# Precise Microdeletion Detection of Prader-Willi Syndrome with Array Comparative Genome Hybridization

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**Objective** Prader-Willi Sydrome (PWS) is a human disorder related to genomic imprinting defect on 15q11-13. It is characterized by a series of classic features such as hypotonia, hyperphagia, obesity, osteoporosis, typical facial and body dysmorphosis, hypogonadism, mental and behaviour disorders. Our study was designed to precisely detect the microdeletions, which accounts for 65%-70% of the PWS. **Methods** Physical and laboratory examinations were firstly performed to diagnose PWS clinically, and to discover novel clinical features. Then the patient was screened with bisulfite-specific sequencing and precisely delineated through high-density array CGH. **Results** With the bisulfite-specific sequencing, the detected CpG island in the PWS critical region was found homozygously hypermethylated. Then with array CGH, a 2.22 Mb type II microdeletion was detected, covering a region from *MKRN3*, *MAGEL2*, *NDN*, *PWRN2*, *PWRN1*, *C12orf2*, *SNURF-SNRPN*, *C/D snoRNAs*, to distal of *UBE3A*. **Conclusions** Array CGH, after the fast screening of Bisulfite-specific sequencing, is a feasible and precise method to detect microdeletions in PWS patients. A novel feature of metacarpophalangeal joint rigidity was also presented, which is the first time reported in PWS.

**Key words**: Prader-Willi Syndrome; array CGH; Bisulfite-specific Sequencing; DNA Methylation; Metacarpophalangeal Joint Rigidity

## INTRODUCTION

Prader-Willi Syndrome (PWS; OMIM 176270) is the first recognized human disorder related to genomic imprinting which causes the failure of expression of paternally inherited imprinted genes in the Prader-Willi Syndrome/Angelman syndrome critical region (PWS/ASCR) of chromosome 15q11-13[1-2]. PWS is characterized by a recognizable complex of dysmorphic features and prominent cognitive, neurologic, motor, endocrine, and behavioural disorders, with a reported prevalence of 1/15 000-1/30 000 and a death rate over 3% annum[1-3]. As for its aetiology, the microdeletions in PWS/ASCR account for 65%-70%, while the maternal uniparental disomy (UPD) for 20%-30% and the imprinting defect only for 2%-5%[1-2].

Array comparative genome hybridization (array- CGH) is a precise method to identify positions and lengths of the microdeletions[4-5] through methylation- specific PCR (MS-PCR), methylation-specific multiplex ligation-dependent probe amplification analysis (MS-MLPA), microsatellite analysis, Real-time PCR, and fluorescence in situ hybridization (FISH) which are all useful techniques to diagnose PWS[6-8]. Hereafter we present the diagnosis of a PWS patient who was screened with DNA methylation analysis and precisely confirmed with array-CGH. A novel hand deformity, metacarpophalangeal joint rigidity, aside from the classic manifestations, was presented in the paper. Status quo of the exploration of microdeletions in the PWS/AS critical region is also discussed.

## MATERIALS AND METHODS

### *Clinical Features*

The patient was noticed for mental and physical development retardation soon after birth. Poor head control, poor suck and feeding difficulty were recorded in her neonatal period. In contrast, since she was 4 years old, the patient has developed into hyperphagia (with food-stealing behavior) and become

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overweighed. Language development delayed, and she spoke her first word when she was 2 years old. The IQ value was 45 according to Wechsler Adult Intelligence Scale (WAIS) at age of 19 without seizure history or menstruation. The patient was admitted to hospital for diabetic ketoacidosis in 2007, and treated with insulin and metaformin thereafter.

The patient was 137 cm and 55 kg, with a body mass index (BMI) of 29.3 kg/m$^2$. (In comparison: father 186 cm, mother 174 cm, sister 176 cm). She had typical PWS appearance, i.e., round face, narrow bifrontal diameter, almond-shaped palpebral fissures, narrow nasal bridge, and thin upper lip with downturned mouth (Fig. 1). In addition, she had no pubic hair but had poorly developed nipples.

The patient had typical small hands and feet deformity. Active and chronic open wounds can be seen on both hands (sign of skin picking behavior). More noticeably, the patient is unable to make a fist. There is rigidity of all the metacarpophalangeal joints which are unable to bend with an angle of more than 30 °, even passively under the help of doctors. In comparison, the angle should be ~90 ° for normal persons. Normal joint spaces exist according to the hands MRI. No evidence of bone malformation, epiphysis enlargement, tendon adhesion or joint fusion of the metacarpophalangeal joints was found (Fig. 2).

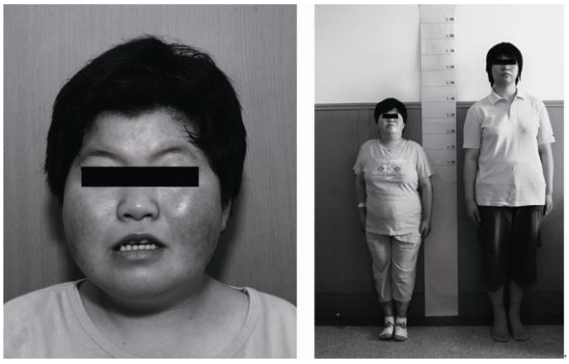

FIG. 1. The typical facial feature (Round face, narrow bifrontal diameter, almond-shaped palpebral fissures, narrow nasal bridge, thin upper lip with downturned mouth), and the short stature (137 cm). On the right side is patient's sister with normal phenotype (176 cm).

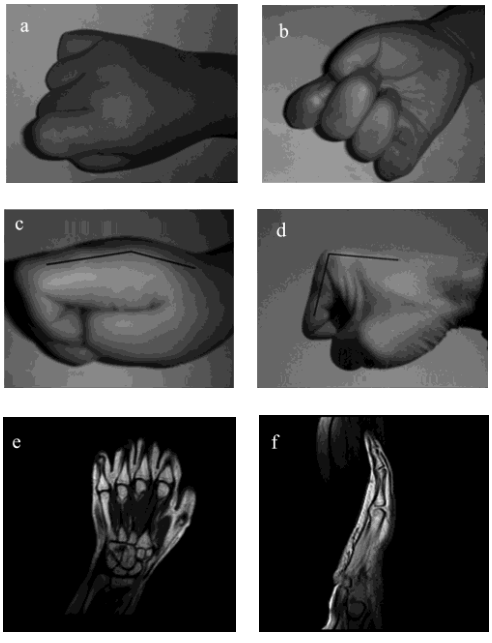


FIG. 2. Rigidity and inability of bendingthe metacarpophal angeal joints, unable to make a fist. It is noticeable that the flatness of opisthenar and the special arrange of fingers in the maximal flexure position (graph a & b). Patient's maximal metacarpophal angeal joint angle is ~30 ° (graph c), in comparison with the normal angle of ~90 ° (graph d). The hand MRI showed good joint spaces without detectable abnormalities on bone, cartilages, muscles or tendons (gragp e & f).

*Bisulfite-specific PCR and Sequencing*

Genomic DNA samples from the patient and her normophenotyped parents and sister were extracted from peripheral blood leukocytes by a standard phenol/ chloroform method, then prepared with methylSEQr™ Bisulfite Conversion Kit from Applied Biosystems Inc. (Foster City, CA, USA). Primers for Bisulfite-specific- PCR were designed with online software Methprimer (http://www. urogene .org/ methprimer/index1.html) accor- ding to exon α of *SNRPN* gene on 15q11.2 on which PWS critical region is located. Forward: 5'-TTTTGATGT ATTTATTTTTATTTATGT-3'; Reverse: 5'-ATTTAAA CCCTAAAATCCTT-3'. PCR was performed with Takara LA Taq and GC I buffer from Takara Bio Inc. (Shuzo, Otsu, Japan) to amplify the high GC-content region, then sequenced with an ABI 3 100 automatic DNA sequencer.

*Array-CGH*

Microarrays used were Agilent SurePrint G3 Human CGH Microarray 244 K with an average distance of 5.3 kb between each probe (Agilent Technologies, Santa Clara, CA). The hybridization and initial analysis were performed commercially at Shanghai Biochip Co. (Shanghai, China). Briefly, the array images were analyzed and the data was extracted by using Agilent's Feature Extraction (FE) software version 9.5 with scan resolution 5 μm, PMT 100%. The chromosomal imbalances within the four family members were identified by using Agilent's CGH analytics software version 3.4.15. The altered

chromosomal regions and breakpoints were detected using Z-scoring (threshold 4.0).

## RESULTS

The results of bisulfite specific PCR and sequencing showed that the amplified 15 pairs of CpG were all homogeneous CG pair in the patient, while all C/TG heterogeneous in her parents and sister (Fig. 3). This means that both of the allelic regions in exon α of gene *SNRPN* were homozygously methylated, which should be heterozygous in normal persons. But this could be consequence of either deletion or maternal uniparental disomy (UPD).

The analysis of array-CGH revealed a 2.22 Mbmicrodeletion (21,122,897-23,348,529 counting from p terminal) in the PWS/AS critical region (PWS/ASCR) on chromosome 15q. Roughly, it covered a region from *MKRN3*, *MAGEL2*, *NDN*, *PWRN2*, *PWRN1*, *C12orf2*, *SNURF-SNRPN*, *C/D snoRNAs* to the distal of *UBE3A* (Fig. 4). Thus, microdeletion was confirmed, with precise size and location.

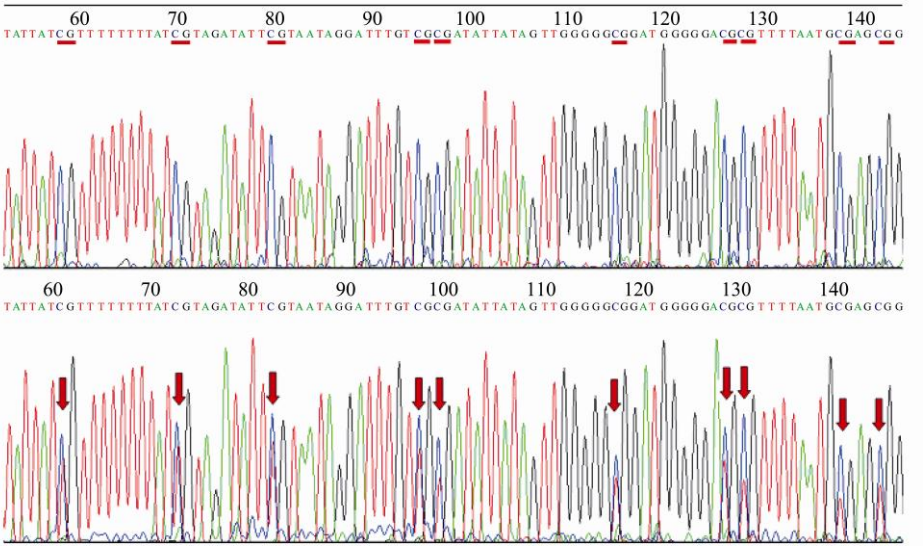


FIG. 3. Results of bisulfite-specific sequencing. Patient's sequence is in upper graph, homogenous CG (hypermethylated) as the red short bars indicated, while in her normophenotype parents and sister they are all heterogenous C/TG, as arrows indicated.

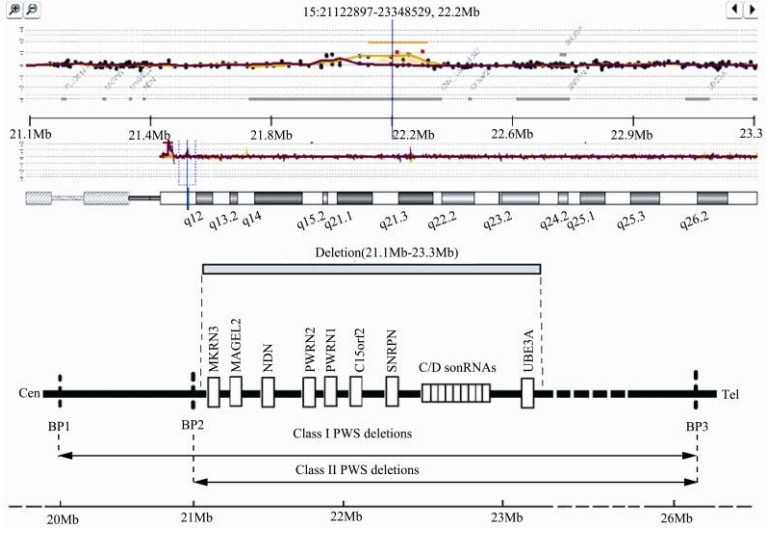


FIG. 4. The array CGH result, showing the location of microdeletion in the PWS critical region. The 2.22 Mbmicrodeletion (21,122,897-23,348,529 counting from pterminal) can be classified as type Ⅱ deletion. Cen: centromeric end, Tel: telomeric end.

## DISCUSSIONS

The PWS region on 15q11.2-q13 includes at least 6 paternal-expressing genes (*MKRN3*, *MAGEL2*, *NECDIN*, *NDN*, *C15ORF2*, and *SNURF-SNRPN*) and a cluster of genes encoding C/D box Small Nucleolar RNAs (snoRNAs) which belong to the same gene family[1-2]. Deletions in PWS/AS critical region accounts for 65%-70% of the PWS, while the maternal uniparental disomy (UPD) accounts for 20%-30% and imprinting defect only for 2%-5%[1-3].

Bisulfite-specific PCR might be the cheapest and quickest way to diagnose PWS. It can analyse DNA methylation which may be the common sequence of all the three categories of PWS, but could not differentiate what the exact defect is[1-2]. So DNA methylation analysis can only be the first step as a quick screening. In our study we detected the hypermethylation of the exon α of *SNRPN*, a classic symbol of PWS with Bisulfite-specific PCR and sequencing, which is almost as simple as common DNA sequencing.

Array CGH has been developed to detect chromosomal copy number variations (CNVs) at a genome-wide and high-resolution scale. It is a promising technique with wide application on human genetics and oncology[5]. Its accuracy depends on the distance between probes used in chip, which was ~5.3 kb in the Agilent chip we used. New microarrays of higher resolutions are under design to achieve better accuracy of detection. Aside from the high accuracy, the second advantage of it is that some array-CGH chips require as little as 250 ng of DNA, making array-CGH a very suitable method to detect directly from clinical samples[5]. The third advantage of array-CGH is its ability to analyse at the whole genome-wide scale when atypical or unknown CNVs are suspected, just like the novel microdeletions found in chromosome 6 and X[9-10]. Here we localized a 2.22 Mb microdeletion (21 122 897-23 348 529 counting from p terminal) in chromosome 15 with array CGH, which is the accurate method to detect CNVs in the whole genome[11]. The newly found microdeletion is a class II PWS deletion or type II deletion, starting from breaking point 2 (BP2) which is approximately on 20.8-21.3 Mb of chromosome 15. The microdeletion is relatively shorter than the commonly reported 4-10 Mb[4,12].

Groups around the world have been struggling to find out microdeletions that are small enough to reveal the critical genes for PWS. Recently, two novel 174 kb and 187 kb microdeletions on snoRNAs clusters, distal to *SNRPN*, were detected, making HBII-85 of C/D snoRNAs be the most possible primary cause of PWS[13-14]. In addition, deletion of HBII-85 snoRNA cluster can lead to growth retardation, neonatal lethality and hyperphagia in mouse model[15-17]. The deletion we found also covered the HBII-85 of C/D snoRNAs cluster region. However, there is still some conflict, because not all microdeletions found in PWS patients are on 15q11.2-q13. Two microdeletions were found on atypical location15q13 and q14, quite distal to the PWS/ASCR[12]. Further distal, a 15q26.2→qter deletion was also reported in order to contribute to PWS[18]. Actually, the smallest deletion ever reported is a 7.5 kb-microdeletion on exon 1 of *SNRPN*[19], which can narrow the PWS critical region to <4.3 kb spanning the *SNRPN* gene CpG island and exon 1. The locations of microdeletions vary greatly in researches, revealing the complexity and heterogeneity of pathogenesis of PWS. In addition, some other chromosomes have even been found by some researches, e.g. chromosome X and 6, associated with PWS manifestations[9-10]. Although HBII-85 of C/D snoRNAs clusters is the most possible cause, we need not be too cautious before making a conclusion.

We reported a novel hand deformity, i.e., metacarpophalangeal joint rigidity, in addition to the classic PWS manifestations. As far as our knowledge, it is the first case with all the metacarpophalangeal joints involved as rigidity in PWS patient. Clinodactyly is a more common form of PWS hand deformity, which is the curvature of the fifth fingers toward the adjacent fourth fingers[20]. The most typical joint problem in PWS patients are hyperlaxity and hyperextensibility of joints derived from hypotonia[21-22]. Scoliosis and delayed bone age are common as well[23-24].

The reason for the joint rigidity in PWS patient is unclear. Generally, it is often the results of malformation of bones, articular cartilages, ligaments or narrowness of the joint cavities, which are not observed in this patient, at least not from physical examination or hand MRI. The symmetry of rigidity of the bilateral metacarpophalangeal joints and lack of injury history indicate the less possibilities of post-trauma articular immobilization. The possible reasons, as we tried to speculate, might be the long-time immobilization derived from hypotonia, or shortness of extensor tendons, or bone malformation from severe osteoporosis. In addition, the reason why the rigidity only involved metacarpophalangeal joints remains to be elucidated. And the relationship between this epigenetic defect and the joint manifestations is unclear yet. Studies are needed to provide more information in clinical, genetic, and epigenetic aspects of PWS.

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