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# CLINICAL CASE REPORTS

## The Challenge of Diagnosing Prader-Willi Syndrome in Preterm Infants

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### ABSTRACT

Hypotonia is often the only finding in newborns with Prader-Willi syndrome. In the most severe cases, it can mimic a neuromuscular disorder. Early diagnosis and treatment are crucial, as they significantly improve the prognosis. However, the absence of characteristic features in the neonatal period, especially in premature infants, makes diagnosis challenging. Inadequate genetic testing may further delay the diagnosis. We report the case of an extremely preterm girl with Prader-Willi syndrome caused by maternal uniparental disomy, in whom severe neonatal hypotonia was the sole presenting feature. Initial fluorescence in situ hybridization testing failed to detect a deletion. Screening for neuromuscular disorders and inborn errors of metabolism was also negative. At six months of age, characteristic physical features became apparent and she was the diagnosis was established by deoxyribonucleic acid methylation analysis. Through this report, the authors sough to review the clinical aspects and diagnostic testing of Prader-Willi syndrome.

**Keywords:** extremely premature; infant; muscle hypotonia; Prader-Willi syndrome; uniparental disomy

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## INTRODUCTION

Prader-Willi syndrome (PWS) is a genetic disorder caused by the absence of expression of paternally inherited genes within the 15q11–q13 region of chromosome 15. Four genetic mechanisms can lead to PWS: deletion (65-75% of cases); maternal uniparental disomy (UPD; 25-41%; heterodisomy, isodisomy, and segmental isodisomy); imprinting defects (ID; approximately 5%; most commonly due to a microdeletion in the imprinting center [IC], though it may also result from an epimutation); and balanced or unbalanced rearrangements (<1%).[1,2] The estimated incidence of PWS is one in 10.000-30.000 births, with an equal gender distribution.[1] The main clinical features of PWS include neurodevelopmental and behavioral disturbances as well as metabolic and endocrine abnormalities. Affected individuals also exhibit characteristic physical features such including ~~as~~ short stature, a narrow bifrontal diameter, almond-shaped palpebral fissures, a narrow nasal bridge, a thin upper lip with downturned corners and small hands and feet.[1]

Early diagnosis and classification is important for two main reasons: early treatment with growth hormone can prevent comorbidity and improve long-term outcomes; second, appropriate and timely genetic counselling, as well as accurate recurrence risk estimation depends on identifying the underlying genetic mechanism.[3]

A definitive diagnosis of PWS requires one or more genetic tests. The recommended first-line tests – methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) and methylation-specific polymerase chain reaction (MS-PCR) – assess the deoxyribonucleic acid (DNA) methylation pattern of the 15q11-q13 region and have a sensitivity of approximately 99%.[2-4] When available, MS-MLPA is the preferred initial test in patients with. In addition to assessing methylation status of the 15q11-q13 region, MS-MLPA also detects deletions (both large deletions and IC microdeletions), providing a definitive diagnosis in 70-75% of cases.[2-4] It also helps exclude Angelman syndrome (AS), which can present with neonatal hypotonia. However, its main limitations are the inability to detect balanced chromosomal rearrangements and to distinguish UPD from ID caused by epimutation. If MS-MLPA is used as the initial test and hypermethylation of the 15q11-q13 region is detected, the diagnosis of PWS is confirmed. In cases with abnormal copy number finding, this may indicate either a large deletion (65-75%) or an ID with an underlying IC deletion (<0.5%).[2] If and IC microdeletion is confirmed, it is import to test the father to determine whether he is a carrier. If so, the recurrence risk for future offspring is 50%.[4] Conversely, if MS-MLPA shows a normal copy number, DNA polymorphism analysis of the proband and both parents should be performed to distinguish UPD from ID due to an epimutation. Suitable tests for this purpose include comparative genomic hybridization microarray with single nucleotide polymorphism array (CGH-SNP) or short tandem repeat analysis via polymerase chain reaction (STR-PCR). ID by epimutation is presumed when UPD is excluded. In rare cases (<1%) of PWS caused by a balanced chromosomal rearrangement, MS-MLPA may yield normal results.[2] If clinical suspicion remains high, fluorescence in situ hybridization (FISH) or chromosome analysis should be considered.

Although MS-PCR is as sensitive as MS-MLPA and is more widely available, it does not provide information about the underlying mechanism, requiring additional testing and associated costs. FISH is generally not recommended as a first-line test when DNA methylation analysis is available, due to its lower sensitivity (65-75%).[2] It detects only deletions and balanced structural abnormalities and cannot distinguish PWS from AS in cases involving deletions. Nevertheless, FISH can be useful in limited-resource settings, as it is less expensive than DNA methylation analysis, rapid and widely available. **Table 1** summarizes the applications and limitations of the most commonly used genetic tests for the diagnosis of PWS.

The authors sought to reviewed the main clinical aspects and diagnose challenges of PWS, contextualizing them through the discussion of a case report.

| Tests | Applications | Limitations |
|---|---|---|
| **MS-MLPA** | Preferred first-line test<br>Sensitivity greater than 99% (including deletion, UPD and ID)[2-4]<br>Detects methylation status and copy number changes<br>Distinguishes PWS and AS deletion<br>Distinguishes deletion and nondeletion PWS<br>Picks up small IC deletions and microdeletions | Cannot distinguish UPD from ID<br>Cannot identify a balanced chromosomal rearrangement (< 1%)[3]<br>Requires specialized labs, potentially limiting accessibility<br>Costs? |
| **MS-PCR** | Sensitivity of 99% (including deletion, UPD and ID)[2-4]<br>Detects abnormal methylation patterns<br>Cost-effective and rapid for initial screening<br>Suitable for small labs with limited resources<br>Distinguishes PWS from AS | Does not give information about the underlying genetic mechanism – further testing is required<br>Unable to identify chromosomal rearrangements |
| **FISH** | Sensitivity of 65–75% [2-4]<br>Detects deletion and balanced structural abnormalities<br>Provides information about the mechanism in case of a deletion<br>Quick and widely available | Cannot distinguish normal, UPD and ID<br>Cannot distinguish PWS and AS deletions<br>Unable to provide information on the entire critical region or on other chromosomes |

| | | |
|---|---|---|
| **Array CGH + SNP** | Sensitivity of 80-90% [2,3]<br>Detects copy number change and single nucleotide polymorphism change<br>Detects deletions and unbalanced chromosome rearrangements<br>Useful for ruling out other chromosomal abnormalities | Cannot distinguish PWS and AS deletions<br>Cannot detect all cases of UPD heterodisomy or balanced chromosome rearrangements (5% of patients)[3]<br>Interpretation of the microarray result may be inaccurate if the patient has a homozygous mutation or mosaicism, both rare in PWS |
| **Cytogenetics** | Detects structural rearrangements | It is not useful to detect deletions or UPD |
| **STR-PCR** | Identifies UPD by analyzing parental inheritance of chromosome 15 markers<br>Useful to confirm UPD after methylation testing | Does not detect deletions or ID defects alone<br>Require parental DNA samples<br>Limited to specialized genetic labs |

**Table 1 -** Summary of the applications and limitations of the most commonly used genetic tests for the diagnosis of Prader-Willi Syndrome.

AS (Angelman syndrome); IC (imprinting center); array CGH-SNP(comparative genomic hybridization microarray with single nucleotide polymorphism array); FISH (fluorescence in situ hybridization); ID (imprinting defects); MS-MLPA (methylation-specific multiplex ligation-dependent probe amplification); MS-PCR (methylations-specific polymerase chain reaction); PWS (Prader-Willi syndrome); UPD (uniparental disomy); STR-PCR (short tandem repeat analysis via polymerase chain reaction).

## CASE REPORT

Family history was unremarkable except for advanced maternal age (40 years old). This was the mother's third pregnancy, following one miscarriage and one live birth of a healthy son. The pregnancy was unrecognized until 22 weeks and was complicated by preeclampsia and intrauterine growth restriction with abnormal doppler flow studies. The mother was treated with methyldopa and nifedipine during pregnancy and reported active smoking. An elective caesarean section was performed at 26 weeks and two days of postmenstrual age (PMA), after completion of antenatal corticosteroids for pulmonary maturation, due to worsening doppler findings and severe fetal growth restriction.

The newborn girl was intubated and given mechanic respiratory support at the delivery room. She was subsequently admitted to the neonatal intensive care unit (NICU). Birth anthropometric measurements (Fenton 2003) were as follows: weight 605 g (3rd percentile), height 33 cm (3rd percentile), head circumference 21.5 cm (10th percentile). No dysmorphic features were noted.

She remained in the NICU for 108 days (PMA of 41 weeks and five days). She required invasive ventilation until day 48, followed by non-invasive support until day 74. At 36 weeks PMA, she continued to require 0.5 L/min of supplemental oxygen and was diagnosed with mild bronchopulmonary dysplasia. Apart from early and late-onset neonatal sepsis and transient hyperbilirubinemia managed with phototherapy, no other short-term complications related to prematurity were observed. Severe axial hypotonia was noted from birth but was underrecognized until 32 weeks PMA, due to prematurity.

Neurological examination showed normal limb tone and strength with antigravity movements and normal deep tendon reflexes (DTR). However, craniofacial features - dolichocephaly, narrow and long face, almond-shaped palpebral fissures, downward labial commissure -and persistent feeding difficulties (poor sucking and swallowing) became apparent. Those aspects combined with difficulty in weaning from mechanical ventilation raised the clinical suspicion of PWS. The differential diagnosis included neuromuscular disorders and inborn errors of metabolism.

A FISH test targeting a deletion in the 15q11.2 region was negative. Neonatal brain ultrasounds (US) showed no abnormalities except for mild enlargement of the frontal and parietal subarachnoid spaces on the day 90 of life. These finding were later confirmed by brain magnetic resonance image (MRI). The national newborn metabolic screening (Portugal) was negative, and blood gas analyses consistently showed no significant metabolic acidosis. Serum levels of creatine kinase, lactate dehydrogenase and aldolase were within normal limits. During hospitalization, the infant's hypotonia gradually improved, respiratory support was successfully weaned, and she achieved full feeding autonomy. Given de clinical stabilization and partial resolution of symptoms, further investigation was deferred at that time. The patient was discharged to the Neuropediatric outpatient clinic.

Over time, the phenotype of PWS became more evident, further increasing clinical suspicion. At six months of age, MS-PCR targeting the PWS locus confirmed the diagnosis. Further genetic analysis, using STR-PCR to compare the STR markers of the proband and her parents in the PWS locus, revealed the presence of only maternal alleles in the proband – confirming maternal UPD as the underlying mechanism.

She maintained a multidisciplinary follow-up and began growth hormone therapy by the age of 15 months (corrected age: 11 months). The overall outcome has been favorable.

At the age of two and a half, the patient had a mild cognitive impairment with good language skills. The body mass index (BMI) was 18 Kg/m$^2$ (percentile 95) with height was within the normal range for age (percentile 5) according to World Health Organization growth charts, although it was lower than expected based on parental height.

## DISCUSSION

Neonatal diagnosis of PWS can be challenging due to the absence of characteristic phenotypic features during this period and the limited sensitivity of the most accessible and affordable genetic tests, such as FISH. Prematurity may further delay the diagnosis.

Gestation is often uneventful, though decreased fetal movements may be reported. Breech presentation and higher incidence of caesarean delivery have been reported. [5] Single cases and a survey also suggested an association with prematurity.[6,7] Birth length and weight are usually 15-20% lower than those of siblings, although they may still fall within the normal range.[5] In this case, both prematurity and elective cesarean section were considered to result from maternal conditions. Breech presentation was not valued due to prematurity. Birth weight and length were below the 10$^{th}$ percentile, likely reflecting fetal growth restriction secondary to abnormal doppler flow studies.

Neonatal axial hypotonia is the hallmark—and often the only—clinical feature of PWS in the neonatal period. It leads to lethargy, hypoventilation (which may result in mild and transient respiratory failure), feeding difficulties requiring assisted feeding for several weeks, and abnormal squeaky, weak cry.[8,9] The severity of the hypotonia varies considerably. Milder cases present with preserved limb strength and normal to brisk DTR, while more severe cases may mimic neuromuscular disorders, with diminished peripheral strength, reduced spontaneous limb movements, and hyporeflexia.[8] Other neonatal features described in PWS include transient bradycardia, thermolability, pallor and/or intense mottling with acrocyanosis, adducted thumbs, hypogonadism (undescended testicles in boys and small clitoris and labia minora in girls), delayed passage of meconium, and thick, viscous saliva.[8] Our patient presented with marked axial hypotonia, while limb tone, strength, and DTRs remained normal, suggesting a central etiology. She also developed respiratory failure required a longer than expected duration of ventilatory support (10 to 21 days for invasive ventilation and 31 days for non-invasive ventilation) together with significant feeding difficulties.[9] These findings were only valued by the 32$^{nd}$ week of PMA. Prematurity worsened the clinical course and worked as a confounding factor.

Typical craniofacial features of PWS described above are well characterized in older children and adults. However, neonatal studies have failed to consistently identify these features, particularly in cases of UPD.[6,7,10] In this patient, typical facial features were not evident at birth, although some physical characteristics became apparent in the following weeks. Prematurity and UPD likely contributed to a more subtle phenotype.

The differential diagnosis of neonatal hypotonia includes both acquired and inherited causes. Perinatal complications such as sepsis or hypoxic-ischemic encephalopathy are more frequent and are usually accompanied by encephalopathy and seizures. Genetic syndromes, congenital myopathies, neuropathies, and inborn errors of metabolism (such as acid maltase deficiency, congenital disorders of glycosylation, and lysosomal disorders) should also be considered.[1,11] Additionally, hypotonia is common in premature infants and usually improves with age. **Table 2** provides an overview of the initial approach to a hypotonic newborn. Although PWS was initially suspected in our case, the severity of hypotonia, along with prolonged ventilatory support and feeding difficulties, raised concerns about a neuromuscular disorder or an inborn error of metabolism, however, investigations for theses causes were all negative. Despite early suspicion, confirmation of PWS was delayed until the phenotype evolved and MS-PCR testing confirmed maternal UPD. A similar case involving a preterm newborn with PWS, and UPD, and delayed diagnosis following negative FISH, has been previously reported.[7]

Although FISH testing was used initially due to local availability, speed, and cost, it lacks the sensitivity of MS-PCR and cannot detect UPD. In contrast, MS-MLPA is currently the most accurate and cost-effective first-line test, providing both diagnosis and information about the underlying genetic mechanism. In resource-limited settings, FISH may still be a practical first step, but clinicians must remain vigilant and pursue further testing if clinical suspicion remains—especially in the context of advanced maternal age, which increases the risk of UPD. [1,2,4]

Cognitive impairment in PWS is generally mild to moderate. Language development is often consistent with the child's cognitive level, and verbal abilities are typically better in patients with UPD.[12,13]PWS is also a major genetic cause of primary obesity and is associated with short stature.[1] By two and a half years of age, the patient's BMI already indicated a tendency toward obesity. Although her height was at the 5$^{th}$percentile, there was a significant discrepancy compared to the familial target height. Nonspecific changes were observed on the neuroimaging. The authors did not find any evidence in the scientific literature of an association between neuroanatomic changes and PWS.

In conclusion, hypotonia may be the only neonatal manifestation of PWS, particularly in premature infants. Severe cases may resemble a neuromuscular disorder. Accurate and early diagnosis is essential, as it enables timely intervention and informs recurrence risk. MS-MLPA is the preferred first-line test. FISH remains an important alternative in resource-limited settings, but clinicians should be aware of its limited sensibility and pursue further testing when indicated by clinical findings or risk factors. Advanced maternal age should prompt heightened suspicion for UPD.

**HYPOTONIA IN THE NEWBORN**

1. Confirm presence of hypotonia;
2. Classify the hypotonia as central or peripheral;
3. If the presentation is acute, C-reactive protein level, a complete blood count and appropriate cultures should be obtained to exclude an infectious etiology.

| **Central** | **Peripheral** |
|---|---|
| Axial tone more affected than appendicular<br>DTRs: normal or brisk<br>Primitive reflexes: normal or exaggerated<br>Muscle strength: typically normal<br>Altered level of consciousness often present<br>Associated features: seizures, dysmorphic features, organ malformations. | Generalized hypotonia<br>DTRs: absent or reduced<br>Weak cry and poor sucking<br>Facial diparesis, ptosis<br>Fasciculations (tongue or appendicular)<br>Difficulty weaning from respiratory support. |
| **Possible causes** | **Possible causes** |
| Genetic (e.g., trisomy 21, PWS, AS)<br>Inborn errors of metabolism<br>Congenital hypothyroidism<br>Brain injury (e.g., hypoxic-ischemic encephalopathy)<br>Structural abnormalities (e.g., malformations, trauma) | Peripheral neuropathies (e.g., hypomyelinating neuropathy)<br>Neuromuscular junction disorders (pre or post-synaptic)<br>Congenital myopathies<br>Muscular dystrophies |

4. If history and physical examination suggest a specific cause, pursue targeted testing accordingly;
5. If no specific cause is identified, proceed with a diagnostic workup (see below).

**Initial investigations**
Creatine kinase, lactate dehydrogenase, aldolase
Thyroid function tests
Blood gas analysis, ammonia, glucose
Cranial ultrasound
Brain MRI (if abnormal exam or suspected central cause)
Spinal MRI (if spinal lesion suspected)

**Additional or target investigations:**
**Genetic Testing:**
Karyotyping (e.g., Trisomy 21)
MS-MLPA (for Prader-Willi / Angelman syndromes)
Chromosomal microarray
Whole exome sequencing
Disease-specific gene panels
**Metabolic Screening:**
Quantitative urine organic acids
Serum amino acids
Acylcarnitine profile
Very long-chain fatty acid profile
**Neuromuscular Evaluation:**
Electromyography and/or nerve conduction studies
Muscle biopsy (in conjunction with genetic testing for congenital myopathies)

**Table 2 -** Overview of the initial approach to a hypotonic newborn

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## REFERENCES

1. Cassidy SB, Driscoll DJ. Prader–Willi syndrome. Eur J Hum Genet. 2009;17(1):3-13. DOI: https://doi.org/10.1038/ejhg.2008.165.
2. Gao Y, Zhong ML, Dai YL, Jiang YH, Zou CC. Overview of genetic testing in Prader-Willi syndrome. EXPERT OPINION ON ORPHAN DRUGS. 2023;11(1):16-25. DOI: https://doi.org/10.1080/21678707.2023.2262104.
3. Goldstone AP, Holland AJ, Hauffa BP, Hokken-Koelega AC, Tauber M. Recommendations for the Diagnosis and Management of Prader-Willi Syndrome. J Clin Endocrinol Metab. 2008;93(11):4183-97. DOI: https://doi.org/10.1210/jc.2008-0649.
4. Smith A, Hung D. The dilemma of diagnostic testing for Prader-Willi syndrome. Transl Pediatr. 2017;5(1):46-56. DOI: http://dx.doi.org/10.21037/tp.2016.07.04.
5. Butler MG. Prader-Willi Syndrome: Obesity due to Genomic Imprinting. Curr Genomics. 2011;12(3):204-15. DOI: https://doi.org/10.2174/138920211795677877.
6. Whitmand, BY. Prader-Willi Syndrome: The More We Know, the Less We Know. No Med. 2024;121(3):235-41.
7. Ciana G, Fertz MC, Pecile V, Demarini S. Premature Birth with Complicated Perinatal Course Delaying Diagnosis of Prader-Willi Syndrome. Case Rep Pediatr. 2011:1-3. DOI: https://doi.org/10.1155/2011/981941.
8. Õiglane-Shlik E, Žordania R, Varendi H, Antson A, Mägi ML, Tasa G, *et al*. The neonatal phenotype of Prader–Willi syndrome. Am J Med Genet. 2006;140(11):1241-4. DOI: https://doi.org/10.1002/ajmg.a.31223.
9. Doyle LW, Carse E, Adams AM, Ranganathan S, Opie G, Cheong JLY. Ventilation in Extremely Preterm Infants and Respiratory Function at 8 Years. N Engl J Med. 2017;377(4):329-37. DOI: https://doi.org/10.1056/NEJMoa1700827.
10. Cataletto M, Angulo M, Hertz G, Whitman B. Prader-Willi syndrome: A primer for clinicians. Int J Pediatr Endocrinol. 2011(1):12. DOI: 10.1186/1687-9856-2011-12.
11. Elissa Y, Leslie D, Solomon LM, Saul RK, Dominick PP. Neonatal Hypotonia. NeoReviews. 2018;19(8):445-455. DOI: https://doi.org/10.1542/neo.19-8-e445.
12. Hirsch HJ, Eldar-Geva T, Bennaroch F, Pollak Y, Gross-Tsur V. Sexual dichotomy of gonadal function in Prader-Willi syndrome from early infancy through the fourth decade. Hum Reprod. 2015;30(11):2587-96. DOI: 10.1093/humrep/dev213.
13. Deal CL, Tony M, Höybye C, Allen DB, Tauber M, Christiansen JS. Growth Hormone Research Society workshop summary: consensus guidelines for recombinant human growth hormone therapy in Prader-Willi syndrome. J Clin Endocrinol Metab. 2013;98(6):1072-87. DOI: https://doi.org/10.1210/jc.2012-3888.

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