Prader-Willi syndrome (PWS) is an imprinting disorder, which arises due to three main mechanisms, and eventually results in total absence of the paternally imprinted genes expression in the 15q11-q13 region. The three genetic mechanisms are paternal deletion of this region (in <70% of cases), maternal uniparental disomy (UPD) (in 25-30%) and imprinting center defect (in 2-5%) (1-3). The paternal copies of the genes are typically expressed in the PWS region; however, due to parent‑of‑origin‑specific imprinting, the maternal copies of these genes are silenced. Almond- shaped and occasional upslanting palpebral fissures, bitemporal narrowing and strabismus are frequent characteristic facial features of individuals with PWS (2,4). Hypogonadism, which manifests as genital hypoplasia (including cryptorchidism) and delayed or incomplete pubertal development, is another characteristic feature (5). With regards to neurobehavior features, PWS is characterized by decreased fetal movement, neonatal hypotonia and feeding difficulties, which lead to the failure to thrive in the postnatal period. The majority of individuals with PWS have mild intellectual disability, particularly behavioral problems, including manipulative behavior, obsessive-compulsive behaviors, compulsive skin picking, stubbornness and temper tantrums. Attention-deficit and hyperactivity symptoms may also occur, along with features suggestive of autism spectrum disorders (2,4,6).

Concerns regarding assisted reproductive technologies (ART) have been raised due to the possible associations between the safety and genetic disorders, particularly imprinting defects (7). Previous studies have provided evidence for an association between imprinting disorders and ARTs. Nine imprinting syndromes have been reported as associated with ART (7,8), whereas other studies have reported no correlation (9,10). For example, Angelman syndrome (AS) and Beckwith-Wiedemann syndrome are two disorders in which an imprinting defect accounts for a significant proportion of affected individuals, with known increased risks for patients born following ART (11-13).

The present study reports a case of a 6-year-old girl with PWS conceived following an ART pregnancy, who presented...
with the clinical features of PWS. Molecular analysis confirmed a de novo microdeletion between breaking point 2 (BP2) and BP3, which occurs from makorin ring finger protein 3 (MKRN3) through HECT and RLD domain containing E3 ubiquitin protein ligase 2 (HERC2) at 15q11.2-q13.1.

Case report

Patient and clinical findings. The patient was a 6-year-old female who was the product of a dizygotic twin pregnancy preceded by in vitro fertilization (IVF). The parents and twin sister were healthy with a normal level of intelligence. Family history was negative for mental retardation, behavioral problems and congenital abnormalities. All the subjects provided written informed consent for clinical and molecular analyses, and the study protocol was approved by the Institutional Review Board (DC15ZISE0114) of The Catholic University of Korea, Daejeon St. Mary's Hospital (Seoul Korea). The determination of twin zygosity was identified by a short tandem repeat (STR) multiplex assay (AmpFLSTR® Identifier; Applied Biosystems, Foster City, CA, USA) that amplifies 15 tetranucleotide repeat loci for autosomal, codominant, unlinked loci and the gender-determining marker amelogenin in a single polymerase chain reaction (PCR) amplification. STR analysis also confirmed the biological association of the father and mother with the proband. Pregnancy was complicated due to small size for the gestational age and delivery was at 31 weeks of gestation. The birth weight of the patient was 1,030 g (below 10th percentile), length was 38 cm (25th percentile) and head circumference was 28 cm (25th percentile). Marked lethargy with no crying and poor reflexes at birth was evident. The patient was intubated immediately and ventilator care was required for 4 weeks. The patient had neonatal feeding difficulties necessitating gavage tube feeding to assure adequate nutrition until 2 months of age. No abnormalities were evident on echocardiogram, urogenital sonogram, audiometric test and brain magnetic resonance imaging. Hypotonia was severe, but gradually improved with age. Sucking power slowly improved over several months and normal eating was possible at 12 months of age. Among laboratory analysis in the first year, biochemical analysis including blood electrolytes, liver and renal function tests, complete blood count, thyroid function tests, serological tests for TORCH infections, amino acid chromatography, transfontanelle ultrasonography, electroencephalography and electromyography were within the normal limits. Motor milestones and language development were delayed; the patient walked independently at 22 months of age and could speak in sentences at 3.5 years of age. The patient had a central obesity with slender limbs and had abnormal lipid profiles, with mild hypertriglyceridemia (triglycerides, 346 mg/dl; normal range 35-110 mg/dl). Facial appearance was characteristic with narrow bifrontal diameter, short upturned nose and downturned mouth with a thin lip. The patient had whitish skin and brown hairs. Upper extremities were notable for the small hands relative to body size. Short stature persisted following birth (below 3rd percentile), but endocrinological investigations including follicle-stimulating hormone, luteinizing hormone, prolactin, human growth hormone, adrenocorticotropic hormone, cortisol, free triiodothyronine, free thyroxine, thyroid-stimulating hormone and insulin were within normal ranges. The patient was borderline mental retardation with an IQ of 75 at 6 years of age. The patient did not present with behavioral problems including temper tantrums, violent behavior and obsessive/compulsive behavior, anxiety or depression. The patient had mild learning disabilities, and so attended a kindergarten for children with normal development. However, the patient received additional educational programs including speech therapy, regular exercise and social skill training. Due to the phenotypic resemblance and the medical records, which were highly indicative of PWS, methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) and array-based comparative genome hybridization (aCGH) analyses detected a de novo microdeletion involving BP2 and BP3 (type 2) at 15q11.2-q13.

MS-MLPA. MS-MLPA was performed using a MS-MLPA probemix ME028-B2 Prader-Willi/Angelman kit (MRC-Holland, Amsterdam, The Netherlands) was performed according to the manufacturer's protocol in the proband and family members (Fig. 1). A total of 32 probes specific for sequences in or near the PWS/AS critical region of chromosome 15q11 were used to detect copy number changes, as well as to analyze CpG island methylation of the 15q11 region for the presence of aberrant methylation patterns either caused by UPD or by imprinting defects in a semi-quantitative manner. The manufacturer's protocols were followed for the DNA preparation, probe hybridization, probe ligation, enzyme digestion and multiplex PCR reaction. Capillary electrophoresis and fragment analysis were conducted on an ABI 3130 DNA analyzer (Applied Biosystems). The resulting peak intensities were normalized to the manufacturer's control probes and to normal DNA as a reference. A probe-peak ratio between 0.7 and 1.3 was considered to represent a normal copy number (wild-type), and a ratio between 0.3 and 0.7 represented a loss of one copy number (deletion). To determine the methylation status, the normalized probe-peak ratio of a ligation-treated sample was compared with the ratio of the same sample treated with ligation and restriction digestion (by Hhal), using the three ligation control probes, four methylation-sensitive probes located on the SNRPN promoter region and one located on the NDN promoter region. MS-MLPA revealed a de novo microdeletion from MKRN3 on 15q11.2 to GABRB3 on 15q12 while sparing APBA2 (there were no MS-MLPA probes for GABRA5, GABRG3, OCA2, and HERC2, which are located between GABRB3 on 15q12 and APBA2 on 15q13.1) in the proband only (Fig. 1A and B).

aCGH. The MS-MLPA result from the proband was verified by a genomic microarray using the SurePrint G3 Human CGH+SNP Microarray 4x180K kit (Agilent Technologies, Inc., Santa Clara, CA, USA) according to the manufacturer's protocol. As a reference sample, the male or female genomic DNA from Agilent was used. The microarray slides were scanned at 3-micron resolution on an Agilent microarray scanner and the raw data were extracted using the Agilent Feature Extraction software V10.7.3.1. Raw data were analyzed using Agilent Genomic Workbench software, CGH.
module 7.0.4.0 (Agilent Technologies). Copy number alterations (CNAs) were reported based on the following criteria: Amplifications and deletions were scored when there was a 10-probe call with a minimum absolute average log\(_2\) ratio of 0.25, minimum genomic sizes of 0.5 Mb and <50% overlap with known CNAs (Database of Genomic Variants; http://dgv.tcag.ca/dgv/app/home). Mosaicism, coexisting minor populations with major diploid population, was detected by visual inspection according to the following criteria: i) A discontinuous line in the copy number state window, compared with a continuous consistent line representing the major clonal population; ii) intermediate values in the smooth signal, such as a minimum of 10 markers with a minimum absolute average log\(_2\) ratio of 0.1. Copy neutral -loss of heterozygosity (LOH) >5 Mb was considered using the LOH algorithm at the default threshold of 6.0. Consequently, the aCGH demonstrated a deletion of ~4.8 Mb extending from \(MKRN3\) through \(HERC2\) at 15q11.2-q13. Therefore, this subject had a type 2 deletion involving BP2 proximally and BP3 distally (Fig. 2).

Discussion

The present study reports the case of a 6-year-old female with PWS caused by a \textit{de novo} microdeletion at 15q11.2-q13.1, which is, to the best of our knowledge, the first PWS case born following ART reported in South Korea. MS-MLPA and aCGH demonstrate type 2 microdeletion between BP2 and BP3 occurring from \(MKRN3\) through \(HERC2\) at 15q11.2-q13.1. MS-MLPA reliably detected an approximation of the BPs and deletion size as evidenced by agreement with aCGH in the present case. Compared with aCGH, the MS-MLPA technique was much more labor and cost-effective, although aCGH

Figure 1. Methylation-specific multiplex ligation-dependent probe amplification analysis for the determination of Prader-Willi syndrome/Angelman syndrome. Probe-peak ratio pattern of the (A and B) proband and the proband’s (C and D) twin sister, (E and F) father and (G and H) mother. Horizontal axis, fragment size in base pairs (bps); vertical axis, probe-peak ratios; green dots, targeted probes; red dots, deleted probes (peak ratio <0.7); blue dots, internal control probes; undigested, samples treated with ligation reaction only; digested, samples treated with the ligation reaction and methylation-sensitive restriction enzyme treated.
provides more precise information regarding the extent of the deletion. In addition, the DNA methylation component of MS-MLPA allows differentiation between PWS and AS, and maternal and paternal 15q11.2 duplications, as well as between uniparental and biparental disomy. Therefore, MS-MLPA is recommended as the first screening test when considering PWS or AS based on clinical criteria.

The microdeletion class in PWS is typically subdivided into type 1 (BP1-BP3) and type 2 (BP2-BP3) based on their proximal breakpoints (14). Type 1 and 2 microdeletions have been reported to be associated with worse adaptive behavior, more severe compulsive behavior and more impairments in reading, math skills and visual perception than those with type 2; the present case could support type 2 microdeletion (15). However, several studies have investigated phenotypic characteristics between type 1 and 2 microdeletions in PWS, and there has been a lack of consensus among the different studies (15,16). Furthermore, the subjects with a unique or an atypical microdeletion revealed distinct phenotypic features (17). Kim et al (17) suggested that the microdeletions in PWS should be characterized by accurately determining their proximal and distal BPs, rather than just their proximal BP, as the distal BPs were not always well delineated in a number of these studies. By contrast, individuals with PWS due to maternal UPD are less severely affected. They have higher verbal IQ and milder physical features than those with microdeletions (2,18).

Whether ART has adverse effects on the fetal genetic status remains to be elucidated. However, a number of complications including imprinting defects have been reportedly associated with ART (19). An increased incidence of aneuploidy and de novo sex chromosome aberrations have been reported previously (20,21) and whether or not an increased risk for imprinting disorders exists remains to be elucidated (22,23). A possible link between ARTs and genomic imprinting disorders has been reported, particularly in AS and Beckwith-Wiedemann syndrome (22). They are mainly caused by four mechanisms: Large deletions or duplications of chromosomal regions that contain imprinted genes, UPD, imprinting mutations and epimutation (24).

Although children with PWS are more likely to be born to parents with fertility problems (22), no significant associations have been described between the incidences of the PWS and ART, such as IVF or intracytoplasmic sperm injection. Several studies found no association between ART and PWS as paternal deletions and maternal UPD account for the majority of PWS cases (13,25,26). A previous study also suggested that the proportion of ART births is not associated with an increased risk of PWS, but did identify a significantly increased proportion of maternal UPD and imprinting defects in the ART-conceived PWS study population (27). As older parents may have experienced infertility issues due to advanced parental ages, maternal UPD is associated with increasing maternal age, so the ART-conceived PWS may be affected due to mechanisms causing UPD and is not due to the ART procedures themselves (28,29).

In conclusion, genotype-phenotype counseling is important for estimating PWS severity due to type 1 or 2, as well as unique, microdeletions due to a novel distal and/or proximal BP. Molecular analyses, including MS-MLPA as a screening method and aCGH as a confirm test, would be more beneficial for the diagnosis and prognosis of PWS. In addition to previous studies, the present study contributes to the consensus regarding genotype-phenotype comparisons in
this respect. Further studies regarding the safety of ART are required to elucidate a possible causal association between 15q microdeletion and ART.

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