

# Loss of Heterozygosity on the Distal Long Arm of Chromosome 15: An Allusion for Prader-willi Syndromes?

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## Research

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# Abstract

## Background

Prader-Willi syndrome (PWS) is a rare neurodevelopmental disorder that is partially caused by maternal uniparental disomy (UPD) of chromosome 15. Copy-neutral loss of heterozygosity (CN-LOH) observed on the distal long arm of chromosome 15 may be an indicator of UPD and may require additional genetic testing as chromosome 15 is known to harbor imprinted genes.

## Methods

Chromosome microarray (CMA) was performed for two children with developmental disabilities or congenital anomalies. The results showed CN-LOH on the distal long arm of chromosome 15. Thereafter, methylation-specific PCR (MS-PCR) or methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) was performed to confirm the diagnosis of PWS.

## Results

MS-PCR did not detect an unmethylated allele for the *SNRPN* gene or MS-MLPA hypermethylation in 15q11.2-q13.1 region, supporting the diagnosis of PWS.

## Conclusions

These data suggested that LOH on chromosome 15, and even the critical region of 15q11.2q13.1 was not involved, perhaps due to partial heterodisomy and partial isodisomy UPD15. Hence, other genetic tests are warranted for the diagnosis of PWS.

# Introduction

Chromosome microarray analysis (CMA), which can detect copy number variations (CNVs), is increasingly used for the genetic diagnosis of unexplained developmental delay/intellectual disability, autism spectrum disorders, or multiple congenital anomalies<sup>[1]</sup>. Furthermore, single nucleotide polymorphism (SNP)-based CMA can confirm the CNVs as well as detect regions of homozygosity<sup>[2]</sup>. Excessive homozygosity may be an indicator of uniparental disomy (UPD).

UPD is defined as the presence of two copies of a whole chromosome inherited from one parent with no copy from the other parent, which was first described by Engel in 1980<sup>[3]</sup> and demonstrated as a mechanism for human genetic disease in 1988<sup>[4]</sup>. UPD can occur as hetero-UPD or iso-UPD depending on whether the two inherited chromosomes are different or identical, respectively. partial heterodisomy and partial isodisomy may arise due to meiotic crossing over<sup>[5]</sup>, which is very rare. UPD does not lead to clinical consequences, unless it uncovers mutations for an autosomal recessive disease<sup>[6]</sup> or the chromosome (e.g., chromosome 6, 7, 11, 14, 15, or 20) carries imprinted genes<sup>[5]</sup>. Prader-Willi syndrome

(PWS; MIM 176270) is the first recognized genomic imprinting disorder, and approximately 25% of individuals with PWS have maternal UPD15 (mUPD15) on chromosome 15q11.2-q13.1 region.

Herein, we reported two children who were identified with LOH on the distal long arm of chromosome 15 by SNP-based CMA, and mUPD of 15q11.2q13.1 was confirmed by methylation-specific PCR (MS-PCR) or methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA), which may be due to partial heterodisomy and partial isodisomy on chromosome 15.

## Materials And Methods

### Subjects

Case 1 was a 9-day-old Chinese boy who presented with hypotonia, poor sucking, no response to noxious stimulation, cryptorchidism and weak cry. He was born to a 33-year-old, G2P2 mother at 40 weeks gestation. Decreased intrauterine movement was noted during the pregnancy. He was delivered by cesarean section due to breech birth and without labor signal. The birth weight was 2.5 kg and birth length was 50 cm. The parents were non-consanguineous and healthy with normal intelligence and no family history of mental retardation, behavioral problems and congenital abnormalities.

Case 2 was an 18-month-old Chinese girl who presented with short stature, feeding problem, and developmental delay. She was born to a G2P2 mother at full-term. Decreased intrauterine movement was noted during the pregnancy. She was delivered by cesarean section without labor signal. The birth weight was 2.6 kg and birth length was 50 cm. Poor sucking, hypotonia and weak cry were noted after birth. The patient exhibited apparent developmental and growth delay compared with children of the same age. She could walk independently by the age of 18 months. Physical examination showed prominent forehead, whitish skin, almond-shaped eyes and hypotonia. The parents were non-consanguineous and healthy with normal intelligence and no family history of mental retardation, behavioral problems and congenital abnormalities.

### Karyotyping and CMA

Chromosome analysis and SNP-based CMA were carried out in accordance with the recommended consensus for evaluating unexplained developmental delay<sup>[1]</sup>. Karyotyping was carried out on peripheral blood samples. Culture initiation, maintenance and harvest were performed using standard methods. Chromosomal abnormalities were designated based on the International System for Human Cytogenetic Nomenclature guidelines [ISCN 2016].

SNP-based CMA was analyzed used the Infinium OmniZhongHua-8 Kit 900k (San Diego, California, USA) according to the manufacturer's instructions. The KaryoStudio (Illumina, Inc) software, CNV partition algorithm and Database of Genomic Variants were used to detect and analyze CNVs. Databases such as OMIM (<http://www.ncbi.nlm.nih.gov/omim>), DECIPHER (<http://decipher.sanger.ac.uk/>), DGV (<http://projects.tcag.ca/variation>), and ISCA

([http://en.wikipedia.org/wiki/International\\_Symposium\\_on\\_Computer\\_Architecture](http://en.wikipedia.org/wiki/International_Symposium_on_Computer_Architecture)) were used as references to evaluate the array data and analyze genotype-phenotype correlations.

## MS-MLPA and MS-PCR

MS-MLPA reagents and kits were obtained from MRC-Holland (Amsterdam, the Netherlands) and MS-MLPA were carried out using the ME028-B2 kit containing sequence specific probes along the length of the 15q11.2-q13 region. The B2 kit contains 48 MLPA probes for copy number detection and methylation status using methylation-sensitive restriction enzymes. For MS-PCR, genomic DNA extracted from the peripheral blood was subjected to bisulfite treatment and pyrosequencing using primers targeting the CpG island of the *SNRPN* gene.

Written informed consent for clinical and molecular analyses were provided by the parents. The study protocol was approved by the Institutional Review Board of The Children's Hospital, Zhejiang University School of Medicine (China).

## Results

Karyotypes of the two cases were normal (Fig. 1). SNP-based CMA results showed 1, 4031Kb block of copy-neutral loss of heterozygosity (CN-LOH) on the distal long arm of chromosome 15 from genomic coordinates 79, 973, 054-94, 004, 138 (hg19) in case 1, while a 26.44 Mb block of CN-LOH on the distal long arm of chromosome 15 from genomic coordinates 42, 284, 351-68, 723, 392 and a 11.02 Mb block of CN-LOH on the distal long arm of chromosome 15 from genomic coordinates 91, 383, 142-102, 398, 631 (GRCh37) in case 2 (Fig. 2). The results of the CMA in both cases indicated partial isoUPD15.

As the region on chromosome 15 is known to include imprinted genes, which can cause Prader-Willi/Angelman syndromes, methylation studies were conducted to further characterize the observed region of CN-LOH. MS-PCR of case 1 showed the presence of methylated alleles only (Fig. 3a), and MS-MLPA of case 2 showed 100% methylation (Fig. 3b). The phenotype and MS-PCR/MS-MLPA indicated the diagnosis of PWS, but there were no copy number alterations on chromosome 15, which suggested mUPD15. Moreover, the CMA findings of the CN-LOH did not overlap with the critical region within the paternal/maternal chromosome region 15q11.2q13.1. Hence, both patients were diagnosed with PWS because of partial heterodisomy and partial isodisomy on chromosome 15.

## Discussion

Herein, we described two children with congenital anomalies/growth retardation who were diagnosed as PWS because of partial heterodisomy and partial isodisomy UPD15 based on karyotyping, CMA and MS-PCR/MS-MLPA as well as the clinical manifestations. The SNP-based CMA results of both patients showed CN-LOH on the distal long arm of chromosome 15, which did not overlap with the critical region of PWS, but the methylation-specific test supported the diagnosis of PWS, which was consistent with the clinical data.

PWS results from the loss of gene expression within the paternally-inherited genes on the 15q11.2-q13 chromosome, and 25% of the cases result from errors in genomic imprinting due to maternal uniparental disomy<sup>[7]</sup>. Gametic complementation, trisomy/disomy rescue, non-disjunction meiosis I/II error could be responsible for UPD<sup>[8]</sup>. partial heterodisomy and partial isodisomy can result from the recombination between chromatids prior to the meiotic segregations. The mechanism of formation of partial heterodisomy and partial isodisomy in the two cases may be the combination of several consecutive events: recombination takes place between chromatids prior to the meiotic segregations, then a disomic gamete arises from nondisjunction in meiosis I/II, and finally, fertilization of the disomic gamete by a normal gamete followed by the loss of chromosome 15 from the normal zygote, which is also called trisomy rescue (Fig. 4).

The International Standard Cytogenomic Array Consortium has proposed that CMA, in place of G-banded karyotype should be considered a first-tier test for patients with unexplained developmental delay/intellectual disability, autism spectrum disorders, or multiple congenital anomalies<sup>[10]</sup>. SNP-based CMA can detect the long stretches of homozygosity, which might represent UPD, but can only detect heterodisomic UPD in the cases with blocks of isodisomy<sup>[10]</sup>, so SNP-based CMA may miss up to one-third of all UPD cases. Hence, methylation-specific test, which is considered as the first-tier test for the diagnosis of PWS, should be conducted when there are large blocks of homozygosity restricted to single chromosome 15.

Recombinant human growth hormone (rhGH) treatment is suggested before the onset of obesity, which often begins by two years of age<sup>[10]</sup>. rhGH is believed to improve the child's body composition, by increasing lean body mass and reducing fat mass. The rhGH treatment along with a healthy lifestyle may prevent obesity, which is a major threat to PWS children. Children who began rhGH treatment before 12 months of age were found to have higher nonverbal and composite IQs than children who began treatment between 1-5 years of age<sup>[11]</sup>. So early diagnosis and treatment will help to improve the quality of life and care of PWS children.

In summary, CN-LOH detected on the distal long arm of chromosome 15 by SNP-based CMA should warrant methylation-specific test for the diagnosis of PWS. Early management may benefit children with PWS. Hence, the detection of CN-LOH on the distal long arm of chromosome 15 may be an allusion for PWS and should prompt immediate follow-up confirmatory tests.

## Declarations

### Acknowledgment

This work was supported by Zhejiang Provincial Program for the Cultivation of High-Level Innovative Health Talents (2014) and Zhejiang Provincial Department of Science and Technology (2021C03094).

### Conflict of interest Statement

There was no conflict of interest.

### **Ethics approval and consent to participate**

Written informed consent for clinical and molecular analyses were provided by the parents. The study protocol was approved by the Institutional Review Board of The Children's Hospital, Zhejiang University School of Medicine (China) Consent for publication

### **Consent for publication**

Written informed consent for publication of their clinical details was obtained from the parents of the patients.

### **Availability of data and materials**

All data generated or analysed during this study are included in this published article.

### **Funding**

This work is supported by Zhejiang Provincial Program for the Cultivation of High-Level Innovative Health Talents (2014) and Zhejiang Provincial Department of Science and Technology (2021C03094).

### **Competing interests**

The authors declare that they have no competing interests.

### **Authors' contributions**

Dai YL, Huang K: diagnosed and managed the case, and drafted the initial manuscript; Zhu MQ and Zhong ML collected and analyzed data; Dong GP, Zou CC: supervised the patient care and reviewed and revised the manuscript. All authors approved the final manuscript and agree to be accountable for all aspects of the work.

### **Acknowledgements**

Not applicable

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## Figures

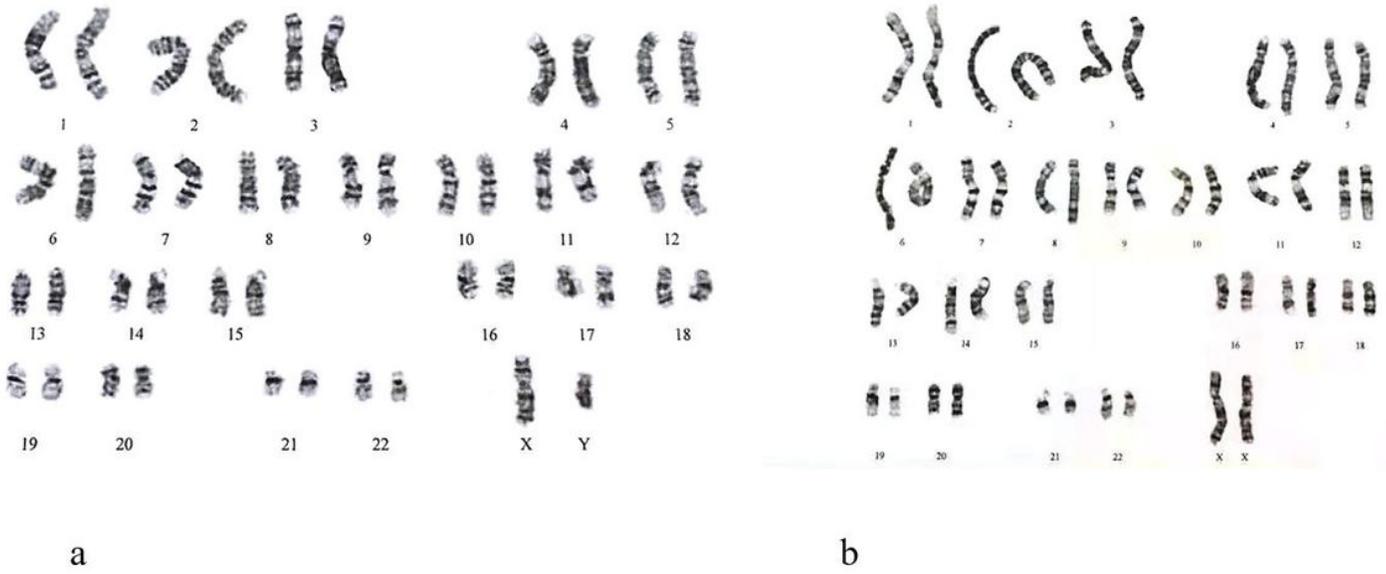


Figure 1

Chromosome analysis showing a normal karyotype. (a) case 1; (b) case 2.

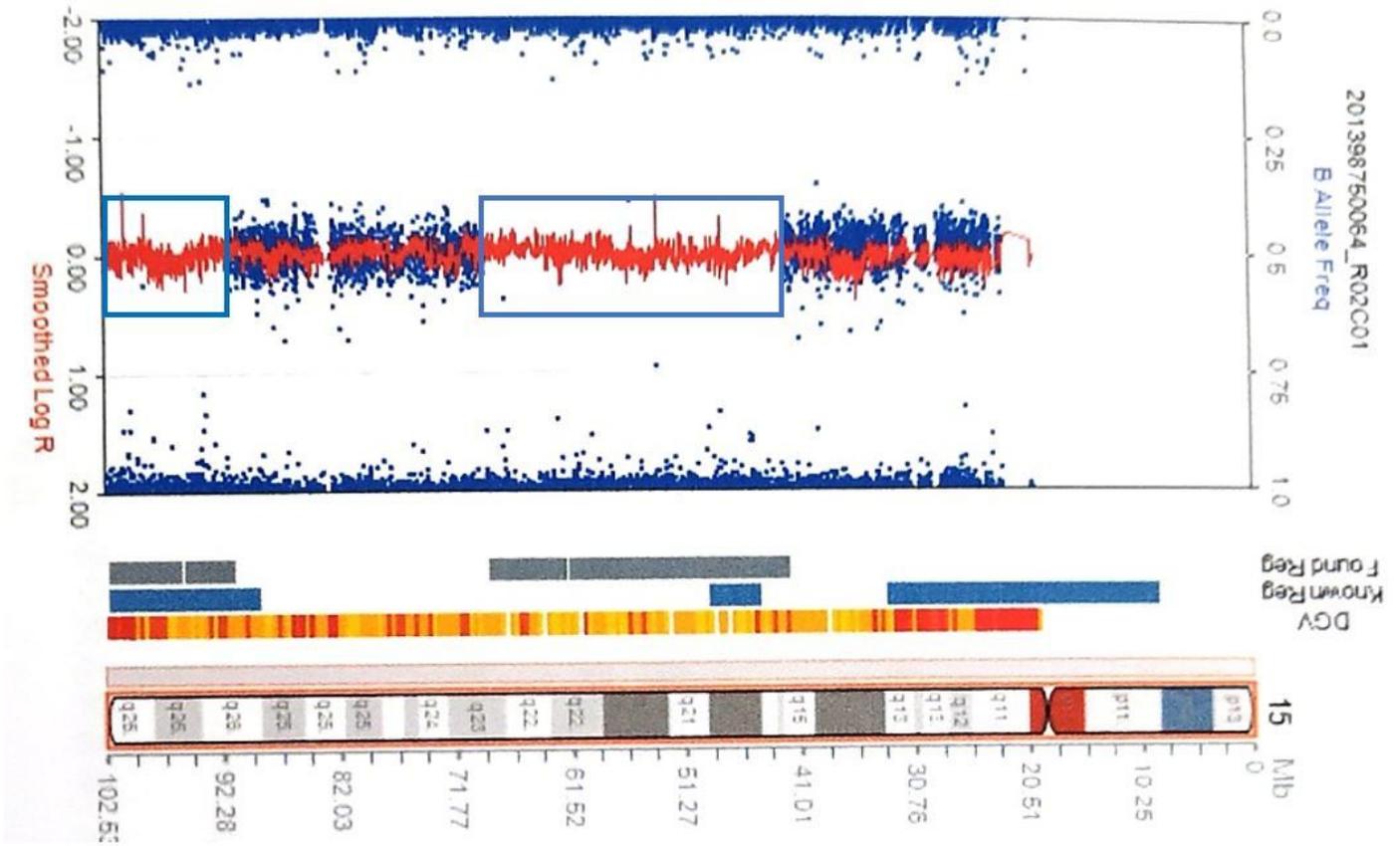
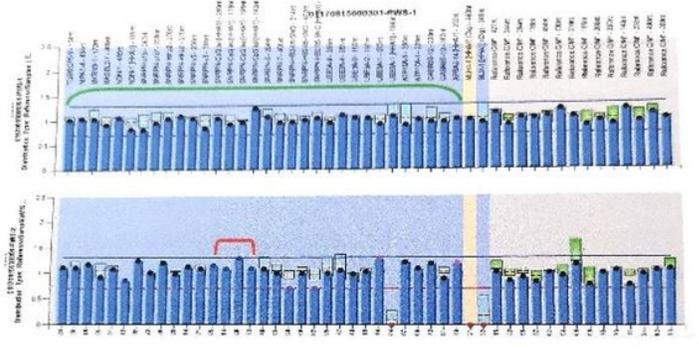
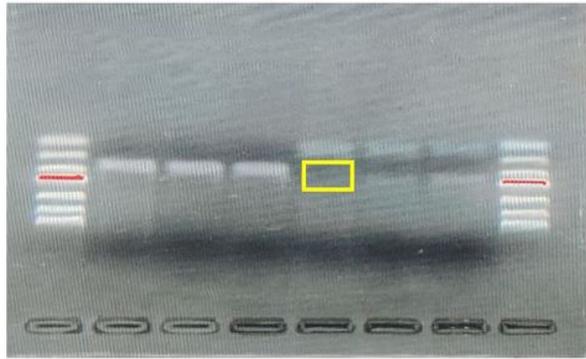


Figure 2

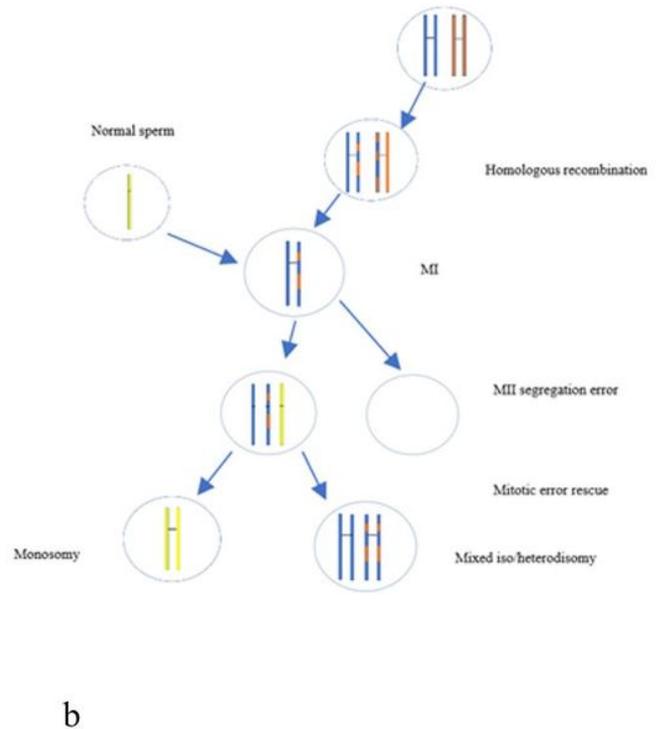
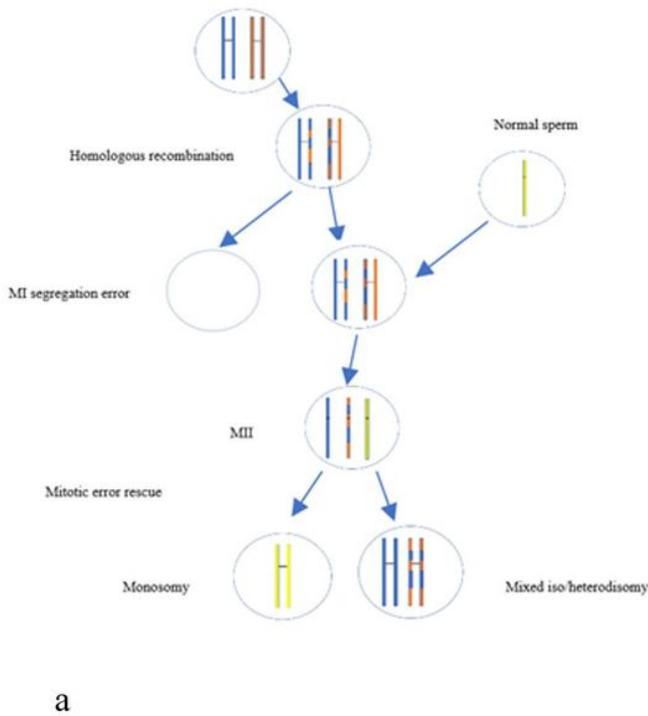
SNP-based CMA showing a 26.44 Mb block of CN-LOH on the distal long arm of chromosome 15 from genomic coordinates 42,284,351-68,723,392 and a 11.02 Mb block of CN-LOH on the distal long arm of chromosome 15 from genomic coordinates 91,383,142-102,398,631 (GRCh37) in case 2 (blue box).



a M 1m 2m 3m 1p 2p 3p M b

**Figure 3**

The results of MS-PCR and MS-MLPA. (A) showing the absence of unmethylated alleles in case 1 (a, yellow box, samples 2 and 3 are control, and m-maternal, p-paternal, M-marker) and MS-MLPA analysis showing hypermethylation in case 2 (b, green line).



**Figure 4**

Possible mechanisms for the generation of the partial heterodisomy and partial isodisomy of chromosome. (a) recombination, non-disjunction at MI, resulting in partial heterodisomy and partial isodisomy; (b) recombination, non-disjunction at MII, resulting in partial heterodisomy and partial isodisomy.