

Growth Regulation, Imprinted Genes, and Chromosome 11p15.5

ADAM C. SMITH, SANAA CHOUFANI, JOSE C. FERREIRA, AND ROSANNA WEKSBERG

Institute of Medical Sciences [A.C.S., J.C.F., R.W.], and Molecular and Medical Genetics [R.W.], University of Toronto, Toronto, Canada, M5S 1A8; Genetics and Genomic Biology, The Research Institute [A.C.S., S.C., J.C.F., R.W.], and Department of Pediatrics [R.W.], The Hospital for Sick Children, Toronto, Canada, M5G 1X8

ABSTRACT: Genomic imprinting refers to parent-of-origin-specific gene expression. Human chromosome band 11p15.5 houses a large cluster of genes that are imprinted. Dysregulation of this gene cluster is associated with the overgrowth and tumor predisposition syndrome, Beckwith-Wiedemann syndrome. Several genes in this imprinted cluster encode proteins involved in growth regulation, *e.g.* the paternally expressed *IGF2* and the maternally expressed cell-cycle regulator cyclin dependent kinase inhibitor, *CDKN1C*. Disruption of imprinted gene expression can result from genetic or epigenetic alterations. Genetic alterations such as duplication, deletion, translocation, inversion, and mutation in imprinted regions have been shown to cause disease. In addition, epimutations that are extrinsic to the primary DNA sequence have also been shown to cause disease. These epimutations usually involve gain or loss of methylation at regulatory differentially methylated regions. Recently, several human diseases in addition to Beckwith-Wiedemann syndrome have been reported to have molecular alterations at chromosome 11p15.5. These include isolated hemihyperplasia, Russell-Silver syndrome, and transient neonatal diabetes mellitus. These molecular alterations and their phenotypic effects on growth are discussed. (*Pediatr Res* 61: 43R–47R, 2007)

Most mammalian autosomal genes are expressed from both the maternally inherited and paternally inherited copies of the chromosomes. However, some genes are expressed in a parent-of-origin-specific manner. This phenomenon, known as genomic imprinting, is regulated by epigenetic mechanisms (1). Epigenetics refers to transmissible changes in gene expression that are not accompanied by a change in primary nucleotide sequence (2). Epigenetic marks include alterations in DNA methylation, chromatin conformation (specified in part by the covalent modifications of histone proteins), and noncoding RNA transcripts. Different combinations of these epigenetic marks have been demonstrated to be critical for normal imprinted gene regulation among various genomic regions. There is considerable evidence of dysregulation of imprinted gene expression in human disorders of growth and development. This can occur *via* primary epigenetic alterations or genetic alterations that change the contribution of parental alleles, such as duplications, deletions, and UPD. UPD refers to the presence of two chromosomal regions from one parent and none from the other.

To date, approximately 50 imprinted genes have been identified in the human genome (3–5). These genes tend to be clustered together in imprinted chromosomal domains. Such domains have been mapped to chromosomes 7q32, 11p15, 15q11, and 20q13. These clusters of genes are characterized by *cis* regulation *via* an IC. IC are regions of DNA that regulate the expression of imprinted genes in *cis* over large distances up to one megabase. IC are also often associated with noncoding RNA transcripts and differential methylation of parental alleles known as DMR (6). One of the best studied clusters of imprinted genes is located on human chromosome 11p15. This cluster contains several imprinted genes that control growth and development (7). Dysregulation of imprinted genes in the 11p15 cluster causes BWS (8–10). Imprinted genes in this cluster can also be dysregulated in IH (11–15). Recently, some patients with RSS, a disorder clinically diagnosed in children with severe pre- and postnatal growth restriction and few dysmorphic features, have also been shown to have methylation alterations in this domain (16–18). Finally, some patients with TNDM with loss of methylation at a locus on chromosome 6q24 have also been shown to have loss of methylation at 11p15 (19). Thus, the 11p15 imprinted region plays an important role in development and growth control. The modulation of epigenetic marks and imprinted gene expression appears now to be important in several conditions other than BWS.

11P15.5 IMPRINTED CHROMOSOMAL REGION

The regulation of imprinted genes on chromosome 11p15.5 is functionally divided into two domains that control the expression of genes over one megabase (Fig. 1). In Domain 1, the imprinted genes *IGF2* and *H19* are found. *IGF2* is a paternally expressed fetal growth factor that has been implicated in the pathomechanism of BWS (20). *H19* is a maternally expressed noncoding RNA for which the function is not well understood. Imprinted expression of these genes is regulated by the action of *H19* DMR or IC. Differential methylation of the *H19* DMR is important in the regulation of *IGF2* and *H19* expression. The *H19* DMR is usually methylated on the paternal allele and this prohibits the binding of CTCF, a

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Correspondence: Rosanna Weksberg, M.D., Ph.D., Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, 555 University Ave., Toronto, ON, M5G 1X8, Canada; e-mail: rweksb@sickkids.ca

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Abbreviations: BWS, Beckwith-Wiedemann syndrome; DMR, differentially methylated region; IC, imprinting center; IH, isolated hemihyperplasia; RSS, Russell-Silver syndrome; TNDM, transient neonatal diabetes mellitus; UPD, uniparental disomy

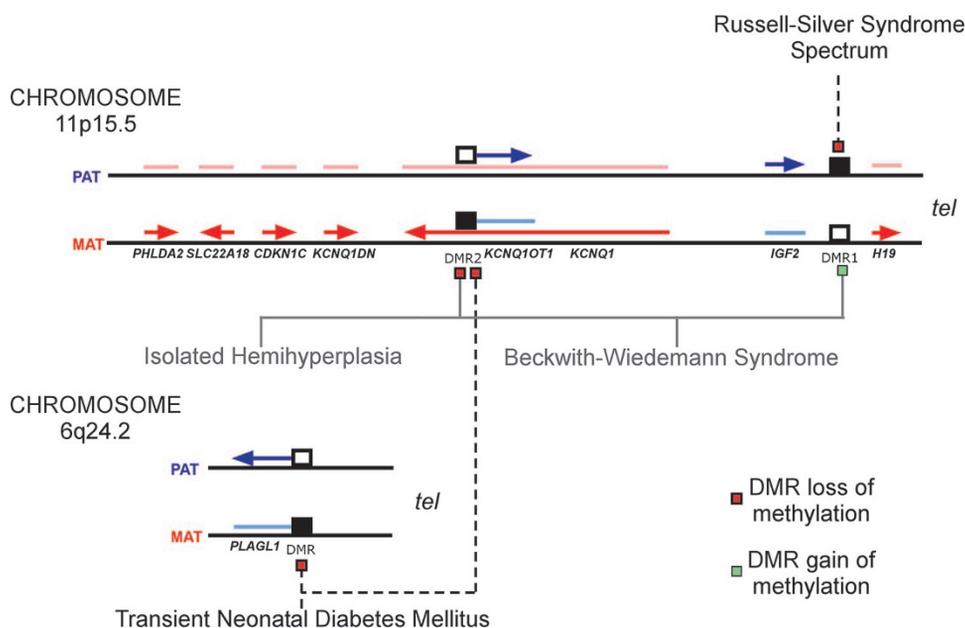


Figure 1. Schematic representation of imprinted gene clusters on human chromosome 11p15.5 and 6q24.2. Maternally expressed genes are indicated as red lines with arrows showing transcription and direction, repressed genes are a light red without arrows. Paternally expressed genes are indicated as blue lines with arrows, and light blue lines are repressed. Hollow squares show the location on normally unmethylated DMR and filled squares indicated the DMR is normally methylated. Methylation alterations, such as loss of methylation (red box) and gain of methylation (green box), show the locations of these changes in each of the four syndromes: BWS, IH, RSS spectrum, and TNDM. This is a simplified representation of these very complex regions, showing the imprinted regions relevant to the discussion in the text. Full details on this region can be seen at UCSC Genome Browser <http://genome.ucsc.edu>, chr11:1,950,000-2,950,000. For detailed information on imprinted genes, see the Catalogue of Imprinted Genes and Parent-of-Origin Effects at <http://igc.otago.ac.nz/home.html>.

zinc finger protein with insulating activity (21). The maternal allele is unmethylated and thus CTCF binding is permitted. *H19* and *IGF2* share a set of downstream mesodermal and endodermal enhancers. Thus, they must compete for access to them for their expression. When CTCF is bound to the maternal allele, it blocks access to the *IGF2* promoters and *IGF2* is expressed solely from the paternal allele where access is not obstructed by CTCF.

Domain 2 contains the imprinted genes *KCNQ1*, *KCNQ1OT1*, *CDKN1C*, *SLC22A18*, and *PHLDA2*. The IC for domain 2 is contained within intron 10 of the gene *KCNQ1OT1* (22,23). This differentially methylated region (*KCNQ1OT1* DMR, formerly KvDMR) contains the promoter for *KCNQ1OT1*, a noncoding transcript, that is thought to regulate in cis the expression of imprinted genes in the domain 2 cluster (24–26)

BWS

BWS (OMIM#130650) is an overgrowth syndrome originally characterized by the presence of macrosomia, macroglossia, and abdominal wall defects. However, many other features are often observed in BWS such as neonatal hypoglycemia, visceromegaly, helical ear pits and creases, hemihyperplasia, and an elevated risk for pediatric embryonal tumors such as Wilms tumor and hepatoblastoma (9,10).

There are several molecular alterations associated with BWS. Genetic alterations that involve both domain 1 and 2 include paternal uniparental disomy for a segment of chromosome 11 that always encompasses 11p15.5. This alteration, which occurs in 20% of BWS patients (9,10), results in a bipaternal pattern of expression, that is, there is a double dose of all paternally expressed genes and no expression of mater-

nally expressed genes. Rarely (1%), BWS patients have duplications of chromosome 11p15.5. Maternally transmitted translocations/inversions also occur rarely (1%), almost always disrupting the gene *KCNQ1* (9).

In domain 1, gain of methylation for the maternal allele of the *H19* DMR occurs sporadically in 5–10% of BWS patients (Fig. 1). Recently, Sparago *et al.* (27,28) reported five heritable partial microdeletions of the *H19* DMR, resulting in gain of methylation presumably due to disruption of CTCF binding to the DMR. Prawitt *et al.* (29) also reported a microdeletion of the *H19* DMR. However, methylation was not altered and was accompanied by duplication of the *IGF2* gene. In this case, the molecular basis for BWS is unusually complex.

In domain 2, sporadic loss of methylation of the *KCNQ1OT1* DMR occurs in 50% of patients (9) (Fig. 1). There has only been one reported microdeletion, deleting 70 kb and likely the entire *KCNQ1OT1* transcript (30). Mutations of the gene *CDKN1C* (*p57^{KIP2}*) itself are seen both sporadically (5%) and in autosomal dominant pedigrees (25%). Loss of methylation at the *KCNQ1OT1* DMR has been shown to be associated with a decreased amount of *CDKN1C* expression indicating a pivotal role for *CDKN1C* in the pathophysiology of BWS (25).

IH

IH (OMIM# 235000) is an abnormality of cell proliferation leading to asymmetric overgrowth of one or more regions of the body. IH occurs with an estimated incidence of 1 in 86,000 (31), with some authors reporting a higher frequency in females (32,33). Hemihyperplasia can occur as an isolated clinical finding or as a feature of other syndromes such as

BWS. In IH, a broad range of tumors has been reported (34) which in some cases are distinct from the profile seen in BWS, supporting the concept that IH is a distinct clinical entity associated with heterogeneous molecular etiologies (15).

A subset of IH cases has been shown to be associated with alterations to the chromosome 11p15.5 region. Mosaic paternal uniparental disomy for chromosome 11, gain of methylation of *H19* DMR, loss of methylation of the *KCNQ1OT1* DMR have all been demonstrated (Fig. 1) (11–15). In all of the above reports, some cases of IH did not demonstrate a detectable alteration on chromosome 11p15. Because uniparental disomy and epigenetic alterations can often be mosaic in somatic cells (15), it is possible that this molecular change was present in those cases of IH but not in the sampled tissue. This is an important consideration given that tissue is often not available directly from the overgrown area and most diagnostic samples are derived from blood. It is also possible that 11p15 is only one of several loci involved in the growth dysregulation observed in patients with IH.

RSS

In striking contrast with BWS, RSS (OMIM 180860) is characteristically diagnosed in individuals with severe prenatal and postnatal growth compromise, usually with normal head size, and a variable combination of facial dysmorphic features (triangular face, down-turned angles of the mouth, prominent forehead/nasal bridge, and small jaw), often with limb or facial asymmetry, with hypotrophy of a limb or side, and developmental delay (35–37). Indeed, several aspects of the phenotype are the reverse of BWS prompting clinicians to hypothesize that the same gene could be involved in both conditions (38).

Most cases of RSS are sporadic; occasionally there are familial cases. There is, indeed, familial and chromosomal evidence for genetic heterogeneity (37). Most cases of RSS have a normal karyotype, however, some reports describe structural chromosomal anomalies of chromosomes 7, 8, 11, 15, 17, and 18 (39,40). Using the information from chromosomal aberrations has been helpful in the identification of genomic regions where genes involved in the etiology of RSS are located. To date, however, no DNA sequence mutations have been found in any of the candidate genes tested (40).

There is considerable controversy regarding the clinical criteria required for a clinical diagnosis of RSS. Prenatal growth compromise, with or without postnatal short stature, has been the most consistent feature described in patients categorized as RSS or RSS-like. Significant variation occurs in the remaining phenotypic features. Molecular results should therefore be examined in the context of the clinical criteria used for ascertainment of the subjects. Maternal uniparental disomy of chromosome 7, a chromosome with known imprinted loci, has been found in 7–10% of RSS cases ascertained using stringent diagnostic criteria. These data demonstrate that imprint dysregulation on human chromosome 7 can cause RSS (40). Further, duplication of maternal 11p15 has also been reported in individuals with reasonably stringent RSS diagnostic criteria (39). Because growth dysregulation in

the two imprinted domains on 11p15 has been observed with BWS, it was therefore postulated that hypomethylation of *H19* promoter and/or IC1 may be a mechanism leading to RSS or RSS-like clinical features. Gicquel *et al.* (18) published a study of nine patients diagnosed using strict RSS criteria, who tested negative for maternal UPD7. In this study, five of the nine showed loss of methylation for several sites in domain 1 of 11p15.5 including the *H19* promoter, IC1, and *IGF2* DMR2 (Fig. 1). For domain 2, no methylation alterations were found at the *KCNQ1OT1* DMR. They concluded that loss of methylation of the telomeric imprinted domain of region 11p15 could be an important cause of RSS.

In RSS, the molecular signature, loss of methylation of the *H19* DMR, is the opposite of that found in BWS, which is gain of methylation of the *H19* DMR. However, the small number of cases studied by Gicquel *et al.* (18) made it difficult to determine the prevalence of this alteration in RSS. Eggermann *et al.* (17) screened 51 patients with prenatal and postnatal growth restriction with three or more phenotypic features of RSS. They found loss of methylation at *H19* DMR in 16/51 (31%) of their patients. Blik *et al.* (16) reported nine further cases with loss of methylation of the *H19* promoter, only two of which were diagnosed with RSS using strict criteria. The remaining cases had prenatal and/or postnatal growth compromise plus asymmetry, and lacked sufficient criteria to be classified as RSS. Blik *et al.* (16) looked for mutations in CTCF binding sites but none were found in their limited series. They concluded that, although it seems that there is a correlation between the methylation index and the severity of the phenotype, hypomethylation at the *H19* DMR can occur in individuals who are just small. In summary, the main phenotypic feature consistently seen among patients with *H19* DMR hypomethylation is the severe prenatal growth restriction with the postnatal maintenance of short stature (more than 2 SD below the mean).

AN IMPRINTED REGION ON 6q24 AFFECTS IMPRINTING ON CHROMOSOME 11P15.5

Recent evidence suggests that imprint regulation of chromosome 11p15.5 may be impacted by expression of imprinted genes on chromosome 6q24. This hypothesis has been developed to explain recent molecular data reported for two imprinting disorders, BWS and TNDM (19,41). TNDM (OMIM# 601410) has an incidence of 1 in 400,000 live births. In this condition, growth-retarded neonates present within the first 6 wk of life with persistent hyperglycemia and low or undetectable insulin levels (42). Another feature noted in the neonatal period by many authors is macroglossia (43) and umbilical hernia (44). The observation of partial pat UPD6 in patients with TNDM suggests that somatic mosaicism may affect the clinical presentation of TNDM. That is, the presence of different genetic mechanisms could contribute to the variability in phenotype seen in patients, as is the case with BWS.

TNDM has been associated with abnormalities involving chromosome 6. To date, three types of molecular abnormalities are identified in patients with TNDM, including paternal uniparental disomy of chromosome 6 (UPD6), paternally in-

herited duplication of 6q24, and loss of methylation at the *PLAGL1* (ZAC) DMR (Fig. 1) (42).

The *PLAGL1* gene, maps to 6q24–25, a region implicated in the origin of several cancers (45). This gene is a widely expressed zinc finger protein that shows transactivation and DNA-binding activity. *PLAGL1* exhibits tumor suppressor function through the induction of both apoptosis and cell cycle arrest (46,47). *PLAGL1* encodes a zinc finger protein and is preferentially expressed from the paternal allele with the maternal allele silent and methylated (48).

A comparison of phenotypes between BWS and TNDM demonstrates concordance for the tongue and umbilical findings but discordance for the growth findings. The overlap in the target tissues affected in these two disorders may reflect a regulatory association between imprinted regions at 6q24 and 11p15. In fact, several studies have reported, in TNDM patients, aberrant methylation at imprinted loci on both chromosomes 6q24 and 11p15.5, respectively. Mackay *et al.* (19) identified, among 43 TNDM patients tested, two with maternal loss of methylation (LOM) at the *KCNQ1OT1* DMR, both of whom also exhibited maternal LOM at the *PLAGL1* DMR. These two TNDM patients presented with clinical manifestations of severe growth restriction at birth, hyperglycemia, umbilical hernia, and macroglossia. None of the 16 BWS patients tested in the same study showed aberrant methylation at the *PLAGL1* DMR. Another report by Arima *et al.* (41) showed similar findings. They examined the methylation status of the *PLAGL1* DMR and *KCNQ1OT1* DMR in 8 BWS and 17 TNDM patients. All BWS patients showed normal methylation at the *PLAGL1* DMR, but two TNDM patients showed loss of methylation at *KCNQ1OT1* DMR. One of these patients was characterized with TNDM complicated with umbilical hernia and macroglossia, features commonly seen in BWS patients. These results support the hypothesis that there is a biologic interaction between these two loci. Further credence for this position can be derived from a comparison of the expression pattern in mouse of the *Zac1* and *p57^{Kip2}* genes, which show a strikingly similar expression pattern in many tissues including the tongue (41). The presence of a zinc finger motif within the *PLAGL1* protein suggests a DNA-binding capability that directed the authors to look for *PLAGL1* binding sites in the CpG island of the human *CDKN1C* (*p57^{KIP2}*) gene. Their work showed that the *PLAGL1* protein binds to, and activates expression of, *KCNQ1OT1* located on chromosome 11p15.5, which is known to negatively regulate the *p57^{KIP2}* gene in cis (41). These data suggest that *PLAGL1* may affect *p57^{KIP2}* gene expression through the *KCNQ1OT1* DMR, forming part of a novel signaling pathway that regulates cell growth.

The interaction of these two regions will require further investigation in terms of both basic molecular biology and clinical phenotype. Mackay *et al.* (49) suggested that methylation defects at more than one locus can modify the clinical presentation of the TNDM phenotype. Most of the TNDM patients presenting with loss of methylation at *KCNQ1OT1* DMR have macroglossia and abdominal wall defects, clinical features commonly seen in BWS.

SUMMARY

Epigenetic alterations of differentially methylated imprinting centers on chromosome 11p15.5 have been linked to several growth-related disorders. These data highlight the fact that interactions among imprinted genes are likely important factors in the expression of clinical disease. Recent observations have demonstrated that many imprinted growth regulatory genes are co-regulated in a network of imprinted genes (50).

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