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Rapid-Onset Obesity With Hypothalamic Dysfunction, Hypoventilation, and Autonomic Dysregulation: Analysis of Hypothalamic and Autonomic Candidate Genes

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ABSTRACT: *R*apid-onset *O*besity with *H*ypothalamic Dysfunction, *H*ypoventilation, and *A*utonomic *D*ysregulation (ROHHAD) is a rare and complex pediatric disorder. Despite increased identification and advancing knowledge of the disease course, the variable onset and timing of phenotypic features in ROHHAD often result in delayed or missed diagnosis, potentially leading to fatal central hypoventilation, cardiorespiratory arrest, and impaired neurocognitive development. The 5-hydroxytryptamine receptor 1A (HTR_{1A}), orthopedia (*OTP*), and pituitary adenylate cyclase activating polypeptide (*PACAP*) genes were targeted in the etiology of ROHHAD based on their roles in the embryologic development of the hypothalamus and autonomic nervous system. We hypothesized that variations of HTR_{1A}, *OTP*, and/or *PACAP* would be associated with ROHHAD. All coding regions and intron-exon boundaries of the HTR_{1A}, *OTP,* and *PACAP* genes, in addition to the promoter region of the HTR_{1A} gene, were analyzed by standard sequencing in 25 ROHHAD cases and 25 matched controls. Thirteen variations, including six protein-changing mutations, were identified. None of these variations were significantly correlated with ROHHAD. This report provides evidence that variation of the HTR_{1A}, *OTP,* and *PACAP* genes are not responsible for ROHHAD. These results represent a further step in the investigation of the genetic determinants of ROHHAD. **(*Pediatr Res* 70: 375–378, 2011)**

Rapid-onset *O*besity with *H*ypothalamic Dysfunction, *H*ypoventilation, and *A*utonomic *D*ysregulation (ROHHAD), formerly known as Late-Onset Central Hypoventilation with Hypothalamic Dysfunction, is a rare and complex pediatric disorder for which rapid weight gain is often a harbinger of potentially fatal central hypoventilation. Since the first published description in 1965 (1), ROHHAD has often been associated and confused with Congenital Central Hypoventilation Syndrome (CCHS), specifically later-onset CCHS (LO-CCHS). However, the clinical distinction of ROHHAD from LO-CCHS (2), coupled with the discovery of mutations in the paired-like homeobox 2B (*PHOX2B*) gene as disease-defining in CCHS and LO-CCHS (3–7), and the subsequent lack of these mutations in ROHHAD subjects (8,9), established the distinction between these clinical entities. Since the summary of Katz *et al.* (2) in 2000, describing the 10 previously reported cases of ROHHAD and one new case, more than 65 new cases have been reported in the literature (2,8–12). Accordingly, knowledge of the clinical presentation and clinical course has significantly advanced with detailed descriptions of the phenotype in the world's largest cohorts (8–10) and creation of the acronym ROHHAD to aid diagnosis (8). Despite these advances, the outcome remains variable for ROHHAD patients and diagnosis outside of the few centers with substantial ROHHAD experience remains difficult. Delayed diagnosis can lead to potentially fatal central hypoventilation and impaired neurocognitive development. In addition, 50–60% of the patients have suffered from cardiorespiratory arrest. The prognosis of these children is greatly improved, however, with early identification of the phenotype. As such, development and advancement of diagnostic methods deserves further investigation.

The consistency in symptoms and presentation of the ROHHAD phenotype, coupled with previous reports of familial occurrence (9), has led to a high suspicion for a genetic etiology. However, previous candidate gene studies have failed to identify a disease-associated genetic variation and are limited by small sample size (Table 1). These investigations have focused on genes involved in development and function of the hypothalamic, autonomic, and/or neuroendocrine systems due to deficiencies of these systems in ROHHAD.

As altered development or maintenance of the hypothalamic, autonomic, and neuroendocrine systems remain strong candidates for the developmental basis of ROHHAD, genes central to the function of these systems continue to be targeted

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Abbreviations: ANS, autonomic nervous system; **CCHS,** Congenital Central Hypoventilation Syndrome; **CMH,** Children's Memorial Hospital; HTR_{1A}, 5-hydroxytryptamine (serotonin) receptor 1A; **LO-CCHS,** later onset-Congenital Central Hypoventilation Syndrome; ***OTP***, orthopedia; ***PACAP***, pituitary adenylate cyclase activating polypeptide; **ROHHAD,** rapid-onset obesity with hypothalamic dysfunction, hypoventilation, and autonomic dysregulation; **RUMC,** Rush University Medical Center

Table 1. *Previously reported candidate gene studies in ROHHAD*

Gene	Function	Reference	Number of subjects
NECDIN	Hypothalamic/respiratory	9	10
ASCL1	Neuroendocrine	9	10
PHOX2B	Respiratory/autonomic	8, 9	15/10
TRKB	Neuronal development/ synaptic plasticity	8	15
BDNF	Neuronal development/ synaptic plasticity	8	15

as candidate genes in ROHHAD etiology. The 5-hydroxytryptamine (serotonin) receptor 1A (HTR_{1A}) shows hypothalamic expression and has a known role in appetite control and energy regulation (13). Furthermore, this gene is densely distributed in medullary regions important for cardiorespiratory regulation and has a key role in the autonomic response to homeostatic stress (14,15). Like HTR_{1A}, the orthopedia (*OTP*) gene shows hypothalamic expression, with an important role in hypothalamic cell specification in the developing hypothalamus (16). The paraventricular nucleus complex of the hypothalamus, important in homeostatic control functions, has also been shown to express *OTP* (16). Pituitary adenylate cyclase activating polypeptide (*PACAP*) is expressed in brain regions important to respiratory, cardiovascular, visceral, and thermoregulatory control including the paraventricular nucleus complex of the hypothalamus (17–20). *PACAP* has also been shown to be essential for normal lipid and carbohydrate metabolism (21) as well as maintenance of normal energy homeostasis (22). PACAP also plays an important role in respiratory chemosensitivity and preventing neonatal hypoventilation at reduced body temperatures (23,24).

The variable onset and timing of phenotypic features in ROHHAD often result in delayed or missed diagnosis. Discovery of a diagnostic test for ROHHAD has great potential to decrease morbidity and mortality and improve the overall patient outcome. In addition, ascertainment of the etiology of ROHHAD would improve understanding of development of the hypothalamic and autonomic systems and their roles in feeding behavior and respiratory control and would allow consideration of mechanistically targeted treatment strategies. The HTR_{1A}, *OTP*, and *PACAP* genes were targeted as candidate genes in the etiology of ROHHAD based on their roles in the embryologic development of the hypothalamic, autonomic, and/or neuroendocrine systems. In addition, these genes have known roles in homeostasis, respiratory control, metabolism, and thermal regulation, all processes which appear to be dysfunctional in ROHHAD. We hypothesized that variations within coding regions of the HTR_{1A}, *OTP*, and/or *PACAP* genes would be associated with ROHHAD.

METHODS

Case identification. Twenty-five individuals with clinical features consistent with ROHHAD who were referred to Children's Memorial Hospital (CMH) or Rush University Medical Center (RUMC) for clinical or genetic assessment were identified for inclusion in this study (15/25 cases included in previous ROHHAD genetics studies; Table 1) (8). Medical records for each proband were reviewed to determine the shared characteristics of ROHHAD. All cases were tested and confirmed negative for any known *PHOX2B* gene mutations (methodology provided below). Additional testing or records were requested on a case-by-case basis to ensure uniform clinical assessments. A subset of the children completed comprehensive evaluation at the Center for Autonomic Medicine in Pediatrics at CMH or previously at the Pediatric Respiratory Physiology Laboratory at RUMC.

Criteria for preliminary diagnosis. The criteria for diagnosis of ROHHAD included: onset of alveolar hypoventilation after the age of 1.5 y and evidence of hypothalamic dysfunction, as defined by ≥ 1 of the following findings: rapid onset obesity, hyperprolactinemia, central hypothyroidism, disordered water balance, failed growth hormone stimulation test, corticotropin deficiency, or delayed or precocious puberty.

Matched control identification. A three-generation family history was obtained for gender- and ethnicity-matched, unrelated living controls to ensure that there was no personal or family history of SIDS, Hirschsprung disease, CCHS, a tumor of neural crest origin, or a primary (nonacquired) disorder of the autonomic nervous system (ANS).

PCR and sequencing. Blood samples were obtained from individuals determined to have ROHHAD and gender- and ethnicity-matched controls. Genomic DNA was isolated using a Puregene reagent kit (Qiagen; Hilden, Germany) from white blood cells. Sequencing of the *PHOX2B* gene was performed as previously described (5). All coding regions and intron-exon boundaries of the HTR_{1A}, *OTP*, and *PACAP* genes, in addition to the promoter region of the HTR_{1A} gene, were PCR-amplified and analyzed by standard sequencing in 25 ROHHAD cases and 25 gender- and ethnicity-matched controls. Primer sequences were designed using PrimerQuest or Primer 3 software (Table 2). All participants signed informed consent for sample donation and participation in genetic studies according to CMH and/or RUMC institutional review board-approved protocols.

Statistical analyses. For each case-control comparison, we computed the standard χ^2 tests of independence between the allelic distributions and ROHHAD phenotype. The difference in the number of index alleles in the cases and controls was compared by the Wilcoxon signed-rank test. Significance was applied at $p < 0.05$.

RESULTS

Cohort identification for ROHHAD cases. The 25 ROHHAD cases identified for inclusion in this study included

Table 2. *PCR and sequencing primers for genes examined in this study*

Gene	Region	Forward (5′-3′)	Reverse (5′-3′)	Sequencing (5′-3′)
HTR_{1A}	Promoter	GCTGGACTGTTAGATGATAACGGAGG	CCTGAAAGCTGCTCCTCGGAGATA	GCTGGACTGTTAGATGATAACGGAGG
				CCTGAAAGCTGCTCCTCGGAGATA
	Exon 1	ATTCCCTTCCTCCGAAACTTCCCA	TCATCACTGGCGGCAGAACTTACA	ATTCCCTTCCTCCGAAACTTCCCA
				GACGCATGCACCATTAGCAAGGAT
OTP	Exon 1	TGGTCAAAGCCTTCTCCTCAAGCA	TATCCACGATACCAAAGCAGGGCA	TGGTCAAAGCCTTCTCCTCAAGCA
				TGCAGCGCCAAGGACTACATTTAC
				TGAACACCGACCACATCCTCTACA
PACAP	Promoter/ Exon 1	AGCGCAGGAACGTGAAGAAG	CAAAGGACTGCAGGAAGGAG	AGCGCAGGAACGTGAAGAAG
	Exon 2	CGCGAGCCCCTTACTTTGGA	AAAATCCTGGGTGGGTCACC	CGCGAGCCCCTTACTTTGGA
	Exon 3	CTCTGGAGGTTTCCCTGTCA	TGCCACTTCAAAATCTCCAA	CTCTGGAGGTTTCCCTGTCA
	Exon 4	GCGATTGAACCTGTGTCTCC	ACACGAGCGATGACTGTTGA	GCGATTGAACCTGTGTCTCC

Table 3. *Polymorphisms identified in 25 ROHHAD cases and 25 matched controls*

Gene	RS*	Location	Allele change	Protein	Protein effect	ROHHAD rare allele frequency†	Control rare allele frequency†
HTR$_{1A}$	rs1799921	Exon 1	82A>G	28	Ile/Val	0.02	0.02
	rs6294	Exon 1	294A>G	98	Val/Val	0.12	0.18
	rs1800043	Exon 1	552C>T	184	Pro/Pro	0.02	0.04
	rs1800042	Exon 1	818A>G	273	Asp/Gly	0.02	0.00
	1*	Exon 1	899A>G	300	Asn/Ser	0.00	0.02
OTP	2*	Intron 1	IVS1-85 C>T	—	—	0.02	0.00
	rs62362480	Intron 2	IVS2-32 T>G	—	—	0.46	0.42
PACAP	rs1893154	Promoter	G>A	—	—	0.14	0.18
	rs1893153	Promoter	T>A	—	—	0.36	0.34
	rs8192597	Exon 2	A>G	42	Ala/Ala	0.40	0.41
	rs2856966	Exon 2	A>G	54	Asp/Gly	0.28	0.28
	rs2231185	Intron 2	A>T	—	—	0.10	0.07
	rs2231187	Exon 4	A>G	575	Lys/Lys	0.22	0.22

* Previously unreported variations.

† $p > 0.05$ for all ROHHAD *vs* control, allele frequency comparisons.

14 females and 11 males (20 Caucasian, 3 Asian, and 2 Hispanic).

HTR$_{1A}$ *gene.* Five protein-changing variations (Table 3) were identified in our cohort. No significant differences in frequency of these variations were observed between cases and controls. Overall, nine ROHHAD cases and 13 controls carried at least one *HTR$_{1A}$* variation.

OTP *gene.* Two variations (Table 3) were identified in our cohort. No significant differences in frequency were observed between cases and controls. Overall, 23 ROHHAD cases and 21 controls carried one or both of these *OTP* variations.

PACAP *gene.* Six variations, including 1 protein-changing missense mutation, were identified in this study (Table 3). Significant differences were not observed between ROHHAD cases and controls for frequency of any of these variations.

DISCUSSION

Based on the common phenotypic profile of known ROHHAD cases and our hypothesis that genes involved in hypothalamic, autonomic, and/or neuroendocrine system development and regulation could cause or contribute to the development of ROHHAD, the *HTR$_{1A}$*, *OTP,* and *PACAP* genes were analyzed for disease-associated variation. Thirteen variations were identified within this cohort of 25 ROHHAD cases and 25 matched controls. However, none of the variants identified in this study was significantly associated with ROHHAD. Although this study did not identify ROHHAD-associated variations in any of these genes, it is possible that variation in genes coding for proteins acting elsewhere in the pathways/networks within which *HTR$_{1A}$*, *OTP*, and *PACAP* function may be involved in the etiology of ROHHAD.

Autoimmune and paraneoplastic mechanisms for ROHHAD have also been suggested. Particular consideration has been given to the possibility that ROHHAD may be a paraneoplastic condition (12,25,26) based on the finding that neural crest tumors occur in ~30–50% of reported cases (8–10). Paraneoplastic conditions are disorders resulting from secondary autoimmune responses triggered by the presence of a tumor in the body and can be successfully treated with immunosuppressive therapy and/or tumor removal. Tumors of neural crest origin are well known to trigger paraneoplastic phenomena, including conditions such as opsoclonus-myoclonus syndrome. Recently, Paz-Priel *et al.* (12) described a single case with some symptoms suggestive of ROHHAD in which high-dose cyclophosphamide, an immunosuppressive drug, was given with broad clinical improvement. As this case did not present with the hypoventilation typical of ROHHAD, it is not clear how relevant this result can be to the broader ROHHAD population. The finding does support the need for additional analysis of the hypothesis that ROHHAD may be a paraneoplastic condition, at least in a subset of cases. However, tumors have not been identified in more than 50% of reported ROHHAD cases. In addition, tumor removal in the subset of ROHHAD cases with tumors has failed to alter the other features of the phenotype, and immunosuppressive strategies have produced mixed results (26). Furthermore, the autoantibodies in serum and cerebrospinal fluid that characterize paraneoplastic syndromes have not yet been identified in the few cases of ROHHAD studied (26). However, tumors are often difficult to detect and in other paraneoplastic conditions cases have presented with disease in which tumors and autoantibodies could not be identified, at least initially, after the onset of disease symptoms (27,28). It is also possible that ROHHAD represents an autoimmune phenomenon precipitated by specific environmental exposures in an individual with a susceptible genetic background, as has been postulated for narcolepsy (29). This alternative could explain the variable timing of onset in the disease. Further research will be needed to ultimately determine the validity of the paraneoplastic and autoimmune hypotheses.

Recently, a case of monozygotic twins discordant for the ROHHAD phenotype was identified (29a). Although this discovery does not exclude the possibility that genetic variation is a causative factor in ROHHAD, it challenges the hypothesized monogenic cause and forces stronger consideration of alternate etiologies. Variation in the epigenomes of identical twins has been shown to accumulate quickly and throughout their lifetime, accounting for a great deal of discordance in phenotypes of twins (30). Variation in the epigenome has previously been found to plays a vital role in development of

pediatric diseases of respiratory and autonomic function such as Prader-Willi and Rett Syndrome (31). The possibility that epigenetic variation plays a role in the development of ROHHAD will be analyzed in future studies.

Although this study is the largest published candidate gene analysis in ROHHAD to date, the small sample size remains a limitation. ROHHAD is a rare disease with only 76 cases reported worldwide (2,8–12). Although this is likely a gross underestimate of the total ROHHAD population, identification and recruitment of patients with this disease is difficult. However, continued identification of patients with ROHHAD and refinement of the phenotype is extremely important due both to the devastating consequences of the phenotype itself (especially with late identification) and to the insight that the study of these patients can provide with regard to hypothalamic development and mechanisms underlying other disorders of obesity and of respiratory control. This study is further limited by the fact that only a subset of the cases in this cohort has been examined in the authors' centers. Although inclusion criteria were strict, and required thorough review of all available medical records in all cases by physicians with ROHHAD experience, classification of the children's symptoms as ROHHAD is best made after extensive in-laboratory clinical testing at centers with substantial experience with this disease.

This report provides evidence that genetic variation of the HTR_{1A}, *OTP,* and *PACAP* genes as studied in our cohort is not responsible for ROHHAD. These results represent a further step in the investigation of the genetic determinants of ROHHAD. Future analysis of genes and networks acting in hypothalamic, autonomic, and neuroendocrine systems development and function will be required to ultimately determine which, if any of these, are dysfunctional in ROHHAD. These studies, in addition to further investigation of ROHHAD as a paraneoplastic, autoimmune, or epigenetic condition and continued study of ROHHAD patients in the clinical setting, will guide future research regarding disease etiology and ROHHAD treatment options.

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