Biochemical Characterization of Single Minded-1 Missense Variants Associated With Severe Obesity

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Summary

- **213 missense *SIM1* variants, including 40 novel loss-of-function (LOF) variants, were identified in ~40,000 individuals with severe obesity in the Rhythm Pharmaceuticals database, which may aid in the diagnosis of individuals with obesity due to SIM1 deficiency**

Introduction

- Single minded-1 (SIM1) is a transcription factor involved in development and function of the hypothalamic paraventricular nucleus, a site critical for the body weight regulating function of the melanocortin-4 receptor (MC4R) pathway[1,2]
- Consistent with the involvement of *SIM1* in the MC4R pathway, rare LOF variants in *SIM1* are associated with early-onset, severe obesity and hyperphagia, hallmark features of rare genetic diseases of obesity[3-5]
- Improved diagnosis in individuals with rare genetic diseases of obesity, such as SIM1 deficiency, may enhance clinical understanding of the disease, lead to specialized management strategies, or inform individual eligibility for clinical trials[6,7]

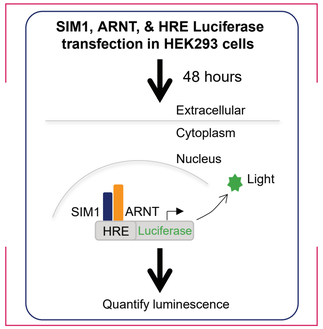
Objective

- To better understand the contribution of *SIM1* variants to severe clinical obesity, we performed functional biochemical characterization of missense *SIM1* variants identified in the Rhythm Pharmaceuticals database of ~40,000 individuals with severe obesity

Methods

- Missense *SIM1* variants were identified in the Rhythm Pharmaceuticals database of ~40,000 individuals with severe clinical obesity
  - The database consists of individuals <18 years of age with ≥97th percentile body mass index (BMI) for age and individuals ≥18 years of age with BMI ≥40 kg/m^2
- Functional biochemical characterization of identified missense *SIM1* variants was assessed using a well-established and controlled hypoxia response element (HRE)-luciferase reporter gene assay (Figure 1)
  - HEK293 cells were transiently transfected with wild-type (WT) or variant *SIM1*, aryl hydrocarbon receptor nuclear translocator (ARNT), HRE-luciferase reporter gene, and *Renilla* (for transfection efficiency)
  - 48 hours after transfection, luciferase activity was measured, and normalized luciferase activity (Firefly/*Renilla*) was expressed relative to WT *SIM1* activity, which was set to 100%

Figure 1. Functional evaluation of *SIM1* variants in an HRE-regulated gene expression assay.



ARNT, aryl hydrocarbon receptor nuclear translocator; HRE, hypoxia response element; SIM1, Single minded-1.

Results

Identification of *SIM1* Variants

- Utilizing the Rhythm Pharmaceuticals DNA database, we identified 233 *SIM1* variants in individuals with severe clinical obesity
- Of the 233 variants, 213 were missense variants (including 209 rare [gnomAD max frequency <1%] variants), 93 were variants that have not been previously described, and 197 were variants that have not been functionally assessed (Table 1)
  - Initially, 189 missense variants were screened; however, following submission of the abstract, additional missense variants were screened, bringing this total to 213

Table 1. Summary of Variants Identified in the Coding Region of *SIM1* in the Rhythm Pharmaceuticals DNA Database

Parameter	N
Total *SIM1* variants (includes frameshift, missense, splice, and stop gained variants)	233
Missense *SIM1* variants	213
Missense *SIM1* variants screened	213
Rare missense *SIM1* variants	209
Novel *SIM1* variants	93
Not previously functionally characterized	197

Functional Evaluation and Validation of *SIM1* Variants

- In addition to the identified *SIM1* missense variants, we evaluated 14 previously characterized *SIM1* variants, including 8 identified in the Rhythm Pharmaceuticals database and 2 common variants (gnomAD max frequency >1%), to validate our in vitro assay (Table 2)

Table 2. Functional Evaluation and Validation of Previously Characterized *SIM1* Variants

SIM1 variant	Publication/gnomAD max frequency	Literature evaluation (% activity relative to WT *SIM1*)	Rhythm evaluation (% activity relative to WT *SIM1*)
T46R	Bonnefond et al[8]	LOF (4%)	LOF (5%)
S71R	Ramachandrappa et al[9]	LOF (50%)	LOF (61%)
I128T	Bonnefond et al[8]; Ramachandrappa et al[9]	WT (91%, 90%)	WT (80%)
Q152E	Bonnefond et al[8]; Ramachandrappa et al[9]	WT (86%, 85%)	WT (81%)
R171H	Ramachandrappa et al[9]	LOF (30%)	LOF (59%)
L238R	Ramachandrappa et al[9]	LOF (60%)	LOF (50%)
H323Y	Bonnefond et al[8]; Sullivan et al[10]	LOF (33%, 40%)	LOF (68%)
P497R	Ramachandrappa et al[9]	LOF (70%)	LOF (71%)
R550H	Ramachandrappa et al[9]	LOF (60%)	LOF (64%)
D590E	Zegers et al[11]	WT (90%)	WT (105%)
D707H	Ramachandrappa et al[9]	LOF (60%)	LOF (51%)
T712I	Ramachandrappa et al[9]	LOF (60%)	LOF (59%)
P352T	0.431199		WT (92%)
A371V	0.430943		WT (89%)

Bonnefond et al[8] percent activity relative to WT *SIM1* is calculated based on supplemental data provided by authors. Ramachandrappa et al,[9] Sullivan et al,[10] and Zegers et al[11] percent activity relative to WT *SIM1* are an approximation based on mean data in publication. LOF, loss-of-function; SIM1, Single minded-1; WT, wild-type.

Functional Evaluation and Classification of 213 *SIM1* variants (Table 3; Figures 3 and 4)

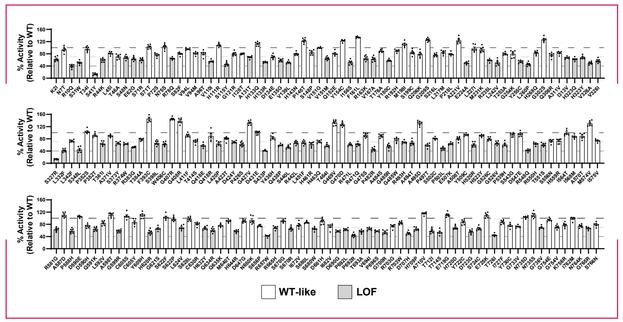
- Functional classification of *SIM1* variants was based on the below criteria
  - WT-like: >70% of WT activity
  - Moderate LOF: 40%–70% of WT activity
  - Complete LOF: <40% of WT activity

Table 3. Summary of Functional Evaluation and Classification of 213 Missense *SIM1* Variants

Parameter	N
Total missense *SIM1* variants screened	213
WT-like variants	117
LOF variants	96
Moderate LOF	93
Complete LOF	3
Novel LOF variants	40

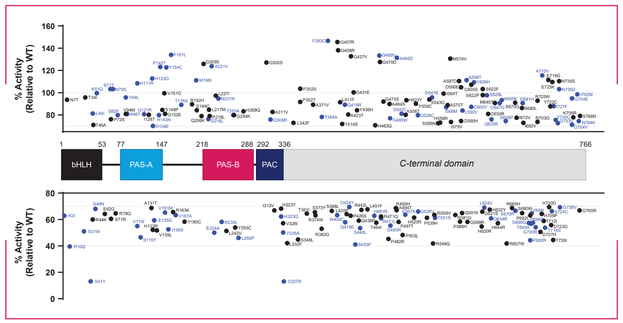
LOF, loss-of-function; WT, wild-type.

Figure 3. Functional assessment of missense *SIM1* variants on the transcriptional activity of *SIM1*.



Results are the mean of at least 2 experiments performed in triplicate ± SEM expressed relative to WT *SIM1* activity, which was normalized to 100%. LOF, loss-of-function; SEM, standard error of the mean; WT, wild-type.

Figure 4. Location and functional evaluation of missense *SIM1* variants.



At the top are variants that have been functionally classified as WT-like based on *SIM1* transcriptional activation of HRE-luciferase. At the bottom are variants that have been functionally characterized as LOF, either moderate or complete. Blue dots indicate novel *SIM1* variants (only present in the Rhythm Pharmaceuticals database). bHLH, basic helix-loop-helix; HRE, hypoxia response element; LOF, loss-of-function; PAC, proline-rich arabino-galactan protein and conserved cysteines; PAS, period (Per)-aryl hydrocarbon receptor nuclear transporter (ARNT)-SIM; SIM1, Single minded-1; WT, wild-type.

Conclusions

- A total of 213 missense *SIM1* variants were identified in individuals with severe clinical obesity
- Of the 213 missense variants, 3 exhibited complete LOF, 93 exhibited moderate LOF, and 117 exhibited WT-like activity
  - Nearly half of the missense *SIM1* variants, including 40 novel variants, observed in individuals with obesity exhibited LOF
- These findings provide important insights into the *SIM1* variant landscape and may help in the future diagnosis and treatment of individuals with obesity due to SIM1 deficiency

*Alastair S. Garfield was an employee of Rhythm Pharmaceuticals, Inc. at the time of abstract submission.

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References: 1. Li et al. *Neuron*. 2019;102:653-667. **2.** Shah et al. *Proc Natl Acad Sci*. 2014;111:13193-13198. **3.** Swarbrick et al. *Obesity*. 2011;19:2394-2403. **4.** Akinci et al. *J Clin Res Pediatr Endocrinol*. 2019;11:341-349. **5.** Huvenne et al. *Obes Facts*. 2016;9:158-173. **6.** Zorn et al. *Molec Cell Pediatr*. 2020;7:15. **7.** Styne et al. *J Clin Endocrinol Metab*. 2017;102:709-757. **8.** Bonnefond et al. *J Clin Invest*. 2013;123:3037-3041. **9.** Ramachandrappa et al. *J Clin Invest*. 2013;123:3042-3050. **10.** Sullivan et al. *Biochem J*. 2014;461:403-412. **11.** Zegers et al. *Int J Obes*. 2014;38:1000-1004.