


CASE REPORT

Esophageal atresia and Beckwith–Wiedemann syndrome in one of the naturally conceived discordant newborn twins: first report

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Introduction

Beckwith–Wiedemann syndrome (BWS) is the most common overgrowth genetic syndrome with a birth prevalence of 1:10,000 live births [1]. BWS phenotype is highly variable for pre- and postnatal occurrence of overgrowth, and it is characterized by possible association of macroglossia, *nevus flammeus*, ear abnormalities, hemihyperplasia, visceromegaly, abdominal wall defects, nephroureteral malformations, neonatal hypoglycemia, and increased tumor risk [2–4]. Several molecular, cytogenetic, and epigenetic defects may determine the abnormal regulation of genes expression at 11p15 related to BWS. Among these pathogenic mechanisms, complex (epi)genetic defects can disrupt imprinted genes included in two domains of the critical region, and found in nearly 70% of BWS patients [5]. Some recent studies report a high incidence of monozygotic twinning in BWS [6]. A phenotypical discordance in monozygotic twins (MT) is a rare but well-recognized phenomenon, even though MT are usually concordant for genetic diseases [7]. Both twinning and BWS show a higher incidence in children born

Key Clinical Message

Recent studies report a high incidence of monozygotic twinning in Beckwith–Wiedemann syndrome. A phenotypical discordance in monozygotic twins is rare. Twinning and Beckwith–Wiedemann syndrome show higher incidence in children born after assisted reproductive techniques. We report on the first observation of esophageal atresia and Beckwith–Wiedemann syndrome in one of the naturally conceived discordant monozygotic twins.

Keywords

Assisted reproductive techniques, buccal swab, hypomethylation, *KCNQ1OT1* gene, phenotypical discordance.

after assisted reproductive techniques (ART) than in naturally conceived ones [8]. We now report on the first observation of MT discordant for esophageal atresia (EA) and BWS, in which a twin–twin transfusion syndrome (TTTS) was also found. We point out the relevance of such complex congenital disorder in naturally conceived discordant newborn MT.

Clinical Report

The proband was a naturally conceived female twin born from the first monochorionic diamniotic pregnancy of a 26-year-old woman, in a peripheral neonatal intensive care unit (NICU) of the town. A cesarean section was performed at 32 weeks, due to the rupture of membranes. The pregnancy was complicated at 26 weeks with TTTS and treated with laser therapy. Birthweight was 1460 g (31st centile); the weight of the other twin was 1670 g (56th centile). Apgar score was 3 and 8; she was intubated and ventilated at birth. Because of a perforated omphalocele, she was admitted to our NICU, which is supplied by a pediatric surgery unit, where a diagnosis of EA was also

established. Medial facial *nevus flammeus*, macroglossia, and bitemporal narrowing were noted. On the second day of life, she underwent surgery for EA with tracheoesophageal fistula and omphalocele. A molecular analysis of DNA from peripheral blood and buccal swab was performed to confirm the clinical suspicion of BWS. A low-level mosaic of about 30% for hypomethylation of *KCNQ1OT1*, with a normal pattern of methylation of the imprinting center (IC) 1, was detected by methylation-sensitive multiplex ligation-dependent probe amplification (MS-MLPA) of both *H19* and *KCNQ1OT1-1-IC loci*, which also excluded microduplication/deletion of the 11p15.5 chromosomal region. The other monozygotic twin was thought to be unaffected, in relation to her normal phenotype. However, molecular testing performed on a blood sample, when she was 3 months of age, showed abnormal methylation of *KCNQ1OT1*, while normal methylation was documented in the DNA isolated from buccal swab. The postoperative course of the affected twin was complicated by severe respiratory distress and oxygen dependency. She died at 2 months of postnatal age for a neonatal sepsis due to an invasive multiresistant coagulase-negative *Staphylococcus epidermidis* infection.

Discussion

Esophageal atresia is less frequent among singletons than MT, and it is usually observed only in one twin [9]. Twinning can be considered as an early blastogenetic defect which may be associated with other midline defects, such as EA with or without tracheoesophageal fistula [10]. A larger number of BWS newborns have been detected among twins than among singletons. The great majority of BWS twins are monozygotic, and they are frequently discordant: if one twin is affected by BWS, the other is not [11]. A possible explanation for WBS discordance in MT is the failure of methylation maintenance during a single cell cycle at the twinning event [12]. In relation to the timing, the failure of methylation maintenance could result in mosaicism or partial/complete discordance for imprinting defects in one or both twins. The present report is the first observation of monochorionic MT discordant for WBS and EA in the same patient, showing a low-level mosaic of about 30% for hypomethylation of *KCNQ1OT1*, both in blood DNA and in buccal swab. No uniparental disomy nor other DNA methylation defects were found. *KCNQ1OT1* hypomethylation was also observed only in the blood cells of the normal twin; her buccal swab DNA profile for BWS was normal. This finding is concordant with other studies on MT discordant for WBS reporting analogous *KCNQ1OT1* hypomethylation in the blood of both the affected and unaffected twin, whereas in the fibroblast and buccal swab

DNA *KCNQ1OT1* hypomethylation was restricted to the affected twin only [6, 11, 12]. Previous studies showed that the concordance for *KCNQ1OT1* hypomethylation in blood cells may be due to blood exchange [7]. This hypothesis is confirmed in current cases by the history of TTTS. As already documented by Zarate and Hopkin [7], our case underlines that TTTS may influence genetic diagnosis when obtaining blood samples for genetic testing in MT. In these cases, due to fetal sharing of circulations, contamination of the recipient's blood (our unaffected twin) with donor's cells (affected twin) can lead to misleading results. The possibility of false negative/positive DNA tests on blood should be thought in cases of TTTS, and testing on tissues other than blood should be taken into consideration [13].

It should also be noted that DNA components derived from the saliva samples primarily contain the same DNA as leukocytes. Therefore, it cannot be used to distinguish discordant twins. In these cases, genetic testing should be performed on other tissues such as buccal swabs, as occurred in our patients. We agree with other studies showing that in cases of twins discordant for BWS, genetic analysis from buccal swabs may provide the definitive diagnosis [11]. In fact, the *KCNQ1OT1* methylation ratio is lower in the buccal swab test than in the blood test, thus confirming/excluding the occurrence of the epimutation. Indeed, the diagnostic detection rate could be improved by introducing the analysis of tissues other than blood, as suggested also by the occurrence of low-rate mosaicism and the variable degree of methylation in different tissues [13]. Therefore, either in negative cases with high clinical suspicion or in cases of TTTS of discordant MT, further testing is mandatory. Our results point out the role of *KCNQ1OT1* in developing the severe BWS phenotype of the affected twin, although additional genomic as well as epigenetic changes cannot be excluded [14]. The high frequency of severe abdominal wall defects in centromeric cases is consistent with the hypothesis that genes expressed by the centromeric domain (IC2), containing also *KCNQ1OT1* and hypomethylated in the newborn here described, are involved in normal midline development [2, 3, 15, 16]. The incidence of WBS is increased in MT, particularly in the female ones [12, 17], due to the ART pregnancies [2–4, 18]. An increased rate of EA with or without tracheoesophageal fistula in twins is reported in several studies [9]. In this perspective, our opinion is that the EA in the present twin depends on the process of twinning, rather than on BWS phenotype.

Authorship

GS: drafted the manuscript. VA: made substantial contributions to acquisition of genetic data. MS: participated in

drafting the article. DV: contributed to interpretation of genetic data. EP: made substantial contributions to acquisition and interpretation of neurologic findings. GC: revised the manuscript for intellectual content and gave final approval of the version to be submitted.

Conflict of Interest

The authors have no conflict of interests to declare. No honorarium, grant, nor other form of payment was given to anyone of the authors to produce the manuscript.

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