INTRODUCTION

More than 100 human genes are currently believed to be imprinted by epigenetic mechanisms that allow expression from only one of the two paternal alleles.

These parental imprints undergo a cycle during the life of an organism that allows their reprogramming at each generation. The imprinted marks are inherited from the parental gametes and are then maintained and realized in the somatic cells of an individual. During early development, methylation modification of the mammalian genome undergoes dramatic changes and is linked to the rapid differentiation and formation of various tissues and organs. The imprint marks are erased in the germline and re-established according to the sex of the contributing individual for the next generation. Among others, one fundamental molecular process in this imprinting cycle is DNA methylation. It is mainly catalyzed by DNA methyltransferases (DNMTs) and is generally associated with gene silencing.

The balanced expression of imprinted genes is needed for a regular development of an individual, and it is therefore not surprising that many imprinted genes are involved in human growth. According to their hypothesized biological function as mediators of the "male-of-sexes" (conflict theory) in the fetal period, paternally- and maternally-imprinted genes have opposite functions: while paternally-expressed factors promote growth, maternally-expressed ones suppress it [1,2].

Indeed, the majority of the known imprinting syndromes are associated with disturbed growth (Table 36.1). The most prominent imprinting disorders (IDs) are Prader–Willi and Angelman syndromes (PWS, AS); in both entities similar but opposite genetic and epigenetic situations of chromosome 15 are present. Further imprinting diseases are transient neonatal diabetes mellitus (TNDM, chromosome 6q24), Silver–Russell syndrome (SRS, chromosomes 11p15), Beckwith–Wiedemann syndrome (BWS, chromosome 11p15) and the maternal/paternal UPD14" (UPD14(mat/pat) syndromes. However, there are overlapping clinical findings in some of these disorders (i.e. TNDM and BWS). Recent studies identified an association between an increased risk for epigenetic defects resulting in IDs and assisted reproduction techniques (ART) [for review, see Ref. 3] but it is not yet clear whether these defects are linked to the subfertility of parents or the technique.


TYPES OF MUTATIONS AND EPIMUTATIONS IN IDs

The regular expression of imprinted genes can be influenced by different types of mutations and epimutations. For nearly all known IDs, the same genetic and epigenetic alterations affecting imprinted genes/gene clusters and their expression and regulation have been reported (Table 36.1). They include genomic mutations (uniparental disomy (UPD), chromosomal imbalances, point mutations) and true epigenetic defects (abnormal DNA methylation but without a genomic alteration) which affect the expression and regulation of imprinted loci. The incidence of the different classes of mutations and epimutations are different in the known IDs (Table 36.1), suggesting that some loci like the ICR1 and the ICR2 in 11p15 are more vulnerable for disturbed methylation than others.

Uniparental Disomy (UPD)

Nearly all human imprinting disorders have been detected through the identification of Uniparental Disomy (UPD). UPD is defined as the abnormal inheritance of both copies of a chromosome or a chromosomal segment from only one parent. Several modes of UPD formation have meanwhile been described; one prominent mechanism is trisomic rescue (for review see Ref. 4) (Fig. 36.1). In the majority of cases, UPD affects the whole chromosome, but meanwhile several UPDs affecting only parts of a chromosome have been reported ("segmental UPD"; 5). In particular in Beckwith–Wiedemann syndrome (BWS) segmental UPD of chromosome 11p15 accounts for up to 20% of cases.

Two types of UPD can be distinguished, uniparental heterodisomy (UPhD) and uniparental isodisomy (UPiD). UPhD means the presence of the two different homologous chromosomes.

![Diagram of UPD formation by trisomic rescue, gamete complementation, post-fertilization error, and monosomic rescue](image)

**Figure 36.1**

Schematic overview on UPD formation by trisomic rescue, gamete complementation, post-fertilization error, and monosomic rescue.
<table>
<thead>
<tr>
<th>Imprinting Disorder</th>
<th>Acronym/ Abbreviation</th>
<th>Frequency</th>
<th>OMIM</th>
<th>Affected Chromosomes/ Imprinted Regions</th>
<th>Types of Mutations/ Epimutations</th>
<th>MLH</th>
<th>Detection Rate</th>
<th>Main Clinical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient neonatal diabetes mellitus</td>
<td>TNDM</td>
<td>1/800,000</td>
<td>601410</td>
<td>6q24: ZAC1/HYMA1</td>
<td>upd(6)pat Paternal duplications Methylation defects upd(7)mat</td>
<td>Yes</td>
<td>85%</td>
<td>Transient diabetes, IUGR, macroglossia</td>
</tr>
<tr>
<td>Silver–Russell syndrome</td>
<td>Russell–Silver syndrome, SRS, RSS</td>
<td>1/10,000</td>
<td>180860</td>
<td>7</td>
<td>upd(11p15)mat Paternal duplication Hypomethylation upd(11p15)mat Chromosomal aberrations</td>
<td>Yes</td>
<td>~10%</td>
<td>Pre- and/or postnatal growth retardation, rel. macrocephaly, asymmetry, triangular face</td>
</tr>
<tr>
<td>Beckwith–Wiedemann syndrome</td>
<td>Wiedemann–syndrome, EMG syndrome, BWS</td>
<td>1/15,000</td>
<td>130650</td>
<td>11p15: IGF2/H19; ICR1: IGF2/H19; ICR2: KCNO1; CDKN1C</td>
<td>Hypermethylation Hypomethylation Point mutations</td>
<td>Yes</td>
<td>~20%</td>
<td>Pre- and postnatal overgrowth, organomegaly, omphalocoele, neonatal hypoglycemia, hemihypertrophy, increased tumor risk in specific molecular subgroups</td>
</tr>
<tr>
<td>UPD(14)mat syndrome</td>
<td>Temple syndrome</td>
<td>Rare</td>
<td>14q32: DLK1/GTL2</td>
<td>upd(14)mat Deletions of the paternal chromosome 14 Aberrant methylation upd(14)mat Aberrant methylation</td>
<td>No</td>
<td>5–10%</td>
<td>IUGR/PNGR, hypotonia, scoliosis, precocious puberty</td>
<td></td>
</tr>
<tr>
<td>UPD(14)pat syndrome</td>
<td>–</td>
<td>Rare</td>
<td>608149</td>
<td>14</td>
<td>Maternal deletion upd(15)pat Aberrant methylation Point mutations</td>
<td>No</td>
<td>70%</td>
<td>IUGR, polyhydramnios, abdominal and thoracic wall defects, bell-shaped thorax</td>
</tr>
<tr>
<td>Angelman syndrome</td>
<td>Happy Puppet syndrome, AS</td>
<td>1/20,000– 1/12,000</td>
<td>105830</td>
<td>15q11–q13: UBE3A</td>
<td>Maternal deletion upd(15)pat Aberrant methylation Point mutations</td>
<td>No</td>
<td>70%</td>
<td>Mental retardation, microcephaly, no speech, unmotivated laughing, ataxia, seizures, scoliosis Mental retardation, neonatal hypotonia, growth retardation, hypogenitalism, hypopigmentation, adipositas/hyperphagia</td>
</tr>
<tr>
<td>Prader–Willi syndrome</td>
<td>Prader–LaBhart–Willi– syndrome, PWS</td>
<td>1/25,000– 1/10,000</td>
<td>176270</td>
<td>15q11–q13</td>
<td>Paternal deletion upd(15)mat Aberrant methylation</td>
<td>No</td>
<td>&lt;30%</td>
<td>Mental retardation, neonatal hypotonia, growth retardation, hypogenitalism, hypopigmentation, adipositas/hyperphagia</td>
</tr>
</tbody>
</table>
TYPES OF MUTATIONS AND EPIMUTATIONS IN IDs

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**FIGURE 36.1**

Schematic overview on UPD formation by trisomic rescue, gamete complementation, post-fertilization error, and monosomic rescue.
from the same parent, while in UPD the same chromosome is duplicated. Up to now, three different possibilities as to how UPD might influence the phenotype have been reported:

1. If UPD affects imprinted genes it will lead to their imbalanced expression. UPD has been reported for nearly all human chromosomes but due to the tendency of imprinted genes to cluster, only some UPDs are associated with specific phenotypes (Table 36.2). Interestingly, on some chromosomes oppositely imprinted genes are clustered (chromosomes 11, 14, 15) whereas for others one parental UPD is risky while the other is not (chromosomes 6 and 7).

2. If one parent carries an autosomal recessive mutation and has a UPID offspring, this child will be homozygous for the same mutated allele and will be affected by the respective disease. Indeed, the first reported case of UPD was a patient suffering from cystic fibrosis caused by maternal UPD of chromosome 7 [6]. Thus, despite the lack of imprinted genes on several human chromosomes, a reduction to homozygosity of recessive alleles has to be considered in any case of UPD.

3. If UPD is derived from a trisomic zygote, some trisomic cells can survive. The clinical outcome of this mosaic constitution can therefore be influenced by the UPD as well as by the trisomic cell line. Then, the phenotype is difficult to predict and a differentiation between symptoms caused by UPD or by the chromosomal mosaicism is often impossible.

The association between UPD and chromosomal disturbances has also to be borne in mind in prenatal diagnosis: in particular, in chorionic villous sampling trisomy mosaicism is a relatively-frequent finding and depending on the chromosome involved UPD testing should be considered after detection of an aneuploidy. Another group prone to UPD are carriers

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Maternal UPD</th>
<th>Paternal UPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
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<td>3</td>
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<td>?</td>
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<tr>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>Transient diabetes melitus</td>
</tr>
<tr>
<td>7</td>
<td>Silver–Russell syndrome</td>
<td>-</td>
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<tr>
<td>8</td>
<td>-</td>
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</tr>
<tr>
<td>9</td>
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</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Silver–Russell syndrome</td>
<td>Backwith–Wiedemann syndrome</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>?</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>UPD(14)mat syndrome</td>
<td>UPD(14)pat syndrome</td>
</tr>
<tr>
<td>15</td>
<td>Prader–Willi syndrome</td>
<td>Angelman syndrome</td>
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<tr>
<td>16</td>
<td>-</td>
<td>-</td>
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<tr>
<td>17</td>
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<tr>
<td>22</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>X</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

584
of (Robertsonian) translocations involving chromosomes 6, 7, 11, 14, and 15. Indeed, the majority of chromosome 14 UPDs (UPD(14)mat, UPD(14)pat) has been detected among Robertsonian translocation carriers.

**Chromosomal Deletions and Duplications**

The functional relevance of chromosomal imbalances affecting imprinted genes is comparable to that of UPD. In PWS for example, a deletion of \(-0.6\) Mb in the paternal chromosome 15 accounts for nearly 70% of cases and affects the region 15q11-q13 while <30% of patients are UPD(15)mat carriers. Functionally, both (epi)mutations result in an inactivation of paternally-expressed genes [for review see Ref. 7]. Vice versa, \( \sim 70\% \) of AS patients show a maternal deletion of the same chromosomal region in 15q. 1-3% a UPD(15)pat. The two chromosome 15 IDs impressively illustrate the profound role of chromosomal imbalances in the etiology of this group of diseases. The situation is similar for TNDM where 6q24 duplications account for approximately one third of cases. Single cases of duplications/deletions, maternal as well as paternal, have also been reported in SRS/BWS and UPD(14)mat/UPD(14)pat (Table 36.1).

**Genomic Point Mutations in ID Patients**

In some cases of IDs point mutations on genomic DNA level are responsible for the clinical course. These mutations might affect either genes underlying a regulation of imprinting centers, or genes involved in establishment or maintenance of methylation marks.

*Point mutations in genes regulated by DMRs* have been reported for BWS and AS and account for a significant number of cases. As aforementioned, CDKN1C mutations are responsible for a large proportion of familial BWS cases; *UBE3A1* mutations can be detected in 10-15% of AS patients (Table 36.1).

In principle, genes responsible for the establishment or maintenance of methylation marks are good candidates to carry mutations causing aberrant methylation. Indeed, a growing number of factors involved in DNA methylation and its regulation have been identified, but so far only two genes have been published to carry point mutations in ID patients. In TNDM patients with multilocus hypomethylation (MLH) autosomal-recessive mutations in the zinc-finger protein ZFP57 have been reported [see below; and Ref. 8]. In a BWS sibship a homozygous mutation in the *NLRP2* gene in the mother was described by Meyer et al. [9] providing evidence for a trans mechanism for the disturbed methylation pattern in 11p15 in the two children. However, screening studies in further enzymes involved in locus specific methylation and regulation such as *DNMT3L* and *PLAGL1* failed to identify pathogenic variants [10,11].

**Imprinting Defects**

Imprinting defects, or epimutations, describe altered DNA methylation patterns at specific differentially-methylated regions (DMRs) which regulate the expression of neighboring genes. The chromosomal region 11p15 represents a typical example of an imprinted region with complex regulation mechanisms (Fig. 36.2) and it is described below as an example.

The region 11p15 contains a number of imprinted genes, the expression of which is regulated by two different imprinting control regions (ICR1 and ICR2), also called H19 DMR and KvDMR1. The telomeric ICR1 confers a differential chromatin architecture to the two parental alleles leading to reciprocal expression of H19 and IGF2. The two genes are co-expressed in endoderm- and mesoderm-derived tissues during embryonic development and compete for the same enhancers. The paternally-expressed IGF2 is involved in fetal development and growth [12,13]. Although H19 was one of the first non-coding transcripts identified, its function is still unknown. Knockout of H19 removing the whole RNA coding
sequence but leaving the promoter and surrounding transcription unit intact had no effect on the imprinted expression of IGF2 [14]. These results indicate that the RNA itself might be non-functional; however, the fact that H19 is a relatively highly conserved gene among mammals (77% identity between human and mouse) suggests a profound functional relevance. A recent study suggests that H19 functions as a primary micro-RNA precursor involved in the post-transcriptional down-regulation of specific mRNAs during vertebrate development [15]. The ICR1 contains seven CTCF target sites in the DMR 2 kb upstream of H19 and shows allele-specific methylation. The Zinc-finger binding factor CTCF binds to the maternal unmethylated ICR1 copy and thereby forms a chromatin boundary. This CTCF binding mechanism blocks IGF2 and promotes H19 transcription of the paternal 11p15 copy. As a result of this complex and balanced regulation, hypo- as well as hypermethylation in the ICR1 in 11p15 cause IDs (SRS and BWS, respectively; Table 36.1).

The centromeric ICR2 in 11p15 regulates the expression of CDKN1C, KCNQ1 (potassium channel KQT-family member 1) and further genes and is methylated only on the maternal allele. Mutations in the paternally-suppressed CDKN1C gene account for up to 40% of familial BWS cases and 5–10% of sporadic patients (Table 36.1). The gene encodes a cyclin dependent kinase inhibitor (p57KIP2) and is part of the p21CDK4Cdk inhibitor family. Functional analysis of CDKN1C germline mutations detected in two BWS patients showed
the loss of cell-cycle inhibition [16]. The gene of another non-coding RNA in 11p15, 
*KCNQ1OT1 (LIT1)*, is localized in intron 10 of the *KCNQ1* gene. *KCNQ1OT1* is expressed 
by the paternal allele and probably represses the expression of the *CDKN1C* gene. Loss of 
methylation of the maternal ICR2 allele correlates with expression of *KCNQ1OT1*. In BWS, 
one central physiological change caused by ICR2 (epi)mutations (hypomethylation at ICR2 
as well as *CDKN1C* point mutations) is the reduced expression of *CDKN1C*.

**MULTILOCUS HYPMETHYLATION (MLH) IN IDs**

For all congenital IDs an association with aberrant methylation or mutations at specific 
loci has been well established. It is therefore amazing that patients with transient neonatal 
diabetes mellitus (TNDM) can exhibit hypomethylation at further imprinted loci in addition 
to the disease-specific in 6q24 [17,18]. Because all affected loci were maternally methylated, 
Mackay et al. [18] defined a maternal hypomethylation syndrome. In some of these patients, 
mutations in the *ZFP57* gene could recently be identified as the cause of the first heritable 
global human imprinting disorder [8]. *ZFP57* point mutations are autosomal recessive, 
a finding that is important for genetic counselling in TNDM. The association between 
mosaicism of hypomethylation and *ZFP57* mutations suggests that this factor is involved 
in the maintenance of imprinted DNA methylation in the early embryo. Recently, also for 
BWS and SRS patients with MLH in blood lymphocytes have been reported [19–22]. In 
these patients both paternally- and maternally-imprinted loci were affected in leukocytes. 
In all studies, a phenotypic difference between BWS or SRS patients with MLH and patients 
carrying isolated 11p15 aberrations was not obvious. A broad range of imprinted loci can be 
affected in the case of MLH; however, it seems that single loci are prone to demethylation, 
i.e. the ICR2 in 11p15 and the *DLK1/GTL2* locus in 14q32.

**GENERAL ASPECTS OF IDs**

At first glance the different IDs are clinically heterogeneous but due to the similar underlying 
molecular defects and the function of the affected genes they often show comparable clinical 
characteristics, i.e.:

- pre- and/or postnatal growth retardation;
- hypo- or hyperglycemia;
- failure to thrive in the newborn and early childhood period;
- neurological abnormalities in childhood.

UPD and epimutations mainly occur *de novo*; in the case of postzygotic origin, mosaicism for 
the disturbances can be observed. As a result, in IDs the following observations are common:

- There is asymmetry of body, head and/or limbs.
- Most cases are sporadic, and familial cases are rare.
- Discordant monozygotic twins occur.
- Genotype-phenotype correlations are difficult to delineate.

In family cases, deviations from Mendelian inheritance can be often observed due to the 
influence of the parental origin of the (epi)mutation and the sex of the contributing parent. 
For genetic counselling of ID families, the knowledge of the nature of molecular mutation or 
mutation subtype is essential to delineate exact risk figures. Patients/carriers with deletions 
the example will have a 50% risk of conceiving a child with a UPD phenotype, depending 
the sex of the patient. In the case of epimutations it might be more difficult to give risk 
figures, and genetic counsellors are therefore advised to continually update their knowledge 
the each disease.
sequence but leaving the promoter and surrounding transcription unit intact had no effect on the imprinted expression of IGF2 [14]. These results indicate that the RNA itself might be non-functional; however, the fact that H19 is a relatively highly conserved gene among mammals (77% identity between human and mouse) suggests a profound functional relevance. A recent study suggests that H19 functions as a primary micro-RNA precursor involved in the post-transcriptional down-regulation of specific mRNAs during vertebrate development [15]. The ICR1 contains seven CTCF target sites in the DMR 2 kb upstream of H19 and shows allele specific methylation. The Zinc-finger binding factor CTCF binds to the maternal unmethylated ICR1 copy and thereby forms a chromatin boundary. This CTCF binding mechanism blocks IGF2 and promotes H19 transcription of the maternal 11p15 copy. As a result of this complex and balanced regulation, hypo- as well as hypermethylation in the ICR1 in 11p15 cause IDs (SRS and BWS, respectively; Table 36.1).

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can be observed. As described above (Fig. 36.2), the region contains two imprinted gene clusters. In contrast to SRS, the ICR2 regulating KCNQ1 and CDKN1C is the preponderant altered region in BWS; nearly 50% of patients carry a hypomethylation at this locus (Table 36.1). UPD(11p15)pat is the second important alteration, while ICR1 hypermethylation is rare. Most BWS cases are sporadic but familial inheritance is observed in 15% of all cases. In BWS families without aberrant 11p15 methylation, CDKN1C point mutations are frequent.

These BWS pedigrees resemble that of an autosomal dominant inheritance with incomplete penetrance. Interestingly, an increased frequency of monozygotic twinning has been reported in BWS families with epimutations (for review, see Ref. 22). It was therefore hypothesized that a methylation error precedes and possibly triggers twinning. BWS twins are nearly always female and discordant for the disorder. In some twin pairs, aberrant methylation was also detectable in leukocytes of the unaffected twin while it was not detectable in buccal swab DNA of the healthy child.

A rudimental genotype/epigenotype–phenotype correlation has recently been established for BWS [34]: hemihypertrophy is strongly associated with UPD(11)pat, exomphalos with ICR2 hypomethylation and CDKN1C mutations. Most importantly, the risk of neoplasias is significantly higher in ICR1 hypermethylation and UPD(11)pat than in the other molecular subgroups. In BWS the determination of the molecular subtype is therefore important for an individual prognosis and therapy. Nevertheless, the phenotypic transitions are fluid and testing for all molecular subtypes should be considered in patients with BWS features.

MATERNAL AND PATERNAL UPD(14) SYNDROMES/UPD(14)mat/pat – CHROMOSOME 14

The so-far called UPD(14)mat and UPD(14)pat syndromes were first described in 1991 by Temple et al. and Wang et al. [35,36]; meanwhile distinct clinical phenotypes have been defined. However, the frequencies of both syndromes are currently unknown.

In both IDs, the original genetic alterations were UPDs associated with Robertsonian translocations. Considering the most important formation mechanism of UPD via trisomy rescue, this observation was consequent because Robertsonian translocations are prone to trisomic offspring. Meanwhile, several cases with isolated IDs and microdeletions affecting the DLK1/GTL2 locus in 14q32 have been described [37,38], resulting in the same phenotypes, and thus new names for these two syndromes are necessary [39].

The UPD(14)mat phenotype is characterized by prenatal and postnatal growth retardation, muscular hypotonia, feeding difficulties, small hands and feet, recurrent otitis media, joint laxity, motor delay, truncal obesity, and early onset of puberty. The facial gestalt comprises a prominent forehead, a bulbous nasal tip and a short philtrum. Patients with UPD(14)-mat show clinical features overlapping with PWS, and thus screening for UPD(14)mat should be performed in patients with PWS-like phenotype after exclusion of the PWS specific (epi) mutations [40].

UPD(14)pat is associated with a severe clinical course with polyhydramnios, a typical small, bell-shaped thorax, abdominal wall defects, and severe developmental delay. The majority of patients die in utero or in the first months of life. In addition to UPD(14)pat, isolated methylation defects at the DLK1/GTL2 locus have meanwhile been identified in UPD(14)pat patients.

All cases reported so far, the DLK1/GTL2 locus is affected. The paternally-expressed gene DLK1 (delta, Drosophila homolog-like 1) encodes a transmembrane signaling protein, maternally-expressed GTL2 (gene trap locus 2) is a microRNA which is involved in transcription regulation. However, the functional link between these genes and the phenotypes is currently unknown.
TRANSIENT NEONATAL DIABETES MELLITUS/TNDM – CHROMOSOME 6

Transient neonatal diabetes mellitus (TNDM) is a rare disease; in addition to hypoglycemia, IUGR and abdominal wall defects are common [23]. Insulin therapy is required for an average of 3 months; afterwards the diabetes resolves. However, the majority of TNDM patients develop type 2 diabetes.

TNDM is associated with an overexpression of the imprinted locus PLAG1/ZAC in 6q. As with the other IDs, three (epi)genetic causes of TNDM have been identified (Table 3 UPD(6)pat, paternal duplications of 6q24, and aberrant methylation at the PLAG1/ZAC locus. The PLAG1/ZAC gene is a maternally-imprinted gene and therefore only expressed from the paternal allele. It encodes a zinc-finger protein which binds DNA and hence influences the expression of other genes [for review see Ref. 24].

SILVER–RUSSELL SYNDROME/SRS – CHROMOSOMES 7 AND 18

Silver-Russell syndrome (SRS) is a congenital disorder mainly characterized by pre- and postnatal growth restriction. The children are relatively macrocephalic and their face is triangular-shaped with a broad forehead and a pointed, small chin. In many cases, asymmetry of limbs and body and clinodactyly V is present. Growth failure is often accompanied by severe failure to thrive, and feeding difficulties are reported. For those children without catch-up growth by the age of two, growth hormone therapy is encouraged.

The genetic basis of SRS is very heterogeneous. In approximately 10% of SRS patients a maternal uniparental disomy for chromosome 7 (UPD(7)mat) can be found [for review see Ref. 25]. The majority of SRS patients (~40%) show a hypomethylation of the ICR1 in the imprinted region 11p15 (Fig. 36.2); in single cases maternal duplications of the whole chromosomal region in 11p15 have been reported ([26]; for review, see Ref. 25). Until recently only one SRS patient with a maternal duplication restricted to the ICR2 has been identified [27]. With the recent identification of a patient with a UPD(11)mat the currently known spectrum of (epi)mutations has been accomplished in SRS [28]. Interestingly, the opposite 11p15 epigenetic and genetic findings can be observed in BWS (Table 36.1). Numerous (submicroscopic) chromosomal disturbances have been described in SRS patients, and screening for cryptic genomic imbalances is indicated after exclusion of UPD(7)mat and 11p15 epimutations [29,30]. Generally, the recurrence risk for SRS is low because the majority of patients are sporadic. Nevertheless, the situation changes in case of familial genetic or epigenetic alterations like chromosomal rearrangements or untypical aberrant methylation.

A genotype-phenotype correlation is difficult in SRS. In general, the 11p15 epimutation carriers show the more characteristic phenotype while UPD(7)mat patients are affected mildly, but exceptions exist [31]. Interestingly, there is evidence for a correlation between genotype/epi-genotype and endocrinological parameters [32].

BECKWITH–WIEDEMANN SYNDROME/BWS – CHROMOSOME 11

Beckwith-Wiedemann syndrome (BWS) was initially called ENS syndrome, from its three main features of exomphalos, macroGLOSSIA, and (neonatal) gigantism. Additional features include neonatal hypoglycemia, hemihypertrophy, organomegaly, earlobe crease, polyhydramnios, hemangiomata, and cardiomyopathy. In 5–7% of children, embryonal tumours (most commonly Wilms’ tumor) are diagnosed. The clinical diagnosis of BWS is often difficult due to its variable presentation and the phenotypic overlap with other overgrowth syndromes [for review, see Ref. 33].

The genetics of BWS are complex, but in the majority of cases an altered expression of mutations of several closely linked genes in 11p15 associated with cell cycle and growth
Nevertheless, prior to genetic testing the significance of genetic testing for the patient and his family should be critically discussed with the families. For each patient and his family, an individual strategy is necessary to avoid misleading and unclear results. Additionally, the putative predictive nature of a genetic test for affected as well as unaffected family members should be considered.

CONCLUDING REMARKS

In summary, there is a growing number of conditions where genomic imprinting effects are recognized to be associated with clinical disorders. Based on the observation that growth disturbance and behavior abnormalities are common features of IDs, genomic imprinting should be suspected in any disorder of unknown etiology characterized by these clinical signs. Furthermore, disorders with an unusual pattern of inheritance should be studied for the possibility that genomically-imprinted genes are involved. In view of the current lack of understanding of the functional basis of the known genetic/epigenetic alterations, identification of these (epi)mutations is of major importance in terms of recurrence risks, prediction of whether offspring will be affected, and risk of malignancies in the case of an ID.

New technologies such as microarrays in conjunction with computational approaches will help us to expand our knowledge and develop an imprinting map of the human genome. Thus, it is likely that many imprinted loci remain to be identified. As shown for the different IDs, epigenetics is a fascinating field of research and will provide us with profound insights into the etiology of many complex biological processes such as growth and we will be able to deduce the contribution of epigenetic changes to complex human disorders such as cancer and psychiatric diseases (see Chapters 33–36 of this book). Additionally, the prevalence and severity of these disorders are probably influenced by environmental factors (see Chapters 28–30 of this book). Thus, future epigenetic research will help us to discover the link between environment, genotype, and phenotype.

References

ANGELMAN AND PRADER–WILLI SYNDROMES – CHROMOSOME 15

Angelman (AS) and Prader–Willi syndrome (PWS) are currently the best-known IDs. Both disorders are associated with mental retardation but further clinical signs are different. Both neurodevelopmental diseases are caused by (epi)mutations in 15q11-q13 (Table 36.1). The lack of the paternal copy of this region results in PWS, while disturbances of the maternal copy of UBE3A lead to AS.

PWS is clinically characterized by neonatal hypotonia and failure to thrive, and then hyperphagia and obesity develop. Hypogonadism, short stature, behavior problems, and mild to moderate mental retardation are further characteristics [for review, see Ref. 41]. AS patients exhibit microcephaly, ataxia, seizures, absence of speech, and sleep disorder [42]. Due to the high percentage of microdeletions in 15q11-q13 in both syndromes, AS and PWS also belong to the so-called microdeletion syndromes. In AS, ~70% of patients have de novo deletions affecting the maternal chromosome. The same frequency can be observed in PWS where the region on the paternal chromosome 15 is deleted. Further AS specific (epi)mutations include UBE3A1 mutations, imprinting defects and paternal UPD(15). In PWS, maternal UPD(15) is frequent and accounts for <30% of patients, while imprinting defects are rare (~1%).

CHROMOSOME 20 AND THE GNAS LOCUS

GNAS is a complex imprinted locus that encodes several transcripts by alternative promoters and splicing. Some loci are expressed biallelically, others exclusively either from the paternal or the maternal GNAS allele [43]. Mutations in the GNAS1 gene are associated with pseudohypoparathyroidism 1a (PHP1A; Albright hereditary osteodystrophy with multiple hormone resistance), pseudopseudoparathyroidism (PPHP), and progressive osseous hypoplasia. PHP1A occurs only after maternal inheritance, whereas PPHP is only paternally inherited.

To date, only three patients with a UPD(20)mat have been reported (for review, see Ref. 4). All patients were characterized by severe pre- and postnatal growth retardation but none of them showed features belonging to the GNAS locus mutation spectrum.

GENETIC TESTING FOR IDs

With the growing knowledge on IDs and the rapid development of new molecular genetic technologies, genetic testing for epimutations and mutations in this group of diseases has been considerably improved. In particular, the development of bisulfite treatment assays to differentiate between methylated and unmethylated DNA was helpful to establish fast, reliable and low-cost strategies. Locus-specific Southern-blot assays have been replaced by different PCR-based methods. With the use of PCR only minimal amounts of patients’ DNA are necessary, this aspect being particularly important for testing of neonates and deceased patients.

While techniques like methylation-specific PCRs and bisulfite sequencing allow the targeted analysis of single loci, methylation-specific Multiplex Ligation Probe-dependent Amplification (MS-MLPA) now allows the parallel characterization of different loci as well as of different types of (epi)mutations (duplications, UPD, aberrant methylation) in a one-tube reaction [for review, see Ref. 45]. The development of methylation-specific microarrays now helps to gain insights into (aberrant) methylation of the total human genome.

The molecular confirmation of a clinical diagnosis is necessary not only for the patient but also for the family. As illustrated for BWS the molecular subtype of an ID allows a better prognosis, and therapeutic management (in the case of BWS the risk for neoplasias exists) but also helps to determine risk figures in the patients’ families.
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