Molecular characterization in chromosome 11p15.5 related imprinting disorders Beckwith–Wiedemann and Silver–Russell syndromes

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Epigenetics deals with modifications in gene expression, without altering the underlying DNA sequence. Genomic imprinting is a complex epigenetic phenomenon that refers to parent-of-origin-specific gene expression. Beckwith–Wiedemann syndrome (BWS) and Silver–Russell syndrome (SRS) are congenital imprinting disorders with mirror opposite alterations at the genomic loci in 11p15.5 and opposite phenotypes. BWS and SRS are important imprinting disorders with the increase of knowledge of genetic and epigenetic mechanisms. Altered expression of the imprinted genes in 11p15.5, especially *IGF2* and *CDKN1C*, affects fetal and postnatal growth. A wide range of imprinting defects at multiple loci, instead of a restricted locus, has been shown in some patients with either BWS or SRS. The development of new high-throughput assays will make it possible to allow accurate diagnosis, personalized therapy, and informative genetic counseling.

Key words: Beckwith–Wiedemann syndrome, Epigenetics, Genomic imprinting, Silver–Russell syndrome, Uniparental disomy.

Introduction

Epigenetics deals with modifications in gene expression, without altering the underlying DNA sequence. Genomic imprinting is a complex epigenetic phenomenon that refers to parent-of-origin-specific gene expression [1]. Genomic imprinting is controlled by chemical switches through genomic DNA methylation, changes in chromatin structure, post-translational histone modification, and interference with non-coding RNAs. Genomic imprinting plays an important role in embryogenesis, reproduction and growth [2]. DNA methylation is a biochemical process that transfers a methyl group (CH_3) from S-adenosylmethionine to the C5 of a cytosine residue to form 5-methylcytosine [3]. DNA methylation of the promoter region suppresses gene expression.

Histone modification and non-coding RNA-associated gene silencing also conduce greatly to imprinting process [4]. More than 100 imprinted genes in human have been identified and most imprinted genes are arranged in clusters, or imprinting domains [5].

There are four causes of molecular alterations in imprinting disorders, which are (1) chromosomal abnormalities such as deletions, duplications or balanced rearrangements, (2) uniparental disomy (UPD), (3) epimutations and (4) point mutations (loss or gain of function) in imprinted genes. UPD is defined as the inheritance of both copies of a chromosome segment from one parent and no copy from the other parent and leads to the imbalanced expression of imprinted genes. Epimutations (aberrant methylation mark) include gain of methylation (GOM) (hyper-

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Conflict of interest: I declare that I do not have any conflicts of interest.

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methylation) and loss of methylation (LOM) (hypomethylation). These causes lead to imbalance gene expression including aberrant silencing of the active allele or expression of the inactive allele. One of the two parental alleles is silenced by allele-specific epigenetic modification whereas the other allele is expressed according to its parental origin [6].

Clusters contain several imprinted genes are located on chromosome 11p15.5 region. Imprinted genes have contributed to fetal growth and development [7]. Thus, aberrant expression of these imprinted genes can be found in congenital anomalies and tumors [7]. Beckwith–Wiedemann syndrome (BWS) and Silver–Russell syndrome (SRS) are congenital imprinting disorders with mirror opposite alterations at the genomic loci in 11p15.5 and opposite phenotypes [8]. This review focuses on the current knowledge of epigenetic and genetic alterations in BWS and SRS.

The Imprinted 11p15 Region

The 11p15 chromosome includes two imprinted domains; the *H19/IGF2* domain in the telomeric region and the *KCNQ1OT1/CDKN1C* domain in the centromeric region (Fig. 1). The monoallelic parent-of origin-specific expression of imprinted genes is regulated by differentially methylated regions (DMRs), also known as imprinting control regions (ICRs) [9]. Each domain is controlled by its ICR (ICR1 in the telomeric region and ICR2 in the centromeric region) and methylated differently.

The first domain in the telomeric region contains insulin growth like factor gene 2 (*IGF2*), *H19*, and ICR1 (*H19/IGF2*:IG-DMR) *IGF2*, a major fetal growth factor, expressed from the paternal allele, whereas *H19* expressed only from the maternal allele during fetal life [7]. ICR1 is located in the intergenic region between *H19* and *IGF2* genes and regulates imprinting through controlling the expression as a transcription insulator. ICR1 domain is organized into two blocks of repeats (A-repeat and B-repeat elements). Several target sites for the transcriptional repressor protein called CCCTC-binding factor (CTCF) present within and around ICR1. The ICR1 is paternally methylated, and CTCF as an insulator, cannot bind to it, thus the downstream enhancers of *H19* can access *IGF2* promoter and expression of *IGF2*. The ICR1 is maternally unmethylated, CTCF can bind to it, and insulates *IGF2* from downstream enhancers, while allowing activation of the *H19* promoter [10,11]. Therefore, *IGF2* is expressed in the paternal allele and silenced in the maternal allele.

The second domain in the centromeric region contains cyclin-dependent kinase inhibitor 1C (*CDKN1C*), potassium channel

KQT family member 1 (*KCNQ1*), and ICR2 (*KCNQ1OT1*:TSS-DMR). ICR2 exists in the promoter region of *KCNQ1OT1* and regulates the *CDKN1C/KCNQ1* locus. ICR2 is methylated on the maternal allele, *CDKN1C* and *KCNQ1* are expressed. *CDKN1C* gene encoding for cyclin kinase inhibitor plays a role in blocking cell proliferation. ICR2 on paternal allele is unmethylated, *KCNQ1OT1* is expressed. *KCNQ1OT1* is a long noncoding RNA and regulates the imprinting in cis of the domain. As *KCNQ1OT1* RNA expression is maternally silenced and paternally expressed, the imprinting genes of the *CDKN1C/KCNQ1* locus are maternally expressed and paternally silenced [12].

In normal individuals, the paternal ICR1 allele is methylated while the maternal ICR1 allele is unmethylated. The paternal ICR2 allele is unmethylated while the maternal ICR2 allele is methylated. Therefore, by the paternal allele, *IGF2* and *KCNQ1OT1* are expressed, whereas by the maternal allele, *H19* and *CDKN1C* are expressed. Altered expression of the imprinted genes in 11p15.5, especially *IGF2* and *CDKN1C*, affects fetal and postnatal growth. These imprinting defects are involved in clinically opposite growth disorders, BWS and SRS [7]. Most cases of both disorders are related to opposite epigenetic or genetic abnormalities in the 11p15 chromosomal region, by altering the expression of the maternally or paternally imprinted genes.

Beckwith–Wiedemann Syndrome

BWS (OMIM #130650) is an overgrowth syndrome, characterized by pre- and postnatal overgrowth, hemihyperplasia, macroglossia, organomegaly, neonatal hypoglycemia, hyperinsulinism, abdominal wall defects, renal abnormalities, ear creases, and increased risk of childhood tumors [13–19]. It has been known that about 10% of BWS patients could develop malignancies, e.g., hepatoblastoma and Wilms tumor in childhood [20]. BWS affects about 1 in 10,500–13,700 individuals worldwide [14,19].

BWS is associated with several epigenetic and genetic defects. A hypomethylation of maternal ICR2 can be identified in 50%–60% of BWS cases [15–17,21]. This abnormality leads reduced expression of *CDKN1C* (a cell cycle inhibitor). Normally, ICR2 in the 11p15 region controls the maternally expressed *KCNQ1* and *CDKN1C*, and the paternally expressed *KCNQ1OT1* gene. A hypermethylation at maternal ICR1 is found in 5%–10% cases of BWS, leading to *IGF2* overexpression [15,17,22,23]. About 20% of these methylation alterations have been associated with genetic defects within the maternal ICR1 [24,25]. Normally, *IGF2* is expressed due to paternal hypermethylation of ICR1 while *H19* is expressed and *IGF2* expression is reduced due to maternal

Fig. 1. Imprinting at the H19/IGF2 (ICR1) locus and KCNQ10T1/CDKN1C (ICR2) locus in the chromosomal region 11p15.5. Genetic and epigenetic disturbances in patients with Beckwith–Wiedemann syndrome and Silver–Russell syndrome.

hypomethylation of ICR1. Hypermethylation of the maternal ICR1 accounts for biallelic expression of the *IGF2* gene. Mosaic segmental paternal UPD (patUPD) in 11p15 region accounts for about 20% of BWS cases [15,17,22,26]. It causes altered expression of both imprinted clusters with ICR2 LOM and ICR1 GOM [19]. Point mutations of the maternal allele *CDKN1C* without methylation abnormalities are detected in 5% cases of sporadic BWS and 50% of familial BWS patients [15,16,27,28]. In about 5% of BWS cases, microdeletions involving ICR1 have been reported and in less than 1% of BWS cases, microduplication of ICR2 are identified [29,30]. These molecular alterations as causes of BWS occur over-expression of paternally imprinted genes (*IGF2* and *KCNQ1OT1*) and/or silenced expression of maternally imprinted genes (*H19* and *CDKN1C*).

Silver–Russell Syndrome

SRS is related to heterogeneous pathogenetic mechanisms. ICR1 hypomethylation in the 11q15 region is the most common epigenetic change causing SRS and accounts for 40% to 50% of SRS cases [31–33]. Hypomethylation of ICR1 leads to the downregulation of *IGF2* and the biallelic expression of *H19* [31,32]. Maternal UPD of chromosome 7 (matUPD7), the first identified molecular cause of SRS, has been found in 5% to 10% of SRS patients [18,31,33,34]. In 1% to 2% of SRS cases, a maternal duplication in 11p15 has been reported as a cause of SRS [35]. Other structural chromosomal aberrations including 1q21 microdeletion, 12q24 microdeletion, ring chromosome 15, and deletion 15qter have been identified in less than 2% of SRS cases [36–40]. Genomic alterations involving ICR2 resulting in GOM have been rarely described [41]. In rare cases of SRS, chromosomal structural mutations in the *H19/IGF2* enhancer region and paternal *IGF2* loss of function mutation have been reported [42,43]. Maternal UPD11, *CDKN1C* gain-of-function mutation, *HMG2* mutation and *PLAG1* mutation have been published in some cases with SRS [44–48].

Genotype–Phenotype Correlations in Beckwith–Wiedemann Syndrome and Silver–Russell Syndrome

A large number of studies have been conducted to define the correlation between genotype and phenotype in BWS and SRS [19,33,49–51]. Tumor predisposition are related with BWS molecular subtypes. It has been known that the higher tumor risk is found in patients with ICR1 GOM and paternal UPD11 than

patients with *CDKN1C* mutation and ICR2 LOM [15,19]. The Wilms tumor prevalence in patients with ICR1 GOM is 21.1% and UPD subgroups is 6.2% [52]. Hepatoblastoma development in paternal UPD cases has higher risk [52]. In BWS, exomphalos is more likely to occur in changes of the ICR2 [19]. It was indicated *KCNQ1* variants predisposing to Long QT disorder associated with ICR2 LOM [50]. SRS patients with 11p15 epimutations have more typical features than in matUPD7 [33]. Delayed neurocognitive development is more frequent in SRS patients with matUPD7 [51]. These findings contribute to allowing more accurate diagnosis, directed therapy, and individualized monitoring.

Multilocus Imprinting Disturbances

A wide range of imprinting defects at multiple loci, instead of a restricted locus, has been shown in some patients with either BWS or SRS [53]. A subset of patients with imprinting disorders exhibits aberrant methylation affecting other imprinted DMRs in addition to disease-specific locus. This condition has been named multilocus imprinting disturbance (MLID) and in particular shows frequently in BWS with ICR2 LOM, while very rare in ICR1 GOM [19,54,55]. Some BWS patients with MLID identify LOM or GOM at maternal and paternal ICRs, while others show a maternal hypomethylation syndrome [54–57]. In SRS, MLID has been reported less frequently [54]. Some BWS and SRS patients with MLID describe the distinct phenotypes. For instance, BWS patients with MLID show lower birth weight and less frequent macrosomia than mono-locus BWS [58]. SRS patients with MLID have growth delay and congenital abnormalities more frequently [59].

Conclusion

Various and overlapping clinical findings make it difficult to recognize and select molecular testing in both BWS and SRS. BWS and SRS are important imprinting disorders with the increase of knowledge of genetic and epigenetic mechanisms.

Molecular or cytogenetic diagnostic tests for BWS and SRS, include DNA methylation testing, sequencing analysis, copy number analysis, chromosome microarray and karyotype. Currently, methylation-specific multiplex ligation-dependent probe amplification and multiplex ligation quantitative polymerase chain reaction allow detection of DMR methylation status and copy number and are more sensitive in cases with low-level mosaicism. A single nucleotide polymorphism-based chromosome microarray analysis can detect a deletion or duplication

and also detect segmental UPD. The development of new high-throughput assays will make it possible to better understand the pathophysiology and molecular disturbances of imprinting disorders. These improvements will contribute to accurate diagnosis, personalized therapy, and informative genetic counseling.

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