Molecular Mechanisms and Clinical Pathophysiologies of Focal ATP-Sensitive Potassium Channel Hyperinsulinism and Beckwith-Wiedemann Syndrome

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Abstract
One of the major advances in the care of patients with congenital hyperinsulinism was the discovery of focal adenomatous hyperplasia since patients can be definitively cured after a limited pancreatectomy. The histological features include hyperplasia and an increased beta-cell mass within the focal lesion. These findings were already reported in the pancreas of patients with Beckwith-Wiedemann syndrome (BWS), a condition which is known to be associated with hyperinsulinism. However, the pathophysiology of focal hyperinsulinism (FoHI) is more complex and includes a ‘two hits’ mechanism: (1) a paternally inherited mutation of an ATP-sensitive potassium (K\textsubscript{ATP}) channel gene located on the 11p15 chromosome; and (2) in a pancreatic endocrine progenitor, a deletion of the maternally inherited 11p15 chromosomal region, compensated by a paternal uniparental disomy as observed in some cases of BWS. The loss of heterozygosity in the imprinted 11p15.5 region bearing genes involved in tumor suppression and cell proliferation explains the histopathological findings of FoHI, while the paternally inherited K\textsubscript{ATP} channel mutation reduced to homozygosity within the beta-cells of the focal lesion causes hyperinsulinemic hypoglycemia unresponsive to diazoxide.

The discovery of the molecular mechanism of focal hyperinsulinism (FoHI) was initially triggered by the striking similarity between the histological findings observed in FoHI and in a particular condition known to cause hyperinsulinemic hypoglycemia: the Beckwith-Wiedemann syndrome (BWS). Indeed, both conditions bear a common chromosomal rearrangement: a paternal uniparental disomy (pUPD) involving the 11p15.5 chromosomal region with a variable extent from the telomere.
toward the centromere. However, specific to FoHI, a second mechanism explains the usual diazoxide-unresponsiveness of FoHI patients: a paternally inherited mutation of an ATP-sensitive potassium (\(K_{ATP}\)) channel gene (located in 11p15.1) is reduced to homozygosity within the focal form because of the pUPD. This chapter will describe the molecular mechanisms underlying BWS and, more specifically, the 11p15 pUPD, to give light on the pathophysiology of FoHI.

**Beckwith-Wiedemann Syndrome**

BWS (OMIM 130650) is a model disorder for the study of imprinting, growth dysregulation, and tumorigenesis. BWS results from various genetic and epigenetic anomalies in the imprinted 11p15.5 region that causes overexpression of paternally expressed genes or a lack of expression of maternally expressed genes. BWS has an incidence of 1 in 13,700. The clinical features as described by Beckwith [1] and Wiedemann in 1969 include the characteristic growth disorder with macrosomia, macroglossia, visceromegaly, abdominal wall defects including omphalocele and umbilical hernia, neonatal hypoglycemia, ear creases/pits, body asymmetry, and an increased risk for embryonal tumor development. Close to 50% of neonates with BWS present with hypoglycemia.

**Pathophysiology of Beckwith-Wiedemann Syndrome**

Molecular mechanisms that lead to BWS are various, but have in common an imbalance in gene expression on chromosome 11p15.5. Most autosomal genes are equally expressed from both alleles, the paternally inherited as well as the maternally inherited one. However, some genes are subjected to imprinting and are thus expressed only with regard to their parent of origin (mother or father).

The 11p15.5 region includes a cluster of several imprinted genes controlled by two imprinting centers (IC1 and IC2), which are differentially methylated on the two parental alleles. These imprinting centers define two domains (fig. 1). One is the telomeric domain controlled by IC1 (paternally methylated), which includes the paternally expressed fetal growth factor IGF2 gene and the maternally expressed H19 gene, considered as a transregulator of genes involved in fetal growth. The other is the centromeric domain controlled by IC2 (maternally methylated), which includes the maternally expressed CDKN1C (cyclin-dependent kinase inhibitor acting as a negative regulator of cell proliferation) and KCNQ1 (potassium channel voltage gated) genes, as well as the paternally expressed KCNQ1OT1 (gene silencer) gene.

**Cytogenetic, Genetic, and Epigenetic Causes of Beckwith-Wiedemann Syndrome**

Familial BWS accounts for about 15% of all cases and are explained by rare cytogenetic abnormalities, with the common finding of involvement of the 11p15 chromosomal region. This consists of (1) maternally inherited translocations or inversions or
(2) paternal duplications (trisomy with a double dose of the paternal 11p15 region) resulting from duplications or unbalanced reciprocal translocation involving the 11p15 region. Familial BWS can also be explained by point mutations of the maternal allele of CDKN1C leading to a loss or a reduced expression of the active allele. In 85% of cases, BWS is sporadic and results from the following: an epigenetic error of one or more of the 11p15.5 imprinted gene clusters, loss of methylation at the imprinting center 2 (IC2 or KvDMR; 50–60% of cases) on the maternal allele leading to the biallelic expression of KCNQ1OT1 and loss of CDKN1C expression, gain of methylation at the imprinting center 1 (IC1 or H19DMR; 5–10%) on the paternal allele leading to the biallelic expression of IGF2 and loss of H19 expression, or from a mosaic paternal 11p15 isodisomy (pUPD, 20% of cases) [2] leading to the overexpression of

Fig. 1. Diagrammatic representation of the chromosome 11. Relevant genes leading to the clinicopathological features of BWS are gathered in the 11p15.5 region. Only alleles represented in large arrows are expressed. Other alleles are silent. In the 11p15.5 region, genes are subdued to imprinting. The triplet arrows over an imprinting center (IC) indicate its methylation. However, genes in the 11p15.1 (ABCC8 and KCNJ11) are expressed from both alleles. The minimal extent of the maternal LOH (thick black lines) is indicated for BWS and for FoHI.
Beckwith-Wiedemann Syndrome and Paternal Uniparental Disomy

pUPD appears to be a postzygotic somatic recombination event since BWS patients with 11p15 pUPD always show mosaicism. The extent of the pUPD always spans from the telomere to a variable breakpoint. It always includes the whole 11p15.5 chromosomal region which contains the genes involved in the pathophysiology of BWS (IGF2, H19, CDKN1C). Recently, a SNP-array study performed on 9 patients did not provide any evidence of a recombination hotspot because all patients had different extent of pUPD (in 4 patients the pUPD spanned from 11p15.5 to different break-points in 11p11.2; in 1 patient to 11p12; in 2 patients to 11p13; and in 2 other patients pUPD was restricted to the 11p15.5 region alone) [3]. During meiosis, specific hotspots for chromosomal recombination are observed; however, the highly variable location of the breakpoints on the short arm of chromosome 11 in BWS patients does not support the hypothesis of a molecular event occurring during meiosis. Finally, the pUPD may be subsequent to an abnormal recombination between nonsister chromatids during mitosis or by a deletion of a region and the further duplication of its homologous region on the other chromosome. The cause of the loss of the maternal allele is not known. Actually, neither gene mutations nor specific DNA sequences are known to provoke or facilitate the loss of the maternal allele.

Some cases with mosaic pUPD for all of chromosome 11 were also described and the clinical finding did not differ from patients with pUPD restricted to a small part of 11p. Cells with pUPD may have a growth advantage. Some exceptional cases of maternal UPD of the 11p15 region have been reported in patients with Silver-Russel syndrome, consistent with the clinical findings of this syndrome (poor fetal growth, etc.). Thus, pUPD of the 11p15.5 region is expected to destabilize the physiological balance between imprinted genes controlling cell growth, thereby permitting overgrowth and tumor growth. The main genes suspected to explain the pathophysiology of BWS are:

- **IGF2**, a paternally expressed gene which has proliferative and antiapoptotic effects. It is mostly expressed during embryogenesis, then its expression drops [4].
- **H19**, a maternally expressed transregulator of genes involved in fetal growth. This gene is expressed only during fetal development and early postnatal growth in mice. The loss of the H19 active allele in mice leads to a fetal overgrowth and probably deregulates IGF2 expression. A recent report by Gabory et al. [5], in the mouse model of BWS, showed that, apart from a cis-effect, the H19 noncoding RNA also regulates the expression of IGF2 by a trans-acting mechanism.
- **CDKN1C**, also a maternally expressed tumor suppressor gene. The encoded protein is an inhibitor of G1 cyclin/Cdk complexes and acts as a negative regulator of cell proliferation. Mutations in this gene were reported in BWS and sporadic cancers.
- **KCNQ1OT1**, a paternally expressed gene that is thought to be noncoding. It may act as a chromatin regulatory RNA (gene silencer) and/or a methylation regulator. It may prevent the expression of CDKN1C from the paternally inherited allele.

pUPD of 11p is expected to increase IGF2 and decrease H19 and CDKN1C expression, thus bestowing a growth advantage to islet cell progenitors which proliferate and ultimately give rise to the histological findings observed in FoHI and the pancreas from BWS patients.

Some genotype-phenotype correlations exist with pUPD. A clear association exists between mosaic pUPD and hemihypertrophy, and between organ enlargement and the proportion of cells with pUPD within each organ [2]. In addition, it has been clearly shown that neoplasias and Wilms tumors are more frequent in BWS with pUPD and gain of methylation (i.e. patients with an overexpression of IGF2) than in patients with other molecular defects.

**Pancreatic Histology in Beckwith-Wiedemann Syndrome**

In the original description by Beckwith [1] in 1969, the pancreas was about 3 times heavier than normal in 2 of the 6 patients. Since then, very little information has been published about pancreatic histology in BWS. The pancreas in BWS is characterized by a florid hyperplasia of ducts, acini, and islets. The pancreatic islets are well organized but abnormally large and have a marked proliferation of beta- and alpha-cells, and a moderate increase of pancreatic polypeptide cells and somatostatin cells [6]. No genotype histology correlation has been published yet. Therefore, it is not known whether the pancreatic histology in BWS is different according to the molecular defect (mosaic pUPD, point mutation in CDKN1C, etc.). Pancreatoblastoma is reported as a rare complication of BWS, and occurs mostly in patients with hemihypertrophy.

**Hypoglycemia in Beckwith-Wiedemann Syndrome**

In 2000, DeBaun et al. [7] reported a series of 263 patients with a clinical diagnosis of BWS. Among them, 52% presented with hypoglycemia at birth. In another series of patients, hypoglycemia required extra feeds or intravenous glucose, with a glucose infusion rate greater than 10 mg/kg/min to maintain euglycemia [8]. Insulin levels were inappropriate in hypoglycemia, ketone bodies were low, and a glycemic response to glucagon injection was found. Thus, hyperinsulinism explains the hypoglycemia observed in BWS. The hypoglycemia usually resolves within the first few days of life. However, in 20% of the patients, hypoglycemia persisted more than 1 week, even beyond the first month of life in 4% of patients and sometimes for several years [7]. Intellectual disabilities were correlated with the severity of hypoglycemia.

Usually hyperinsulinemic hypoglycemia in BWS is easily treated with diazoxide. However, several cases of diazoxide-unresponsive hyperinsulinism have been described in BWS, with some even resistant to octreotide and requiring surgery. In the report by Hussain et al. [9], the patient was diazoxide- and octreotide-unresponsive, required a glucose infusion rate of 20 mg/kg/day, and finally underwent a pancreatectomy. After
surgery, hypoglycemia resolved without any treatment at 14 months of age. However, the relation between BWS and hyperinsulinism was not clear in this case because 11p15 pUPD was found in blood cells, but not in the pancreatic beta-cells.

The mechanism of hyperinsulinism related to BWS is still unclear. Hyperplasia of the beta-cells has been suggested to explain hypoglycemia. This hyperplasia may be due to the overexpression of a growth factor gene (IGF2) observed in 20% of patients [10] and/or an imbalance between IGF2 and tumor suppressor genes (H19, CDKN1C) [11]. IGF2 is known to weakly bind the insulin receptor. However, mice models of BWS overexpressing IGF2 display clinical features of BWS except hypoglycemia. Another hypothesis is that the duplication of the region containing the insulin locus (11p15.5) leads to an overexpression of the INS gene. However, no evidence of duplication of INS, HRAS1, and IGF2 [12], or overexpression of the INS and IGF2 genes [13], has been found to cause hyperinsulinism in BWS. Thus, it is not known whether the cause of hyperinsulinism in BWS is either the increased mass of the beta-cells, dysregulation of the insulin secretion by the beta-cells, or a combination of these two hypotheses.

The role, level, and influence of paracrine secretions from the other cells types of the hyperplastic pancreatic islets (glucagon, somatostatin) on the beta-cells are not known.

Abnormalities of the Ploidy
We reported 2 cases of unusual BWS associated with mosaic abnormalities of the ploidy [14]. Both cases were suspected to unbalance the 11p15 region and were diazoxide-unresponsive, leading to surgery despite the absence of K ATP channel gene mutations. The histological features of the focal lesions were unusual, and one was suspected of malignancy. A genome-wide loss of the maternal alleles and a paternal isodisomy was found in the lesional pancreas of the first patient, whereas we observed a triploidy with one maternal allele and two distinct or identical paternal alleles within the lesions of the second patient.

Focal Hyperinsulinism
When hyperinsulinism is resistant to all available treatments (diazoxide, octreotide, and diet), surgery is required to avoid recurrent severe hypoglycemia and a risk of subsequent neurological disabilities. In the mid-1980s, pathologists studied very severe patients who were surprisingly cured after a hemipancreatectomy. Surprisingly, the histological analysis of the pancreas was normal. A careful second look, using anti-insulin antibodies, revealed a small focal endocrine ‘tumor-like’ lesion which showed evidence of beta-cell proliferation. By contrast, other hyperinsulinism patients, now classified as diffuse hyperinsulinism (DiHI), had in their pancreatic islets a normal number of beta-cells, which exposed signs of hyperactivity. Thus, the two distinct
types of pancreatic beta-cell involvement, focal and diffuse, were described for the first time [1, 15].

In 1997, we found a striking similarity between beta-cell proliferation in FoHI and what was observed in the pancreas of BWS patients [16]. This observation led us to investigate whether the genes in the 11p15.5 region (especially the proliferation and tumor suppressor genes) were involved in FoHI.

The focal adenomatous hyperplasia of the pancreatic islets in FoHI does not suggest a tumor since it does not invade or push at the margins and has no pseudocapsule. It is more likely that it suggests an abnormal developmental process because an organoid pattern remains and other endocrine cells are present within the foci (positive immunohistochemistry with antisomatostatin antibodies). Finally, this hyperplastic lesion is restricted to a small portion of the pancreas and is surrounded by normal tissue, suggesting a clonal process. The common histological features between BWS and FoHI led to the search for a common mechanism.

**Loss of the Maternal Allele from the 11p15 Chromosomal Region**

A variable loss of maternal chromosomal material was detected at the expense of the short arm of chromosome 11 [16]. Microsatellite studies of other chromosomes were normal, confirming the exclusive involvement of chromosome 11 in FoHI. This loss of heterozygosity (LOH) was not found in the surrounding normal pancreas, but was restricted to the pancreatic focal lesion, consistent with a proliferative monoclonal lesion. Moreover, the maternal 11p LOH was not found in islets cells from DiHI [16].

The studies by our team (10 patients) [16] and by Suchi et al. (11 patients) [17] using microsatellite markers showed a constant loss of the maternal alleles in the 11p15.1 region, which contains the ABCC8 and KCNJ11 genes. Some evidence from hyperinsulinism as well as from BWS led to the hypothesis of a unique (but variable in its location) breakpoint on the short arm of chromosome 11 leading to the loss of the telomeric extremity. The extent of this LOH is variable, but involves: *constantly* (1) subtelomeric microsatellites (e.g. D11S922 in 11pter), (2) the 11p15.5 region (e.g. D11S1758, D11S569) where the genes involved in BWS are located and that contains imprinted domains, and (3) the 11p15.1 region which contains the nonimprinted ABCC8 and KCNJ11 genes (e.g. D11S902, D11S921); and *variably* a seemingly random extent toward the centromere. The loss of the maternal allele was observed almost constantly in the 11p14 region and, in 3 out of 10 patients on the long arm of the 11 chromosome (e.g. D11S1365 in 11q14.1). In these 3 patients, the LOH involved almost two thirds of the whole maternal chromosome.

As a consequence, the expression of maternal genes appears decreased or absent as shown by the loss of p57kip2 expression demonstrated by immunohistochemistry [17]. This protein is encoded by the imprinted and maternally expressed CDKN1C gene from the 11p15.5 region. Using p57kip2 antibody, Suchi et al. [17] showed a moderate labeling of normal tissues, whereas 48 out of 56 FoHI exposed a clear loss of the