

## Genetics of Childhood Disorders: XII. Genomic Imprinting: Breaking the Rules

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Genomic imprinting refers to a process that distinguishes paternally derived chromosomes from maternally derived chromosomes. Imprinting plays a critical role in gene expression, mammalian development, and human disease. However, the biological requirement for imprinting remains a mystery. In the first 2 columns on the topic, we will review how imprinting was initially identified, present some hypotheses about the mechanisms of imprinting, and speculate on the evolutionary forces maintaining this phenomenon. The subsequent 2 columns will discuss the molecular bases for 2 disorders in which imprinting is involved, namely, Prader-Willi and Angelman syndromes.

Genomic imprinting might be considered the exception that proves the rule. Until the late 1980s, Gregor Mendel's laws of inheritance were thought to be inviolate. All autosomal genes were believed to be expressed equally, regardless of whether they were inherited from the mother or the father. For most genes, this is true. However, it is now recognized that a small subset of genes are violators of Mendel's laws and are expressed differently depending on the parent from whom they are inherited.

Genomic imprinting refers to the normal process whereby specific genes or DNA segments are reversibly modified during gametogenesis in a parent-specific fashion. Although research on exactly how this occurs is not yet understood, one modification that is believed to play a role is the reversible addition of methyl groups to specific cytosine residues within the DNA sequence, a process that occurs differently in generation of the egg and the sperm. Genomic imprinting is called an epigenetic phenomenon, since the gene structure—the actual sequence of nucleotides—is not affected as occurs during the mutations that were discussed in previous columns. Rather, the “imprint” is erased during gametogenesis and must be reapplied in a gender-specific manner. For example, in the normal situation, a methylated gene inherited by a male from his mother will be unmethylated during spermatogenesis, and this unmethylated gene, when passed on to his daughter, must be remethylated during oogenesis.

As a result of this differential methylation, the maternally inherited copy of an imprinted DNA segment differs from the paternally inherited copy. What has recently been discovered is that these differences may also be reflected in differences in gene expression, even though the nucleotide sequences of the 2 segments are identical. The imprinting process leads to an inactivation of either the paternally or maternally inherited

copy of some genes within some of the body's cells. Genetic mutations that change this pattern and lead to either increased or decreased expression of imprinted genes may upset the normal amounts of proteins that are expressed.

The discovery of genomic imprinting as a normal mechanism of genetic regulation has provided dramatic insights into previously puzzling human diseases. Within the past decade, mutations of imprinted genes on several different chromosomes have been found to cause a wide range of phenotypic effects. This genetic mechanism underlies 3 well-known genetic disorders, namely, Prader-Willi, Angelman, and Beckwith-Wiedemann syndromes.

Prader-Willi and Angelman syndromes were the first disorders recognized as occurring because of genomic imprinting. Both disorders affect approximately 1 in 10,000 persons. Prader-Willi syndrome is a multisystem disorder characterized by neonatal hypotonia and failure to thrive, followed by early childhood-onset hyperphagia and resultant obesity. Symptoms also include short stature, typical facial features, small hands and feet, and hypogonadotropic hypogonadism. Mild mental retardation is present as is a characteristic neurobehavioral profile with temper tantrums, obsessive-compulsive disorder, and occasionally psychosis. Hypothalamic dysfunction is thought to underlie many of the major features of this disorder.

In contrast, Angelman syndrome is a disorder featuring microcephaly, severe mental retardation, minimal or absent speech, hyperactivity, sleep disturbance, seizures, hypertonia, and gait ataxia. Often described are characteristic jerky movements, a flexed arm posture, and a happy disposition with frequent inappropriate outbursts of laughter.

The first insight into the causation of Prader-Willi syndrome and Angelman syndrome came in the 1980s. Both conditions were noted to have a cytogenetically detectable deletion involving the proximal long (q) arm of one copy of chromosome 15. Curiously, the chromosome 15 deletions seen in both patient groups seemed to involve the identical chromosomal region, extending from bands 15q11 to 15q13.

That 2 such strikingly diverse phenotypes could be caused by the same chromosomal deletion was unexplainable by classical concepts of inheritance. In the late 1980s, new molecular genetic techniques resolved this apparent paradox by revealing that the chromosome 15 deletions in these syndromes differed in a fundamental way, namely, by their parent of origin. The

deletions occurring in patients with Prader-Willi syndrome were found to involve the paternally inherited copy of chromosome 15, while those in Angelman syndrome involved the same region but in the maternally inherited copy. In both cases, the relevant parents had normal chromosomes themselves.

This parent-of-origin effect on the phenotype is now known to be attributable to the phenomenon of genomic imprinting. The chromosome 15q11-q13 region contains multiple genes that are differentially expressed, depending on the parent of origin. As was mentioned, one mechanism that is thought to be involved is methylation within the chromosomal region that prevents the expression of the relevant gene(s). The extent of methylation is under the control of a specific DNA sequence within this region called the imprinting center. Other mechanisms that may play a role in the differential expression of genes will be discussed in the subsequent column.

Although it is not yet known which genes are critical for the phenotypic effects in Prader-Willi syndrome, it is clear that this disorder occurs when there is loss of expression of genes in the 15q11-q13 region that are normally expressed only from the paternally inherited copy of the chromosome. While the same genes are also present on the maternally inherited copy, they are not expressed. It is now known that this loss of paternal gene expression can occur by 3 different genetic mechanisms—deletion, uniparental disomy, and mutations to the imprinting center (Fig. 1).

In approximately 70% of cases, Prader-Willi syndrome is caused by a paternal interstitial chromosome 15 deletion. These sporadically occurring deletions may or may not be microscopically visible by high-resolution chromosome analysis. In about 28% of cases, Prader-Willi syndrome results from maternal uniparental disomy, whereby affected individuals inherit both copies of chromosome 15 from their mother and none from their father, usually as the consequence of abnormal chromosome segregation during meiosis. In these cases, the disorder occurs in spite of the presence of 2 complete and normal copies of chromosome 15, because the relevant genes are silent on both maternally inherited copies and the paternally inherited copy that would be active is missing.

In the remaining cases (2%) of Prader-Willi syndrome, there is an intrinsic abnormality in the mechanism by which imprinting occurs. This sometimes involves a detectable mutation in the imprinting center and can be inherited in an autosomal dominant manner. As a result, both the maternal and paternal copies of chromosome 15 have a maternal imprint, resulting in lack of expression of the paternally inherited genes. In such situations, it appears that the imprint from the paternal grandmother could not be erased in the paternal germline. This is the only genetic category of Prader-Willi syndrome associated with a significant recurrence risk. Rarely, a translocation involving a chromosome break within the 15q11-q13 region will disrupt normal imprinting.

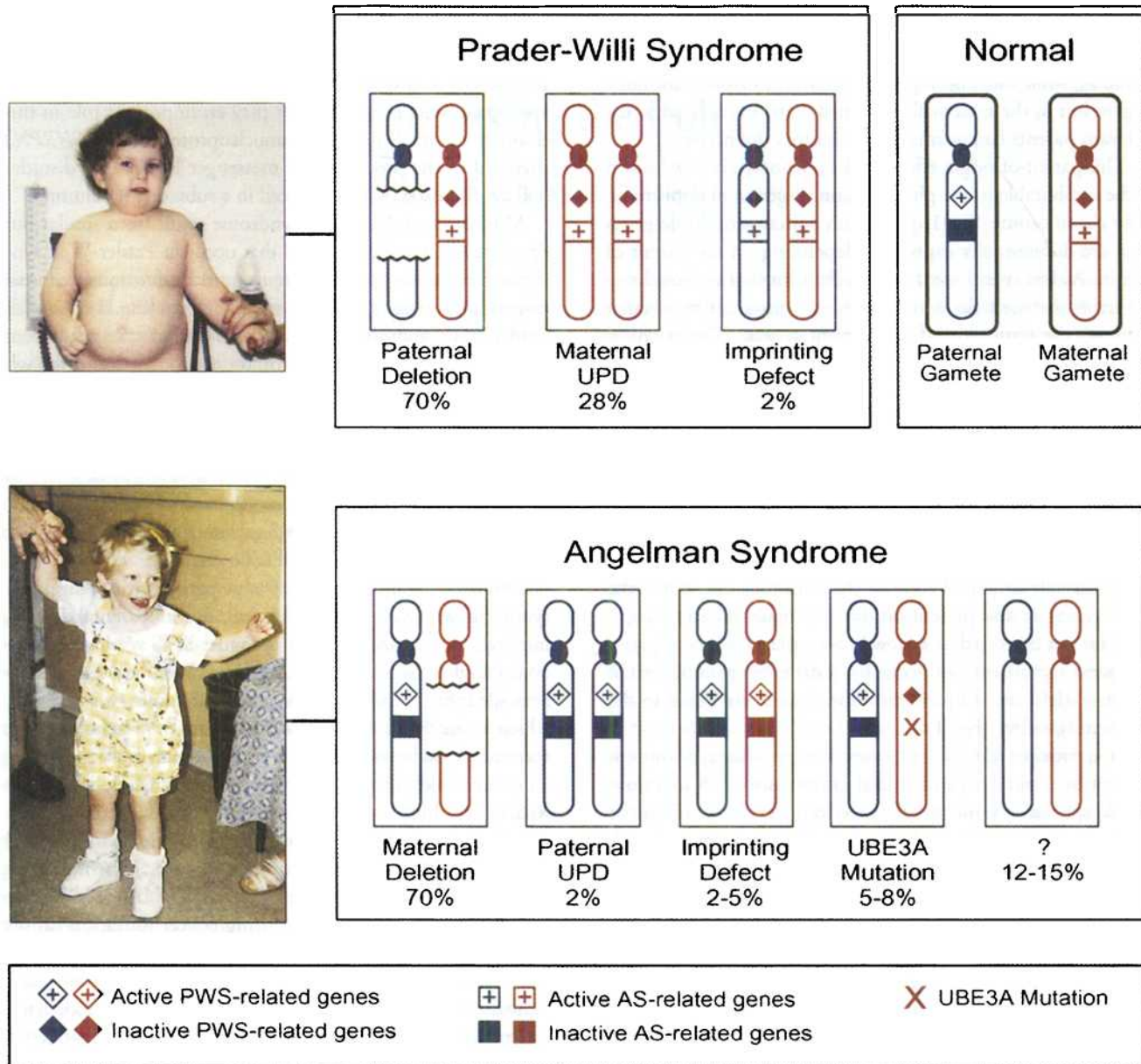
Despite the fact that several maternally imprinted genes have been identified in the 15q11-q13 region, there is currently no consensus as to which of these are critical to Prader-Willi syndrome. One gene that may play an important role in this disorder is a small nuclear ribonucleoprotein (called *SNRPN*) involved in the processing of messenger RNA. This disorder will be discussed in greater detail in a subsequent column.

Most cases of Angelman syndrome result from similar but opposite phenomena to those that occur in Prader-Willi syndrome, namely maternal chromosome 15 deletions, paternal uniparental disomy, or imprinting defects (Fig. 1). As with Prader-Willi syndrome, the majority (70%) of cases result from a visible or submicroscopic chromosome 15 deletion, although in Angelman syndrome the deletion involves the maternally inherited chromosome. Similarly, a small proportion (2%–5%) of cases of Angelman syndrome are due to abnormalities that affect the imprinting process. Only about 2% of cases of Angelman syndrome result from paternal uniparental disomy, probably because abnormal segregation of chromosomes is less likely in the male than in the female germline.

This leaves approximately 25% of patients with Angelman syndrome who have neither deletion, nor uniparental disomy, nor imprinting defects. Unlike in Prader-Willi syndrome, however, a single causative gene for Angelman syndrome has recently been identified. This gene, *UBE3A*, is a ubiquitin-protein ligase, which normally functions to add ubiquitin to proteins to target them for degradation. *UBE3A* is expressed only from the maternally inherited chromosome 15 and is specifically imprinted within the brain. Many, although not all, patients with Angelman syndrome who do not have deletions, uniparental disomy, or imprinting defects have now been shown to have mutations in the maternally inherited copy of *UBE3A*. When a *UBE3A* mutation or an imprinting center mutation causes Angelman syndrome, it is associated with a 50% recurrence risk to the parents of the affected individual. Finally, there are some individuals with Angelman syndrome in whom the underlying genetic mechanism of their disorder has not been identified.

Advances in the understanding of the genetic abnormalities responsible for Prader-Willi syndrome and Angelman syndrome have led to the development of highly sensitive and specific diagnostic tests for these disorders. The one test that can detect Prader-Willi syndrome due to any cause and Angelman syndrome due to all detectable causes except a *UBE3A* mutation is known as methylation analysis. This is a DNA-based test that can distinguish between the maternal methylated and paternal unmethylated copies of a gene within the 15q11-q13 region. An exclusively maternal methylation pattern occurs in individuals with Prader-Willi syndrome, whereas an exclusively paternal pattern is seen in most cases of Angelman syndrome.

If an abnormal methylation pattern is identified, then additional tests are available to determine the specific genetic mechanism underlying either disorder in a given patient. High-



**Fig. 1** Pictured are a 2-year-old boy with Prader-Willi syndrome and a 2-year-old girl with Angelman syndrome. Genes, imprinting patterns, and mutations involving chromosome 15 in both disorders are indicated by the key at the bottom. The relative proportion of each type of genetic abnormality in each syndrome is indicated below a schematic of the abnormality. UPD denotes uniparental disomy. The normal paternal (blue) and maternal (red) copies of chromosome 15 are shown on the upper right. Parents provided written permission to use photographs.

resolution chromosome analysis with a fluorescence in situ hybridization (FISH) probe for a gene within 15q11-q13 (usually *SNRPN*) can identify those cases of Prader-Willi or Angelman syndrome arising from the characteristic chromosome 15 deletion. Chromosome analysis is also a valuable diagnostic test in patients who are being evaluated for Prader-Willi syndrome and Angelman syndrome because sometimes other chromosome anomalies are found to be the cause of their mental retardation and neurobehavioral dysfunction.

Testing known as microsatellite analysis is also available to verify the presence of uniparental disomy by analyzing the pattern of DNA sequence variations on the copy of chromosome 15 inherited from each parent. This testing requires that DNA samples be obtained from the patient and both parents in order to determine whether polymorphisms in the affected individual are present in both parents or only in one. Finally, research-based testing for imprinting center mutations is available for patients with either disorder who have abnormal methylation studies

without evidence of a chromosome 15 deletion or uniparental disomy and for *UBE3A* mutations in patients with suspected Angelman syndrome who have a normal methylation analysis.

Some clinical differences have recently been identified between patients who have uniparental disomy versus those who have a deletion. To a large degree, these differences are thought to result from the loss of other, nonimprinted genes in patients who have deletions.

Beckwith-Wiedemann syndrome is another genetic disorder related to genomic imprinting. It is an overgrowth condition featuring large body size, large tongue, omphalocele, organomegaly, neonatal hypoglycemia, and a predisposition to embryonic tumors, especially Wilms tumor. Although the molecular basis of this disorder is not fully understood, it is known to arise from abnormal dosage of specific imprinted genes located on chromosome 11p15, including an insulin-like growth factor gene. Several different genetic mutations have been identified as causing Beckwith-Wiedemann syndrome, including chromosomal abnormalities, uniparental disomy, and mutations within a single gene.

Other medical conditions are now recognized to arise from abnormalities of genomic imprinting. For example, a small proportion of cases of Russell-Silver syndrome, a growth disorder leading to very short stature and body asymmetry, results from uniparental disomy of chromosome 7. Similarly, transient neonatal diabetes is caused by uniparental disomy of chromosome 6, and a pattern of multiple anomalies is found in the presence of uniparental disomy of chromosome 14.

Only a small number of human genes have thus far been shown to be imprinted, but discovery of the imprinting mechanism as essential to normal human development represents an important step forward in the genetics of childhood psychiatric illnesses. Indeed, it has helped to broaden the understanding of

genetic disorders that do not follow Mendel's laws of inheritance and has forced an expansion of thinking about the complexities of human development. Finally, it is hoped that research in this area will provide further insights that may ultimately lead to the development of novel treatments for these disorders.

#### WEB SITES OF INTEREST

<http://www.geneimprint.com/>  
<http://www.genes.uchicago.edu/upd/upd.html>  
<http://www.informatik.uni-rostock.de/HUM-MOLGEN/journals/HMG/0014.html>

#### ADDITIONAL READINGS

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