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Gene Silencing Approach for an orphan GNAO1-related neurodevelopmental disorder

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GNAO1 DISORDER

GNAO1-related neurodevelopmental disorder is a fatal ultra-rare genetic disease:

- Characterized by early-onset epileptic encephalopathy and/or dyskinetic movement disorders
- Caused by heterozygous *de novo* mutations in *GNAO1* gene, that is highly expressed in the brain and plays role in neuronal signaling [1]
- <100 registered patients worldwide

Currently no effective treatment is available for this pathology.

OBJECTIVES

Our aim at Marlin Biotech is to find gene therapy cure for GNAO1 disorder. Considering autosomal dominant condition of this disease, we suggest a strategy of **allele-specific suppression** of GNAO1 transcript:

- Allows selective inhibition of abnormal GNAO1 product synthesis in brain tissues
- Leaves functional GNAO1 gene unaffected

METHODS

To test gene therapy approach *in vitro*, we developed an assay with expression of exogenous wild type or mutant (c.607 G>A) GNAO1 variants in cultured cells.

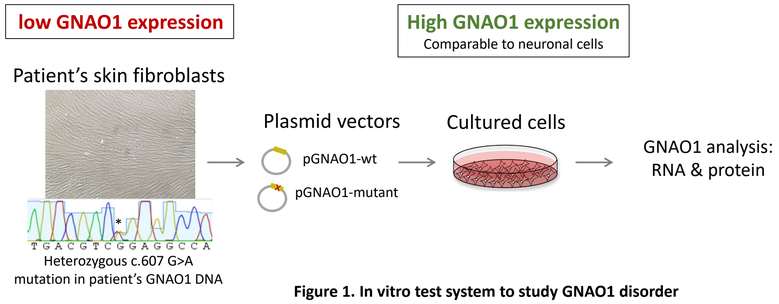

Figure 1. In vitro test system to study GNAO1 disorder

RESULTS

We screened synthetic siRNA duplexes that target mutation site in GNAO1 RNA and downregulate expression of mutated gene through RNA interference (RNAi) pathway (Fig 2A and 2B). Our data demonstrates that at least one RNAi effector reduces accumulation of mutant GNAO1 transcripts in allele-specific manner (Fig 2C). These results were confirmed at RNA and protein levels in heterozygous assay where both wild type and mutant GNAO1 variants were introduced into cells simultaneously in 1:1 ratio to mimic heterozygous condition of the patients (data not shown).

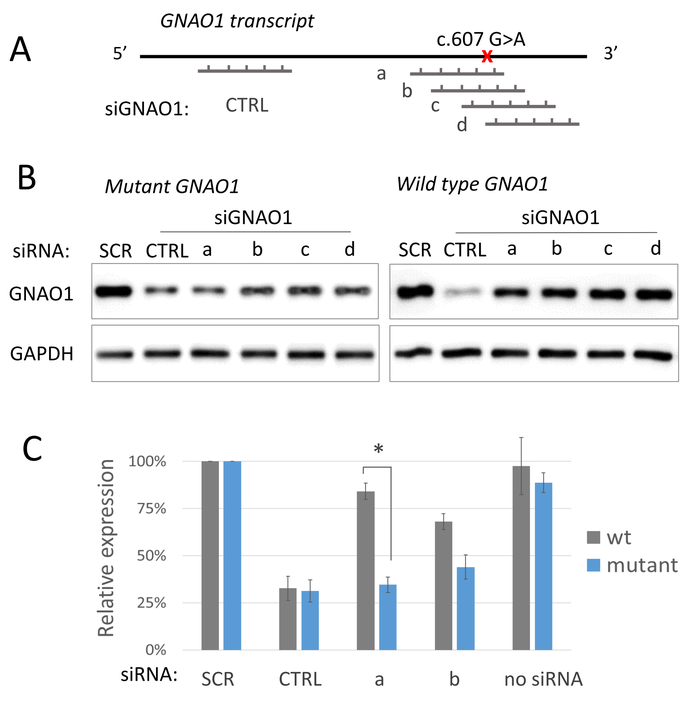

Figure 2. Allele-specific downregulation of mutant GNAO1 transcript by siRNA in cultured cells
Position of siRNA target sequences in GNAO1 transcript is schematically shown (**A**). siRNAs were screened in HEK293T cells expressing exogenous copies of mutant or wild type (wt) GNAO1. Suppression of GNAO1 variants at the protein (**B**) and RNA (**C**) levels was assessed by Western blotting and RT-qPCR, respectively. Scrambled siRNA (SCR) served as a negative control for GNAO1 downregulation and was set at 100%. siGNAO1-CTRL served as positive control and reduced the expression of both GNAO1 variants to 20-30%. siGNAO1a-d were designed to c.607 G>A mutation site; siGNAO1a selectively suppressed mutant variant while native GNAO1 was only moderately affected. Data is an average ± SD, n=4, * p<0.001, one-way ANOVA.

CONCLUSIONS

Our pilot experiments demonstrate the potential of allele-specific silencing approach for gene therapy of GNAO1-related neurodevelopmental disorder. Identified target region in GNAO1 can be used for further development of gene suppression technologies.

FUTURE PROSPECTIVE

Our next step is designing RNAi-based therapeutics for GNAO1 disorder that are compatible with delivery via adeno-associated virus (AAV) vectors to brain tissues. To validate beneficial effect of AAV-RNAi technology *in vivo*, we are also developing humanized mouse model of GNAO1 disorder using CRISPR/Cas9 technology.

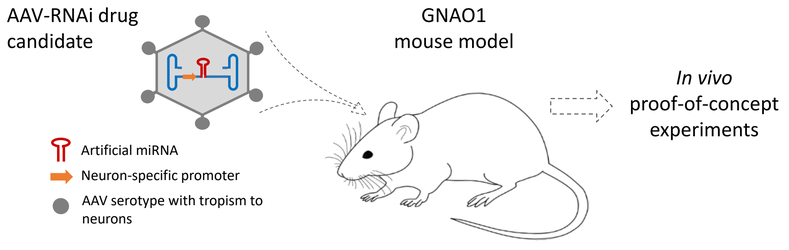

Figure 3. Proposed development of AAV-based gene therapy for GNAO1 disorder and testing in a relevant mouse model

REFERENCES

[1] Nakamura et al. (2013). De Novo mutations in GNAO1, encoding a Gαo subunit of heterotrimeric G proteins, cause epileptic encephalopathy. *Am J Hum Genet.* 93(3):496-505.

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