

# 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<sup>1,2</sup>
- 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<sup>3-5</sup>
- 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<sup>5-7</sup>

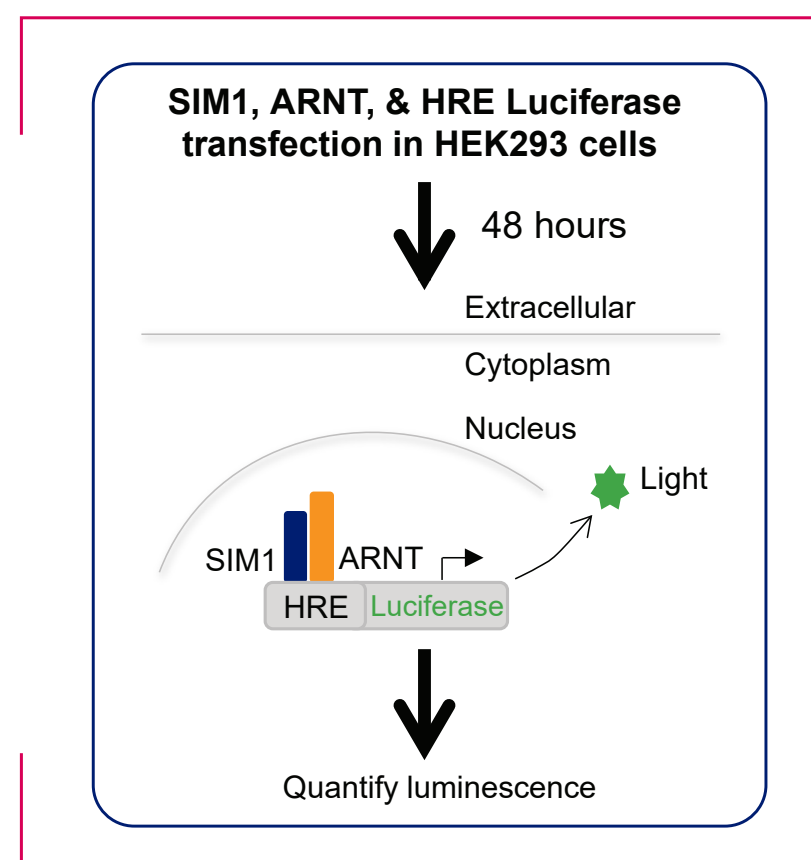
## Objective

- 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<sup>2</sup>
- 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.



\*Ida H. Moeller, Alastair S. Garfield, and Bhavik P. Shah were employees 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. Ramachandrapa 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.

## 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 <i>SIM1</i> variants (includes frameshift, missense, splice, and stop gained variants)	233
Missense <i>SIM1</i> variants	213
Missense <i>SIM1</i> variants screened	213
Rare missense <i>SIM1</i> variants	209
Novel <i>SIM1</i> 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

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

Bonnefond et al<sup>8</sup> percent activity relative to WT *SIM1* is calculated based on supplemental data provided by authors. Ramachandrapa et al<sup>9</sup>, Sullivan et al<sup>10</sup>, and Zegers et al<sup>11</sup> 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.

## 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

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

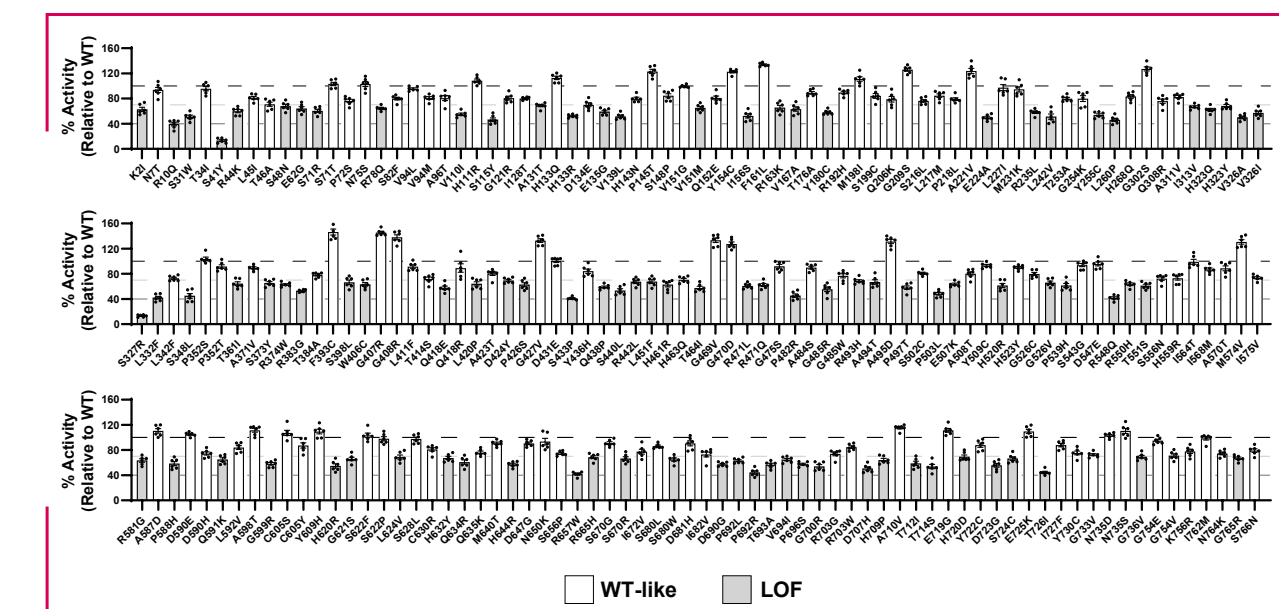
- Functional classification of *SIM1* variants was based on the below criteria
  - WT-like: >70% of WT activity
  - Moderate LOF: 40%–70% of WT activity
  - Complete LOF: <40% of WT activity

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

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

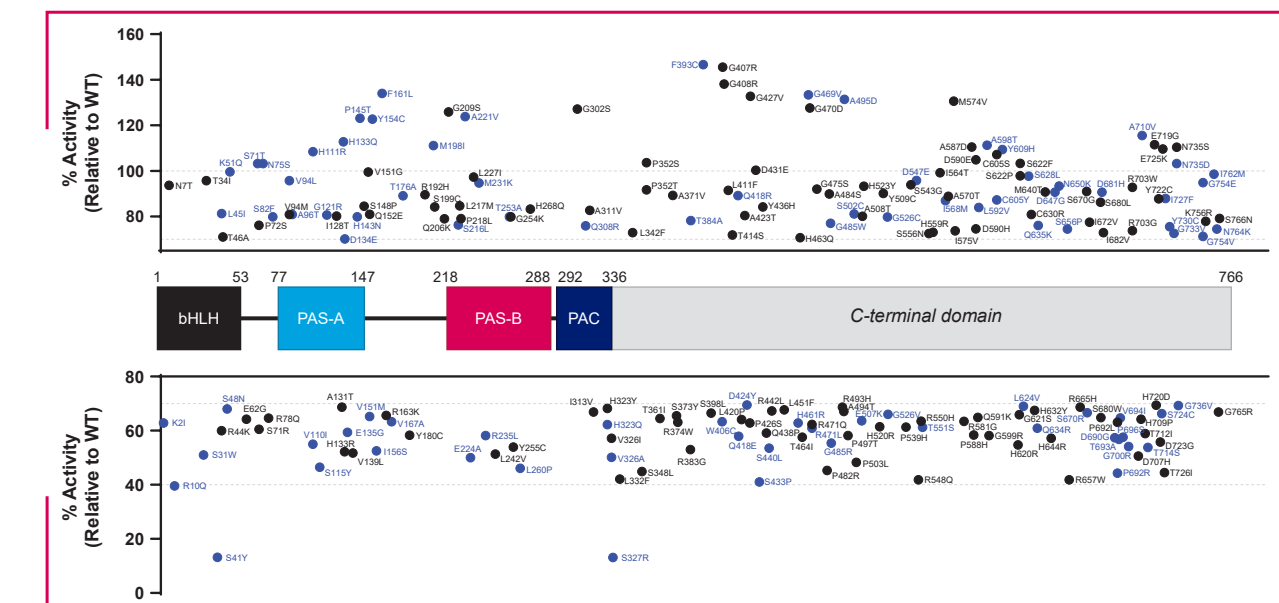
LOF, loss-of-function; WT, wild-type.

**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 arabinogalactan protein and conserved cysteines; PAS, period (Per)-aryl hydrocarbon receptor nuclear transporter (ARNT)-*SIM1*; *SIM1*, Single minded-1; WT, wild-type.