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What is This?

Prader-Willi Syndrome With a Long-Contiguous Stretch of Homozygosity Not Covering the Critical Region

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Abstract
Prader-Willi syndrome is a common and complex disorder affecting multiple systems. Its main manifestations are infantile hypotonia with a poor sucking reflex, a characteristic facial appearance, mild mental retardation, hypogonadism and early-onset obesity. Prader-Willi syndrome is due to the absence of paternally expressed imprinted genes at 15q11.2-13, and 3 main mechanisms are known to be involved in its pathogenesis: paternal microdeletions, maternal uniparental disomy events, and imprinting defects. DNA methylation analysis can detect almost all individuals with Prader-Willi syndrome but is unable to distinguish between the molecular classes of the disease. Thus, additional methods are necessary to identify the molecular classes. Here, we employed chromosomal microarray analysis–single nucleotide polymorphism for diagnosis and detected a long-contiguous stretch of homozygosity on chromosome 15, which is highly predictive of maternal uniparental disomy on chromosome 15. Other methods, including fluorescence in situ hybridization, chromosomal microarray analysis–comparative genomic hybridization, genotyping and family linkage analysis, were performed for further validation. In conclusion, our study highlights the use of long-contiguous stretch of homozygosity detection for the diagnosis of Prader-Willi syndrome.

Prader-Willi syndrome is a multisystem disorder with an estimated prevalence of 1/15 000 to 1/30 000.[1] It is characterized by severe hypotonia with a poor sucking reflex and feeding difficulties in early infancy. In late infancy or early childhood, Prader-Willi syndrome patients can become morbidly obese if the disease is uncontrolled. Moreover, all Prader-Willi syndrome patients have some degree of cognitive disability. Of note, Prader-Willi syndrome is the first human disorder that has been linked to genomic imprinting.

Prader-Willi syndrome is caused by an absence of paternal gene expression on chromosome 15q11.2-q13. This deficiency is mainly caused by chromosome microdeletion or maternal uniparental disomy.[2,3] Several genes in the 15q11.2-q13 region are controlled by genomic imprinting. In Prader-Willi syndrome, these genes are active in the paternal chromosome 15 but are inactive in the maternal chromosome 15. Generally speaking, the failure of paternally inherited genes is required for the Prader-Willi syndrome phenotype. Approximately 65% to 75% of individuals affected with Prader-Willi syndrome carry deletions in the paternally contributed 15q11.2-q13,[4,5] while 20% to 30%

are affected by maternal uniparental disomy on chromosome 15.[3] The remaining 2% to 5% of individuals are affected by imprinting defects, which could disrupt the imprinting process on the paternally inherited chromosome 15.[6]

Although the published consensus clinical diagnostic criteria allow for the accurate diagnosis of Prader-Willi syndrome, genetic testing is the main means of diagnosis. DNA

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methylation analysis has been considered the first-line diagnostic test, but it is unable to distinguish between the molecular classes of Prader-Willi syndrome. To solve this problem, other methods, such as high-resolution karyotyping, fluorescence in situ hybridization, or chromosomal microarray analysis–comparative genomic hybridization, can be employed.[7,8] However, none of these methods can detect uniparental disomies and imprinting defects other than deletions. As supplementary methods, chromosomal microarray analysis–single nucleotide polymorphism and DNA sequencing could identify maternal uniparental disomies and imprinting defects in Prader-Willi syndrome patients.[9,10]

Of these diagnostic methods, chromosomal microarray analysis–single nucleotide polymorphism analysis can detect not only deletions but also uniparental disomies in cases with a long-contiguous stretch of homozygosity. A long-contiguous stretch of homozygosity, or a long-contiguous stretch of homozygosity, is an uninterrupted region of homozygous alleles with a genomic copy number of 2. Minimal thresholds for long-contiguous stretch of homozygosity calls are generally set at approximately 0.5 to 1 Mb in population genetic analyses[11-13] or more conservatively at 3 to 10 Mb in clinical analyses.[14] Here, we report a case of a Prader-Willi syndrome patient with long-contiguous stretch of homozygosity on 15q14-q21.1, strongly suggesting uniparental disomy on chromosome 15. Subsequent genotyping and linkage analysis of the family was performed for validation. Other than the regions affected by the maternal uniparental disomy, no deletions were found in the chromosomes. Our research, therefore, highlights the use of long-contiguous stretch of homozygosity in the diagnosis of Prader-Willi syndrome and possibly sheds light on a convenient method of diagnosing uniparental disomy in Prader-Willi syndrome/Angelman syndrome.

Materials and Methods

Clinical Features

The described male patient was delivered by a 28-year-old woman in the 35th week of gestation by cesarean section, which was required due to the development of polyhydramnios (weight 1860 g). As a 4-month-old, he had down-slanting palpebral fissures, strabismus, mild muscular hypotonia, and breathing difficulties.

He also had repeated infections of the respiratory tract and pneumonia. Color Doppler ultrasound of the heart showed an exceedingly narrow aortic bifurcation diaphragm (mild) with secondary diaphragmatic atrial defects (5.4 mm) and a patent foramen ovale. Cranial ultrasound showed moderate bilateral lateral ventricle broadening and bilateral lateral ventricle narrator flake with mild echogenicity; the head magnetic resonance ectocinerea and nucleus accumbens in T1-weighted imaging was somewhat high, while in T2-weighted imaging, it was slightly low. Dysplasia was observed in the eyes and retinal vasculature. The patient's hypotonia presented with decreased movement and lethargy, with decreased spontaneous arousal, a weak cry, and poor reflexes, including a poor sucking reflex.

The patient's birth history was significant for intrauterine growth retardation observed on ultrasound at gestational week 28, Apgar scores of 5 at 1 minute and 8 at 5 minutes, and admission to the neonatal intensive care unit for hypotonia and feeding difficulties after birth. The diagnosis of Prader-Willi syndrome was suspected on the basis of this clinical manifestation. At 19 months of age, he died of respiratory failure.

Cytogenetic Analysis

After informed consent was obtained, peripheral venous blood was collected from both the patient and his parents. Karyotyping analysis was performed on the peripheral blood samples using conventional G-banding techniques (550-band resolution). Peripheral blood (2 mL) was collected and subjected to lymphocyte culture according to standard cytogenetic protocols.[15] Fluorescence in situ hybridization on nuclei with a Prader-Willi syndrome/Angelman (Prader-Willi syndrome/Angelman syndrome) specific probe (Vysis, Abbott Laboratories, Abbott Park, IL) was used in the cytogenetic analysis following the manufacturer's instructions.

Chromosomal Microarray Analysis–Comparative Genomic Hybridization Array Analysis

After informed consent was obtained, peripheral venous blood was collected. Genomic DNA was isolated from peripheral blood leukocytes using the QIAamp DNA Blood Mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The final concentration of genomic DNA was adjusted to 100 ng/μL. After fluorescence in situ hybridization analysis, an array comparative genomic hybridization was performed using a 44k oligo array (Agilent, Santa Clara, CA; mean resolution: 20 kb) for a general screening approach of any other microdeletion syndromes throughout the whole genome. DNA Analytix software was used for the data analysis (Agilent).

Chromosomal Microarray Analysis–Single Nucleotide Polymorphism Array Analysis

With a higher resolution, chromosomal microarray analysis–single nucleotide polymorphism array analysis was performed with the patient's and his parents' peripheral blood samples using an Affymetrix cyto HD Array. DNA was amplified, labeled, and hybridized to the CytoScan HD array (Affymetrix) platform according to the manufacturer's protocol. The array has been designed specifically for cytogenetic research, and it includes more than 2 million markers across the genome, including single nucleotide polymorphism probes and probes for detecting copy number variations (Cyto-arrays). CEL files obtained by scanning CytoScan arrays were analyzed with Chromosome Analysis Suite Software (Affymetrix). Genome version GRCH37 (hg19) was used for the annotations. Only those achieving the manufacturer's quality cut-off measures were included in our analysis. Gains and losses that affected a minimum of 50 markers within a 100-kb region were initially considered.

Genotyping and Linkage Analysis of the Family

Small tandem repeat marker genotyping was performed on the patient and his parents by means of the quantitative fluorescent polymerase chain reaction (q-PCR). Seven small tandem repeat markers specific for chromosome 15 were chosen. Of these small tandem repeat markers, D15S128 was located in the typical Prader-Willi syndrome deletion region, while D15S205 was located in the distal region (Figure 1). Small tandem repeat markers were amplified from the genomic DNA of 3 family members, and the primers used are shown in Table 1. The
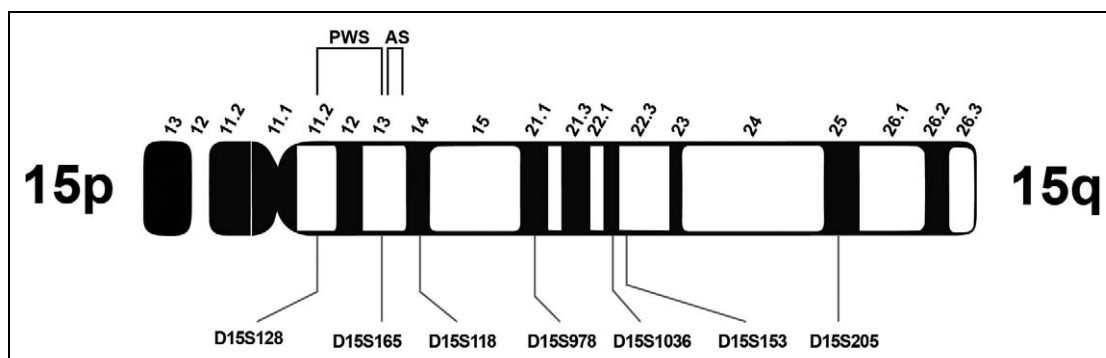
Figure 1. Schematic diagram of chromosome 15 and the small tandem repeat markers. Seven small tandem repeat locations are marked according their location. Abbreviations: AS, Angelman syndrome region; PWS, Prader-Willi syndrome region.

Table 1. Primers of the 7 Small Tandem Repeat Loci.

STR marker	Site	Primers (5′-3′)
D15S128	15q11.2	F: GCTGTGTGTAAGTGTGTTTTATATC
		R: GCAAGCCAGTGGAGAG
D15S165	15q13.3	F: GTTTACGCCTCATGGATTTA
		R: GGGCACACAGTCCCAA
D15S118	15q14	F: TCAAAGACCCATATCAACCA
		R: GTGCTGAAAAGCGACACTTA
D15S978	15q21.1	F: AGCTTCATACACTGAAATTGTTG
		R: CACCGGGAAACCTTGAT
D15S1036	15q22.2	F: CTCTGAGACCACTTTCAAGC
		R: ATGTTACCCATTTGTGGAGA
D15S153	15q22.31	F: AGTACCTGAAAGGGTGGG
		R: GATCAGTGTAGGCTCCAAA
D15S205	15q25.2	F: CTTAATGGTTTGGCAGGATA
		R: AGCTTAAAANCAAAATCTCCC

Abbreviations: F, forward primer; R, reverse primer; STR, small tandem repeat.

methods are consistent with those used in previous work.[16] Linkage analysis results were obtained by analyzing the core sequences of the 7 small tandem repeat loci and the phenotypes of family members.

Results

Karyotyping and Fluorescence In Situ Hybridization

Peripheral blood samples from the patient and his parents were subjected to karyotyping analysis. The conventional G-banding technique (550-band resolution) was employed. The results revealed a normal karyotype of 46,XY in all samples. Subsequently, fluorescence in situ hybridization was used with the Prader-Willi/Angelman region probe, which specifically hybridizes to 15q11-q13. However, no abnormalities in this region were found in either the patient (Figure 2B) or his parents by fluorescence in situ hybridization. These negative results suggested that the chromosomal structure was intact in the 15q11-q13 region.

Chromosomal Microarray Analysis–Comparative Genomic Hybridization Analysis

To detect smaller microdeletions across the whole genome, a chromosomal microarray analysis–comparative genomic

hybridization assay was performed. In accordance with the karyotyping and fluorescence in situ hybridization results, the chromosomal microarray analysis–comparative genomic hybridization analysis did not identify any microdeletions in either the patient or his parents (data not shown).

### Chromosomal Microarray Analysis–Single Nucleotide Polymorphism Analysis

In addition to the chromosomal microarray analysis–comparative genomic hybridization analysis, chromosomal microarray analysis–single nucleotide polymorphism analysis was also employed. Intriguingly, a long-contiguous stretch of homozygosity on chromosome 15 [arr 15q14q21.1 (35,025,426-48,697,676) x 2 hmz] was detected by chromosomal microarray analysis–single nucleotide polymorphism array in the patient (Figure 2A). This observation, combined with the parents' chromosomal microarray analysis–single nucleotide polymorphism array results, suggested that maternal uniparental disomy had occurred in the patient. Of note, the chromosomal microarray analysis–single nucleotide polymorphism analysis did not detect any other long-contiguous stretch of homozygosity on chromosomes other than chromosome 15 (data not shown).

Genotyping and Linkage Analysis of the Family

The linkage analysis results are shown in Figure 2C. The results demonstrate that the patient inherited 2 copies of chromosome 15 from his mother but no copies from his father. In other words, the patient carried a maternal uniparental disomy of chromosome 15.

Discussion

Prader-Willi syndrome and Angelman syndrome are distinct human neurogenetic disorders involving several disease-causing mechanisms. The principal genetic defects in Prader-Willi syndrome are 15q11.2-13 deletions of paternal origin or maternal chromosome 15 uniparental disomy.[2] In contrast,
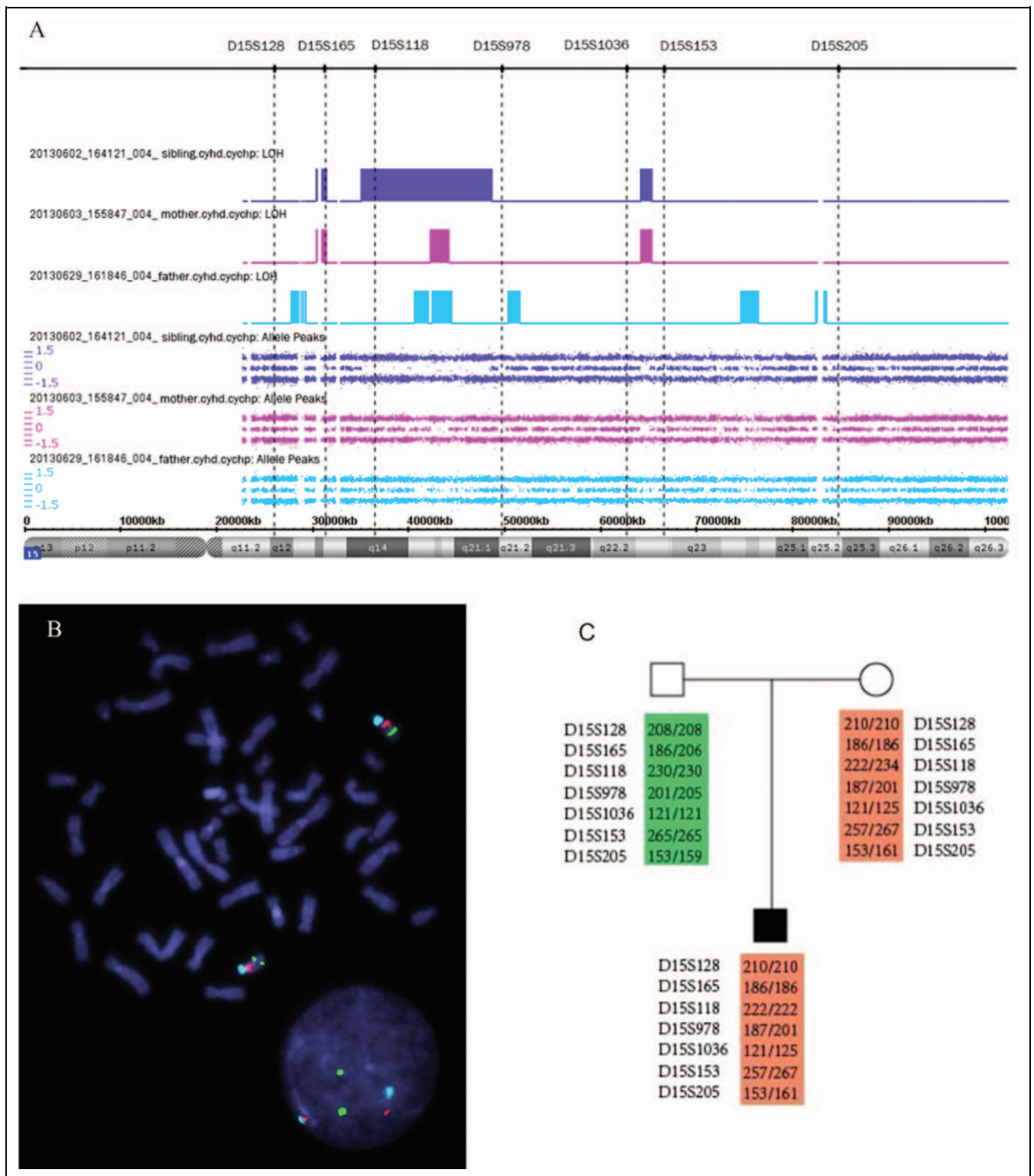
Figure 2. (A) Long-contiguous stretch of homozygosity at 15q14-q21.2. The long-contiguous stretch of homozygosity at 15q14-q21.2 is represented by the colored box. (B) Results of fluorescence in situ hybridization in the patient. Metaphase fluorescence in situ hybridization study of cultured lymphocytes and amniocytes with CEP15 probes (shown in blue), SNRPN probes (SNRPN is in the critical reign of Prader-Willi syndrome/Angelman syndrome, shown in orange), and PML probes (shown in green). No deletion was detected on chromosome 15 in the cultured lymphocytes. (C) Results of linkage analysis in the family. Linkage analysis of the patient's chromosome 15 suggested that he inherited both chromosomes from his mother and none from his father.

maternal deletions and uniparental disomy of the paternal chromosome 15 are instead associated with Angelman syndrome.[17]

It is known that DNA-based methylation testing can detect both deletion and abnormal parent-specific imprinting in the region of chromosome 15 involved in Prader-Willi syndrome pathogenesis; therefore, this method is considered the first-line test for Prader-Willi syndrome diagnosis.[7] However, methylation testing is incapable of distinguishing the molecular classes of Prader-Willi syndrome. Additional genetic studies, therefore, are necessary to identify the molecular classes of Prader-Willi syndrome. As the traditional cytogenetic means of diagnosis, karyotyping combined with fluorescence in situ hybridization analysis can detect almost all Prader-Willi syndrome deletions.[18] The current diagnostic options are now more diverse because of improvements in the available techniques. For example, chromosomal microarray analysis–comparative genomic hybridization can detect deletions and copy number variations without any prior information or predicted candidate genes/loci, making unbiased whole-genome investigations convenient. Of note, chromosomal microarray analysis–comparative genomic hybridization analysis has been widely used by clinical centers to rule out unsuspected copy number variations.[19] As another array-based method, chromosomal microarray analysis–single nucleotide polymorphism analysis can not only detect deletions/copy number variations but it can also identify long-contiguous stretch of homozygosities, which are usually caused by uniparental disomies. In addition to chromosomal microarray analysis–single nucleotide polymorphism analysis, uniparental disomies can also be detected by other methods for DNA polymorphism analysis. Of these methods, family linkage analysis based on small tandem repeat markers is a powerful and credible option.[16,20] If family polymorphism studies reveal that the proband inherited a copy of chromosome 15 from each parent, an imprinting deficit should be considered. Imprinting defect detection can be accomplished by sequence analysis or by using the recently developed methylation-specific multiplex-ligation probe amplification assay.[21,22] In our case, we first detected no deletion with cytogenetic karyotyping/fluorescence in situ hybridization and chromosomal microarray analysis–comparative genomic hybridization array. Instead, we found a long-contiguous stretch of homozygosity using a chromosomal microarray analysis–single nucleotide polymorphism array, which suggested maternal uniparental disomy 15 in our patient. The maternal uniparental disomy on chromosome 15 was further validated by linkage analysis of the family.

After the 15q11.2-q13 deletion, maternal uniparental disomy 15 is the second most common cause of Prader-Willi syndrome, accounting for approximately 20% to 30% of cases. This condition is characterized by having 2 maternal copies and no paternal copies of chromosome 15.[3,23] Constitutional uniparental disomy can arise from problems in chromosome segregation or from chromosomal structure aberrations. Generally speaking, trisomy rescue is the main reason for maternal uniparental disomy. In trisomy rescue, a maternal nondisjunction event produces an ovum that is disomic for chromosome 15. The disomic egg is then fertilized with normal sperm, generating trisomy 15, a condition that is lethal for blastocysts. However, lethal trisomy 15 can be rescued by the loss of 1 chromosome 15 during mitosis. This produces maternal uniparental disomy 15 if the paternal chromosome is lost.[24] In addition to trisomy rescue, other possible causes of uniparental disomy have also been reported, including nullisomic gamete complementation, monosomic rescue, and nonhomologous recombination. Nullisomic gamete complementation involves fertilization between a nullisomic gamete and a disomic gamete to generate a zygote with uniparental disomy. Monosomic rescue refers to fertilization between a nullisomic gamete and a monosomic gamete, which leads to uniparental disomy following duplication of the monosomic chromosome. Nonhomologous recombination can lead to segmental uniparental disomy.

In this study, data from the 3 family members suggested that uniparental disomy occurred in chromosome 15 of the proband. First, the oocyte underwent homologous recombination in meiosis I and subsequent nondisjunction events in meiosis II, resulting in an ovum of 23,X,+15. In the very early stages of implantation or embryogenesis, the aberrant egg was fertilized with a normal 23,Y sperm cell, and trisomy 15 rescued the onset of embryogenesis. The paternal chromosome 15 was then lost, allowing the embryo to survive and producing a maternal uniparental disomy 15. The mechanism mediating homologous recombination involves the nonallelic homologous recombination of low copy repeats in chromosome 15.[25,26] It has been reported that low copy repeats might make the DNA unstable through nonallelic homologous recombination during cell division, either during meiosis or mitosis.[27] However, as a result of meiotic recombination, uniparental disomy events involving an entire chromosome are not commonly purely isodisomic or heterodisomic; they are instead composed of a mixture of both segment types. The isodisomy could be distinguished by chromosomal microarray analysis–single nucleotide polymorphism array but not by chromosomal microarray analysis–comparative genomic hybridization array. In our study, the patient carried a heterodisomy of chromosome 15, which was discovered by comparing the patient's single nucleotide polymorphism-array results with the parents' single nucleotide polymorphism-array results.

Compared with DNA microsatellite polymorphism analysis, single nucleotide polymorphism analysis can genotype thousands of single nucleotide polymorphisms distributed along the proband's putative uniparental disomy chromosome. Although numerous well-defined microsatellite markers exist, this technique may not be suited for the detection of segmental uniparental disomy, given that the established (clinically validated) assay may not have sufficient markers within the specific region of isodisomy to confirm the presence of a small segmental uniparental disomy. Thus, the chromosomal microarray analysis–single nucleotide polymorphism array appears to have an exclusive advantage for the detection of segmental uniparental disomy if parents' array data exist.

In conclusion, long-contiguous stretch of homozygosity in imprinted chromosomes detected by a single nucleotide

polymorphism–based microarray strongly suggests uniparental disomy. Thus, subsequent microarray-based single nucleotide polymorphism genotyping of parents or specific diagnostic testing of methylation patterns for the corresponding imprinting disorder should be used for confirmation, particularly when the clinical features of the patient are consistent with the syndrome of interest. In contrast, the molecular classes of Prader-Willi syndrome are not easy to distinguish. Here, we used a chromosomal microarray analysis–single nucleotide polymorphism array to identify maternal uniparental disomy 15 in the proband, highlighting the importance of long-contiguous stretch of homozygosity in the diagnosis of uniparental disomy–related disease such as Prader-Willi syndrome/Angelman syndrome. However, single nucleotide polymorphism–based analysis is limited when the parents are close relatives (eg, first-degree cousins).

Author Contributions

XY and ZY contributed equally to the manuscript. XY was responsible for the design of the project, data analysis, and manuscript write-up. XY also facilitated the panel with assistance from WJ and SY. XY and ZY drafted the first versions of the manuscript. SY, SY and FQ were the neurologists on the team; they reviewed relevant abstracts and articles and contributed to the final critical review of the write-up. WJ, ZL and CY assisted in literature review, perform experiment, data entry, and data analysis phases of the project.

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