Genetics of Childhood Disorders: XIII. Genomic Imprinting: The Indelible Mark of the Gamete

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The phenomenon of genomic imprinting might never have been recognized but for its effects on gene expression. As was discussed in the last column, certain chromosomal subregions undergo imprinting. Genes in those regions are expressed in a manner that depends on the parent of origin for the chromosome. Some genes are thus expressed only if they lie on one chromosome, even though a second normal copy of the gene is present on the chromosome derived from the other parent. At some loci, it is the maternal copy of the gene that is expressed, while at other loci it is the paternal copy. This is in contrast to what happens for the vast majority of genes in which both the maternal and paternal copies of the gene are expressed.

The first indication that imprinting was occurring in mammals arose from nuclear transplantation experiments carried out in the early 1980s. The nuclei from fertilized mouse oocytes were removed and replaced with either a pair of sperm-derived or a pair of oocyte-derived haploid nuclei to reconstitute a diploid chromosome number. Despite the normal chromosome number, the androgenetic (sperm-derived) and gynogenetic (oocyte-derived) embryos did not survive embryogenesis. The embryos survive only when half the chromosomes derived from the mother and half from the father.

A further notable finding in these experiments was the distinctive growth patterns of the embryos. Those derived from androgenic cells formed extraembryonic tissue well but embryonic tissue poorly, whereas those of gynogenic origin formed embryonic tissue well and extraembryonic tissue poorly. These experiments demonstrated that simply having the correct number of chromosomes was not sufficient for normal mammalian development. Contrary to nearly 100 years of dogma, the results showed that maternally and paternally derived chromosomes were not functionally equivalent.

In the wake of these surprising results, efforts focused on identifying the specific genomic regions responsible for these

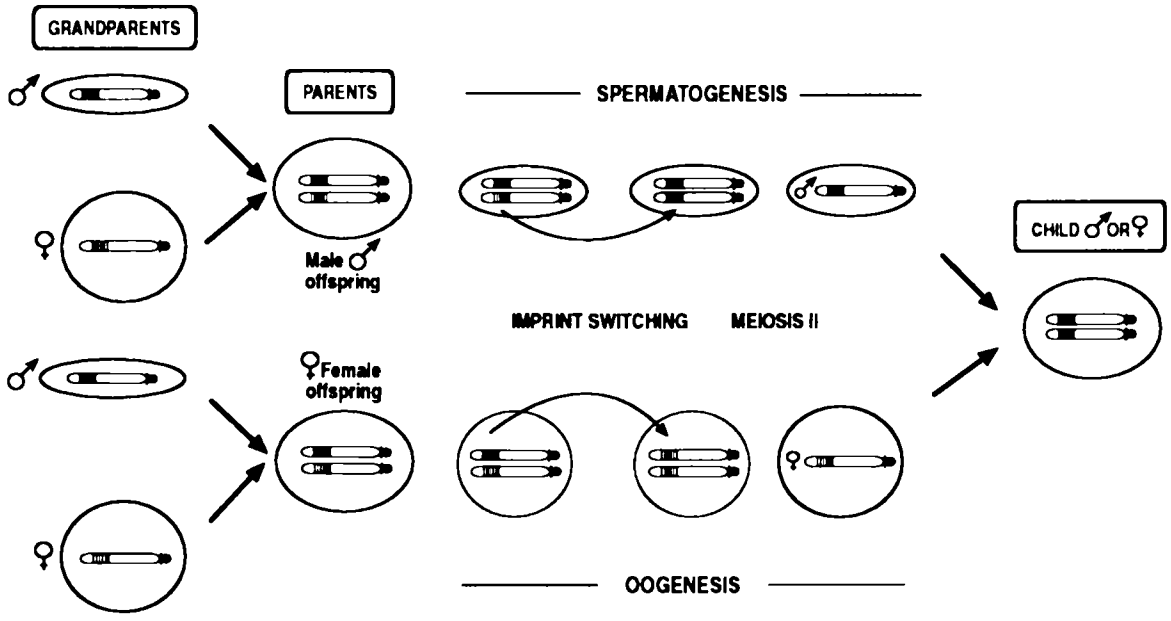

Fig. 1 Imprinting is a stable and reversible event that depends on the parental origin of the chromosome and not on the sex of the offspring. The phenomenon is illustrated by depicting an imprinted region within the chromosome shown. A paternal imprint is depicted as black, maternal as striped. For some genes it is the paternal gene that is silenced; for other genes the maternal gene is silenced. Grandparental gametes are shown on the left and give rise to the parental generation in the middle of the diagram. A male offspring has to switch his mother's imprint (striped) to a paternal imprint (black) during spermatogenesis. The female situation requires the paternal imprint (black) to switch to a maternal pattern (striped) during oogenesis. The child born as the third generation in this family will thereby receive the appropriate imprints on each parental chromosome.

phenotypic effects. Mice were bred to generate offspring with 2 copies of a specific chromosomal region derived from a single parent. The effects on embryonic viability or gross morphology could be studied in this manner. A number of genomic regions were thus identified to be undergoing imprinting. One copy or the other was silenced and did not express proteins.

In subsequent experiments, many of these regions have been found to contain one or more genes that are indeed imprinted. At present, more than 30 imprinted genes have been identified in mammals. Importantly, there is no common functional characteristic to these genes, although many are required for normal development.

Genomic imprinting is not merely a gene expression phenomenon, however. When an imprinted locus on the paternal and maternal chromosome is compared, differences other than gene expression are also found. Chemical alterations may occur on the maternal copy of a gene and not the paternal gene. For example, methylation of cytosines can vary between the 2 genes. Nuclease accessibility to DNA can also be different, indicating differences in the way the DNA is packaged as chromatin. The timing of DNA replication at these loci has been found to differ on the paternal and maternal chromosomes. These processes are interesting, as they reflect the epigenetic organization of the DNA. Epigenetic (from *epi-* above, upon, higher than) processes are those that determine how the DNA is regulated to produce useful patterns of gene expression.

We have already discussed in these columns how one form of gene regulation occurs through the promoter regions. Certain nucleotide sequences are present that either enhance, or repress, transcription of the gene. Regulation of gene expression is also mediated by epigenetic processes such as chromatin organization and cytosine methylation. It is not surprising, then, that differences in these regulatory elements are found at imprinted loci. The contribution of certain epigenetic regulators to imprinting is discussed below. Taking into account that gene expression is regulated by its epigenetic organization, we are able to define genomic imprinting as follows: *If the epigenetic organization of a locus is dependent on its gamete of origin, then that locus is subject to genomic imprinting.*

Mutations of imprinted genes cause disease in unusual ways. A mutation of an imprinted gene may have no obvious effect. This will happen if it is inherited on the usually silenced chromosome. However, if the mutation is inherited on the normally active chromosome, then the individual will be unable to produce functional protein. The mutated gene is unable to produce functional protein, while the other nonmutated but imprinted or silent gene is unable to compensate. As the parental origin of the mutation determines whether there is a phenotypic effect, the resulting family histories can be distinctive.

A particularly interesting and unusual mechanism of disease-causing mutation involving imprinted genes is that of uniparental disomy (UPD). UPD refers to the inheritance of both copies of a chromosome (or chromosomal region) from a single parent. The mechanism by which this occurs is presumed to involve an initial trisomy that would be lethal if transmitted. The loss of one of the trisomic chromosomes at random in totipotent cells may occur early in embryogenesis and allows for the further development of the embryo. However, if 2 paternal chromosomes and 1 maternal chromosome comprised the initial trisomy and the maternal chromosome were to be lost, the resulting fetus would have inherited 2 normal-appearing chromosomes, both of which would be from the father. A similar mechanism would give rise to a maternal UPD.

In the situation of paternal UPD, difficulties would arise for those genes that are normally expressed only if they lie on the maternal chromosome. Although 2 normal copies of the gene are present, both are on paternally derived chromosomes and in this scenario these genes are silenced. No functional protein can be synthesized.

Maternal UPD would conversely give rise to silencing of paternally expressed imprinted genes on that chromosome. The translocation-bearing mice referred to earlier were used to generate offspring with UPD of either parental origin for chromosomal subregions, indicating that certain defined regions contain imprinted genes. Cases of UPD in humans have been serendipitously identified, allowing subregions of the human genome to be likewise defined as likely or unlikely to contain imprinted genes.

Mutations of single imprinted genes, as well as deletions or UPD for whole or parts of chromosomes containing imprinted genes, have been found to give rise to human diseases. Just as there is no common family of genes that are imprinted, neither is there a typical category of human disease caused by mutations involving imprinted genes. The broad categories of human diseases involving imprinted genes include neoplasia, neurodevelopmental disorders, metabolic disorders, dysmorphic conditions, and possibly psychiatric disorders (for instance, various authors have proposed that Tourette's disorder and autism may involve imprinted genes).

These disorders are due to inherited mutations involving imprinted loci. A separate category of disease involves the disruption of the gametic imprint in somatic cells. In other words, the imprint is established normally in gametes but is disrupted later in somatic cells, so that genes that were expressed only from one chromosome either fail to be expressed from either chromosome or are expressed from both chromosomes. The phenotypic consequence of such an event is typically found to be neoplasia.

There are several current models for the mechanism of genomic imprinting. The first hypotheses arose from the observed differences of methylation between imprinted loci on homologous chromosomes. This chemical modification of the DNA (the addition of a methyl group to cytosine nucleotides) is reversible and stable and was therefore an attractive candidate for being what was "imprinted" in genomic imprinting. The

disruption of imprinting in mice that lacked the enzyme necessary to add methyl groups to DNA added support to this idea.

However, a number of more recent observations suggest that methylation, while necessary, is not sufficient to govern imprinting in mammals. X inactivation, a process that has many similarities to imprinting, has been studied extensively in somatic cells during the period when the inactivation occurs. It has been found that methylation was established only subsequent to inactivation, and therefore it appeared to be involved in consolidating some other primary process rather than being the primary process itself. Second, the discovery that imprinting could occur in domains of hundreds of kilobases changed the focus from processes such as methylation of promoter regions of individual genes to much larger domains of DNA that affect multiple genes. Finally, the methylation patterns seen at imprinted genes are rarely found to be stably maintained throughout development, thus casting doubt on the notion that methylation is the primary determinant of the epigenetic regulation of an imprinted region.

A second proposed mechanism for imprinting involves competition for regulators of gene expression. This phenomenon has been found recently to occur for 2 imprinted regions, the *H19/Igf2* region of mouse chromosome 7 and the *Igf2r* region of mouse chromosome 17. In each case, there are 2 genes close to each other on the same chromosome. One of the genes is expressed on one chromosome and is imprinted on the other. The second gene is expressed only when it, and not the first, can use local enhancers. Normally, what occurs is that on one of the chromosomes the first gene is not imprinted and therefore is expressed and competes successfully for use of the local enhancers. The second gene is therefore silenced. On the other chromosome, however, the first gene is not expressed, allowing the second gene to use the local enhancers and be expressed.

Deletion of the primarily imprinted gene in each of the cases mentioned (either by knockout or by deletion) leads to alteration in expression of the secondarily imprinted gene, confirming this interrelationship. While this explains the interrelationship of imprinting of these specific genes, it neither explains how the first gene is imprinted to establish the process nor explains how the many other nearby imprinted genes are regulated.

A third model of imprinting is based on the imprint switch mutations observed in Prader-Willi and Angelman syndrome patients. A male should derive half of his chromosomes from his mother. When he passes them on to the next generation, these same chromosomes must now know that they derive from the paternal side. Normally, a male will "switch" his maternally inherited chromosomes to a paternal imprint in his gametes, with the converse for females (Fig. 1).

Imprint switch mutations have been found that interfere with this process and prevent the establishment of the correct imprint in the gamete. Very small deletions can lead to these imprint-switching problems, suggesting that there are discrete gamete-responsive segments of DNA in imprinted regions that interact with the nuclear environment to initiate the imprinting process. While the mechanism by which such elements might work is not yet known, the effect of this "imprinting center" is to regulate chromatin structure and methylation, resulting in imprinting of the adjacent hundreds of kilobases of DNA. Such an idea represents a hierarchical model in which the activity of an imprinting center dictates a number of biochemical and structural changes, including methylation, resulting in the silencing of genes in the immediate region.

It is not known why imprinting occurs in mammals. Several explanations have been proposed for its biological necessity. For instance, it has been suggested that imprinting allows the mother to tolerate the fetal growth without a catastrophic expense of energy. This theory is based on the observation that genes promoting embryonic growth are frequently paternally expressed, while genes that appear to be required to moderate fetal growth are maternally expressed. As the first imprinted genes to be identified fit this pattern, this theory carried a great deal of weight, but subsequently a large number of imprinted genes were identified with less immediately obvious links to embryonic growth. Moreover, an assumption underlying this model is that genomic imprinting is purely a mammalian phenomenon, as egg-laying species would not be subject to the same energy expenditure. However, there is accumulating evidence that insects such as *Drosophila, Sciara,* and *Planococcus* have an imprinting process with similar effects on gene expression, but, being insects, they lay eggs and do not carry fetuses.

The simplest conclusion regarding the biological importance of genomic imprinting is based on some of the earliest experimental observations: the male and female genomes are rendered complementary to each other, and certain genes are reduced in dosage by up to 50%. These observations alone suggest that significant reasons for imprinting to occur in mammals include the prevention of asexual reproduction and the repression of expression of certain dosage-sensitive genes. The failure of asexual reproduction is clearly illustrated by the experiments described earlier involving androgenetic and gynogenetic mouse embryos, which are derived from one parent's gametes only. In addition, the notion that imprinting is maintained in order to regulate levels of gene expression is supported by the example of certain imprinted genes that cause overgrowth and malformations if overexpressed.

It is clear that imprinting plays an essential role in gene expression in very diverse species. The discovery of imprinting helped explain patterns of transmission of human diseases that had confounded generations of geneticists. It was one of a handful of advances over the past 2 decades that challenged long-held notions about how genetic traits are passed from one generation to the next. The further clarification of the underlying mechanisms of imprinting is likely to have an equally dramatic effect on our understanding of how genes and large regions of the

genome are regulated and how disruptions in this process contribute to human disease.

WEB SITES OF INTEREST

http://www.geneimprint.com/
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Health and Behaviour Problems at 8 Weeks as Predictors of Behaviour Problems at 8 Months. N.J. Spencer, C. Coe

Objective: To assess the value of health and behavioural problems at 8 weeks as predictors of behavioural problems at 8 months in a whole year birth cohort. *Study Design:* Prospective birth cohort study. *Setting:* The socially and ethnically diverse city of Coventry. *Main Outcome:* Parent reported behavioural problems at 8 months. *Method:* Parent reported infant health and behaviour data were collected, using a validated questionnaire administered by the family health visitor at 8 weeks and 8 months, on 1541 infants participating in the Coventry cohort study. Sociodemographic data were collected at the health visitor's initial visit. Unadjusted relative risks (with 95% confidence intervals (CI)) of behaviour problems at 8 months by sociodemographic variables and health and behavioural problems at 8 weeks were estimated. Adjustment for confounding was made by logistic regression. *Results:* Infants reported to have behavioural problems at 8 weeks had a significant risk of parent reported behavioural problems at 8 months (adjusted relative risk, 3.44; 95% CI, 1.95 to 6.09) after adjustment for other health outcomes and sociodemographic factors. Of infants with behavioural problems by 8 weeks of age, 19.1% were reported to have behavioural problems at 8 months. *Conclusions:* Infants whose parents report behaviour problems by 8 weeks of age are at higher risk of behavioural problems at 8 months. However, despite the higher risk, the proportions of infants identified by behaviour at 8 weeks were too small for the early outcomes to be useful as predictors of behaviour at 8 months in the whole infant population. Arch Dis Child 1999;81:166–168. Reproduced with permission from the BMJ Publishing Group.