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Mini Review

GENOMIC IMPRINTING AND ITS RELEVANCE TO GENETIC DISEASES

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Summary Genomic imprinting is a biological phenomenon determined by an evolutionally acquired, underlying system that may control harmonious development and growth in mammals. It is also relevant to some genetic disorders in man. In this article, lines of biological evidence of imprinting, characteristics of the mouse and human imprinted genes, and findings and mechanisms on the occurrence of several human imprinting disorders are reviewed.

Key Words genomic imprinting, *IGF2, H19, SNRPN,* imprinting center, Prader-Willi syndrome, Angelman syndrome, Beckwith-Wiedemann syndrome

Introduction

Genomic imprinting is defined as an epigenetic process by which male and female germlines confer a parent-of-origin specific mark or modification (primary imprint) on a chromosome region, so that each of parental alleles is differentially expressed in offspring. Although equivalency of each parental allele is one of the principles of the Mendel law, genomic imprinting is an exception to the rule. The modification is valid with generations, *i.e.*, another mark is made in the next generation. In this view, the modifications differ from gene mutations. Genes or a chromosomal regions modified are referred to as imprinted genes or regions. Expressions of such genes are usually suppressed (or inactivated), and inactivation of the paternal allele is "paternal imprinting" (in other words, maternal expression) and that of the maternal allele "maternal imprinting" (paternal expression).

Evidence of Genomic Imprinting

Mammalian parthenogenotes never reach birth and generally represent unique phenotypes. Hydatidiform mole, the human androgenote that is composed of two copies of the paternal genome (Kajii and Ohama, 1977), develops only the placental tissue without an embryo, while benign ovarian teratoma, the human

gynogenote consisting of two copies of the maternal genome, has only the embryonic tissue lacking the placenta. Similar findings came from pronucleus transplantation experiments in the mouse. In androgenotes of this species, development of the placenta and yolk sac is nearly normal but that of the embryo is very poor, and reverse results are observed in the gynogenote (Cattanach, 1991). These findings indicate that the parental alleles are differentially expressed in early mammalian development and both alleles are absolutely necessary for normal development.

A status that both homologous chromosomes are inherited from a parent is called uniparental disomy (UPD). The mouse with UPD for a given chromosome segment is made by mating between a parent who has a duplication of the segment and the other parent lacking the same segment. Since a gene dose in such a UPD mouse is not different from that in the wild-type mouse, a phenotype would not be different between the two. However, this complementation rule is not applied to several chromosomal regions. For example, maternal UPD mice for a proximal region of mouse chromosome 11 show growth deficiency, while paternal UPD mice for the same region represent overgrowth (Cattanach and Kirk, 1985). The maternal UPD mouse for a distal region of chromosome 7 shows lethal growth deficiency, and the paternal UPD mouse dies at an earlier stage (Ferguson-Smith *et al.*, 1991). At least 10 imprinting regions from 6 different mouse chromosomes have been identified, and an imprinting map has been made (Cattanach, 1991). In man, 9 (chromosomes 2, 6, 7, 11, 14, 15, 16, 20, and X) of the 16 UPDs observed represent phenotypic effects (Ledbetter and Engel, 1995) (Table 1). The fact that some of these regions show homology to those in the mouse suggests that genomic imprinting is completely or partially conserved in the evolutional process.

More direct evidence for imprinting in the mouse was provided by experiments with hairpin tail mice, T^{hp}. A half of the offspring of a T^{hp} mother are lethal, while those of a T^{hp} father have a hairpin-like short tail. This "T-associated maternal effect, *Tme*" is caused by a DNA deletion on chromosome 17 (Barlow *et al.*, 1991). It was suggested that the presence of a maternal copy of *Tme* is essential for normal development of the mouse embryo, namely, *Tme* is the maternally expressing locus and the paternal *Tme* copy is inactive in offspring.

Identification of Imprinted Genes

At least 14 imprinted genes have been identified in the mouse by means of a variety of searching methods (Table 1). Transgenic mouse experiments contributed to the establishment of the concept of imprinting. Heterozygotes for a mutant insulin-like growth factor II gene (*Igf2*) that is mapped to a distal imprinted region of mouse chromosome 7 showed growth deficiency when the mutant allele came from the father, while those whose mutant allele was derived from the mother represented normal growth, the result indicating that the mouse *Igf2* is a maternally imprinted (or paternally expressing) gene (DeChiara *et al.*, 1991). On the other hand, the gene for the receptor of Igf-II, *Igf2r*, was identified to be paternally

Table 1. Imprinting genes/markers in the human and mouse.

Human					Mouse		
Gene	Location	Polymorphism	Expression	Methylation imprint	Gene	Location	Expression
IGF2R	6q25-27	+	M		*Igf 2r*	17 prox	M
MAS	6q25-27		?		*Mas*	17 prox	P
IGF2	11p15.5		P	+	*Igf 2*	7 dis	P
H19	11p15.5		M	+	*H 19*	7 dis	M
MASH2	11p15.5		?		*Mash 2*	7 dis	M
INS	11p15.5		biparental		*Ins 2*	7 dis	P
$p57^{KIP2}$	11p15.5		?	+	$p57^{KIP2}$	7 dis	M
WT1	11p13	+	M		*Wt 1*	2	biparental
SNRPN	15q11.2-12		P	+	*Snrpn*	7 prox	P
PAR5	15q11.2-12		P				
IPW	15q11.2-12		P				
PAR1	15q11.2-12		P				
ZNF127	15q11.2-12		?	+	*Znf 127*	7 prox	P
					Peg 3	7 prox	P
					Peg1/Mest	6 prox	P
					Ins 1	6 prox	P
					CDC 25Mm	9	P
U2af1-rs1	5, 19, X		biparental ?		*U2af1-rs1*	11 prox	P

imprinted, and this gene is responsible for T^{hp}/*Tme* (Barlow *et al.*, 1991). Thus, *Igf2* and *Igf 2r* is reversely imprinted. Likewise, a maternally expressing gene, *H19* (Bartolomei *et al.*, 1991), and a paternally expressing gene, *Snrpn* (the small nuclear ribonucleoprotein polypeptide N gene) (Leff *et al.*, 1992), were isolated from the mouse chromosome 7. *H19* is highly expressed in the mesoderm and extraembryonic tissues. It is most likely that *H19* plays an important role in the fetal growth and has a tumor suppressor potential. *Snrpn* was isolated from a mouse imprinting region that corresponds to the human Prader-Willi syndrome critical region (*PWCR*). Searching in an *Igf2-H19* imprinting gene cluster in the mouse genome, two more imprinted genes were identified: the insuln II gene (*Ins2*) and its retroposon, *Ins1*. These insulin genes are expressed biallelically (not imprinted) in the pancreas, but paternal monoallelic expression was evident in the yolk sac (Giddings *et al.*, 1994). The mouse homolog (*Mash2*) of the Drosophila *achaete-scute* gene, *Ascl2*, which encodes a transcription factor, is maternally expressed in the trophoblast cell and preimplantation zygote, and thus it is considered to be essential for the development of trophoblasts (Guillemot *et al.*, 1995). *Igf2*, *H19*, *Ins2*, *Mash2*, *Snrpn*, *ZNF127*, and another imprinted gene, $p57^{KIP2}$, are all located on the mouse chromosome 7. With the restriction landmark genome scanning (RLGS) method, the paternally expressed mouse gene, *SP2* (*U2afbp-rs*), was isolated (Hatada *et al.*, 1993; Hayashizaki *et al.*, 1994), assigned

to an imprinting region of chromosome 11, and possibly plays a role in the determination of body size. Another approach, a screening of the genome by means of the subtraction hybridization method made it possible to isolate two novel mouse paternally expressed genes: *Peg1* (paternally expressed gene 1), which was mapped to chromosome 6 (Kaneko-Ishino *et al.*, 1995), and the other, *Peg3*, mapped to the proximal region of chromosome 7 (Kuroiwa *et al.*, 1996). Until now, 4 mouse maternally expressing genes, *Igf2r*, *H19*, *Mash2* and $p57^{KIP2}$ (Hatada and Mukai, 1995), and 10 paternally expressing genes, *Mas*, *Igf2*, *Ins2*, *Snrpn*, *Zuf127*, *Peg3*, *Peg1*, *Ins1*, *CDC25Mm* and *SP2* have been identified (Table 1).

Human imprinted genes were also identified either independently or by utilizing homology to the mouse imprinted genes (Table 1). Although *IGF2*, *H19*, *SNRPN* and $p57^{KIP2}$ (Hatada *et al.*, 1996a) were confirmed to be imprinted as are their mouse homologs, *INS* has been shown to be expressed biallelically. Searching around the *SNRPN* region identified 4 novel imprinted genes, *ZNF127*, *PAR5*, *PAR1* and *IPW* (Sutcliffe *et al.*, 1994; Wevrick *et al.*, 1994), all expressing paternally. The human Wilms tumor gene, *WT1*, shows an imprinting polymorphism in the placenta, *i.e.*, there are two populations, one with maternal monoallelic expression and the other with biallelic expression (Jinno *et al.*, 1994). The expression of the human *H19* is biallelic in the placenta before 9 weeks of pregnancy, but maternal monoallelic expression is observed after 10 weeks, indicating that the human *H19* imprinting depends upon developmental stages (Jinno *et al.*, 1995). Because of the difficulty of observations of gene expression in an early human development, it has not yet been confirmed whether other genes are imprinted in man.

Genomic Imprinting-Related Genetic Diseases

Prader-Willi syndrome (PWS) and Angelman syndrome (AS). PWS is a neurogenetic disorder characterized by severe muscular hypotonia in early infancy, hypopigmentation of the skin, hypogonadism, hyperphagia and subsequent obesity, and mental retardation. AS is another disorder characterized by severe mental retardation, epilepsy, ataxic gait, and paroxysmal laughter. Each disorder occurs in about 1/15,000 newborns. A majority (73%) of PWS patients have a deletion at the q11-q13 region on the paternally-derived chromosome 15 [del(15)(q11.1q13)pat] and 25% maternal UPD for the chromosome [upd(15)mat], while 73% of AS patients have a deletion at the same region on the maternally-derived chromosome and 2% paternal UPD for chromosome 15 (Fig. 1). This finding is the analogy of the above mentioned "maternal effect, *Tme*" in T^{hp}. Normally, *PWCR* is a paternally expressed domain, and when the function of gene(s) in the domain is lost, PWS may occur. On the other hand, the Angelman syndrome critical region (*ASCR*) is a maternally expressed region, of which "loss of function" may result in AS. By molecular studies, the smallest deletion overlap (SRO) responsible for PWS is estimated to be from *D15S63* to *D15S174*, to which several genes have

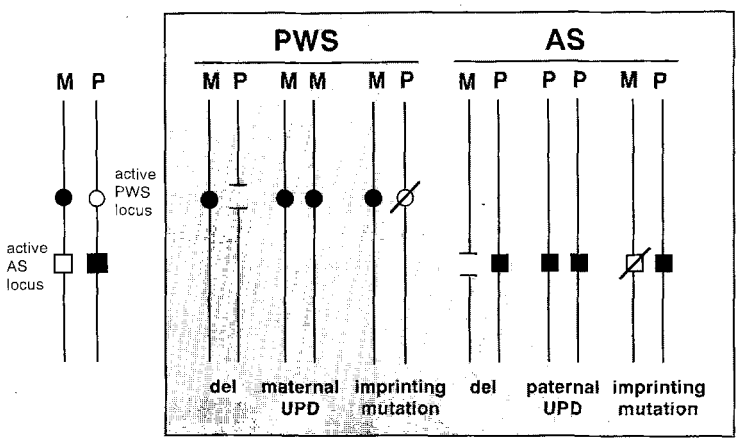

Fig. 1. Three classes of mutations in Prader-Willi syndrome and Angelman syndrome.

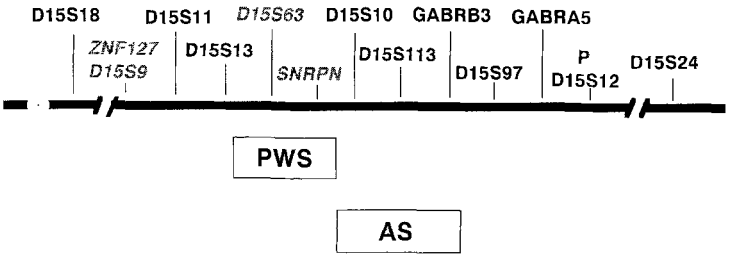

Fig. 2. Genetic map of loci at 15q11-q13 region. Loci with italic letters show differential methylation patterns between parental alleles. Box depicts the putative PWS and AS loci.

been assigned (Fig. 2). *SNRPN* is one of them and may act in the nerve system during early development by participating in mRNA-splicing. Paternal monoallelic expression of *SNRPN* has been observed in the brain of normal fetuses, but no such expression is detected in PWS patients (Glenn *et al.*, 1993). In addition, a PWS patient who has t(15;19), a breakpoint of which is located within *SNRPN*, was recently found. From these characteristics, *SNRPN* is a strong candidate PWS gene. Similarly, in *PAR5* which is located 50-kb downstream to *SNRPN*, paternal monoallelic expression is observed in the normal fetal tissues but no expression in PWS patients (Wevrick *et al.*, 1994). Two other transcripts, *IPW* and *PAR1*, located 150-kb and 170-kb distal to *SNRPN*, respectively, are expressed only on the paternally derived chromosome. Methylation patterns specific for maternally and paternally derived chromosomes 15 are observed, *i.e.*, the maternal alleles at the *D15S9* (ZNF127) located 1-Mb proximal to *SNRPN*, *D15S63* (PW71) and the *SNRPN* loci are usually hypermethylated, and the paternal alleles at these loci remain undermethylated. PWS patients with del(15)pat or upd(15)mat retain only

the methylated maternal alleles, losing the active paternal loci (Driscoll *et al.*, 1992; Dittrich *et al.*, 1993).

There have been a few PWS/AS patients who have biparentally-derived chromosomes 15 but show abnormal methylation patterns. Both alleles at the *D15S63* locus in such PWS patients showed maternal methylation patterns as if they came from the mother, and *vice versa* in AS patients. Similar results were obtained at the *D15S9* locus. The abnormality in these patients is referred to as an imprinting mutation. Detailed study demonstrated that three such PWS patients with imprinting mutations actually had a minute deletion involving exon 1 of *SNRPN* (Buiting *et al.*, 1994; Sutcliffe *et al.*, 1994; Dittrich *et al.*, 1996). On the other hand, 7 AS patients with an imprinting mutation had either a minute deletion or a base substitution at a region more proximal to *SNRPN*-exon 1. Novel alternative transcripts of *SNRPN* were identified from this region (Dittrich *et al.*, 1996). Imprinting mutations do not disturb the whole imprinting phenomenon but may specifically involve only the PWS/AS region. Minute deletions in PWS patients at exon 1 of *SNRPN* may cause a change of the methylation pattern at both *D15S9* and *D15S63* loci and involve the inactivation of the *ZNF127*, *SNRPN*, *PAR5*, *IPW* and *PAR1* genes. Since these loci/genes are located within

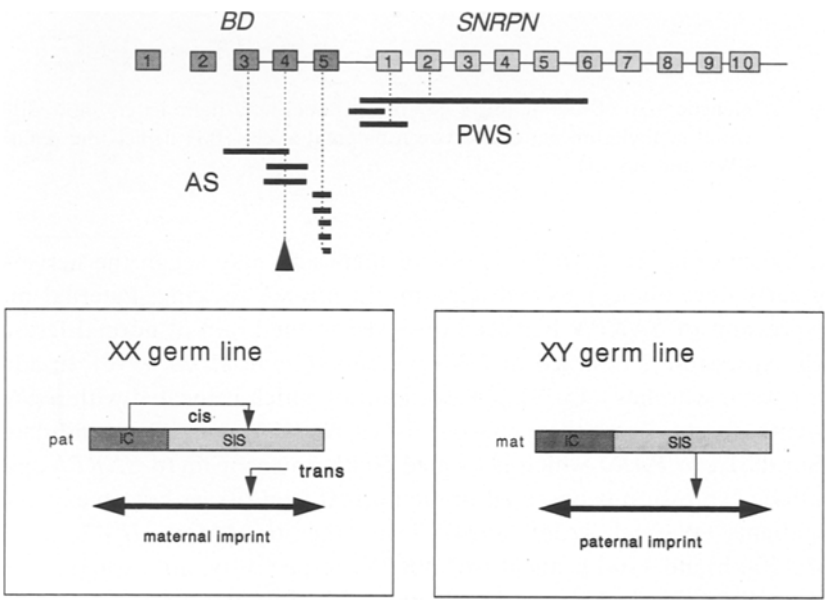

Fig. 3. Microdeletions in PWS and AS patients with imprinting mutations (upper) and a model for reset of parental imprint (lower). Solid bars and triangle in the upper row depict microdeletions and a point mutation observed. The putative imprintor (*IC*) at the *BD* region may act as a suppressor for the putative imprint switch initiation site (*SIS*) at exon 1 of *SNRPN*.

a 1.5-Mb extent, the imprinting may involve this 1.5-Mb domain. From these findings, a hypothesis was proposed: at this 5′ region of *SNRPN,* there must be an imprinting center (*IC*) or imprintor which controls in a *cis*-acting manner a putative imprinting switch initiation site (*SIS*) located at exon 1 (Fig. 3) (Dittrich *et al.*, 1996). Normally, in the female germline, the paternally-derived *IC* suppresses the *SIS* function, and therefore the paternal imprint would switch to that for the maternal chromosome by a *trans*-acting factor in the germ cell. On the other hand, in the male germline, since *IC* on the maternally-derived chromosome is inactive, *SIS* would mark the chromosome with the paternal imprint. This model was successful to explain complex genetic findings on PWS/AS patients. In PWS patients, an imprinting mutation having occurred in a grandmother is transmitted to a father. Because the maternal imprint had normally occurred in the grandmother, the father is phenotypically normal. However, as the imprinting was not reset in his germ cells because of a lacking of *SIS*, the chromosome 15 retained the maternal imprint, PWS occurs in his child (Fig. 4). The reverse is true in AS.

Beckwith-Wiedemann syndrom (BWS). BWS is an overgrowth syndrome

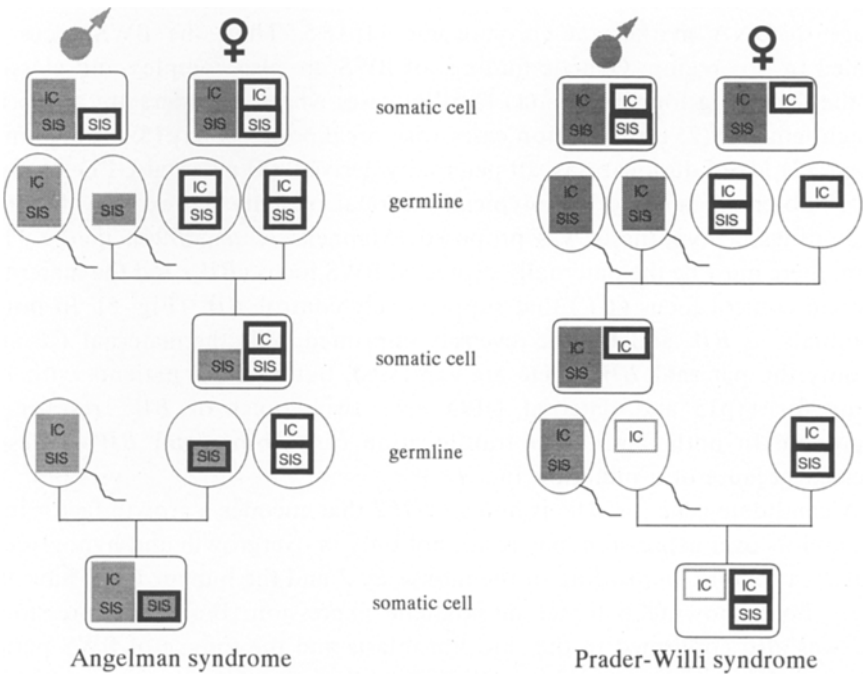

Fig. 4. Mechanisms of imprinting mutations in PWS and AS patients. Shaded and open boxes depict paternal and maternal imprints, respectively, while shaded and thick-black margins of boxes paternally and maternally derived chromosomes 15.

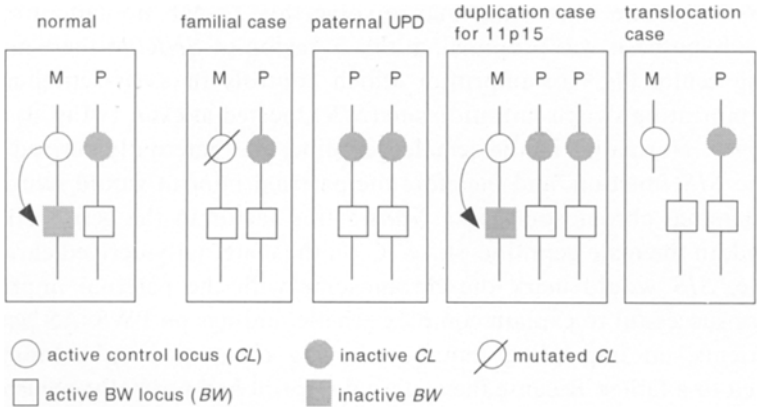

Fig. 5. A two-locus imprinting model for the occurrence of Beckwith-Wiedemann syndrome. BWS occurs when two *BW* alleles become active.

characterized by gigantism, macroglossia, exomphalos, hypoglycemia in infancy, and embryonal tumors including Wilms tumor. A linkage analysis in familial cases with an autosomal dominant mode of inheritance demonstrated a strong linkage to DNA markers at chromosome 11p15.5. Thus, the BWS locus was assigned to this region. Genetic findings of BWS are also complex and classified into the following four classes: (1) familial cases whose disorders are all derived through females; (2) translocation cases with breakpoints at 11p15, all maternally derived; (3) 11p15 duplications, all paternally derived; (4) paternal UPD involving 11p15.5 observed in some karyotypically normal patients. To explain the above four findings, a hypothesis was proposed (Mannens *et al.*, 1994): in the 11p15 region, there must be the paternally expressed BWS locus (*BW*) and the maternally expressed control locus (*CL*) that suppressively control *BW* (Fig. 5). In normal individuals, as *BW* and *CL* are reversely imprinted, only the maternal *CL* allele and only the paternal *BW* allele are expressed, but in BWS patients with both trisomy for 11p15 and paternal UPD have two copies of *BW*, resulting in overgrowth. In both familial or translocation cases, biparental *BW*-alleles are expressed because of a mutation in *CL*.

A candidate gene for *BW* is human *IGF2* that encodes a growth factor in the fetus, and its over expression may result not only in overgrowth and hypoglycemia but also in tumors. Imprinting of the mouse *Igf2* and the human *IGF2* have been proven, both showing paternal monoallelic expression. Biallelic expression of *IGF2* was also confirmed in the skin fibroblasts and the tongue of BWS patients (Weksberg *et al.*, 1993), and also in Wilms tumor tissue (Ogawa *et al.*, 1993). One of the candidate for *CL* is the $p57^{KIP2}$ gene which is maternally expressed (Hatada *et al.*, 1996a). The $p57^{KIP2}$ protein is a potent tight-binding inhibitor of several G1 cyclin/Cdk complexes and is a negative regulator of cell proliferation. Hatada *et*

al. (1996b; unpublished data) recently demonstrated that a nonsense mutation in the $p57^{KIP2}$ gene was identified in 4 of 15 familial or sporadic BWS patients analyzed. Furthermore, in one family, the phenotypically normal mother had the same mutation as the patient. The findings are well consistent with the maternal transmission of mutations in familial BWS cases and with the characteristic of *CL* in the previous model. Furhter studied are required how this gene interacts with *IGF2*.

Prospects

Genomic imprinting may play a role in the occurrence of other genetic disorders. They are listed in Table 2, in which an inheritance mode cannot well be explained by formal genetics. Observations of UPD in human chromosomes may also provide suggestive evidence of imprinting, *i.e.*, recent studies demonstrated

Table 2. Possible imprinting disorders.

Disorders	Unique features
Prader-Willi syndrome	paternal deletion/maternal UPD
Angelman syndrome	maternal deletion/paternal UPD
Wiedemann-Beckwith syndrome	maternally derived familial case/paternal UPD/ paternally derived 11p15 duplication
Huntington disease	early onset when paternally transmitted/anticipation
Myotonic dystropy	congenital form when maternally derived/anticipation
Fragile X syndrome	Sherman paradox
Neurofibromatosis type I	severe form when maternally transmitted
Neurofibromatosis type II	early onset when maternally transmitted
Cerebellar ataxia	early onset/several from when paternally transmitted
Non-specific epilepsy	frequent maternal transmission
IDDM	frequent paternal transmission
Albright hereditary osteodystrophy	frequent maternal transmission
Growth deficiency due to Pit-1 mutation	phenotypically normal father/grandmother with the same mutation
Wilms tumor	LOI of maternal IGF2 allele/LOH of maternal allele
Osteosarcoma	LOH of maternal allele
Ph1-positive CML	t(9pat;22mat)
Hereditary Glomus tumor	paternal transmission
Hydatidiform mole	androgenesis/development of placenta
Benign ovarian teratoma	gynogenesis/development of fetal tissues
Triploidy	extra paternal copy/overgrowth of placenta
Cardiac anomaly	frequent maternal transmission
Sotos syndrome	frequent maternal transmission
Psoriasis vulgaris	frequent paternal transmission
Silver-Russell syndrome	UD7MA
Anomaly/mental retardation	UD9MA
Multiple anomalies	UD14MA/UD14PA
Total blindness	UD14MB

that imprinting is relevant to Silver-Russell syndrome (Kotzot *et al.*, 1995) and myeloid leukemia in a patient with von Recklinghausen disease (Stephens *et al.*, 1996). Thus, it is of great interest not only to find new imprinting disorders but to dissect the mechanisms of genomic imprinting, especially how the primary imprint is determined and why this phenomenon exists in the mammals.

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ADDENDUM

During proofreading, there appears a work on the isolation of the gene, *UBE3A*, responsible for Angelman syndrome (Kishino *et al.*, 1997) *UBE3A*-mutations identified in 3 AS patients may cause a frameshift and premature translocation termination of the ubiquitin-mediated protein and may lead to degradation of the protein during brain development in AS.

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