Epigenetic Anomalies in Childhood Growth Disorders

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Abstract

Fetal growth is a complex process involving environmental, epigenetic and genetic factors. Fetal growth restriction is associated with morbidity among small for gestational age (SGA) neonates as well as in children and adults who were former SGA. Imprinted genes (whose expression is restricted to a single parental allele) have a critical role in controlling mammalian fetal growth. The human chromosome 11p15 encompasses two imprinted domains regulated by their own differentially methylated imprinted control region (ICR1 at the H19/IGF2 domain, and ICR2 at the KCNQ1/CDKN1C domain). Loss of imprinting at these two domains is implicated in two clinically opposite growth disorders. Indeed, our group has identified a loss of DNA methylation (LOM) at ICR1 in over 50% of patients with Russell-Silver syndrome (RSS) characterized by intrauterine and postnatal growth retardation with spared cranial growth, dysmorphic features, frequent body asymmetry and severe feeding difficulties. By contrast, gain of methylation at ICR1 is found in 10% of patients with Beckwith-Wiedemann syndrome (BWS), an overgrowth syndrome with an enhanced childhood tumor risk. We have now identified over 130 RSS patients with 11p15 LOM. This 11p15 epimutation is a frequent and specific cause of RSS as it has not been identified in non syndromic SGA patients. These new findings in the pathophysiology of RSS allow long-term follow-up studies to be performed based on molecular diagnosis. This will help to define appropriate clinical guidelines regarding growth, rapid bone age advance during puberty and feeding difficulties. Remarkably, we have also recently found that ~10% of RSS patients and ~25% of BWS patients showed multilocus LOM at imprinted regions other than ICR1 or ICR2 11p15, respectively. Several clinical studies demonstrated that assisted reproductive technology significantly increased the risk of human imprinting diseases including BWS and RSS, suggesting that the environment may favor imprinting disorders.
Introduction

Fetal growth is a complex process involving multiple environmental, epigenetic and genetic factors. Intrauterine growth retardation or small for gestational age (IUGR/SGA) represents about 5% of the births, and is associated with severe morbidity. Indeed, IUGR patients are exposed to an enhanced risk to develop cardiovascular or metabolic diseases in adult life (fetal programming or the adaptation hypothesis, developmental origins of health and disease) [1]. By contrast, fetal overgrowth syndromes are associated with developmental abnormalities, tissue and organ hyperplasia and a higher risk of childhood tumors [2]. It has been recognized that faithful genetic and epigenetic programming has a critical role in controlling mammalian fetal and postnatal growth.

The most studied epigenetic mark is DNA methylation of CpG dinucleotide cytosine residues within gene promoters, transposons and imprinted regions. Genomic imprinting is an epigenetic mechanism whereby expression of a subset of genes is restricted to a single parental allele. The majority of imprinted genes are organized in clusters throughout the genome and are regulated by an imprinting control region (ICR) [3]. The methylation of the genome undergoes dynamic reprogramming during fetal development. The first step takes place in primordial germ cells where methylation is erased from the ICRs and further reestablished, on cytosine residues of the CpG dinucleotides, according to the gender-specific gamete [4]. The second important widespread epigenetic reprogramming occurs in the preimplantation period, where the whole genome undergoes a wave of general demethylation soon after fertilization, while the imprinting is maintained during this period and then followed by a progressive wave of lineage-specific de novo methylation beginning at the blastocyst stage. The mechanisms leading to the establishment and maintenance of allele-specific DNA methylation at ICRs throughout fetal development and adulthood are very complex and not fully understood. They involve not only cis- and transacting regulatory factors [5, 6], but also influenced by environmental conditions such as dietary factors or assisted reproductive technology (ART) [7–9]. Loss of imprinting (LOI) through gain (GOM) or loss (LOM) of DNA methylation is implicated in several human diseases and cancer [8]. The human chromosome 11p15 encompasses two imprinted domains (fig. 1) important in the control of fetal and postnatal growth. Each domain is differentially methylated and regulated by its own ICR (ICR1 at the telomeric region for the H19/IGF2 domain which is methylated on the paternal allele, and ICR2 at the centromeric region for the KCNQ1OT1/CDKN1C domain which is methylated on the maternal allele). LOI at these two domains is involved in two clinically opposite growth disorders. Indeed, LOM at ICR1 is identified in over 50% of Russell-Silver syndrome (RSS; a growth restriction syndrome) patients [11, 12], whereas GOM at ICR1 is found in 10% of Beckwith-Wiedemann syndrome (BWS; an overgrowth syndrome with an increased tumor risk)
patients with identified molecular anomalies [13]. The great majority of BWS cases are associated with other molecular defects within the 11p15 region (see above), but this clinical and molecular mirror demonstrates the crucial role of the imprinted *IGF2* gene in fetal growth. Several other human diseases are explained by deregulation of imprinted gene expression, but epimutations are less frequent than in RSS and BWS (table 1).

**Fig. 1.** Normal 11p15 epigenetic organization and molecular defects in RSS and BWS. The telomeric ICR1 domain regulates the expression of *IGF2* and *H19* during fetal life. *IGF2* is expressed exclusively from the methylated paternal allele (P), and *H19* is expressed exclusively from the unmethylated maternal allele (M). The maternally methylated centromeric ICR2 regulates the expression of *CDKN1C* (maternal expression) and *KCNQ1OT1* (paternal expression). Expressed genes are represented by white boxes and non-expressed genes by gray boxes. In RSS patients, ICR1 LOM leads to loss of *IGF2* expression and *H19* biallelic expression. In BWS patients, ICR1 GOM leads to loss of *H19* expression and *IGF2* biallelic expression; ICR2 LOM leads to loss of *CDKN1C* expression and *KCNQ1OT1* biallelic expression; pUPD leads to *IGF2* overexpression. Maternal *CDKN1C* mutations also lead to BWS.
Russell-Silver Syndrome

Clinical Diagnosis

RSS is a clinically heterogeneous syndrome characterized by severe IUGR with relative macrocephaly, postnatal growth retardation, a distinctive triangular face with prominent forehead and body asymmetry [14–16]. Many other minor signs have been added including clinodactyly of the fifth finger, café-au-lait spots, genital abnormalities, hypoglycemia, excessive sweating, blue sclera and severe feeding difficulties. No consensus definition has yet been adopted. Recent findings in the molecular diagnosis of the syndrome (see above) prompted several teams to propose more or less complex clinical scores to establish genotype/phenotype correlations [12, 17–20]. Based on a large cohort of >150 RSS patients [12 and unpubl. data] with an identified molecular defect, we proposed a validated clinical scoring system for the diagnosis of RSS:

- the patient must be born SGA (birthweight and/or length ≤−2 SDS for gestational age), and
- the patient must present at least 3 of the following 5 criteria: (1) postnatal growth retardation at 2 years of age or at the nearest measure available, (2) relative macrocephaly (i.e. arbitrarily defined when the head circumference at birth is at least 1.5 SDS above the birthweight and/or length), (3) body

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>OMIM</th>
<th>Growth phenotype</th>
<th>Frequency of epimutation, %</th>
<th>Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient neonatal diabetes mellitus</td>
<td>601410</td>
<td>IUGR</td>
<td>20</td>
<td>6q24</td>
</tr>
<tr>
<td>RSS</td>
<td>180860</td>
<td>IUGR, short final height</td>
<td>0</td>
<td>mUPD</td>
</tr>
<tr>
<td>BWS</td>
<td>130650</td>
<td>macrosomia</td>
<td>60</td>
<td>11p15</td>
</tr>
<tr>
<td>mUPD14-like syndrome</td>
<td></td>
<td>IUGR, overweight, precocious puberty, short final height</td>
<td>?</td>
<td>14q32</td>
</tr>
<tr>
<td>Angelman syndrome</td>
<td>105830</td>
<td>&lt;5</td>
<td></td>
<td>15q11-13</td>
</tr>
<tr>
<td>Prader-Willi syndrome</td>
<td>176270</td>
<td>severely overweight, short final height</td>
<td>1–2</td>
<td>15q11-13</td>
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<tr>
<td>Pseudohypoparathyroidism 1a/1b</td>
<td>103580</td>
<td>short final height</td>
<td>?</td>
<td>20q13</td>
</tr>
</tbody>
</table>

Table 1. Frequency of epimutation in different imprinting disorders
asymmetry, (4) prominent forehead and (5) feeding difficulties during early childhood and/or postnatal body mass index (BMI) below –2 SDS at 2 years of age or at the nearest measure available.

Of note, we have included feeding difficulties and/or low BMI as one of the criteria as they are reported to be particularly frequent and severe in RSS during infancy and early childhood. This manifestation can be associated with frequent gastrointestinal disorders, such as constipation, gut dysmobility, gastroesophageal reflux disease [21]. We have retained the prominent forehead as the main characteristic of facial dysmorphism, but it should be assessed before 3 years of age since the characteristic facial features of the RSS seem to change, and are not as marked in late childhood and adulthood [12, 19].

Molecular Diagnosis
The genetic cause of RSS was unknown for a long time. Most of the cases are sporadic, both genders are equally affected. The first molecular abnormality identified in a significant proportion of patients was maternal uniparental disomies (mUPD) for chromosome 7 (mUPD7), present in around 7–10% of the cases [22]. mUPD7 involves either iso- or heterodisomy of the whole chromosome in most subjects. This finding implies that one or more genes on this chromosome are imprinted, and that disturbed imprinting is responsible for the phenotype. So far, two candidate regions on chromosome 7 have been the focus of research: 7p11.1-p14 and 7q31, for which a number of RSS patients with duplications (or inversions) or segmental UPD have been reported. These regions harbor imprinted genes possibly involved in human growth and development like GRB10 and PEG1/MEST. However, their implication in the physiopathology of the syndrome has not been demonstrated yet.

In 2005, our group described the main molecular defect associated with RSS: ICR1 LOM, present in more than 50% of the cases [11, 12]. In most of the cases, the LOM is partial, reflecting the mosaic distribution of the epimutation. In RSS patients, the paternal allele switches to a maternal epigenotype resulting in biallelic expression of H19 and loss of IGF2 expression in pathologic cells (fig. 1). The cause of this epimutation remains unknown. Mosaic distribution and LOM at multiple loci (see later) argue for a postzygotic occurrence of the epimutation.

Genotype/Phenotype Correlations
RSS patients with ICR1 LOM often display a more severe phenotype regarding growth parameter with a more typical dysmorphism (relative macrocephaly, prominent forehead) and body asymmetry [12, 19, 20].

Specific features of mUPD7 RSS patients are mild developmental delay mainly consisting in speech difficulties, predisposition to myoclonus dystonia and a putative susceptibility to develop autism traits [20, 23, 24]. All these features are thought to be related to disruption of expression of specific imprinted genes on chromosome 7.
**Beckwith-Wiedemann Syndrome**

**Clinical Diagnosis**

BWS is a rare overgrowth disorder involving developmental abnormalities, tissue and organ hyperplasia and an increased risk of childhood tumors [25]. It is generally accepted that diagnosis of BWS requires at least 3 clinical findings including at least 2 major signs which are macroglossia, macrosomia at birth or postnatal overgrowth, abdominal wall defect (ranging from umbilical hernia to exomphalos) and organomegaly. Minor findings include neonatal hypoglycemia, ear creases and pits, facial nevus, hemihyperplasia and embryonal tumors.

**Molecular Defect**

BWS results from molecular or chromosomal alterations that cause overexpression of the paternally expressed genes or a lack of expression of the maternally expressed genes within the 11p15 region [25, 26] (fig. 1):

- LOM at ICR2 (50–60% of cases)
- UPD of paternal origin (pUPD; 20% of cases)
- GOM at ICR1 (5–10%)
- Genic mutation on the maternal allele of the CDKN1C gene (5% of cases)
- Cytogenetic anomalies (1–2% of cases) consisting of maternally inherited balanced rearrangements (translocations or inversions) and trisomy with double dose of the paternal 11p15 region (resulting from duplications or unbalanced reciprocal translocation involving the 11p15 region)

All those molecular defects display a mosaic pattern except CDKN1C mutation and cytogenetic anomalies.

**Genotype/Phenotype Correlations**

Genetic diagnosis and molecular characterization are important to determine the outcome of BWS and perform reliable genetic counseling. Particularly, pUPD and GOM at ICR1 result in a high risk of nephroblastoma while CDKN1C mutations can be inherited and cause familial recurrence [25, 26]. CDKN1C mutations are strongly associated with exomphalos, whereas macrosomia is more frequent in ICR1 GOM [27 and unpubl. data].

**Human Multilocus Imprinting Disorders**

Imprinting disorders are complex syndromes and display a high degree of clinical heterogeneity. Until a few years ago, it was believed that LOI leading to various human syndromes were isolated events affecting only a given locus involved in a particular syndrome. However, we found that a subset of BWS patients, including some born after ART, displayed LOM at imprinted loci other than the ICR2 11p15 region [27]. Concomitantly, Mackay et al. [28] reported a subset of
transient neonatal diabetes mellitus patients with 6q24 LOM (ZAC1 DMR) also
displaying multilocus imprinting defects. Since these two reports, other groups
have described multilocus imprinting defects in BWS and in TNDM patients.
More recently, we revealed that about 10% of RSS patients exhibit multilocus
LOM at both paternally and maternally methylated loci, and that over two thirds
of these patients exhibit LOM at DLK1/GTL2 1G-DMR 14q34 in addition to that
at ICR1 11p15 [29]. These regions are two of the three paternally methylated ICRs.
Comparisons between the clinical characteristics of monolocus LOM patients and
multilocus LOM patients did not reveal any statistically significant differences.
First idea was that the dominant phenotype is determined by the locus of more
extensive demethylation. However, we observed that some RSS patients have simi-
lar degrees of LOM at different loci but only the RSS phenotype. This led us to sug-
gest an epidominance effect exerted by the 11p15 region in the expression of the
phenotype. However, imprinting disorders have complex phenotypes, and some of
them share some clinical characteristics, for example TNDM and BWS (abdomi-
nal defects and macroglossia) and RSS and chromosome 14-related syndromes
(IUGR). For these overlapping features, it is difficult to relate the observed clinical
abnormality to one or another given locus. Most multilocus defects are partial,
thus reflecting a degree of mosaicism. The expression of one syndrome rather
than another may possibly therefore be explained by tissue-specific mosaicism.

**Assisted Reproductive Technologies and Loss of Imprinting**

Several studies reported the increased incidence of ARTs conception in BWS
and Angelman syndrome patients [9, 30]. This finding seems also relevant for
RSS patients with ICR1 LOM [19 and unpubl. data]. In mice, IVF and embryo
culture lead to LOI of several genes including Igf2 and h19 [30]. To date, no
specific procedure has been identified. It appears that the environment acts
through epigenetic mechanisms to modulate development. Patients born after
ART exhibiting an imprinting defect illustrate the link between early concep-
tion environment and epigenetic changes. Larger series are needed to investi-
gate this further. Identification of the factors involved in the maintenance and/
or the establishment of imprinting are undoubtedly crucial for understanding
both the mechanisms underlying imprinting regulation and which disruptions
lead to complex diseases such as RSS and BW.

**Conclusion**

The identification of ICR1 LOM and mUPD7 in nearly 70% of patients with RSS
is a considerable step towards understanding the pathophysiology of this disor-
der. It involves imprinted genes like IGF2 and H19 within the 11p15 region. It
also involves imprinted genes on chromosome 7 that remain unidentified. In the same way, it is not established if there is an interaction between chromosome 7-encoded factors and the growth-relevant region 11p15. Long-term, systematic endocrine follow-up of cohorts of RSS patients with ICR1 LOM and mUPD7 will help to clarify phenotypic specificity and to draw appropriate guidelines for the clinical management. In 30% of patients with a clinical diagnosis of RSS, the underlying molecular defect is unknown. However, the relatively non-specific features of RSS present a continuing challenge to clinical diagnosis. This is why the use of a clinical score could help to reduce the heterogeneity of patients with idiopathic RSS. Identification of the factors involved in the maintenance and/or the establishment of imprinting is undoubtedly crucial for understanding both the mechanisms underlying imprinting regulation and which disruptions lead to complex diseases such as RSS and BWS.

References


