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Maternal gametic transmission of translocations or inversions of human chromosome 11p15.5 results in regional DNA hypermethylation and downregulation of *CDKN1C* expression

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ABSTRACT

Beckwith–Wiedemann syndrome (BWS) is an overgrowth syndrome associated with genetic or epigenetic alterations in one of two imprinted domains on chromosome 11p15.5. Rarely, chromosomal translocations or inversions of chromosome 11p15.5 are associated with BWS but the molecular pathophysiology in such cases is not understood. In our series of 3 translocation and 2 inversion patients with BWS, the chromosome 11p15.5 breakpoints map within the centromeric imprinted domain, 2. We hypothesized that either microdeletions/microduplications adjacent to the breakpoints could disrupt genomic sequences important for imprinted gene regulation. An alternate hypothesis was that epigenetic alterations of as yet unknown regulatory DNA sequences, result in the BWS phenotype. A high resolution Nimblegen custom microarray was designed representing all non-repetitive sequences in the telomeric 33 Mb of the short arm of human chromosome 11. For the BWS-associated chromosome 11p15.5 translocations and inversions, we found no evidence of microdeletions/microduplications. DNA methylation was also tested on this microarray using the HpaII tiny fragment enrichment by ligation-mediated PCR (HELP) assay. This high-resolution DNA methylation microarray analysis revealed a gain of DNA methylation in the translocation/inversion patients affecting the p-ter segment of chromosome 11p15, including both imprinted domains. BWS patients that inherited a maternal translocation or inversion also demonstrated reduced expression of the growth suppressing imprinted gene, *CDKN1C* in Domain 2. In summary, our data demonstrate that translocations and inversions involving imprinted domain 2 on chromosome 11p15.5, alter regional DNA methylation patterns and imprinted gene expression *in cis*, suggesting that these epigenetic alterations are generated by an alteration in "chromatin context".

1. Introduction

Beckwith–Wiedemann syndrome (BWS) is an overgrowth disorder involving macroglossia, omphalocele, and an increased risk (1000-fold) of embryonal tumors [1–3]. This condition is associated with dysregulation of gene expression in an imprinted gene cluster on chromosome band 11p15.5 (Fig. 1). The chromosome 11p15.5 region houses two imprinted domains, each controlled by an imprinting center (IC). Imprinting centers are characterized by differential, parent of origin-specific methylation. Regulation of imprinted genes also involves non-coding RNA transcripts that regulate the expression of neighboring genes *in cis* over distances up to one megabase [4–6]. Molecular changes observed in patients with BWS include both genetic and/or epigenetic alterations on chromosome 11p15.5 [7,8]. The epigenetic alterations include changes in DNA methylation or histone modifications in at least one of the two imprinting centers (IC1, IC2) [8–10].

In Domain 1, IC1 is associated with the genes *H19* (a non-coding RNA of unknown function) and insulin-like growth factor 2 (*IGF2*) (Fig. 1). Gain of methylation at the maternal IC1 accounts for 5% of BWS cases [11], with repression of the maternal *H19* transcript and de-repression of the maternal *IGF2* transcript [12].

In Domain 2, IC2 overlaps the 5′ region of *KCNQ1OT1*, a non-coding RNA found within intron 10 of the *KCNQ1* gene [13]. Also in this domain are several other imprinted genes implicated in BWS, including the *CDKN1C* gene, a cyclin-dependent kinase inhibitor. *KCNQ1OT* is a paternally expressed, non-coding RNA, which downregulates the expression

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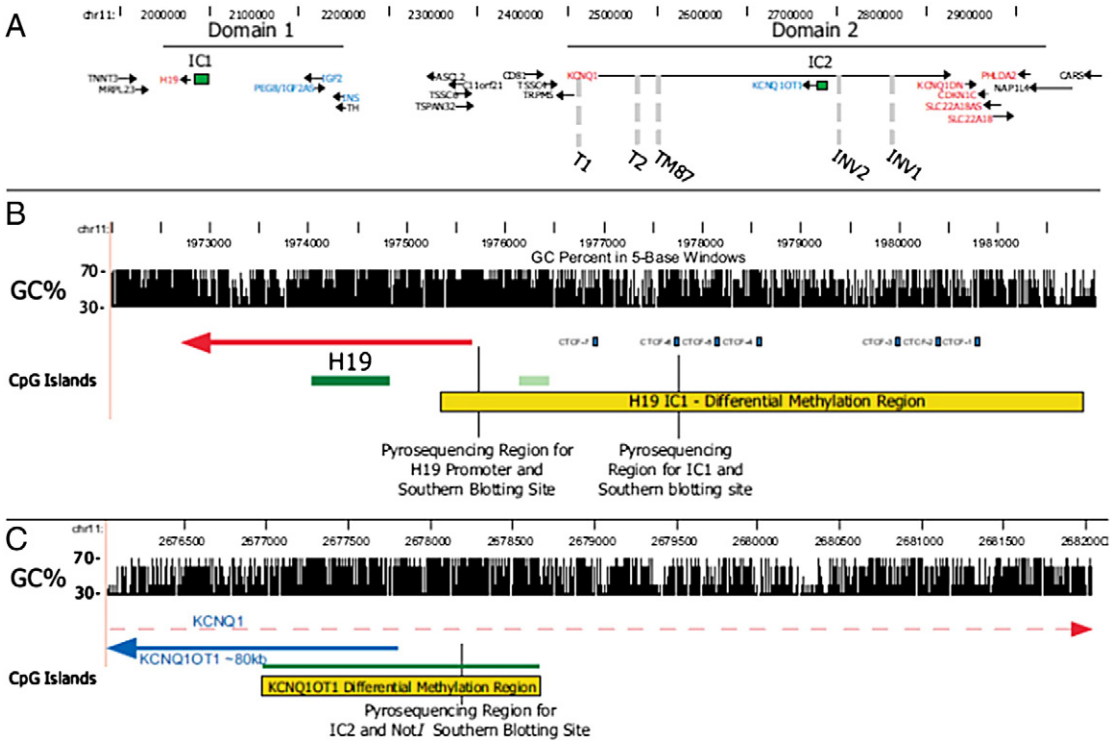

Fig. 1. Schematic map of the 11p15.5 region. A) Schematic representation of the chromosome 11p15.5 imprinted cluster from the UCSC genome browser coordinate chr11:1 900 000–3 000 000 (http://genome.ucsc.edu). The 11p15.5 imprinted cluster is divided into two domains (Domain 1 is telomeric, while Domain 2 is more centromeric) with an imprinting center for each Domain. Biallelically expressed genes are shown in black. Maternally expressed imprinted genes are noted by red text color and paternally expressed imprinted genes are noted by blue text color. Direction and approximate length of the transcript are indicated by arrows. IC1 is a differentially methylated region upstream of the *H19* transcription start site. IC2 is a differentially methylated region upstream of the non-coding RNA *KCNQ1OT1*. The approximate locations of the translocation breakpoints are shown on the map based on previously published mapping [19, 25, 26] and validated by our BAC FISH studies. B) The IC1 differentially methylated region in detail. The location of the *H19* transcript is noted by the red arrow (maternal expression) and the location of CpG islands and the *H19* DMR are shown by green and yellow boxes respectively. The location of the seven putative CTCF binding sites are shown by small blue boxes and the location of the targets for pyrosequencing and Southern assays are indicated. C) The IC2 differentially methylated region in detail. The location of the *KCNQ1OT1* transcript is noted by the blue arrow (paternal expression) and the location of CpG island and the *KCNQ1OT1* DMR is shown by green and yellow boxes respectively. The location of the targets for the pyrosequencing and Southern assays is indicated. The *KCNQ1* (maternally expressed) transcript passes through this region in the antisense orientation although there is no coding sequence as this region is contained within intron 10 of *KCNQ1*.

of nearby genes on the paternal chromosome e.g. *KCNQ1* and *CDKN1C* [5, 14] (Fig. 1). Loss of maternal methylation of IC2 is seen in 50% of patients with sporadic BWS [2, 7, 15, 16]. Deletion of the orthologous sequence in mouse results in loss of imprinting of several neighboring genes including *KCNQ1*, indicating that this IC is critical for maintaining imprinted gene expression in Domain 2 [4]. In humans, loss of methylation at the maternal IC2 on chromosome 11p15.5 has been shown to be associated with reduction of *CDKN1C* expression, thereby explaining the pathophysiology of such cases of BWS [5]. Other genetic alterations associated with BWS include paternal uniparental disomy (UPD) of chromosome 11 (~20%), mutations in the *CDKN1C* gene (5–10%), and microdeletions involving IC1 (~5%) and infrequently microduplications of IC2 (<1%) [17, 18].

In <1% of BWS cases, cytogenetically visible chromosomal changes are associated with the BWS phenotype. These include paternally transmitted duplications of chromosome 11p15.5 and maternally transmitted translocations or inversions [19–27]. Unbalanced chromosome rearrangements involving chromosome 11p15.5 alter the copy number of imprinted genes thereby presumably changing the dosage of growth regulatory genes such as *IGF2* and *CDKN1C*.

BWS patients with maternally transmitted, apparently balanced translocations or inversions of chromosome 11p15.5 exhibit typical features of BWS. The mechanism by which such balanced translocations and inversions of the imprinted cluster on 11p15.5 result in the BWS phenotype is not understood. In the case of such balanced rearrangements there are no known associated changes in copy number and all the translocations/inversions are maternally inherited.

The cause of the BWS phenotype in apparently balanced translocations and inversions is not clear. FISH mapping studies have indicated that the chromosome 11p15.5 translocation and inversion breakpoints cluster near the *KCNQ1* gene — encompassing a region of over 400 kilobases. Such BWS-associated translocations and inversions also demonstrate breakpoints that lie several megabases centromeric to *KCNQ1* (such as sample B10.1 in [19]). No alterations in single copy genomic sequences have been reported in these apparently balanced translocation and inversions. We hypothesized that position effects over large distances, leading to altered expression of genes in the 11p15.5 imprinted cluster, might result in BWS.

However, recent literature indicates that chromosomal rearrangements that are apparently balanced by standard cytogenetic methods may involve unexpected complexity when investigated using high resolution technologies [20, 21]. Therefore we proposed an alternative hypothesis that microdeletion/microduplication of regulatory elements when passed through the maternal germline could lead to the BWS phenotype. Further, since apparently balanced translocations and inversions only produce a BWS phenotype upon maternal transmission, we also hypothesized that an epigenetic mark that could not be reset in the female germline could cause the BWS phenotype. Through molecular investigations of the latter possibility, *cis*-acting elements important in the establishment or maintenance of imprinting on chromosome 11p15.5, could be discovered.

We report here that translocations and inversions disrupting the centromeric imprinted domain on chromosome 11p15.5 are not associated with microdeletions or microamplifications, but can result in regional changes in DNA methylation. Further, we show that dysregulation of the maternally expressed growth suppressing gene, *CDKN1C*, occurs in patients with BWS that have a maternally derived translocation or