Molecular Diagnosis of Prader-Willi Syndrome

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Abstract

Background : Prader-Willi syndrome (PWS) is characterized by neonatal hypotonia and feeding problems in infancy, developmental delay, hyperphagia with obesity, short stature, hypogonadism, characteristic facial appearance, and behavior problems. The diagnosis of PWS is based on clinical findings that change with age. PWS has proved to be a difficult condition to recognize with the diagnosis often being delayed until later childhood or even adulthood. Therefore, a molecular testing for PWS is needed to confirm the diagnosis.

Objective : To study the clinical features of Prader-Willi syndrome patients and confirm diagnosis by molecular testing.

Material and Method : Eighteen Prader-Willi syndrome patients who were diagnosed between March, 1997 and February, 2002 at the Genetic Unit, Queen Sirikit National Institute of Child Health, Bangkok. Peripheral blood lymphocytes were obtained and cultured using the standard technique for chromosome analysis. For fluorescence in situ hybridization (FISH) studies, the specific DNA probes for loci small nuclear ribonucleoprotein polypeptide N (SNRPN) were used to detect deletion. Non-deleted cases were confirmed to have PWS by methylation analysis.

Results : The diagnosis of eighteen PWS patients was confirmed by FISH using DNA probes for loci SNRPN demonstrating a deletion of chromosome 15q11-q13 in fourteen cases (77%). Four cases (23%) were confirmed to have PWS resulting from maternal uniparental disomy by demonstrating exclusively maternal specific DNA methylation patterns.

Conclusion : The clinical diagnosis of PWS should be confirmed by molecular testing especially in the infancy period to avoid needless invasive diagnostic testing.

Key word : Prader-Willi Syndrome, Fluorescence in Situ Hybridization, Uniparental Disomy, Methylation Study

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Prader-Willi syndrome (PWS) is characterized by neonatal hypotonia and feeding problems in infancy, developmental delay, hyperphagia with obesity, short stature, hypogonadism, characteristic facial appearance, and behavior problems[1]. This condition results from absence of the normally active paternally inherited genes on chromosome 15q11-q13, due to deletion of the paternally derived 15q11-q13 in 70 per cent of cases or by maternal uniparental disomy (UPD) of chromosome 15 (both copies of chromosome 15 are inherited from the mother and none are inherited from the father) in approximately 28 per cent of cases[2-8]. About 2 per cent of cases have apparently normal chromosomes of biparental origin, but lack expression of the paternal gene in the PWS critical region thought to be caused by imprinting mutation[8-11].

The diagnosis of PWS is based on clinical findings that change with age. Although well defined diagnostic criteria has been established, it is often difficult to make the clinical diagnosis of PWS, especially in the newborn and children under 3 years of age[12]. PWS has proved to be difficult to recognize and the diagnosis of this condition is often delayed until later childhood or even adulthood. Therefore, a molecular testing for PWS is needed to confirm the diagnosis. In the past, deletions had involved high resolution G-banding techniques with the difficulties in microscopically assessing the 15q11-q13 region, but currently application of molecular cytogenetic technique using fluorescence in situ hybridization (FISH) analysis with specific DNA probes have proved to be a more sensitive approach[2]. Deletion, UPD and abnormalities in the imprinting process can be documented through identification of an exclusively maternal contribution to 15q11-q13 by means of parent-of-origin differences detectable by methylation analysis[13-18]. In the present study, the author reports the results of the molecular analysis and the clinical evaluation in eighteen patients with Prader-Willi syndrome.

PATIENTS AND METHOD

Eighteen PWS patients were clinically diagnosed by the clinical geneticist at the Queen Sirikit National Institute of Child Health during a 5 year period (March, 1997 - February, 2002). Complete molecular testing and medical history of interest for the study were available for 18 of the 29 patients followed. Patients without molecular testing were excluded from the study. All patients were examined by the author and the diagnosis was made using the well defined diagnostic criteria for PWS[12]. The cardinal PWS manifestations include hypotonia, severe feeding difficulty in infancy, characteristic facial features, small hands and feet, hypogonadism, hypopigmentation of the skin, hyperphagia, obesity and mental retardation. Obesity was not taken into the diagnostic criteria for infants because it usually becomes evident only during childhood. Hypogonadism is identified by genital hypoplasia (cryptorchidism, scrotal hypoplasia and a small penis in males, and hypoplasia of the labia minora and clitoris in females).

Cytogenetic and fluorescence in situ hybridization (FISH) studies

Peripheral blood lymphocytes from all patients were cultured for 72 hours using RPMI culture media supplemented with fetal calf serum, L-glutamate, and phytohemagglutinin. Then, colchicine was added for 15 minutes and the cultures were harvested using standard procedures. G-banding was done on the next day after aging. For each case, a total of 20 metaphases was counted, 5 analyzed and 2 karyotyped. For fluorescence in situ hybridization (FISH) studies, lymphocytes were cultured using standard methods before hybridization. The specific DNA probes for loci within the 15q11-q13 region (SNRPN) were obtained from VYSIS, USA. Each probe also contained chromosome 15 marker cosmids that hybridize to specific sequences in 15p11.2 and 15q22 to facilitate the identification of both 15 homologues. In situ hybridization detection was carried out according to the protocol recommended by the supplier (VYSIS, USA). Detection of the signals was done using a fluorescence microscope. For each case a minimum of 10 metaphases was scored for the presence or absence of paired signals on both homologues for each of the probes using a Zeiss photomicroscope equipped with epifluorescence. If FISH studies detected a deletion, no further studies were done. If the FISH did not show a deletion, methylation studies were performed.

RESULTS

The results of the clinical findings and investigations are summarized in Table 1. Conventional cytogenetic studies (G-banding) of peripheral blood lymphocyte metaphases were normal in all patients except patient no 2 who had a balanced translocation with the karyotyped 46,XX,t(6;9)(p25;q33) which was

Table 1. Clinical and molecular data of patients with PWS.

Case no.	Sex	Age	Hypotonia	Feeding problems	Characteristic facies	Hypogonadism	Hyperphagia	Delayed development/ mental retardation	FISH	Methylation analysis
1	F	2 mo	+	+	-	+	-	+	Normal	Positive
2	F	4 mo	+	+	+	+	-	+	Deletion	ND
3	M	4 y	+	+	+	+	+	+	Normal	Positive
4	F	1 y 9 mo	+	+	+	-	+	+	Deletion	ND
5	F	6 y	+	+	+	+	+	+	Deletion	ND
6	F	9 d	+	+	-	-	-	ND	Deletion	ND
7	F	3 y 4 mo	+	+	+	+	+	+	Deletion	ND
8	M	11 y 8 mo	+	+	+	+	+	+	Deletion	ND
9	M	4 y 9 mo	+	+	+	+	+	+	Deletion	ND
10	M	7 mo	+	+	+	+	+	+	Deletion	ND
11	M	4 y 2 mo	+	+	+	+	+	+	Deletion	ND
12	F	4 y 2 mo	+	+	+	+	+	+	Deletion	ND
13	M	4 y 7 mo	+	+	+	+	+	+	Deletion	ND
14	M	1 y	+	+	+	+	+	+	Deletion	ND
15	F	7 mo	+	+	+	+	-	+	Deletion	ND
16	M	8 mo	+	+	+	+	+	+	Normal	Positive
17	M	16 d	+	+	-	+	-	ND	Normal	Positive
18	F	3 mo	+	+	+	+	-	+	Deletion	ND

Abbreviations are as follows : + = present , - = absent, ND = not determined

inherited from her father. FISH analysis using DNA probes recognizing loci within the common PWS region (SNRPN) was performed to assess the integrity of the 15q11-q13 region. Deletion of the 15q11-q13 region was detected in fourteen of the eighteen PWS patients (77%). Four PWS patients (23%) showed normal FISH results but were confirmed to have PWS resulting from maternal UPD by demonstrating exclusively maternal specific DNA methylation patterns. It is conceivable that in the four patients with normal FISH and maternal pattern only, methylation results could have had an imprinting mutation, though UPD is far more likely. There were 9 females (8 with deletion and 1 with UPD) and 9 males (6 with deletion and 3 with UPD) with a mean age of 2.6 years. The age ranged from 9 days to 11 years and 8 months. Age range of deleted cases was 9 days to 11 years and 8 months. Age range of UPD cases was 1 month to 4 years. Nine cases were first diagnosed under 1 year and two cases were recognized in the newborn period.

DISCUSSION

Prader-Willi syndrome, with an incidence of 1 in 15,000 live births[19], is characterized by neonatal hypotonia and failure to thrive in infancy followed by hyperphagia and obesity beginning in early childhood, hypogonadism, mild to moderate mental retardation, acromiria (small hands and feet), and characteristic facial features. The dysmorphic features seen in PWS consist of a narrow bifrontal diameter, almond-shaped eyes with upslanting palpebral fissures, and thin upper lip. PWS was first described in 1956 by Prader et al. In 1976, Ledbetter et al identified a small deletion of chromosome 15 as a cause of the PWS[15]. Cytogenetic studies demonstrate a deletion of a proximal portion of chromosome 15q11-q13 in approximately 70 per cent of affected cases. Most of them usually arise from de novo deletions in chromosome 15q11-q13 of paternal origin. In 1989, Nicholls et al reported maternal UPD (both copies of chromosome 15 are inherited from the mother and none are inherited from the father) in PWS patients in whom a deletion could not be detected[7]. Normal people usually have an active paternal and inactive maternal imprint of chromosome 15q11-q13. PWS is caused by the absence of normally active paternally inherited genes at chromosome 15q11-q13. The maternally inherited genes are normally inactive owing to genetic imprinting (also called genomic imprinting which refers to differential expression of genes depending on the parent of origin of the genetic information) [20]. Patients with PWS who have either a deletion or UPD inherit only maternal genes from this region. This finding suggests that the region of chromosome 15q11-q13 are not expressed equally from maternally and paternally inherited chromosomes[21,22]. Therefore, the PWS phenotype results from the loss of expression of paternally expressed genes within 15q11-q13 and shows that the inheritance of both maternal and paternal genetic material from the critical region of 15q11-q13 is essential for normal human development. The parent of origin differences in the deletion and uniparental disomy in this syndrome implicate genetic imprinting in this chromosome region. Finding in PWS shows that for some genes it matters whether the genes are inherited from the mother or the father[23]. Alleles at a gene locus are differentially expressed depending on the parent of origin. Thus, the PWS clinical phenotypes result from the lack of the expressed paternal allele. At present, the gene or genes for PWS have not been identified, but there are a number of candidate genes that are expressed from the paternal allele only : zinc-necdin gene 127 (ZNF), human necdin gene (NDN), SNRPN, and imprinted in Prader-Willi gene (IPW). It is likely that PWS results from the loss of function of at least two of these or additional genes[9].

Currently, high resolution chromosome analysis is not routinely performed to detect deletion owing to its high rate of false positive and false negative results and thus is not recommended for the diagnosis of PWS[24]. FISH analysis is required as an additional test to confirm the presence or absence of chromosome 15q11-q13 deletions in PWS patients. Non-deleted PWS cases require methylation pattern studies to assess UPD. The cases which do not show evidence of deletion or UPD should be further investigated to identify mutational mechanisms underlying PWS[3]. Although the methylation test detects all cases of PWS caused by deletions, uniparental disomy, and imprinting mutations, it gives no information about the nature of the genetic defect. For genetic counseling purposes, conventional chromosome analysis, fluorescence in situ hybridization and DNA polymorphism studies should be performed[14].

Recognition of PWS in older children and in adults is easily accomplished by those familiar with this condition. However, infants with PWS have proved to be difficult to recognize and the diagnosis of this condition is often delayed until later childhood

or even adulthood. The clinical diagnosis of PWS in infancy may be problematic because many of the features are subtle and evolve with time. The characteristic craniofacial features were not noted in newborn patients and small hands and feet were not present at birth. Of the major diagnostic criteria, only hypotonia, feeding problems and poor weight gain are clinically present in the neonate[25,26]. Delay in diagnosis of PWS may have an influence on the management, the progression and severity of the manifestations of the disorder. Molecular testing for clinically suspected cases of PWS may lower the age at diagnosis and avoiding needless invasive diagnostic tests. Furthermore, the early diagnosis of PWS has important genetic implications and offers therapeutic options for the prevention and treatment. Obesity is the main cause of morbidity and mortality resulting in cardiopulmonary compromise, type II diabetes mellitus, sleep apnea, hypertension, thrombophlebitis and chronic leg edema[20]. Early diagnosis should lead to improved early dietary, behavioral and therapeutic interventions, and thus, to potentially better outcomes for patients and their families.

Based on the present study, fourteen cases were confirmed by FISH and the rest were confirmed by methylation analysis. Among major clinical signs of PWS, neonatal hypotonia and feeding problems with poor weight gain in infancy were noted in all patients. A number of recognized features of PWS in infancy were confirmed in the present study. Laboratory evaluation of patients suspected to have PWS can be helpful particularly in infants or patients presenting at the time of evaluation without several of the major manifestations of the syndrome. The author suggests that the FISH technique to detect deletion should be adopted as a confirmatory diagnostic test. If no deletion is detected, methylation analysis is necessary. This approach easily adaptable to a cytogenetic laboratory, is the quickest method for the diagnosis of the deletion which is the cause of PWS in most cases.

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การวินิจฉัยกลุ่มอาการพราเดอร์-วิลลี่โดยวิธีทางอณูวิทยา

*สุทธิพงษ์ ปังคานนท์, พบ**

ความเป็นมา : กลุ่มอาการพราเดอร์-วิลลี่มีลักษณะสำคัญของโรคคือผู้ป่วยจะมีภาวะตัวอ่อนและมีปัญหาทางด้านการกินในระยะทารก ต่อมาจะมีพัฒนาการล่าช้า กินเก่งและอ้วน ตัวเตี้ย ลักษณะแสดงออกทางเพศที่ล่าช้า รูปร่างใบหน้าที่มีลักษณะเฉพาะและมีปัญหาทางด้านพฤติกรรม การวินิจฉัยโรคนี้ส่วนใหญ่ต้องอาศัยอาการทางคลินิกซึ่งจะมีการเปลี่ยนแปลงตามอายุของผู้ป่วย การวินิจฉัยโรคนี้ทำได้ยากโดยเฉพาะในทารก ดังนั้นการตรวจวินิจฉัยเพื่อยืนยันโรคนี้จึงจำเป็นต้องใช้วิธีการตรวจทางอณูวิทยามาช่วย

จุดประสงค์ : เพื่อศึกษาลักษณะและอาการแสดงทางคลินิกของกลุ่มอาการพราเดอร์-วิลลี่และยืนยันการตรวจวินิจฉัยโดยวิธีการตรวจทางอณูวิทยา

วัสดุและวิธีการ : ผู้ป่วยกลุ่มอาการพราเดอร์-วิลลี่ จำนวน 18 รายซึ่งได้รับการวินิจฉัยจากอาการทางคลินิกที่หน่วยพันธุกรรม สถาบันสุขภาพเด็กแห่งชาติมหาราชินี ในช่วงระยะเวลาตั้งแต่เดือนมีนาคม พ.ศ. 2540 ถึงเดือนกุมภาพันธ์ พ.ศ. 2545 การเพาะเลี้ยงเซลล์เพื่อตรวจโครโมโซมใช้วิธีมาตรฐาน การตรวจด้วยวิธีฟลูออเรสเซนต์ อินไซด์ตู ไฮบริไดเซชั่นเพื่อตรวจหาตำแหน่งที่มีการขาดหายของโครโมโซม ใช้ตัวตรวจหาดีเอ็นเอที่ตำแหน่ง SNRPN ในรายที่ผลการตรวจไม่พบว่ามีการขาดหายของโครโมโซมจะทำการตรวจยืนยันโรคโดยใช้วิธีการตรวจเมทิลเลชั่น

ผลการศึกษา : ผู้ป่วยซึ่งได้รับการวินิจฉัยว่าเป็นกลุ่มอาการพราเดอร์-วิลลี่จากอาการทางคลินิกจำนวน 18 ราย ได้รับการตรวจยืนยันโดยวิธีฟลูออเรสเซนต์ อินไซด์ตู ไฮบริไดเซชั่นโดยใช้ตัวตรวจหาดีเอ็นเอที่ตำแหน่ง SNRPN พบว่ามีการขาดหายของโครโมโซมที่ตำแหน่ง 15q11-q13 จำนวน 14 รายคิดเป็นร้อยละ 77 ของผู้ป่วยทั้งหมด ผู้ป่วยที่เหลือจำนวน 4 รายคิดเป็นเป็นร้อยละ 23 ของผู้ป่วยทั้งหมดได้รับการตรวจยืนยันว่าเป็นกลุ่มอาการพราเดอร์-วิลลี่ซึ่งมีสาเหตุมาจากยูนิ-พาเรนทอลไดโซมี โดยผลการตรวจเมทิลเลชั่นพบเฉพาะส่วนของโครโมโซมที่มาจากแม่

สรุป : การวินิจฉัยกลุ่มอาการพราเดอร์-วิลลี่จากอาการทางคลินิกควรได้รับการตรวจยืนยันโดยวิธีการตรวจทางอณู-วิทยา โดยเฉพาะอย่างยิ่งในผู้ป่วยทารกเพื่อหลีกเลี่ยงการตรวจวินิจฉัยอื่น ๆ ที่ไม่จำเป็น

คำสำคัญ : กลุ่มอาการพราเดอร์-วิลลี่, ฟลูออเรสเซนต์ อินไซด์ตู ไฮบริไดเซชั่น, ยูนิพาเรนทอลไดโซมี, การตรวจเมทิลเลชั่น

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