CASE DESCRIPTION

A 5-month-old boy was referred to the genetics and neurology services for evaluation of developmental delay, “albinism,” and possible seizures. He was born to a 19-year-old G1P1 (one gestation, one delivery) mother at 36 weeks of gestation via spontaneous vaginal delivery. His birth weight was 2.6 kg (5.7 lbs), and length was 49.5 cm (19.5 in). He had a traumatic delivery and developed left subdural hemorrhage. He was in the neonatal intensive care unit for approximately 3 weeks (his being there was secondary to postnatal respiratory distress and pneumonia), but passed the newborn hearing and extended metabolic screens. During the first months of life, he developed an umbilical hernia and exhibited feeding difficulties. At 4 months of age, he was admitted for evaluation of “spells.” His electroencephalogram was unremarkable. Echocardiography showed normal anatomy and function and a small patent foramen ovale. He had 2 brain MRIs, 1 in the first month, which showed left subdural hemorrhage, and another at 4 months, which showed subarachnoid widening. Ophthalmology evaluated him and ruled out ocular albinism.

His review of systems was significant for abnormal muscle tone, fair skin color, loud breathing, constipation, and gastroesophageal reflux. Family history was unremarkable other than that his mother required speech therapy as a child. His two paternal half siblings and parents were healthy. On physical examination at 5 months of age, his length and weight were below the first percentile, and his occipitofrontal circumference was at the 66th percentile (relative macrocephaly). He was alert and interactive and showed no signs of acute distress. He had a long face with bitemporal narrowing, almond-shaped eyes, and slightly flat nasal bridge. Neurological examination demonstrated truncal hypotonia and increased muscle tone in the lower extremities. His developmental assessment at 5 months of age showed that he was able to control his head and started reaching out and grasping, but did not roll over. He started smiling at 3 months and cooing at 2.5 months. There was no developmental regression. His initial laboratory studies were unremarkable except for mild acidosis with bicarbonate 17–19 mmol/L (reference interval 20–30 mmol/L) and slightly increased ammonia at 108 μmol/L (reference interval 10–50 μmol/L).

Overall, his neurologic tests and clinical evaluations did not suggest a specific syndrome. In addition, his metabolic workup (acylcarnitine profile, serum amino acids, urine organic acids, and lactate) was unremarkable. Chromosomal microarray analysis (CMA) was performed because the diagnostic yield with CMA is significantly higher than classic chromosome analysis and professional societies recommend it as the first-tier diagnostic test for patients with unexplained developmental delay/intellectual disabilities or congenital anomalies (1). CMA testing revealed a 5-Mb deletion on 15q11-q13 between genomic coordinates 23,615,768 and 28,644,578 (hg19) (Fig. 2A).

BACKGROUND

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are complex genetic disorders with clinically distinct

QUESTIONs TO CONSIDER

1. What 2 genetic disorders are caused by lack of gene expression in 15q11-q13?
2. What are the classic clinical features of these genetic disorders?
3. What laboratory tests should be performed to identify and differentiate between these 2 disorders?

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3 Nonstandard abbreviations: CMA, chromosomal microarray analysis; PWS, Prader-Willi syndrome; AS, Angelman syndrome; IC, imprinting center; UPD, uniparental disomy; FISH, fluorescence in situ hybridization.
phenotypes, although both map to 15q11-q13. The primary phenotypes are attributed to loss-of-expression of imprinted genes within this critical region.

PWS (OMIM #176270) is characterized by severe hypotonia and feeding difficulties in early infancy, followed later in life by excessive eating and gradual development of morbid obesity. Physical characteristics are more evident in infancy and may include a disproportionately long head (dolichocephaly), narrow bifrontal diameter, almond-shaped eyes, thin upper lip with downturned mouth, and small hands and feet. However, the phenotype exhibits significant clinical variability with age (2).

Clinical findings in AS (OMIM #105830) include severe developmental delay, speech impairment, gait ataxia and/or limb tremors, and a unique behavior of happy demeanor with frequent, inappropriate laughter, smiling, or excitability. Microcephaly and seizures are also common (3, 4). Manifestation of the unique AS neurodevelopmental features becomes more evident after the first year of life. Patients with AS and PWS commonly present in early infancy with nonspecific clinical findings, which makes the differential diagnosis broad and complicated. In fact, it can take several years before the correct diagnosis is suspected and established. Before the introduction of molecular tests, consensus clinical diagnostic criteria were the only tool for the diagnosis of AS and PWS (4, 5). Currently, these criteria are extremely valuable in selecting the group of patients who need molecular investigation to confirm their suspected diagnosis.

On the basis of initial clinical indications including truncal hypotonia, poor weight gain, feeding difficulties, relative macrocephaly, and some facial features, it was thought that this patient’s abnormal genetic findings were consistent with PWS.

Genomic imprinting refers to differential expression of a locus between the maternally and paternally inherited alleles. There is often transcriptional silencing or repression of 1 allele, and normal development requires inheritance of both maternal and paternal alleles. PWS and AS are classic examples of genomic

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**Fig. 1. Molecular diagnostic algorithm for AS/PWS.**

DNA-based methylation analysis provides a conclusive diagnosis of AS/PWS in most cases. Methylation-specific PCR, multiplex ligation-dependent probe amplification, or Southern blot analysis should be used as first-line tests. On the basis of the initial findings, subsequent UBE3A gene sequencing, FISH, UPD, and IC analyses are performed to identify the genetic mechanisms for estimating the recurrence risk, parental testing, and counseling. In clinically challenging cases, high-throughput genome-wide studies (CMA or next generation sequence (NGS) analysis) may be performed to identify the possible genetic cause. *If there is a strong clinical suspicion of PWS, additional studies are needed to rule out HBII-85 microdeletion, MAGEL2 mutations, or PWS-like conditions (e.g. UPD 14, 14q32 deletion) [Sahoo et al. (8)].*
imprinting disorders in which the phenotype depends on whether the deletion or mutant allele is inherited from the father or mother. A specific region of chromosome 15, the imprinting center (IC), regulates a gene cluster such that some genes are expressed paternally \([\text{including MKRN3 (makorin ring finger protein 3)}, MAGE-like 2, NDN (neacidin, melanoma antigen [MAGE] family member), PWRN1 (Prader-Willi region non-protein coding RNA 1), and SNRPN (small nuclear ribonucleoprotein polyepitope N)]\), whereas others are expressed maternally (includ-\[\text{ing UBE3A (ubiquitin protein ligase E3A)}\] ) (6). For example, \(\text{NDN}\) and \(\text{SNRPN}\) must be paternally expressed because they have promoter CpG islands that are methylated, and therefore silenced, on the maternal chromosome (7, 8). Although multiple genetic mechanisms can cause PWS, all result in loss of paternally expressed genes at 15q11-q13. These mechanisms include a paternally derived de novo deletion of this region (approximately 75%–80%), maternal uniparental disomy (UPD) of chromosome 15 (approximately 20%–25%), or paternal imprinting defects that silence paternal alleles (approximately 1%). UPD occurs when both copies of a given genomic region are inherited from only 1 parent. In AS, the disease mechanism may be de novo deletion of maternally derived chromosome 15q11-q13 (approximately 70%–75%), mutations in \(\text{UBE3A}\) (approximately 10%), paternal UPD of chromosome 15 (approximately 3%–7%), an imprinting defect on the maternal chromosome (approximately 2%–3%), or unknown etiology (approximately 10%). AS-like phenotypes have also been described with mutations in \(\text{SLC9A6 [solute carrier family 9, subfamily A (NHE6, cation proton antiporter 6)]}\), \(\text{TCF4}\) (transcription factor 4), and \(\text{MECP2}\) (methyl CpG binding protein 2) (9, 10).

The recurrence risk for PWS and AS families depends on the genetic mechanism. It is <1% in cases with a deletion, UPD, or imprinting defect without IC.
deletion. If chromosomal rearrangement or an imprinting defect with IC deletion is involved, then familial risk can be as high as 50%. Paternal UPD with predisposing parental translocations can be associated with up to 100% recurrence risk. Additionally, recurrence risk may be as high as 50% when a UBE3A mutation is present in AS patients. The offspring of PWS patients have approximately 50% risk of being affected with AS or PWS depending on the sex of the child (2–5).

Genetic testing for AS/PWS is an essential tool to confirm clinical diagnosis, facilitate management, and enable appropriate genetic counseling for recurrence risk. Several consensus molecular testing approaches for AS/PWS genetic diagnosis have been described (Fig. 1). DNA-based testing should initially be performed to assess parent-specific methylation in the 15q11-q13 region (10). Methylation analysis identifies approximately 80% of AS and >99% of PWS patients. Abnormal methylation can be determined by Southern blot analysis, methylation-specific PCR, or methylation-specific multiplex ligation-dependent probe amplification. If abnormal methylation is detected, the genetic mechanism must be identified through additional testing. Fluorescence in situ hybridization (FISH) and CMA are used to identify or confirm deletion of this critical region. Additionally, microsatellite analysis and CMA can detect UPD by loss of heterozygosity. In suspected AS cases without a deletion, sequencing of UBE3A is performed. If biparental inheritance is established, IC analysis can be performed. Identifying the genetic mechanism of these diseases is critical to estimate probable recurrence risk and offer both familial studies and counseling.

RESOLUTION OF THE CASE

Our patient presented with truncal hypotonia, poor weight gain, feeding difficulties, relative macrocephaly, long face, bitemporal narrowing, flat nasal bridge, and developmental delay. These findings were initially more suggestive for PWS than AS. However, young AS patients may also exhibit feeding difficulties and muscle hypotonia. In addition, absence of seizures and macrocephaly, likely due to the patient’s young age, made AS/PWS differentiation particularly difficult. CMA was therefore chosen as the first-line genetic test, which revealed deletion of chromosomal segment 15q11-q13 (Fig. 2A). CMA cannot distinguish parental alleles or methylation status of the imprinted domain on 15q11-q13, which are important to establish a definitive diagnosis. To determine parental origin of the deletion and its methylation status, consensus algorithms recommend methylation analysis, which can identify or exclude PWS in >99%. Therefore, Southern blot analysis was performed (Fig. 2B), and loss of the maternal allele was observed, which is consistent with AS. This case illustrates the difficulty in clinically distinguishing AS/PWS at a young age.

CASE FOLLOW-UP

The patient is currently 10 months of age, and neurology and genetics closely monitor his development and growth. He receives physical, occupational, and speech therapies. He continues to exhibit developmental delay, but has normal growth parameters and relative macrocephaly and remains seizure-free. He has started displaying inappropriately happy behavior, which is characteristic of AS. Mothers of AS patients with deletions are counseled to have chromosomal and FISH analyses to rule out a chromosomal rearrangement, but the proband’s mother declined testing. Assuming normal cytogenetic studies, her estimated recurrence risk is <1%.

POINTS TO REMEMBER

- PWS and AS are imprinting disorders of chromosome 15q11-q13, with PWS caused by loss of paternally expressed genes and AS caused by loss of maternally expressed genes.
- PWS is traditionally characterized by hypotonia, short stature, excessive eating, obesity, intellectual delay, and small hands and feet. AS is characterized by severe developmental delay, speech impairment, seizures, gait ataxia and/or limb tremors, frequent laughing or smiling, and a unique behavior of happy demeanor.
- If AS or PWS is clinically suspected, DNA-based methylation analysis should be performed as the first-line genetic test. CMA and other molecular tests are used to clarify the underlying molecular mechanism.
- If DNA methylation is negative in the context of highly suspected clinical diagnosis, molecular testing of UBE3A or next generation sequencing should be considered.
- Assessment of the recurrence risk depends on the molecular mechanism causing PWS or AS, and clinical testing should be performed according to established consensus algorithms.
Commentary

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Angelman syndrome (AS)² and Prader-Willi syndrome (PWS) are rare genetic disorders with reported prevalence ranging from approximately 1:10000 to 1:30000. Although these are rare disorders, it is important not to miss these diagnoses because there are therapeutic and reproductive implications for the patients and parents. In PWS, recombinant human growth hormone therapy results in improvements not just in physical growth, but in cognitive and motor development as well. Ideally, growth hormone should be started as soon as the diagnosis has been confirmed. In AS, the use of certain antiepileptic medications such as carbamazepine and phenobarbital may worsen seizures, whereas other antiepileptic medications, such as valproate and clonazepam, and low-glycemic-index diets have been found to be particularly effective treatments for seizures in these patients.

However, infants with PWS and AS often present with nonspecific findings such as global developmental delay and hypotonia. Although feeding difficulties and poor weight gain in early infancy may prompt consideration of PWS, and the presence of seizures may prompt consideration of AS, these findings are not specific. In fact, in this case study, the infant had facial features thought to be reminiscent of PWS, as well as feeding difficulties and poor weight gain. It should also be noted that the hyperphagia and obesity associated with PWS do not present until late childhood. Similarly, some patients with AS do not have clinical seizures, but almost all children with AS have electroencephalograph abnormalities. The diagnostic challenge is that chromosomal microarray analysis, the first-line investigation for infants with such presentations, will not detect all of these cases; unless chromosome 15 methylation analysis is performed, some cases will be missed. As noted by the authors, if methylation analysis is normal, molecular testing for mutations in UBE3A (ubiquitin protein ligase E3A) may be necessary to confirm the diagnosis of AS.

References