Genomic Imprinting and Brain Function

62

Anthony R. Isles

Abbreviations

AG	Androgenetic
ART	Artificial reproductive technology
AS	Angelman syndrome
CNV	Copy number variant
GG	Gynogenetic
IC	Imprinting center
mUPD	Maternal uniparental disomy
PG	Parthenogenetic
pUPD	Paternal uniparental disomy
PWS	Prader-Willi syndrome
snoRNA	Small nucleolar RNA
VTA	Ventral tegmental area

Brief History

Genomic imprinting is a relatively new phenomenon. Until the mid-1980s, the understanding of genetics very much followed the original laws laid down by Gregor Mendel, the Austrian monk who first systematically examined and described the inheritance of traits. In 1984, two separate groups, one led by Davor Solter in the USA and another led by Azim Surani in the UK, used experimental embryology to demonstrate that completion of mouse development required a copy of the genome

A.R. Isles
Medicine/Psychological Medicine & Neurology, Neuroscience and Mental Health Research Institute, Cardiff University, Cardiff, UK
e-mail: islesar1@cardiff.ac.uk

D.W. Pfaff (ed.), *Neuroscience in the 21st Century*,
DOI 10.1007/978-1-4614-1997-6_68,

from both the father (paternal) and mother (maternal) and that two copies of either the paternal or maternal genome was not sufficient. This was the first suggestion that the different parental genomes were not equivalent; a finding that disobeyed aspects of Mendel's second law of inheritance ("the law of independent assortment"). Around this time, a group led by Bruce Cattanach also demonstrated that duplication of specific chromosomal regions of the mouse genome gave rise to differential phenotypes depending on their parent of origin. Later, with the advancement of molecular biology techniques, the first of the genes responsible for the non-equivalence of the parental genomes were identified. These genes were termed "imprinted genes," due to the fact that one of the two parental copies of these genes (alleles) was "marked" (or "imprinted"). This imprinting referred to epigenetic marks, such as the addition of a methyl group to the DNA, which resulted in expression of these genes being from one parental allele only. Throughout the 1990s and 2000s, more and more imprinted genes have been identified, and it is now known that they play a significant role in key aspects of physiology. Genomic imprinting seems to be of particular importance in the brain. This was first recognized by Barry Keverne and colleagues in the University of Cambridge, UK, who demonstrated that the maternal and paternal genomes influence neurodevelopment in distinct regions of the brain. There is now a growing body of evidence describing the contribution of imprinted genes to behavior and neurodevelopmental illness. A recent genome-wide screen using cutting-edge DNA sequencing technology has underlined the importance of genomic imprinting in the brain by identifying hundreds of imprinted gene candidates representing between 5% and 10% of all the genes expressed in the mammalian brain.

The Imprinting Process

Unlike the majority of genes where there is equivalent expression from both inherited copies of a gene (alleles), imprinted genes are subject to epigenetic modifications that lead to paternal and maternal alleles having different levels of activity. Over the past two decades, the techniques required to analyze these molecular process have been developed and refined, and it is now recognized the complexity and breadth of epigenetic mechanisms. DNA methylation represents the simplest and most robust epigenetic process and, usually, increased methylation levels at a gene loci equates to decreased expression of a gene.

Far more complex are other epigenetic mechanisms related to chromatin modification. The DNA within each cell is wrapped around histone proteins. These are then packed together to form what is known as chromatin, the basic material from which chromosomes are composed. This provides stability and protection for the DNA. The histone proteins around which DNA is bound can be modified by the addition of different chemical groups to their protein "tails." Differing histone protein modifications result in altered 3-D chromatin structures (open or closed), meaning the DNA sequences underneath are more or less accessible to transcription enzymes, which in turn leads to more or less gene

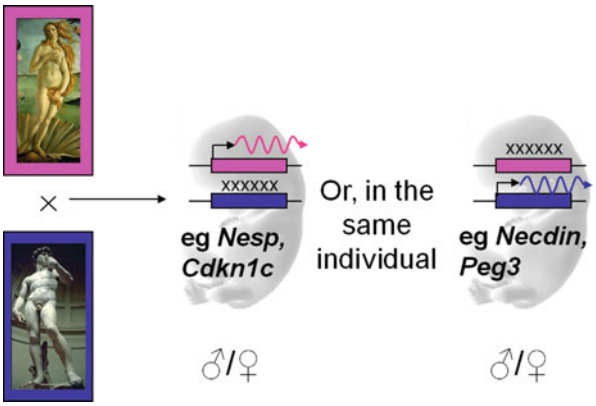

Fig. 62.1 A schematic showing the basic expression properties of imprinted genes. Like all autosomal genes, two copies (alleles) of every gene are inherited; one from the mother and one from the father. In the vast majority of cases, expression from these two parental alleles is, on average, equal. However, in the case of imprinted genes, although a copy of each gene is inherited from mother and father, epigenetic marking leads to expression from only one parental allele. This is developmentally determined and robust, so, for instance, expression of *Nesp* and *Cdkn1c* only occurs from the copy inherited from the mother. Other imprinted genes, such as *Necdin* and *Peg3*, are only ever expressed from the copy inherited from the father. Importantly, imprinted gene expression is generally independent of the sex of the offspring and is solely due to the parental origin of the alleles

expression. However, unlike DNA methylation, the histone code is less easy to "read," due to the many different types of histone proteins and possible chemical modifications.

The genomic imprinting process is initiated in the developing germ cells (sperm and eggs). Here DNA methylation of a DMR (*d*ifferentially *m*ethylated *r*egion) provides the primary or gametic imprint. Some of these gametic DMRs are found to be key control regions that are able to regulate expression of several genes within a cluster. These control regions are known as imprinting centers (ICs), or imprinting control elements (ICEs). After fertilization, these gametic imprints are maintained in somatic cell lineages and built upon by additional epigenetic marks including modification of core histone proteins surrounding the genes and regulation by noncoding RNAs. In the somatic cell lineages, differential epigenetic marking results in differential reading by the transcriptional machinery, and as a consequence, gene expression is exclusively (or predominantly) from one parental allele. This means that despite the physical presence of a maternal and paternal allele in the DNA, one of these parental alleles is silenced and expression is monoallelic (Fig. 62.1).

However, a key element about imprinted genes is that the pattern of silencing is robust and stable across generations. That is to say, a paternally expressed imprinted gene, such as *GnasXl*, is always expressed from the copy inherited

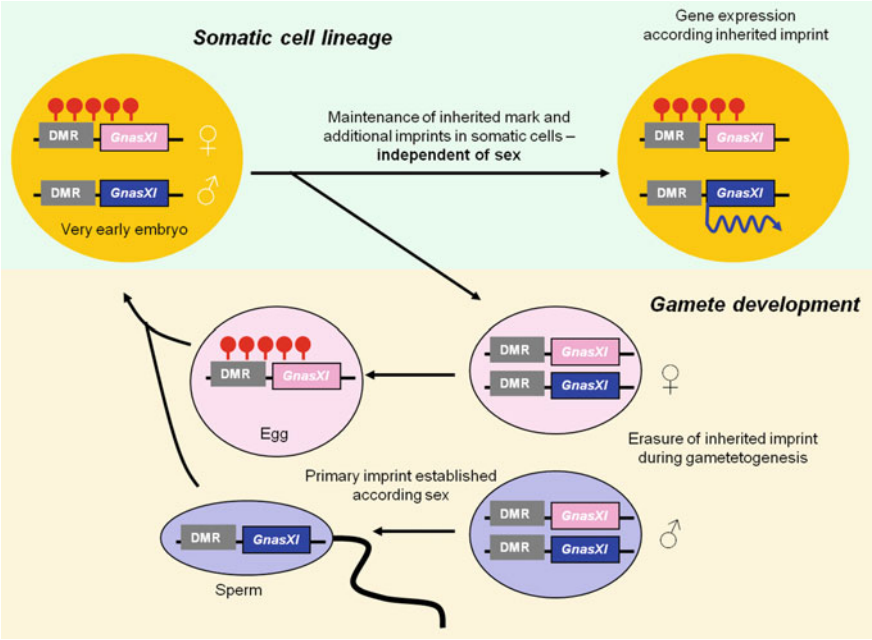

Fig. 62.2 A graphic illustrating the developmental cycle of genomic imprinting. The main epigenetic mark that is inherited is based on DNA methylation of a specific "differentially methylated region" (DMR, illustrated by red "lollipops"). The inherited epigenetic mark is then maintained and built upon (via recruitment of modified chromatin and noncoding RNA) in the somatic cell lineages. However, in the developing germ cells, the DNA methylation is erased and the epigenetic mark reset according to the sex of the developing embryo

from the father. Therefore, genomic imprinting is a developmentally determined process with the somatic epigenetic marks inherited from the parents being wiped and a new primary imprint being established in gametogenesis, according to the sex of the developing offspring. For example, in a developing female, the paternal copy of *GnasXl* will be expressed in somatic cells, with the maternal copy silenced. However, the epigenetic mark will be reset in the developing eggs, and all copies of *GnasXl* marked for silencing as this individual will pass their copy of *GnasXl* on as a mother (Fig. 62.2).

Nevertheless, it is becoming increasingly clear that there are variations in the control of expression of imprinted genes both temporally and/or spatially, such that some genes only show imprinting and parent-specific expression (i.e., monoallelic) in particular tissues whereas in other tissues, the same genes show nonimprinted (biallelic) expression. For instance, the gene *UBE3A* is imprinted and expressed from the maternal copy in brain but nonimprinted in other tissues where it is expressed from both parental copies. Even more complex is the degree of epigenetic control exerted over the imprinted gene *Grb10*. This gene is expressed from the maternal copy only in the body tissues, the paternal copy being silenced.

However, in the central nervous system, expression is solely from the paternal allele, with the maternal copy being silenced. Nonetheless, even these complex and subtle variations of imprinting and monoallelic expression of certain genes within an individual are still robust and stable from generation to generation (i.e., these patterns are observable from one generation to the next).

Developmental Consequences of Imprinting

Despite imprinted gene representation in mammalian genomes being small (there are currently approximately 145 recognized imprinted genes and noncoding RNAs in mice and a further 1,300 candidate imprinted genes), they are absolutely crucial for normal development and function. Mouse embryos manipulated to have either only a paternal, or only a maternal genome, are malformed and die before midgestation. Moreover, parthenogenetic (PG, two maternal genomes) and androgenetic (AG, two paternal genomes) embryos show very different phenotypes; PG embryos were normally developed but much smaller, probably as a consequence of reduced extraembryonic tissue; AG embryos were severely growth retarded with relatively expanded trophoblastic tissue.

Since these pioneering experiments, a number of individual imprinted genes have been characterized using various molecular biology techniques. It is clear, as hinted at by the early embryology studies, that a large number of imprinted genes are expressed in the placenta and/or fetus and influence in utero growth (this is touched upon further below). However, it is now known that there are a number of other key areas of physiology in which imprinted genes are recognized to play a role. These include maternal care, preweaning growth, and nutrient acquisition; energy metabolism; brain development; and behavior. The latter area of physiology is the topic of this chapter and is discussed in greater detail below.

Evolutionary Ideas

One of the most fascinating aspects of imprinted genes is their very existence. Genes subject to genomic imprinting are (at least part of the time) effectively haploid, thus negating the apparent benefits of sexual reproduction and diploidy, namely, increased genetic diversity and reduced exposure to the effects of deleterious alleles. This has led to much debate as to why genomic imprinting has arisen and resulted in many findings relating to imprinted genes being couched in terms of their evolution.

There have been many attempts to explain the occurrence of genomic imprinting. However, all theories are limited by what is known of the function of imprinted genes, and there is an argument that until there is a better overall understanding of this functionality, there is little point developing a theory. However, two theories – the "intragenomic conflict" and "coadaptation" theories – do provide an explanation for the many widely observed physiological roles of imprinted genes. Moreover, these are the only two ideas thus far, which address the role of genomic imprinting in the brain.

Intragenomic Conflict

The intragenomic conflict theory was developed with kinship ideas in mind and is an extension of the classic "parent-offspring" conflict. Intragenomic conflict suggests that where asymmetries of relatedness between maternal and paternal genes exist, there will be a conflict of interests between parental genomes over certain aspects of physiology. The classic example where such asymmetries of relatedness occur is in the developing fetus. As a consequence of multiple paternities, either within or between pregnancies, maternally derived genes will always be shared between siblings, but this is not necessarily true for paternal genes. In this scenario, paternal genes within the developing offspring would be predicted to attempt to increase resources from the mother. In contrast, maternally derived genes within the developing offspring would limit any effect in order to maximize the mother's (and therefore the maternal genes) overall reproductive output. This imbalance leads to an "arms race" involving expression levels of maternal and paternal copies of a gene, ultimately resulting in the silencing of one parental allele.

The idea that maternal and paternal genomes may be in conflict over resource allocation in utero is mostly borne out by the data. Greater than half of the known imprinted protein coding genes and noncoding RNAs are expressed in the placenta and/or the developing fetus. Furthermore, a variety of targeted deletions and transgenic studies in mice have shown that absence or overexpression of these imprinted genes has consequences for fetal growth and size. Although early embryology studies clearly demonstrated their global importance in order for normal development to proceed, many imprinted genes are not involved in development per se but instead are primarily concerned with acquisition of nutrients. Strikingly, the generally observed pattern of paternally silenced/maternally expressed genes limiting and maternally silenced/paternally expressed genes enhancing growth is predicted by the intragenomic theory.

Intragenomic conflict can be seen at a physiological level as well as an epigenetic level. This is best illustrated in the mouse by the antagonistic components of the *Igf2*-*Igf2r* system. *Igf2* and *Igf2r* were two of the very earliest confirmed imprinted genes. The insulin-like growth factor type 2 (*Igf2*) is a paternally expressed growth enhancer, whereas the insulin-like growth factor type 2 receptor (*Igf2r*) is a maternally expressed growth inhibitor that binds Igf2 and targets it for degradation. Mice targeted to be null for *Igf2* were growth retarded, whereas those null for *Igf2r* showed growth enhancement. Further analysis has revealed that Igf2 signals both demand in the fetus and supply via the placenta and interacts with placental transporter systems, including a system A amino acid transported encoded by the imprinted *Slc38a4* gene.

Similar arguments can be made for the role of imprinted genes in the early postnatal period when most mammals are reliant upon their mothers for nutrient and other resources. Again the prediction from intragenomic conflict is that paternally expressed genes in the offspring will attempt to maximize resources from the mother, whereas maternally expressed genes will act to limit resource acquisition. Here it can be seen, from mouse and studies of human disease, how paternally expressed imprinted genes influence suckling.

Coadaptation

In contrast to intragenomic conflict, the coadaptation theory suggests that genomic imprinting occurs in mammals as a means to coordinate the evolution of provisioning of offspring pre- and postnatally. In this context, the monoallelic expression of imprinted genes is a means by which their "evolvability" is accelerated. This is because haploid expression, while increasing exposure to deleterious alleles, also has the advantage over diploid expression of rapid fixation of an advantageous trait in a population. It is thought that the development of a placenta and maternal care in mammals is an area of function where such rapid fixation of any selective advantage is important, leading to the evolution of imprinted genes influencing these aspects of physiology. As has been seen, this is an area of physiology in which imprinted genes do play a major role.

The coadaptation idea was originally developed in light of the identification of two separate paternally expressed genes in the mouse (*Peg1* and *Peg3*), both of which were found to effect placental function and fetal growth, and maternal care in adult females. Further analysis also revealed that *Peg3* was not only important for mothers providing postnatal care and feeding but was also involved in the neonates' ability to suckle. The idea proposed was that the high turnover of these two genes down the paternal line would provide the platform for the rapid coadaptation of in utero growth and postnatal provisioning and care. A similar argument, based on the adaptive integration of offspring and maternal genomes, can be made for the coadaptive evolution of maternally expressed imprinted genes.

How Have Imprinted Genes Evolved to Influence Adult Brain Function?

The evolution of genomic imprinting in mammals appears to be driven by the development of in utero fetal development and the placenta and the associated parental care. This is illustrated by the fact that true imprinting is found in the eutherian and marsupial mammalian lineages but not the egg-laying monotremes. Moreover, many of the first studied imprinted genes had clear roles in placental function and in utero growth and/or the interaction between mother and offspring in the preweaning period. Both the intragenomic conflict and coadaptation evolutionary theories of genomic imprinting provide an explanation for a large proportion of these observed physiological effects of imprinted gene; although no one theory can account for all occurrences. Nevertheless, with regard to the adult brain, at present, the coadaptation theory offers only an explanation for the role of imprinted genes controlling maternal behavior (something the intragenomic conflict theory struggles with). However, as shall be seen, imprinted genes are known to be expressed in many brain regions and influence much other behavior than simply maternal care.

In terms of adult brain function, intragenomic conflict has been theorized to contribute to the evolution of motivated behaviors, in particular social behavior. The asymmetries of relatedness between maternal and paternal genomes that are

required for intragenomic conflict can also be established in animal societies in which individuals of one or other of other sexes move away from the natal group (sex-biased dispersal). For instance, as occurs in many animal social groups, males leave the group on reaching sexual maturity. This leads to a preponderance of shared maternally derived genes as the group consists of maternally related females: sisters, maternal half-sisters, maternal cousins, maternal aunties, etc. This leads to the situation in which there is a conflict of interest between the parental alleles shared in the group (in most case, maternally derived) favor behaviors that promote the social group and these shared genes, whereas those parental alleles which are not as extensively shared promote more "selfish" behavior. What is clear is that such an internal conflict would be manifest at the behavioral level, impacting on how individuals interact socially within the group. The range of behaviors predicted to be influenced is extensive and could include one or many functions, such as kin recognition, alarm calls, risk taking, grooming, communal nursing, and aggressive behavior.

Overall Patterns of Genomic Imprinting in the Brain

Early Brain Development Studies

Seminal mouse studies by Barry Keverne and colleagues in the mid-1990s revealed that imprinted genes are likely to contribute significantly to brain development and also indicated potentially dissociable (and possibly antagonistic) influences of paternally and maternally expressed genes on this process. As indicated earlier, mouse embryos composed solely of two copies of the maternal genome (parthenogenetic: PG; gynogenetic: GG) or two copies of a paternal genome (androgenetic: AG) die in midgestation. However, chimeras made up of a mixture of either normal and PG cells (N ↔ PG/GG) or normal and AG cells (N ↔ AG) can survive to term as long as the uniparental cell contribution is <35%. Keverne and colleagues generated such chimeric mice and studied them in terms of their brain development. N ↔ PG/GG displayed relatively large brain to body size ratios, while N ↔ AG had a relatively small brain to body size ratios, implying that one or more imprinted genes have profound effects on brain size. Specifically, the data seem to indicate that the overall effect of maternally expressed genes is to enhance brain size, while the combined effect of paternally expressed genes is to limit brain growth. More interestingly perhaps was the distribution of PG/GG and AG cells within the brains of the two types of chimera. By labeling the PG/GG and AG cells with a reporter gene, it was possible to follow where these cells ended up in the brains of N ↔ PG/GG and N ↔ AG chimeras. Initially, both PG/GG and AG cells were found throughout the brain. However, postnatally, there was a clear reciprocal pattern, with PG/GG cells contributing mainly to the neocortex and AG cells contributing more to the hypothalamic, septal, and preoptic areas (Fig. 62.3a).

These data provided the first suggestion that imprinted genes of different parental origins may have differential "interests" with regard to the brain. These effects

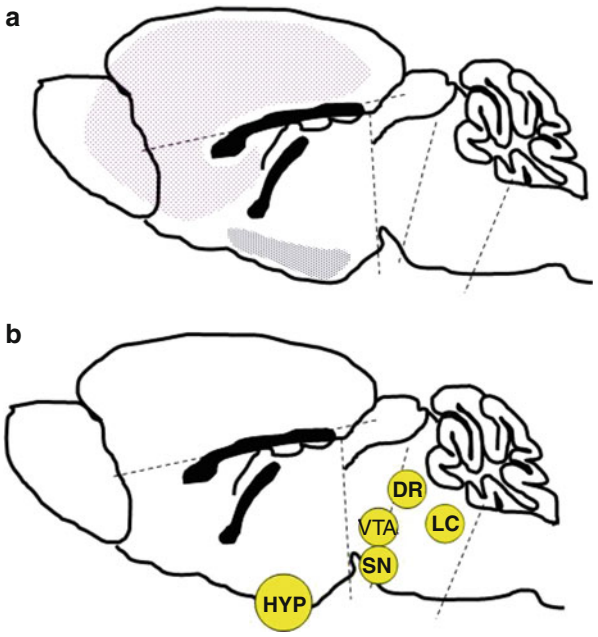

Fig. 62.3 A schematic illustrating the main areas of the brain in which genomic imprinting is thought to contribute. The first studies, using chimeras of normal and uniparental cells, demonstrated a difference in the pattern of distribution of parthenogenetic/gynogenetic and androgenetic cells in the brains of adult mice (**a**). Parthenogenetic/gynogenetic cells, containing two copies of the maternal genome only (in *pink*), were found in the forebrain and striatal areas of the adult mouse but were excluded from the hypothalamic regions. In contrast, androgenetic cells, which contained two copies of the paternal genome only (in *blue*), were excluded from the forebrain but found in abundance in the hypothalamus. Very recently, a genome-wide screen for imprinted gene expression in the brains of mice did not entirely support these original observations. Instead, this study found that imprinted genes, while expressed throughout the brain, were particularly enriched in key "hot spots" (**b**). These were the hypothalamus (HYP), substantia nigra (SN), ventral tegmental area (VTA), dorsal raphe (DR) nucleus, and the locus coeruleus (LC). However, there was no apparent parental bias in these regions

may represent either the combined effects of many paternally and maternally expressed imprinted genes or the actions of one or two imprinted genes of major effect. If the former is the case, it may be expected that maternally expressed imprinted genes would be disproportionately expressed in neocortical regions and paternally expressed imprinted genes would be disproportionately expressed in hypothalamic and septal regions; it is now known that this is not exactly the case, and the answer is probably somewhere in between. In reality, the distribution of AG and PG/GG cells in the two types of chimera probably does point to where the interests of maternal and paternal imprinted genes in the brain lie when unfettered by the action of opposing parental alleles. Nevertheless, probably most importantly, this work demonstrated that imprinted genes are not only involved in placental function and fetal growth but also have a major role in the brain.

Imprinted Gene Expression in the Brain

Since those groundbreaking embryology experiments, many imprinted genes (~90%) have been shown to be brain expressed. Some of these have been discovered and identified as a result of efforts to characterize imprinted gene clusters and early imprinted gene screens. These include two key brain-expressed imprinted genes, *Peg1* and *Peg3*, which were isolated on the basis of the fact that they showed parent-of-origin-specific expression. However, many other brain-expressed imprinted genes have been examined due to their association with the human neurodevelopmental disorders Prader-Willi syndrome (PWS) and Angelman syndrome (AS). In fact, these disorders were linked to genomic imprinting before the first imprinted genes were even characterized, due to the fact that the same genetic mutation (a large deletion on human chromosome 15) gave rise to either PWS or AS, two very different syndromes, depending on whether it was inherited from the father or mother, respectively. The genes within the PWS/AS interval include *NECDIN, MAGEL2* and *UBE3A* as well as number of noncoding RNA species. More recently, a number of new brain-expressed imprinted genes have been identified, many of these in humans, as a result of taking account of parent-of-origin factors in genomics studies of psychiatric illness, such as autism and schizophrenia.

In 2010, a study taking advantage of second-generation sequencing techniques demonstrated the existence of hundreds of putative imprinted genes in the mouse brain. It has been known for some time that a number of imprinted genes, particularly in the brain, show complex patterns of epigenetic regulation and parental allele expression. The result of this is that some genes are only imprinted in certain tissues (or brain regions) and/or certain developmental time points, while being expressed from both parental alleles in all tissues at all other times. In the past, this subtle regulation will undoubtedly have been a hindrance to the identification of new imprinted genes. However, second-generation sequencing technologies are capable of producing tens of millions of sequence reads from very little input material. This is expected to revolutionize how gene expression is analyzed and would, for instance, allow the rapid quantification of allelic expression from discrete tissue samples.

These studies investigating parental-specific expression in mouse brain took advantage of these techniques and combined them with old-fashioned reciprocal crosses between different substrains of mice (Fig. 62.4). These crosses provided the genetic diversity to distinguish alleles of different parental origin, both at the genomic and expression (RNA) level. RNA sequencing was used to investigate whether there was a bias in the parental allele expression. The study demonstrated the existence of approximately 1,300 genes showing some evidence for parent-of-origin-specific bias in their expression. The most likely explanation for this is obviously genomic imprinting, but until a specific epigenetic mechanism has been shown, these genes remain "candidate imprinted genes." Nevertheless, these studies are of great excitement, not just because of the massive increase in numbers (representing between 5% and 10% of all gene expression in the mouse brain) but also because they allowed the first global view of where imprinted genes are

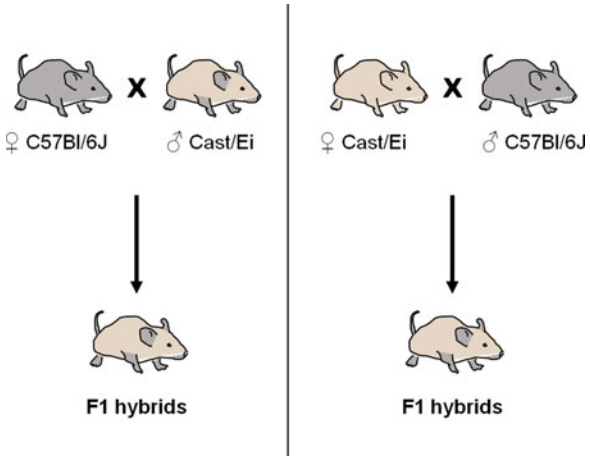

Fig. 62.4 Reciprocal F1 crosses have been used for many years to investigate the parent-of-origin effects associated with genomic imprinting. Most recently, crosses between the standard laboratory strain of mice, C57Bl/6 J, and a wild derived strain, CAST/Ei, have been used to examine genome-wide parent-of-origin effects on brain gene expression. Second-generation sequencing techniques were used to identify genetic variation (polymorphisms) between these two strains. Then F1 crosses were generated, and this genetic information was used to determine whether there was a bias in which parental allele was expressed. Importantly, both reciprocal crosses between C57Bl/6 J and CAST/Ei were analyzed (i.e., when C57Bl/6 J was the mother and CAST/Ei the father; then, separately, when CAST/Ei was the mother and C57Bl/6 J the father). This disambiguated strain effects from true parent-of-origin effects on gene expression

expressed within the brain since the studies of N ↔ PG/GG and N ↔ AG chimeras over a decade previously.

By comparing the list of "candidate imprinted genes" to existing genome-wide brain expression databases, this screen was also able to suggest "hot spots" of imprinted gene expression within the brain. Although there are examples of imprinted gene expression throughout the mouse brain, it seems that there is an overabundance of imprinted genes expressed in the hypothalamus and the areas of monoaminergic neuron projections (locus coeruleus, dorsal raphe nucleus, ventral tegmental area, substantia nigra) and activity (nucleus accumbens) (Fig. 62.3b).

These findings differ from the earlier neurodevelopmental studies of "PG/GG chimeras" and "AG chimeras" in that there was no apparent maternal or paternal brain regions and also in that in the overall scheme of the 1,300 imprinted candidate genes discovered, a smaller proportion were expressed in the neocortical regions. Indeed, this imprinted gene screen indicated that maternally expressed genes were more abundant in early neurodevelopment and that there was a preponderance of paternally expressed gene throughout the adult brain (including the cortical regions). However, comparisons between the early neurodevelopmental studies and this genomic screen are probably not valid. As outlined above, the neurodevelopmental studies may represent the outcome of the action of one or two imprinted genes of major effect, rather than a general pattern of imprinted gene expression in

the brain. Nonetheless, what all these studies illustrate is that genomic imprinting is of importance to neurodevelopment and seems to be key to the function of certain regions of the brain.

Behaviors Influenced by Genomic Imprinting

Based on the pattern of imprinted gene expression found in the brain, genomic imprinting can be predicted to influence a number of key behaviors. These include quite specific influences, over maternal behavior and feeding for instance and more generally what can be termed "motivated behavior." In this section, I will give examples of where imprinted genes influence these from studies using mouse models in which the expression of imprinted genes have manipulated and human disorders. However, this is not an exhaustive list of behavioral studies of imprinted genes. Moreover, future studies are required in order to examine the extent to which genomic imprinting influences those behaviors predicted by the hot spots of imprinted gene expression in the brain.

Maternal Care and Reproductive Behavior

One area of the brain strongly implicated to a "hot spot" of genomic imprinting by both the earlier neurodevelopmental studies and the recent genome-wide imprinted gene screen is the hypothalamus. This area of the brain is associated with the regulation of a number of "basic" behavioral drives, including feeding, sex, and maternal care. The latter function was found to be disrupted in one of the first studies linking a specific imprinted gene with a behavioral deficit. Fascinatingly, two separate studies, examining different paternally expressed genes, emerged at the same time, both showing similar results.

Females who carry a targeted (null) copy of *Peg1* or *Peg3* inherited from their fathers show gross deficits in maternal care that are independent of any possible effects in the pups. *Peg1* mutant females were deficit in normal aspects of maternal behavior, such as placentophagia, retrieval of pups, nest building, and crouching (suckling). Needless to say, the consequence of these abnormal behaviors is a reduced survivability of the offspring. Similarly, *Peg3* mutant females also displayed deficits in aspects of normal maternal care (retrieval, nest building, and crouching). Like the *Peg1* mutant females, this maternal care deficit was not due to olfactory dysfunction, and furthermore, the *Peg3* mutant females showed normal reaction to newly introduced pups (sniffing). Additionally, *Peg3* mutant females showed reduced milk letdown, despite having histologically normal mammary glands. This suggests a deficit in the neurobiological control of lactation, and investigations demonstrated a reduced number of oxytocin-positive neurons in the hypothalamus of *Peg3* mutant females. Given the central role played by oxytocin in both maternal behavior and milk letdown, the indication is that this is the neurobiological pathway via which the Peg3 protein is exerting an effect, an idea that sits

nicely with this protein's involvement in signaling pathways affecting apoptosis and cell survival.

Intriguingly, studies of *Peg3* null males have also revealed a role for this imprinted gene in sexual behavior, pointing to an involvement of genomic imprinting in all aspects of reproductive behavior. Although *Peg3* null males are fertile, they do not improve copulatory ability with sexual experience and behave like virgins even after mating with several females. The improved copulatory ability normally seen in male mice makes them more efficient reproductively, and they develop a preference for the odor of receptive estrus female. This altered olfactory preference also fails to develop in *Peg3* null mice.

PWS and Feeding Behavior

Imprinted genes are also strongly expressed in the arcuate nucleus of the hypothalamus, an area which is involved in the regulation of feeding. However, in addition to the basic drive to eat, feeding behavior is also influenced by the motivation for the rewarding properties of food. The role of genomic imprinting in both these aspects of feeding behavior is nicely illustrated by the neurodevelopmental disorder Prader-Willi syndrome (PWS).

PWS is caused by loss of paternal gene expression from the imprinted cluster on human chromosome 15q11-q13 (see Fig. 62.5). Many of the genes within this interval are strongly expressed in the hypothalamus, and PWS presents with multiple neuroendocrine abnormalities related to hypothalamic insufficiency. Clinically, PWS is characterized by severe hypotonia at birth and a failure to thrive in infancy due to poor suckling ability. Individuals also have growth retardation and hypogonadism/hypogenitalism, incomplete and delayed puberty, and infertility. On emerging from infancy (2–5 years old), there is a switch in the feeding problems, with individuals now showing hyperphagia and obsession with food that if not managed correctly leads to central obesity and related health problems.

Behaviorally, the hyperphagia seen in PWS primarily manifests as a failed satiety response. However, behavioral and neuroimaging experiments suggest that individuals are not "hungrier," but in fact, there is some disruption of satiety signaling once feeding has begun, meaning they do not feel full. In addition, there also appears to be an abnormal processing of the rewarding properties of food with altered activation of "reward centers" in the brain, such as the nucleus accumbens. It is not clear as yet whether these two processes are interrelated, and it has been suggested that the failed satiety response leads to the development of abnormal food reward processing. However, the PWS interval contains a number of genes, many of which are expressed in both the hypothalamus and other areas of the brain, including the nucleus accumbens. Consequently, it may also be that there are two separate neural mechanisms contributing to altered feeding behavior in this disorder.

The neural bases of hyperphagia in PWS are not completely understood, although they may be due, in part, to increased circulating levels of the hormone

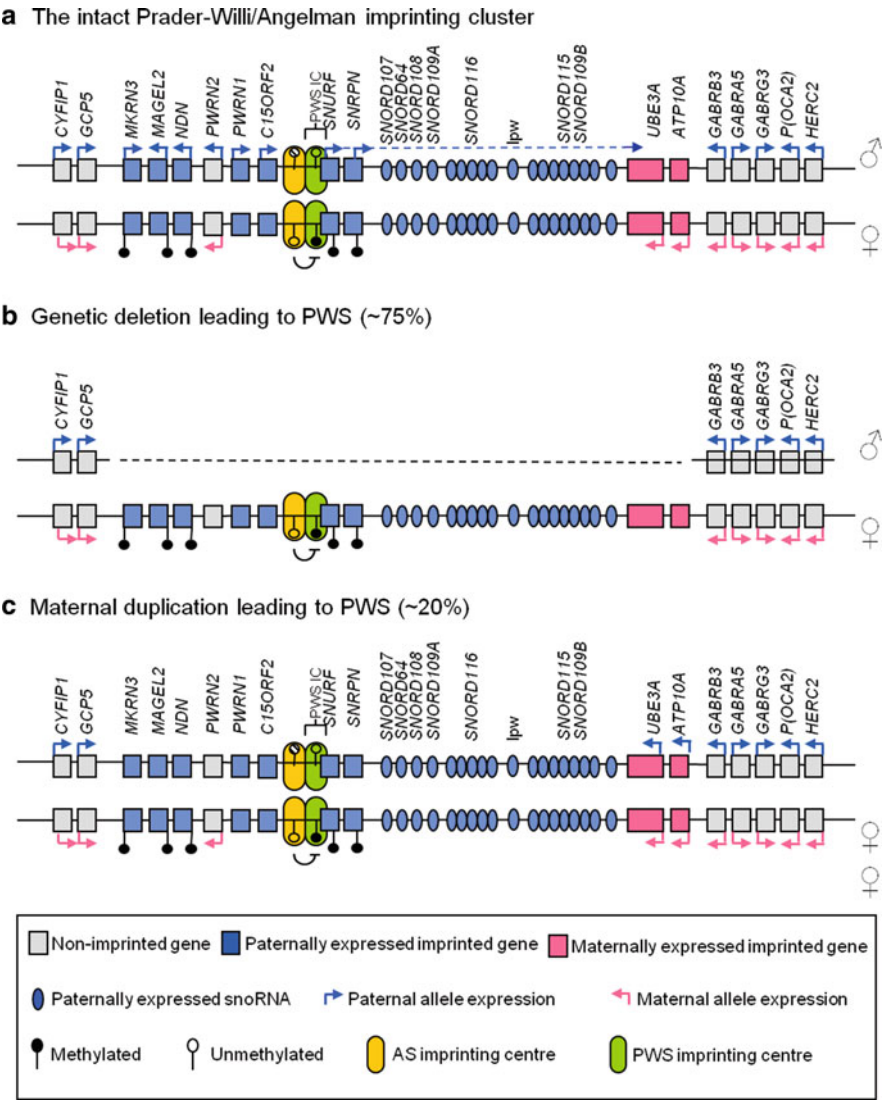

Fig. 62.5 A graphic representation of the Prader-Willi/Angelman imprinted gene cluster of human chromosome 15q11-q13 (**a**). The region contains several protein coding and noncoding paternally expressed imprinted genes. There are also two maternally expressed genes implicated in the etiology of Angelman syndrome. Loss of expression of the paternally expressed genes, either through deletion of the DNA (**b**) or by inheriting two maternal copies of chromosome 15 (maternal uniparental disomy: mUPD; **c**), results in Prader-Willi syndrome (PWS). As well as loss of paternal gene expression, mUPD leads to an overexpression of the normally maternally expressed genes. Deletion and mUPD account for the vast majority of cases of PWS. However, a smaller deletion encompassing the imprinting center (IC) also results in loss of paternal gene expression (and overexpression of normally maternally expressed genes) and PWS due to changes in the epigenetic regulation of the interval. IC deletion accounts for <5% of PWS cases

ghrelin which do not fall as rapidly as normal in the transition from infancy. Using a mouse model null for the gene *Snord116*, it has been suggested that lack of expression of this noncoding RNA species may be a fundamental contributor to this aspect of the phenotype. *Snord116* null mice show longer mealtimes and have hyperghrelinemia. However, experimentally lowering ghrelin does not completely resolve the hyperphagia in PWS, pointing to more than one abnormality in the neural circuitry involved in regulating feeding. Interestingly, another noncoding RNA molecule in the PWS interval, *Snord115*, has a regulatory role over the functionality of the serotonin 2C receptor ($5HT_{2C}R$). $5HT_{2C}Rs$ play a number of roles in mediating feeding, both in terms of the ability of serotonin to stop feeding and in regulating dopamine release in response to reward. As for the behavioral manifestation for hyperphagia in PWS, it may be that a number of neural circuitries regulating feeding are disrupted by different genes with the PWS imprinting cluster.

Genomic Imprinting and the Monoamine System

As outlined above, the other strong areas of imprinted gene expression are those with monoamine neurotransmitter projections to the rest of the brain. These are the noradrenergic *locus coeruleus*, serotonergic *dorsal raphe nucleus*, and dopaminergic *substantia nigra* and ventral tegmental area (VTA). In addition to *Snord115* which regulates $5HT_{2C}R$ function (see above), two other key imprinted genes are involved in the monoamine system and are strongly expressed in these key brain areas: *Grb10* and *Nesp*.

Nesp is a maternally expressed gene within the *Gnas* imprinting cluster, encoding neuroendocrine secretory protein 55 (Nesp55). *Nesp* shows strong, but discrete, expression in the locus coeruleus, dorsal raphe nucleus, and Edinger-Westphal nucleus. It is also widely expressed in the hypothalamus. The *Nesp* gene transcript is an important RNA regulatory element of other gene expression within the Gnas cluster. However, a targeted mutation that eliminated the translation and production of Nesp55 also revealed a role for this protein in the brain.

Mice lacking Nesp55 show an altered behavioral reactivity to novel environments as indicated by the fact that they display increased locomotor activity when placed in a new location. When given the choice between a novel and familiar location, the Nesp55 null mice show less motivation to spend time in the new environment, a behavior which seems to be independent from fear per se. Although there is no defined neural basis for this behavior, the expression of *Nesp* in the locus coeruleus strongly implicates the ascending noradrenaline neurons which are known to exert control over reactivity to novelty.

As described previously, *Grb10* shows a complex pattern of regulation, with the maternal copy being expressed in the body tissues and the paternal copy expressed in the brain. Paternal *Grb10* shows a similar discrete pattern of expression in the brain to that of *Nesp*. However, in addition to the locus coeruleus, dorsal raphe nucleus, and Edinger-Westphal nucleus, *Grb10* also strongly expressed VTA and

substantia nigra pars compacta, another area indicated as an imprinting hot spot in the brain.

Mice lacking maternal expression of *Grb10* have metabolic and physiological abnormalities, including increased weight gain. However, as is expected from the pattern of expression, mice null for a paternal copy of *Grb10* demonstrate behavioral changes. Specifically, one function of *Grb10* appears to be the regulation of social dominance. Mice null for paternal *Grb10* show abnormalities in social grooming and other behaviors which point to these mutant animals being more socially dominant. Again the neural basis of this behavioral change has yet to be explored. However, it is known that *Grb10* is neurally expressed and colocalizes with the dopamine transporter, serotonin, and choline acetyltransferase.

Obviously, the work on both *Nesp* and *Grb10* is, at present, in its early stages. However, these hint at the kinds of behaviors, social and motivational, that might be influenced by imprinted genes that are expressed in these hot spots of genomic imprinting in the monoaminergic system.

Genomic Imprinting and the Human Brain

Brain Evolution

It is generally accepted that the original driving force behind the evolution of genomic imprinting was probably the development of an extended pregnancy and parental care. However, once established, other selective pressures may have led genomic imprinting to be co-opted to act on other functions, such as brain and behavior. There is some evidence for this from a number of brain-expressed imprinted genes. Some imprinted genes, such as *neuronatin*, only appear later in the eutherian mammalian lineages, after genomic imprinting has evolved. This kind of enrichment appears to be accelerated in humans, with the genes *LRRTM1*, *C15ORF2*, and *5HTR2A* only occurring, or becoming imprinted, in the human brain. Although these data are limited, they do provide a tantalizing suggestion that genomic imprinting in the brains of humans may be particularly important.

Obviously, of direct relevance to this question is the analysis of imprinted genes in other species of primates; unfortunately, much of the data simply allows a comparison between mouse and human. Nonetheless, two separate studies of brain-expressed imprinted genes have directly addressed the question of whether these genes are particularly important for humans. As mentioned above, paternally expressed *C15ORF2* is only found in man, but more detailed analysis has also pointed to the fact that it has been positively selected through human evolution. Similarly, the maternally expressed *Klf14* has also undergone accelerated evolution in humans. However, this gene is also present (and imprinted) in mice, suggesting that the positive selection is very much related to some key change in the human lineage.

What selective pressures have led to this is not clear at present. One idea links back to the original work with mouse chimeras demonstrating differential

distribution of PG/GG (maternal genomes only) and AG (paternal genome only) cells in the brain. The distinct brain regions to which PG/GG and AG cells contribute have also evolved differentially in primates. While the frontal cortex and striatal areas have expanded relative to the rest of the brain, the hypothalamus and septum have contracted in size. The forebrain areas that have expanded are thought to have done so due to the selective pressure of living in social groups and all complexities of social behavior which that entails. In the majority of primate societies, the maintenance of social cohesion and group continuity over successive generations is dependent on the matriline; these are also in the "interests" of the maternal genome, which is more likely to be shared among the members of the group. It seems more than coincidental that the areas of brain expansion required for living in social groups are also those to which the maternal genome makes a substantial developmental contribution. In humans, social behavior is taken to another level, and so it may be the case that the importance of imprinted genes in the brain has also increased.

Neurodevelopmental and Psychiatric Disorders

Whether or not they are adaptive, the increased numbers of imprinted genes expressed in the brain may still be important contributors to abnormal brain function in humans. This has been illustrated by the study of classic genomic imprinting syndromes, such as PWS described above and its sister disorder Angelman syndrome (AS). These two neurodevelopmental syndromes were some of the first to be linked with genomic imprinting. AS is characterized by severe learning difficulties, ataxia, seizures, and EEG abnormalities, whereas PWS, described above, is characterized by a failure to thrive in infancy, mild learning difficulties, and on emerging from infancy a grossly abnormal satiety response to food intake. Although very different phenotypically, both are caused by disruption of the cluster of imprinted genes on human chromosome 15q11-q13, which contains a number of paternally expressed genes and brain-specific small nucleolar (sno) RNA species, and two maternally expressed genes (Fig. 62.5). The key point however is that despite being due to disruptions of the same region, AS and PWS have distinct molecular genetic underpinnings. AS is due to a lack of expression of *maternally* derived genes (in particular *UBE3A*), caused by maternally derived de novo deletions of the cluster, chromosome 15 paternal uniparental disomy (pUPD), and disruption of the imprinting mechanism itself. Conversely, PWS results as a consequence of lack of expression of *paternally* derived genes, caused, again, by de novo deletions of the cluster (this time paternally derived), chromosome 15 maternal uniparental disomy (mUPD), and IC mutations.

In addition to the main phenotypic characteristics, both AS and PWS display a high incidence of neuropsychiatric abnormalities. Individuals with PWS in particular are prone to an affective disorder, including mood instability, nonpsychotic depression, and psychosis. What is emerging is that different

genotypes, all resulting in the core deficits in PWS, can give rise to different patterns of mental illness. Specifically, those individuals with PWS as a result of maternal chromosome 15 mUPD genetic subtype in particular, are far more prone to experiencing a psychotic episode than the paternal 15q11-q13 deletion subtype. These data allow us to start assigning specific gene(s) to particular parts of the phenotype; for instance, the predicted overdosage of maternally expressed *UBE3A* and *ATP10C* gene products due to the presence of two maternal copies in the mUPDs suggests that these gene products are important in the etiology of psychosis in PWS.

The possible role of imprinted genes in the development of psychiatric illness more generally is now being recognized. Although gene variation association studies have not linked imprinted genes with the occurrence of mental illness particularly, other studies focusing on genomic alterations affecting more than one gene do implicate genomic imprinting. Such changes have been revealed in genome-wide studies linking copy number variations (CNVs) to neuropsychiatric problems. CNVs are microduplication or deletions that occur throughout the genome affecting the copy number of a number of genes. CNVs occur naturally but are sometimes directly implicated in the development of disease. Due to their tight regulation, imprinted genes may be particularly vulnerable to the effects of mutations which alter copy number, and a number of clusters of imprinted genes have been implicated in the development psychiatric illness via the studies of CNVs. Again the PWS imprinted gene interval, 15q11-q13, seems to be important here. This region has been linked in a number of independent studies to both schizoaffective disorders and autism, with maternal duplications being particularly problematic. Again this data points to the normally maternally expressed genes, *UBE3A* and *ATP10C*, playing a central role in the development of neuropsychiatric illness.

Summary and Conclusions

Genomic imprinting is a robust, developmentally determined, epigenetic process that results in expression of imprinted genes from one parental copy only. The existence of imprinted genes provides an evolutionary conundrum, and their evolution may be due to development of in utero growth and extended parental care seen in the eutherian and marsupial mammals. This idea is supported by the fact that many imprinted genes are expressed in the developing fetus and placenta and also have a role in nutrient acquisition from the mother both pre- and postnatally.

In addition, genomic imprinting is important for brain development and function. The number of imprinted genes expressed in the mouse brain exceeds 1,000, representing between 5% and 10% of all brain transcription. Within the brain, key regions, such as the hypothalamus and areas of importance to monoaminergic innervation, are key hot spots for imprinted gene expression. This is reflected in the behaviors influenced by imprinted genes, which include feeding, maternal care, and motivated and social behaviors.

Imprinted genes are also important for normal brain function in people. There are a number of explicit genomic imprinting disorders, including the neurodevelopmental disorders Prader-Willi and Angelman syndromes. More generally, genome-wide studies of copy number variations are linking imprinted genes with neuropsychiatric illnesses, such as schizophrenia and autism.

There is still much to learn about the role of imprinted genes in the brain. This will be elucidated using mouse models and, hopefully in the future, the application of modern sequencing techniques coupled with neuropsychological testing and neuroimaging in people. Furthermore, the nature of imprinted genes means they maybe prime candidates for exploring how epigenetics may mediate environmental effects on brain and behavior.

Outlook

In reality, understanding of what imprinted genes are doing in the brain is in its infancy. The knowledge of genomic imprinting in the brain will no doubt increase over the coming years with further behavioral and neural analysis of mice carrying genetic modifications of imprinted genes. As the number of models and behaviors assessed expands, so an understanding of the overall pattern of brain functions influenced by genomic imprinting will develop.

An important development for the understanding of genomic imprinting will be the analysis of other animal models. In particular, an assessment of genomic imprinting in nonhuman primates is needed in order for an insight into whether the physiological functions in which imprinted genes are involved are changing over evolutionary time. In the same way, by exploiting new sequencing methodologies, it will be possible to begin to address the extent and importance of genomic imprinting in humans.

Gene-Environment Interaction and Genomic Imprinting

One key future development in the genomic imprinting field will be the extent to which the epigenetic process that regulate imprinted genes can be modified by environmental insults and life events. It has been known for some time that the environment plays a big role in shaping behavior; it is now known that these effects are sometimes encoded at a molecular level via latent or active changes in the epigenetic status of certain genes. Given the potential sensitivity of imprinted genes to changes in the level of expression, they may be particularly vulnerable to any alteration in epigenetic status that occurs through environmental effects. Indeed, there are already examples of where the environment impacts upon imprinted gene expression.

One crucial period of life in which variations from the norm can have dramatic long-lasting effects is in utero. There are examples of how a compromised in utero environment has implications for imprinted gene expression in placenta and the

fetus, where of course they naturally play an important role. Studies of the "Dutch Hunger Winter" famine show that prenatal exposure to dietary stress resulted in persistent epigenetic changes at the *IGF2* gene.

This can also have effects on brain-expressed imprinted genes with lasting consequences. For example, restricting the protein intake of the mother during pregnancy has dramatic effects on development of the dopaminergic neurons in the offspring, resulting in behavior abnormalities in later life. These changes are driven in part by decreased DNA methylation and increased expression of the imprinted gene *Cdkn1c*, which has an established role in midbrain dopamine neuron maturation.

A more artificial manipulation, but a very important one in terms of human health, is the in vitro culture of early stage embryos. A number of animal studies have established that processes such as in vitro culture of early stage embryos disrupts the normal developmentally programmed epigenetic processes resulting in aberrant gene expression. This has been found to have pronounced phenotypic effects, particularly in ruminants, and may provide an explanation for the fetal overgrowth and other problems seen in cloned animals, such as "Dolly" the sheep. Although these effects are not exclusive to imprinted genes, the nature of the degree of regulation seen in this class of genes renders them particularly sensitive to any alteration in epigenetic status that occurs through in vitro culture.

It is against this background that attention has recently turned to examining the consequences of assisted reproductive technologies (ART) on imprinted gene-related disorders. There is an accumulating body of evidence suggesting that this is indeed the case, although it must be stressed that as many imprinted gene disorders are in themselves quite rare, the absolute risk is extremely low. A number of studies have suggested increased incidence of offspring born with AS following ART, and in all cases, there was loss of DNA methylation at the key imprinting center (IC), demonstrating that alteration of the epigenetic status was causal (as opposed to a de novo gene deletion). However, the extent to which epigenetic changes at imprinted (and other) genes occur with ART remains to be seen; for instance, two recent studies have failed to find an increased incidence of PWS. Nevertheless, taken together, animal studies and initial clinical work do suggest that the epigenetic code may change with embryo culture in vitro.

Further Reading

Garfield AS, Cowley M, Smith FM et al (2011) Distinct physiological and behavioral functions for parental alleles of imprinted Grb10. Nature 468:534–538

Gregg C, Zhang J, Weissbourd B et al (2010) High resolution analysis of parent of origin expression in the mouse brain. Science 329(5992):643–648

Haig D (2002) Genomic imprinting and kinship. Rutgers University Press, New Brunswick

Isles AR (2010) Evolution of genomic imprinting in humans: does bipedalism have a role? Trends Genet 25:495–500

Keverne EB, Martel FL, Nevison CM (1996) Primate brain evolution: genetic and functional considerations. Proc Biol Sci B 263:689–696

Li L et al (1999) Regulation of maternal behavior by paternally expressed *Peg3*. Science 284:330–333

Meaney MJ, Fergusson-Smith AC (2010) Epigenetic regulation of the neural transcriptome: the meaning of the marks. Nat Neurosci 13:1313–1318

Reik W, Walter J (2001) Genomic imprinting: parental influence on the genome. Nat Rev Genet 2(1):21–32

Wilkinson LS, Davies W, Isles AR (2007) Genomic imprinting effects on brain development and function. Nat Rev Neurosci 8:832–843