Loss-of-function mutations in SIM1 contribute to obesity and Prader-Willi–like features

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Sim1 haploinsufficiency in mice induces hyperphagic obesity and developmental abnormalities of the brain. In humans, abnormalities in chromosome 6q16, a region that includes SIM1, were reported in obese children with a Prader-Willi–like syndrome; however, SIM1 involvement in obesity has never been conclusively demonstrated. Here, SIM1 was sequenced in 44 children with Prader-Willi–like syndrome features, 198 children with severe early-onset obesity, 568 morbidly obese adults, and 383 controls. We identified 4 rare variants (p.I128T, p.Q152E, p.R581G, and p.T714A) in 4 children with Prader-Willi–like syndrome features (including severe obesity) and 4 other rare variants (p.T46R, p.E62K, p.H323Y, and p.D740H) in 7 morbidly obese adults. By assessing the carriers’ relatives, we found a significant contribution of SIM1 rare variants to intra-family risk for obesity. We then assessed functional effects of the 8 substitutions on SIM1 transcriptional activities in stable cell lines using luciferase gene reporter assays. Three mutations showed strong loss-of-function effects (p.T46R, p.H323Y, and p.T714A) and were associated with high intra-family risk for obesity, while the variants with mild or no effects on SIM1 activity were not associated with obesity within families. Our genetic and functional studies demonstrate a firm link between SIM1 loss of function and severe obesity associated with, or independent of, Prader-Willi–like features.

Introduction

The basic helix-loop-helix–PER-ARNT-SIM (bHLH-PAS) transcription factor single-minded 1 (SIM1) plays a key role in neuronal differentiation within the paraventricular nucleus of the hypothalamus, which is critical for food intake regulation (1). Sim1-haploinsufficient mice display hypodevelopment of the paraventricular nucleus of the hypothalamus and are hyperphagic, obese, and highly sensitive to diet-induced obesity, whereas Sim1 overexpression in mice leads to a decrease in food intake (2–4).

In humans, several deletions in the chromosome 6q16, the region that includes SIM1, have been identified in obese patients presenting with a Prader-Willi–like (PWL-like) syndrome (5), which is characterized in early infancy by global developmental delay, hypotonia, and feeding difficulties and later in life by hyperphagia, obesity, and facial dysmorphisms (6). The 4.1-Mb critical region for PWL syndrome includes SIM1 but also 11 other genes or gene predictions, and the specific role of SIM1 haploinsufficiency in the development of PWL syndrome has not definitively been established (6). Nonetheless, a de novo balanced translocation disrupting SIM1 has been described in a patient presenting with severe obesity but no other clinical features suggestive of a PWL syndrome (7). Another study reported 6 rare non-synonymous SIM1 mutations in extremely obese patients only, but no functional analyses were performed (8).

In the present study, we aimed to assess the contribution of rare mutations in SIM1 to monogenic and syndromic forms of obesity. Coding regions of SIM1 were sequenced in 44 children presenting with PWL syndrome features, 198 children with severe early-onset obesity, 568 morbidly obese adults, and 383 normal-weight controls. Subsequently, we functionally characterized the effects of the mutations on SIM1 activity and analyzed phenotypes in SIM1-deficient patients.

Results and Discussion

By focusing on rare coding variants only [minor allele frequency (MAF) <1%], we identified 4 substitutions (p.I128T, p.Q152E, p.R581G, and p.T714A; Figure 1) in 4 children presenting with
clinical features of PWL syndrome, including severe obesity that started between 1 and 2 years of age (Table 1 and Supplemental Table 1; supplemental material available online with this article; doi:10.1172/JCI68035DS1), and 4 other substitutions (p.T46R, p.E62K, p.H323Y, and p.D740H; Figure 1) in 7 morbidly obese adults (Table 1 and Supplemental Table 2).

With regard to the obese child SIM1 mutant carriers presenting with PWL syndrome (Supplemental Table 1 and Supplemental Figure 1), the girl carrying the p.I128T variant received the substitution from her normal-weight father. The mother of the girl carrying the p.Q152E variant did not carry this substitution; the father’s DNA and phenotypes were not available. The girl carrying the p.R581G variant was an adopted child. In contrast, we were able to investigate the family of the boy carrying the p.T714A variant. His 3 siblings, his mother, and a maternal aunt also carried this substitution. The mother and her 4 children were severely obese and presented at least one other clinical feature associated with PWL syndrome, while the aunt was only overweight and without clinical abnormalities (Supplemental Table 3).

Among the morbidly obese adult SIM1 mutant carriers (Supplemental Table 2 and Supplemental Figure 2), 4 unrelated patients carried the same p.T46R variant. In the first mutated participant, the p.T46R variant had arisen de novo. In the second mutated participant, the p.T46R variant had been inherited from his obese mother. Furthermore, this participant had an obese sister who also carried the p.T46R variant. Although parental DNA samples were not available, the father’s DNA was not available.

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AThe total risk score (0 = lowest risk → 5 = highest risk) is the sum of each score calculated per software (see Supplemental Methods): 0 if Polyphen-2 = “Benign,” Sift = “Tolerated,” SNAP = “Neutral,” Pmut = “Neutral,” Align GVGD = “C0”; 1 if different than “benign,” “tolerant,” “neutral,” or “C0.” *F0 = no effect; 1 = mild effect on SIM1 activity with ARNT or ARNT2; 2 = strong effect on SIM1 activity with ARNT and ARNT2. MAF is according to the NHLBI Exome Project. NA, not available.

Table 1

Genetic and functional description of the 8 SIM1 rare substitutions
not available for the third p.T46R-carrying participant, her obese
son carried the substitution, while her normal-weight sister and
normal-weight daughter did not. The family of the fourth p.T46R-
carrying participant was not available. The patient carrying the
p.E62K variant had a normal-weight mother and 2 normal-weight
daughters, none of whom carried the substitution. His severely
obese father has died, and no DNA was available. The carrier of
the p.H323Y variant had a morbidly obese sister who was also a
p.H323Y carrier, while both parents had died. No DNA was avail-
able from relatives of the p.D740H carrier.

In summary, we identified 21 (adults and children) carriers of a
rare heterozygous SIM1 variant (11 probands and 10 of their rela-
tives) and 16 non-carrier relatives, and we found a strong contri-
bution of SIM1 rare variants to intra-family risk for obesity (odds
ratios [95% confidence interval]: OR = 20.9 [3.5–126.5]; \( P = 9.3 \times 10^{-4} \)).

Of note, 3 of the 8 rare variants have been listed in the Single
Nucleotide Polymorphism database (dbSNP 137) — p.E62K/
rs201038781, p.I128T/rs138546433, and p.Q152E/rs140908824
— but are very rare in the general population, according to the
NHLBI Exome Project and the 1000 Genomes Project (MAF
<0.1%). When we sequenced 383 normal-weight controls, we found
a single carrier of the p.I128T variant (in the heterozygous state).
A previous study reported that p.I128T did not cosegregate with
either obesity or overweight phenotype in a family of European
descent (9). Therefore, this variant is unlikely to have a signifi-
cant effect on obesity or PWL-related clinical features. We then
genotyped 2,896 normal-weight individuals but did not find any

Subsequently, we aimed to functionally assess the 8 rare substi-
tutions. Using five types of in silico prediction software, we found
that the mutation with the highest risk score of damaging effect
was p.T46R, while the substitutions with the lowest risk score
were p.Q152E and p.T714A (Table 1). One of the aryl hydrocar-
bon receptor nuclear translocators, either ARNT or ARNT2, is
required as a dimerization partner for SIM1 to function as a tran-
scription factor, with mouse knockout studies suggesting that
ARNT2 is the in vivo partner in the hypothalamus (10, 11). We
constructed a homology model to estimate structural features of
the SIM1:ARNT2 dimer at the bHLH and PASA domains, which
was based upon the crystal structure for the N-terminal half of
the CLOCK:BMAL1 dimer (12). We found that the N-terminal
amino acids (T46, E62, I128, and Q152) likely lie on the surface
of the protein (Figure 2A). E62, I128, and Q152 amino acids were
located in unstructured loops (Figure 2, B and C); thus, their pos-
sible contributions to perturbations of SIM1 structure could not
be estimated. However, T46 lay in the middle of a helix that was in
close proximity to a helix of ARNT2 (Figure 2B); thus, the larger
arginine residue may critically perturb the dimerization interface.

We then assessed the functional effects of the 8 substitu-
tions on the transcriptional properties of the SIM1:ARNT and
SIM1:ARNT2 dimers in vitro using luciferase gene reporter assays
in human 293 Flp-In T-Rex stable cell lines expressing the WT
protein or one of the 8 SIM1 mutants (Figure 3, A and B). Impor-
tantly, with this system, only 1 copy of the mutated (or WT) SIM1
is integrated at a specific and predefined insertion site in the cell
genome. This renders activity levels independent of the number
of inserted copies and the insertion site, and allowed an accurate
comparison of the mutants’ activities. We found a strong loss-
on SIM1 transcriptional activity with both ARNT and ARNT2
(\( P < 0.001 \); Figure 3C). These mutations were located in the bHLH,
PASB, and C-terminal domains, respectively (Figure 1). The 3
mutations were identified in morbidly obese adults (\( n = 9 \) carriers
of p.T46R or p.H323Y), in 1 overweight adult (carrier of p.T714A),
and in severely obese subjects with PWL syndrome (\( n = 4 \) carriers
of p.T714A). Together, these loss-of-function mutations were asso-
ciated with a strong intra-family risk for obesity (OR = 28.0\( \times 10^{-7} \);
\( P = 5.6 \times 10^{-4} \)). The p.I128T and p.Q152E variants, which were
identified either in normal-weight participants (\( n = 2 \) carriers
of p.I128T) or in severely obese subjects with PWL syndrome (\( n = 2 \)
carriers of p.I128T or p.Q152E), had a milder loss-of-function
effect on SIM1 activity with ARNT or ARNT2 (Figure 3C). Two
substitutions (p.E62K and p.D740H) which were identified in 2
morbidly obese participants, had a gain-of-function effect on SIM1
activity with ARNT only (Figure 3C). We did not detect any effect
of p.R581G on SIM1 activity (Figure 3C), which was identified in a
severely obese participant with PWL syndrome. Together, the

Figure 2
Homology model of the SIM1:ARNT2 heterodimer. SIM1 is shown in yellow and ARNT2 in green. (A) Surface representation of SIM1, with amino acids that harbor variants shown in red. (B and C) Ribbons diagram of the SIM1:ARNT2 heterodimer, with amino acids that harbor variants depicted in pink.
rare variants with mild or no effects on SIM1 activity were not significantly associated with obesity within families (OR = 7.5[0.5–122.7]; P = 0.158). When we compared our functional data with the in silico predictions, only 62.5% concordance was found.

Our genetic and functional studies, together with the findings of Ramachandrappa et al. (13), convincingly demonstrate a link between SIM1 loss of function and severe to morbid obesity that may also be associated with PWL-related clinical features including developmental delay (or intellectual disability) and facial dysmorphism. The observed effects of SIM1 loss-of-function mutations on both development and body weight regulation are in line with the key role of SIM1 in development of the paraventricular nucleus of the hypothalamus (1). Importantly, the neclin protein (NDN), which is believed to be involved in the Prader-Willi syndrome (14), regulates the activity of the SIM1:ARNT2 dimer (15), reinforcing the putative role of SIM1 in the PWL syndrome.

SIM1 non-synonymous mutations were present in numerous patients with severe obesity associated with, or independent of, PWL-related clinical features. Some mutations, such as p.T46R, dramatically reduced SIM1 activity on a reporter gene and were only found in obese individuals, suggesting that loss-of-function SIM1 mutants may underpin a monogenic form of obesity. However, some substitutions with marked loss-of-function effect (e.g., p.T714A) did not always associate with severe obesity or PWL syndrome. Moreover, the p.I128T variant, which caused mild loss of SIM1 function, was unlikely to contribute to obesity. Therefore, mutation of SIM1 is not always responsible for a fully penetrant form of obesity. Similar features have been repeatedly found in MC4R loss-of-function mutation carriers, where a permissive role of the environment on disease expression has been suggested (16, 17). The degree of penetrance is expected to be determined by the severity of loss of function of a particular mutant in combination with environment and genetic background. In obese patients presenting with the clinical features associated with Prader-Willi syndrome, if chromosomes 15q11 and 6q16 are found not to contain abnormalities, then SIM1 sequencing and subsequent molecular characterization should be performed, in order to demonstrate the presence of causative loss-of-function mutations.

Methods
Further information is available in Supplemental Methods.

Statistics. For the genetic study, effects of mutation on obesity risk were calculated through univariate logistic regression models using SPSS 14.0. For the functional analysis, univariate ANOVA was performed on the log values generated for each mutant with reference to WT using SPSS 17.0. Separate analyses were performed for ARNT- and ARNT2-transfected cells. A P value less than 0.05 was considered significant.

Study approval. The study protocol was approved by the ethics committees of Lille II University, the University of Antwerp, the Canton of Zurich, and the University of Paris-Sud and the Institutional Review Board of the University of Florida, and study participants signed informed consent agreements. For children younger than 18 years, oral consent was obtained, and parents provided written informed consent.

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