Novel siRNA-based therapeutic approach for Megacystis Microcolon Intestinal Hypoperistalsis Syndrome (MMIHS)

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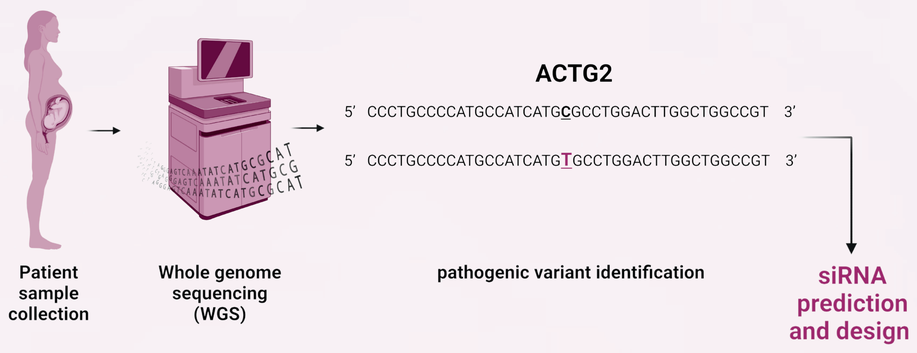
Introduction

MMIHS is a rare form of functional intestinal obstruction in the newborn characterized by megacystis, microcolon, and intestinal dysmotility. A classic feature of MMIHS is represented by *ACTG2* mutations that can be both inherited in an autosomal dominant manner or occur as de novo events during oogenesis, spermatogenesis, or early embryonic development. (1,2) *ACTG2* encodes γ-2 enteric actin and its mutations disrupt actin polymerization leading to impaired contraction of intestinal smooth muscle cells. (3) Even though survival has improvedin recent years, MMIHS is a condition that requires invasive palliative treatments. (2)

Objective

Our study proposes a therapeutic approach based on siRNAs as a novel treatment for MMIHS

Methods



Whole genome sequencing (WGS) was performed to identify mutations in *ACTG2* gene. DNA was isolated from chorionic tissue of a 13th week pregnant patient presenting an echography with alteration of gut and bladder of the newborn and sequenced on Illumina Novaseq6000. Variant analysis was performed using Variant Call Files (VCFs) computed with DRAGEN (Illumina) on the human reference genome GRCh37 and the pathogenic variant was identified with EXTENSA™ software. A Machine a (ML) software, fed with siRNA silencing information retrieved from experimental data, has been used as prediction tool for the identification of siRNAs with the most discriminatory power between ACTG2^{mutant} and ACTG2^{WT} mRNA. *In vitro* preclinical studies have been performed on HEK293 cells to evaluate the specific silencing of the ACTG2^{mutant} mRNA and to test the reduction of its expression in an efficient and highly specific manner.

Conclusions

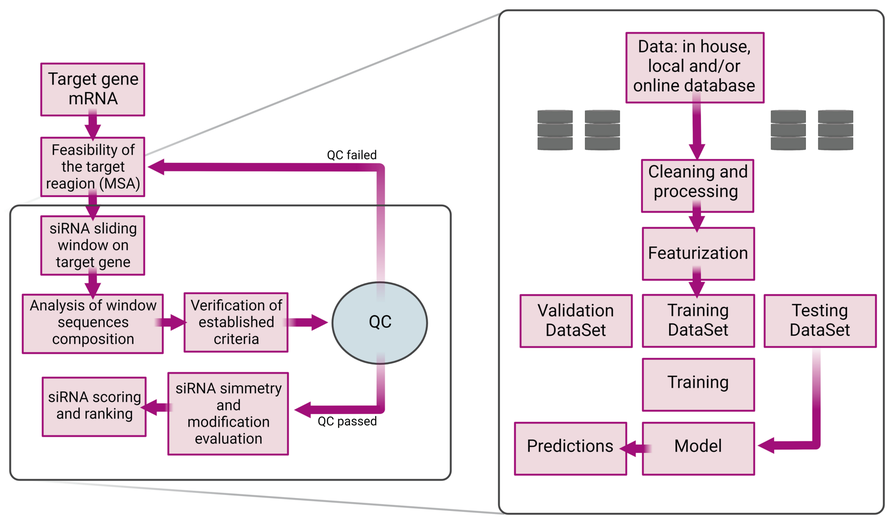
Our study identified siRNA sequences as good candidates for the development of a new therapeutic approach in MMIHS and underscore a translational impact for future strategy to treat the underlying cause of this disease.

References

1. *Gosemann JH, Puri P. Megacystis microcolon intestinal hypoperistalsis syndrome: systematic review of outcome. Pediatr Surg Int. 2011;27(10):1041-6.*
2. *Ambartsumyan L. Megacystis-Microcolon-Intestinal Hypoperistalsis Syndrome Overview. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Gripp KW, et al., editors. GeneReviews(®). Seattle (WA): University of Washington, Seattle Copyright © 1993-2022, University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.; 1993.*
3. *Halim D, Hofstra RM, Signorile L, Verdijk RM, van der Werf CS, Sribudiani Y, et al. ACTG2 variants impair actin polymerization in sporadic Megacystis Microcolon Intestinal Hypoperistalsis Syndrome. Hum Mol Genet. 2016;25(3):571-83*

Results

1. siRNAs sequence prediction and design



b

Mutation	siRNA name	N° mismatches vs WT	siRNA sequence	Efficacy vs WT	Efficacy vs mutant	Patent status
ACTG2^{R178C}	11A	1	3′ dTdT -ACGGUAGUAC**A**CGGACCUGAA 5′ 5′ UGCCAUCAUGUGCCUGGACUU -dTdT 3′	/	++	√
	11B	2	3′ dTdT -ACGGUAGUAC**A**CGGACCUGAA 5′ 5′ UGCCAUCAUGUGCCUGGACU***A*** -dTdT 3′	/	+	√
	11C	4	3′ dTdT- ACGGUA***C***UAC**A**CGGACCUGAA 5′ 5′ UGCCAU***G***AUGUGCCUGGACU***A*** -dTdT 3′	/	+++	√
	11D	3	3′ dTdT- ACGGU***U***GUAC**A**CGGACCUGAA 5′ 5′ UGCCAU***G***AUGUGCCUGGACUU -dTdT 3′	/	+	√
	11E	3	3′ dTdT- ACGGUA***C***UAC**A**CGGACCUGAA 5′ 5′ UGCCA***A***CAUGUGCCUGGACUU -dTdT 3′	/	+	√
	11F	1	3′ dTdT- ACGGUAGUAC**A**CGGA***G***CUGAA 5′ 5′ UGCCAUCAUGUGCCUCGACUU -dTdT 3′	++	+	√
	11G	1	3′ dTdT- ACGGUAGUAC**A**CGGA***G***CUGAA 5′ 5′ UGCCAUCAUGUGCCU***C***GACU***A*** -dTdT 3′	++	+	√
	18	1	3′ dTdT- UAC**A**CGGACCUGAACCGACCG 5′ 5′ AUGUGCCUGGACUUGGCUGGC -dTdT 3′	/	+	√

Fig 1. Prediction of ACTG2^{mutant}–specific siRNAs
A) *Software data workflow and analysis process. The software has the ability to release as output a list of sequences that discriminate between variants of the same gene given two alleles and a target SNP. The software is based on Machine Learning (ML) approach and it has been fed with siRNA silencing information retrieved from experimental data. The ML model with such data allowed us to set rules and parameters to score the sequences. The more the data inside the software the more the accuracy in spotting the right sequences.*
B) *Selected siRNAs with the corresponding proposed mutations, main parameters used by our software to select the appropriate siRNA sequences and, discriminatory power between ACTG2^{mutant} and ACTG2^{WT} mRNA sequence. Patent application have been filed for all the selected siRNAs (patent-pending status).*

2. HEK293 transfection for hACTG2^{WT} and hACTG2^{mutant} overexpression

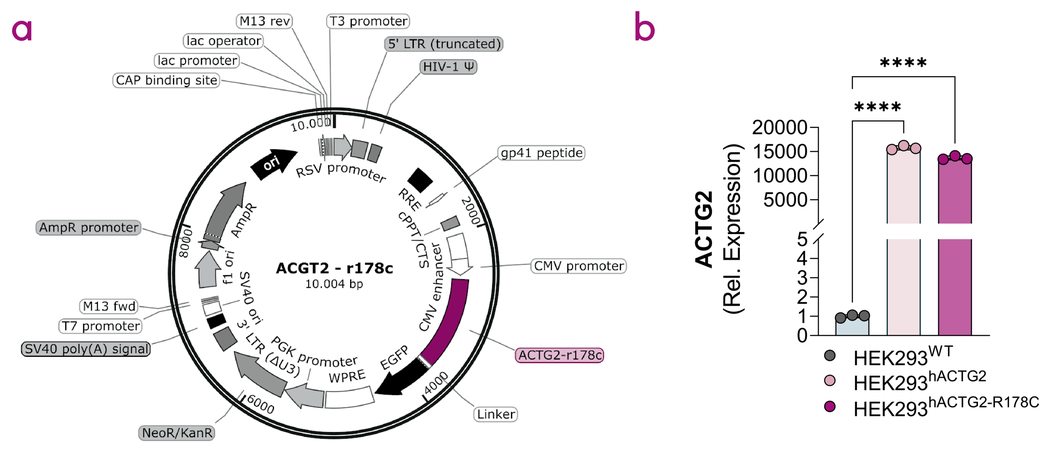


Fig. 2 Set up of HEK293 cell model
A) *Cartoon depicting the pEGFP vector carrying the constructs with the desired mutant ACTG2 sequence (Vector ID: VB211020-1174xrj) used in the study to transfect human HEK293 cells with lentiviruses particles for the generation of HEK293 stable cell lines for hACTG2/R178C overexpression.*
B) *HEK293 cells stably transfected with the pEGFP vector for the overexpression of hACTG2 and hACTG2-R178C. Expression of the ACTG2 gene was quantified by qRT-PCR on HEK293^{WT}, HEK293^{hAcTG2} and HEK293$^{hAcTG2/R178c}$. Data (mean +/- SD) represent the results of at list three independent in vitro experiments. Statistical analysis has been performed using one-way ANOVA with post-hoc Tukey's test.*

3. hACTG2^{mutant} silencing in transfected HEK293

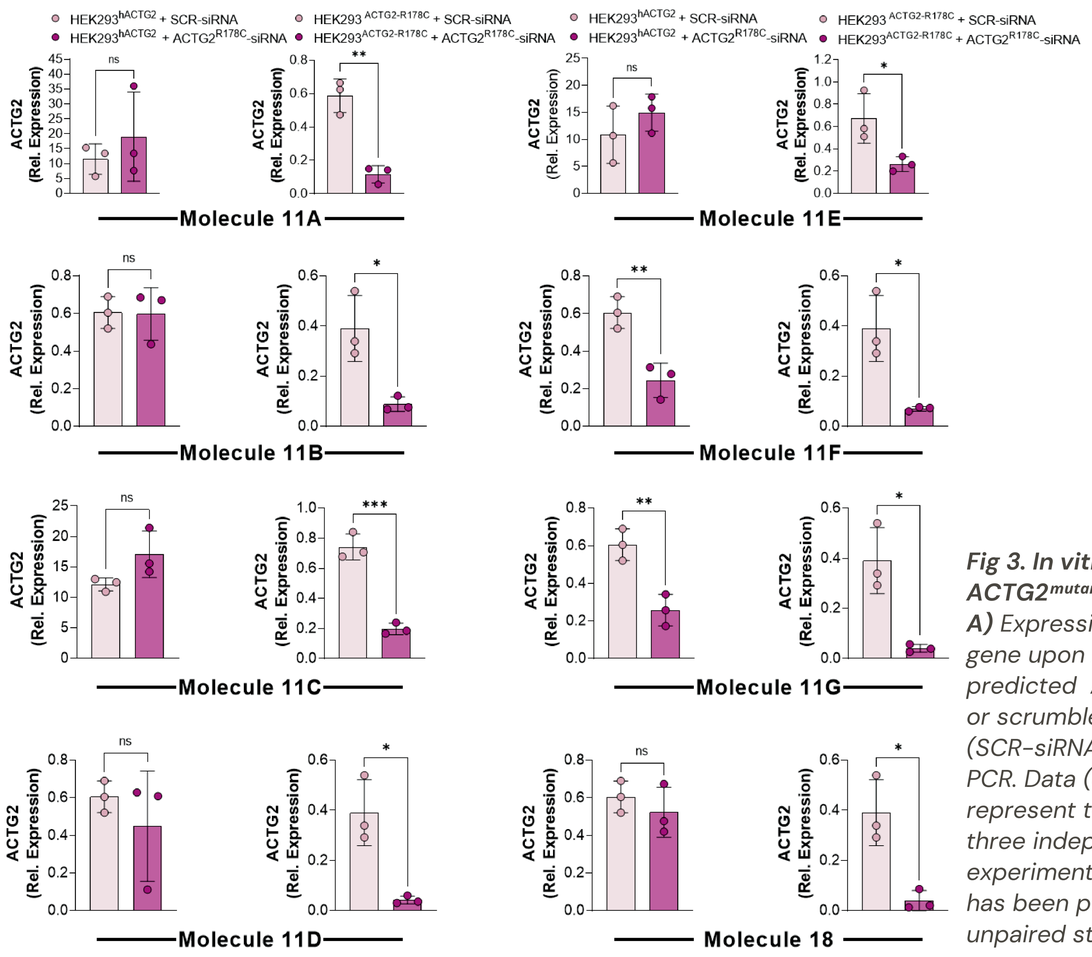


Fig 3. In vitro testing of ACTG2^{mutant}–specific siRNAs.
A) *Expression of the ACTG2 gene upon 24 h silencing with predicted ACTG2^{mutant} siRNAs or scrumble negative control (SCR-siRNA) quantified byqRT-PCR. Data (mean +/- SD) represent the results of at list three independent in vitro experiments. Statistical analysis has been performed using unpaired student t-test.*

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Authors declare no conflict of interests