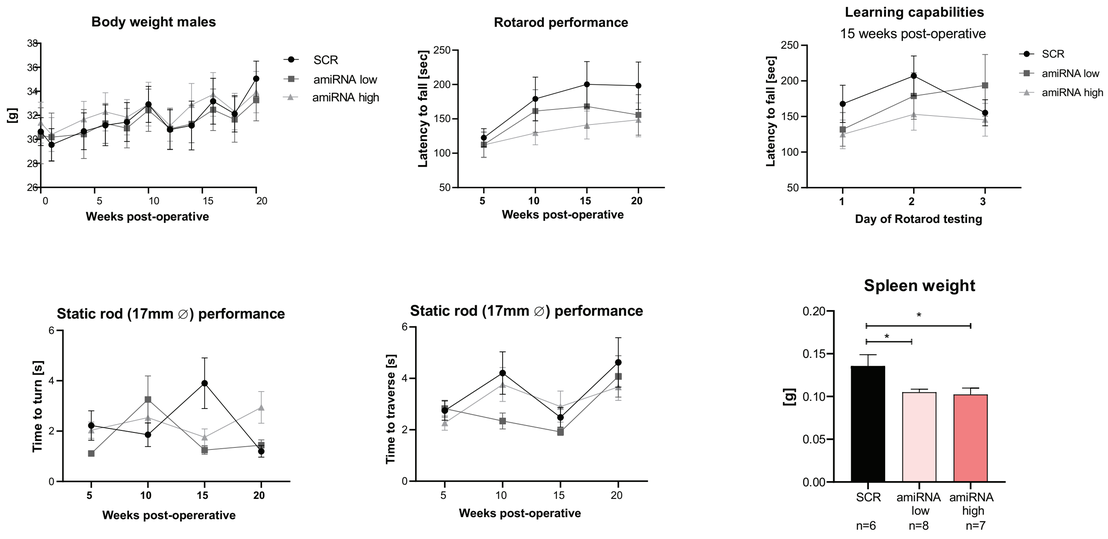
shA2 and amiR136-A2 lower mHTT level in vivo in an allele-selective manner



amiR136-A2 is well tolerated up to 20 weeks post-injection



amiR136-A2 improves some motor and cognitive deficits



Conclusions

- amiRNA **reduces mHTT** protein level *in vivo*
- HTT reduction is **allele-selective**
- amiRNA is **not toxic** (up to 20 weeks post injection)
- amiRNA **reduces HTT aggregates** in the striatum
- amiRNA treatment **influences the weight of the spleen** (back to normal);
- **improved** confidence of walking and turning on a static rod

Acknowledgments

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