# MOLECULAR MARKERS FOR DIAGNOSIS OF PRADER-WILLI SYNDROME IN THAI PATIENTS BY FISH

Sirilak Wiriyaukaradecha[1], Pimpicha Patmasiriwat[1], Pornswan Wasant[2] and Pornsri Tantiniti[1]

[1]Department of Microscopy, Faculty of Medical Technology, Mahidol University, Bangkok;
[2]Department of Pedriatrics, Faculty of Medicine, Mahidol University, Bangkok, Thailand

**Abstract.** Paternal microdeletion of chromosome 15 at q11-q13 has been reported in 75 % of Prader-Willi syndrome (PWS) patients in western countries. Diagnosis of PWS in Thailand is mainly based on clinical observation and, in some cases, confirmed by conventional cytogenetic analysis. Loss of a tiny segment in this region (microdeletion) has made it difficult to discriminate from the normal karyotype. An attempt to solve this problem has been made by using a high resolution chromosome culture. However, this method is a tedious and time-consuming technique which is suitable for only experienced cytogeneticists. We report molecular cytogenetic analysis for PWS in Thai patients using FISH in addition to standard GTG- banding chromosome analysis. Nine Thai patients clinically diagnosed or with a suspicion of PWS were investigated. The FISH probes consist of the region-specific probes (SNRPN or D15S10 probe) and two chromosome 15-specific control probes (D15Z1 centromeric and PML chromosome 15 long arm probe). Bright field and FISH programs of an automatic karyotyper were applied to facilitate the efficiency of the chromosome analysis. We found that 2 out of 9 patients showed a deletion at 15q11-q13 region by standard GTG chromosome analysis while 4 out of 9 patients showed a delation in this region by FISH. Consistent losing of SNRPN and D15S10 signals in FISH was observed in these patients. This forty-four per cent deletion is considerably lower than those reported from western countries. We propose that DNA methylation at SNRPN promoter as well as structural abnormalities in other chromosome regions might also play a role in the etiology of this disorder in Thais, which should be investigated further.

## INTRODUCTION

Selective loss of expression of maternal and paternal genetic material has been referred to as genomic imprinting (Engel, 1980; Hall, 1990). Loss function of the remaining homologous gene is an important etiology of certain genetic disorders such as Prader-Willi syndrome (PWS) and Angelman syndrome (AS). These conditions are neurobehavioral disorders that result from the loss of expression of imprinted genes in the paternal (PWS) and maternal (AS) chromosome 15. Several genes believed to be involved in PWS. They are active only on the chromosome inherited from the father. Thus, a paternally inherited deletion removes the single active copy of these genes. One of these genes is SNRPN which encodes a small nuclear riboprotein that is expressed in brain tissue. A gene that causes Angelman syndrome encodes protein involved in ubiquitin-mediated protein degradation during brain development. This gene is active only on the chromosome inherited from the mother (Jiang *et al*, 1998; Mann and Bartolomei, 1999). PWS and AS can occur through different mechanisms, such as deletion of 15q11-q13, uniparental disomy (UPD), imprinting mutation and, in some AS cases, mutation in UBE3A (Nicholls *et al*, 1998). Parental microdeletion of chromosome 15q11-q13 has been observed in approximately 75% of PWS patients in western countries (Connor and Ferguson-Smith,1997) and maternal UPD accounts for about 25% of PWS patients (Mascari *et al*, 1992; Robinson *et al*, 1991).

Prader-Willi syndrome occurs with the frequency of 1 in 10,000-20,000 livebirths. The disorder is characterized by hypotonia, swallowing difficulties in the newborn, short stature and char-

Correspondence: Pimpicha Patmasiriwat, Department of Microscopy, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand.
Tel: (66) 2419 7169, (66) 1809 9927; Fax: (66) 2412 4110
E-mail: mtppm@mahidol.ac.th

acteristic face, including narrow bifrontal diameter, almond-shaped eyes, and triangular mouth. A representative Thai child with PWS is shown in Fig 1. In adult patients, external genitalia are usually hypoplastic. Hyperphagia and severe obesity are common features of PWS. The hands and feet of PWS patients are usually small. The IQ of PWS patients varies between 20 and 80 (Butler, 1990; Connor and Ferguson-Smith, 1997). Consensus diagnosis criteria for PWS were developed in 1993 (Holm *et al*, 1993) and have proven to be accurate. Details of the criteria are described in Table1. Fifty percent of PWS cases have a cytogenetic microdeletion within the long arm of chromosome 15 at 15q11-13. It is a tiny interstitial deletion at the limits of optical resolution, using the conventional cytogenetic technique. A high resolution technique can sometimes be used to detect the microdeletion (Yunis,1976; Ikeuki, 1984), however, it is not always applicable for Prader-Willi syndrome because it is time consuming and has a high false positive and false negative rates (Hoo *et al*, 1990; Bettio *et al*, 1995; Smith *et al*, 1995). Deletions in chromosome 15q of variable sizes have been reported in a further 25% of cases by FISH or DNA analysis with probes from the deleted region. Although molecular techniques exhibit higher sensitivity than the standard (conventional) one, it is still necessary to perform the paired standard cytogenetic analysis to rule out Prader-Willi-Like syndrome. A condition with signs and symptoms similar to PWS with the exception of the 15q11-13 deletion (Stein *et al*, 1996; Monaghan *et al*, 1998). In this work, the FISH was performed with two DNA specific probes: the DNA probe that recognizes the region within the common PWS region (D15S10) and the probe for the minimal critical PWS region (SNRPN = Small Nuclear Ribonucleoprotein Polypeptide N) (Butler *et al*, 1996; Ishikawa *et al*, 1996). Clinical and laboratory results from

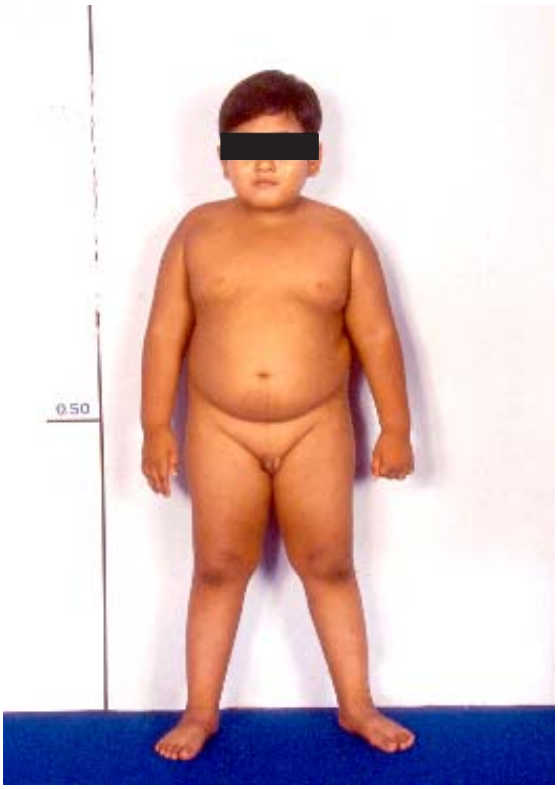


Fig 1–Thai patient at 9 years of age.

Table1
The criteria of PWS presented by Holm, 1993.

| Major criteria | Minor criteria | Supportive criteria |
|---|---|---|
| Neonatal and infantile central hypotonia | Decreased fetal movement and infantile lethargy, improving with age | High pain threshold |
| Feeding problem | Typical behavior problems | Decreased vomiting |
| Onset of rapid weight gain | Sleep disturbance/ sleep apnea | Scoliosis and/ or kyphosis |
| Hyperphagia | Short stature for the family | Early adrenarche |
| Characteristic facial features | Hypopigmentation | Osteoporosis |
| Hypogonadism | Small hands and feet | Unusual skill with jigsaw puzzles |
| Developmental delay/ mild to moderate mental retardation | Narrow hands with straight ulnar border | Normal neuromuscular studies |

this work will certainly be useful for diagnostic process of PWS as well as other genetic disorders.

## METHODS

### Cytogenetic and FISH experiments

Cytogenetic analysis for GTG banding chromosomes was performed on peripheral blood specimens. At least twenty metaphases were analysed in each sample. Analysis of chromosomes was based on the International System for Human Cytogenetic Nomenclature (ISCN, 1995) (Mitelman, 1995). FISH were performed using the two specific cosmid probes for loci within the 15q11-q12 region (SNRPN and D15S10 (VYSIS, Inc)). Each probe also contained CEP15 (D15Z1) and PML (chrom 15 p and q control probes). FISH were performed according to the manufacturer's instructions with slight modifications. The analysis was facilitated with CytoVision FISH software. Approximately 30 metaphases were analysed in each patient's sample for the FISH analysis.

## RESULTS

Clinical data and laboratory results are demonstrated in Table 2 and Table 3. Of the nine patients clinically diagnosed as PWS, five cases were female and four were male. The age range was between 1 and 10 years. Seven of nine cases demonstrated delayed development and/or mental retardation. Patients 4, 6 and 9 showed delayed development and/or mental retardation, typical facies and hypotonia. Obesity was observed in 6 of 9 patients while small hands and feet were ob-

Table 2
Clinical findings on PWS patients referred for chromosome 15 analysis.

| | Patient ID | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Clinical feature | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Delayed development/ mental retardation | + | + | + | + | NA | + | NA | + | + |
| Typical face | - | - | - | + | - | + | - | - | + |
| Hypotonia | NA | NA | + | + | + | + | NA | NA | + |
| Obesity | + | + | NA | NA | + | + | + | + | NA |
| Small hands / feet | -/- | -/- | -/- | +/+ | -/- | -/- | -/- | -/- | -/- |

NA, non available: the NA indicates that the clinical feature was not notified in the medical record or the test was not carried out.

Table 3
Result of cytogenetic and FISH analysis of clinically diagnosed PWS patients.

| Patient ID | Name | Sex | Age (year) | Standard method | FISH | |
|---|---|---|---|---|---|---|
| | | | | | SNRPN | D15S10 |
| 1 | CHY.I | Male | 10.3 | 46,XY | Normal | Normal |
| 2 | PCH.J | Male | 10.3 | 46,XY | Normal | Normal |
| 3 | PN.S | Female | 1.0 | 46,XX | Normal | Normal |
| 4 | PM.B | Female | 2.6 | 46,XX | Normal | Normal |
| 5 | PW.N | Female | 2.5 | 46,XX,del(15)(q11-13) | Deleted | Deleted |
| 6 | CHL.S | Male | 6.0 | 46,XY | Deleted | Deleted |
| 7 | TN.CH | Male | 6.5 | 46,XY | Normal | Normal |
| 8 | KM.E | Female | 10.3 | 46,XX,del(15)(q11-13) | Deleted | Deleted |
| 9 | WR.A | Female | 1.0 | 46,XX | Deleted | Deleted |

served in only patient 4. Both standard GTG chromosome banding and FISH, of patient 1 to patient 4 showed no microdeletion at 15q (negative result). Patient 5 was a 2.5-year-old girl with hypotonia and obesity. Results from standard chromosome analysis indicated 15q deletion in some metaphases analysed. The microdeletion (loss of FISH signal) at SNRPN and D15S10 regions could be confirmed by FISH with both region specific and chromosome specific probes (positive result). Patient 6 was a 6-year-old boy. He has developmental delay, typical facies, hypotonia and obesity. His conventional cytogenetic finding could not detect a 15q deletion, but loss of signal at SNRPN and D15S10 regions was detected by FISH analysis with both SNRPN and D15S10 probes. Patient 7 was a boy age 6.5 years. He has only obesity. Chromosome analysis and FISH at 15q11-q13 were normal. Patient 8 was a 10.3-year-old girl with developmental delay, and obesity. Patient 9 was a 1-year-old girl. She has developmental delay, typical facies and hypotonia. Laboratory chromosome analysis of patient 8 showed a microdeletion at the 15q11-13 region could be seen in some metaphases using GTG (Fig 2) whereas all metaphases from FISH showed loss of the region specific signal. For patient 9, as in patient 6, microdeletion could not be seen by GTG but was positive by FISH. Representative pictures of non-deleted and microdeleted chromosomes at 15q11-q12 as analysed by FISH are shown in Fig 3.

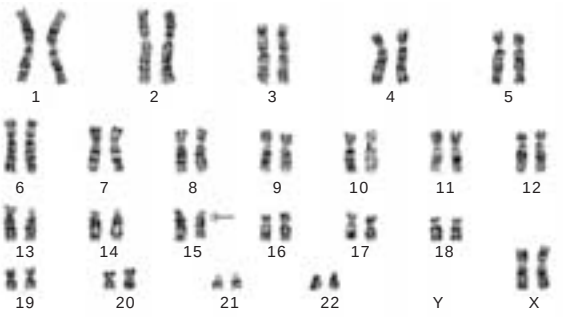


Fig 2–Representative G-banded karyotype of patient 8 showing 46,XX with deleted chromosome 15q11-13.

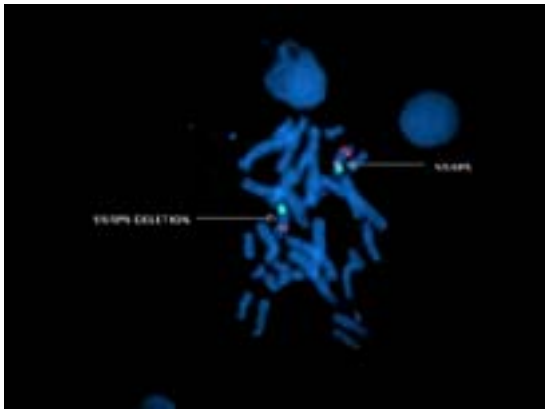

Fig 3–Representative fluorescent metaphase from PW-8 showing deleted fluorescent signal within PWS region by SNRPN probe in one of chromosome 15.

## DISCUSSION

The parental origin of deletion and UPD are evidences of genomic imprinting known to involve in PWS. Our result shows that FISH analysis has diagnostic value for confirming PWS, while the conventional cytogenetic method shows a lower resolution for this microdeletion. Four out of nine patients (44.4%) clinically diagnosed as PWS have a microdeletion on a region of at least 275 kb in length. This deleted area included the *SNRPN* gene as well as the D15S10 locus. While deletion at 15q11-13 was seen in two of the four cases, these four cases showed the same pattern of FISH deletion. The other 5 cases did not show the deletion at the 15q11-13 region by either the GTG or the FISH techniques. Fridman and Koiffmann (2000) reported thirty PWS patients in Brazil who were previously diagnosed by methylation pattern studies of SNRPN exon 1. Eighteen out of 30 patients (60%) had a paternal deletion of 15q11-q13, 8 (26.6%) had maternal UPD and another 4 patients were uninformative of the genetic mechanism. The lower percentage of patients with microdeletions at chromosome 15q11-q13 found in our series (44.4%) could be attributed to several reasons. First, although clinical diagnostic criteria are very important to rule out patients of other similar signs and symptoms from PWS, it is difficult to discriminate the true PWS from PWS-like patients by sole clinical diagnosis without screening with methylation pattern studies. In addition, clinical investigation of PWS is not easy because of the

long duration of the clinical history from birth to early childhood. In some cases, such as patient 7, the clinical data were incompleted and recorded as obesity and hyperphagia, without other important criteria for the diagnosis of PWS. This might be a reason for the negative result (non-deletion) found in the cases who were clinically diagnosed as PWS with less concrete diagnostic criteria for the disease. In fact, some of these patients might have some other conditions not related to PWS. Second, it is possible that maternal UPD or gene mutation is an alternative cause of PWS in the non-deleted patients group expressing the same phenotypes as the deleted one as has been found by other investigators (Mascari *et al* 1992; Mutirangura *et al*, 1993; Robinson *et al*, 1993; 1996). It is possible that patient 4 had this machanism of PWS because she had clinical features of PWS without detection at 15 q 11-13 region. Thus, further molecular study of the non-deleted cases is necessary to search for the various genetic etiologies of PWS or to rule out this disorder. Sakdikul and Mutirangura from Thailand (personal communication) had previously analysed Thai patients clinically diagnosed with PWS by using the methylation specific PCR (MSPCR) and they found that one-third of the Thai patients diagnosed as PWS showed positive results for MSPCR. Although Kubota *et al* (1997) reported that the MSPCR is the most efficient technique and it will show positive results in almost all PWS and majority of AS, this conclusion might not be applicable for Thai patients. Our results show that 4 out of 9 Thai patients who were clinically diagnosed as PWS at Siriraj Hospital exhibited microdeletion of chromosome 15q11-12 by FISH technique (44.4%) (Table 3). This result is in agreement with the work of Sakdikul S and Mutirangura A at Chulalongkorn University in Bangkok (Personal communication). Apart from the incomplete clinical data, we also suggest that there may be some other genetic changes that can give rise to the PWS phenotype in Thai patients. On the other hand, the lower positive result in Thai patients with PWS when tested with either MSPCR or FISH techniques compared to other countries might be the result of the differences in the clinical diagnostic criteria for PWS in each institution. We suggest that both clinical diagnosis and MSPCR screening are necessary to rule out conditions other than PWS before further study of the genetic alteration of PWS.

In conclusion, nine patients clinically diagnosed as Prader-Willi syndrome were studied for detecting the deleted region by molecular cytogenetics (FISH). Standard cytogenetic and FISH techniques were performed in all probands. Positives for microdeletion were detected in four patients when confirmed with FISH with the SNRPN and D15S10 probes. The results support the advantage of FISH as a necessary diagnostic tool for diagnosis of microdeletion syndrome such as PWS. Furthermore, the study of non-microdeleted patients with varying molecular probes is likely to provide further knowledge of the PWS pathogenesis and the other gene(s) involved in Thai patients. Study of more PWS cases in Thais, in conjunction with genetic study of different regions, is open for further investigation.

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