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An atypical case of hypomethylation at multiple imprinted loci

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Abstract

Angelman syndrome (AS) and Prader-Willi syndrome (PWS) are caused by genetic and epigenetic mutations of the imprinted gene cluster on chromosome 15q13.

Although the imprinting mutations causing PWS and AS are essentially opposite in nature, remarkably, a small number of patients have been reported with clinical features of PWS but epigenetic mutations consistent with AS.

We report here a patient who presented with clinical features partially consistent with both PWS and BWS (Beckwith-Wiedemann syndrome). Epimutations were found at both the AS/PWS and BWS loci, and additionally at the H19, PEG3, NESPAS and GNAS loci. This patient is therefore the first described case with a primary epimutation consistent with AS accompanied by hypomethylation of other imprinted loci (AS-HIL).

Key words: Angelman, Prader-Willi, Beckwith-Wiedemann, Imprinting, hypomethylation

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Introduction

Imprinted genes are limited in expression potential by their parent of origin, which often coincides with parental-allele specific DNA methylation. Imprinting disorders typically have heterogeneous clinical presentations. Some features, such as prenatal overgrowth, macroglossia, or truncal obesity, are associated with more than one imprinting disorder, suggesting that imprinted genes control many and overlapping facets of human development. Moreover, in recent years, patients have been identified with imprinting disorders affecting more than one imprinted locus, and their

clinical features may diverge from the 'classical' presentation of the disorder, possibly reflecting the complement of genes affected.¹

Angelman syndrome (AS) and Prader-willi syndrome (PWS) are distinct genetic conditions caused by abnormalities at the 15q11-13 imprinted region. Patients with classical Angelman syndrome present with severe mental retardation, speech impairment, ataxia and seizures. They have a characteristic behaviour pattern of hyperactivity and a happy personality and episodes of inappropriate bursts of laughter. Dysmorphic features include microcephaly, macrostomia and prominence of the jaw.² Prader-Willi syndrome is characterised by global developmental delay, hypotonia and feeding difficulties with failure to thrive in infancy. There is rapid weight gain usually between the ages of 1 and 4 years. This is due to hyperphagia and results in truncal obesity. Patients have short stature and small hands and feet, and males have hypogonadism. The face is characterised by almond shaped eyes and an upward slant of the palpebral fissures. Temper tantrums and a tendency to an obsessive compulsive phenotype are commonly seen.³

AS is most commonly (~70%) caused by interstitial deletion of the maternally derived chr15q11-13. Other causes include paternal uniparental disomy (UPD) (1-2%), imprinting defect (3-5%) and mutations in *UBE3A* (5-10%). Prader-Willi syndrome is caused by a paternal deletion of the same region (~70%) or maternal UPD (~29%). A primary imprinting abnormality is rare.⁴

There are a small number of reported cases of patients with clinical features of PWS but a molecular diagnosis of AS.⁵⁻⁷ In the majority of these cases partial loss of maternal methylation is observed.

We report here a three year-old girl who presented with features of both PWS and a second imprinting disorder, Beckwith-Wiedemann syndrome (BWS). It was hypothesised that a methylation anomaly at more than one locus might explain the phenotype. Therefore, imprint status was investigated at both the PWS/AS and BWS regions, and thereafter at other imprinted loci.

Case report

The patient is one of 4 children born to unrelated Sri Lankan parents. Conception was natural and the pregnancy uncomplicated. Her birth weight at term was 3640g (55th centile). A large, protruding tongue was noted in the neonatal period, the umbilicus was normal. There were no feeding problems, normal tone and no history of hyper- or hypoglycaemic episodes. Parathyroid, calcium and phosphate levels were normal. There was no history of organomegaly. Motor development was normal and she walked independently at 14 months. She was a shy child with some developmental delay particularly with speech, having no words by 2 years. She had excessive weight gain in her second year and by 3.5 years her OFC was 53.3 cm (98th centile), height 96.9cm (<25th centile) and weight 19.5kg (95th centile). At the age of three and a half years she has remained seizure free and is not ataxic. In addition to her large tongue, she has a round face and bilateral single palmar creases . She has two words and points to indicate her needs. In light of her macroglossia, Beckwith-Wiedemann syndrome (BWS) was suspected; however, because of the developmental delay and increased BMI, Prader-Willi syndrome (PWS) also seemed a possibility.

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Materials and Methods

Ethical permission for this study was obtained from Southampton and South West Hampshire Research ethics committee, REC reference number: 07/H0502/85.

DNA was extracted from peripheral blood by standard procedures.

Initially methylation was determined at the PWS/AS by Methylation specific PCR (MS-PCR) according to Kubuta et al⁸, and by methylation specific MLPA (MS-MLPA) at 11p15 using MRC Holland BWS kit ME030B according to the manufacturer's instructions (as described by Scott et al⁹). *KCNQ1OT1* and *H19* results were confirmed by Methylation-sensitive high resolution melt analysis according to Alders et al¹⁰ (MS-HRM).

Subsequently, methylation specific PCR (MS-PCR) analysis of the patient and parental samples was carried out at the *ARHI*, *PLAGL1*, *MEST1*, *GRB10*, *KCNQ1OT1*, *H19*, *IGF2P0*, *DLK1*, *SNRPN*, *PEG3*, *GNAS*, *NESP55* and *NESPAS* loci as described previously^{1, 11}. Pyrosequencing of *SNRPN*, *GNAS*, *NESPAS* and *PEG3* was performed as previously described^{1, 12}.

Microsatellite analysis around the *SNRPN*, *KCNQ1*, *H19* and *PEG3* regions was performed using published microsatellites by standard methods. The markers investigated were: D15S817, D15S128, D15S1506, D15S122, D11S4177, D11S922, D11S4046, D11STH2, D11S1318, D11S4088, D11S4146, D19S210, and D19S887. *ZFP57* sequencing was performed as previously described¹.

Results

Initial testing of the *SNRPN* DMR by MS-PCR and the 11p15.5 region using MS-MLPA revealed a reduced intensity of the methylated maternal band at the *SNRPN* DMR, partial hypomethylation at *KvDMR* and subtle hypomethylation at *H19*. The results at *KvDMR* and *H19* were confirmed by MS-HRM (data not shown).

To extend these findings, MS-PCR was carried out at 13 imprinted loci across the genome. In addition to confirming the abnormalities at 11p15 and 15q13, partial loss of maternal methylation was seen at *GNAS*, *NESPAS* and *PEG3* (**Figure 1**). The

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SNRPN epimutation and the additional epimutations at *GNAS*, *NESPAS* and *PEG3* were confirmed by pyrosequencing.

Methylation analysis of the parental samples revealed normal methylation levels at all the loci investigated.

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Microsatellite analysis of the patient and her parents around the regions of methylation abnormality showed biparental inheritance, excluding multiple regions of uniparental disomy as a cause of the imprinting anomalies. Sequencing of the *ZFP57* gene revealed no mutations (data not shown).

Discussion

We describe here a patient with clinical features suggestive of both PWS and BWS, who was found to exhibit hypomethylation of multiple imprinted loci (HIL).

The clinical features of this patient were remarkably mild given the extent of her methylation abnormalities. Macroglossia and single palmar creases are both diagnostic features of BWS, and consistent with the imprinting anomaly observed. However, the normal birth weight, umbilicus, glycaemia and lack of hemihypertrophy were inconsistent with BWS. The patient's high BMI and developmental delay were consistent with the initial clinical suspicion of PWS, though normal tone and normal facial appearance were inconsistent with PWS. Moreover, she had no ataxia, microcephaly or seizures to suggest AS. We suggest that this case is an example of the clinical picture that may emerge when hypomethylation affects multiple imprinted loci.

Methylation abnormalities at multiple imprinted loci have been described previously in other imprinting syndromes including Transient Neonatal diabetes (TND^{1,13}), BWS¹⁴⁻¹⁶ and Russell-Silver syndrome¹⁷⁻¹⁸. The only gene mutation so far associated

with such disorders is mutation of *ZFP57*, which was found in approximately 50% of patients with TND HIL¹. This patient showed no evidence of a *ZFP57* mutation, and her pattern of methylation abnormalities seems distinct from that seen in either TND HIL or BWS HIL. Interestingly, the epimutations observed include mosaic hypomethylation of the maternally methylated *SNRPN*, *KCNQ1OT1*, *PEG3* and *NESPAS* loci and the paternally methylated H19 locus. This pattern of abnormalities suggests failure of imprint maintenance, rather than its establishment.

It would be interesting to study further cases with an epimutation consistent with AS but presenting with features of PWS, as it is likely that some of these cases will also harbour additional methylation changes.

This case is a significant addition to the weight of evidence suggesting that patients with HIL may go undiagnosed because their presentation may blur the boundaries of recognised imprinting disorders. We therefore suggest that epigenetic analysis at multiple loci should be carried out in patients presenting with clinical signs that could be consistent with more than one of the classical imprinting disorders. It is becoming clear that the clinical picture in patients with HIL is extremely variable and that epigenetic as well as genetic mutations should be suspected in any patient with an unusual syndromic presentation.

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Conflict of interest

The authors declare no conflict of interest.

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Titles and legends

Legend to Figure 1.

Electropherograms of methylation-specific PCR amplicons. The successive panels represent analysis of the SNRPN, KCNQ1OT1, H19, PEG3, GNAS and NESPAS DMRs, respectively.

In each panel, the top trace illustrates a normal control, and the lower trace the patient.

Bars labelled me and un indicate “methylated” and “unmethylated” peaks, respectively. Hypomethylation in the patient is indicated by a reduction in the methylated peak height relative to the unmethylated peak when compared to the control.

