Endocr (2009) 36:37–44
DOI 10.1007/s12020-009-9203-1

CASE REPORT

Clinical and genetic analysis for four Chinese families with Prader–Willi syndrome

Yu-wen Zhang · Hui-ying Jia · Jie Hong · Yan Ge · Hui-jie Zhang · Chun-fang Shen · Lei Ye · Bin Cui · Xiao-ying Li · Wei-qiong Gu · Yi-fei Zhang · Wei-qing Wang · Guang Ning

Received: 17 February 2009 / Accepted: 9 April 2009 / Published online: 7 May 2009

Abstract Prader–Willi syndrome (PWS) is a complex, genetic, multisystem disorder. Its major clinical features include neonatal hypotonia and failure to thrive, mental retardation, hypogonadism, short hands and feet, hyperphagia-caused obesity, and characteristic appearance. The genetic basis of PWS is also complex. It is caused by the absence of expression of the active paternal genes such as the *SNRPN*, *NDN*, and possibly others in the PWS critical region on 15q11–13. PWS is in effect a contiguous gene syndrome resulting from deletion of the paternal copies of the imprinted. Consensus in clinical diagnostic criteria was established in 1993. However, identifying relevant patients for tests remains a challenge for most practitioners, as many features of the disorder are nonspecific, and others can be subtle or evolved over time. Consequently, molecular genetic tests can be used to diagnose PWS accurately, allowing early diagnosis of the syndrome. High resolution G-banding, high resolution cytogenetic methylation-specific PCR (MS-PCR), and fluorescence in situ hybridization (FISH) are routinely used to diagnose PWS. In this study, four Chinese patients, with typical PWS features, were detected by MS-PCR and FISH. Three were cytogenetically normal, but lacked paternal expression of proximal chromosome 15q because of maternal uniparental disomy (UPD). The other one, however, demonstrated an unbalanced de novo translocation 46, XX, t (7; 15).

Keywords Prader–Wlli syndrome · Obesity · Genomic imprinting · *SNRPN* gene · Uniparental disomy maternal · Methylation

Yu-wen Zhang and Hui-ying Jia have contributed equally to this work.

Y. Zhang · H. Jia · J. Hong (✉) · Y. Ge · H. Zhang · L. Ye · B. Cui · X. Li · W. Gu · Y. Zhang · W. Wang · G. Ning
Department of Endocrine and Metabolic Diseases, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai Key Laboratory for Endocrine Tumors, Endocrine and Metabolic Division of Shanghai Universities E-Institutes, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, 197 Ruijin Er Road, Shanghai 200025, People's Republic of China
e-mail: hongjie13d@hotmail.com

B. Cui · X. Li · G. Ning
Health Science Center, Shanghai Institute of Biological Sciences, Chinese Academy of Science and Shanghai Jiao Tong University Medical School, Shanghai, People's Republic of China

C. Shen
Department of Endocrine and Metabolic Diseases, Minhang Hospital, Ruijin Conglomerate, Shanghai, People's Republic of China

Introduction

Prader–Willi syndrome (PWS) is a genetic disorder that was first described by Down [1] and was reported by Prader et al. [2]. It is characterized by severe hypotonia and feeding difficulties in early infancy, followed by infancy or early childhood excessive eating (hyperphagia) that leads to gradual development of morbid obesity, variable mental retardation, delayed development, distinctive behavior, hypogonadism, or even infertility. Short stature and characteristic facial features are often present [2, 3]. Although symptoms caused by excessive food intake are a hallmark of the disorder, other psychiatric manifestations are common and can lead to significant interference in the development and normal functioning of the affected individual. Characteristic behavioral symptoms may include temper tantrums, rebelliousness and stubbornness, emotional

lability, obsessive ruminations, and compulsive behaviors such as skin-picking [3]. In light of its distinctive behavioral, psychiatric, and genetic findings, PWS has emerged as a model among genetic and neurodevelopmental disorders. The genetic basis of PWS is known to be associated with the chromosome 15q11–13 region, also known as the Prader–Willi Syndrome/Angelman Syndrome (PWS/AS) region; which contains genes that are epigenetically imprinted. In the 15q11–13 region, normally a set of maternal genes and a set of paternal genes are active. If an abnormal occurrence such as a chromosomal deletion or maternal uniparental disomy (UPD) would inactivate the active paternal genes in this region, leading to PWS [4]. The consensus criteria for diagnosis were first established by Holm et al. [5] and subsequently simplified according to the age of early onset; PWS is the most frequent cause of syndromatic obesity occurring in 1/25000 births, however, this statistic is likely an underestimate; more realistic estimated prevalence of this disease is 1/10,000–22,000 [6]. PWS affects both sexes equally without racial boundary. Early diagnosis and multidisciplinary care with growth hormone (GH) treatment have been shown to improve the developmental outcome of affected children and particularly to reduce the incidence of obesity [7]. Morbility and mortality of PWS are mainly related to severe obesity. Because of the wide phenotypical variability of the PWS, in which severe obesity [8], childhood polyphagia, sleep apnea, and hyperinsulinemia have been reported [9], molecular testing to rule out the syndrome would have been useful or, probably, should be mandatory. Cytogenetic and molecular genetic diagnosis can detect and confirm this disease at an early stage and provide useful information for genetic counseling for families of the affected [10]. Approximately, in 70% of the cases, PWS is the result of deletion of the paternal 15q11–13 region; approximately 28% attribute to maternal UPD; in <2%, it is the result of mutation, deletion, or other defect in the imprinting center [4, 11–15]. Currently, methylation-specific PCR (MS-PCR) and high resolution cytogenetic analysis are commonly used for PWS diagnosis, but FISH is performed for identifying maternal UPD.

In this study, we report four Chinese patients with typical PWS features. Combining MS-PCR with FISH and high resolution G-banding, we detected maternal UPD of chromosome 15 in three of the patients, and the other one, however, demonstrated an unbalanced de novo translocation 46, XX, t (7; 15).

Materials and methods

The study protocols were approved by the Hospital Ethics Committee for Human Research, and informed consent was obtained from every subject participating in the study. The study was approved by the Institutional Review Board of Rui-Jin Hospital, Shanghai Jiao Tong University Medical School, P.R. China.

Subjects

Patient 1

The patient was a 16-year-old girl who was the second child born to nonconsanguineous parents, weighing 2.5 kg at birth, with Apgar's score at 7. The mother remembered that the fetus had not been as active as her healthy elder sister. For the first couple of months, she presented with weak cry and neonatal hypotonia with poor sucking motion, and she was therefore drip-fed. Since the age of 4, she had insatiable appetite, food-foraging behavior, and an excessive sleeping pattern, gaining weight at an alarming speed, and at the age of 6, she had onset of obesity, but with delayed development. She could sit up, walk, and say the first words at the ages of 1, 3, and 4, respectively. She had behavior problems that included temper tantrums and severe aggressiveness. She suffered from massive obesity (height 142 cm, weight 61 kg, BMI 30.25 kg/m^2), diabetes mellitus (FBG 12 mmol/l, PBG 16 mmol/l), and gonadotropin deficiency (LH 0.63 IU/l, FSH 1.53 IU/l) with primary amenorrhea, short distal extremities, and mental retardation. Growth hormone arginine stimulation test showed that GH cannot be stimulated. No specific endocrine causes were found. She also had other dysmorphisms: narrow bifrontal diameter, almond-shaped eyes, downturned mouth with thin upper lip, and small hands and feet. There were no special neuropathological features observed. The family history recorded no unusual event, and the patient has an older sister who was still healthy at the age 25 years. Genetic testing corroborated the diagnosis of PWS. The results of high-resolution chromosome analysis and FISH with the *SNRPN* probe were normal. Deletion of the paternal 15q11–13 region was detected by MS-PCR.

Patient 2

The patient was a 15-year old girl who was the second child of a full-term gestation born to nonconsanguineous parents, weighing 2.6 kg at birth. In retrospect, the pregnancy was unusual because of decreased fetal movement. Her Apgar's score was 6. She also presented with weak cry, neonatal hypotonia, and poor sucking ability; she was then bottle-fed at the first couple of months. She had insatiable appetite and food-foraging behavior since the age of 4 and onset of obesity at the age of 5, gaining weight at an alarming speed, yet her development was delayed. She could sit up at age 4, and at age 5, she could walk, and say

the first words in the same year. She had behavior problems that included temper tantrums and severe aggressiveness. She suffered from massive obesity (height 144 cm, weight 69.1 kg, BMI 33.3 kg/m^2), gonadotropin deficiency (LH 0.46 mIU/l, FSH 1.63 mIU/l) with primary amenorrhea, short distal extremities, and mental retardation. She also had other dysmorphisms: narrow bifrontal diameter, almond-shaped eyes, down-turned mouth with thin upper lip, and small hands and feet. There were no special neuropathological features that were observed. The family history recorded no unusual event, and the patient has an elder sister who was healthy. Genetic testing confirmed the diagnosis. The results of high-resolution chromosome analysis and FISH with the *SNRPN* probe were normal. Deletion of the paternal 15q11–13 was detected by MS-PCR.

Patient 3

The proband was a boy of 12 years of age, about 130 cm tall, and weighed 55 kg (BMI 32.54 kg/m^2). He had neonatal hypotonia and lethargy, with poor sucking ability, requiring special feeding techniques. He had alternating esotropia as an infant. His development was also delayed; he could sit up at age 18 months, at age 5, he could walk, and say the first used words at age 8. He had onset of obesity at age 8 with insatiable appetite and food-foraging behavior. He had behavior problems that included temper tantrums and severe aggressiveness. The patient had a squared nasal tip, narrow bifrontal diameter, and down-turned mouth with thick viscous saliva. Initial examination revealed micrognathia, a 2.5-cm penis, and cryptorchidism. Endocrinological evaluation revealed diabetes mellitus (FBG 11.2 mmol/l, PBG 14.1 mmol/l) and gonadotropin deficiency (LH 0.15 mIU/l, FSH 2.66 mIU/l, T 0.18 ng/ml) with descended testicles. The family history recorded no unusual event, and the patient has two older sisters who are healthy. The results of a high-resolution chromosome study were normal (46, XY), including those for FISH analysis with probe A (*D15S11*) and probe B (*GABRB3*) (Oncor). Deletion of the paternal 15q11–13 was also detected by MS-PCR.

Patient 4

She was a 20-year-old girl with short and morbidly obese (height 145 cm, weight 73.5 kg, BMI 34.96 kg/m^2). She was born to nonconsanguineous parents, and her Apgar's score was 6. The pregnancy was noted for decreased fetal movement. Her medical history showed neonatal failure to thrive and hypotonia. For the first couple of months, she presented with weak cry and neonatal hypotonia with poor sucking motion and hence, she was drip-fed. She had onset of obesity with insatiable appetite and food-foraging behavior since age 4. She gained weight at an alarming speed but with delayed development. She could sit up at age 4, and at age 5 she could walk and say the first words in the same year. She had behavior problems that included temper tantrums and severe aggressiveness. She had periods of somnolence at the age of 6, becoming more frequent with age. Then she suffered from massive obesity, short distal extremities, and mental retardation. She also had many dysmorphisms: obesity, small hands and feet, narrow bifrontal diameter, scoliosis. She was mentally retarded and had primary amenorrhea, diabetes mellitus, hypogonadism, and some other metabolic syndromes such as hypertension and albuminuria. There were no special neuropathological features observed. The family history recorded no unusual event. The results of a high-resolution chromosome study were normal (46, XX). MS-PCR of *SNRPN* gene was also normal. However, FISH with the *SNRPN* probe found an unbalanced de novo translocation 46, XX, t (7; 15).

Cytogenetic studies

For each patient, complete karyotype analysis of high-resolution chromosomes G-banding was performed, and special attention was paid to the possibility of chromosome 15 abnormalities.

MS-PCR

Genomic DNA from peripheral blood leukocytes of the four patients and their family members were extracted using a commercially available kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. MS-PCR was performed using the CpGenome^{TM} Prader–Willi/Angelman Amplification Kit of Chemicon (Temecula, CA, USA). PCR products were obtained using either the M or the P primer sets (GenBank accession No. L 32702) after the bisulfite modification of genomic DNA. M primer sets contained M-F (5′-TAAATAAGTACGTT TGCGCGGTC-3′) and M-R (5′-AACCTTACCCGCTCCATCGCG-3′). P primer sets contained P-F (5′-GTAGGTTGGTGTGTATGTTTAGG T-3′) and P-R (5′-ACACAAACATCTCCAACAACCA-3′). Thermo-Start DNA polymerase was commercially obtained from AB gene (Epsom, KT199AP, UK). The bisulfite-modified genomic DNA was amplified in 25-μl volume of mixture containing standard PCR buffer 2 μl, 25 mM dNTP 0.5 μl, genomic DNA 200 ng, 0.5 μl of each specific primer, and 2.5 μ/μl Taq DNA polymerase 0.3 μl (Sangon, Shanghai, China)., with an initial denaturation for 15 min at 95°C. Then, 35 cycles were run with denaturation for 30 s

at 95°C, annealing for 30 s at 62°C, and extension for 30 s at 72°C. The final extension was for 10 min at 72°C. The PCR products obtained after the bisulfite modification of normal control DNA generated a maternal 174-bp product and a paternal 100-bp product. The PCR products obtained after the bisulfite modification of PWS patients' genomic DNA generated only the maternal 174-bp product.

FISH

MS-PCR was used for routine screening, FISH then followed for a positive test to determine whether a deletion was present. PWS and AS are genetically distinct and have been linked to deletion of the 15q11–13 region. Both syndromes may also have resulted from UPD which is not detectable by FISH analysis. DNA FISH probes can be used in metaphase and interphase cells to detect deletion in these specific regions. The LSI Prader–Willi/Angelman Region *SNRPN* probe identifies deletion of the SNRPN locus, also located within the 15q11–13 region. The Spectrum Green CEP 15 (D15Z1) and Spectrum Orange LSI *PML* serve as control probes for the chromosome 15 p and q arms and may be helpful in detecting deletions or translocations of 15q. The LSI *SNRPN* Spectrum Orange probe is available for detection of the 15q11–13 region and does not contain the control probes CEP 15 or LSI *PML*. The normal chromosome 15 shows two fluorescent signals, one from the fluorescence-tagged SNRPN probe within 15q11–13 and the other from a fluorescence-tagged control probe (PML) localized to 15q22.

Results

Typical findings on physical examination and characteristic facial features of these patients with PWS are summarized (Table 1). All the four patients (3 girls and 1 boy, age range 12–14 years) were clinically assessed as PWS after careful examination and scoring; they all demonstrated delayed development, typical facial features, hypotonia, obesity, and small hands and feet. The results of the diagnostic testing are summarized in Table 1. Standard chromosome analysis was performed and all the patients showed normal karyotypes. FISH showed two normal signals for the *SNRPN* region in three patients (P-1, P-2, P-3). Further MS-PCR results showed that the PCR products obtained, after the bisulfite modification of genomic DNA of the three PWS patients, generated only the maternal 174-bp product, suggesting that these three patients were mUPD (Fig. 1). Whereas MS-PCR of *SNRPN* gene was normal (Fig. 1) in patient 4, while FISH of the *SNRPN* probe found an unbalanced de novo translocation 46, XX, t (7; 15) (Fig. 2).

Discussion

PWS is a genetic disorder characterized by mental retardation, dysmorphic features, and behavioral dysfunction, most notably food-related problems such as hyperphagia, food-seeking obsession, and a high risk for obesity [2]. It is a sporadic genetic disorder representing the most common diagnosable genetic obesity syndrome.

Initially, as reported by Prader et al. [2], PWS was conceptualized as a syndrome of obesity, short stature, cryptorchidism, and mental retardation following severe hypotonia in the neonatal period (decreased activity in utero, "floppy" at birth, marked feeding difficulties). With accumulated clinical experience and research studies, behavioral characteristics such as hyperphagia, outbursts of temper, obsessional traits, and stubbornness, and clinical features such as central adiposity, sleep disorders, abnormalities of temperature, and pain perception were added in the Consensus Diagnostic Criteria [5]. These criteria are grouped into major (hypotonia and feeding problems during infancy, dysmorphic facial features, hypogonadism, developmental delay, and hyperphagia with obesity), minor (reduced foetal movements, behavioral problems, sleep disturbance, short stature, hypopigmentation, small hands and/or feet, eye abnormalities, thick viscous saliva, articulation defects, skin picking), and supportive (high-pain threshold, decreased vomiting, temperature-control problems, scoliosis, kyphosis, early adrenarche, osteoporosis, unusual skill with jigsaw puzzles, and normal neuromuscular studies) accordingly. The presence of a manifestation, "small hands and feet," in the patients was judged using the same criteria if their hands sizes were <25th percentile and/or feet sizes <10th percentile compared to height–age standards. Questionnaire-based studies have shown that people with PWS have a characteristic behavioral profile in which outbursts of temper (including aggressive behavior and screaming), self-harm (especially skin picking), mood swings, and repetitive speech are common [16], whereas repetitive questioning, compulsive behaviors, and hoarding are relatively more common; it has been proposed that people with PWS are at increased risk for obsessive/compulsive disorder [17]. Adaptive behaviors (Vineland Adaptive Behavior Scales) [18] show strengths in the Daily Living Skills domain and weaknesses in the Socialisation domain [19]. The most striking behavior is severe and persistent overeating which, if unrestricted, leads to life threatening obesity. It has been proposed that this is the result of a failure of the normal feedback mechanisms that leads to a state of satiation following food intake [20]. Otherwise, many individuals with PWS show a distinctive, although not necessarily unique, profile of cognitive strengths and weaknesses. Some PWS patients show relative strengths in spatial-perceptual organization and visual

Table 1 The diagnostic testing of the four patients

Diagnostic Criteria for PWS/subjects	1	2	3	4
Major Criteria (1 point)				
1. Neonatal and infantile central hypotonia with poor suck reflex, which improves over time	√	√	√	√
2. Characteristic behavior problems—temper tantrums, violent outbursts, and obsessivecompulsive	√	√	√	√
3. Excessive or rapid weight gain (crossing two centile channels in weight for length charts) after 12 months but before 6 years of age; central obesity in the absence of intervention	√	√	√	√
4. Short stature for genetic background by age 15 (in the absence of growth hormone intervention)	√	√	√	√
5. Hypogonadism—with any of the following depending on age: genital hypoplasia or delayed or incomplete gonadal maturation with delayed pubertal signs in the absence of intervention after 16 years of age	√	√	√	√
6. Global developmental delay in children younger than 6 years of age; mild to moderate mental retardation or learning problems in older children	√	√	√	√
7. Hyperphagia, food foraging, or obsessions with food	√	√	√	√
8. Deletion of 15q11–13 on high resolution (greater than 650 bands) or other cytogenetic or molecular abnormality of the Prader–Willi chromosome region, including maternal disomy	√	√	√	
Minor Criteria (0.5 point)				
1. Decreased fetal movement or infantile lethargy or weak cry in infancy, improving with age	√	√	√	√
2. Characteristic behavior problems—temper tantrums, violent outbursts, and obsessive compulsive behavior; tendency to be argumentative, oppositional, rigid, manipulative, possessive, or stubborn; perseverating, stealing, or lying (more than five required)	√	√	√	√
3. Sleep disturbance or sleep apnea	√	√	√	√
4. Short stature for genetic background by age 15 (in the absence of growth hormone intervention)	√	√	√	√
5. Hypopigmentation—fair skin and hair compared to family	√			
6. Small hands (<25 percentile), feet (<10 percentile), or both, for height and age	√	√	√	√
7. Narrow hands with straight ulnar border	√	√		√
8. Eye abnormalities (esotropia, myopia)	√	√	√	√
9. Thick, viscous saliva with crusting at corners of mouth	√	√	√	√
10. Speech articulation defects	√	√	√	√
11. Skin picking			√	
Score	13	12.5	12.5	11.5

Note: A diagnosis of Prader–Willi syndrome should be suspected in children younger than 3 years with a score of at least 5; and in children 3 years and older with a score of at least 8, with 4 points from major criteria

processing tasks; these strengths may relate to the profi- supportive finding in the clinical criteria for PWS. By contrast, common weaknesses are noted in sequential processing and short-term memory tasks, including visual, motoric, and auditory short-term memory [17].

Genomic imprinting refers to differential epigenetic modification dependent on the sex of the parent from which the gene allele is inherited; it determines the activity of the gene [21]. PWS is a well-studied genomic imprinting disease and caused by the absence of the paternally derived PWS/AS region of chromosome 15. PWS is considered the most common syndroma cause of life-threatening obesity. Because of the presence of repeated DNA elements flanking the chromosome region 15q11–13, the region is unstable and many types of rearrangements may occur in this region of the genome. Several genes in this area have been implicated as causative of PWS, including *SNRPN*, *ZNC127*, *NDN*, and *IPW* [22, 23]. The exact role of these genes is still controversial, however. Several cytogenetic and molecular methods have been developed in PWS diagnosis. High-resolution G-banding can detect the chromosomal deletion and rare translocation or ring chromosomes in the PWS/AS region. In a significant minority of PWS cases, deletions are too small to be visualized in this manner. In such instances, DNA

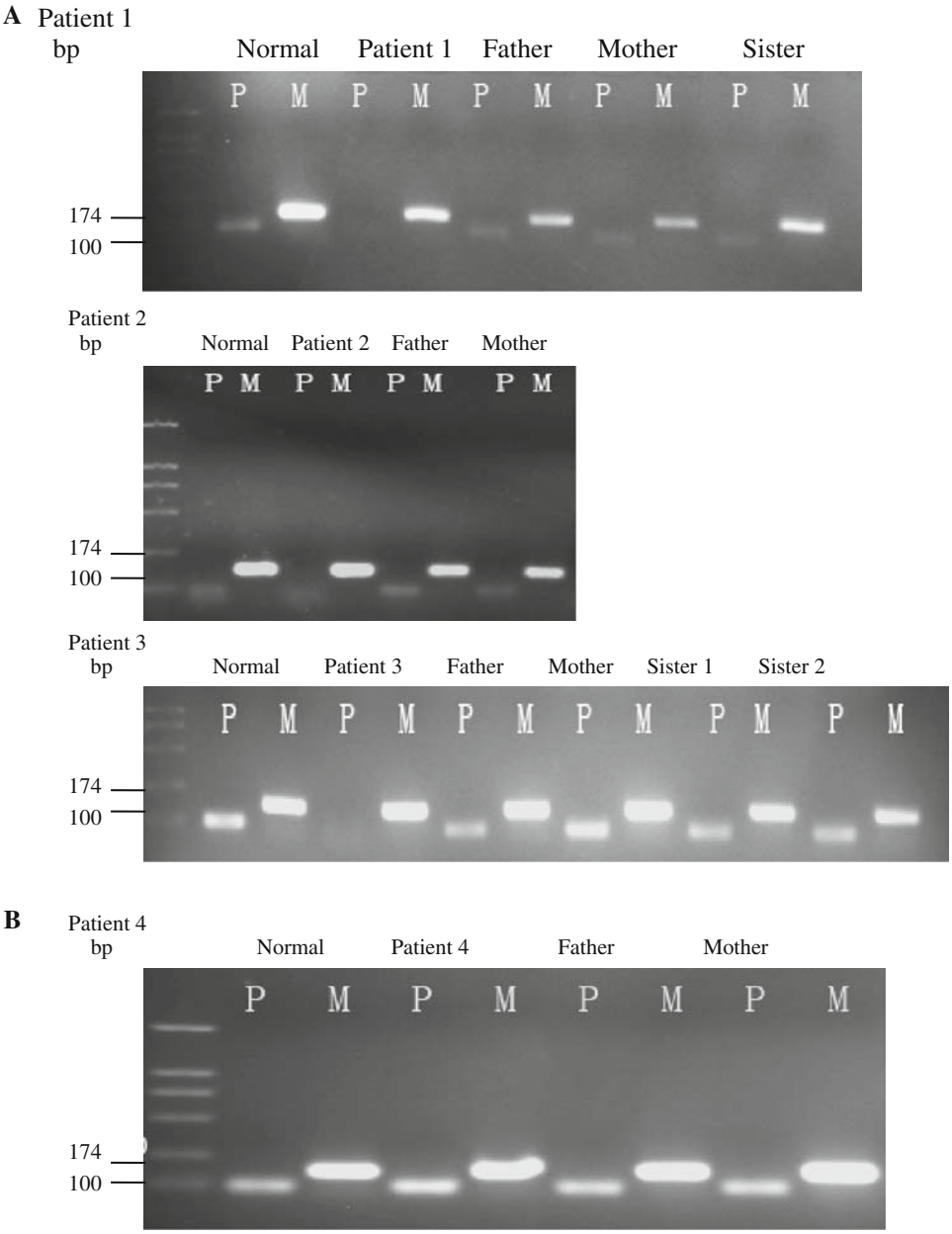

Fig. 1 MS-PCR shows that the three patients (P1, P2, P3) lacked the paternal 100-bp band. **a** MS-PCR of Patient 4 was normal which showed both the paternal and the maternal bands, **b** *M* DNA marker, *P* and *M* in the samples indicated the paternal and maternal products, respectively

probes are available to search for defects in 15q11–13. Probes with nucleotide sequences complementary to those in the Prader–Willi region, including *SNRPN*, can be visualized when linked to a fluorescent marker, a technique called FISH. Despite the much greater resolution afforded by FISH, the approach cannot identify cases of UPD [24]. MS-PCR [25] could then be used to detect abnormal parent-specific imprinting within the Prader–Willi critical region on chromosome 15, increasing the detection capability to more than 99% of the affected individuals. With MS-PCR, the three patients were confirmed with PWS.

Unfortunately, diagnosis of PWS is overlooked, despite the clinical criteria. Management of the syndrome requires a multidisciplinary approach; treatment implications involve medical and dietary management as well as psychiatric intervention. Infants may require supplemental tube feedings to avoid failure to thrive. Early intervention for motor skills, speech, and language is necessary, and individualized education plan at the start of school should be arranged for the affected children. GH therapy in children with PWS increases muscle tone and enhances growth [26, 27]. On the other hand, GH is thought to have other significant benefits, such as allowing certain patients to moderately increase daily caloric intake without incurring substantial weight gain, thus mitigating some of the difficulties associated with maintaining highly restrictive diets. For nonfood-related behavioral problems in PWS, there are few guidelines from psychopharmacology. The relatively

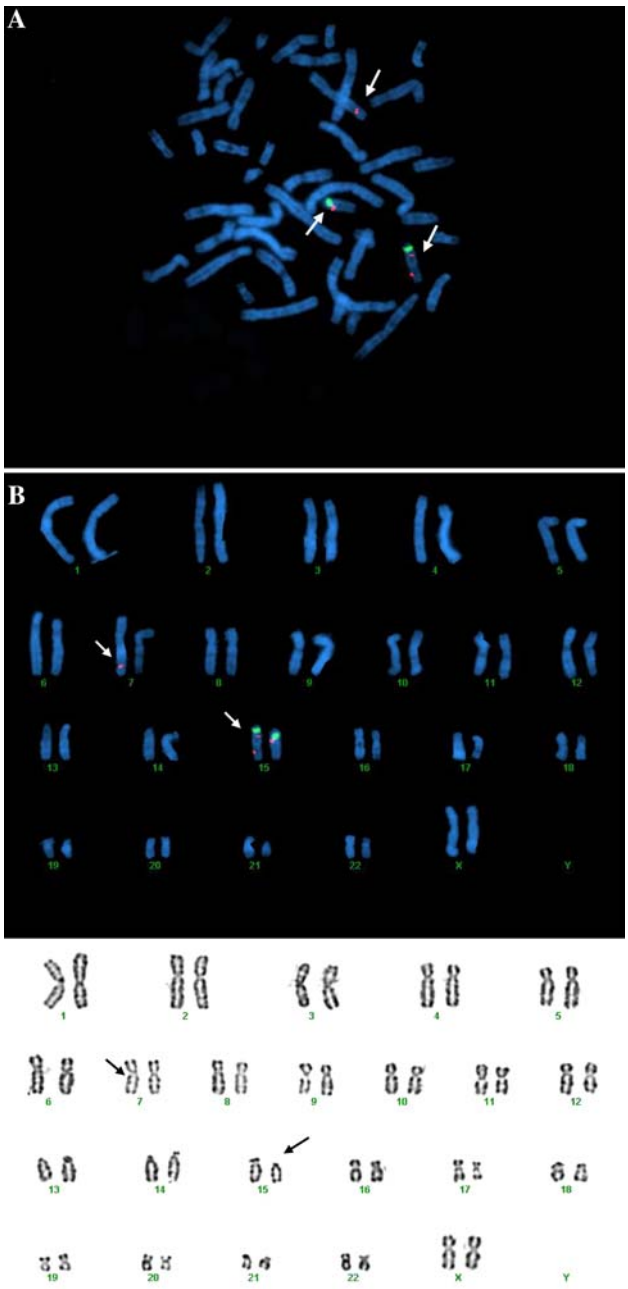

Fig. 2 A balanced de novo translocation 46, XX, t (7; 15) was found on patient 4 (*arrow*). **a** Representative G-banded karyotype of patient 4 showing 46, XX, with deleted chromosome 15q11–13. An unbalanced de novo translocation t (7; 15) (*arrow*) (**b**)

high rate of anxious and depressive presentations among Prader–Willi syndrome patients has led to the frequent use of serotonin re-uptake inhibitors (SSRIs). Even with the clinical success seen with SSRIs and GH, the mainstay of management for behavioral difficulties—both food- and non-food-related—remains with behavior modification. Efforts to restrict food intake by careful dietary planning, close supervision, and limiting food access must extend from home into school, work, and community settings.

The four patients in this study presented with almost all the clinical features of PWS with severe hypotonia and feeding difficulties in early infancy, persisted in later infancy or early childhood by excessive eating (hyperphagia) and gradual development of morbid obesity, variable mental retardation, delayed development, distinctive behavior, hypogonadism, or even infertility, fulfilling the diagnostic consensus criteria for PWS. Standard chromosome analysis was performed for all the cases, and all showed normal karyotypes. After performing FISH and MS-PCR of *SNRPN*, we found that three cases with nondeleted FISH and the absence of the paternal band in the MSP test. The cytogenetic results subsequently confirmed mUPD of chromosome 15. However, MS-PCR of *SNRPN* gene in patient 4 was normal, while FISH of the *SNRPN* probe found an unbalanced de novo translocation 46, XX, t (7; 15).We did not continue the investigation on this de novo translocation, as there might be other abnormalities on some other genes which may require much further investigations, which is beyond our ability. Our patients were treated symptomatically for their diabetes mellitus, albuminuria, and hypertension. Simultaneously, we made efforts to help restrict their food intake by careful dietary planning, close supervision advice given to the parents, and suggested that limiting food access must extend from home into school, work, and community settings. They were not treated with GH or/and SSRIs, since the patients were too old to be effectively treated and not to present many mental symptoms.

In summary, we describe the clinical and genetic features of four patients with PWS. Although previous studies had gained some advances in PWS, our knowledge about this disease is still limited. PWS is a complicated genetic disorder with many manifestations, which are being discovered increasingly with new investigative technology, as well as improvement in life expectancy. Therefore, more researches should focus on understanding the molecular basis of the disease, and a more complete picture with respect to the global distribution of PWS correlative genes mutations is required. We hope this information we show here will open new horizons to future genetic and molecular investigations that may lead to a better elucidation of the pathophysiology of PWS, and hopefully, help improve in the prevention and treatment of this devastating disease.

Acknowledgments We thank all the members of the participating families for their cooperation. This study was supported by grants from Shanghai Leading Academic Discipline Projects (No. Y0204) and Chinese National Natural Science Foundation for Excellent Young Scientist (No. 30725037).

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