Partial KCNQ1OT1 hypomethylation: A disguised familial Beckwith–Wiedemann syndrome as a sporadic adrenocortical tumor

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1. Introduction

Beckwith–Wiedemann syndrome has a wide spectrum of complications such as embryonal tumors, namely adrenocortical tumor. Tumor predisposition is one of the most challenging manifestations of this syndrome. A 45-day old female with a family history of adrenocortical tumor presented with adrenocortical tumor. The case raised suspicion of a hereditary Beckwith–Wiedemann syndrome, therefore molecular analysis was undertaken. The results revealed partial KCNQ1OT1 hypomethylation in the infant’s blood DNA which was associated with a complete loss of methylation in the infant’s adrenocortical tumor tissue. It is unique for familial Beckwith–Wiedemann syndrome caused by KCNQ1OT1 partial hypomethylation to manifest solely through adrenocortical tumor. Incomplete penetrance and specific tissue mosaicism could provide explanations to this novel hereditary Beckwith–Wiedemann syndrome presentation.

2. Clinical report

A 45-day old female of healthy parents was referred to the Pediatric Department for investigation of rapid weight gain. She was born at 39 weeks of gestation by cesarean section in response to a fetal distress. The birth weight was 4 kg (97 percentile) and clinical findings at birth were normal. In a physical examination, the subject weighed 5.6 kg (>97th percentile), and her blood pressure was 95/46 mm Hg. The infant had cushingoid features: a “buffalo hump” and a “moon face” with hypertrichosis. A lump was felt in the left upper quadrant of the abdomen. We found neither abdominal wall defect nor organomegaly. The genital organs were normal.

In the family history, her paternal aunt (65 years) was diagnosed with benign left adrenal tumor, which was surgically removed at the age of 60. Laboratory findings showed normal 24-h glucose profile. Endocrine investigations revealed a high level of both urinary free cortisol and serum cortisol in circadian rhythm (serum cortisol at 8 a.m. was 1640 μg/l). It was also noted that serum testosterone level was high, while the ACTH level was within normal range. The level of VMA in the urinary excretion of 24 h was also normal. CT-scan detected a left isolated adrenal necrotic tissue mass of 6 cm × 4 cm. A surgical resection was undertaken. The histopathological examination showed a typical benign ACT. Immediate post-operative outcomes were satisfactory and the cushingoid appearance was gradually improving. After 9 months of follow-up, the infant weighed 9 kg (75th percentile) and serum cortisol at 8 a.m. was down to 39 μg/l. The thoracic and abdominal CT scan control was normal. This list of findings raised suspicion of a hereditary BWS, hence molecular analyses were undertaken.
3. Genetic investigation

In order to investigate BWS epigenetic alterations, we opted for BWS specifically designed MS-MLPA (methylation-sensitive multiplex ligation probe amplification) kit (ME030 BWS/RSS). MS-MLPA reaction is a semi-quantitative method for methylation profiling, in which copy number detection is combined with the use of methylation-sensitive restriction enzyme.

DNA was extracted from blood samples from the baby, her parents and paternal aunt using FlixiGene DNA kit. DNA was also extracted from the tumor and skin tissues of the baby using the phenol/chloroform DNA extraction protocol. All 6 DNA samples were analyzed using the ME030 BWS/RSS MS-MLPA kit.

While MS-MLPA analysis of the ACT tumor tissue revealed a complete loss of methylation at KCNQ1OT1 gene, the baby’s blood DNA showed a partial loss of methylation at the same imprinting center, and the baby’s skin tissue showed a normal methylation profile (Fig. 1, Table 1). The aunt’s blood DNA MS-MLPA analysis unexpectedly showed a normal methylation profile (Table 1). As such, tumor DNA was not available. Blood DNA analysis from both parents was strictly consistent.

4. Discussion

BWS has a wide phenotypic spectrum including pre and postnatal overgrowth, macroglossia and anterior abdominal wall defects (Thorburn et al., 1970). Additional and variable complications may occur such as organomegaly, hypoglycemia, hemihypertrophy, genitourinary abnormalities and rare embryonal tumors like Wilms tumors and adrenocortical tumor which are reported in approximately 5% of cases (Reik and Maher, 1997; Ribeiro et al., 2000). Given the numerous and adrenocortical tumor which are reported in approximately 5% of cases (Reik and Maher, 1997; Ribeiro et al., 2000). Given the numerous genetic alterations that involve both IC1 and IC2 are reported in 20% of cases and indicate paternal uniparental disomy (UPD) (Cooper et al., 2005; Choufani et al., 2010). CDKN1C point mutations concern a small fraction of BWS patients (Lam et al., 1999). Although most patients have a normal karyotype, a number of BWS patients display chromosomal abnormalities including translocations, inversions, partial 11p15 trisomy, IC2 microdeletions and IC1 microduplications (Pettinati et al., 1986).

Most BWS cases are sporadic but family inheritance has been reported in 15% of cases (Bliek et al., 2001). The imprinting genomic defects are reported in patients with hereditary BWS at a higher prevalence of CDKN1C point mutations, IC2 microdeletions and IC1 microduplications (Niemitz et al., 2004). Most hereditary KCNQ1OT1 methylation defects are either due to IC2 microdeletion or associated with IC1 hypermethylation, hence to UPD (Bliek et al., 2001).

Nonetheless KCNQ1OT1 hypomethylation associated with a normal IC1 methylation status has already been reported in familial BWS (Bliek et al., 2001). Jet Bliek reported 31 BWS patients, including 4 familial cases, all displaying normal IC1 methylation and hypomethylation of KCNQ1OT1. Interestingly, none of those patients had developed a tumor.

Although ACT is known to correlate to H19 hypermethylation, hence to IC1 hypermethylation, few cases of sporadic ACT displaying KCNQ1OT1 hypomethylation were reported. Partial KCNQ1OT1 hypomethylation reported in our patient’s blood DNA has already been reported in 29 out of 40 non-UPD patients (Bliek et al., 2001).

The infant’s paternal aunt with surgically managed ACT remains the only evidence of BWS hereditary transmission in our patient. Lindor et al. already suggested that, besides several other clinical criteria, other family members affected with ACT may raise suspicion of BWS (Lindor et al., 2008).

The present study reports a unique familial BWS caused by partial KCNQ1OT1 hypomethylation, solely expressed through ACT. Our patient is likely to have inherited the imprinting defect from her mother. As previously described, a milder phenotype is assigned to paternal transmission of BWS. Incomplete penetrance could provide an explanation to under-diagnosis of familial cases. Although the father (II.1, Fig. 1) and the aunt (I.3, Fig. 1) did not show KCNQ1OT1 hypomethylation in their blood DNAs, a specific familial tissue mosaicism could underlie this special constellation.

### Table 1

| DNA Sample            | IC1 Methylation Index | IC2 Methylation Index |
|-----------------------|-----------------------|-----------------------|
| Blood DNA Father      | 0.53                  | 0.54                  |
| Blood DNA Mother      | 0.56                  | 0.56                  |
| Blood DNA Aunt        | 0.53                  | 0.56                  |
| Blood DNA Baby        | 0.51                  | 0.35                  |
| Baby tumor DNA        | 0.56                  | 0.05                  |
| Baby skin DNA         | 0.56                  | 0.54                  |

Footnotes:
- Methylated status is determined according to MS-MLPA recommendations.
- Methylated index (MI) below 0.5 indicates a loss of methylation. MI between 0.6 and 0.5 indicates a normal methylation pattern and MI above 0.6 indicates a hypermethylation.

Table 1

| DNA Sample | IC1 Methylation Index | IC2 Methylation Index |
|------------|-----------------------|-----------------------|
| Blood DNA  | 0.53                  | 0.54                  |
| Mother     | 0.56                  | 0.56                  |
| Aunt       | 0.53                  | 0.56                  |
| Baby       | 0.51                  | 0.35                  |
| Baby tumor | 0.56                  | 0.05                  |
| Baby skin  | 0.56                  | 0.54                  |

**Fig. 1.** Pedigree of the three generation family showing IC1 and IC2 methylation pattern: complete methylation; partial methylation; patients with ACT; P: paternal allele; M: maternal allele. Generation order: I, II and III.
5. Conclusion

Tumor predisposition is a challenging manifestation of BWS. It is unique for familial BWS caused by KCNQ1OT1 partial hypomethylation to express solely through adulthood and childhood ACT. Incomplete penetrance and specific familial tissue mosaicism could provide explanations to this mild hereditary BWS presentation.

Acknowledgment

Authors are grateful to the patient and her family. Special thanks go to Miss Ahlem Msakni and Miss Safa Bouker for their technical support.

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