Biochemical Loss-of-Function Data for *SIM1* Increases Probability of Clinical Weight Loss Response to the MC4R Agonist Setmelanotide

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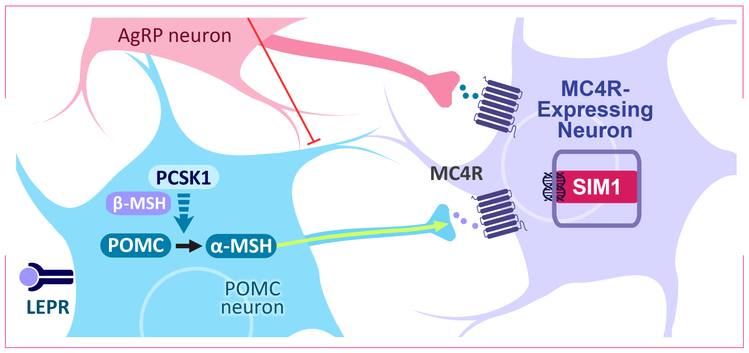
Introduction

- Single-minded homolog 1 (SIM1) is a transcription factor expressed by most neurons of the paraventricular nucleus of the hypothalamus (PVH) and is critical for their development (Figure 1)[1,2]
  - Within the PVH^{SIM1} neuron population, melanocortin-4 receptor (MC4R) neurons are key regulators of satiety and body weight[3-5]

Figure 1. SIM1 in the MC4R pathway.



LEPR, leptin receptor; MC4R, melanocortin-4 receptor; MSH, melanocyte-stimulating hormone; PCSK1, proprotein convertase subtilisin/kexin type 1; POMC, proopiomelanocortin; SIM1, single-minded homolog 1.

- Rare loss-of-function (LOF) variants in *SIM1* are associated with early-onset, severe obesity and hyperphagia[6,7]
- DAYBREAK (NCT04963231) was a 2-stage Phase 2 study designed to evaluate the efficacy of setmelanotide, an MC4R agonist, in individuals carrying a confirmed variant in ≥1 gene with strong or very strong relevance to the MC4R pathway[8]
- Setmelanotide has been shown to reduce weight and hunger in patients with certain MC4R pathway diseases caused by variants in genes central to this pathway[9-14]

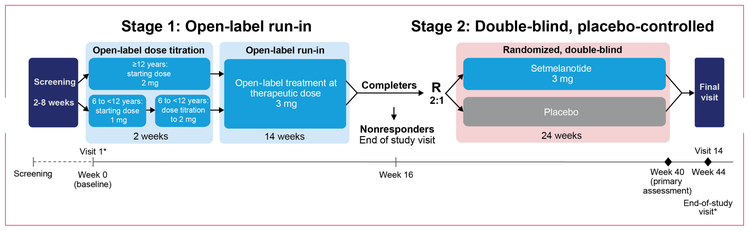
Objective

- The objective of this retrospective analysis was to test if experimentally derived biochemical data assessing the impact of missense variants on SIM1 function (American College of Medical Genetics [ACMG] criteria PS3) are predictive of response of an individual after 16 weeks of setmelanotide treatment

Methods

- DAYBREAK is a 2-stage, double-blind, placebo-controlled trial conducted at 37 sites across 8 countries (Figure 2)

Figure 2. DAYBREAK study design.



*Virtual visit. R, randomization.

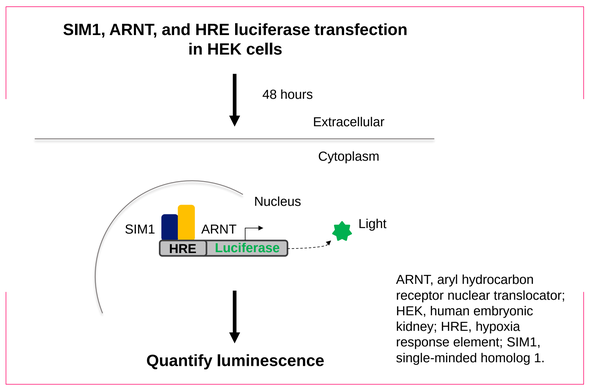
- Individuals aged 6 to 65 years with body mass index (BMI) ≥40 kg/m^2 (aged ≥18 years) or ≥97th percentile (aged ≥6 to <18 years) and hyperphagia who carried variants classified as uncertain significance (VUS), likely pathogenic, or pathogenic according to ACMG criteria in ≥1 of the 31 genes were eligible
- Individuals meeting prespecified weight loss criteria from baseline at the end of the 16-week, open-label run-in period (stage 1) were eligible to enter a 24-week, double-blind, randomized, placebo-controlled period (stage 2)
- The primary endpoint was the proportion of individuals by genotype who achieved a BMI reduction of ≥5% from baseline at the end of stage 1
  - The percent change in BMI from baseline to the end of stage 1 (Week 16) of individuals with *SIM1* variants was used in these analyses
  - An individual was classified as a responder if they met the primary endpoint (a BMI reduction of ≥5% from baseline at the end of stage 1)

Acknowledgments: This study was funded by Rhythm Pharmaceuticals, Inc. Editorial assistance was provided under the direction of the authors by MedThink SciCom and funded by Rhythm Pharmaceuticals, Inc.

Disclosures: PS, DK, JG, and ASG are employees of and own stock or stock options in Rhythm Pharmaceuticals, Inc. BPS was an employee of Rhythm Pharmaceuticals, Inc. at the time of contribution to the study. GO has received study funding and compensation for speaking engagements from Rhythm Pharmaceuticals, Inc. MR has received honoraries for lectures from and been an investigator in clinical trials for Rhythm Pharmaceuticals, Inc. ISF has received compensation for consulting, advisory board participation, and speaking engagements from Rhythm Pharmaceuticals, Inc. and Novartis.

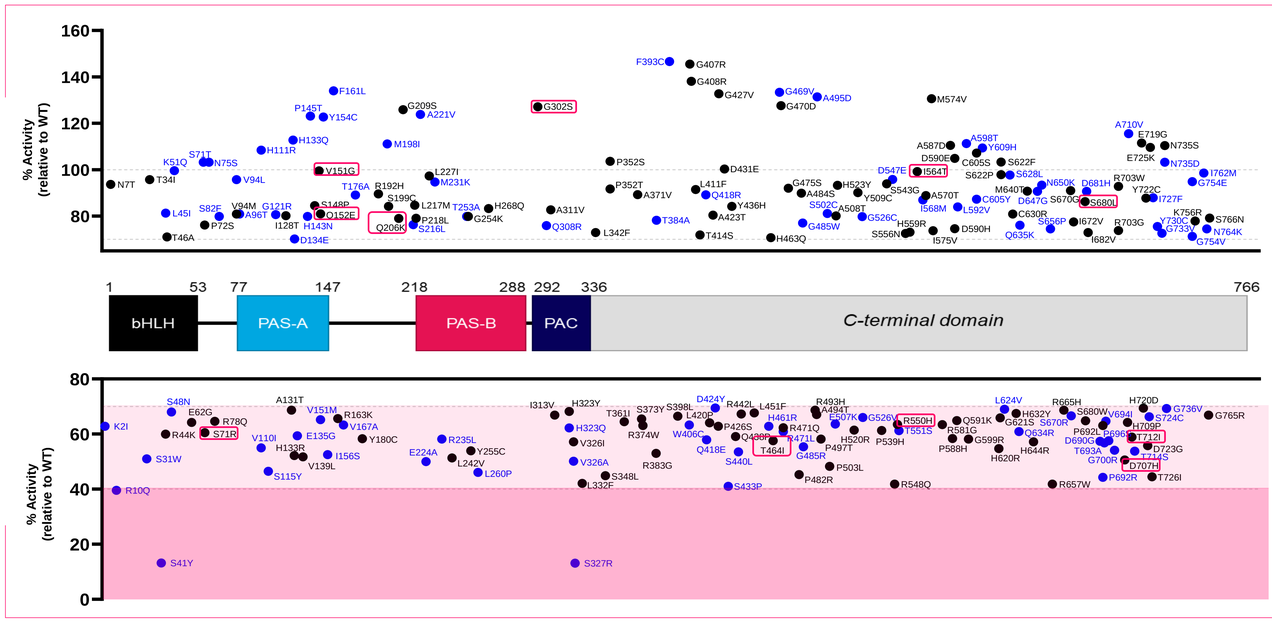
- In a prior study, the functional impact of 213 of 243 unique *SIM1* missense variants, identified from ~50,000 deidentified individuals with early-onset obesity who were sequenced through a Rhythm-sponsored genetic testing program, was assessed using a previously reported luciferase reporter gene assay[1,15]
  - SIM1 is a transcription factor that requires heterodimerization with aryl hydrocarbon receptor nuclear translocator (ARNT) to mediate the transcription of hypoxia response element (HRE)-regulated genes
  - Briefly, SIM1 function was assayed by cotransfecting HEK293 cells with wild-type or variant *SIM1* constructs, an ARNT plasmid, and an HRE-luciferase reporter gene followed by luciferase quantification after 48 hours (Figure 3)
  - Results of the assay were validated against previously published data and expressed as a percentage of wild-type function (Figure 4)

Figure 3. In vitro SIM1 functional assay.



ARNT, aryl hydrocarbon receptor nuclear translocator; HEK, human embryonic kidney; HRE, hypoxia response element; SIM1, single-minded homolog 1.

Figure 4. Results of the functional characterization of 213 *SIM1* missense variants.



Blue dots represent variants that were observed only in the Rhythm database (at the time the assay was performed). The percent activity of the WT protein is plotted on the y-axis, with variants plotted on the bottom panel showing some loss of function compared with WT. Of two VUS-SP variants with functional data, one was predicted to be LOF while the other was a splice variant and was not assayed. Variants in pink boxes represent those in ≥1 of the 20 patients enrolled in DAYBREAK with *SIM1* variants. bHLH, basic helix-loop-helix; LOF, loss-of-function; mLOF, moderate LOF; PAC, proline-rich, arabinogalactan proteins, conserved cysteines; PAS, Per-ARNT-Sim; SIM1, single-minded homolog 1; sLOF, severe LOF; SP, suspected pathogenic; VUS, variant of uncertain significance; WT, wild-type.

Results

- Twenty individuals carrying one of 17 unique, rare *SIM1* variants were enrolled in the DAYBREAK trial (Table 1)
  - Of these 17 *SIM1* variants, 15 were classified as VUS and 2 as VUS-suspected pathogenic according to ACMG criteria
- In vitro functional data, from our assay or previously published studies, were available for 13 of the *SIM1* variants carried by 16 individuals
  - Seven of 13 variants were predicted to retain wild-type activity
  - Six of 13 were predicted to result in moderate or severe LOF

Correlating the Functional Data With Setmelanotide Response From the DAYBREAK Trial

- Four of 5 responders in the trial carried variants that had been functionally characterized; all 4 responders carried variants that were predicted LOF via published assays or the Rhythm functional assay (Table)
  - All 7 individuals carrying wild-type–like variants either discontinued the trial or were nonresponders
  - Incorporating the functional assay data to predict pathogenicity would therefore increase the response rate for *SIM1* variant carriers in DAYBREAK from 25% to 44%
    - Of the 5 nonresponders who carried predicted LOF variants, 2 carried p.Asp707His, which has been reported to show variable penetrance
  - Similar results were obtained in an analysis of the 16 individuals who completed the trial; ad hoc analysis of the trial on the basis of functional predictions increased the response rate from 31% to 50%
    - Including only functionally defined variants, the mean change in percent BMI in the responder group was –11.3% (SD, 4.6%; n=4) versus –0.76 (SD 2.4; n=9) in the nonresponder group
    - Two of the 4 nonresponders with LOF variants carried the variable-penetrance p.Asp707His allele

Table. Response to setmelanotide treatment in individuals with *SIM1* variants.

	% BMI at 16 weeks	Variant	ACMG classification	Published functional assays[1,6]	Rhythm functional assay
Responders	-15.1	p.Asp707His	VUS	Moderately damaging[1]	Moderate LOF
	-14.5	p.Thr712Ile	VUS	Severely damaging[1]	Moderate LOF
	-10.4	p.Ser71Arg	VUS	Severely damaging[1]	Moderate LOF
	-5.8	p.Glu14Asp	VUS	NA	NA
	-5.1	p.Leu238Arg	VUS-SP	Severely damaging[1]	NA
Nonresponders	-2.9	p.Pro352Ser	VUS	NA	WT
	-1.2	p.Gln152Glu	VUS	Mild effect[6]	WT
	0.4	p.Ser680Leu	VUS	Uncertain[1]	WT
	0.6	p.Ile564Thr	VUS	NA	WT
	3.6	p.Gly302Ser	VUS	NA	WT
	-3.7	p.Thr712Ile	VUS	Severely damaging[1]	Moderate LOF
	-3.3	p.Asp707His	VUS	Moderately damaging[1]	Moderate LOF
	-1.5	p.Arg550His	VUS	Severely damaging[1]	Moderate LOF
	1.1	p.Asp707His	VUS	Moderately damaging[1]	Moderate LOF
	-2.8	p.Leu479Pro	VUS	NA	NA
	-3.6	GT donor	VUS-SP	NA	NA
Discontinued trial	NA	p.Gln206Lys	VUS	NA	WT
	NA	p.Val151Gly	VUS	NA	WT
	NA	p.Thr464Ile	VUS	NA	Moderate LOF
	NA	p.Met513Val	VUS	NA	NA

ACMG, American College of Medical Genetics; BMI, body mass index; LOF, loss of function; NA, not available; SP, suspected pathogenic; VUS, variant of uncertain significance; WT, wild-type.

Conclusions

- These data highlight the importance of experimentally derived functional data for informing the clinical significance of *SIM1* variants in patients living with severe early-onset obesity
- Confirmed *SIM1* LOF variants are more likely to impact MC4R pathway function and contribute to clinical presentation
- Loss of normal MC4R pathway activity due to confirmed *SIM1* LOF variants increases the probability of response to the MC4R agonist setmelanotide
- A better understanding of the factors influencing variable penetrance would be required to accurately predict the functional consequence of variants such as p.Asp707His

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