ARTICLE

Prader–Willi Syndrome and Angelman Syndrome

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Prader–Willi syndrome (PWS) and Angelman syndrome (AS) are two distinct neurogenetic disorders in which imprinted genes on the proximal long arm of chromosome 15 are affected. Although the *SNORD116* gene cluster has become a prime candidate for PWS, it cannot be excluded that other paternally expressed genes in the chromosomal region 15q11q13 contribute to the full phenotype. AS is caused by a deficiency of the *UBE3A* gene, which in the brain is expressed from the maternal allele only. The most frequent genetic lesions in both disorders are a de novo deletion of the chromosomal region 15q11q13, uniparental disomy 15, an imprinting defect or, in the case of AS, a mutation of the *UBE3A* gene. Microdeletions in a small number of patients with PWS and AS have led to the identification of the chromosome 15 imprinting center (IC). The IC consists of two critical elements, which act in *cis* to regulate imprinting in the whole chromosome 15q11q13 imprinted domain.

KEY WORDS: genomic imprinting; imprinting defects; Prader–Willi syndrome; Angelman syndrome; DNA methylation

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INTRODUCTION

The Prader–Willi syndrome (PWS) and Angelman syndrome (AS) are the first known examples of human diseases involving imprinted genes. Each occurs with a frequency of ~1:15,000–1:25,000 live births. De novo interstitial deletions of the same chromosome region [del(15)(q11q13)] in patients with PWS and AS, were first identified by high-resolution chromosome banding and later confirmed by molecular studies [Ledbetter et al., 1981; Kaplan et al., 1987; Magenis et al., 1987; Donlon, 1988; Knoll et al., 1989; Tantravahi et al., 1989; Nicholls et al., 1989a]. At that time it was hard to explain why indistinguishable deletions of the same chromosomal region could lead to two clinically distinct disorders, each with characteristic cognitive, behavioral, and neurologic phenotypes. The first evidence for a parent-of-origin effect came from a study performed by Butler and Palmer [1983], who demonstrated, by studying the inheritance of chromosomal polymorphisms, that the deletion always occurs on the chromosome 15 inherited from the father. This finding was confirmed at the molecular level by Knoll et al. [1989], who also demonstrated that chromosome 15q11q13 deletions in AS always occur on the maternal chromosome. Based on the study of polymorphic DNA markers, Nicholls et al. [1989b] provided the first evidence that some PWS individuals inherit both chromosome 15s from the mother. The first patients with AS and a paternal uniparental disomy were identified by Malcolm et al. [1991] indicating that AS results from the absence of the maternal contribution of at least one gene in the chromosomal region 15q11q13. And indeed, further studies revealed that the chromosomal region 15q11-q13 contains a cluster of genes that are expressed from the paternal or maternal chromosome only. It is now known that AS results from the loss of function of the *UBE3A* gene, which in brain is expressed from the maternal chromosome only [Kishino et al., 1997; Matsuura et al., 1997]. In PWS, the situation is less clear, but a deficiency of the paternally expressed *SNORD116* snoRNAs can result in a PWS or PWS-like phenotype [Sahoo et al., 2008; de Smith et al., 2009]. However, this does not exclude the involvement of other genes in defining the phenotype.

It is now known that AS results from the loss of function of the UBE3A gene, which in brain is expressed from the maternal chromosome only. In PWS, the situation is less clear, but a deficiency of the paternally expressed SNORD116 snoRNAs can result in a PWS or PWS-like phenotype.

A small number of patients with PWS (~1–3%) and AS (2–4%) who have apparently normal biparental chromosomes 15 but were found to have

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aberrant DNA methylation throughout the imprinted domain on chromosome 15 were first described in 1993 and 1994 [Glenn et al., 1993; Buiting et al. 1994; Reis et al., 1994]. In this group of patients the disease is due to aberrant imprinting and gene silencing. In patients with PWS and an imprinting defect, the paternal chromosome carries a maternal imprint, whereas in patients with AS and an imprinting defect the maternal chromosome carries a paternal imprint, leading to a loss of paternal or maternal contribution of imprinted genes in the chromosome 15q11q13 region, respectively. In a few of these patients, the imprinting defect is caused by microdeletions defining a bipartite imprinting center (IC) [Buiting et al., 1995] which acts in *cis* and regulates DNA methylation, chromatin modification, and gene expression of the entire imprinted domain on proximal 15q.

CLINICAL FINDINGS

Prader–Willi Syndrome

The clinical features of PWS include low birth weight, severe hypotonia and feeding difficulties in early infancy, followed by hyperphagia and obesity starting in early childhood [for review see Cassidy and Driscoll, 2009]. Short stature, small hands and feet, a characteristic facial appearance (e.g., narrow bifrontal diameter, almond-shaped eyes, triangular mouth), a distinctive behavioral phenotype with temper tantrums, obsessive–compulsive characteristics and sometimes psychiatric disturbance are common findings. Motor milestones and language development are delayed. In both sexes hypogonadism is present and manifests as genital hypoplasia, incomplete pubertal development, and infertility in most cases. The vast majority of individuals with PWS have mild to moderate mental retardation. A non-insulin-dependent diabetes mellitus often occurs in obese individuals. Consensus diagnostic clinical criteria for PWS have been developed by Holm et al. [1993]. However, revised criteria, designed to trigger diagnostic testing, were published based on the frequency of criteria in molecularly proven individuals [Gunay-Aygun et al., 2001].

Angelman Syndrome

AS is characterized by microcephaly, gait ataxia, severe mental retardation, and absent or severely limited speech. In addition, sleep disorder, seizures, and a characteristic electroencephalography (EEG) appearance with striking high voltage slow-wave activity are frequent findings. Affected individuals exhibit unique behaviors with an apparent happy demeanor that includes inappropriate laughter and excitability. Developmental delay is first noted around 6 months of age but development does progress. Diagnostic consensus criteria for AS have been developed [Williams et al., 1995, 2006] and are helpful in understanding the spectrum of abnormalities in AS and in deciding which individuals are appropriate candidates for genetic testing.

STRUCTURE AND ORGANIZATION OF THE IMPRINTED DOMAIN IN THE 15q11q13 REGION

The chromosomal region 15q11q13 contains a cluster of imprinted genes, which are expressed either from the paternal or maternal chromosome only (Fig. 1). The paternally expressed genes are located in the more centromeric part of the region. These are *MKRN3*, *MAGEL2*, *NDN*, *C15orf2*, *SNURF-SNRPN*, and the C/D box small nucleolar (sno-)RNA genes *SNORD 107* (previously *HBII-436*), *SNORD64* (previously *HBII-13*), *SNORD108* (previously *HBII-437*), *SNORD109A* (previously *HBII-438A*), *SNORD116* (previously *HBII-85*), *SNORD115* (previously *HBII-52*), and *SNORD109B* (previously *HBII-438B*). *SNORD115* and *SNORD116* are present as multi copy gene clusters, whereas the other snoRNA genes are single copy genes. In contrast to other C/D box snoRNAs, which are usually involved in the modification of ribosomal RNAs, these snoRNAs do not have a region complementary to ribosomal RNA and might be possibly involved in the modification of mRNAs, probably by modulating alternative splicing [Cavaille et al., 2000; Kishore and Stamm, 2006; Bazeley et al., 2008]. Paternal-only expression of *MKRN3*, *NDN*, and *SNURF-SNRPN* is regulated by parent-of-origin-specific DNA methylation of the promoter regions of each gene. Whereas the active paternal allele is unmethylated, the inactive maternal allele is methylated. Parent-of-origin-specific DNA methylation can be used to confirm the clinical diagnosis of PWS and AS patients with a deletion of 15q11q13, uniparental disomy, and an imprinting defect (see Diagnostic Testing). Although *C15orf2* has been reported to be biallelically expressed in testis, in fetal brain expression was found to be restricted to the paternal allele [Farber et al., 2000; Wawrzik et al., 2010].

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The most complex gene in the 15q11q13 region is *SNURF-SNRPN*. The original gene, found to consist of 10 exons, encodes 2 different proteins. Exons 1–3 encode SNURF, a small polypeptide of unknown function [Gray et al., 1999], while exons 4–10 encode SmN, a spliceosomal protein involved in mRNA splicing in the brain [Ozcelik et al., 1992]. Exon 1 and the promoter region overlap with the IC (see below). Many 5′ and 3′ exons of *SNURF-SNRPN* have been identified. These exons have two peculiar features: they do not have any protein coding potential and they occur in many different splice forms of the primary transcript. Alternative transcripts containing

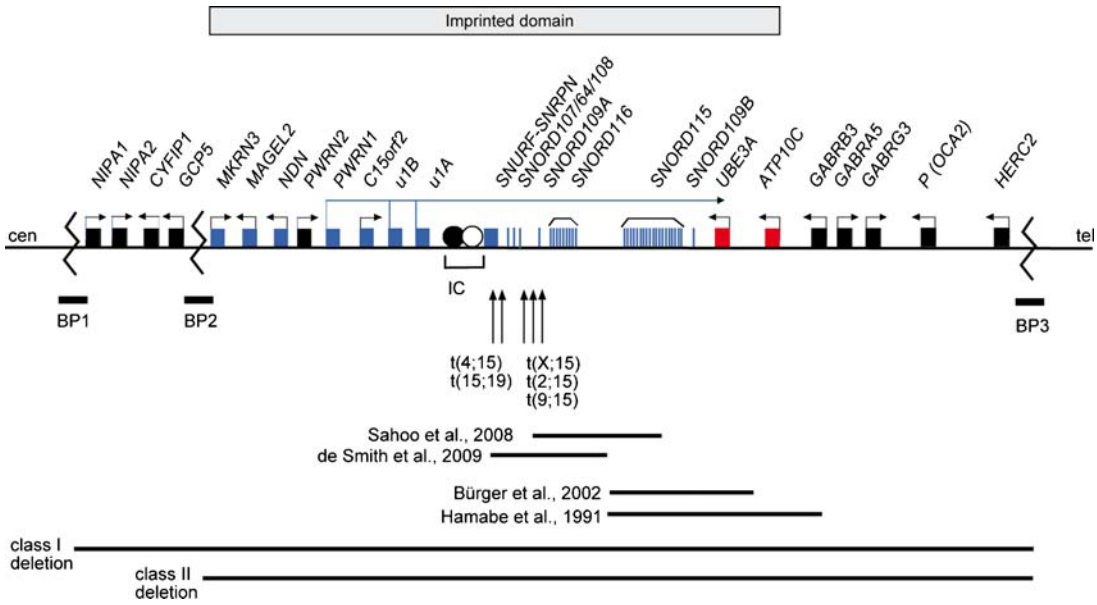

Figure 1. Schematic overview of human chromosomal region 15q11q13. Genes expressed from the maternal chromosome only are drawn as red boxes, genes expressed from the paternal chromosome only are drawn as blue boxes, snoRNAs are drawn as vertical lines, genes expressed from both parental alleles are drawn as black boxes. Orientation of transcription is indicated by horizontal arrows. The two critical IC elements, the AS-SRO and PWS-SRO are drawn as black or open circles, respectively. Class I and class II deletions and atypical familial deletions in patients with AS as well as atypical deletions in patients with PWS affecting the *SNORD116* locus are drawn as horizontal lines. Translocation breakpoints in PWS-like patients with a balanced translocation are indicated by vertical arrows.

novel 5′ exons were described by Dittrich et al. [1996] and characterized in detail by Farber et al. [1999]. The upstream transcripts have three alternative start sites, *u1A*, *u1B*, and *PWRN1* [Dittrich et al., 1996; Farber et al., 1999; Wawrzik et al., 2009]. Additional 3′ exons were described by Buiting et al. [1997] and Runte et al. [2001]. Some of these splice variants are found predominantly in brain and span the *UBE3A* gene in an antisense orientation. The *SNURF-SNRPN* region also serves as a host for all snoRNA genes (Fig. 1). The snoRNA genes are encoded within introns of the *SNURF-SNRPN* gene and lack a direct methylation imprint. Imprinted expression of these genes is indirectly regulated by *SNURF-SNRPN* methylation. They are expressed from the paternal allele only, because they are processed from the paternally expressed *SNURF-SNRPN* sense/*UBE3A* antisense transcript [Cavaille et al., 2000; Runte et al., 2001].

The relative contribution of the genes (reviewed above) to the PWS phenotype is still unclear. Balanced translocations as well as atypical deletions may help to elucidate the contribution of each of these genes. So far, six cases with typical PWS or a PWS-like phenotype and a balanced translocation, involving the *SNURF-SNRPN* locus, have been described [Schulze et al., 1996; Sun et al., 1996; Conroy et al., 1997; Kuslich et al., 1999; Wirth et al., 2001; Schule et al., 2005]. Two translocation breakpoints are located in intron 2, disrupting the *SNURF-SNRPN* coding region. The other breakpoints lie inside exon 17 (one case) or exon 20 within the 3'-untranslated region of the *SNURF-SNRPN* transcription unit. In all these cases the methylation at the *SNURF-SNRPN* locus or expression of the genes centromeric to *SNURF-SNRPN* is not affected. However, a lack of expression of the C/D box snoRNA *SNORD116* genes has been shown in three of the six translocation patients [Wirth et al., 2001; Gallagher et al., 2002; Schule et al., 2005].

Three patients with all the characteristics of PWS, but with atypical deletions that do not include *MKRN3*, *MAGEL2*, and *NDN* have been recently reported [Kanber et al., 2009]. In contrast, these three genes are deleted on the paternal chromosome in a patient with an unbalanced translocation 45,X,der(X)t(X;15)(q28;q11). This patient does not show any of the typical features of PWS, except for obesity and mental retardation, suggesting that these three genes do not play a major role in PWS.

For two other patients with paternally derived atypical deletions between *SNURF-SNRPN* and *UBE3A* it has been demonstrated that deficiency of *SNORD116* snoRNAs can cause key characteristics of the PWS phenotype [Sahoo et al., 2008; de Smith et al., 2009] (Fig. 1). Similar to the six patients with balanced translocations, the deletions in both patients have no effect on methylation at the *SNURF-SNRPN* locus or on expression of the genes centromeric to *SNURF-SNRPN*. Although one of these patients showed most of the major revised clinical criteria including neonatal hypotonia, feeding difficulties, failure to thrive in infancy, hyperphagia, developmental delay, and excessive weight gain in early childhood, he has additional atypical features including

high birth weight, a height at the 95th centile, and a head circumference above the normal range, which are usually not observed in PWS [Sahoo et al., 2008]. This patient was found to have a paternally derived deletion of 175 kb affecting the snoRNA gene *SNORD109A*, the entire *SNORD116* locus and half of the *SNORD115* gene cluster. However, the absence of a PWS phenotype in individuals in previous studies has excluded the *SNORD115* gene cluster from a major role in the PWS phenotype [Runte et al., 2005]. An additional patient, who presented with hyperphagia, obesity, hypogonadism, and other features associated with PWS, has a ~187 kb deletion encompassing all 27 *SNORD116* gene copies, *SNORD109A*, *SNORD108*, *SNORD64*, *SNORD107* with its proximal breakpoint in exon 2 of the *SNURF-SNRPN* gene [de Smith et al., 2009]. Although the centromeric and telomeric extension of both deletions is different, both overlap the *SNORD116* locus. Thus, *SNORD116* is probably a major gene contributing to the PWS phenotype. It is likely, however, that one or more additional genes in the region also contribute to the phenotype.

Based on the finding of point mutations in patients with AS, *UBE3A* has been identified as the critical gene leading to the AS phenotype. In contrast to the paternally active genes, the maternally active *UBE3A* gene lacks differential DNA methylation. Another striking difference is that imprinted *UBE3A* expression is tissue specific and is restricted to certain cells in the brain. At present it is unclear how tissue-specific imprinting of *UBE3A* is regulated, but the paternally expressed *SNURF-SNRPN* sense/*UBE3A* antisense transcript may be involved in silencing the paternal *UBE3A* allele [Rougeulle et al., 1998; Chamberlain and Brannan, 2001]. As shown by Runte et al. [2001], the *UBE3A* antisense RNA is the 3′ end of the *SNURF-SNRPN* transcript.

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So far, there is no evidence that *ATP10C* plays a role in AS. *ATP10C*, which maps ~200 kb telomeric to *UBE3A*, was first reported to be expressed from the maternal chromosome only in brain [Herzing et al., 2001; Meguro et al., 2001]. Similar to *UBE3A*, the promoter region of this gene is completely unmethylated. However, in a recent study, imprinted expression could not be detected in all individuals tested; indicating that imprinted expression in brain is polymorphic [Hogart et al., 2008]. In contrast to *UBE3A*, *ATP10C* is not imprinted in mice [Dubose et al., 2009].

GENETIC LESIONS

PWS and AS can result from various chromosome 15q11q13 alterations including a typical 5–7 Mb de novo deletion, uniparental disomy of chromosome 15, an imprinting defect, or in cases of AS from, a mutation of the *UBE3A* gene (Fig. 2). In contrast to PWS, ~10–15% of patients suspected of having AS have a genetic defect of unknown nature.

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In rare familial cases of AS, atypical deletions affecting either all or part of the *UBE3A* gene have been reported [Hamabe et al., 1991; Burger et al., 2002; Boyes et al., 2006] (Fig. 1). In such families, the mothers may carry the same deletion as the affected children and therefore are at 50% risk for having another child with AS.

De Novo Interstitial Deletions of the Chromosomal Region 15q11q13

An ~5–7 Mb de novo interstitial deletion of the proximal region of chromosome 15 [del(15)(q11-q13)], which includes the entire imprinted domain plus several non-imprinted genes, is found in the majority (~70%) of patients with PWS and AS. The deletions are fairly common, occurring at a frequency of about 1/10,000 newborns. In PWS, the deletion is always on the paternal chromosome whereas in AS the deletion is always on the maternal chromosome. In some patients, the region is deleted as the result of an unbalanced translocation.

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The deletions result from non-homologous recombination events mediated by 250–400 kb repetitive sequence

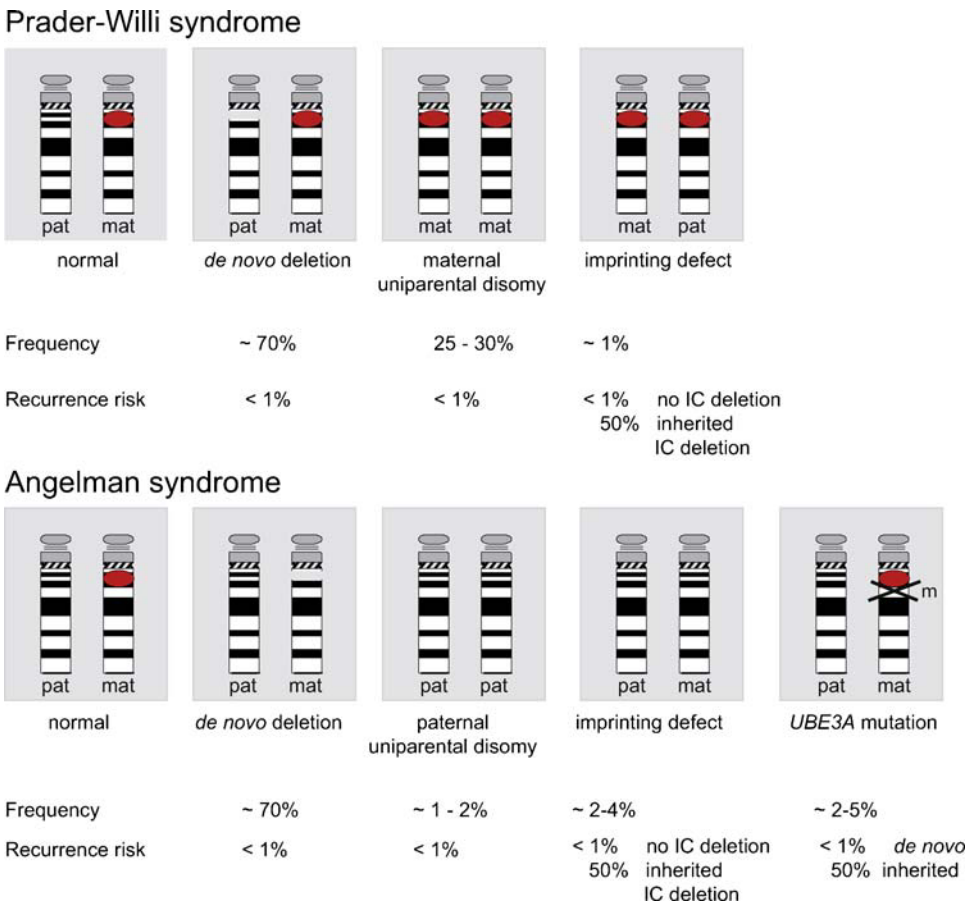

Figure 2. Molecular defects in PWS and AS. The frequency of the underlying defect in both disorders and the recurrence risk are given below. Methylation of paternally expressed genes is indicated by a red dot. Mat, maternal; pat, paternal.

blocks which define the common breakpoint regions BP1-3 [Christian et al., 1998, 1999; Amos-Landgraf et al., 1999]. At the molecular level, usually two classes of deletions (class I and II) can be distinguished, one spanning from breakpoint 1 (BP1) to breakpoint 3 (BP3) and the other from breakpoint 2 (BP2) to BP3 (see Fig. 1). In rare cases patients can harbor larger deletions extending telomeric beyond BP3. The breakpoints involved in these large deletions are the less common distal breakpoints BP4 and BP5 [Sahoo et al., 2007]. A precise localization of the deletion breakpoints and the determination of the deletion sizes have been performed by array-CGH analysis during the last few years [Sahoo et al., 2005, 2007; Butler et al., 2008]. Carrozzo et al. [1997] and Robinson et al. [1998] demonstrated that the deletions can occur via cross-over events between the two homologous chromosomes 15 (interchromosomal) or between different regions of one chromosome 15 (intrachromosomal). The recurrence risk is very low, if the parents have normal chromosomes.

Uniparental Disomy of Chromosome 15

The second most common genetic abnormality in PWS (~25–30%) is a maternal uniparental disomy of chromosome 15 [upd(15)mat], which most often arises from maternal meiotic non-disjunction followed by mitotic loss of the paternal chromosome 15 after fertilization. Upd(15)mat leads to the lack of expression of imprinted genes that are active on the paternal chromosome only. Paternal uniparental disomy 15 [upd(15)pat], resulting in loss of an active maternal *UBE3A* gene copy, occurs in ~2–5% of AS cases, but since almost all cases are isodisomic, the likely origin is maternal non-disjunction with postzygotic duplication of the

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sperm-derived chromosome 15 [Robinson et al., 2000]. The recurrence risk for uniparental disomy is very low when parental chromosomes are normal.

UBE3A Mutations

Mutations in the *UBE3A* gene were first described by Kishino et al. [1997] and Matsuura et al. [1997] and are present in about 10% of the patients with AS. *UBE3A* encodes an E3 ubiquitin ligase involved in the ubiquitination pathway of protein degradation. Mutations can be identified in 20% of sporadic patients with normal methylation and in around 75% of familial patients. Mutations affecting the maternal allele have been found throughout the entire coding region with clusters in exons 9 and 16; exon 16 contains a highly conserved HECT domain. Frameshift, nonsense, and splice site mutations have been identified but missense mutations have also been reported. The majority of mutations are de novo and only ~20% of mothers carry the same mutation [Clayton-Smith and Laan, 2003]. A mother carrying such a mutation has a 50% recurrence risk for another child with AS. In cases with a de novo mutation of *UBE3A*, the recurrence risk is low. However, germ line mosaicism was described in several families with affected siblings but without a mutation in the mother [Malzac et al., 1998].

Imprinting Defects

In some patients (~1–3% in PWS and 2–4% in AS) the disease is due to an imprinting defect. In patients with PWS and an imprinting defect, the paternal chromosome carries a maternal imprint leading to gene silencing of the paternally expressed genes in the chromosomal region 15q11q13. In patients with AS, the maternal chromosome carries a paternal imprint leading to silencing of the *UBE3A* gene.

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Imprinting Defect Caused by an Imprinting Center Deletion

The identification of small deletions in a subgroup of patients (8–15%) with an imprinting defect has led to the definition of a bipartite IC that regulates in *cis* imprint resetting and imprint maintenance in the whole chromosome 15q11q13 imprinted domain [Sutcliffe et al., 1994; Buiting et al., 1995]. To date, ~21 IC-deletions in patients with PWS and 13 IC-deletions plus one inversion in patients with AS and an imprinting defect have been identified [Sutcliffe et al., 1994; Saitoh et al., 1996; Schuffenhauer et al., 1996; Ohta et al., 1999a,b; Bielinska et al., 2000; Buiting et al., 2000; McEntagart et al., 2000; Ming et al., 2000; El-Maarri et al., 2001; Raca et al., 2004; Camprubi et al., 2007; Newkirk et al., 2008; Ronan et al., 2008; Buiting et al., unpublished work]. Two smallest regions of overlap (SRO) define two critical elements in the IC region, the AS-SRO and the PWS-SRO [Buiting et al., 1995]. The PWS-SRO is 4.3 kb in size and overlaps with the *SNURF-SNRPN* exon1/promoter region [Ohta et al., 1999b]. The IC element affected by these deletions is required for the maintenance of the paternal imprint during early embryonic development [Bielinska et al., 2000; El-Maarri et al., 2001].

Approximately 50% of microdeletions found in patients with PWS are familial mutations. The deletions are without any phenotypic effect when transmitted through the female germ line, but lead to an incorrect, maternal imprint on the paternal chromosome when transmitted through the male germ line (see Fig. 3). Thus, in some families only a few, sometimes distantly related

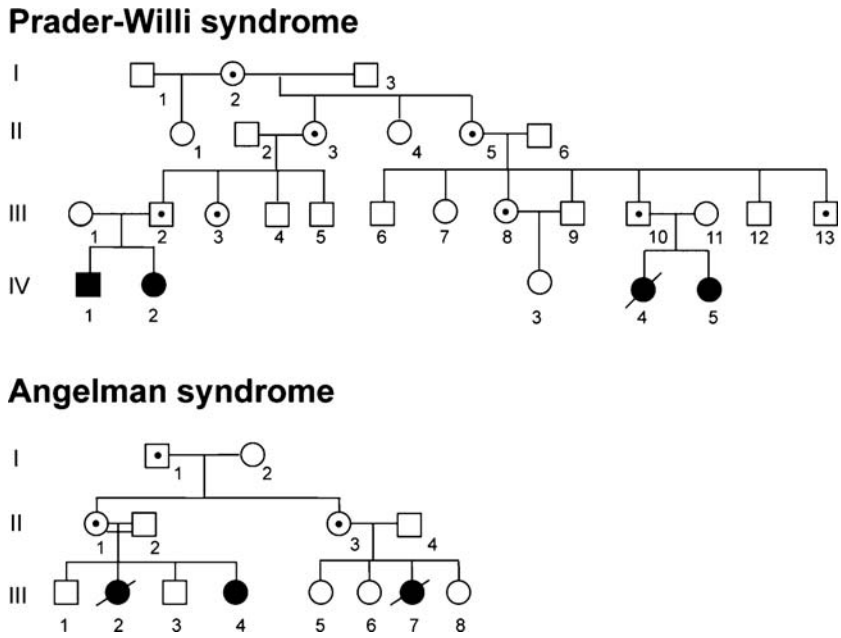

Figure 3. Segregation of imprinting center (IC) deletions. Unaffected deletion carriers are indicated by a dot. A deletion of the PWS-SRO in the PWS family [PWS-AT, El-Maarri et al., 2001] is silent when transmitted through the female germ line. After transmission through the male germ line the deletion chromosome acquires a maternal methylation imprint in early embryogenesis leading to PWS. Thus, distantly related family members may be affected, although their parents are not affected. Note that the individual III-13 is at 50% risk for a child with PWS, whereas individuals III-3 and III-8 are not. In contrast, an IC deletion of the AS-SRO in the AS family is silent when transmitted through the male germ line, but prevents the establishment of a maternal imprint when transmitted through a female, leading to AS.

family members can be affected. Familial IC deletions are associated with a 50% recurrence risk. In the case of a de novo deletion, the recurrence risk is not increased if it occurs after fertilization, but it can be increased if the father has germ line mosaicism.

In contrast to the PWS IC deletions, none of the IC deletions found in patients with AS affects the *SNURF-SNRPN* promoter/exon 1 region. The smallest region of overlap in patients with AS and an IC deletion (AS-SRO) is 880 bp in size and maps ~35 kb proximal to *SNURF-SNRPN* exon 1 [Buiting et al., 1999; Horsthemke and Buiting, 2008]. Two out of the 13 IC deletions described so far have occurred de novo on the maternal chromosome, but in most of the cases they have been inherited from the mother [Horsthemke and Buiting, 2008]. The deletions are without any phenotypic effect when transmitted through the male germ line, but lead to an incorrect paternal imprint when transmitted through the female germ line. It appears that the AS-SRO has an important role in the establishment of the maternal imprint in the female germ line; possibly by interacting with the PWS-SRO and that a deletion of this element prevents maternal imprinting of the deletion chromosome. There is only one familial case where the imprinting error is not due to an IC deletion but the result of an inversion spanning ~1.5 Mb with a breakpoint inside the IC [Buiting et al., 2001]. As a consequence, the IC is disrupted and the AS-SRO has been removed from the PWS-SRO to the proximal border of the imprinted domain in an inverted orientation. When this inversion is transmitted through the female germ line, it prevents maternal imprinting in the whole domain, suggesting that close proximity and/or the correct orientation of the AS-SRO and the PWS-SRO are necessary to establish a maternal imprint [Buiting et al., 2001].

Imprinting Defects Without Imprinting Center Deletions

IC deletions are found only in a small fraction of patients with PWS or AS and an imprinting defect. In the vast majority of patients (85% in PWS and 92% in AS) the imprinting defect represents a primary epimutation [Buiting et al., 2003; Horsthemke and Buiting, 2008]. Such epimutations can occur during imprint erasure in primordial germ cells, imprint establishment during later stages of gametogenesis, or imprint maintenance after fertilization. If it occurs in the germ line, all cells of the patient are affected. If it occurs after fertilization, it results in somatic mosaicism [see Horsthemke, this issue].

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In AS patients with a primary epimutation, the maternal chromosome carrying an incorrect paternal imprint is inherited either from the maternal grandfather or from the maternal grandmother [Buiting et al., 1998, 2003]. This finding suggests that the imprinting defect occurred after erasure of the parental imprints and results from an error in imprint establishment or imprint maintenance (see below). In contrast, in PWS patients with a primary epimutation, the paternal chromosome carrying an incorrect maternal imprint is always derived from the paternal grandmother [Buiting et al., 2003], suggesting that the incorrect imprint in the PWS patients results from a failure of the paternal germ line to erase the grandmaternal imprint (epigenetic inheritance).

Somatic mosaicism in patients with PWS and an imprinting defect appears to be very rare. Only three cases have been described to date [Buiting et al., 2003; Wey et al., 2005]. These patients were found to have a reduced proportion of non-methylated *SNURF-SNRPN* alleles in peripheral blood DNA. In the patient described by Wey et al. [2005], the critical paternal 15q11q13 region in this proband was found to be inherited from the paternal grandmother, compatible with the findings in all the other informative PWS imprinting defects cases and indicative of an error in imprint erasure during early spermatogenesis [Buiting et al., 2003]. However, if the imprinting defect in this proband also resulted from such an error, it would be difficult to explain why it is present in a mosaic form. Since somatic mosaicism typically results from a postzygotic event, a more likely explanation would be that in such cases the imprinting defect occurred after fertilization, when the genome undergoes massive epigenetic reprogramming and that possibly the protection against remethylation occasionally fails so that in one cell the paternal *SNURF-SNRPN* allele is methylated.

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The postzygotic loss of the maternal imprint [imprint maintenance error, for details see Horsthemke, this issue] is a significant cause of AS, since, in contrast to PWS, more than 40% of AS patients with an imprinting defect are found to have somatic mosaicism [Buiting unpublished work] These patients were found to have a small amount of methylated alleles, as they show a weak maternal band in methylation analysis for the *SNURF-SNRPN* locus using various techniques. Nazlican et al. [2004] have shown that the faint band reflects somatic mosaicism, that is, a mixture of normal cells and cells with an imprinting defect. Furthermore, Nazlican et al. could show by studying the X inactivation patterns in fibroblast clones of one such patient that the imprinting defect occurred very early in the embryo prior to the blastocyst stage. In the same

study, 24 AS patients with a somatic mosaic were investigated for the degree of somatic mosaicism. The percentage of normally methylated cells was found to range from <1% to 40%. Patients with a higher percentage tended to have milder clinical signs, but the correlation was not statistically significant [Nazlican et al., 2004].

GENOTYPE–EPIGENOTYPE–PHENOTYPE CORRELATIONS

In addition to imprinted genes, several non-imprinted genes are located in the chromosomal region affected by the common large deletions in 15q11q13 (Fig. 1). These genes may modify the PWS and AS phenotype, and some are responsible for other genetic disorders.

In addition to imprinted genes, several non-imprinted genes are located in the chromosomal region affected by the common large deletions on chromosome 15q11q13. These genes may modify the PWS and AS phenotype, and some are responsible for other genetic disorders.

One of these genes is the oculocutanous albinism type II (*OCA2*) gene. Hypopigmentation is a frequent finding in PWS and AS patients with a common large deletion. This non-imprinted phenotype is associated with the deletion of one OCA2 gene copy and may be caused by a gene doses effect [Spritz et al., 1997].

Typical facial appearance or skill with jigsaw puzzles are reported less frequently in individuals with PWS and upd(15)mat [Gillessen-Kaesbach et al., 1995; Cassidy et al., 1997], and they have been described to have somewhat higher verbal IQ compared to those with a common large deletion of the 15q11q13 region [Dykens and Rosner, 1999; Dykens, 2002]. The frequency of psychosis and autism is however significantly higher in cases with upd(15)mat [Veltman et al., 2004; Vogels et al., 2004; Whittington et al., 2004]. Poor adaptive behavior, intellectual ability, compulsions, and other subtle differences between individuals with a class I and class II deletion are suggested from other reports [Roof et al., 2000; Butler et al., 2004; Milner et al., 2005; Varela et al., 2005].

Genotype–phenotype analysis of the molecular subclasses of AS have shown that individuals with AS and a common large deletion of the chromosomal region 15q11q13 are most severely affected. Haploinsufficiency for co-deleted genes that are not imprinted may contribute to a more severe AS phenotype, and *GABRB3* may have a role in the susceptibility to severe seizures in conjunction with *UBE3A* deficiency [Minassian et al., 1998]. Among the deletion cases there is a higher frequency of seizures, microcephaly, dysmorphic features, delayed motor milestones, and language impairment compared to those with upd(15)pat or an imprinting defect. In contrast, obesity is a more common finding in the latter cases. Individuals with a *UBE3A* mutation frequently have seizures and microcephaly but have better motor and communication skills compared to individuals with a deletion. A higher frequency of obesity when getting older for this group has been reported by Lossie et al. [2001]. Atypical phenotypes have been described in individuals with AS and a complete or mosaic imprinting defect [Gillessen-Kaesbach et al., 1999; Nazlican et al., 2004]. These patients present with muscular hypotonia at birth and obesity, clinical signs reminiscent of PWS [Gillessen-Kaesbach et al., 1999].

Individuals with PWS or AS and a class I deletion (Fig. 1), but not those with a class II deletion, are hemizygous for four non-imprinted genes located between the deletion breakpoint cluster regions BP1 and BP2. These are the genes *NIPA1*, *NIPA2*, *CYFIP1*, and *GCP5* [Chai et al., 2003]. Heterozygous mutations in *NIPA1* lead to autosomal dominant spastic paraplegia [Rainier et al., 2003]. Since spastic paraplegia has never been observed in PWS or AS and a class I deletion, it is likely that a mutant protein and not reduced gene dosage leads to the disease. For the genomic region containing these four genes, copy number variation has been observed, attributing to deletions and duplications which complicates the definition of class I and class II deletions. This copy number variation has been found in healthy individuals and seems to be non-pathogenic [Jiang et al., 2008]. However, it cannot be excluded as contributing to the phenotype in some individuals. Clinical differences within individuals with PWS and a class I deletion compared to those with a class II deletion has been described by Butler and collegues [Butler et al., 2004; Bittel et al., 2006]. Patients with a class I deletion seem to have generally more behavioral and psychological problems than individuals with the class II deletion. Doornbos et al. [2009] reported evidence for an association between behavioral disturbances in nine individuals with a deletion affecting the region between BP1 and BP2. Doornbos and collegues discussed a possible pathogenic nature of this deletion with an obviously incomplete penetrance, since the deletion is often inherited from a healthy parent.

DIAGNOSTIC TESTING

The most sensitive approach in diagnosing PWS and AS is to study DNA methylation in particular at the *SNURF-SNRPN* locus. The promoter/exon 1

The most sensitive approach to diagnosing for PWS and AS is to study DNA methylation in particular at the SNURF-SNRPN locus.

region of *SNURF-SNRPN* is unmethylated on the paternally expressed allele and methylated on the maternally

repressed allele. Normal individuals have both a methylated and an unmethylated allele. By methylation analysis, de novo deletions of the chromosomal region 15q11q13, uniparental disomy, and imprinting defects can be detected for both PWS and AS disorders. In cases with PWS, only a maternally methylated allele can be detected, whereas in cases with AS and one of the above-mentioned molecular defects, only a paternally unmethylated allele can be detected.

A normal methylation pattern makes the diagnosis of PWS highly unlikely, since in more than 99% of cases the disease is caused by one of the above-mentioned molecular defects. In contrast, only 70–75% of patients with AS can be detected by methylation analysis. In patients with suggestive clinical features and a normal methylation pattern, it is recommended to perform U*BE3A* mutation screening as mothers of patients with a mutation in *UBE3A* will have a recurrence risk of up to 50% depending on their carrier status.

Depending on the method used for methylation analysis, further investigations may be necessary to determine the underlying defect in order to estimate the recurrence risk. One approach is to study the DNA methylation pattern at one position in the *SNURF-SNRPN* promoter/exon 1 region by methylation-specific PCR. This method is based on sodium bisulfite treatment of DNA followed by PCR using primers specific for methylated and unmethylated alleles [Kubota et al., 1997; Zeschnigk et al., 1997]. This approach will confirm a diagnosis but cannot provide any further information with regard to the underlying defect. Fluorescence in situ hybridization and/or microsatellite analysis are necessary to find out more about the molecular subclass.

Efficient testing is first provided by the use of methylation sensitive multiplex ligation-dependent probe amplification (MLPA, ME028-B1 kit, MRC, Holland) of the chromosome 15q11q13 region. This test provides a simultaneous analysis of methylation pattern and gene dosage at several sites across the region. This approach will confirm the diagnosis and further identify the presence of a de novo deletion of the chromosome 15q11q13 region which is the most common molecular defect in PWS and AS. However, this method does not allow one to distinguish between uniparental disomy and an IC defect. Of note, IC deletions, which can be associated with a 50% recurrence risk, are directly identified using the MLPA method.

A karyotype is important for determining recurrence risk in some cases of PWS/AS. In cases with a de novo deletion or uniparental disomy the recurrence risk is very low (<1%) when parental chromosomes are normal. Cases with de novo deletions should be further investigated by cytogenetic analysis to rule out the presence of an unbalanced translocation. Furthermore, rare cytogenetic rearrangements in the parents that may predispose to a deletion should be excluded by molecular cytogenetic analysis. In cases with uniparental disomy, a Robertsonian translocation in the appropriate parent, which increase the recurrence risk should be excluded by cytogenetic analysis.

DIFFERENTIAL DIAGNOSES

PWS

One of several disorders that can strongly resemble PWS presenting with neonatal hypotonia and later onset obesity is upd(14)mat, which can be caused by uniparental disomy 14, imprinting defects or deletions affecting the imprinted *DLK1-MEG3* locus in the chromosomal region 14q32. A number of other conditions associated with obesity and developmental disability including Cohen syndrome, Bardet–Biedl syndrome, Alstrom syndrome, and the 1p36 microdeletion syndrome should be considered.

AS

A differential diagnosis which should be considered in girls with features of AS is Rett syndrome. In infants it can be difficult to distinguish between AS and Rett syndrome because of overlapping features such as microcephaly and ataxia.

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The Mowat–Wilson syndrome is caused by mutations affecting the *ZFHX1B* gene on chromosome 2, is associated with severe mental retardation, microcephaly, seizures, short stature, and characteristic facial features that resemble those of AS. Mutations of the *SLC9A6* have been reported in patients with an AS-like phenotype. Further, single gene conditions mimicking AS are the ATR-X syndrome, Gurrierie syndrome and methylenetetrahydrofolate reductase (MTHFR) deficiency. Another differential diagnosis with an AS-like condition is the 22q13 microdeletion syndrome. There are many other microdeletions and chromosome abnormalities with a wide spectrum of clinical features that overlap with AS [Williams et al., 2001].

CONCLUSIONS

PWS and AS, two distinct neurogenetic disorders with different clinical presentations were the first known examples of human diseases involving imprinted genes. In both syndromes, imprinted genes located in the chromosomal region 15q11q13 are affected. De novo deletions of proximal 15q, uniparental disomy and an imprinting defect are common genetic lesions in both disorders, leading to the loss of maternal (AS) or paternal contribution (PWS) of the imprinted domain in chromosome 15q11q13. In contrast to other imprinting diseases, that is, Beckwith–Wiedeman syndrome (chromosome 11p15), Silver–Russell syndrome (chromosome 11p15) or patients with transient neonatal diabetes mellitus (chromosome

6q24), where imprinting defects account for the majority of patients, only in a small number of patients with PWS and AS (1–4%) the disease is caused by an imprinting defect. The high incidence of large de novo deletions in PWS and AS and the relative low frequency of an epimutation in these syndromes are likely due to the fact that the chromosomal region 15q11q13 contains low copy repeats that are targets of non-homologous recombination. Such repeats are not known to flank the imprinted domains on chromosomes 11p15 or 6q24.

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