

ARTICLE



Testing for rare genetic causes of obesity: findings and experiences from a pediatric weight management program

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BACKGROUND: Genetic screening for youth with obesity in the absence of syndromic findings has not been part of obesity management. For children with early onset obesity, genetic screening is recommended for those having clinical features of genetic obesity syndromes (including hyperphagia).

OBJECTIVES: The overarching goal of this work is to report the findings and experiences from one pediatric weight management program that implemented targeted sequencing analysis for genes known to cause rare genetic disorders of obesity.

SUBJECTS/METHODS: This exploratory study evaluated youth tested over an 18-month period using a panel of 40-genes in the melanocortin 4 receptor pathway. Medical records were reviewed for demographic and visit information, including body mass index (BMI) percent of 95th percentile (%BMIp95) and two eating behaviors.

RESULTS: Of 117 subjects: 51.3% were male; 53.8% Hispanic; mean age 10.2 years (SD 3.8); mean %BMIp95 157% (SD 29%). Most subjects were self- or caregiver-reported to have *overeating to excess or binge eating* (80.3%) and *sneaking food or eating in secret* (59.0%). Among analyzed genes, 72 subjects (61.5%) had at least one variant reported; 50 (42.7%) had a single variant reported; 22 (18.8%) had 2–4 variants reported; most variants were rare (<0.05% minor allele frequency [MAF]), and of uncertain significance; all variants were heterozygous. Nine subjects (7.7%) had a variant reported as *PSCK1* “risk” or *MC4R* “likely pathogenic”; 39 (33.3%) had a Bardet-Biedl Syndrome (BBS) gene variant (4 with “pathogenic” or “likely pathogenic” variants). Therefore, 9 youth (7.7%) had gene variants previously identified as increasing risk for obesity and 4 youth (3.4%) had BBS carrier status.

CONCLUSIONS: Panel testing identified rare variants of uncertain significance in most youth tested, and infrequently identified variants previously reported to increase the risk for obesity. Further research in larger cohorts is needed to understand how genetic variants influence the expression of non-syndromic obesity.

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INTRODUCTION

Obesity is a heterogeneous disorder that develops from a complex interplay of risk factors, which include environmental, biologic, genetic, and social determinants of health [1]. Twin studies have estimated the heritability of obesity to be between 40 and 75% [2]. Genome wide association studies (GWAS) have demonstrated that polygenic obesity is caused by the cumulative influence of many genes whose effect is amplified in an obesogenic environment [1, 3, 4]. In the United States (US), 7.9% of children and adolescents aged 2–19 years have severe obesity, which has been defined as having a body mass index (BMI) \geq 120th percent of the 95th BMI percentile (%BMIp95) for age and sex [5]. Children with severe obesity are at increased risk for cardio-metabolic and psychological comorbidities [6, 7].

Genetic testing for children with syndromic early onset obesity, such as Prader-Willi or Bardet-Biedl syndromes (BBS) [8, 9] may occur at early ages, however, genetic screening for children with obesity in the absence of syndromic features has not been a routine part of pediatric weight management (PWM) care. Rare

genetic forms of obesity in children are poorly understood and likely underdiagnosed [10], despite being responsible for 5–7% of early onset obesity in children [10, 11]. Because genetic variants may influence physiological pathways involved in energy homeostasis, it is important to consider polygenic and monogenic forms of obesity in children with early onset obesity and hyperphagia [12, 13]. In children with early onset obesity, genetic screening is recommended for those who have clinical features of genetic obesity syndromes (including hyperphagia) and/or a family history of severe obesity [13, 14]. If the child presents with developmental delay, dysmorphic features, hormonal deficiencies (e.g., hypogonadism, adrenal insufficiency), congenital anomalies, or vision loss, the threshold for genetic testing should be lowered [14]. Children with underlying genetic disorders may be at greater risk for significant sequelae requiring targeted, thorough, and specialized approaches [15]. The aims of this study are to: (1) describe the findings and experiences from one PWM program implementing targeted sequencing analysis for genes known to cause rare genetic disorders of obesity; (2) provide insight for PWM programs

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considering genetic testing; and (3) identify areas for future research.

SUBJECTS AND METHODS

This exploratory study reports on 117 youth evaluated for rare disorders of obesity using genetic screening. The study included youth seen at a PWM program over 18 months (July 2019 – December 2020). The hospital institutional review board (IRB) determined this study to be secondary research exempt from consent.

PWM program clinicians include medical providers, registered dietitians, and social workers. Approximately 2/3 of the patients receiving care in the program have severe obesity [16]. During the initial visit, detailed family history, including parent-reported weight and height or obesity status are collected. Parental consanguinity is not routinely assessed.

For the first 7 months of the study, providers queried families about child eating behaviors, and caregivers completed a paper survey used in the clinical assessment of hyperphagia or disordered eating (Table 1). For the last 9 months of the study, some patients received care remotely via telemedicine due to COVID-19 risks, so responses were collected through provider interview. After resuming in person visits, parents additionally completed questions 3–7 (Table 1) on a paper survey. Hyperphagia is difficult to assess and measure, and a single definition is evolving in the literature [17]. Patient and/or family reports of frequently feeling hungry were recorded. Genetic testing was offered based on results of the eating behavior screen, concerns raised during the visit, child weight gain patterns, and family history. Study activity tracked which PWM provider tested subjects. Providers were interviewed at the end of data collection to assess their perceptions of and rationale for offering testing.

Individual electronic medical records were reviewed for demographic information (sex, parental-reported race/ethnicity) and visit information (child physical exam, age and %BMIp95). Subjects with %BMIp95 \geq 120% were considered to have severe obesity [5]. Progress notes were reviewed as well as patient/parent responses to eating behavior questions. Subjects with records affirming “Eats in secret or sneaks food” were reported in the variable *sneaking food or eating in secret*. Subjects with records affirming “Continues to eat even though he/she is not hungry”, “Eats to the point of stomach pain or vomiting” or having binge eating behaviors were reported in the variable *overeating to excess or binge eating*. Race and ethnicity information were categorized into 4 groups: non-Hispanic Black (hereafter, Black); Hispanic; non-Hispanic White (hereafter, White); and non-Hispanic Other (hereafter, Other). Youth in the Other race and ethnicity grouping were of Asian and multiracial ancestry. Self-reported weight and height of biological parents were obtained from records and BMI was calculated when data were available. Parental BMI \geq 30 kg/m² or noted as having obesity were recorded to have obesity [18].

The clinic completed testing at no cost to patients through a program sponsored by Rhythm Pharmaceuticals (Boston, MA, USA), a company developing drug therapy for treatment of rare disorders of obesity [19]. Buccal swab or saliva samples were collected and sent to a clinical lab for analysis (PreventionGenetics, Marshfield, WI, USA). Reports were sent to the providers, who shared results with families. The program offers free testing to parents and siblings of youth to clarify inheritance and family phenotype patterns, for example for youth having a variant of uncertain significance in a gene known to cause autosomal dominant (AD) disease or when two changes to the same gene known to cause autosomal recessive (AR) pathology are found.

Table 1. Eating behavior questions.

1. Do you frequently feel very hungry?
2. Eats or drinks anything during the night (besides water)?
3. Eats in secret or sneak food?
4. Is very focused on food?
5. Continues to eat even though they are not hungry?
6. Eats in response to a feeling?
7. Tendency to be anxious?
8. Eats a large amount of food in short time?
9. Eats to the point of stomach pain or vomiting?

Clinical indications for testing include youth \leq 18 years with BMI \geq 97th percentile for age and sex. This panel sequences 40 genes for which obesity is a common feature, including genes in the melanocortin 4 receptor (MC4R) pathway [10, 20], including 22 Bardet-Biedl (BBS) genes (Table 2) [21]. Methods used in the testing process can be found at the PreventionGenetics Website [22].

The genetic testing reports issued to the clinical team include the following information: the specific gene(s) involved; Online Mendelian Inheritance in Man (OMIM) number; mode of inheritance established for pathogenic variants in that gene to cause disease (autosomal recessive [AR], autosomal dominant [AD], both AR/AD, or unknown); genetic variant (i.e., nucleotide change with predicted amino acid sequence change); clinical variant identification number (ClinVar ID) [23] highest allele frequency among any population gnomAD; [24] in silico missense predictions; and variant interpretation. The specific in silico algorithms used by the lab are Polymorphism Phenotyping V-2 (PolyPhen-2), Sorting Intolerant From Tolerant (SIFT), Mutation Taster, and Functional Analysis Through Hidden Markov Models (FATHMM) [25, 26]. Per American College of Medical Genetics guidelines [27], gene variants are interpreted as “pathogenic”, “likely pathogenic”, “variant of uncertain significance” (VUS), “likely benign”, and “benign”. The “likely benign” and “benign” variants are not listed in the reports. The testing lab applies the classification of “risk” alleles for variants that are common in the general population, but have been shown to be a risk factor for obesity in laboratory and population/family studies as well as by expert consensus of well-established low penetrance variants reported in ClinVar [28].

Reports from genetic testing for each subject (and family members if tested) were reviewed and collated. To explore differences in evaluated characteristics, subjects were placed into four groups based on known inheritance patterns for target genes and risk/pathogenicity: (1) negative (no variant identified); (2) subjects with a variant reported as *PCSK1* “risk” or *MC4R* “likely pathogenic”; (3) subjects having at least one variant in a gene known to cause disease in AD or AD/AR inheritance patterns with or without other gene variants (excluding group 2); and (4) subjects with variants in genes known to cause disease in AR inheritance patterns only (excluding group 2).

We report descriptive data overall and by gene variant group for categorical variables, including child sex, age group, race, and ethnicity group, two eating behavior variables (*sneaking food or eating in secret* and *overeating to excess or binge eating*), and biological maternal and paternal obesity status, and for the continuous variables %BMIp95 and age. Fisher’s Exact and Kruskal-Wallis tests, as appropriate, were applied to evaluate between group differences. Analyses were done using IBM SPSS Statistics, version 27.

RESULTS

Overview, subject characteristics, and provider response

During the 18-month study period, 117 children with obesity and hyperphagia were screened for rare genetic causes of obesity. Two subjects were siblings. About half of subjects were male (51.3%), half were Hispanic (53.8%), mean age was 10.2 years (SD, 3.8), mean %BMIp95 was 157% (SD 29), and almost all subjects (95.7%) had severe obesity (Table 3). No subjects had syndromic findings on physical exam. Seventy-two subjects (61.5% of individuals tested) had at least one variant reported among the analyzed genes; 50 (42.7% of individuals tested; 69.4% of those with a variant) had a single variant, and 22 (18.8% of the total tested; 30.1% of those with a variant) had more than one variant reported. Seventeen youth had 2 variants, 3 youth had 3 variants, and 2 had 4 variants reported (Table 4). All variants found were heterozygous with no complex heterozygosity. Of the 5 providers in the PWM, the majority (78%) of testing was offered by one provider with the remaining distributed among the other providers. Providers reported feeling unprepared to explain the results to families, had concerns about the added workload of contacting families with results, and thought that results would not influence care.

Reported variants

Among 72 subjects with a variant identified, 93 unique variants were reported in 34 different genes (Supplementary Table 1).

Table 2. Genes Analyzed.

Gene	Gene name	Gene transcript NCBI # ^a
<i>ADCY3</i>	Adenylate cyclase type 3	NM_001320613.1
<i>ALMS1</i>	Alstrom 1	NM_015120.4
<i>ARL6¹ (BBS3)</i>	ADP-ribosylation factor-like-6	NM_032146.5; NM_001323513.1
<i>BBIP1¹ (BBS18)</i>	BBSome-interacting protein 1	NM_001195306.1; NM_001195304.1
<i>BBS1¹</i>	Bardet-Biedl Syndrome 1	NM_024649.4
<i>BBS2¹</i>	Bardet-Biedl Syndrome 2	NM_031885.3
<i>BBS4¹</i>	Bardet-Biedl Syndrome 4	NM_033028.4
<i>BBS5¹</i>	Bardet-Biedl Syndrome 5	NM_152384.2
<i>BBS7¹</i>	Bardet-Biedl Syndrome 7	NM_176824.2; NM_018190.3
<i>BBS9¹ (PTHB1)</i>	Bardet-Biedl Syndrome 9	NM_001348041.2; NM_198428.2
<i>BBS10¹</i>	Bardet-Biedl Syndrome 10	NM_024685.3
<i>BBS12¹</i>	Bardet-Biedl Syndrome 12	NM_152618.2
<i>BDNF</i>	Brain derived neurotrophic factor	NM_001143810.1; NM_170734.3; NM_001143809.1; NM_170731.4
<i>CEP290¹ (BBS14)</i>	Centrosomal protein 290	NM_025114.3
<i>CFAP418¹ (BBS21)</i>	Chromosome 8 open reading frame 37	NM_177965.3
<i>CPE</i>	Carboxypeptidase E	NM_001873.3
<i>GNAS</i>	Guanine nucleotide binding protein, alpha stimulating complex locus	NM_016592.3; NM_080425.3; NM_000516.5; NM_001309842.1; NM_001077488.3
<i>IFT27¹ (BBS19)</i>	Intraflagellar transport 27	NM_006860.4; NM_001177701.2
<i>IFT74¹ (BBS22)</i>	Intraflagellar transport 74	NM_025103.2; NM_001099224.1; NM_001349928.1
<i>IFT172¹ (BBS20)</i>	Intraflagellar transport protein 172	NM_015662.2
<i>KSR2</i>	Kinase Suppressor of Ras 2	NM_173598.4
<i>LEP</i>	Leptin	NM_000230.2
<i>LEPR</i>	Leptin receptor	NM_002303.5; NM_001198688.1; NM_001003680.3; NM_001003679.3
<i>LZTFL1¹ (BBS17)</i>	Leucine zipper transcription factor-like protein 1	NM_020347.3; NM_001276379.1
<i>MC3R</i>	Melanocortin-3 receptor	NM_019888.3
<i>MC4R</i>	Melanocortin-4 receptor	NM_005912.2
<i>MKKS¹ (BBS6)</i>	McKusick-Kaufman syndrome	NM_018848.3
<i>MKS1¹ (BBS13)</i>	MKS transition zone complex subunit 1 (Meckel syndrome, type 1)	NM_001321269.1; NM_017777.3; NM_001165927.1
<i>NCOA1</i>	Nuclear receptor coactivator 1	NM_003743.4; NM_001362950.1
<i>NTRK2</i>	Neurotrophic receptor tyrosine kinase 2	NM_006180.4; NM_001007097.2; NM_001018065.2
<i>PCSK1</i>	Proprotein convertase subtilisin kexin type 1	(NM_000439.4; NM_001177875.1)
<i>PHF6</i>	PHD Finger Protein 6	NM_032458.2; NM_032335.3
<i>POMC</i>	Proopiomelanocortin	NM_000939.3
<i>RAI1</i>	Retinoic Acid Induced 1	NM_030665.3
<i>SDCCAG8¹ (BBS16)</i>	Serologically defined colon cancer antigen 8	NM_006642.3; NM_001350248.1
<i>SH2B1</i>	Src homology 2B 1	NM_001145795.1; NM_001145797.1; NM_001145796.1
<i>SIM1</i>	Single-minded homolog 1	NM_005068.2
<i>TRIM32¹ (BBS11)</i>	Tripartite Motif Containing 32	NM_012210.3
<i>TTC8¹ (BBS15)</i>	tetratricopeptide repeat domain 8	NM_144596.3; NM_001288782.1; NM_001288781.1

^aEntrez Gene is the gene-specific database at the National Center for Biotechnology Information (NCBI), a division of the National Library of Medicine, located on the campus of the US National Institutes of Health in Bethesda, MD, USA. Entrez Gene generates unique integers (GeneID) as stable identifiers for genes and other loci for a subset of model organisms.

¹Bardet-Biedl Syndrome (BBS) gene.

Among those screened, variants were found almost equally among all age groups (Table 3). Approximately one-quarter of the sample were aged 2–6 years, but among children in this age group 22/29 (75%) had a variant reported. Among older children, just over half had a variant reported. There was a significant difference in groups by race and ethnicity ($p = 0.019$), with 66.7%

of White youth in the group having a *PCSK1* “risk” or *MC4R* likely pathogenic variant. Frequency of White ethnicity among other groups ranged from 5.0% to 31.1%. Many children were reported to be *sneaking food or eating in secret* (59.0%) and most (80.3%) were *overeating to excess or binge eating*. Most of the youth had a biological parent with obesity. Frequencies were similar across the

Table 3. Characteristics by gene variant group.

Characteristic	Total Sample (N = 117)		Gene variant group		At least 1 AD or AD/AR variant ^b (n = 20, 17.1%)		All AR variant(s) including BBS ^c (n = 43, 36.8%)		p value ^e
	n	(%) ^d	n	(%) ^d	n	(%) ^d	n	(%) ^d	
Sex									0.902
Male	60	(51.3)	22	(43.3)	4	(44.4)	10	(50.0)	(55.8)
Female	57	(48.7)	23	(56.7)	5	(55.6)	10	(50.0)	(44.2)
Age Group									0.126
2–6 years	29	(24.8)	7	(40.0)	5	(55.6)	8	(40.0)	(20.9)
7–11 years	47	(40.2)	20	(33.3)	3	(33.3)	6	(30.0)	(41.9)
12–17 years	41	(35.0)	18	(26.7)	1	(11.1)	6	(30.0)	(37.2)
Race/Ethnicity									0.019
Black	14	(12.0)	5	(11.1)	1	(11.1)	1	(5.0)	(16.3)
Hispanic	63	(53.8)	23	(51.1)	2	(22.2)	15	(75.0)	(53.5)
Other	6	(5.1)	3	(6.7)	0	(0.0)	3	(15.0)	(0.0)
White	34	(29.1)	14	(31.1)	6	(66.7)	1	(5.0)	(30.2)
Sneaking food or eats in secret									0.077
Yes	69	(59.0)	22	(48.9)	5	(55.6)	10	(50.0)	(74.4)
No	48	(41.0)	23	(51.1)	4	(44.4)	10	(50.0)	(25.6)
Overeating to excess or binge eating									0.875
Yes	94	(80.3)	37	(82.2)	7	(77.8)	17	(85.0)	(76.7)
No	23	(19.7)	8	(17.8)	2	(22.2)	3	(15.0)	(23.3)
Biological mother with obesity									0.763
Yes	75	(64.1)	28	(70.0)	5	(71.4)	12	(66.7)	(78.9)
No	28	(23.9)	12	(30.0)	2	(28.6)	6	(33.3)	(21.1)
Unknown	14	(12.0)							
Biological father with obesity									0.671
Yes	60	(51.3)	23	(71.9)	6	(85.7)	9	(64.3)	(78.6)
No	21	(17.9)	9	(28.1)	1	(14.3)	5	(35.7)	(21.4)
Unknown	36	(30.8)							
Median									
Age, years	10.2	2.6–17.9	10.2	4.3–16.5	6.3	2.8–13.5	9.8	3.9–17.9	10.6
%BMIp95	153	100–274	153	100–274	156	103–173	146	116–177	155

^aBMIp95, percent of 95th percentile body mass index for age and sex.

^bwith or without other gene variants.

^chaving at least one variant in a gene known to cause disease following an AD or AD/AR inheritance pattern, with or without other gene variants, not having PCSK1 “risk” variant.

^dincludes those with AR disease gene variant scoring as “pathogenic”, “likely pathogenic”, and “uncertain” interpretation, not having PCSK1 “risk” variant.

^ePercentages may not add to 100% due to rounding.

^fFisher’s Exact tests for categorical data and Kruskal-Wallis test for continuous data.

Table 4. Gene variants by subject ($N = 72$). All variants are heterozygous.

# Subjects	Subject ID	Established inheritance in OMIM				Risk assessment
		AD	AD, AR	AR	AR, BBS	
Youth with Multiple Gene Variants Reported ($n = 22$, 18.8%)						
1	16	<i>KSR2</i>	<i>MC4R</i>	<i>PCSK1</i>	<i>BBS1</i>	<i>PCSK1</i> – risk Others VUS
1	36	<i>KSR2</i>			<i>MKS1</i>	Both VUS
1	50	<i>MC3R</i>		<i>ADCY3</i>		Both VUS
1	8	<i>NCOA1</i>		<i>PCSK1</i>	<i>ITF74</i>	<i>PCSK1</i> – risk Others VUS
1	26	<i>NTRK2</i> <i>RAI1</i>				Both VUS
1	11	<i>RAI1</i>		<i>ALMS1</i> <i>LEPR</i>		All VUS
1	37	<i>RAI1</i>		<i>PCSK1</i>		Both VUS
1	7	<i>SH2B1</i> <i>SIM1</i>				Both VUS
1	9		<i>MC4R</i>	<i>PCSK1</i>		<i>MC4R</i> – likely pathogenic <i>PCSK1</i> – risk
1	23		<i>POMC</i>		<i>TTC8</i>	Both uncertain
1	40			<i>ALMS1</i> <i>LEPR</i>	<i>TRIM32</i> <i>WDPCP</i>	<i>WDPCP</i> – likely pathogenic Others VUS
1	18			<i>LEPR</i>	<i>BBS9</i> ^a <i>BBS9</i>	All VUS
1	2			<i>LEPR</i>	<i>IFT172</i>	Both VUS
1	53			<i>LEPR</i>	<i>LZTFL1</i>	Both VUS
1	46			<i>PCSK1</i>	<i>BBS9</i>	Both VUS
1	62			<i>PCSK1</i>	<i>BBS12</i>	<i>PCSK1</i> – risk <i>BBS12</i> – likely pathogenic
1	54			<i>PCSK1</i>	<i>CEP290</i>	<i>PCSK1</i> – risk Other VUS
1	5			<i>PCSK1</i>	<i>IFT74</i>	<i>PCSK1</i> – risk Other VUS
1	31			<i>PCSK1</i>	<i>TTC8</i>	<i>PCSK1</i> – risk Other VUS
2	38, 48				<i>BBIP1</i> ^a <i>BBIP1</i>	Both VUS
1	61				<i>CEP290</i> <i>ITF172</i>	Both VUS
Youth with Single Gene Variants Reported ($n = 50$, 42.7%)						
1		<i>BDNF</i>				VUS
1		<i>GNAS</i>				VUS
3		<i>RAI1</i>				VUS
1		<i>SH2B1</i>				VUS
4			<i>MC4R</i>			VUS
3			<i>POMC</i>			VUS
2				<i>ADCY3</i>		VUS
4				<i>ALMS1</i>		VUS
3				<i>CPE</i>		VUS
1				<i>LEPR</i>		VUS
2				<i>PCSK1</i>		Risk
2				<i>PCSK1</i>		VUS
1					<i>BBIP1</i>	VUS
1					<i>BBS1</i>	VUS
3					<i>BBS9</i>	VUS
1					<i>BBS10</i>	Pathogenic
1					<i>CEP290</i>	Likely pathogenic

Table 4. continued

# Subjects	Subject ID	Established inheritance in OMIM				Risk assessment
		AD	AD, AR	AR	AR, BBS	
3					CEP290	VUS
3					IFT172	VUS
1					IFT174	VUS
1					LZTFL1	VUS
1					MKKS	VUS
5					SDCCAG8	VUS
1					TRIM32	VUS
1					TTC8	VUS

AR autosomal recessive, AD autosomal dominant, BBS Bardet-Biedl Syndrome, OMIM Online Mendelian Inheritance in Man, VUS variant of uncertain significance.

^aindividual with 2 *BBS9* variants and one individual with 2 *BBIP1* variants confirmed to be *in cis* on the same haplotype, inherited from 1 parent; no parental testing available on the other individual with 2 *BBIP1* variants, that individual does not meet BBS clinical diagnostic criteria.

gene groupings for sex, eating behavior variables, and parental obesity status (Table 3). All groups had similar %BMIp95.

Nine youth (7.7%) had the same variant reported as *PCSK1* “risk” (see Supplementary Table 1) [29–31]. One youth with a *PCSK1* “risk” variant also had an *MC4R* variant reported as “likely pathogenic”. Among these 9 youth, 2 (22.2%) had a single variant, 5 (55.6%) had 2 variants, 1 (11.2 %) subject had 3 and 1 (11.2 %) subject had 4 variants. Other reported variants of *PCSK1* ($n = 4$) were VUS. Detailed information (e.g., ClinVar ID, known inheritance mode for which that gene causes disease, nucleotide alteration and amino acid change, etc.) on each gene variant per subject can be found in Supplementary Table 1.

There were 20 youth (Table 4) who had at least one heterozygous VUS in genes associated with AD or AD/AR inheritance in Mendelian forms of obesity. Seven of these youth had more than one VUS including 5 with one or more variants in genes that cause disease through AR inheritance; 2 of these 5 youth had a Bardet-Biedl syndrome (BBS) gene variant. Of the 20 youth in this group, 14 youth had variants in genes associated previously with AD inheritance, including *BDNF*, *GNAS*, *KSR2*, *MC3R*, *NCOA1*, *NTRK2*, *RAI1*, *SH2B1*, and *SIM1* (Table 4, Supplementary Table 1). *MC4R*, and *POMC* are associated with both AD and AR disease. Variants in *MC4R* ($n = 6$) and *POMC* ($n = 4$) were reported for 11 youth: including one *MC4R* “likely pathogenic” variant noted above.

We had 22 subjects whose immediate families qualified for genetic testing at no cost and counseling under the sponsored program [21]. Families of 14 subjects completed testing of one or more additional family members, 3 declined and 5 were not offered as they were lost to follow-up.

There were 43 youth (Table 4) with at least one variant in a gene that causes obesity disorders through AR inheritance, including 5 youth with two or more variants in genes known to cause AR disease. In addition, 31 of these 43 youth had one or more BBS gene variants (26 had a single BBS variant, 5 had two BBS variants). Twenty-nine youth had other gene variants in non-BBS AR disease genes (*ADCY3*, *ALMS1*, *CPE*, *LEPR*, and *PCSK1*). These include the *PCSK1* variants assessed as “risk” noted above. Six individuals had an *ALMS1* variant, all reported as VUS. Two subjects with *ALMS1* variants also had a *LEPR* variant and one subject with an *ALMS1* variant also had 2 BBS gene variants (Table 4). *CPE* variants ($n = 3$) were reported for 3 youth; all reported as VUS.

There were 39 youth (33%) with a BBS gene variant across the whole sample. There were a total of 43 variants reported in 16 different genes of the 22 tested that have been associated with BBS [9], an AR condition (*BBIP1*, *BBS1*, *BBS9*, *BBS10*, *BBS12*, *CEP290*, *IFT74*, *IFT172*, *IFT174*, *LZTFL1*, *MKKS*, *MKS1*, *SDCCAG8*, *TRIM32*, *TTC8*,

WDPCP) (Table 2). One subject had a single heterozygous variant in *BBS10* assessed as “pathogenic” and 3 subjects had a single heterozygous variant assessed as “likely pathogenic” (*WDPCP*, *BBS12*, *CEP290*). There were 3 youth identified to have 2 BBS gene variants within the same BBS gene (Table 4). To confirm carrier status and segregation of variants, testing of family members was recommended; only 2 of 3 families completed testing. One youth with 2 *BBIP1* variants assessed as VUS inherited both *in cis* from a single parent with obesity, ruling out AR inheritance. Similarly, the youth with two *BBS9* variants inherited both from a single parent with obesity.

DISCUSSION

Main findings

This study describes the findings and experiences of targeted sequencing analysis for genes that cause rare disorders of obesity in youth with obesity and hyperphagia in a PWM program. Among youth screened, 61.5% ($n = 72$) had a variant fulfilling objective criteria reported. Fifty subjects (42.7%) had one variant and 22 subjects (18.8%) had multiple variants. Most variants were of uncertain significance. Only 9 subjects (7.7%) had a “risk”, or “likely pathogenic” variant interpreted as increasing risk for obesity. Serra-Juhe et al. [32], reported that ~5% (23/463) of children with early onset obesity had a likely pathogenic variant contributing to obesity risk. Loid et al. [33], identified pathogenic/likely pathogenic variants in 8% (7/92) of subjects with severe early-onset obesity before age 10 years. Variants in several genes known to cause rare forms of monogenic obesity have also been shown to contribute to polygenic obesity [34, 35]. Notably, variant pathogenicity interpretation can evolve over time; new clinical, genetic, or functional data may influence pathogenicity calls and result in differences between labs. For example, the genetics lab used for this study reported the *PCSK1* (ClinVar ID 14040) variant as “risk” and the *LEPR* (ClinVar ID 631614) variant as uncertain which is discrepant from current ClinVar reports [29, 30, 36–51] (Supplementary Table 2), highlighting the importance of variant reanalysis. Without extensive efforts to provide genetic and functional evidence of variant effect, such as segregation analysis to establish inheritance patterns, more extensive examination of phenotypic characteristics, assessment in physiologically relevant experimental systems, and transcriptomic analysis of subject biospecimens, interpretation of whether these variants of uncertain significance do or do not impart risk for obesity is limited.

MC4R pathogenic variants have been reported as the most common cause of early onset obesity with prevalence rates up to

7% in primarily White European populations [1, 11]. However, among the 6 subjects who had a *MC4R* variant in our sample, only one youth (1%) had a “likely pathogenic” variant. This discrepancy in prevalence may reflect differences in frequencies between racial and ethnic groups as our subjects were 29% White [52, 53], or may be due to the modest size of our study cohort. A total of 9 (7.7%) youth had genetic changes (*PCSK1* “risk”, *MC4R* “likely pathogenic”) established as increasing risk for obesity. There were 4 youth (3.4%) identified as carriers for BBS (“pathogenic” or “likely pathogenic”: *WDPCP*, *BBS12*, *BBS10*, *CEP290*). One of these 4 youth also had a *PCSK1* “risk” gene in addition to being a carrier for a BBS gene variant.

There were 39 (33.3%) youth with a rare (MAF < 0.5%) heterozygous BBS gene variant, including 4 with “pathogenic” or “likely pathogenic” variants. This equates to a BBS gene variant carrier frequency of ~1 in 29. Assuming an estimated incidence of 1/100,000 for BBS, Sapp, et al. [54], estimated carrier frequency for pathogenic variants in 12 different BBS loci ranging from 1 in 250 to 1 in 2,200. BBS is a clinically heterogeneous disorder hallmarked by obesity, polydactyly, retinitis pigmentosa, renal anomalies, and learning difficulties [55]. It is also genetically heterogeneous, following a predominantly autosomal recessive inheritance pattern of at least 27 genes that play crucial roles in ciliary function [55–57]. There have been varying reports of BBS gene carrier status affecting risk for obesity [58]. Pathogenic alteration in BBS genes decreases leptin sensitivity, increases *LEPR*, and causes overactivity of the *LEPR* signaling pathway leading to leptin resistance [34, 35]. Balanced leptin signaling is needed for a normal satiety response [59, 60]. Heterozygous carriers of pathogenic BBS genes variants are expected to lack syndromic and phenotypic characteristics, but it is possible that monoallelic variants may contribute to increased risk for obesity in certain contexts [35, 61]. Our preliminary data suggest a potential enrichment of rare protein-coding variants in BBS genes compared to the general population. Notably, the BBS gene variants identified in our cohort are rare when compared to ethnically-matched populations in gnomAD (9% of variants ≤ 0.5% and > 0.1% MAF; 66% of variants ≤ 0.1% and < 0.001%); and 25% of variants ≤ 0.001% MAF; (Supplementary Table 1). Although we recognize that a majority of variants were interpreted as VUS, previous reports have shown that rare allele frequency correlates with a greater likelihood for variant pathogenicity [62]. Even so, family member testing, expanded testing to include all causal BBS and/or known ciliopathy genes, targeted clinical evaluation for clinical characteristics associated with BBS [55], and expanded cohort size are needed to formally substantiate the clinical significance of observations from this study [63].

Everyone in our cohort with variants in genes associated with AD or AR/AD inherited disease and the two youth with 2 *BBIP1* and 2 *BBS12* variants qualified for family testing though not all were completed due to difficulties in follow-up. For individuals with variants in genes associated with AD inheritance, family studies are indicated to delineate inheritance. For example, for individuals harboring *GNAS* variants, family testing and testing for calcium metabolism disorders may be indicated [64]. Individuals with some *CPE* variants are at increased risk for early onset type 2 diabetes [65], and those with variants of *KSR2* may have severe insulin resistance that responds well to metformin [66]. Thus, identification of variants associated with rare causes of obesity may provide insight into clinical care and further evaluation. The high frequency of obesity among family members in our cohort suggest further data on inheritance patterns within families may be helpful.

Lessons learned and recommendations

At this PWM program gene variants were common in our cohort, though most were VUS. Several subjects had more than one gene variant reported. Our current understanding is limited regarding

how gene variants, regardless of significance, influence obesity and hyperphagia [67]. Findings from his study suggest that identification of particular variants which may be contributing to the child’s obesity can help guide more personalized obesity care and help identify patients who may or may not respond to drug therapies or bariatric surgery [9, 15, 68].

We identified differences in how testing was employed across providers. While some providers routinely offered testing others were more selective. Development of clinical tools to increase provider confidence in offering testing and sharing the results may be helpful. Innovative ways to collaborate with colleagues in genetics, including genetic counselors as ad hoc consults embedded in PWM programs may prove to be both time and cost effective.

While information gained from genetic testing did not lead to a specific diagnosis, the structured hyperphagia assessment was helpful for providers to understand what families are experiencing. Currently there is not a standardized way to assess for hyperphagia and more research is needed [17]. Likewise, most genetic variants found were VUS, which meant providers and families were left with test results that were indeterminate based on current evidence. Though inconclusive results may not change current treatment, providers can communicate how our understanding of genetic data is evolving. Future research partnerships are needed to understand provider and family concerns regarding the testing process, their understanding of the results, and their perceptions of how the results may influence their decision-making regarding treatment and management.

We provided free genetic screening through the sponsoring lab [21], otherwise testing would have been costly for the families and the healthcare facility. This suggests that it may be important to develop comprehensive diagnostic and management strategies for youth with hyperphagia that include testing for genetic disorders [8, 15]. These strategies should include insurance coverage for genetic screening and genetic counseling given the potential for ambiguous results.

LIMITATIONS

The gene panel used for this study [21] is not comprehensive and other genes not evaluated may be contributing to null findings [69]. The reporting of the risk for obesity associated with each variant may differ between clinical labs. Due to the small sample size and lack of comparison of our findings to larger population database phenotypic and genotypic findings, it is not possible to determine if the subjects tested have a higher or lower prevalence of gene variants associated with obesity. Our findings can neither establish nor refute causality for any of the variants, nor was that the intent of this study. GWAS, mutational burden analysis, and functional studies to understand the impact of rare variants are required to understand significance, if any, of many of the variants found. Our study did not routinely include family phenotype or testing which is needed to establish causality of specific variants. These evaluations were beyond the scope of this clinical study.

A series of 9 questions were used to assess hyperphagia. Though not validated, they are the result of clinical experience in this field. A validated hyperphagia questionnaire has been used in studies of children with Prader-Willi [17], but translation of this to the clinical PWM setting has not been reported. Only children with obesity and hyperphagia were tested, so the frequency of genetic variants among youth receiving care at this PWM program with obesity not assessed as having hyperphagia is not known. Nor did we collect data on other characteristics which have been associated with increased risk of obesity (such as neurodevelopmental concerns and medications). Information was not collected to track youth offered genetic screening, but whose caregiver refused. Finally, interruptions/alterations in care due to

COVID-19 restrictions and access to clinic resources contributed to a non-uniform application of testing by clinic providers.

CONCLUSIONS

Our study reports findings and experiences of genetic testing for genes in the MC4R pathway among youth with obesity and hyperphagia being seen at a PWM program. Implementation of testing identified VUS in most youth tested, and variants previously identified to increase risk for obesity were infrequently found. Thus, providers and families were left with indeterminate test results based on the current state of knowledge. Further research in larger cohorts, which include familial and functional studies, is needed as to better understand how genetic variants associated with risk for obesity influence the expression of non-syndromic obesity. In addition, inclusion of provider, patient/family experiences with genetic testing and how it may impact treatment should be considered.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are not publicly available due to IRB restrictions but are available from the corresponding author on reasonable request and approval of the IRB of record.

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AUTHOR CONTRIBUTIONS

KR and HB conceptualized the project, conducted data collection and analysis, and wrote the initial manuscript draft. All authors (AA, KS, MQ, CM, SN, EED) were involved in the writing, review and editing of the manuscript and had final approval of the submitted and published versions. Specifically, KR: Conceptualization, methodology, validation, investigation, writing original draft, project administration, AA: writing-review & editing, KS: investigation, writing-review & editing, MQ: investigation, writing-review & editing, CM: writing-review & editing, SN: writing-review & editing, EED: writing review & editing, HB: Conceptualization, methodology, validation, investigation, formal analysis, writing-original draft, supervision.

COMPETING INTERESTS

The authors declare no conflict of interest and have not received any compensation from PreventionGenetics or Rhythm Pharmaceuticals.

ADDITIONAL INFORMATION

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