

EPIGENETICS AND HUMAN DISEASE

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■ **Abstract** Epigenetics is comprised of the stable and heritable (or potentially heritable) changes in gene expression that do not entail a change in DNA sequence. The role of epigenetics in the etiology of human disease is increasingly recognized with the most obvious evidence found for genes subject to genomic imprinting. Mutations and epimutations in imprinted genes can give rise to genetic and epigenetic phenotypes, respectively; uniparental disomy and imprinting defects represent epigenetic disease phenotypes. There are also genetic disorders that affect chromatin structure and remodeling. These disorders can affect chromatin in *trans* or in *cis*, as well as expression of both imprinted and nonimprinted genes. Data from Angelman and Beckwith-Wiedemann syndromes and other disorders indicate that a monogenic or oligogenic phenotype can be caused by a mixed epigenetic and genetic and mixed de novo and inherited (MEGDI) model. The MEGDI model may apply to some complex disease traits and could explain negative results in genome-wide genetic scans.

DEFINITIONS AND HISTORICAL PERSPECTIVE

In this review, we define epigenetics as the study of stable and heritable (or potentially heritable) changes in gene expression that do not entail a change in DNA sequence. For example, because neurons and hepatocytes from an individual have identical genomes at the level of nucleotide sequence but large differences in gene expression, there must be mechanisms providing stable or semistable regulation of gene expression apart from nucleotide sequence. The processes of developmental biology depend largely on epigenetic mechanisms to orchestrate the formation of many different tissues and organs from a fertilized egg because all cells in an individual, save a few exceptions such as B and T lymphocytes, have the same nucleotide sequence.

Regulation of gene expression has two components. First, labile regulation is the moment-to-moment control by transcriptional activators and repressors, whose nuclear concentrations, covalent modifications, and subunit associations fluctuate extensively. Second, epigenetic regulation is the control of gene expression that has sufficient stability to be transmitted from parental cell to daughter cells; this

occurs by altering the chromatin structure and includes covalent modification of DNA and histones. These two components of regulation act in concert on a gene, and the boundary between labile and epigenetic regulation is not always distinct. Changes in gene regulation during the cell cycle include both labile and epigenetic regulation and demonstrate the complexity of the boundary between the two because chromatin remodeling occurs across different portions of the cell cycle.

Genes in eukaryotic cells do not function in a vacuum but within a milieu determined by the developmental and environmental history of the cell; the chromatin structure with its covalent modifications and stable protein associations that result from this historical and contextual information provides epigenetic regulation and constitutes the epigenotype. An epigenotype is established primarily through the folding of DNA into chromatin and the architecture of that chromatin within the three-dimensional space of the nucleus. Scientists have known for years many important aspects of chromatin structure as exemplified by DNA methylation and the folding of DNA into euchromatin versus heterochromatin, but recent discoveries include the identification of covalent modification of histones and the characterization of nonhistone proteins that modulate DNA-histone interactions. The covalent modification of histones constitutes a potential "histone code" that can be stably transmitted from parent cell to daughter cells (74). Because genomic DNA must exist in a particular chromatin configuration, the genotype can only give rise to phenotype through the prism of the epigenotype (Figure 1, left) and, in this context, the epigenotype can be compared to the variations in font that can be added to a primary text. This analogy is suitable for describing how a second layer of regulatory information can be laid down on a primary nucleotide sequence (Figure 1, right). The epigenotype shows far greater plasticity than the genotype in the normal development of an individual, and it is reasonable to speculate that epigenetic errors could be a major contributor to human diseases. Thus, the epigenotype may be intrinsically less stable than the genotype. The genotype must exert its effects in the context of the epigenotype, and this position makes it an excellent candidate to modify the effects of the genotype and play a role in mediating penetrance and variation in expression.

Genomic imprinting is a distinct subset of epigenetic regulation in which the activity of a gene is reversibly modified depending on the sex of the parent that transmits it. This leads to unequal expression of the maternal and paternal alleles for a diploid locus. Until now, many examples of epigenetic abnormalities contributing to human disease involve imprinted genes. Although it may be that epigenetic dysregulation of imprinted genes is easier to detect and correlate with disease than that at nonimprinted genes, it is more likely that the additional complexity of epigenetic regulation for imprinted genes makes them more susceptible to epigenetic dysregulation compared to nonimprinted genes. For imprinted genes, the parent-specific information on the chromosome from the parent of the opposite sex must be erased, reset, and maintained at every generation, and errors that can cause disease in this context include uniparental disomy (UPD, the inheritance of two chromosomes from one parent and none from the other) and imprinting defects

(Figure 2). An imprinting defect is an abnormality of the parent-of-origin-specific gene regulation, often in the form of an epigenetic reversal, such that a maternal allele or genomic domain has the epigenotype (chromatin structure and resulting pattern of gene expression) of a paternal allele or genomic domain, or vice versa.

Biologists are comfortable with the concept that DNA sequence is replicated through Watson and Crick base pairing to transmit the genome from one somatic cell to another or from a human parent to a child. However, biological and medical research has given much less attention to the epigenetic mechanisms that can transfer stable biological properties from one cell to the next or from one generation of organism to the next. There is now a fairly good understanding of how the state of DNA methylation can be transmitted from a parent cell to a daughter cell by a maintenance mechanism that methylates hemimethylated CpG dinucleotides (Figure 3). Similar conservative mechanisms for replicating the structure of chromatin and the histone code must exist, but they are currently poorly understood. This review provides a current perspective on the role of epigenetics in human biology and disease, taking advantage of comparative genomics, particularly in the mouse. There has been an increasing recognition of the importance of epigenetics in cancer biology, but we focus primarily on the role of epigenetics in nonneoplastic disease.

Much of the earliest definitive evidence of the role of epigenetics in human disease came from genes subject to genomic imprinting (52, 132, 138). A reasonable starting point for discussing genomic imprinting in mammals is the work of McGrath & Solter in 1984 (103), which demonstrated that mouse eggs manipulated to contain two maternal pronuclei (gynogenotes) or two paternal pronuclei (androgenotes) did not develop normally, and did not survive to birth. Gynogenotes give rise to better formation of the embryo, but very poor development of extraembryonic tissues. In contrast, androgenotes have better formation of the extraembryonic tissues, but poor development of the embryo and rarely develop beyond implantation. In the 1980s, Cattanach and colleagues systematically bred mice to obtain offspring with two maternal copies and no paternal copy or vice versa for each mouse chromosome (20, 21). A mouse with two paternal copies and no maternal copy of chromosome 1 has paternal UPD or maternal deficiency for chromosome 1. Extensive breeding studies demonstrated that mice with UPD for many chromosomes appeared entirely normal, but UPD for other chromosomes or regions within chromosomes caused a broad range of phenotypic abnormalities, often including abnormal growth and/or embryonic lethality. In 1987, the first case of uniparental disomy in a human was described in a patient with cystic fibrosis and short stature related to maternal UPD for chromosome 7 (143).

With a growing body of knowledge relating to imprinted genes in mice and humans, in 1996 Pembry (120) proposed that transgenerational modulation of gene expression might be possible through genomic imprinting, and he detailed how this might apply to human growth. Through the 1990s, the major role of epigenetics in cancer was increasingly recognized. In the mid-1990s, Holiday (66,

67) wrote extensively about the importance of DNA methylation and other aspects of epigenetics in the regulation of mammalian gene expression. He emphasized that “there are not four bases in human DNA, but at least five and very likely others” and bemoaned the lack of attention to DNA methylation in the sequencing of the human genome. In 1995, Strohmaier (145) suggested that we “redirect our attention to epigenetic regulation as a second informational system in parallel with the genome” to understand complex disease traits. There have been many reviews and commentaries regarding the potential role of epigenetics in complex disease traits, psychiatric disorders, and behavioral abnormalities (7, 36, 72, 76, 123, 124, 144), but firm evidence for these hypotheses is generally lacking.

Currently, there is considerable knowledge regarding disease phenotypes caused by abnormalities of specific imprinted domains as exemplified by Prader-Willi syndrome (PWS), Angelman syndrome (AS), and Beckwith-Wiedemann syndrome (BWS) (38, 140). An entire book detailing the phenotypic findings associated with UPD and imprinting defects for each human chromosome is available (38). These disorders clearly demonstrate how a single phenotype can be a genetic disease in some patients and an epigenetic disease in others (Figure 4). For example, genetic forms of a disease (e.g., common deletion 15q11-q13 causing PWS) are indistinguishable from epigenetic forms of the same disease (e.g., maternal UPD causing PWS). In deletion cases, about 4 megabases of genomic DNA are lost, whereas the entire genomic sequence is normal for UPD cases.

Epigenetics also plays a role in disease processes involving nonimprinted genes, and these instances can be divided into disorders affecting chromatin in *trans* or affecting chromatin in *cis*, as discussed below. Previous reviews and commentaries focusing on epigenetics and disease are available (12, 27, 36, 69, 109, 157).

BIOCHEMISTRY AND REMODELING OF CHROMATIN

In mammals, DNA methylation is found predominantly at the carbon-5 position of about 80% of all cytosines that are part of symmetrical CpG dinucleotides. Because most 5-methylcytosines lie within retrotransposons, endogenous retroviruses, or repetitive sequences (11, 172), methylation may have evolved as a host defense mechanism to prevent the mobilization of these elements and to reduce the occurrence of chromosomal rearrangements. Unmethylated CpG dinucleotides are found mainly in short CpG-rich sequence domains known as CpG islands that are in the vicinity of gene promoters (113). The phenomenon of genomic imprinting requires multiple steps during development to distinguish maternal from paternal chromatin in imprinted domains, and differential DNA methylation is usually prominently involved. In each generation, the imprint inherited from the parent of the opposite sex must be erased and then reestablished in developing germ cells so that the maternal or paternal imprint is appropriate for the sex of the individual and can be correctly transmitted to the next generation. After fertilization, a mechanism must maintain the imprint in the developing embryo and in somatic

cells during mitosis (30). Because most imprinted genes are characterized by differentially methylated regions (DMRs) in which one of the two parental alleles is methylated in somatic cells, DNA methylation may serve as an allelic mark to distinguish the chromosomes throughout this process. In the mouse, a programmed progression of DNA methylation takes place during gametogenesis in both sexes. For both the sperm and the egg, imprinted genes and repeat sequences start to undergo demethylation before E10.5 and are completely demethylated when primordial germ cells migrate to the gonads (E12.5–13.5), followed by remethylation either on day E15.5 or after birth in the male or female germ lines, respectively (133, 148). The establishment of the methylation patterns for the entire genome as found in adult somatic cells also entails genome-wide reprogramming in the early embryo with a wave of demethylation at the time of cleavage, and subsequent de novo methylation occurring after implantation when new methyl groups are added to unmethylated CpG pairs (51, 89, 133, 170). Intracisternal A-type particle (IAP) sequences in the murine genome resist demethylation during preimplantation development (86), and this may be analogous to the processing of methylation marks for genomic imprinting. A definitive role for DNA methylation in development and the monoallelic expression of imprinted genes was first demonstrated through the analysis of mice with a partial loss of function mutation in the *Dnmt1* gene encoding DNA methyltransferase 1 (Dnmt1). Dnmt1 acts preferentially on hemimethylated DNA substrates during DNA replication to maintain methylation patterns through cell division, as shown in Figure 3 (50). Mice homozygous for this *Dnmt1* mutation died before day E11 and showed a threefold reduction in the level of 5-methylcytosine, as well as the dysregulated expression of several imprinted genes including *H19* and *Igf2* (91, 92). To identify other proteins that might provide de novo methyltransferase activity in the early embryo, homology searches for proteins containing the catalytic domain of Dnmt1 identified three candidate proteins: Dnmt2, Dnmt3a, and Dnmt3b (116, 117). Using gene targeting to generate mutations, distinct functions for each enzyme were partially characterized by virtue of different developmental defects and different DNA methylation abnormalities. Dnmt2 is not essential for global de novo or maintenance methylation of DNA in ES cells (117). Contrary to initial reports, Dnmt2 does have weak DNA methyltransferase activity (64). Dnmt3a and Dnmt3b are essential for de novo methylation and for normal embryogenesis in the mouse (115). Some distinction of the functions of Dnmt3a and Dnmt3b was achieved with the recognition that minor satellite repeats located in centromeres were unmethylated in *Dnmt3b* $-/-$ ES cells and day E9.5 embryos, but not in those lacking *Dnmt3a* (115). Detailed descriptions of the phenotypes of several other mouse methyltransferase mutations generated by gene targeting provided additional insight into the role of individual enzymes. An oocyte-specific isoform of Dnmt1 (Dnmt1^o) is involved in maintaining maternal imprints (68), and Dnmt3L, a protein lacking enzyme activity, colocalizes with Dnmt3a and Dnmt3b and is required to establish imprinting in the female germ-line (15, 58). In addition to possibly serving as the mark that can distinguish the two parental alleles, DNA methylation can also repress transcription

and play a role in allele-specific silencing of imprinted genes such as *H19* or *Snrpn* that are methylated on the silenced allele (6, 42, 141, 147) or in X-inactivation where methylation spreads across most of the inactive chromosome (126). Methylation could potentially interfere with the binding of transcription factors or other regulatory proteins to DNA, and this type of mechanism is found for the differentially methylated murine *H19/Igf2* imprinting control region on chromosome 7 (57, 137). The CCCTC-binding factor (CTCF) was named for its ability to bind to CCCTC sequences in DNA; it is an evolutionarily conserved transcription factor that includes 11 zinc finger motifs that function in different combinations and confer the capacity to bind to sites of widely divergent nucleotide sequences. A role for this protein in specifying a boundary between active and inactive chromatin domains was first observed at the chicken β -globin locus where the binding of CTCF to a 42-base pair (bp) sequence prevented interaction of the enhancer with the promoter (10, 129). In the *H19/Igf2* domain, CTCF regulates imprinted expression by binding to the unmethylated maternal but not to the methylated paternal imprinting control region; when bound, CTCF provides a barrier for interaction of the *H19* enhancers with *Igf2* (57, 137). Additionally, CTCF appears to be involved in the process of selecting a murine X chromosome for inactivation by regulating the expression of the murine antisense transcript from the *Tsix* gene (22).

Methylation of DNA at specific sites can also modulate histone covalent modification through the recruitment of methyl-binding proteins such as methyl-CpG-binding protein 2 (MeCP2). MeCP2 is an abundant nuclear protein encoded on the X chromosome; it is part of a complex including histone deacetylase (HDAC) and the corepressor Sin3a that controls transcription through the deacetylation of core histones (77, 111). MeCP2 has an N-terminal, methyl-CpG binding domain (MBD) and a transcriptional repressor domain (TRD) that associates with the corepressor complex (110). Additional evidence for the link between DNA methylation and histone acetylation comes from the finding that two other methyl-binding proteins, MBD2 and MBD3, also associate with a complex containing an HDAC as well as the helicase motif protein Mi-2 (61, 62, 114, 156, 173).

In eukaryotic organisms, DNA is packaged with histones within the nucleus of the cell and is localized in nondividing cells within either condensed regions known as heterochromatin or in a more accessible conformation known as euchromatin. Identification of these two types of chromatin led to the hypothesis that differences in chromatin structure might be associated with tissue-specific or temporal differences in gene expression. A first level of compaction (5-10x) of DNA is achieved by organization into nucleosomes in which approximately 146 bp of DNA are wound twice around an octamer of core histones consisting of two of each of the four histones H2A, H2B, H3, and H4. Adjacent nucleosomes are connected by a segment of linker DNA to form a 30-nm fiber, and the addition of histone H1 to each nucleosome lends stability to and facilitates the formation of further higher-order structures. For proteins such as transcription factors to gain access to DNA within the nucleosomes, ATP-dependent chromatin remodeling

complexes introduce transient conformational changes in the positions of histone octamers. These chromatin remodeling complexes contain 2–12 subunits, including a switch/sucrose nonfermenting (SWI/SNF)-related ATPase, in combination with cofactors that mediate the specificity of the complex for either a particular transcription factor and/or a given region of chromatin.

Amino acid residues within the histone tails or amino-terminal ends of the histone proteins are also chemically modified by methylation of lysine or arginine, acetylation or ubiquitination of lysine, and phosphorylation of serine. In general, acetylation of histones H3 and H4 is associated with unfolding and accessibility of chromatin, and is catalyzed by histone acetyltransferases (HATs) that are usually part of multiprotein complexes; HDACs promote the condensation of chromatin and repress gene transcription. Similarly, methylation of Lys4 of H3 (H3-K4) is correlated with active gene expression, whereas methylation of Lys9 of H3 (H3-K9) directed by homologues of the site-specific Su(var)3–9 enzyme first described in *Drosophila* is more often associated with gene silencing. An emerging paradigm in this area of gene regulation is that there are interactions between these different types of chemical modifications, and that the covalent marks provide binding sites for effector proteins containing conserved sequences such as the bromo- or chromodomains. The entirety of these chemical modifications is called the “histone code” and endows a mammalian cell with another layer of gene regulation that complements the primary DNA sequence (41, 74, 93). Only recently has attention focused on the potential importance of the distinction of histone H3 and H3.3 variants, emphasizing that the relationship among modifications, histone variants, and nucleosome assembly pathways is complex and currently unclear (104). There is ambiguity as to whether DNA methylation might direct chromatin remodeling or whether the histone methylation might direct DNA methylation, and the two are not mutually exclusive. Recent work suggests that methylated alleles of DMRs are marked by hypermethylation on lysine-9 of histone H3 (H3-K9), whereas the unmethylated alleles have H3 lysine-4 (H3-K4) methylation and H4 acetylation; this suggests that maintaining DMRs involves interdependence and mutual exclusion of different epigenetic modifications (45). Furthermore, H3-K9 methylation is required for maintaining and perhaps establishing CpG methylation at the *Snrpn* DMR and allele-specific expression in mouse ES cells (167). In many cases, DNA methylation provides a genetic mark for genomic imprinting, as with the imprint control region of the *H19/IGF2* domain. In addition, treatment with azacytidine or deoxy-azacytidine, inhibitors of DNA methylation, can activate expression of alleles silenced by genomic imprinting or X-inactivation. Inhibitors of HDACs tend to be weaker activators of silenced alleles. In contrast to evidence that DNA methylation might be a primary determinant, data from *Neurospora* clearly indicates that DNA methylation depends on histone methylation (149). It is possible that DNA methylation and histone modification may work in concert to reinforce the effect of the other. Detailed reviews of the complex processes of chromatin remodeling are available (9, 74, 84, 85, 96).

ROLE OF NONCODING RNAs IN EPIGENETICS

Historically, RNAs were not considered to play a major role in chromatin remodeling, but this view has changed dramatically over the last five years. As the sequencing of mammalian genomes gained momentum in the late 1990s, there was increasing evidence for noncoding RNAs based on comparing the genomic sequences with EST databases. In 2001, Eddy provided a good review of the growing awareness of noncoding RNAs (37), including these definitions: noncoding RNA (all RNAs other than mRNA), fRNA (functional RNA; synonymous with noncoding RNA), microRNA (putative translational regulatory gene family), siRNA (small interfering RNA; active molecules in RNA interference), snRNA (small nuclear RNA; includes spliceosomal RNAs), and snoRNA (small nucleolar RNA; some known snoRNAs are involved in rRNA modification). Another perspective is the growing evidence of highly conserved genomic sequences across species (e.g., between mouse and human) linked to the possibility that many of these regions might produce noncoding RNA products. Mattick (100) provides a detailed review of evidence that noncoding RNAs occur more frequently than previously assumed, and he proposes that these RNAs play a major role in regulating gene expression. There is a recent report that microRNAs play a role in the control of murine hematopoiesis (23). Two microRNAs expressed from the maternal chromosome are implicated in control of the *Dlk1-Gtl2* imprinted cluster on mouse chromosome 12 (94). In studies of FMRP, the product of the fragile X gene, and its *Drosophila* homolog, there is evidence that FMRP interacts with microRNAs and other components of the related pathways, including Dicer and the mammalian ortholog of Argonaute 1 (*AGO1*) (71, 75). It is unclear if this represents a form of stable (epigenetic) as opposed to labile gene regulation, and the findings raise the question of whether epigenetic regulation extends to the translational level. Reviews are available on the subjects of noncoding RNAs and the evidence that some of these play a role in chromatin remodeling (63, 100, 165). It is likely that genetic disorders involving the role of noncoding RNAs in the epigenetics of human disease processes will be increasingly recognized over the next few years.

MONOALLELIC GENE EXPRESSION

The strategy of expressing only one of two available alleles in the diploid state has evolved in a variety of biological circumstances and is called monoallelic expression or allelic exclusion. Monoallelic expression was initially recognized in mammals in the form of X chromosome inactivation to achieve dosage compensation and as exclusive expression of a single rearranged immunoglobulin allele to achieve specificity of antigen recognition. In genes for immunoglobulins and T-cell receptors, somatic cell recombination contributes to the gene regulation, but epigenetic mechanisms also play an important role (108). For most other forms of monoallelic expression, the regulatory mechanisms are entirely epigenetic and do

TABLE 1 Monoallelic expression of genes*

Genes	Chromosome	Selection of allele	Biological function
X-linked genes in females, somatic tissues	X	Random by chromosome	Dosage compensation
X-linked genes in females, extraembryonic	X	Paternal silencing	Dosage compensation
Immunoglobulins and T cell receptors	Autosomes	Random	Antigen specificity
Odorant receptors	Autosomes	Random	Receptor specificity?
NK cell receptors	Autosomes	Random	Receptor specificity?
Interleukin-2	4	Random	Tight regulation?
<i>SYBL1</i> in females	X	Random	Dosage compensation?
<i>SYBL1</i> in males	X and Y	Y-specific silencing	Dosage compensation?
Imprinted genes	Autosomes	Parent of origin with opposite imprinted domains on same chromosome	Parental conflict model, rheostat or evolvability model, other

*See Reference 26 for additional details.

not involve somatic recombination (Table 1). For this review, genomic imprinting according to parent of origin (defined above) is particularly relevant because of its role in human disease processes. Monoallelic expression is reviewed in greater detail elsewhere, including discussion of odorant receptors, interleukin-2, and natural killer (NK) cell receptors (26). The monoallelic expression of the synaptobrevin-like 1 (*SYBL1*) gene is unique; it lies on the long arm pseudoautosomal region of the X and Y chromosomes and is unique in that it is subject to random X-inactivation in females, but the allele on the Y is specifically silenced in males (33).

The evolutionary advantage of monoallelic expression typically relates to dosage compensation or to an advantage of singular molecular specificity (Table 1), although the understanding of the advantage of monoallelic expression of interleukin-2 is less clear. The evolutionary advantage of monoallelic expression for imprinted genes is uncertain, although the genetic conflict model and the rheostat or evolvability model are two of many hypotheses (8, 162).

On the X chromosome, monoallelic expression is determined at the whole-chromosome level via random X-inactivation, and the two chromosomes are replicated asynchronously with the expressed chromosome replicating early. For imprinted genes, monoallelic expression is determined at the whole-chromosome level in the sense that the entire chromosome is either of maternal or paternal origin, but oppositely imprinted domains regularly occur in adjacent regions of a single chromosome. Asynchronous replication is common in imprinted chromosomal domains. For genes not subject to genomic imprinting according to parent

of origin, but monoallelically expressed (e.g., odorant receptors, immunoglobulins, and interleukin-2), there is evidence in the mouse that monoallelic expression is accompanied by asynchronous replication, and that the early replicating allele is preferentially expressed, as with X-inactivation (91). This asymmetry of replication and expression is determined randomly at the single-cell and whole-chromosome levels such that all of the monoallelically expressed genes on a particular chromosome (e.g., chromosome 1) are replicated early and expressed from either the maternal or the paternal copy of chromosome 1 (142). This suggests that there is some form of epigenetic distinction between the two members of each chromosome pair. This “autosome-pair nonequivalence” also was found in human cells, again with monoallelic expression associated with asynchronous replication for genes or domains (39). There are hints that monoallelic or unbalanced allelic expression may play a much wider role in gene regulation and perhaps be especially important at a single-cell level and/or in a brain-specific manner (16, 95, 119). This may be combined with single-cell specificity for alternatively spliced isoforms (112).

SPECIAL ASPECTS OF IMPRINTED GENE EXPRESSION

The pattern of inheritance for a phenotype that is caused by a mutation in an imprinted gene is distinct from that observed for autosomal dominant, autosomal recessive, or X-linked traits. Regardless of whether the mutation involves loss of function, gain of function, or some other mechanism, the phenotype is only present when the mutant allele is inherited on the parental chromosome that is expressed as opposed to silenced. Examples include hereditary paragangliomas (OMIM 168000), in which the presumed relevant gene is silenced on the maternal allele and expressed on the paternal allele, and AS (OMIM 105830), in which the Angelman ubiquitin ligase is expressed in brain (see below) from the maternal allele and silenced on the paternal allele. This gives rise to unique pedigrees where distantly related family members may have the same genetic disorder, although they are separated by many healthy family members (53).

Genomic imprinting can be tissue specific or cell type specific or specific to alternative transcripts derived from a single locus. Insulin-like growth factor 2 (*Igf2*) was one of the first imprinted genes described in the mouse, and it was noted that silencing of the maternal allele occurs in most tissues, but not in choroid plexus (35). Expression of *Igf2* in the mouse also changes during development (40), and there is evidence for promoter-specific imprinting of *IGF2* in human (154). With the Angelman ubiquitin ligase, expression is monoallelic in brain but not somatic tissues (1, 135, 155) and, even more specifically, expression is monoallelic in neurons but not glia (169). There are now many examples of tissue-specific and transcript-specific imprinting.

The expression of imprinted genes is made additionally complex if erasure and resetting of the imprint is not complete in a single generation. There is considerable

evidence from studies in mice that the genomic imprint is often not erased and reset in a single generation (81, 130). Some of these observations are based on the study of transgenic mouse lines, and there is little data as to whether the epigenotype of most or all imprinted genes is fully erased and reset in a single generation in humans. The possibility of incomplete erasure and resetting of genomic imprints in a single generation as a normal situation raises the potential for chromosomes of differing grandparental origin to demonstrate differences in expression. An allele for a given locus inherited from the mother may behave differently depending on whether it originated from the maternal grandfather or from the maternal grandmother. Each imprinted domain/allele has a unique history of paternal versus maternal transmission in the immediate preceding generations (Figure 5). In most existing forms of statistical genetic analysis, there is little, if any, attention given to whether the grandparental origin of alleles might be a significant variable based on incomplete erasure and resetting of genomic imprints in a single generation.

Errors in the mechanisms for resetting and maintaining the genomic imprint lead to imprinting defects with or without nucleotide sequence abnormalities, as detailed below. There is at least one clear example where imprinting defects in the absence of detectable sequence abnormalities arise preferentially from the paternal grandmother, where erasure and resetting is required, and not from the paternal grandfather, which already carries a paternal imprint; this results in imprinting defects of the 15q11–q13 region, causing PWS (18).

There are special issues to consider in the case of epigenetics and monozygotic (MZ) twins. In addition, considerations of *de novo* mutations and epimutations raise special issues for MZ twins. The epigenotype undergoes rapid and cataclysmic change from the time of fertilization to the blastocyst stage of embryogenesis, and extensive further changes occur throughout development (see 133). These changes involve dramatic swings in DNA methylation and chromatin structure. There are two related implications with MZ twinning, which occurs near the blastocyst stage. These relate in part to the assumption that the epigenotype may be intrinsically less stable than the genotype, as noted above. First, any *de novo* change or epimutation affecting the epigenotype that arises during gametogenesis or in the embryo prior to MZ twinning will likely affect both twins in a concordant manner. Second, any phenotypic discordance between MZ twins could be attributed to epigenetic changes that arise after twinning (36, 125); similarly, mosaic epigenetic differences existing prior to MZ twinning might be distributed unevenly to the twins. It is often assumed that a high ratio of concordance in MZ twins compared to dizygotic (DZ) twins implies a polygenic etiology. For example, the concordance for MZ twins with autism is much higher than that for DZ twins (87). For autism, “The very high (25-fold) MZ:DZ concordance ratio is indicative of at least several interacting loci and, potentially, of many such loci” (134). Alternatively, this high ratio could be explained by *de novo* genetic or epigenetic events occurring prior to the time of MZ twinning (7). As Table 2 shows, genetic new mutations causing trisomy 21, achondroplasia, or Rett syndrome all result in

TABLE 2 Concordance for monozygotic (MZ) compared to dizygotic (DZ) twins for various conditions

Disorder	MZ	DZ
Trisomy 21	~100%	<5%
Achondroplasia or Rett de novo mutation	~100%	nil
Autism narrow definition	~60%	nil
Autism broad definition	~90%	~10%
Hypothetical de novo gametic or preMZ twinning imprinting defect	>90%?	low?

high ratios of concordance in MZ versus DZ twins, indicating that de novo events represent an alternative mechanism to a polygenic cause for such high ratios.

DISORDERS OF IMPRINTED GENES

The increasing recognition of UPD in patients with various phenotypes and the systematic breeding of mice to search for phenotypes related to UPD have led to the identification of many abnormal phenotypes caused by altered expression of imprinted genes. In the mouse, it is feasible to systematically breed mice with maternal or paternal UPD for virtually all chromosomes (20). This enables the recognition of numerous embryonic lethality and postnatal phenotypes associated with maternal or paternal UPD for specific chromosomal segments. A Web site (<http://www.mgu.har.mrc.ac.uk/research/imprinted/imprin.html>) provides up-to-date information on imprinted genes in the mouse, and a different site (<http://www.geneimprint.com>) lists 75 imprinted transcripts arising from 13 different human chromosomes as of March 2004. There are numerous examples where phenotypic abnormalities in the human correlate well with the findings in mice, although there is generally less information regarding embryonic lethality in humans. In addition, more subtle phenotypic abnormalities of learning or behavior in humans might easily go undetected in the mouse.

Some of the most extensively studied disorders involving imprinted genes are PWS, AS, BWS, pseudohypoparathyroidism, and Russell-Silver syndrome (Table 3). There are numerous reviews and chapters and an entire book that describe these disorders (19, 38, 90, 160, 163) (see specific disorders at <http://www.genereviews.org/>), and the clinical phenotypes are not reviewed here. An exhaustive survey of parent-of-origin effects in humans is available from 1998 (107), and a more recent overview of the physiology, phenotypic effects, and molecular data for mammalian imprinted genes is available (151). In this section, we draw on specific disorders for examples of the various mechanisms and principles whereby epigenetics contributes to diseases of imprinted gene expression.

TABLE 3 Selected disorders of imprinted genes

Disorder	Mechanisms in approximate order of frequency	Chromosome region	Specific gene(s)	OMIM
Prader-Willi syndrome	Deletion, UPD, imprint defect	15q11-q13	snoRNAs?, other?	176270
Angelman syndrome	Deletion, UPD, imprint defect, point mutation	15q11-q13	<i>UBE3A</i>	105830
Beckwith-Wiedemann syndrome	Imprint defect, UPD, duplication, translocation, point mutation	11p15.5	<i>IGF2</i> , <i>CDKN1C</i>	130650
Pseudohypoparathyroidism	Point mutation, imprint defect, UPD	20q13.2	<i>GNAS</i>	103580
Russell-Silver syndrome	UPD, duplication, translocation, inversion	7p11.2	Various candidates	180860

A diverse set of molecular events can lead to phenotypic abnormalities involving imprinted genes. UPD is a general mechanism that can theoretically affect any portion of the genome. In regions harboring imprinted genes, phenotypic abnormalities may arise from overexpression or underexpression associated with maternal or paternal UPD. Another general mechanism for disorders of imprinted genes involves the potential for large deletions removing one or more genes or point mutations affecting a specific locus. Point mutations most commonly represent loss of function, but may involve gain of function or other mechanisms as well. As noted above and in Figure 4, UPD (an epigenetic defect) and genetic mutations can give rise to the same phenotype.

A variety of other mechanisms can lead to abnormal phenotypes involving imprinted genes. These include so-called imprinting defects, in which a chromosome of one parental origin has an abnormal epigenotype (DNA methylation, chromatin structure, and gene expression pattern), often that for a chromosome of the opposite parental origin. These imprinting defects may be associated with mutations (DNA sequence abnormalities), often in the form of deletions of an imprinting center. This is particularly well characterized for PWS and AS. Imprinting defects also are found in instances where no genetic mutation can be identified (18), and it is virtually certain that at least some of these represent epigenetic defects with no abnormality of nucleotide sequence. Again, PWS and AS provide excellent examples of imprinting defects both with and without deletions, affecting a bipartite imprinting center that exists in the PWS/AS 15q11–q13 chromosomal domain. There is evidence that *in vitro* fertilization and, in particular, intracytoplasmic sperm injection (ICSI) can cause imprinting defects in the absence of genetic

mutations (i.e., abnormalities of DNA sequence). This has been reported for AS and BWS (31, 34, 97). Interstitial duplication of 15q11–q13 or an extra isodicentric chromosome 15q11–q13 accounts for a small fraction of patients with autism; in these cases, the autism phenotype is present when the abnormality is of maternal origin, but not when the abnormality is of paternal origin. Imprinting defects can represent a form of position effect, when the protein coding gene giving rise to the phenotype is located hundreds of kilobases away from the imprinting center, as with AS. Other examples of position effect include apparently balanced translocations of maternal origin giving rise to BWS and the spreading of X-inactivation to an autosome in X/autosomal translocations.

The heterogeneity of mechanisms causing AS and BWS is particularly instructive (Figure 6). There are at least five known mechanisms leading to the AS phenotype. The most common is large ~4-Mb deletions of 15q11–q13. These are routinely de novo and can be considered genetic because there is a major alteration of DNA sequence. Another mechanism involves paternal UPD for 15q11–q13. Again, the abnormalities are routinely de novo, but in this instance, they are epigenetic rather than genetic because there is no alteration in DNA sequence in the UPD cases of AS. There are two classes of imprinting defects causing AS. Some patients have small deletions of the bipartite imprinting center, and these are frequently inherited. This represents a class of abnormality that is primarily genetic, but has secondary epigenetic effects on the region, and the abnormality can be either de novo or inherited. Other AS patients with imprinting defects have no identifiable DNA sequence abnormality, and they represent an epigenetic effect, which is usually de novo in origin. Finally, there are patients with loss-of-function mutations in *UBE3A*, and these genetic cases of AS can be either de novo or inherited. This provides a clear precedent for a model of causation that involves both epigenetic and genetic abnormalities as well as de novo and inherited defects that give rise to a single phenotype of AS.

Similar information exists for BWS, where again the etiology involves a mixture of genetic and epigenetic abnormalities as well as de novo and inherited defects. BWS involves a particularly complex set of imprinted domains on human chromosome 11p15.5. The reciprocally imprinted *H19* and *IGF2* genes have been studied intensively in human and mouse. The overgrowth phenotype in BWS is caused at least in part by overexpression of the paternally expressed *IGF2*, but there is a lack of understanding as to how overexpression of *IGF2* and loss-of-function mutations in the maternally expressed p57^{KIP2} encoded by the *CDKN1C* gene give rise to a seemingly similar BWS phenotype. The BWS defects include (a) paternal duplications encompassing the *IGF2* locus presumably contributing to the overgrowth that is part of the phenotype, (b) translocations on the maternal chromosome likely causing position effects on the imprinted genes, (c) loss of function point mutations in the maternal allele for p57^{KIP2}, (d) paternal UPD for 11p15.5 (usually mosaic), and (e) most commonly imprinting defects associated with loss of imprinting for the *KCNQ1OT1* gene. Russell-Silver syndrome, a relatively nondescript phenotype of short stature often with asymmetry, can be

caused by maternal UPD for chromosome 7. It is uncertain whether the phenotype is caused by maternal overexpression of a growth-suppressing gene or paternal loss of expression for a growth-promoting gene. Other cytogenetic abnormalities suggest that the gene or genes causing the phenotype map to 7p11.2, but the exact molecular basis is not currently known.

Pseudohypoparathyroidism represents a group of phenotypes (types Ia, Ib, Ic, and II and Albright hereditary osteodystrophy; OMIM 103580) reflecting various forms of resistance to the action of parathyroid hormone. Most phenotypes are related to mutations in the guanine nucleotide-binding protein, α -stimulating activity polypeptide 1 (*GNAS1*) locus on chromosome 20q13.2. This is a complex locus producing multiple transcripts, some of which are maternal specific, some are paternal specific, and some are biallelically expressed (90).

GENETIC DISORDERS AFFECTING CHROMATIN STRUCTURE AND REMODELING IN *TRANS*

Disorders in this category can affect both imprinted and nonimprinted chromosomal domains or genes. There is a growing list of disorders with mutations in the genes encoding proteins that are essential for normal epigenetic regulation (Table 4), and these conditions are a group of Mendelian diseases affecting the genes essential for chromatin structure and remodeling; these disorders were previously reviewed (4, 12, 60, 69). The disorders that affect chromatin in *trans* often have pleiotropic clinical presentations and affect many different organ systems. The best-characterized disorders represent defects in proteins directly related to DNA methylation or defects in proteins related to chromatin remodeling. As discussed above, there are complex links between DNA methylation and chromatin structure (13, 73). Therefore, mutations of these genes can alter the chromatin structure and result in either activation or repression of gene expression, although the target genes that are affected have not been identified and related to the phenotype for the most part. Disorders that affect chromatin in *trans* often have widespread effects, whereas those that affect chromatin in *cis* tend to affect one or a few genes locally (Table 4).

Immunodeficiency-centromeric instability-facial anomalies syndrome (ICF syndrome; OMIM 242860) is one of the more straightforward examples of a Mendelian disorder affecting the biochemistry of DNA and chromatin remodeling. ICF is a rare autosomal recessive disorder in which patients are mentally retarded and have instability of pericentromeric heterochromatin (particularly for chromosomes 1, 9, and 16). Historically, hypomethylation of DNA in classical satellites 2 and 3 was described in ICF patients well before the identification of the mutated gene. In 1999, three groups independently reported mutations in *DNMT3B*, a de novo DNA methyltransferase, in patients with ICF syndrome (56, 115, 168). The mutations in *DNMT3B* indicated that this enzyme is important for de novo methylation of centromeric satellites 2 and 3. As noted above, mice with homozygous null

TABLE 4 Genetic disorders of chromatin structure and remodeling not specific to imprinted genes

Disorder	Comment	OMIM
Defects affecting chromatin structure and remodeling <i>in trans</i>		
ICF syndrome	<i>DNMT3B</i> mutations	242860
Rett syndrome	<i>MECP2</i> mutations	312750
α -thalassemia/mental retardation, X-linked (ATR-X)	X-linked helicase-2 mutations	301040 309590
Immunoosseous dysplasia, Schimke type	SMARCAL1, SWI/SNF-related protein	242900
Rubinstein-Taybi syndrome	<i>CREBBP</i> mutations	180849
<i>MTHFR</i> deficiency	<i>MTHFR</i> mutations	236250
Recurrent hydatidiform mole	Unidentified gene in 19q13.4	231090
See Bickmore & van der Maarel (12) for additional listings.		
Defects affecting chromatin structure and remodeling <i>in cis</i>		
Fragile X mental retardation syndrome	Triplet repeat in <i>FMRI</i> with DNA methylation and silencing	309550
Deletion LCR $\gamma\delta\beta$ - and $\delta\beta$ -thalassemia	Deletion of LCR causes loss of globin expression	141900
FSH dystrophy	Shortened repeat derepresses adjacent genes	158900
Disorders of XIC	Skewing mutations and ring X deletions of XIC	314670, 300087
For position effects, see Kleinjan & van Heyningen (83).		

mutations for *Dnmt3b* also have hypomethylation of centromeric minor satellite repeats and have multiple defects resulting in embryonic lethality (115). The mechanisms whereby the defect in a DNA methyltransferase results in a distinct and complex phenotype remain elusive. The DNA of the inactive X chromosome in females with ICF is much less methylated than is normal for an inactive X, and there are changes in replication timing and gene expression that vary among genes subject to X-inactivation. However, the phenotype in females is not dramatically different from that in males, which suggests that abnormalities of X-inactivation are not a major component of the pathogenesis of the disorder. The gene for synaptobrevin-like 1 (*SYBL1*) is located in the pseudoautosomal regions of the long arms of the X and Y chromosomes, and its normal inactivation on the X in females and on the Y in males is disrupted in ICF syndrome (99). The extent to which the pathophysiology of ICF syndrome involves disturbed expression of imprinted versus nonimprinted genes is currently unknown. One interesting possibility is that the altered methylation status in repetitive sequences may alter the

chromatin structure by sequestering the protein complexes that normally activate or repress specific genes.

The discovery of mutations in *MECP2* in Rett syndrome (OMIM 312750) provides another clear example of a mutation in a gene related to recognition of DNA methylation causing a human disease (3). Rett syndrome is a relatively frequent form of mental retardation accompanied by ataxia, seizures, and purposeless hand wringing caused in most cases by de novo mutations in females; the same mutations cause embryonic lethality in hemizygous males. Mutation analysis has led to the recognition of other phenotypes including neonatal encephalopathy and nondistinctive X-linked mental retardation in males (54). As discussed above, MeCP2 is a well characterized methyl-CpG-binding protein that can bind to a single methylated CpG site in genomic DNA. MeCP2 also can act in a large chromatin protein complex and function as a transcriptional repressor. As with *DNMT3B* and ICF syndrome, the specificity of the MeCP2 protein in the regulation of downstream targets is poorly understood. There are two reports that MeCP2 binds to one promoter and represses transcription of brain-derived neurotrophic factor (*BDNF*), a secreted protein that is essential for neural function. Both groups report that depolarization of neurons, which is known to induce *BDNF* expression, results in dissociation of MeCP2 from the *BDNF* promoter, providing a neuronal activity-dependent form of epigenetic gene regulation (24, 98). The evidence that neuronal activity regulates gene expression by epigenetic mechanisms provides a new direction for investigating the function of MeCP2.

The α -thalassemia X-linked mental retardation (*ATRX*) syndrome is another example of a disorder affecting chromatin structure in *trans*, as reviewed elsewhere (4). Affected males have relatively severe mental retardation, and most or all are unable to walk, whereas heterozygous females are usually asymptomatic. The *ATRX* protein contains a plant homeodomain (PHD)-like zinc finger motif as well as an ATPase domain of the SNF2 family of helicase/ATPases. Mutations in *ATRX* cause down-regulation of the α -globin locus and presumed dysregulation of many other genes, thereby giving rise to the complex phenotype. Mutations also cause changes in the pattern of methylation of several highly repeated sequences, including the ribosomal DNA arrays, a Y-specific satellite, and subtelomeric repeats (47). Numerous other syndromic diagnoses are sometimes caused by mutations in *ATRX* (see OMIM and <http://www.genereviews.org/>). Associated phenotypes include males with mild to moderate retardation (171) and acquired α -thalassemia in myelodysplastic syndrome (*ATMDS*) due to somatic mutations (48).

Deficiency of methylenetetrahydrofolate reductase (*MTHFR*) is an interesting disorder from the perspective of DNA and histone methylation. Extreme deficiency of *MTHFR* is a rare disorder that results in mental retardation (136). There is a very common hypomorphic allele for *MTHFR* that is associated, in homozygotes particularly, with increased levels of homocysteine; the relationship of this allele to the risk of atherosclerosis and neural tube defects has been studied extensively (139). Mice heterozygous or homozygous for *MTHFR* deficiency have decreased levels of S-adenosylmethionine (SAM) and decreased global DNA methylation

(25). It is possible that part of the phenotype in severe *MTHFR* deficiency could be mediated through disturbances of chromatin in *trans* involving decreased methylation of DNA or histones; thus, its inclusion in Table 4.

Complete hydatidiform moles (CHM) and ovarian teratomas represent growths of cells with two paternal or two maternal genomes, respectively. Both represent biological aberrations in which the inability to give rise to a live birth is determined in whole or in part by genomic imprinting. No fetus is present with CHMs; they most often are XX and homozygous, apparently arising by duplication of a haploid sperm in an anucleate egg, and a minority are XY and heterozygous, apparently arising through dispermy in an anucleate egg (88). Partial moles are distinct from CHMs and are triploid, usually with one maternal and two paternal genomes resulting from dispermy; a fetus is present (88). Ovarian teratomas arise through parthenogenic activation of ova containing two maternal genomes; one study suggests that “65% of teratomas are derived from a single germ cell after meiosis I and failure of meiosis II (type II) or endoreduplication of a mature ovum (type III); 35% arise by failure of meiosis I (type I) or mitotic division of premeiotic germ cells (type IV)” (146).

Although most CHM are uniparental with two paternal genomes, a small number of women have an autosomal recessive disorder characterized by recurrent CHM of biparental origin (78). This represents a disorder in which many, but not all, imprinted loci spread across the maternal genome have a paternal epigenotype; therefore, this is an example of a condition affecting chromatin structure in *trans*. The gene encoding the putative factor maps to chromosome 19q13.4 (65), but has not been identified as of this writing.

There may be secondary epigenetic alterations in the Mendelian disorders caused by polyglutamine repeat expansions (e.g., Huntington disease and spinocerebellar ataxia). When *Drosophila* models of these diseases are used to screen for genetic modifiers, many of the genes identified are involved in chromatin remodeling (43). Treatment with a histone deacetylase inhibitor reduced polyglutamine toxicity in a study using cultured cells (102), and treatment using the histone deacetylase inhibitor sodium butyrate extended survival in a mouse model of Huntington disease (44).

Genetic disorders definitely or likely affecting chromatin structure in *trans* also include Rubinstein-Taybi syndrome, Coffin-Lowry syndrome, Schimke immunosseous dysplasia, and others (see reviews 4, 12, 60, 69).

GENETIC DISORDERS AFFECTING CHROMATIN STRUCTURE AND REMODELING IN *CIS*

The fragile X mental retardation syndrome (*FMRI* gene encodes FMRP protein; OMIM 309550) is an excellent example of a genetic disorder affecting chromatin structure in *cis*; this involves altered DNA methylation, chromatin abnormalities, and transcriptional repression caused by a primary genetic abnormality

(trinucleotide repeat expansion) (158). A full mutation of the *FMR1* gene results in hypermethylation at the promoter region and repression of gene expression. The 5'-end of *FMR1* is associated with acetylated histones H3 and H4 in cells from normal individuals, but acetylation is reduced in cells from fragile X patients (28, 29).

There are examples where deletions of regulatory elements can act over a great distance to alter chromatin structure and abolish expression of protein coding genes. With the nonimprinted β -globin cluster, deletions in the locus control region (LCR) tens of kilobases upstream of the β -globin coding region cause $\gamma\delta\beta$ - or $\delta\beta$ -thalassemia with loss of expression in *cis* associated with an altered chromatin state (49). With the imprinted Angelman ubiquitin ligase, deletions of the AS portion of the bipartite imprinting center (AS-IC) hundreds of kilobases from the coding exons also cause loss of expression in *cis* and are almost certainly associated with an altered chromatin state (166). These represent forms of position effects. Kleinjan & van Heyningen (83) tabulate position effects for at least ten additional loci and discuss molecular mechanisms.

The molecular basis of facioscapulohumeral muscular dystrophy (FSHD) is a still unfolding story of a likely position effect (see Bickmore and van der Maarel 12). Most cases of this common myopathy are dominantly inherited and map to chromosome 4qter. Near the telomere of the chromosome is a low-copy GC-rich repeat of 3.3 kb called *D4Z4*, with 11–150 repeat units in the normal population, but fewer repeats in affected individuals (161). Only some of the chromosomes with shortened repeats are disease causing, and these alleles are associated with an increased abundance of transcripts mapping just centromeric to the repeat. Thus, some mutant chromosomes bring about a less repressive chromatin structure centromeric to the repeat and up-regulate expression from one or more 4qter genes. Bickmore & van der Maarel (12) report that it is possible that the FSHD mutations affect the normal chromosome in *trans* as well as the mutant chromosome in *cis*, but this is speculative at present.

There is one report that an antisense transcript generated by deletion and juxtaposition of an α -globin gene to another locus causes a stable silencing of the globin gene (82, 150). Numerous forms of epigenetic regulation involve the participation of a noncoding RNA as exemplified in yeast, *Drosophila*, *C. elegans*, fungi, and plants (2, 17, 84, 101, 118). These studies define an unexpected link between transcription elongation and histone methylation (55). X-inactivation in females is a highly complex form of epigenetic regulation in which the *Xist* transcript and the antisense counterpart *Tsix* contribute in varying ways to X chromosome counting, choice, silencing, and maintenance (14). In terms of human diseases or variants involving X-inactivation, mutations in the promoter for *XIST* can cause severe skewing of X-inactivation (127), and ring X chromosomes lacking an X-inactivation center (XIC) cause a severe phenotype in females due to inability of the ring chromosome to undergo inactivation (106).

For almost all Mendelian disorders where the disease gene has been identified, there is a small or modest fraction of patients in whom mutations are not found when

all known exons, coding and noncoding, are sequenced. One possibility is that there are epigenetic or genetic abnormalities that affect gene expression in *cis*. Although it is difficult to discover mutations having regulatory effects over a great distance, it is feasible to determine if an allele is producing a transcript of appropriate structure and abundance, so long as the gene is expressed in an accessible cell source such as leukocytes or cultured cells. This approach is effective for characterizing mutations causing familial polyposis (128). If deficiency of the transcript from a specific allele is detected, it is possible to use chromatin immunoprecipitation (ChIP) to determine if the chromatin structure for a suspect allele has normal properties, and this type of analysis demonstrates a defect in the case of deletions of the LCR causing $\gamma\delta\beta$ - or $\delta\beta$ -thalassemia (49), as noted above.

ROLE OF DIET AND ENVIRONMENT IN EPIGENETICS

Because the epigenotype normally displays more developmental and temporal variability within a normal individual than the genotype, the epigenotype might be more susceptible to environmental influences than the genotype. There are many perspectives that suggest this is the case. As noted above, ICSI and in vitro fertilization may increase the risk of epigenetic imprinting defects, and this would represent a potential iatrogenic environmental effect. There are various reports of potential interactions between epigenetics and aging, and it is reasonable to consider the hypothesis that aging involves cumulative epigenetic changes and that this interaction could be susceptible to environmental effects; a recent review with extensive bibliography is available (5). Particularly intriguing is the evidence that increased expression of the histone deacetylase encoded by the *SIR2* gene increases life span in yeast and *C. elegans* (59). These authors suggest that in both yeast and *C. elegans* “*SIR2* genes are involved in sensing environmental conditions and, in the face of scarcity or stress, trigger specialized survival forms,” through a form of epigenetic regulation. In terms of nutritional effects, cardiovascular and diabetes mortality may be affected by nutrition during the slow growth period of the parents and grandparents (79). The potential for complex relationships involving the epigenetic history of an allele (Figure 5), nutrition, and transgenerational effects is intriguing (121).

In terms of nutritional effects on epigenetics, folic acid is of particular interest. There are reports that the intake of folic acid and related metabolites can influence the expression of imprinted genes, both in mice and in humans. Folic acid has multiple metabolic effects contributing to biosynthesis of purines and pyrimidines, and is also essential for the production of adequate amounts of SAM, which is the primary donor in most or all methylation reactions (Figure 7). There is evidence that increased folic acid intake with added choline or betaine in mice can lead to increased DNA methylation of a retroviral insertion element within an agouti allele, and silencing of agouti expression accompanies the methylation (Figure 8) (159, 164); the expression of this mutated agouti allele varies

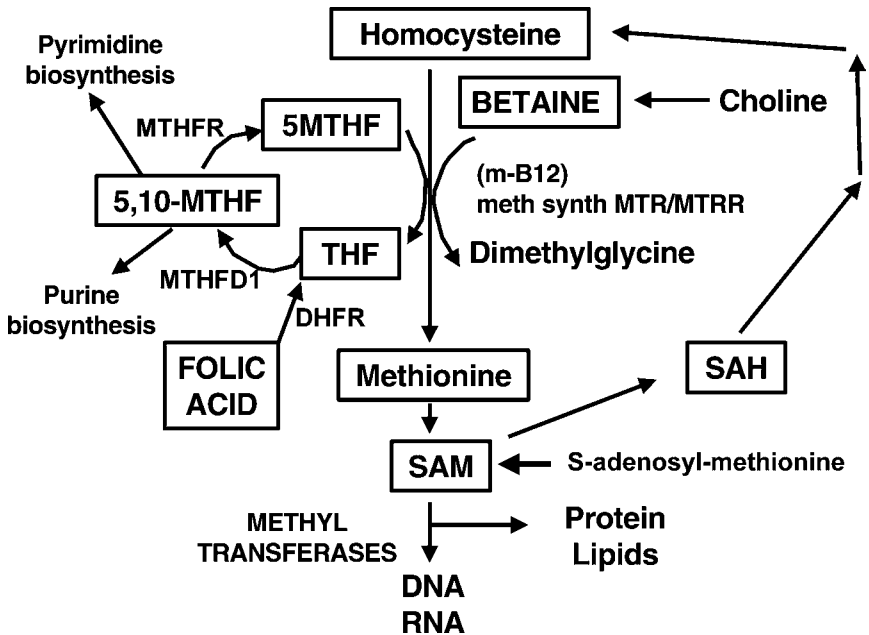


Figure 7 Folic acid and related pathways for production of S-adenosylmethionine (SAM). SAM is required for methylation of DNA and histones. SAH, S-adenosyl-homocysteine; DHFR, dihydrofolate reductase; MTHFD1, methylenetetrahydrofolate dehydrogenase; THF and MTHF, tetrahydrofolate and methyltetrahydrofolate.

according to parental lineage and strain background (105). There is also evidence that the folate effect may be transmitted beyond a single generation, again suggesting that imprints may not be completely erased and reset in a single generation. It is currently unknown whether folic acid intake can alter expression of nonimprinted genes.

As noted above, mice lacking *MTHFR* have decreased global DNA methylation. There also is evidence that folic acid intake in humans can affect the level of global DNA methylation, and that the genotype for *MTHFR* may affect the level of global DNA methylation (46). In one report, global DNA methylation decreased on a folate-depleted diet, but levels did not rise when folate intake was raised (131). Recently, there was a report that folic acid status might have substantial effects on imprinted gene expression in humans (70). In a study of adult males on hemodialysis, numerous patients had elevated blood homocysteine levels, presumably related to folate depletion. Both global and locus-specific DNA methylation were reduced in these individuals. Administering a substantial dose of folic acid increased both global and locus-specific DNA methylation. There was evidence for conversion of abnormal biallelic expression for a number of loci to the more normal monoallelic expression. This included data for *H19*, *IGF2*, and *SYBL1*.

Another study reported that approximately 10% of the normal population demonstrates biallelic expression for *IGF2* in peripheral blood cells (32). It is unknown if these individuals would convert to monoallelic expression if given high doses of folic acid, and the interrelationships between environment and genotype in regards to monoallelic versus biallelic expression has hardly been explored.

The evidence that folic acid intake and *MTHFR* genotype can influence DNA methylation suggests that the effects of folic acid and *MTHFR* phenotype on the risk for neural tube defects might be mediated through an epigenetic mechanism involving methylation of DNA or perhaps methylation of histones. There is conclusive evidence that increased intake of folic acid by child-bearing women can reduce the risk of neural tube defects. There is relatively strong evidence that *MTHFR* genotype is also a risk factor for neural tube defects; a meta-analysis tends to support the conclusion that women and fetuses homozygous for the hypomorphic mutation are at increased risk of neural tube defects (153). Folic acid could affect the risk of neural tube defects through its role in purine and pyrimidine metabolism, through effects on DNA or histone methylation, or through other effects, and it would be of great interest to determine the molecular basis for the effect of folic acid intake and *MTHFR* genotype on the risk of neural tube defects.

A MIXED EPIGENETIC AND GENETIC AND MIXED DE NOVO AND INHERITED MODEL FOR COMPLEX TRAITS

Our laboratory has worked extensively on the role of chromosome 15q11–q13 in PWS and AS, and more recently on the relevance of this chromosomal region to the occurrence of autism. As discussed above, the extensive knowledge about the molecular basis for the etiology of AS is particularly informative in regards to providing a precedent for a monogenic but mixed epigenetic and genetic and mixed de novo and inherited (MEGDI) model for the etiology of a phenotype (Figure 9). If this model for etiology holds for less common disorders such as AS, PWS, and BWS, it might be important in some more common disorders, particularly those ordinarily classified as complex disease traits. We propose that this mixed model of etiology might be relevant to autism (174). Apparent complexity can arise from many circumstances involving genetic, epigenetic, environmental, and stochastic interactions. Complexity can occur due to locus heterogeneity with individual families typically representing a single gene disorder as exemplified by maturity onset diabetes of youth (MODY). Monogenic or digenic inheritance involving a few loci can give rise to moderate complexity in a single family as exemplified by Hirschsprung disease. As noted above, BWS and AS are excellent examples of mechanistic complexity involving MEGDI factors affecting a single gene or chromosomal domain. The etiology of coronary artery atherosclerosis provides

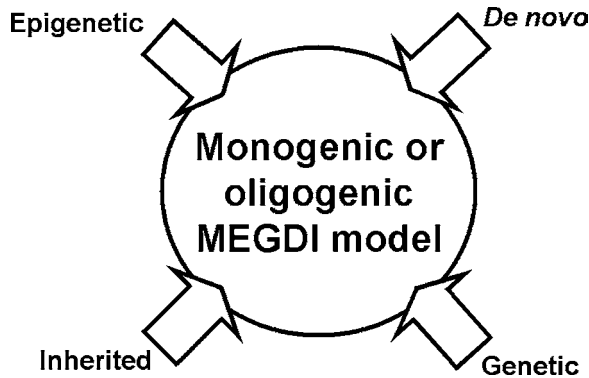


Figure 9 A mixed epigenetic and genetic and mixed de novo and inherited model for a monogenic or oligogenic disease. See Figure 6 for known examples.

another perspective on complexity with many loci and strong environmental factors in play in a single family and even in a single individual.

There are numerous disorders of unknown etiology that are often described as representing complex disease traits with little insight as to the nature of the complexity that might be involved beyond the fact that there is some familial clustering, but family structures are not compatible with simple Mendelian inheritance. In considering other complex disease traits and disorders of unknown etiology, psychiatric and behavioral disorders may merit special consideration. Petronis (80, 122, 124) proposed that epigenetic mechanisms may be important in various psychiatric disorders, including schizophrenia, bipolar illness, and depression. We speculate that models involving imprinted genes and disorders with evidence for an effect of folic acid might merit attention. There is a relatively extensive, although somewhat older, literature regarding folic acid levels and *MTHFR* genotype in schizophrenia (152). There are similar reports regarding *MTHFR* genotype in bipolar disease and depression, although these have not been confirmed and are relatively inconclusive. However, we propose that an MEGDI disease model deserves consideration in a variety of complex disease traits including psychiatric disorders of unknown etiology.

The mixture of epigenetic and genetic and mixed de novo and inherited mechanisms is widely appreciated in the etiology of cancer, where the concept of multistep pathogenesis is well established. We propose that epigenetic contributions will be increasingly recognized as contributing to the causation of complex disease traits whose etiology is currently unknown. It is important to emphasize that the genotype cannot affect the phenotype except through the prism of the epigenotype, and that genetic, epigenetic, environmental, and stochastic factors can lead to very complex mechanisms that cause dysregulation of gene expression. We suggest

that some disorders will conform to an oligogenic MEGDI model (Figure 9) that impacts one or a few genes.

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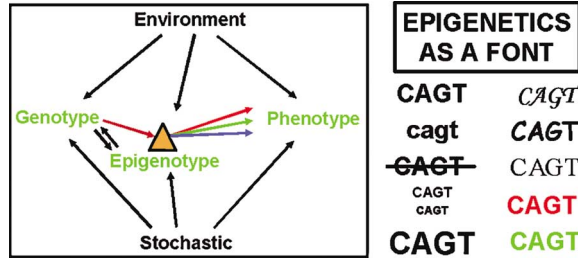


Figure 1 The role of the epigenotype in determining phenotype. (Left) The genotype must flow through the prism of the epigenotype to contribute to the determination of phenotype. Environmental and stochastic factors can impact the genotype, epigenotype, and phenotype. (Right) The epigenotype functions as an additional layer of regulatory information superimposed on the primary nucleotide sequence in a manner similar to the font superimposed on a primary text. Modified from Beaudet (7), with permission.

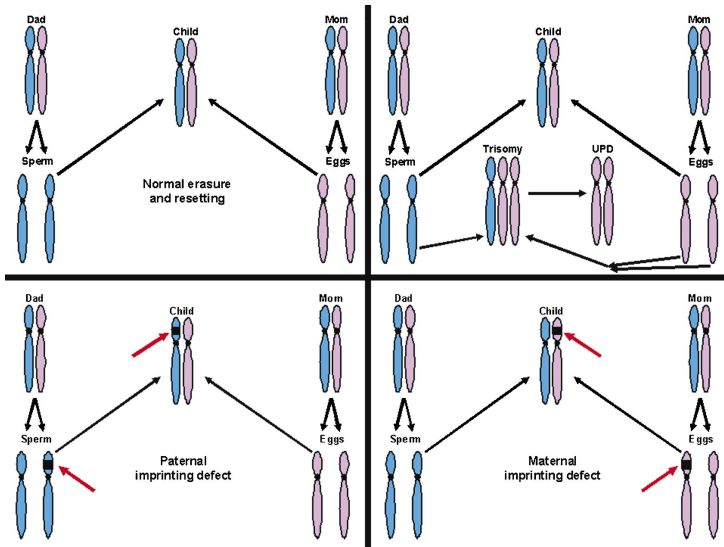


Figure 2 Normal erasure and resetting of genomic imprints and related errors. The upper left depicts the normal erasure and resetting process with the maternal epigenotype shown as a pink chromosome and the paternal epigenotype shown as a blue chromosome. The upper right depicts maternal nondisjunction leading to a trisomic embryo with subsequent chromosomal loss to produce maternal UPD. The lower left depicts a paternal imprinting defect with the abnormal segment of a chromosome in black indicating a maternal or other abnormal epigenotype on a chromosome of paternal origin. The lower right depicts a maternal imprinting defect with the abnormal segment of a chromosome in black indicating a paternal or other abnormal epigenotype on a chromosome of maternal origin.

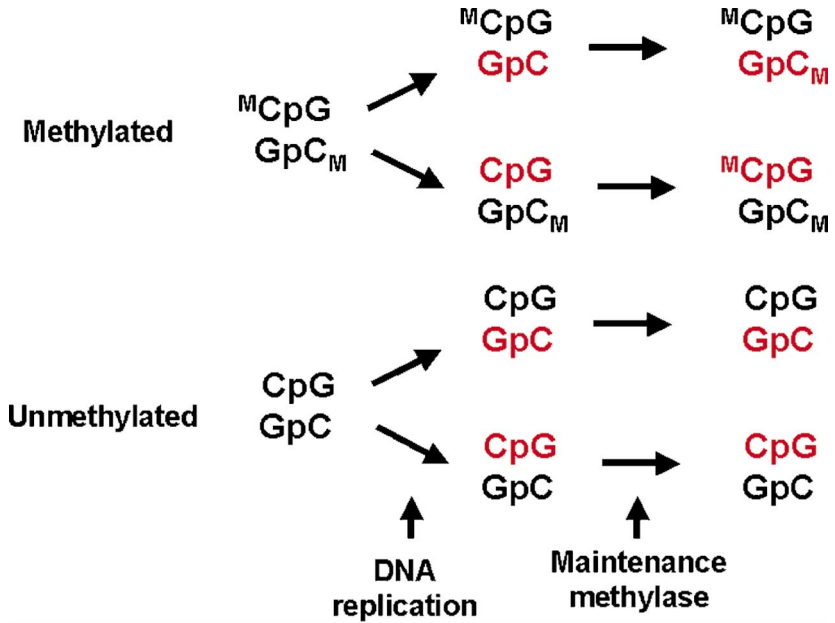


Figure 3 The maintenance methylase system preserves methylation status of CpG dinucleotides through mitosis. The parental strand of DNA is in black with the daughter strand in red. M = methyl group on C nucleotide.

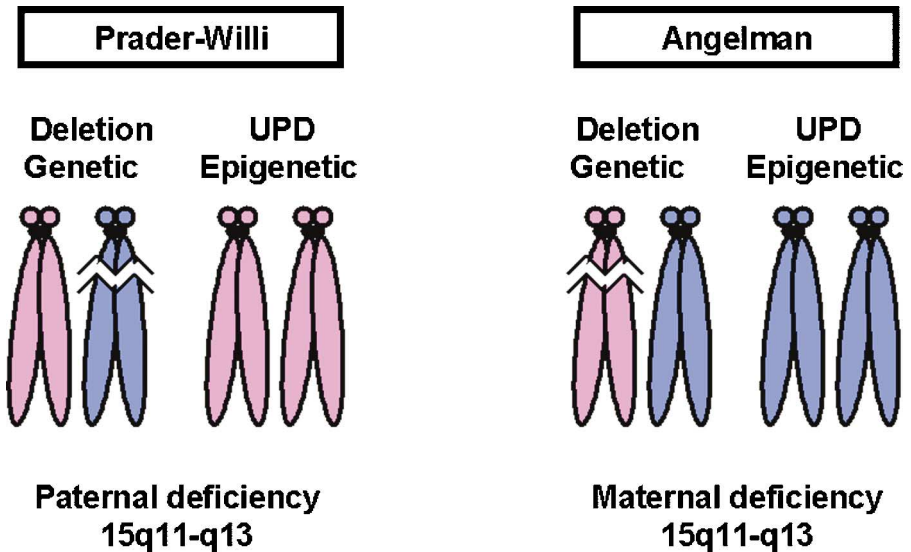


Figure 4 Genetic deletion or epigenetic UPD give rise to indistinguishable phenotypes.

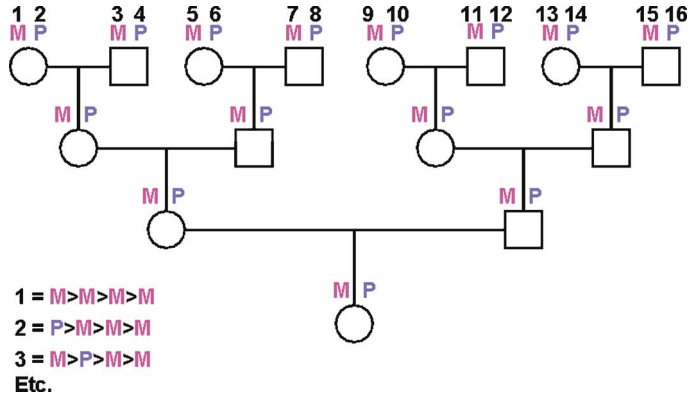


Figure 5 Switching of the genomic imprint for a domain or allele in a four-generation pedigree. The maternal and paternal alleles inherited in the fourth generation can each have any one of eight historical paths, which may not be equivalent if erasure and resetting of imprints is not always complete in a single generation.

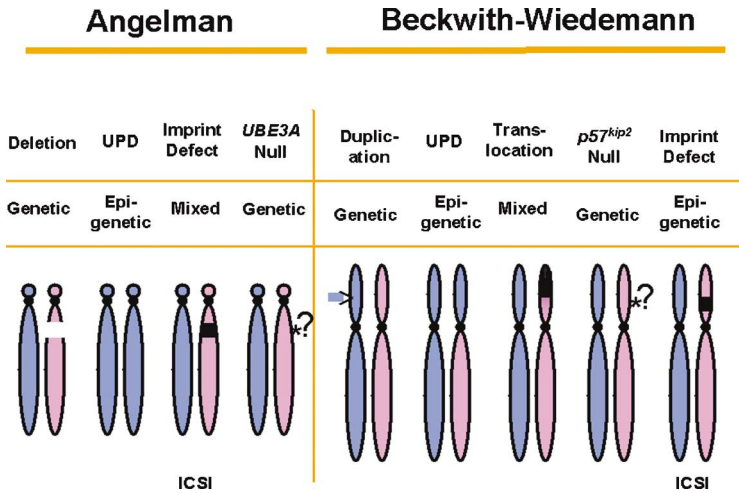


Figure 6 Heterogeneity of molecular mechanisms giving rise to the Angelman or Beckwith-Wiedemann phenotype. A blue chromosomal segment represents a paternal chromosome with a normal paternal epigenotype, a pink chromosomal segment represents a maternal chromosome with a normal maternal epigenotype, and a black chromosome segment represents the involved region with an imprinting defect or a portion of another autosome in a translocation. The asterisk indicates a loss-of-function point mutation. Imprinting defects that cause AS either may be associated with deletions of the imprinting center or have no detectable nucleotide sequence abnormality. ICSI indicates that this type of defect has been associated with intracytoplasmic sperm injection.



Figure 8 Range of coat color seen for agouti allele subject to variable DNA methylation and expression. Folic acid combined with choline or betaine administered to the pregnant mother can affect the coat color of the offspring. From Waterland & Jirtle (159) with permission.