A maternally methylated CpG island in \textit{KvLQT1} is associated with an antisense paternal transcript and loss of imprinting in Beckwith–Wiedemann syndrome


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ABSTRACT Loss of imprinting at \textit{IGF2}, generally through an \textit{H19}-independent mechanism, is associated with a large percentage of patients with the overgrowth and cancer predisposition condition Beckwith–Wiedemann syndrome (BWS). Imprinting control elements are proposed to exist within the \textit{KvLQT1} locus, because multiple BWS-associated chromosome rearrangements disrupt this gene. We have identified an evolutionarily conserved, maternally methylated CpG island (\textit{KvDMRI1}) in an intron of the \textit{KvLQT1} gene. Among 12 cases of BWS with normal \textit{H19} methylation, 5 showed demethylation of \textit{KvDMRI1} in fibroblast or lymphocyte DNA; whereas, in 4 cases of BWS with \textit{H19} hypermethylation, methylation at \textit{KvDMRI1} was normal. Thus, inactivation of \textit{H19} and hypomethylation at \textit{KvDMRI1} (or an associated phenomenon) represent distinct epigenetic anomalies associated with biallelic expression of \textit{IGF2}. Reverse transcription–PCR analysis of the human and syntenic mouse loci identified the \textit{KvDMRI1}-associated RNA transcript exclusively from the paternal allele and in the opposite orientation with respect to the maternally expressed \textit{KvLQT1} gene. We propose that \textit{KvDMRI1} and/or its associated antisense RNA (\textit{KvLQT1-AS}) represents an additional imprinting control element or center in the human 11p15.5 and mouse distal 7 imprinted domains.

Genomic imprinting describes the process by which a subset of mammalian genes is “marked” during gametogenesis such that they are expressed differentially in somatic cells depending on their parental origin (1–3). This mark may be differential methylation, because DNA methylation is necessary for the proper regulation of imprinted genes (4). Furthermore, some differentially methylated regions (DMRs) are thought to represent genomic imprints, because they are differentially methylated in male and female germ cells and remain so throughout development (5–10). Nevertheless, the mechanism by which the primary information in these DMRs is used to regulate genomic imprinting is understood only partially. The DMRs of most imprinted genes are associated with short, G-rich, direct repeat sequences (11, 12), which have been postulated to facilitate heterochromatization and gene silencing at imprinted loci (11). A more recently identified characteristic of imprinted genes is their association, in some cases, with imprinted antisense RNA transcripts. At the paternally expressed mouse \textit{Igf2} and \textit{Zfp127} (and human homologue) loci, antisense transcripts that are also expressed paternally have been identified and overlap with the protein coding gene (13–15). For the maternally expressed \textit{Igf2r} and \textit{UBE3A} genes, overlapping antisense transcripts have been found and are oppositely imprinted with respect to the protein coding gene (16, 17). It has been proposed that antisense transcripts serve to regulate overlapping genes by promoter or transcript occlusion or by competing with these loci for regulatory elements such as transcription factors or enhancers (18). An allele-specific competitive advantage for each transcript on the respective alleles would lead to imprinted gene expression (19).

Normal mammalian development requires the correct parental contribution of imprinted genes. A lack of biparental contribution or aberrant expression of imprinted genes leads to a variety of developmental abnormalities in the mouse (20–22) and humans (22–27). Human chromosome 11p15.5 contains at least seven imprinted genes (Fig. 1), six of which are preferentially or exclusively expressed from the maternally derived chromosome and one (\textit{IGF2}) of which is expressed primarily from the paternal allele (22). BWS, an overgrowth and cancer predisposition condition that maps to this region, results from the aberrant expression of one or more of these imprinted loci. BWS has a complex genetic etiology and can arise from paternal uniparental disomy (UPD), paternal duplication of 11p15.5, maternally inherited coding mutations in the \textit{p57KIP2} gene, or maternal chromosome rearrangements (26, 28). However, the most common mechanism resulting in BWS is the loss of imprinting (LOI) of \textit{IGF2} without apparent chromosomal abnormalities (26, 29–32). In contrast to Wilms’ tumors where LOI at \textit{IGF2} is usually accompanied by hypermethylation and silencing of the \textit{H19} gene (33, 34), biallelic expression of \textit{IGF2} in BWS usually occurs independently of changes in methylation or expression at \textit{H19} (32).

The identification of an \textit{H19}-independent pathway for LOI at \textit{IGF2} in patients with BWS suggests that at least one additional imprinting control region exists in chromosome band 11p15.5 (31, 32, 35). The existence of additional imprinting control elements is supported by the finding that, although

This paper was submitted directly (Track II) to the Proceedings office. Abbreviations: BWS, Beckwith–Wiedemann syndrome; DMR, differentially methylated region; EST, expressed sequence tag; LOI, loss of imprinting; LOH, loss of heterozygosity; RT-PCR, reverse transcription–PCR; RT, reverse transcriptase; UPD, uniparental disomy. Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF119385).

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targeted deletion of H19 affects the imprinting of Igf2 and Ins2 (36), the more distant Mash2, Kvlqt1, and p57Kip2 genes are unaffected (37). Although effects at more removed loci cannot be excluded, the disruption of the Kvlqt1 gene in multiple cases of BWS patients with chromosome rearrangements suggests that the Kvlqt1 locus may harbor imprinting control elements (38, 39). This report describes the identification and characterization of a region within the human and mouse Kvlqt1 genes that has characteristics of an imprinting control element. We find that the imprinted methylation at this locus is disrupted in a majority of patients with BWS lacking other known genetic defects, and we suggest that this loss of methylation may be causally related to the disease phenotype.

**MATERIALS AND METHODS**

**Cell Lines, Patient Samples, and Mice.** Cell lines with names containing the prefix “GM-” were obtained from the NIGMS Human Genetic Mutant Cell Repository. Peripheral blood lymphocytes were obtained from laboratory staff. Wilms’ tumor samples were provided by the National Wilms’ Tumor Study Group Tissue Bank. Cell lines from patients with BWS carrying the inv(11)(11;16), and inv(11;22) rearrangements as well as a cell line from the rhabdoid tumor with the inv(11;22) rearrangement have been described by Sait et al. (40). Fibroblast or lymphocyte DNA samples from nonrarrangement BWS cases were from patients who have been described (31, 32). Human testis, sperm, and ovaries were obtained as described by Driscoll and Migeon (41). Other fetal tissues were acquired from the Brain and Tissues Banks for Developmental Disorders (University of Maryland). C57BL/6J and PWK inbred mouse strains were provided by Rosemary Elliott (Roswell Park Cancer Institute).

**DNA Preparation and Southern Hybridization.** Genomic DNA was prepared from cell lines and most tissues by standard proteinase K digestion and phenol extraction. Sperm DNA was isolated from semen as described (41). Probes BX2 and DMRP were generated by PCR from PAC clone pdl-74K15 (39) by using primers BX2f (5′-TCTAGAGGGAGATCGG-3′) plus BX2r (5′-GCAGTCCATAGGGCGCAAGACG-3′) and DMRPf (5′-TCTCTGGGAGGTGAAATG-3′) plus DMRPr2 (5′-TGCTGCGTCTCTGCTC-3′), respectively. PCR products were cloned into pCRII-TOPO (Invitrogen). Southern hybridizations were generally carried out as described (40) with two final washes of 0.2× SSC/0.5% SDS at 65°C. Hybridization signal was detected by using a Storm PhosphorImager (Molecular Dynamics). Quantitation of band intensity was done by using National Institutes of Health IMAGE software (rsb.info.nih.gov/ij/image/).

**Isolation and Characterization of the Mouse Locus.** A portion (subclone 34; sc34) of the corresponding mouse locus was isolated by low-stringency (final washes: 2× SSC/0.5% SDS at 35°C) hybridization of the human locus to a PAC subclone library of a murine PAC clone known to span the syntenic region on mouse distal chromosome 7 (42). The locus was expanded by rescreening the subclone library with a probe generated from the mouse PAC by using a linker-mediated PCR method (39) and a unique primer (sc34:3: 5′-GGTCTGTAATAACTGAAAACC-3′) designed from the sequence of sc34.

**Reverse Transcription–PCR (RT-PCR).** Total RNA was isolated from cell lines and tissues by using RNeasy Mini Kits (Qiagen, Chatsworth, CA), and ∼6 μg of RNA was treated with RNase-free DNase (GIBCO/BRL). Half of the treated RNA was used for first-strand cDNA synthesis with SuperScript II (GIBCO/BRL) reverse transcriptase (+RT) by using either oligo(dT) or an antisense strand-specific (sense with respect to Kvlqt1 mRNA) primer. The remainder of the treated RNA was incubated in a similar manner but without reverse transcriptase (−RT). The sequence of primers used for expressed sequence tag (EST) “connection,” “transcript sampling,” and expression analysis in single chromosome 11 hybrids (see Fig. 2 for primer positions) as well as those used for strand-specific reverse transcription are available on request from the authors.

**Analysis of Allelic Expression Patterns in the Mouse.** An expressed polymorphism in the mouse antisense transcript was detected between C57BL/6J and PWK mice by single-strand conformation polymorphism analysis by using genomic DNA and primers sc34.1 (5′-TTGCTCGAGATGGCTGTG-3′) and sc34.2 (5′-CTTGCTGTAACCTTTGCTCT-3′) with 0.5× MDE gels (FMC; 32 W; 3.5 h; 4°C). Cloning into pCRII-TOPO and sequencing identified a single base pair (A→G) polymorphism (position 4,231 in GenBank accession no. AF119385) between C57BL/6J and PWK mice, respectively. Allelic expression analysis was performed by using the same primers but with cDNA made from F1 fetal tissue RNA. Polymorphism characterization and assessment of imprinting at Kvlqt1 were carried out as described above but with primers Kvlqt1.5 (5′-GGGTAGAGCCTGACTCCTTC-3′) and Kvlqt1.6 (5′-TAGGGTGGACAGTGGACAATCC-3′). Two polymorphisms between C57BL/6J and PWK mice were found: a nucleotide substitution, GC/CA in C57BL/6J and PWK, respectively, at position 2,460–2,461 in the 3′ untranslated region of the Kvlqt1 mRNA (GenBank accession no. U70068) and an insertion–deletion polymorphism (C) at position 2,481.

**Bioinformatics.** CpG islands were located by using GRAIL (avalon.epn.orl.n/.Grail-bin/EmptyGrailForm). Database searches were performed by using BLAST at the National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov/blast/nph-blast?Jform=0). Direct repeat structures (see Fig. 2 for locations) were identified by dot matrix analysis by using the PUSTELL DNA MATRIX module (window = 40 min; percentage score = 57) in MACVECTOR (Oxford Molecular Group, Campbell, CA).
RESULTS

Maternal-Specific Methylation at KvDMRI. One-third (>325 kb) of the 1-Mb imprinted domain in 11p15.5 is occupied by the KvLQT1 gene, in which protein-coding mutations result in Romano–Ward and Jervell and Lange-Nielsen syndromes (43). The human and mouse (KvLQT1) genes are regulated by genomic imprinting in a developmental and tissue-specific manner (37, 38, 44–46); however, features characteristic of imprinted genes, such as differentially methylated CpG-rich regions (DMRs) and short direct tandem repeat structures (11, 12), have not been reported. Large-scale sequencing of PAC clone pdj-74K15 (GenBank accession no. U90058; ref. 39) and computer analysis identified a CpG island (designated KvDMRI) in intron 10 of KvLQT1, which also contained two direct repeat sequence motifs (Figs. 1 and 2a). Two probes, DMRP and BX2 (Fig. 2a), were developed by PCR and hybridized to Southern blots of normal human DNA digested with the methylation-sensitive restriction enzyme NotI by using EST-specific primers. The bidirectional arrows (primer names indicated above) correspond to positive RT-PCR assays used to “sample” the genomic DNA sequence for expressed sequences. (b) Physical map of the syntenic mouse locus (same symbols as in a). A region of homology (83% identity over 437 bp) between the mouse and human loci is indicated by the striped box (the open end indicates that the downstream extent of homology is unknown, as the mouse sequence is not complete).

To confirm the parental origin of the methylated KvDMRI allele and to show that this methylation was regulated by genomic imprinting, the mouse locus was identified and used to analyze DNA isolated from aborted kidney, sc34 (Fig. 2b) detected a HindIII restriction fragment length polymorphism between C57BL/6J and PWK mice. In C57BL/6J × PWK F1 interspecific animals, only the 11.0-kb PWK (paternal) allele was cleaved by the methylation-sensitive restriction enzyme EagI, whereas, in F1 DNA from the reciprocal cross, the paternal allele was detected. (c) Hybridization of sc34 to HindIII (H) and HindIII/EagI (H/E) double digests of adult kidney DNA from reciprocal C57BL/6J (B6) × PWK F1 animals (for F1 hybrids, the parental parent is specified first). (d) The BX2 probe was hybridized to EcoRI/NotI digests of human DNA from somatic tissues (peripheral blood lymphocytes or brain), testes (ts), sperm (sp), and fetal ovaries (ov). The ratio of the intensity of the upper and lower bands is shown in the accompanying histogram. The faint band at 3–3.5 kb present in all lanes is a cross-hybridizing locus observed when experiments are done at reduced stringency. The additional band seen in the sperm lane is likely due to a heterogeneous methylation at this cross-reacting locus.

To determine whether the methylation at KvDMRI represents an imprinting mark established in the germ line, BX2 was hybridized to EcoRI/NotI digests of human somatic and germ-line DNA samples (Fig. 3d). In this experiment, the average ratio of the intensity of the uncut (methylated) to cut (unmethylated) band for somatic cell DNA was 0.4. Methylation at this locus was virtually absent in sperm DNA but enriched in two (ratio = 0.8 and 1.1) of three fetal ovary DNA samples. Because ovary samples typically contain 70% somatic cells (41), these results are consistent with the Not1 site being methylated in human oocytes. The lack of enrichment in one ovarian specimen may reflect a larger than average contribution of somatic tissue in this dissection. Although definitive proof of maternal methylation during gametogenesis awaits analysis of purified oocytes, these results are consistent...
with maternal-specific methylation at KvDMR1 and, together with the finding of differential methylation at this site in murine embryonic stem cells (data not shown), suggest that this epigenetic difference represents a true gametic imprinting mark.

**Identification of Human (KvLQT1) and Mouse (Kvlqt1) Antisense Transcripts.** Further characterization of the mouse locus (GenBank accession no. AF119385) located the differentially methylated EagI site(s) within a CpG island and identified a direct repeat sequence (Fig. 2b) in a position analogous to the human CpG island. Sequence alignment uncovered a region of 83% identity over >400 bp between human and mouse loci (see Fig. 2); however, no consensus splice sites could be found. Blast analysis of KvDMR1 and flanking sequences identified several ESTs in human and one in mouse representing sequences transcribed in the opposite orientation with respect to KvLQT1 (Fig. 2). RT-PCR analysis with oligo(dT)-primed cDNA from human fetal liver RNA suggested that these cDNAs represented fragments of the same transcript (Fig. 4a). All EST and RT-PCR sequences were continuous with genomic DNA and showed no evidence of exon–intron boundaries. For each RT-PCR experiment, identical results were obtained when cDNA synthesis was carried out with primers specific for transcripts from the antisense strand (with respect to the direction of transcription of KvLQT1). Although RT-PCR detected transcripts in all human fetal tissues tested (Fig. 4a), corresponding transcripts were not detectable on Northern blots (CLONTECH) made from fetal or adult RNAs. Mouse EST 1265245 and human EST 68627 contained potential ORFs (150–400 bp) but are unlikely to reflect protein-coding potential, considering that no homology (at the nucleotide or amino acid level) exists between them. Although its length and potential overlap with KvLQT1 exons remain to be determined, we have designated this transcript KvLQT1-AS (Kvlqt1 antisense).

**Imprinted Expression of KvLQT1/Kvlqt1-AS.** Because of the proximity to KvDMR1, we wished to determine whether KvLQT1-AS was imprinted. However, no polymorphisms were detected in 12 individuals after single-strand conformation polymorphism scanning of 450 bp and restriction-endonuclease-fingerprinting analysis of 2,600 bp (data not shown). We therefore took advantage of a recently developed panel of single human chromosome 11 somatic cell hybrids that have been characterized with respect to their expression of the imprinted H19 and IGF2 genes and methylation at KvDMR1 (47). Primers designed for ESTs 68627, 592241, and 435896 (Fig. 2a) were used in oligo(dT)-primed RT-PCR analysis of these hybrids, and, as illustrated for the EST 592241 primer pair (Fig. 4b), expression was observed only in the six hybrids shown to contain an unmethylated chromosome 11 (i.e., paternal). Identical results were obtained when this experiment was repeated with a primer designed for reverse transcription of the antisense RNA. By using an expressed sequence polymorphism, RT-PCR analysis with either strand-specific or oligo(dT)-primed RT-PCR analysis of these hybrids and, as illustrated for the EST 592241 primer pair (Fig. 4b), expression was observed only in the six hybrids shown to contain an unmethylated chromosome 11 (i.e., paternal). Identical results were obtained when this experiment was repeated with a primer designed for reverse transcription of the antisense RNA. By using an expressed sequence polymorphism, RT-PCR analysis with either strand-specific or oligo(dT)-primed RT-PCR analysis of these hybrids and, as illustrated for the EST 592241 primer pair (Fig. 4b), expression was observed only in the six hybrids shown to contain an unmethylated chromosome 11 (i.e., paternal). Identical results were obtained when this experiment was repeated with a primer designed for reverse transcription of the antisense RNA. By using an expressed sequence polymorphism, RT-PCR analysis with either strand-specific or oligo(dT)-primed RT-PCR analysis of these hybrids and, as illustrated for the EST 592241 primer pair (Fig. 4b), expression was observed only in the six hybrids shown to contain an unmethylated chromosome 11 (i.e., paternal). Identical results were obtained when this experiment was repeated with a primer designed for reverse transcription of the antisense RNA. By using an expressed sequence polymorphism, RT-PCR analysis with either strand-specific or oligo(dT)-primed RT-PCR analysis of these hybrids and, as illustrated for the EST 592241 primer pair (Fig. 4b), expression was observed only in the six hybrids shown to contain an unmethylated chromosome 11 (i.e., paternal).

**Loss of imprinting at KvDMR1 in Patients with BWS.** Because all BWS chromosome rearrangements in BWS breakpoint cluster 1 (BWSCR1) are located within the KvLQT1 gene (38, 39), it has been postulated that the disruption of the KvLQT1 genomic region affects the imprinting of IGF2 and perhaps other genes in the 11p15 domain (38). Because epigenetic changes at KvDMR1 might be related to this deregulation, the methylation status of this locus was tested in several classes of patients with BWS. Reduced methylation at KvDMR1 was observed in three patients with paternal UPD (Fig. 5a and not shown), reflecting the mosaic nature of UPD in BWS (28). Of 12 patients without UPD and with normal methylation at H19 (31, 32), 5 showed complete loss of the methylated band, whereas all 4 patients with BWS and hypermethylation at H19 (31, 32) showed normal methylation at KvDMR1 (Fig. 5a and data not shown). Of the five patients with loss of methylation at KvDMR1, two were informative for the Apal/AvaII polymorphism described in IGF2 (48) and both showed LOI at IGF2 (32). All seven samples with normal methylation at KvDMR1 and H19 were uninformative at IGF2, precluding assessment of imprinting in these patients. However, three of these have been shown to have mutations in the p57kip1 gene (A.C.S. and E.R.M., unpublished work). DNA from an aborted fetus with BWS, a maternally inherited inv(11)(p13;p15.5) (40), and LOI at IGF2 also showed loss of methylation at KvDMR1 (Fig. 5b), indicating that the inv(11) affected imprinted loci separated by 500 kb and on either side of the breakpoint. Two additional BWS translocations and one rhabdoid tumor translocation (40) showed normal methylation at KvDMR1 (Fig. 5b); the allelic expression pattern of IGF2...
protein-coding gene and therefore conforms to the expression competition model of genomic imprinting (19, 49). On the other hand, if the overlapping antisense transcripts associated with the similarly imprinted Igf2 and Zpf127/Zpf227 loci carry out regulatory functions, these functions may not be related to genomic imprinting and/or are likely to act through a different mechanism. It is interesting to note that the three oppositely imprinted antisense RNAs described to date are associated with maternally expressed imprinted genes (16, 17, and this study).

Based on expression analysis in Dmnt1−/− mice, Caspary et al. (37) showed Kvlqt1 to be an indirect target of methylation and predicted the existence of a maternally methylated locus and associated paternally expressed RNA within Kvlqt1, with the paternal transcript competing with Kvlqt1 for expression. One possibility is that Kvlqt1-AS is an imprinted gene that competes with the target-imprinted gene (19) Kvlqt1 for expression and is silenced directly by DNA methylation. Down-regulation of Kvlqt1-AS expression during developmental relaxation of Kvlqt1 imprinting (37, 44–46) would lend support to the notion of a functional role for the antisense RNA transcription in Kvlqt1 imprinting. A second possibility is that KvDMR1 acts as an insulator or boundary element as recently suggested for the core element upstream of the H19 gene (50, 51). In this model, KvDMR1 would block the promoter of Kvlqt1 and/or other genes in the vicinity from interacting with enhancers, and antisense RNA transcript levels would not necessarily change during development. Although Kvlqt1 has maternal-specific expression during early embryonic growth in all mice tested, the developmental regulation of Kvlqt1 imprinting varies considerably between strains (37, 44–46). Whether this variability is related to differences in methylation at KvDMR1 or elsewhere within the gene is unknown; however, consistent with KvDMR1 being an imprinting control element, differential methylation is maintained (at least at the Eegl sites tested; Fig. 3c) in adult kidney DNA where biallelic expression of Kvlqt1 is evident in the same tissue from C57BL/6J×PWK/F1 offspring (G.V.F. and M.J.H., unpublished work). In this respect, it will also be important to compare the expression of Kvlqt1 and Kvlqt1-AS in BWS patients with and without methylation at KvDMR1.

The H19 locus is not a domain-wide imprinting control element, because, unlike Insm2 and Igf2, the imprinting of p57kiwi, Kvlqt1, and Mash2 is unaffected in H19 deletion mice (37). This finding supports earlier conclusions resulting from the observation that the majority of informative patients with BWS have LOI at Igf2 (32) but retain normal methylation and monoallelic expression at H19 (30–32). This lack of reciprocity is also noted for the BWS inv(11) reported by Brown et al. (35) as well as the inv(11) case described here. These observations suggest the existence of an H19-independent mechanism for the regulation of Igf2 imprinting and, together with the results of the study of the H19 knockout mouse (36, 37), predict the existence of a separately regulated imprinted domain and at least one additional cis-acting imprinting control element or center in 11p15.5 (and mouse distal 7). A subset of DMRs have been termed gametic imprints because of their establish-ment in the germ line and maintenance throughout development (2). Transgenic and targeted mutation analyses in the mouse have shown the importance of at least two of these sites in the regulation of genomic imprinting, namely the upstream DMR of H19 and region 2 in Igf2 (16, 50). Furthermore, the maternally methylated DMR at exon 1 of the SNRPN gene is included in the smallest microdeletions found in patients with Prader–Willi syndrome showing imprinting-center defects (52). Although direct studies of purified oocytes are needed for confirmation, the lack of methylation at KvDMR1 in sperm and its enrichment in ovaries suggest that this locus is also a gametic imprinting mark and could therefore represent a critical control element or imprinting center in 11p15.5 (and distal chromosome 7 in the mouse). The loss of methylation at KvDMR1 in patients with BWS with normal H19 methylation and biallelic expression of Igf2 and the existence of could not be determined in these samples, because none were informative at Igf2. The BWS inv(11) has, however, been shown to disrupt the asynchronous replication pattern at Igf2 (28), suggesting that rearrangements in this domain might affect imprinting by additional mechanisms not connected to aberrant methylation at KvDMR1.

**DISCUSSION**

This work describes an imprinted CpG island in an intron of KvLQT1 that is methylated on the active (maternal) allele of this gene and associated with an oppositely oriented RNA transcript expressed from the repressed (paternal) KvLQT1 locus. This situation is reminiscent of the “imprinting box” in region 2 of the mouse Igf2r locus (8), which has recently been shown to be necessary for the correct imprinted expression of Igf2r transgenes (16). Moreover, transgenes showing repression of Igf2r after paternal transmission expressed an antisense transcript dependent on this CpG island (16). Thus, KvLQT1 can be added to the increasing number of endogenous imprinted genes shown to overlap with imprinted antisense transcripts (13–17). Similar to the situation at the Igf2r and UBE3A loci, and unlike that at Igf2 and Zpf227/Zpf227 (13–15), KvLQT1-AS/Kvlqt1-AS is imprinted in the opposite direction compared with the associated
the associated paternally expressed antisense RNA suggest the hypothesis that this locus regulates the imprinted expression of \(KvLQT1\), \(IGF2\), and perhaps other imprinted genes in the domain. Functional disruption of \(KvDMR1\), as evidenced by loss of methylation, may account for a majority (five of nine patients studied here) of non-UPD, nonrearrangement BWS cases without mutations in \(p57^\text{Kip2}\). A limitation of this study is the general unavailability of patient tissues affected by overgrowth. It is formally possible that changes in methylation at \(KvDMR1\) in fibroblasts and lymphocytes from patients with BWS may reflect tissue-specific differences or occasional loss of methylation in these cell types, and may not accurately reflect the methylation status in affected tissues. However, in an analysis of 17 normal individuals (Fig. 3a and data not shown) including DNA from four lymphoblast and two fibroblast cell lines, three lymphocyte samples, and eight fetal hearts, no departure from the differentially methylated pattern shown in Fig. 3a has been observed. On the other hand, the proportion of patients with BWS and epigenetic changes at \(KvDMR1\) might even be greater if analysis of the tissues actually affected by overgrowth were available for molecular examination. Analysis of genomic sequence indicates the presence of multiple CpG islands within the 11p15.5 imprinted domain (C.D.D., G.V.F., and M.J.H., unpublished work). The assessment of methylation at these sites in both normal and patient DNA will help to determine whether their methylation patterns show allelic specificity and are as stringently controlled as the methylation pattern for \(KvDMR1\). Definitive answers to questions regarding the function of \(KvDMR1\) and its associated paternally transcribed generation of mouse models with specific mutations in this region and further detailed study of the molecular pathology of patients with BWS.

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