Whole Exome Sequencing Identifies *RAI1* Mutation in a Morbidly Obese Child Diagnosed With ROHHAD Syndrome

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Context: The current obesity epidemic is attributed to complex interactions between genetic and environmental factors. However, a limited number of cases, especially those with early-onset severe obesity, are linked to single gene defects. Rapid-onset obesity with hypothalamic dysfunction, hypoventilation and autonomic dysregulation (ROHHAD) is one of the syndromes that presents with abrupt-onset extreme weight gain with an unknown genetic basis.

Objective: To identify the underlying genetic etiology in a child with morbid early-onset obesity, hypoventilation, and autonomic and behavioral disturbances who was clinically diagnosed with ROHHAD syndrome.

Design/Setting/Intervention: The index patient was evaluated at an academic medical center. Whole-exome sequencing was performed on the proband and his parents. Genetic variants were validated by Sanger sequencing.

Results: We identified a novel de novo nonsense mutation, c.3265 C>T (p.R1089X), in the retinoic acid-induced 1 (*RAI1*) gene in the proband. Mutations in the *RAI1* gene are known to cause Smith-Magenis syndrome (SMS). On further evaluation, his clinical features were not typical of either SMS or ROHHAD syndrome.

Conclusions: This study identifies a de novo *RAI1* mutation in a child with morbid obesity and a clinical diagnosis of ROHHAD syndrome. Although extreme early-onset obesity, autonomic disturbances, and hypoventilation are present in ROHHAD, several of the clinical findings are consistent with SMS. This case highlights the challenges in the diagnosis of ROHHAD syndrome and its potential overlap with SMS. We also propose *RAI1* as a candidate gene for children with morbid obesity. **(*J Clin Endocrinol Metab* 100: 1723–1730, 2015)**

The rising prevalence of severe childhood obesity is attributed to complex genetic and environmental influences. However, a percentage of early-onset extreme obesity can be attributed to single-gene defects (1). There is increasing awareness of a syndrome associated with rapid-onset obesity with hypothalamic dysfunction, first described by Fishman et al (2) and recently renamed ROHHAD (rapid-onset obesity with hypothalamic dys-

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received November 25, 2014. Accepted March 13, 2015.
First Published Online March 17, 2015

Abbreviations: BDNF, brain-derived neurotropic factor; MRI, magnetic resonance imaging; RAI1, retinoic acid-induced 1; ROHHAD, rapid-onset obesity with hypothalamic dysfunction, hypoventilation and autonomic dysregulation; SDS, SD score; SMS, Smith-Magenis syndrome; WES, whole-exome sequencing.

function, hypoventilation, and autonomic dysregulation) by Ize-Ludlow et al (3). Several reports have now described additional cases with a diverse spectrum of clinical manifestations (3–6). Obesity and alveolar hypoventilation typically start after 1.5 years of age, with hypothalamic and/or pituitary hormone dysfunction that may encompass GH deficiency, central hypothyroidism, diabetes insipidus, adrenal insufficiency, pubertal disturbances, and hyperprolactinemia. Additional features include autonomic dysregulation, behavioral or developmental disorders, and neuroendocrine tumors (3–6). Unambiguous identification of ROHHAD syndrome has been challenging; confirmatory laboratory testing is not yet available, and the patient population may represent a heterogeneous group of underlying etiologies. Hence, the diagnosis is based exclusively on the clinical findings.

Due to the high morbidity and mortality associated with ROHHAD, genetic causes are being actively investigated. Mutations have been ruled out in several candidate genes, including *PHOX2B*, linked to congenital central hypoventilation syndrome, and *BDNF*, *TRKB*, *NECDIN*, *ASCLI*, *HTR1A*, *OTP*, and *PACAP*, associated with the development and function of hypothalamic, autonomic, and neuroendocrine systems (3, 7–10).

We report a de novo mutation in the retinoic acid-induced 1 (*RAI1*) gene in an 11-year-old boy with severe rapid-onset obesity and developmental delay who was clinically diagnosed with ROHHAD syndrome. This study broadens the spectrum of clinical features associated with *RAI1* mutations and highlights the importance of whole-exome sequencing (WES) in the determination of genetic causes of rare undefined syndromes.

Case Report

The proband is the male product of a dizygotic twin pregnancy conceived from in vitro fertilization to a 31-year-old Irish Gravida 8 Para 4 mother and a 33-year-old Portuguese father; their first conception together. The twins were induced at 33 weeks gestation due to fetal compromise in the sister. The proband had a birth weight of 2.30 kg (+0.8 SD score [SDS]) and a length of 48.2 cm (+1.9 SDS), whereas his twin sister's birth weight was 1.8 kg (−0.4 SDS) and her length 43.2 cm (+ 0.2 SDS). His Apgar scores were normal, and he had prematurity-related issues including apnea, bradycardia, temperature irregularities, and feeding difficulties that resolved during early infancy. There was no evidence of intraventricular hemorrhage or asphyxia, and he did not require ventilator therapy.

The proband was delayed in achieving motor and language milestones and was enrolled in an early intervention program. The genetics team evaluated him at 20 months of age for hypotonia and dysmorphic facial features that included macrocephaly (head circumference, 52 cm; +3.12 SDS), hypertelorism, flat nasal bridge, prominent forehead, and anteverted nares (Figure 1A). Additionally, his mother noted a high tolerance for pain with no crying during needle pricks, inability to mount fever with infection, lack of tears, and excessive sweating. A karyotype, chromosomal microarray, and tests for inborn errors of metabolism were normal; tests for fragile X and familial dysautonomia were also negative. A magnetic resonance image (MRI) of the brain showed the presence of prominent subarachnoid spaces and ventricles.

At 2.5 years of age, he underwent a sleep evaluation for difficulty in initiating and maintaining sleep, with consistent awakening between 3 and 4 AM. The study showed one episode of mixed apnea with maximal end-tidal CO_2 of 49 Torr. Additional sleep studies were performed at later ages due to persistent sleep issues. At 5 years of age, he had an apnea-hypopnea index of 10/h (normal, <5/h), lasting up to 20 seconds each, and a reduction in O_2 saturation to 77%. He was started on bilevel positive airway pressure (BiPAP) by mask at 13/5 cm of water. A sleep study at 8 years of age revealed hypoventilation with peak end-tidal CO_2 of 65 Torr during spontaneous breathing, hypoxemia to 77%, and an apnea-hypopnea index of 27/h during the rapid eye movement (REM) and non-REM phases of sleep. At 10 years, his daytime venous CO_2 level was 69 mm Hg (normal, 38–52 mm Hg). He underwent a tracheostomy, which he pulled out within 3 months.

During the second and third year of his life, he gained 12.5 kg each year (Figure 1E). Endocrinological evaluation at 4 years of age revealed a normal IGF-1 level with mildly elevated IGF binding protein-3 (IGF-BP3) at 5.3 μg/mL (normal, 1–4.7 μg/mL), and normal free T_4 at 1.04 ng/dL (0.8–1.90) and TSH level, fasting insulin level of 6 μIU/L (normal, 3–12 μIU/L), with a blood glucose of 81 mg/dL (normal, 61–99 mg/dL). The leptin level was 88.9 ng/mL (appropriate for the adiposity; normal, 0.5–12.7 ng/mL). A low-dose dexamethasone suppression test was normal. Follow-up annual pituitary function screening tests did not show any dysfunction.

His neurological evaluation was concerning for developmental delay and behavioral disorder. He was diagnosed with autism spectrum disorder at 3 years of age. Psychological evaluation at 8 years showed a nonverbal IQ of 53 with a verbal IQ of 43 on the Stanford-Binet Intelligence scale. His overall assessment showed marked impairment in cognitive abilities and adaptive skills, with aggressive, self-injurious behavior and tantrums. His individualized education plan includes occupational, speech, social, and behavioral support since kindergarten.

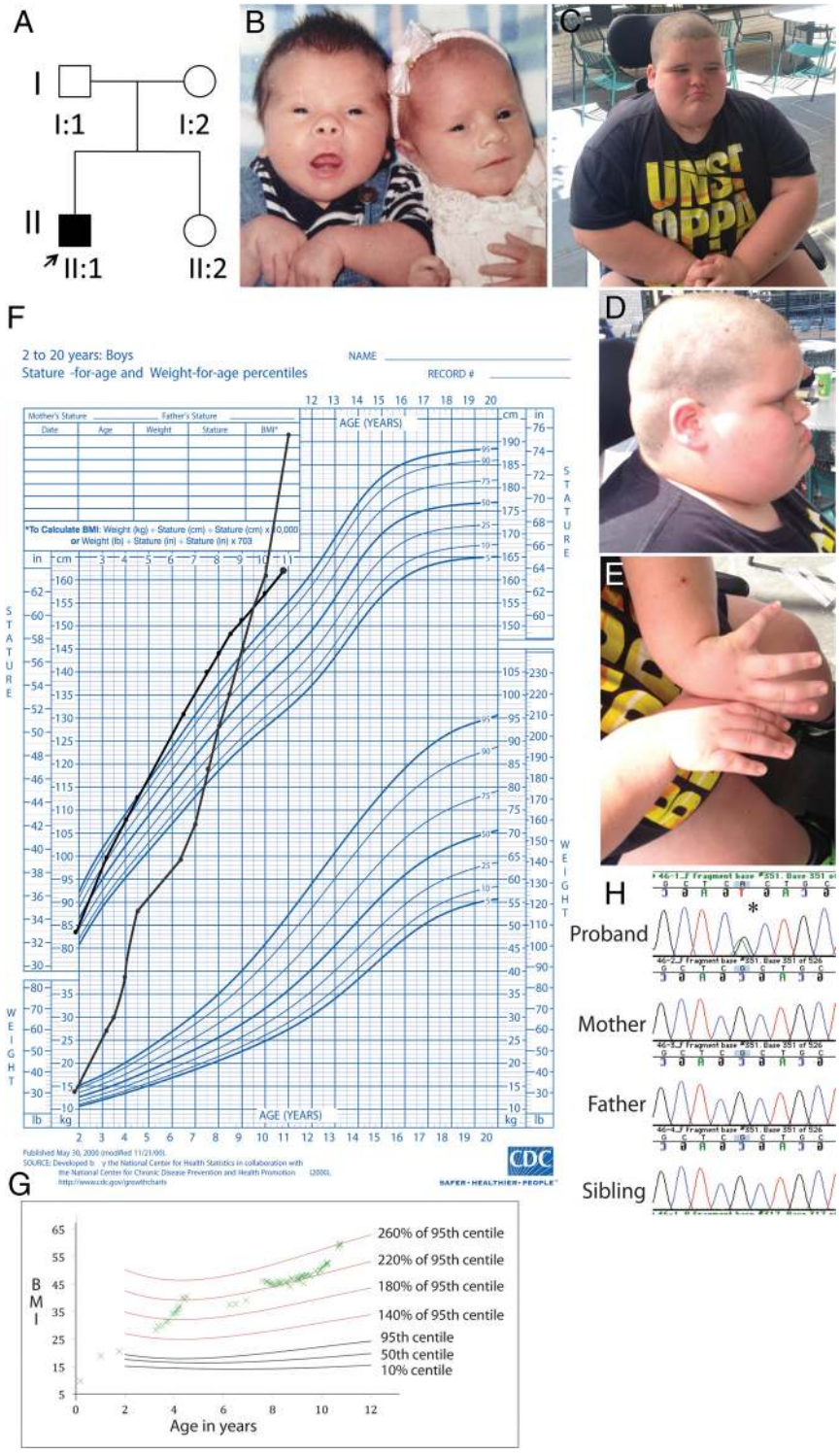

Figure 1. Phenotypic, anthropometric, and genetic findings in the proband. A, Pedigree of the family; the proband is II:1. B–E, Physical findings of the proband. B, Facial features of the proband as an infant (left) with the unaffected sister (right). C and D, Facial features of the proband at 11 years—front (C) and side view (D). E, Image of the hands, showing small hands. F and G, Anthropometric measurements. F, Height and weight trajectory on the CDC 2000 growth curves. G, BMI curves for age. H, Sanger DNA sequencing of genomic PCR products showing the de novo c.3265C>T mutation (noted with asterisk) in the proband, absent in both parents and unaffected sibling.

He developed constipation and gastroesophageal reflux requiring medical management. The result of an extensive biochemical and molecular investigation to find a unifying diagnosis was inconclusive (Table 1).

During early childhood, he developed hypertension (150/110 mm Hg) needing antihypertensive therapy. Cardiac function was normal on echocardiogram. His family reported an excessive thirst and urination, but serum sodium and glucose levels were normal. He could not tolerate a water deprivation test as part of definitive testing for diabetes insipidus due to his behavioral disturbances. His bone age was significantly advanced to 12 years and 6 months at a chronological age of 7 years and 9 months. Tests for precocious puberty were negative, and in the absence of other plausible etiologies, obesity was thought to contribute to the advanced bone age. At 8 years of age, he was given a clinical diagnosis of ROHHAD syndrome based on the constellation of symptoms of rapid weight gain, hypoventilation, autonomic dysregulation, and behavioral manifestations (Table 2). Testing for *PHOX2B* mutations to rule out congenital central hypoventilation syndrome was normal.

Due to continued severe weight gain, he required a motorized chair for ambulation. At 11 years of age, his weight was 166.3 kg (+4.1 SDS), height was 163.8 cm (+2.8 SDS), and BMI was 62.0 kg/m^2 (+3 SDS) on Centers for Disease Control 2000 growth curves. A serial screening for neuroendocrine tumors with whole-body MRI has been normal. He has developed staring spells without electroencephalogram abnormalities. He was diagnosed with sensory-neural hearing loss. He has now developed hypercholesterolemia (low-density lipoprotein, 112 mg/dL), diabetes (glycated hemoglobin, 7.5%), and

Table 1. Etiological Diagnostic Testing for the Proband

	Result	Cost (in US dollars)[a]
Genetic tests (suspected diagnosis)		
Karyotype	46,XY	315
Chromosomal microarray	Normal	2972
FMR1 gene testing (Fragile X syndrome)	Normal	1414
Deletion/duplication 15q11-q13 (Prader-Willi syndrome)	Negative	1570
MAGEL2 gene (Prader-Willi syndrome)	Negative	1575
IKBKAP gene testing (familial dysautonomia)	Normal	1500
NSD1 gene sequencing (Sotos syndrome)	Negative	892
CDKN1C, *H19*, *KCNQ1OT1* methylation studies (Beckwith-Wiedeman syndrome)	Negative	1800
GPC3 gene sequencing (Simpson-Golabi-Behmel syndrome)	Negative	1000
PTEN gene (Bannayan-Riley-Ruvalcaba syndrome)	Negative	1768
MECP2 gene (Rett syndrome)	Negative	1550
PHOX2B gene testing (central hypoventilation)	Negative	921
Total cost of genetic testing		17 277
Clinical whole exome sequencing (2013)		7000
Other diagnostic tests		
Urinary organic acids, ammonia, lactate, pyruvate, electrolytes, glucose, uric acid (inborn errors of metabolism, mitochondrial disease)	Normal	333
Biotinidase enzyme activity (biotinidase deficiency)	Normal	115
O-glycan profile and quantification (congenital disorders of glycosylation)	Mild changes; repeat, normal	474
N-glycan and carbohydrate-deficient transferrin (congenital disorders of glycosylation)	Normal	120
Serum glutaric acid level (glutaric acidemia type 1)	Normal	169
MRI with magnetic resonance spectroscopy (cerebral creatine deficiency, Canavan disease)	Large subarachnoid space, prominent ventricles	3272

[a] Costs are based on the Laboratory Medicine Rate Book at Boston Children's Hospital and are comparable with various commercial laboratories in the United States.

elevated transaminases (alanine aminotransferase, 77 U/L; aspartate aminotransferase, 83 U/L), and continues on antihypertensive therapy. The other family members, including his twin sister, are unaffected.

Subjects and Methods

The proband, both parents, and the unaffected sibling were enrolled in an Institutional Review Board approved study at Boston Children's Hospital (BCH). DNA extraction from blood samples was performed by the Research Connection Biobank Core, and WES by the Genetics Diagnostics Laboratory at BCH. Library preparation was performed using the SureSelectXT Human All Exon V4 kit (Agilent Technologies), and sequencing was performed on a HiSeq platform (Illumina, Inc) as paired-end 2 × 100-bp runs. The reads were mapped to the human genome assembly (hg19; UCSC browser) using Burrows-Wheeler Alignment version 0.5.8, and single nucleotide polymorphisms and indels were detected using SAMTOOLS 0.1.18 (http://samtools.sourceforge.net) and GATK 1.6–7 (https://www.broadinstitute.org/gatk/). The resulting variant call format files filtered to include nonsynonymous, splice site and indel variants with an allele frequency <0.001 in the NHLBI Exome Variant Server database (http://evs.gs.washington.edu/EVS/) or <0.01 in the 1000 Genomes project, phase 3 (http://www.1000genomes.org). We screened for all currently described genes for monogenic obesity in the Human Obesity Gene Map (1).

Results

WES was performed on genomic DNA from the proband and both parents. The mean read depth was 100–115X, and >10X coverage of the target region was 98.1–98.7%. A total of 1387 nonsynonymous, splice site and indel variants in the proband satisfied the filtration criteria described above. Of those variants, 28 were de novo dominant, recessive (homozygous or compound heterozygous), autosomal, or X-linked. These genes were further evaluated for human disease or animal models overlapping with the phenotype. Variants in three genes satisfied the above criteria. On Sanger sequencing, one variant was confirmed, the other two being false positive. The confirmed variant was a de novo nonsense *RAI1* mutation in the proband (chr 17: 17699527; NM_030665: exon3: c.3265C>T: p.R1089X) that was absent in both parents and the unaffected sister (Figure 1H). This variant was not present in either the Exome Variant Server or 1000 Ge-

Table 2. Comparison of the Clinical Findings in the Proband With Those Described With ROHHAD Syndrome and SMS

Clinical Features	Proband	ROHHAD Syndrome, %	SMS
Hypothalamic dysfunction			
Rapid-onset obesity	+	100	+ (Present, not rapid onset)
Hyperphagia	+	53	+
Polydipsia	+	53	−
Hypernatremia	47	NA	
Hyperprolactinemia	−	47	NA
Diabetes insipidus	−	33	−
Hypothyroidism	33	+	
Adrenal insufficiency	−	27	+/−
Hypodipsia	−	27	+
Polyuria	27	NA	
Short stature	−	20	+/−
Delayed puberty	−	13	−
Hyponatremia	13	NA	
Low IGF-1 and IGFBP-3 levels	−	13	NA
Precocious puberty	−	13	+
Premature adrenarche	13	+/−	
Transient SIADH	−	13	NA
Hypogonadotropic hypogonadism	−	6	−
Transient diabetes insipidus	6	NA	
Respiratory manifestations			
Alveolar hypoventilation	+	100	−
Cardiorespiratory arrest	60	−	
Reduced CO_2 ventilatory response	+	60	−
Obstructive sleep apnea	+	53	+
Cyanotic episodes	27	−	
Neurological findings			
Developmental delay	+	20	+
Developmental regression	20	+	
Sleep disturbances	+	13	+
Seizures	−	33	+
Hypotonia	27	+	
Abnormal brain MRI scans	+	47	NA
Behavioral disorders			
Depression	−	13	+
Flat affect	+	13	+
Psychosis	−	13	+
Behavioral outbursts	+	6	+
Bipolar disorder	−	6	+
Emotional lability	+	6	+
Obsessive-compulsive disorder	−	6	+
Oppositional-defiant disorder	+/−	6	+
Tourette syndrome	−	6	NA
Hallucinations	−	6	NA
Self-injury	+	NA	+
Autonomic dysregulation			
Ophthalmological manifestations	+	87	+
Thermal dysregulation	+	73	NA
Gastrointestinal dysmotility	+	67	+
Altered perception of pain	+	53	+
Altered sweating	+	53	NA
Cold hands and feet	+	40	+
Bradycardia	−	33	NA
Syncopal episodes	−	6	+/−
Other findings			
Hearing loss	−	NA	+
Enuresis	+	27	+
Asthma	−	20	+/−
Hypercholesterolemia	+	20	+
Scoliosis	+	20	+
Recurrent pneumonia	+	13	+
Brachydactyly	+	NA	+
Impaired glucose tolerance	+	6	−
Type 2 diabetes mellitus	−	6	−
Tumor of neural crest origin	−	33	NA

Abbreviations: IGFBP, IGF binding protein; SIADH, syndrome of inappropriate antidiuretic hormone; NA, information not available; +, present; −, absent; +/−, rarely described. ROHHAD syndrome adapted from Ref. 3, and SMS with *RAI1* mutation adapted from Refs. 12–15, 17, 25, 26.

nomes database. The mutation truncates the *RAI1* at amino acid 1089, and the resultant protein does not contain the nuclear localization signals or the functional PHD domain (11, 12). Consequently, the truncated *RAI1*, if stable, will remain in the cytoplasm, unable to regulate transcription, and effectively result in haploinsufficiency. *RAI1* haploinsufficiency without 17p11.2 deletions has been shown to cause Smith-Magenis syndrome (SMS) (13–16).

Discussion

In this study, we report a de novo mutation in *RAI1*, c.3265 C>T (p.R1089X) in a patient who presented with early-onset morbid obesity, developmental delay, and sleep disturbances. In light of the identified *RAI1* mutation, the proband's clinical features were reevaluated, revealing a significant overlap between both SMS and the ROHHAD syndrome (Table 2). A reassessment of the chromosomal microarray did not reveal any evidence of deletion. This study emphasizes the challenges in the diagnosis of ROHHAD syndrome. We concur that the diagnosis of ROHHAD syndrome should be considered early in children with acute onset obesity due to the wide spectrum of evolving clinical features and the high morbidity and mortality without optimal ventilatory support. Due to the lack of definitive diagnostic criteria, individual clinical judgment will likely play an important role in making the diagnosis.

The *RAI1* gene is located on chromosome 17, has six exons, and more than 90% of the coding region is located in exon 3 (11, 17, 18). This gene is highly conserved across species and is widely expressed at low levels throughout the body (11, 12, 19, 20). Available data support *RAI1* as a transcriptional regulator or epigenetic code reader, with an N-terminal transactivation domain, centrally positioned nuclear localization signals, and a C-terminal PHD domain (20, 21). *RAI1* dosage abnormalities are the primary cause of contiguous gene syndromes. A 17p11.2 microdeletion can lead to SMS (OMIM no. 182290), whereas a microduplication of the same region can lead to Potocki-Lupski syndrome (OMIM no. 610883).

SMS was first described in 1986, and the prevalence is estimated to be between 1/15 000 and 1/25 000 (22). The clinical presentation includes craniofacial anomalies, intellectual disabilities, and behavioral abnormalities with sleep disturbances, self-injury, and aggressive behavior (15). Less common manifestations include ophthalmological and otolaryngological anomalies (~80%), hearing impairment (~70%), cardiac defects (~40%), and renal defects (~20–30%) (23, 24). Molecular studies in SMS have revealed a common deleted region of approximately 3.7 Mb in most SMS patients (>70–80%) (22). In patients with a clinical phenotype of SMS without a deletion, several de novo and familial mutations have been identified in the *RAI1* gene (12–17), with a somewhat different spectrum of clinical features (25). The key features of SMS, craniofacial abnormalities, intellectual disability, and neurobehavioral disturbances, including derangement of the sleep cycle, are consistent between the two groups. However, certain other abnormalities such as short stature, cardiovascular, renal, and dental aberrations are much less common in patients with *RAI1* point mutations. Obesity is a common, but not consistent, finding (13–16, 19). A phenotypic investigation of eating behavior in a large cohort with SMS has demonstrated significant hyperphagia in patients with *RAI1* mutation (26). *Rai1* haploinsufficiency in mice leads to hyperphagia, obesity, and altered fat distribution, possibly related to reduced brain-derived neurotropic factor (*Bdnf*) expression, a gene associated with hyperphagia and obesity (19). Furthermore, the weight gain in mice with *Rai1* haploinsufficiency is dependent on the composition of their diet, with the highest gain in mice fed with high-carbohydrate and high-fat diets (27). This observation emphasizes the role of early diagnosis and formulation of a targeted nutritional modification plan for patients with *RAI1* mutations. A recent *Xenopus laevis* model with targeted knockdown of the *rai1* gene has demonstrated a role for *rai1* in neuronal and cartilage precursor migration (20). In addition to changing the facial structure, a reduction in *rai1* causes increased apoptosis of neural cells mediated by reduced *bdnf* expression (19).

Four nonsense mutations (c.238C>T, p.Arg80*; c.1297C>T, p.Gln433*; c.1973G>A, p.Trp658*; and c.2878C>T, p.Arg960*) and several frameshift *RAI1* mutations have been described previously (13, 14, 17) in patients with obesity, hyperphagia, dysmorphic facies, brachydactyly, developmental delay, altered circadian rhythm, and behavioral disturbances, features also seen in our patient, albeit with a different mutation. In comparison, the rapid-onset obesity, hypoventilation, polydipsia, autonomic disturbances of alacrimia, temperature instability, altered sweating, and hypertension seen in our patient, have been reported in the ROHHAD syndrome (3), but not in SMS patients, complicating a priori diagnosis. However, our patient did not have any pituitary function abnormalities or neuroendocrine tumors, features that have been described in patients with ROHHAD syndrome.

In summary, identification of *RAI1* mutation in our patient demonstrates that *RAI1* should be considered a candidate gene in children with morbid obesity, especially when presenting with SMS or ROHHAD-like phenotypic

manifestations. Although a possible genetic basis for ROHHAD syndrome remains unknown, this case highlights the value of WES in the identification of a genetic cause of rare and atypical disease phenotypes. In addition to arriving at the diagnosis, WES was cost-effective when compared to the extensive diagnostic testing performed in the patient (Table 1). Although WES is often described as being "hypothesis-free," it does incorporate an underlying hypothesis—that there is a major monogenic or oligogenic cause for a patient's phenotype. Multiple patients with rare dysmorphic syndromes have had their disease cause determined by WES. The fact that expansion of the clinical phenotype is a frequent outcome of such investigations challenges the traditional approach to differential diagnosis focused on "classical" signs for each disorder, especially in undiagnosed patients with complex neurodevelopmental syndromes (28). This is more than an academic issue in this particular case. The long-term risk for respiratory failure and/or neuroendocrine tumors in our patient remains unknown, but in light of the *RAI1* mutation finding, follow-up assessment including imaging procedures and electrophysiological studies must be weighed against the inherent risks of sedatives and/or anesthetics and economic considerations.

Acknowledgments

The authors thank the proband and his parents for their assistance and participation in this study. We thank Drs Timothy Yu and David Margulies of the Research Connection of Boston Children's Hospital for pipeline and infrastructure development, Paula Maness for exome quality data, and Dr Erinn Rhodes for clinical input.

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This work was supported by the Research Connection at Boston Children's Hospital and the Gene Discovery Core of The Manton Center for Orphan Disease Research. Sanger sequencing was performed by the Molecular Genetics Core Facility of the Intellectual and Developmental Disabilities Research Center at Boston Children's Hospital, supported by National Institutes of Health (NIH) Grant P30 HD18655. V.V.T. was supported by Grants T32DK007699 and P30-DK040561 (Nutrition and Obesity Research Center at Harvard), both from the National Institute of Diabetes and Digestive and Kidney Diseases/NIH. P.B.A. was supported by Grant K08AR055072 from the National Institute of Arthritis and Musculoskeletal and Skin Diseases/NIH. P.B.A. and A.H.B. were supported by Grant U19HD077671 from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development/National Human Genome Research Institute/NIH.

Disclosure Summary: The authors have nothing to disclose.

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