Case Report

Prenatal diagnosis of hypomethylation at KvDMR1 and Beckwith–Wiedemann syndrome in a pregnancy conceived by intracytoplasmic sperm injection and in vitro fertilization and embryo transfer

Chih-Ping Chena,b,c,d,e,f,*, Yi-Ning Sug, Shee-Uan Chenh, Tung-Yao Changi, Pei-Chen Wui, Schu-Rern Cherb, Peih-Shan Wuj, Yu-Ling Kook, Wayseen Wangbl

aDepartment of Obstetrics and Gynecology, Mackay Memorial Hospital, Taipei, Taiwan
bDepartment of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan
cDepartment of Biotechnology, Asia University, Taichung, Taiwan
dSchool of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan
eInstitute of Clinical and Community Health Nursing, National Yang-Ming University, Taipei, Taiwan
fDepartment of Obstetrics and Gynecology, National Taiwan University Hospital, Taipei, Taiwan

Case report: A 34-year-old, primigravid woman was referred to the hospital at 21 weeks’ gestation because of advanced maternal age and an isolated omphalocele in the fetus. Her husband had the fertility problem of oligospermia. This pregnancy was achieved by intracytoplasmic sperm injection and in vitro fertilization and embryo transfer. Prenatal ultrasound revealed a 2.1 cm × 1.6 cm isolated omphalocele.

The woman underwent amniocentesis. Array comparative genomic hybridization and methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) were applied to the DNA extracted from the uncultured amniocytes. Conventional cytogenetic analysis and high-resolution melting analysis were performed on cultured amniocytes. Array comparative genomic hybridization revealed no genomic imbalance. MS-MLPA analysis revealed H19DMR(IC1) normal methylation and KvDMR1(IC2) hypomethylation. Conventional cytogenetic analysis revealed a karyotype of 46,XX. High-resolution melting analysis using a methylation-specific polymerase chain reaction assay confirmed normal methylation at H19DMR(IC1) and hypomethylation at KvDMR1(IC2). The altered methylation status at 11p15.5 and the phenotype of omphalocele were consistent with the diagnosis of BWS.

Conclusion: In case of prenatally detected omphalocele associated with an obstetric history of assisted reproductive technology, a differential diagnosis of BWS should be considered. Methylation assays such as MS-MLPA and methylation-specific polymerase chain reaction using uncultured amniocytes are useful for rapid diagnosis of BWS under such circumstances.

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macromelia, visceromegaly, macroglossia, hemihyper trophy, adrenocortical cytomegaly, nephromegaly, cardiomegaly, placentomegaly, placental mesenchymal dysplasia, polyhydramnios, omphalocele/umbilical hernia, ear creases/pits, cleft palate, facial nevus flammeus, midface hypoplasia, renal medullary dysplasia, nephrocalcinosis, nephrolithiasis, cardiac anomalies, diastasis recti, advanced bone age, hemangiomata, and a 7.5% risk of developing embryonal Wilms’ tumor, neuroblastoma, adrenocortical carcinoma, hepatoblastoma and rhabdomyosarcoma [1–8].

The chromosome 11p15.5 imprinting cluster is functionally divided into domain 1 and domain 2 [1,2,6–8]. Domain 1 contains two imprinting genes of IGF2 (OMIM 147470) and H19 (OMIM 103280). IGF2 is expressed from the paternal allele, whereas H19 is expressed from the maternal allele. Domain 2 contains three imprinting genes of CDKN1C (OMIM 600856), KCNQ1 (OMIM 607542) and KCNQ1OT1 (OMIM 604115). KCNQ1OT1 is expressed from the paternal allele, whereas CDKN1C and KCNQ1 are expressed from the maternal allele. The H19-associated imprinting center (IC) 1 or differentially methylated region (DMR)1 (H19DMR) is methylated on the paternal allele and unmethylated on the maternal allele. The KCNQ1OT1-associated IC2, or DMR2 or KvDMR1 (KvDMR) is methylated on the maternal allele and unmethylated on the paternal allele. In patients with BWS, loss of methylation (hypermethylation) at IC2 on the maternal allele occurs in 50%; gain of methylation (hypermethylation) at IC1 on the maternal allele occurs in 5%; CDKN1C mutations occur in 10% (in 5% of patients with no family history and in 40% of patients with positive BWS family history); paternal uniparental disomy 11p15.5 occurs in 20%; and duplication, inversion or translocation of 11p15.5 occurs in 1% [8].

Assisted reproductive technologies (ARTs) have been associated with epigenetic syndromes such as BWS, Angelman syndrome, Prader–Willi syndrome, transient neonatal diabetes mellitus, and Russell–Silver syndrome (RSS) [9–13]. Halliday et al [9] reported a 1/4000 risk of developing BWS in children conceived by in vitro fertilization (IVF), or nine times greater than that of the general population. Here, we present our experience of prenatal diagnosis of altered methylation status at 11p15.5 and BWS in a pregnancy conceived by ART.

Case presentation

A 34-year-old, primigravid woman was referred to the hospital at 21 weeks’ gestation because of advanced maternal age and an isolated omphalocele in the fetus. The woman had experienced fertility problems because of oligosperma in the husband. This was her first pregnancy, and it was achieved by intracytoplasmic sperm injection (ICSI) and in vitro fertilization and embryo transfer (IVF-ET). Two blastocysts were cultured, two embryos had been implanted, and a singleton pregnancy was achieved. Level II ultrasound examination at 21 weeks’ gestation revealed a normal amount of amniotic fluid, a female fetus with a fetal biometry equivalent to 21 weeks, and a 2.1 cm × 1.6 cm isolated omphalocele (Fig. 1). The woman underwent amniocentesis. Array comparative genomic hybridization (aCGH) and methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) were applied on the DNA extracted from the uncultured amniocytes, and conventional cytogenetic analysis and high-resolution melting analysis were performed on cultured amniocytes. aCGH revealed no genomic imbalance. MS-MLPA analysis using SALSA MS-MLPA BWS/RSS ME030-C1 probemix (MRC-Holland, Amsterdam, The Netherlands) revealed H19DMR(IC1) normal methylation (methylation index = 0.5) and KvDMR1(IC2) hypomethylation (methylation index < 0.5) (Fig. 2). Conventional cytogenetic analysis of cultured amniocytes revealed a karyotype of 46,XX. High-resolution melting analysis of cultured amniocytes using a methylation-specific polymerase chain reaction (PCR) assay confirmed altered methylation status at 11p15.5 with hypomethylation at KvDMR1(IC2) and normal methylation at H19DMR(IC1) (Fig. 3). The molecular finding of altered methylation status and the abnormal phenotype of omphalocele were consistent with the diagnosis of BWS.

Discussion

The present case shows the usefulness of methylation assays such as MS-MLPA and methylation-specific PCR in rapid prenatal diagnosis of aberrant CpG methylation of the imprinting control region KvDMR1 associated with ART.

MS-MLPA has been proven to be a specific and sensitive technique for detecting all chromosome 11p15.5 imprinting defects of BWS and RSS in an easy and low-cost test [14–16]. In the present case, the MS-MLPA probe mixture of SALSA MS-MLPA BWS/RSS ME030-C1 probemix was used to identify the hypomethylation at KvDMR1 and BWS using uncultured amniocytes. The ME030-C1 probemix contains a total of 42 MLPA probes including 26 probes that are specific to the BWS/RSS 11p15.5 region; 13 reference probes that detect genes outside the BWS/RSS region; two probes in the NSD1 region associated with Sotos syndrome; and one digestion control probe. The 26 probes specific to the BWS/RSS 11p15.5 region include nine H19 probes, of which five are methylation insensitive, and four are methylation sensitive, with 50% methylated in normal blood DNA and located inside H19DMR(IC1); four KCNQ1OT1 probes, all of which are methylation sensitive with 50% methylated in normal blood DNA and located inside H19DMR(IC1); two CDKN1C probes, of which two are methylation insensitive, and one is methylation sensitive with 10% methylated in normal blood DNA; eight KCNQ1 probes, all of which are methylation insensitive; and two IGF2 probes, of which one is methylation insensitive, and one is methylation sensitive at 5’DMR0, and with 0% methylated in normal blood DNA. Ten of the 26 probes specific to the BWS/RSS 11p15.5 region contain the methylation-sensitive HhaI endonuclease recognition site and provide information about the 11p15.5 methylation status. The 13 reference probes include the probes at the regions of 2p25, 2q24, 3q29, 7q31, 9q21, 10q21, 10q25, 12q13, 14q24, 16q22, 17p12, 18q21 and 22q11, respectively. The digestion control probe is located at
8p21, contains an HhaI recognition site that is unmethylated in most control samples, does not generate a signal after HhaI digestion, and is used to confirm complete digestion by the HhaI enzyme.

The methylation-specific PCR assay can specifically distinguish the paternal and maternal alleles, and identify the differential methylation of the imprinted loci of H19DMR and KvDMR1 at 11p15.5 without requiring the parental samples. The methylation-specific PCR assay can be performed by sodium bisulfite treatment. In CpG methylation, a methyl group can be attached to cytosine (C) located at 5’ to guanine (G), and sodium bisulfite can...
convert unmethylated cytosine to uracil, while the methylated cytosine in the CpG dinucleotide is resistant to the chemical modification treatment of sodium bisulfite.

Our case had hypomethylation at KvDMR1 (IC2) and belonged to the most common type of abnormal methylation status associated with BWS. In a study of molecular alteration of the 55 cases with BWS analyzed by MS-MLPA, Priolo et al [14] found IC2 hypomethylation in 43.6% (24/55), IC1 hypermethylation in 10.9% (6/55), 11p15.5 paternal uniparental disomy in 21.8% (12/55), 11p15.5 paternal duplication in 1.8% (1/55), and normal methylation in 21.8% (12/55). More generalized DMR hypomethylation is more frequent in post-ART BWS cases than non-ART BWS cases [11]. In a study of 25 children with post-ART BWS, Lim et al [11] found IC2 epimutation of KvDMR1 loss of methylation in 24 of the 25 patients. Gomes et al [10] reported hypomethylation at KvDMR1 (IC2) in three of 18 clinically normal children conceived by ART. Hori et al [17] also detected abnormal hypomethylation at KvDMR1 (IC2) in ART-produced calves with large offspring syndrome. Various hypotheses have been raised to explain the epimutations associated with ART, such as: aberrant DNA methylation of imprinted loci in superovulated oocytes [18,19]; differential effect of culture on methylation pattern in the embryos [20,21]; epigenetic
abnormalities in sperms or oocytes in the parents undertaking ART [22,23]; aberrant DNA methylation of imprinted genes in human sperms associated with oligospermic patients [24,25]; and advanced parental age in the parents undergoing ART [26].

In conclusion, in case of prenatally detected omphalocele associated with an obstetric history of ART, a differential diagnosis of BWS should be considered. Methylation assays such as MS-MLPA and methylation-specific PCR using uncultured amniocytes are useful for rapid diagnosis of BWS under such circumstance.

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References