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RESEARCH REPORT

The tale of two genes: from next-generation sequencing to phenotype

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Abstract An 18-yr-old man with a history of intellectual disability, craniofacial dysmorphism, seizure disorder, and obesity was identified to carry a de novo, pathogenic variant in *ASXL1* (c.4198G>T; p.E1400X) associated with the diagnosis of Bohring–Opitz syndrome based on exome sequencing. In addition, he was identified to carry a maternally inherited and likely pathogenic variant in *MC4R* (c.817C>T; p.Q273X) associated with monogenic obesity. Dual genetic diagnosis occurs in 4%–6% of patients and results in unique clinical phenotypes that are a function of tissue-specific gene expression, involved pathways, clinical expressivity, and penetrance. This case highlights the utility of next-generation sequencing in patients with an unusual combination of clinical presentations for several pillars of precision medicine including (1) diagnosis, (2) prognosis and outcome, (3) management and therapy, and (4) utilization of resources.

INTRODUCTION

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Ontology terms: childhood-onset truncal obesity; intellectual disability, profound; microretrognathia; prominent glabella

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De novo splice-site, nonsense, and frameshift variants in *ASXL1* (additional sex combs-like 1) cause the multisystemic, autosomal dominant Bohring–Opitz syndrome (BOS; OMIM 605039) (Dangiolo et al. 2015). *ASXL1*, located at 20q11.21, is an enhancer of trithorax and Polycomb (ETP) gene that encodes a protein that regulates transcription of HOX (Fisher et al. 2003, 2010). Members of the *ASXL* family of genes also regulate adipogenesis through modulation of PPARγ activity; specifically, ASXL1 represses the adipogenesis (Park et al. 2011). ASLX1 protein has 1541 amino acids, and protein truncation due to loss of function variants causes reduction of transcription factor zinc finger ZIC1, which contributes to the BOS phenotype (Matheus et al. 2019). Somatic variants in *ASXL1* have been reported in myeloid malignancies. Evidence suggests that this gene may act as a tumor suppressor (Gelsi-Boyer et al. 2009).

The clinical phenotype of BOS is characterized by recognizable craniofacial dysmorphism including microcephaly or trigonocephaly, prominent metopic ridge, synophrys, glabellar and eyelid nevus flammeus, exophthalmus, hypertelorism, palate anomalies, and micrognathia. Other features include severe intrauterine growth retardation, poor feeding, failure to thrive, profound intellectual disability, flexion of elbows with ulnar deviation, and flexion of wrists and metacarpophalangeal joints (Russell et al. 1993; Hoischen et al. 2011; Visayaragawan et al. 2017). Minor cardiac anomalies and increased risk of Wilms' tumor have been reported (Russell et al. 2015). Although the phenotype of BOS can be highly variable, severe prenatal and postnatal growth deficiencies have consistently been noted in all reported cases (Russell et al. 2015). Obesity, on the other hand, has only been reported in

one case when the patient's body mass index (BMI) increased significantly in early childhood (Pierron et al. 2009).

Although obesity is a complex and multifactorial disorder, several genes have been identified as causes for monogenic obesity. Leptin (*LEP*) (Montague et al. 1997; Strobel et al. 1998) and its related genes (*Leptin receptor* [*LEPR*], *pre-pro-opiomelanocortin* [*POMC*] [Krude et al. 1998], *and melanocortin-4 receptor [MC4R]* [Vaisse et al. 1998]) are the first genes that have been associated with monogenic obesity (Geets et al. 2018). *MC4R* variants are the most common cause of monogenic form of obesity in European populations (Hinney et al. 2013). The MC4R protein has 332 amino acids, and its loss-of-function variants causing protein truncation are known to lead to obesity (Vaisse et al. 1998; Yeo et al. 1998). Penetrance and expression of *MC4R* variants are variable within and between family members. Among family members with *MC4R* variants, some have no obesity at all, some have early-onset severe childhood obesity, and some have adult-onset obesity (Dubern et al. 2001; Stutzmann et al. 2008).

We report a patient with features of BOS and morbid obesity who was diagnosed through exome sequencing (ES) following a diagnostic odyssey. In this case, ES not only identified a de novo pathogenic variant in *ASXL1* but also a likely pathogenic, maternally inherited variant in *MC4R*, a known monogenic cause for morbid obesity.

RESULTS

Clinical Presentation and Family History

An 18-yr-old male was referred for evaluation of intellectual disability, craniofacial dysmorphism, short stature, and obesity.

On physical exam he was found to have hypertelorism with broad and thick eyebrows and left exotropia because of a prior injury. His glabella was prominent with a nevus flammeus covering most of his forehead. He had mild micrognathia with simple, normally set ears (Fig. 1). His achieved ranges of motion were as follows: glenohumeral forward flexion and abduction to ~150°; full elbow extension; forearm supination to neutral left and full supination right; and bilateral neutral dorsiflexion of the wrist. His knees were fixed at 90° flexion and his hips at 120°, which required him to sit in a wheelchair. He had severe planovalgus foot deformities bilaterally. His muscle tone was reduced, although his muscle strength was preserved. The remainder of his physical exam was within normal limits. His weight was 86.8 kg (>90 centile), height was 162.4 cm (<3rd centile), and occipitofrontal circumference was 57 cm (65th centile). His BMI was >98th centile (moderate obesity, BMI 32.9 kg/m^2). His growth curve is shown in Figure 2.

He was born full-term to healthy, nonconsanguineous parents following an uncomplicated pregnancy. The family history was noncontributory. The maternal BMI is within normal limits (currently 24.3). Early infancy was complicated by muscular hypotonia and feeding difficulties, resulting in failure to thrive during the first year of life (Fig. 2). His cognitive and speech development were significantly delayed. At 10 yr, he developed generalized epileptic seizures and responded well to levetiracetam. At 14 yr, he exhibited self-injurious behavior including head banging that led to retinal detachment in one eye. The self-injurious behavior improved following treatment with gabapentin and topiramate, respectively, although never completely resolved to date. A brain MRI at 15 yr of age showed a 7 mm × 5 mm × 5 mm lesion in the right pontine without enhancement following contrast, and it was thought not to be clinically significant. Magnetic resonance spectroscopy was within normal limits. At 15 yr of age, he was also noted to gain significant weight despite dietary intervention and continued to do so until the present day.

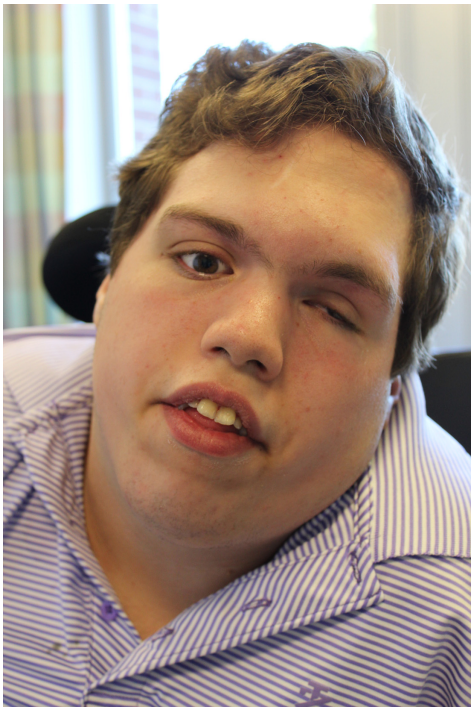
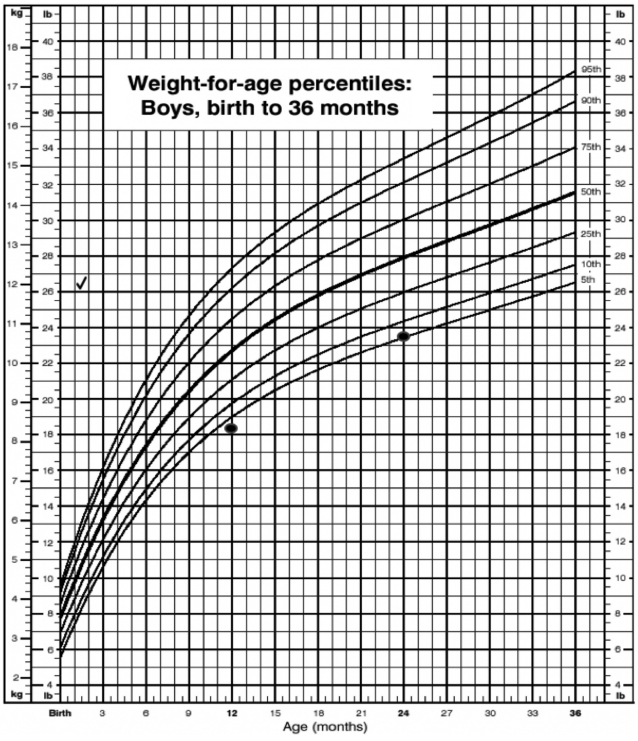


Figure 1. Proband at 18 yr of age.



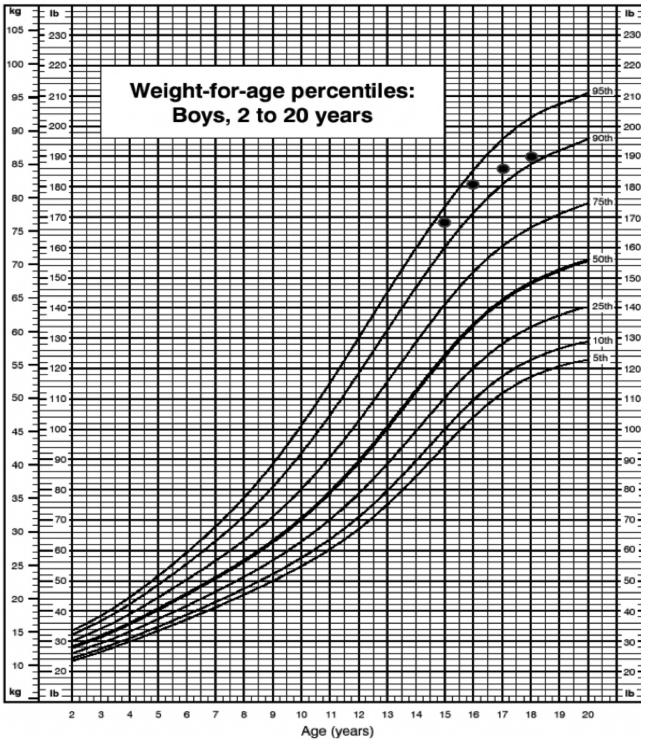


Figure 2. Weight charts.

Prior to ES, he underwent extensive metabolic and genetic testing, which were all unremarkable. Testing included plasma total/free carnitine and acylcarnitine profiles, amino acids, lactic and pyruvic acids, 7-dehydrocholesterol, urine amino acids, acylglycines, mucopolysaccharides, oligosaccharides, and organic acids in addition to chromosomal microarray, karyotype, methylation studies for Prader–Willi syndrome, mitochondrial DNA sequencing and deletion/duplication testing, single-gene testing for *SLC9A6* (Angelman-like syndrome), *MECP2* (Rett syndrome), *STK9/CDKL5* (atypical Rett syndrome), *ATP6AP2* and *SYN1* (epilepsy), *SLC6A8* (creatine transporter deficiency), and *OPHN1* (intellectual disability).

At 18 mo of age, he underwent a muscle biopsy. Microscopy showed mild myofiber size variation with mild increase in type I fibers. Immunohistochemistry staining for complexes II, III, IV, and V of the respiratory chain showed normal distribution. Oxidative phosphorylation enzyme assay revealed complex I activity of 0 nmol/min/mg (reference > 33; 5th percentile) and low complex IV activity of 210 nmol/min/mg (reference > 923; 5th percentile). It was thought that these deficiencies in complexes I and IV could be primary or secondary.

Genomic Analyses

ES was run on nuclear and mitochondrial genome, as part of the diagnostic workup on peripheral blood samples of the proband and his parents. Variants were confirmed by Sanger sequencing (XomeDx). Coverage information for ES is provided in Table 1. We used American College of Medical Genetics (ACMG) guidelines for variant interpretation in order to classify the variants (Richards et al. 2015). ES revealed heterozygous variants in *ASXL1* and *MC4R*: one de novo, pathogenic variant in exon 13 of *ASXL1*, c.4198G>T (p.E1400X) (NM_015338.5) and one maternally inherited, likely pathogenic variant in exon 1 of *MC4R*, c.817C>T (p.Q273X) (NM_005912.2) (Table 2). Neither variant was listed in either the Human Genome Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) (Stenson et al. 2017), ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar), or gnomAD database (https://gnomad.broadinstitute.org/). Both variants in this case were predicted to cause loss of normal protein function through protein truncation with loss of at least an 142-amino acid residue for the c.4198G>T variant and loss of the last 60 amino acids for the c.817C>T variant. We did not have any cell lines from the patient to check the protein function.

There were no exons that were called as deletions by the pipeline, and manual review of the normalized coverage data showed that no exons were significantly deviated from the normal-copy expectation, indicating that an exon-level deletion is unlikely.

DISCUSSION

Dual genetic diagnoses are rare but have been reported in the literature (Wallis et al. 2016). It is estimated that ~4%–6% of children diagnosed with a genetic disease also have a second,

Table 1. Sequencing coverage information for *ASXL1* and *MC4R* variants; c.4198 G>T and c.817 C>T

Sample	Mean RefSeq CDS coverage	RefSeq CDS ≥1 0× (%)	ASXL1 c.4198G>T (var/total)	MC4R c.817C>T (var/total)	Mean coverage	c.4198G>T (reads)	c.817 C>T (reads)	Sequence read length (bp)	Sequence read type
Proband	145	98.57	152/258	102/206	145	258	206	2 × 150	Paired end
Mother	264	98.68	0/432	196/366	264	432	366	2 × 150	Paired end
Father	159	98.68	0/241	0/190	159	241	190	2 × 150	Paired end

Table 2. Variant table

Gene	Chromosome	HGVS DNA	Accession no.	HGVS protein	Variant	Effect	ClinVar	Inheritance
ASXL1	GRCh37:Chr 20:31024713 GRCh38:Chr 20:32436910	c.4198G>T	NM_015338.5	p.E1400X	Nonsense	Path (PVS1,PS2, PM2, PM4,PP4)	450465	De novo
MC4R	GRCh37:Chr 18:58038766 GRCh38:Chr 18: 60371533	c.817C>T	NM_005912.2	p.Q273X	Nonsense	Likely path (PVS1, PM2,PM4)	450466	Maternal

(Chr) Chromosome, (no) number, (path) pathogenic, (PVS) pathogenic very strong, (PS) pathogenic strong, (PM) pathogenic moderate, (PP) pathogenic supporting.

independent genetic diagnosis (Yang et al. 2013; Stavropoulos et al. 2016; Posey et al. 2017; Trujillano et al. 2017). These concurrent diagnoses can present with unique phenotypes; this aggravates the difficulty of making a diagnosis solely based on the phenotype of the patient. This can be particularly challenging and misleading when the two genetic conditions present with contradicting clinical features. Therefore, single-gene tests or even gene panel testing may prove futile for solving such cases. In contrast, clinical ES is recommended as a comprehensive approach for identification of underlying genetic disorders (Yang et al. 2013). ES has the advantage of enabling us to diagnose two or more pathogenic variants in different parts of the genome; in comparison, targeted genetic diagnostic methods such as panel testing only focus on narrow differential gene lists.

Although ES is particularly advantageous in diagnosis of dual-gene disorders, it may still be difficult to delineate the contribution of each variant to the phenotype, especially when overlapping features exist between syndromes. In fact, this makes the treatment and management plan even more puzzling. In our case, this was not a limitation because the case had presented with a distinctly unusual phenotype (i.e., severe obesity in the presence of BOS).

BOS is a rare genetic syndrome with dysmorphic features, severe intellectual disability, poor growth, and skeletal abnormalities. Virtually all patients with this condition struggle with poor feeding and growth restriction, which causes significant morbidity and mortality (Russell et al. 2015). Obesity is not a usual feature and has only been reported in one case previously. This patient had significant increase in her BMI from 10.8 to 23.9 between age 3 and 5.5, corresponding to a change from a *Z*-score of −4.5 to +7. This rapid weight gain was not explained by medications or endocrinological problems (Pierron et al. 2009). In contrast, our case indeed had all the classic features of BOS, but the *MC4R* variant likely caused abnormal accumulation of body fat, thereby rescuing the patient from failure to thrive at age 15 and putting him on the other end of the spectrum for weight (i.e., >98th percentile for BMI), contradicting the classical presentation of BOS. The role of *ASXL1* in the regulation of adipogenesis (Park et al. 2011) can also be possibly affected by a second variant and may cause enhanced adipogenesis instead of suppression in classic BOS.

Our proband is also one of the oldest known patients with BOS, with only one other patient reported in the literature who lived until 24 yr of age; that patient also suffered from feeding difficulties and failure to thrive in infancy (Hoischen et al. 2011). The exact impact of the *MC4R* variant on the *ASXL1* variant and his prognosis is unclear, but it may be the reason for his nonclassical phenotype (i.e., obesity in teen years).

The case reported herein signifies how the presence of a second genetic diagnosis can modify the classic presentation of a well-described syndrome, and why the possibility of a genetic dual diagnosis should be considered in cases with unusual phenotypical features. This case highlights the utility of ES in patients with unique, unrecognizable phenotypes for several domains of precision medicine including (1) diagnosis, (2) prognosis and outcome, (3) management and therapy, and (4) utilization of resources.

METHODS

Exome Sequencing

Exome sequencing was performed at GeneDx following targeted exon capture with the Clinical Research Exome kit (Agilent Technologies) on an Illumina HiSeq 2500 2 × 100 bp. Both the sequencing technology and variant interpretation protocols have previously been described (Tanaka et al. 2015). The general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page (http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/). The GeneDx pipeline also detects exon-level copy-number changes with single-exon resolution for deletions (Retterer et al. 2015).

ADDITIONAL INFORMATION

Data Deposition and Access

Our patient consent does not permit patient sequence data to be uploaded to a data repository. The variants reported have been deposited in the ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) database and can be found under accession numbers SCV000619055.1 (*ASXL1*:c.4198G>T (p.Glu1400Ter)) and SCV000619056.2 (*MC4R*:c.817C>T (p.Gln273Ter)).

Ethics Statement

The family was enrolled in The Manton Center for Orphan Disease Research, Gene Discovery Core under informed consent governed by the Institutional Review Board of Boston Children's Hospital (IRB number is 10-02-0053). Written informed consent was provided by both parents for research and publication.

Competing Interest Statement

The authors have declared no competing interest.

Referees

Peter N. Robinson
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Author Contributions

M.R., A.S., and O.B. contributed to patient recruitment and phenotyping. M.R., A.S., C.H., and O.B. contributed to writing the initial draft of the manuscript. All authors contributed to revising the manuscript and reviewing the final draft.

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