

**ASHG/ACMG Report**

**Diagnostic Testing for Prader-Willi and Angelman Syndromes: Report of the ASHG/ACMG Test and Technology Transfer Committee**

**American Society of Human Genetics/American College of Medical Genetics Test and Technology Transfer Committee**

**Overview**

Prader-Willi syndrome (PWS) is a complex disorder whose diagnosis may be difficult to establish on clinical grounds and whose genetic basis is heterogeneous. Slightly >70% of cases are due to a 15q11-q13 deletion in the paternally contributed chromosome. These deletions are optimally detected by FISH utilizing SNRPN (small nuclear ribonucleoprotein N) and alpha-satellite DNA probes. Approximately 28% of cases of PWS are due to maternal uniparental disomy (UPD). This abnormality can best be documented using microsatellite probes. Less than 2% of cases have an abnormality in the imprinting process, which causes nonexpression of paternal genes in the PWS critical region. The latter group is detectable through identification of parent-of-origin differences by using methylation-sensitive SNRPN or PW71B probes, a process called methylation analysis. Chromosome analysis is usually a routine part of the evaluation of these patients, in order to rule out other abnormalities, and will also detect rare instances of translocations or other chromosome rearrangements. Although high-resolution chromosome analysis will reveal many interstitial deletions, it is no longer considered sufficient.

Angelman syndrome (AS) is a clinically distinct disorder from PWS that can also be difficult to diagnose, particularly in the first few years of life. Approximately 70% of cases of AS have a deletion of 15q11-q13 in the maternally contributed chromosome 15. In most cases, this is the same deletion as that identified in PWS. About 3%---5% of cases of AS are due to paternal UPD. An abnormality in the imprinting process has been described in about one-third of the remaining 25% of patients; others in that group are hypothesized to have AS on the basis of a single gene mutation whose genetic locus is unknown. This category includes some familial cases that are mapped to 15q11-q13.

None of the recurrences of PWS or AS, to date, have involved the typical deletion or UPD, but rather have involved translocations, imprinting mutations, or AS patients with no detectable molecular abnormality.

The following are the recommendations of a Joint American College of Medical Genetics/American Society of Human Genetics Test and Technology Transfer Committee working group, which was convened to identify scientifically and clinically

valid approaches to the diagnosis of PWS and AS and determination of their genetic basis.

## **Assessing the Scientific Validity of Diagnostic Testing for PWS and AS**

### *High-Resolution Chromosome Analysis*

The typical 15q11-q13 interstitial chromosome deletion seen in 70% of those with PWS and in 70% of those with AS is usually detectable by high-resolution chromosome analysis (550 bands). However, recently developed molecular analysis techniques demonstrate that both false-negative and false-positive results occur with this technique (Kuwano et al. 1992; Delach et al. 1994). Therefore, high-resolution chromosome analysis is insufficient for deletion detection. Any deletion suspected by cytogenetics should be confirmed by FISH or other molecular techniques. Cytogenetic analysis alone will, however, identify translocations predisposing to 15q deletion and will also identify some disorders that mimic PWS and AS. Many patients will have had at least a routine chromosome analysis before molecular diagnostic techniques are considered.

### *FISH*

FISH analysis is scientifically and clinically valid for the detection of the 15q11-q13 deletion found in 70% of people with typical PWS and in 70% of those with typical AS (Kuwano et al. 1992; Delach et al. 1994). Currently commercially available probes within the common deletion boundaries (e.g., SNRPN probe) should be used in combination with any probe outside these boundaries. Use of a probe centromeric to the typical deletion site (e.g., a 15 alpha-satellite probe) allows discrimination between those cases that are due to an interstitial deletion (the majority of patients) and those cases in which a translocation caused the deletion. Recurrence risk within the family may be significant in cases with a translocation. Two-color FISH using one or two probes within the typical deletion boundaries and a centromeric probe is optimal.

### *PCR to Detect UPD*

This scientifically and clinically valid technique uses informative markers to trace the transmission of chromosome 15 from each parent to the child in order to determine whether the child demonstrates normal biparental inheritance, has only maternal markers (PWS), or only paternal markers (AS) (Mutirangura et al. 1993; Ledbetter and Engel 1995). This process can be used to identify UPD, when combined with FISH, methylation analysis (see below), or markers outside the PWS/AS region. UPD is identified by a failure to inherit chromosome 15 alleles from one parent when deletion can be ruled out. Molecular genetic analysis of short tandem repeats within the usual deletion boundaries or in other parts of chromosome 15 is used in most molecular laboratories. Dosage analysis is not a reliable method to distinguish deletion from UPD. UPD analysis requires samples from the proband and both parents, making it impractical in some cases. Confirmation of stipulated paternity can be obtained through the use of markers on other chromosomes.

## *Methylation Analysis*

This analysis based on Southern hybridization detects the parent-of-origin--specific methylation imprint at loci that show differential methylation (Glenn et al. 1993). It is a scientifically and clinically valid test for SNRPN and PW71B probes (Gillissen-Kaesbach et al. 1995), both of which are available through ATCC. Parental samples are not required for methylation analysis. If methylation analysis demonstrates only the maternal pattern, PWS is confirmed; if it shows only the paternal pattern, AS is confirmed. To distinguish between deletion and UPD for genetic-counseling purposes, FISH and/or PCR studies must be done. Methylation analysis will identify deletion cases that are due to translocation as being affected but will not distinguish these from the common interstitial deletion, which requires a chromosome analysis and/or FISH. In addition, some cases of PWS and AS will have a maternal only or paternal only methylation imprint, respectively, but will lack deletion and UPD as demonstrated by FISH and PCR. These cases should be referred to a research lab, since they may have a microdeletion in the putative imprinting center (Nicholls 1993; Reis et al. 1994; Buiting et al. 1995; Sutcliffe et al. 1995).

## *Exceptions*

If the above analyses fail to demonstrate a genetic abnormality, and the clinical diagnosis is still suspected, referral to a clinician experienced in the disorder is indicated. Some cases of AS (approx. 15%--20%) do not demonstrate paternal specific inheritance or methylation imprint and will be missed when any of the above techniques are used (White and Knoll 1995). Referral to a research lab should be considered for further molecular investigation in this situation.

## **Approaches to Diagnostic Testing For PWS/AS**

Two different approaches to the laboratory diagnosis of PWS or AS are appropriate. The one used for a specific patient will depend on a number of factors, including local availability of testing, previously accomplished studies on the patient, and the level of diagnostic expertise of the referring physician.

### *Approach I*

- A. Conduct analysis of parent-of-origin status by using Southern hybridization with the methylation-sensitive SNRPN or PW71B probes.
  1. If biparental inheritance is identified, PWS is ruled out on the basis of all cases known to date. Most identifiable causes of AS are also ruled out.
  2. If only maternal alleles are present, the diagnosis of PWS is confirmed. FISH and PCR can be used to determine whether deletion, UPD, or an imprinting mutation is the cause, for genetic-counseling purposes. If the PWS is due to deletion, do FISH on the patient's father to rule out a balanced insertion or an inherited deletion not expressed in the father.

3. If only paternal alleles are present, the diagnosis of AS is confirmed. FISH and PCR can be used to determine whether deletion, UPD, or an imprinting mutation is the cause, for genetic-counseling purposes. If the AS is due to deletion, do FISH on the patient's mother to rule out a balanced insertion or an inherited deletion not expressed in the mother.
- B. Chromosome analysis (equal or greater than 550 band level) should be performed to seek possible chromosomal translocation or rearrangement.

### *Approach II*

- A. Perform chromosome analysis and FISH using SNRPN or other probe in the common deletion region, along with a centromeric probe.
- B. Perform methylation analysis, which will detect both UPD and imprinting mutations.
  1. If methylation analysis is normal, PWS and most identifiable causes of AS are ruled out.
  2. If methylation analysis is abnormal, PWS is confirmed if only the maternal band is present and AS is confirmed if only the paternal band is present.
- C. If methylation analysis is abnormal and there is no deletion by FISH, do UPD studies by PCR using microsatellite markers from 15q11-13.
  1. If maternal UPD is present, the diagnosis of PWS is confirmed.
  2. If paternal UPD is present, the diagnosis of AS is confirmed.
  3. If biparental inheritance is identified, an imprinting mutation is suggested. Referral to a research lab should be considered for further molecular investigation.

### *If Above Studies Are Normal*

- A. If these studies fail to demonstrate a laboratory abnormality and the clinical diagnosis of PWS or AS is still strongly suspected, consultation with a clinician experienced with the disorder being considered is indicated.
- B. Consider evaluation for other conditions with phenotypes overlapping with PWS and AS that can be studied by high-resolution chromosome analysis, molecular studies (e.g., fragile X), FISH (e.g., elastin probe for Williams-syndrome or Smith-Magenis-syndrome probe), or metabolic studies (e.g., for Albright hereditary osteodystrophy), etc.
- C. Some cases of AS (approx. 15%---20%) do not demonstrate parent-specific methylation and will be missed if any of the above techniques are used. Referral to a research lab should be considered in these situations for further molecular investigation.

### *Prenatal Detection*

- A. In certain circumstances when prenatal diagnosis is done for other purposes (e.g., advanced maternal age), an abnormality may be found that raises the question of possible PWS or AS. Prenatal detection is possible as follows:
1. When cytogenetic deletion is suspected with chorionic villus sampling (CVS) or amniocentesis, perform FISH with SNRPN or other PWS/AS---critical region probes for confirmation.
  2. When trisomy 15 mosaicism is detected on CVS or amniocentesis, perform PCR to detect UPD 15. Check paternity status, using probes from other chromosomes.
    - a. If maternal UPD is present, PWS is confirmed.
    - b. If paternal UPD is present, AS is confirmed.
  3. In cases in which there is prenatal identification of a familial or de novo translocation or marker involving chromosome 15, deletion 15q or UPD is possible. Perform FISH and UPD analysis, as above.
- B. When one individual in a family has PWS on the basis of the typical deletion or UPD, recurrence risk is low (probably 1%). However, for reassurance, prenatal testing using FISH or PCR and microsatellite analysis is possible.
- C. In cases of PWS or AS associated with a translocation present in one parent, a significant recurrence risk exists and prenatal detection by cytogenetics including FISH or microsatellite studies is possible.
- D. In cases of PWS or AS due to an imprinting mutation, recurrence risk is substantial (probably 50%). Prenatal diagnosis of an imprinting mutation previously identified in a proband is possible. Methylation analysis of CVS or amniocentesis samples has not yet been validated so that prenatal testing is not currently available in families with abnormal methylation but normal FISH and UPD analysis who do not have a detectable imprinting mutation.
- E. Cases of AS without an identifiable cause could have a substantial recurrence risk (up to approx. 50%), but prenatal detection is not currently available in such cases.

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## **Endorsements**

This statement has been approved by the ASHG/ACMG Test and Technology Transfer Committee (M. Watson, Chair) and the Boards of Directors of the ASHG and ACMG.

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