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Diagnosing Angelman and Prader-Willi Syndromes Prenatally: DNA Methylation Outcomes Following Varied Initial Indications

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I. Introduction

Angelman Syndrome (AS) and Prader-Willi Syndrome (PWS) are neurodevelopmental conditions that involve imprinting errors of the 15q11.2-q13 AS/PWS critical region by mechanisms such as deletions, uniparental disomy(UPD), imprinting center defects (ID), and DNA sequence changes, with most cases being sporadic. PWS results from lack of expression of the paternally derived allele, whereas AS results from lack of expression of the maternally derived allele. Prenatal indications for both include family history and confirmation following a positive cell-free DNA (cfDNA) screening or diagnostic testing results indicating suspicion of AS/PWS.[1,2] Additionally, some literature suggests an increased risk for imprinting disorders for children conceived using assisted reproductive technology.[3] Therefore, another common indication for AS/PWS methylation testing is in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). While ultrasound (U/S) findings are not typically found in AS, recent literature suggests that U/S findings of decreased fetal movement, abnormal limb position, polyhydramnios, and small fetal abdominal circumference may indicate increased risk for PWS.[1] Given the perinatal issues surrounding AS/PWS, prenatal diagnosis may lead to better management during and after delivery. The purpose of this study is to review the experience of a diagnostic laboratory over the course of 99 consecutive samples with regard to prenatal AS/PWS DNA methylation analysis in order to better understand the likelihood of a positive result given the indication for testing. Indications, other relevant testing results, and methylation results are examined.

II. Methods

For the purpose of this study, 99 consecutive cases received for AS/PWS methylation testing were analyzed. Cases were collected from a database used to track molecular fetal cases and various reporting systems. Molecular analysis of the *SNRPN* gene was performed by methylation-specific PCR and gel electrophoresis on DNA obtained from direct amniotic fluid or cultured amniotic fluid. This method detects all cases of AS/PWS arising from UPD, microdeletions and imprinting center defects, but does not define the nature of underlying genetic defect.

III. Results

LabCorp received 99 consecutive samples ordered for prenatal AS/PWS DNA methylation analysis. Testing was cancelled for one sample. The indications for methylation testing for the remaining 98 samples were: 34 (34.7%) due to an increased risk based on cfDNA screening, 20 (20.4%) follow-up to an abnormal fetal microarray or karyotype, 20 (20.4%) due to a family history, 16 (16.3%) because the pregnancy was a result of IVF/ICSI, and the remaining 8 (8.2%) were sent for other indications. Eight samples (8.2%) had positive methylation results; 7 positive for PWS and 1 positive for AS. Of the 8 samples with positive methylation results, 5 (62.5%) were follow-up to abnormal diagnostic results and 3 (37.5%) were follow-up to an increased risk on cfDNA screening. For samples submitted as follow-up to abnormal fetal diagnostic results, two had deletions involving the AS/PWS critical region, two were found to have possible UPD of chromosome 15, and one showed an unbalanced translocation (**Table 1**).

Of the 34 samples that were submitted due to increased risk based on cfDNA, the cfDNA screening methodology was identified for 21 (61.8%), **Figure 3**. All 17 samples from patients that had cfDNA screening using SNP-based methodology had negative AS/PWS methylation testing (false positive cfDNA results). Four samples came from patients that had cfDNA screening using massively parallel sequencing (MPS); 3 of these 4 samples (75.0%) had positive PWS methylation results.

Table 1. Findings for samples sent as follow-up to abnormal array/chromosome analysis

Cytogenetic Result	Methodology	Total	Positive AS/PWS
ROH/Possible UPD	Array	9	2(PWS)
Gain/Duplication	Array	5	0
Deletion involving AS/PWS critical region	Array	3	2(PWS)
Chromosomal Rearrangement/Marker	Chromosome/FISH	3	1(AS)

Figure 1. Indications for fetal AS/PWS methylation testing

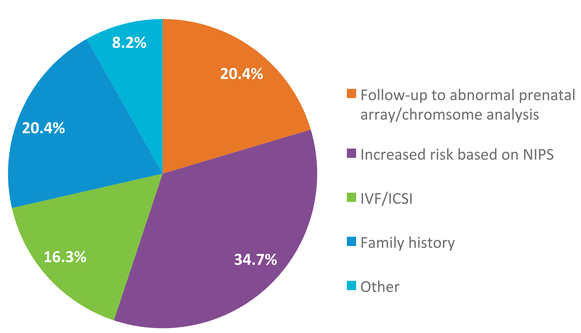


Figure 2. AS/PWS methylation results per indication

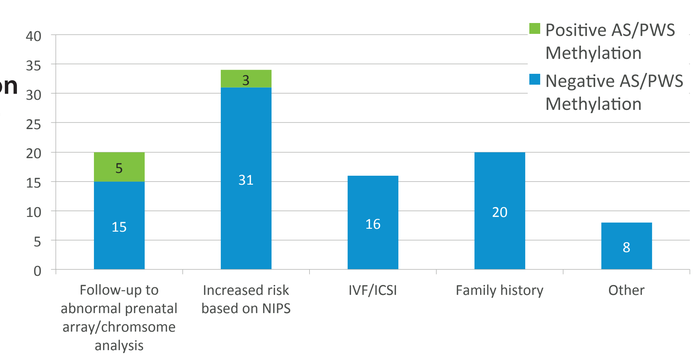
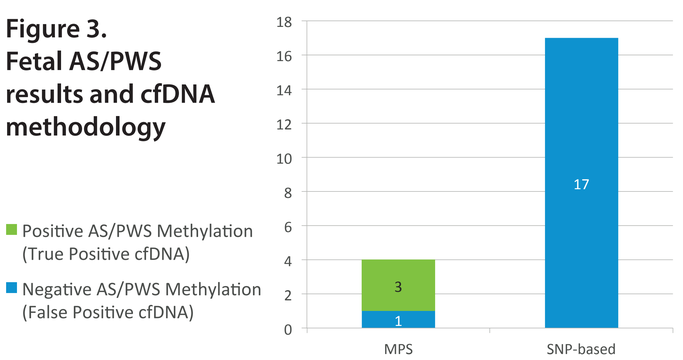


Figure 3. Fetal AS/PWS results and cfDNA methodology



IV. Discussion

Increased risk for AS/PWS based on cfDNA screening was the most common indication, but only 3/34 (8.6%) samples received for this indication were confirmed by AS/PWS methylation testing. All 3 samples that confirmed were identified as being from 2 labs that use MPS. MPS based cfDNA methodology has been previously shown to have a reported PPV of 75.0-100% for deletions associated with 15q AS/PWS[4] and the present study supports this finding. While 50% of the samples were identified as having cfDNA screening done using SNP-based technology, none of those samples resulted in positive AS/PWS methylation testing. It is likely that the SNP-based technology is detecting regions of homozygosity in the AS/PWS critical region rather than a microdeletion[5]. This highlights that positive predictive values may vary based upon the methodology and laboratory employed. Further studies are warranted to determine if this difference exists for other microdeletions commonly tested on cfDNA screening.

The indication with the highest rate of positive results was follow-up to abnormal cytogenetic testing. However, 75.0% of samples submitted for this indication resulted as negative. This is likely due to the variety of microarray findings that can be interpreted as suspicious for AS/PWS, including duplications which are less likely to cause AS/PWS. As suspected, due to the *de novo* nature of both conditions, samples with an indication of family history were associated with no positive results. Finally, there were more positive results for PWS than AS even though the prevalence for the conditions is similar. This could be due to underlying mechanisms of each condition and ascertainment bias. Array can detect 80-90% of PWS cases and 68% of AS cases.[6,7] This is likely related to the observation that more AS cases are caused by point mutations as compared to PWS cases and these point mutations would be not be identified by a cytogenetic microarray.

Key Points:

- Prenatal methylation analysis for AS/PWS is an important follow-up for a pregnancy with suspected AS/PWS
- PPV for AS/PWS is different depending on the cfDNA methodology, with some labs utilizing MPS-based platforms delivering a high PPV and other labs employing SNP-based platforms delivering a much lower PPV
- Fewer cases of AS may be diagnosed prenatally because a portion of AS is caused by point mutations which would not be detected by cfDNA, karyotype, or microarray

V. References

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