Genome Imprinting

The Silencing of Genes and Genomes

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Gregor Mendel, the Father of Genetics was, fortunately for us, very lucky in that the characters studied by him presented a very neat pattern of inheritance. This enabled him to generalize from his observations and to give us the basic tenets of inheritance. Genetic investigations in the 20th century have, however, revealed many contradictions to the principles enunciated by Mendel. One of the important challenges facing geneticists is to explain the mode of inheritance of traits and conditions that do not appear to follow Mendel's laws. One of the principles of Mendel is 'The Principle of Equivalence in Reciprocal Crosses': no matter which parent contributes a gene to its offspring, the gene will behave in the same way in producing the phenotype. Even though this principle holds true most of the time, there are a few exceptions. They are (1) traits linked to genes on the sex chromosomes – X and Y, (2) traits controlled by genes outside the cell nucleus (mitochondrial and chloroplast genomes), and (3) traits governed by a phenomenon called genome imprinting.

The term 'imprinting' was probably first used in biology in the late 1930's in connection with animal behaviour. Helen Crouse first used it in cytogenetic context in her study of chromosome elimination in *Sciara* in 1960. Recently, genomic imprinting has been used to refer to the differential expression of genetic material, depending on whether the genetic material has come from the male (father) or the female (mother) parent, suggesting that paternally and maternally derived genes may behave differently even though they may otherwise be identical. In recent years, genome imprinting has been implicated in a variety of pathological conditions in humans. In this article, we will discuss a few examples that illustrate the drastic effects that genome imprinting can have on the phenotypes of progeny.

Pronuclear Transplantation

It is possible, by careful microsurgical procedures, to remove either the paternal or maternal nucleus from a freshly fertilized mouse zygote and to inject a nucleus of choice into the zygote. By doing this, zygotes in which all nuclear genes (i.e., both sets of haploid chromosomes) are derived entirely from the father (androgenetic) or entirely from the mother (gynogenetic) can be obtained. It is important to note that such zygotes have the entire genome of the mouse represented in them. If such reconstituted zygotes are allowed to develop, it is seen that the androgenetic zygotes have relatively normal development of membranes and placenta, but very poor development of embry-

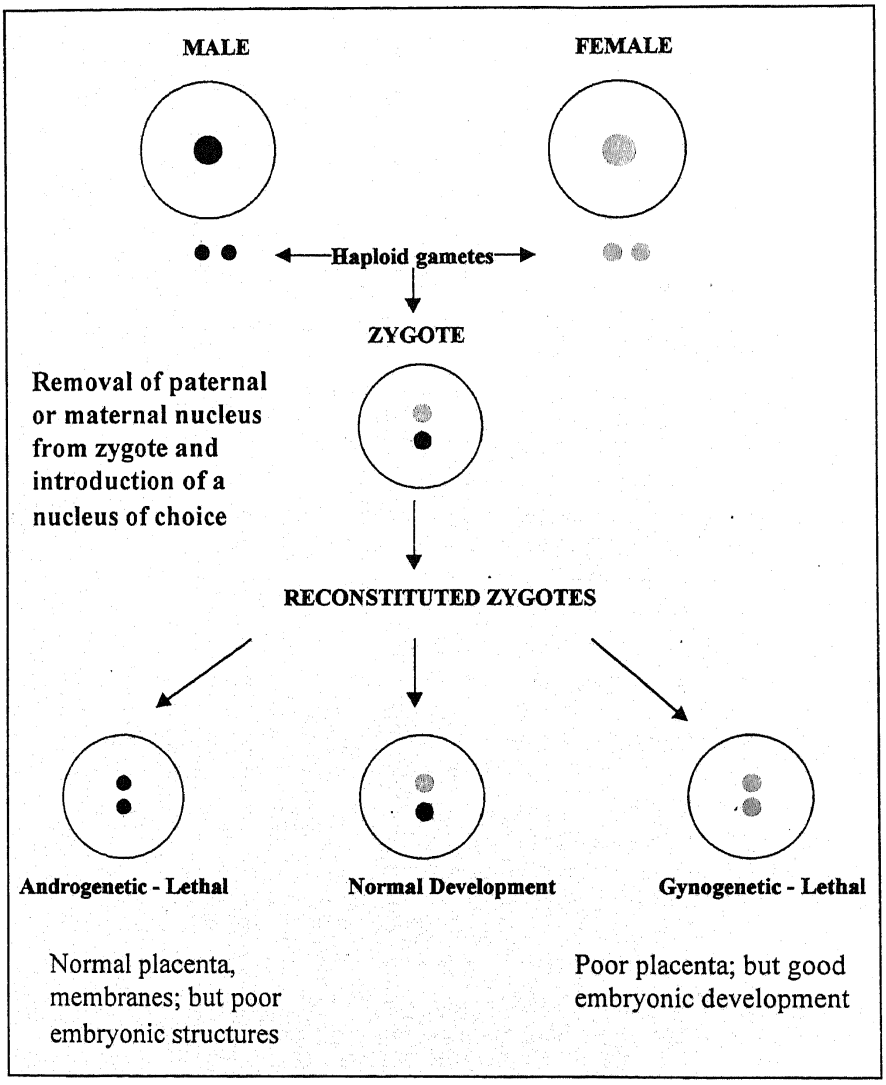

Figure 1. Pronuclear transplantation experiments in mouse. Even though androgenetic and gynogenetic embryos have total complement of the genome, their development is not normal.

onic structures. Conversely, the gynogenetic zygotes have relatively good embryonic development but very poor development of membranes and placenta. Both these conditions are lethal. This experiment, carried out in the 1980's, suggests that both maternally and paternally derived chromosomes are necessary for normal development: merely possessing the full diploid complement of genes does not suffice. Since the DNA sequences in the chromosomes of gynogenetic, androgenetic and normal embryos were all the same, this experiment suggests that the genes had somehow been modified or imprinted differentially because of their maternal or paternal origin.

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Human homologies for the type of situation described above are naturally occurring placental malformation, the hydatidiform mole, and the embryonically derived tumor, the teratoma. The complete mole is seen to have two sets of paternally derived chromosomes (androgenetic), whereas ovarian teratomas are gynogenetic and have two sets of maternally derived chromosomes.

Uniparental Chromosomal Disomies

In diploid organisms, one member of each pair of homologous chromosomes is typically inherited from the mother, and the other from the father. Mice have 20 pairs of homologous chromosomes ($2n=40$), and all of them are acrocentric. In one strain of mice, a centric fusion was seen to have occurred between one or more pairs of acrocentric chromosomes. In a centric fusion, the two members of the homologous pair are held together and hence they cannot separate during meiosis. Consequently, one daughter cell receives two copies of that chromosome and the other daughter cell receives none. If an egg with two copies of that chromosome is fertilized by a sperm lacking that chromosome, the resulting embryo will contain the normal quota of chromosomes, but both copies of that particular chromosome will have come from the mother. This is referred to as uniparental inheritance of chromosomes. In mice, the phenotypic consequences of inheriting both copies of chromosome no. 2 from the

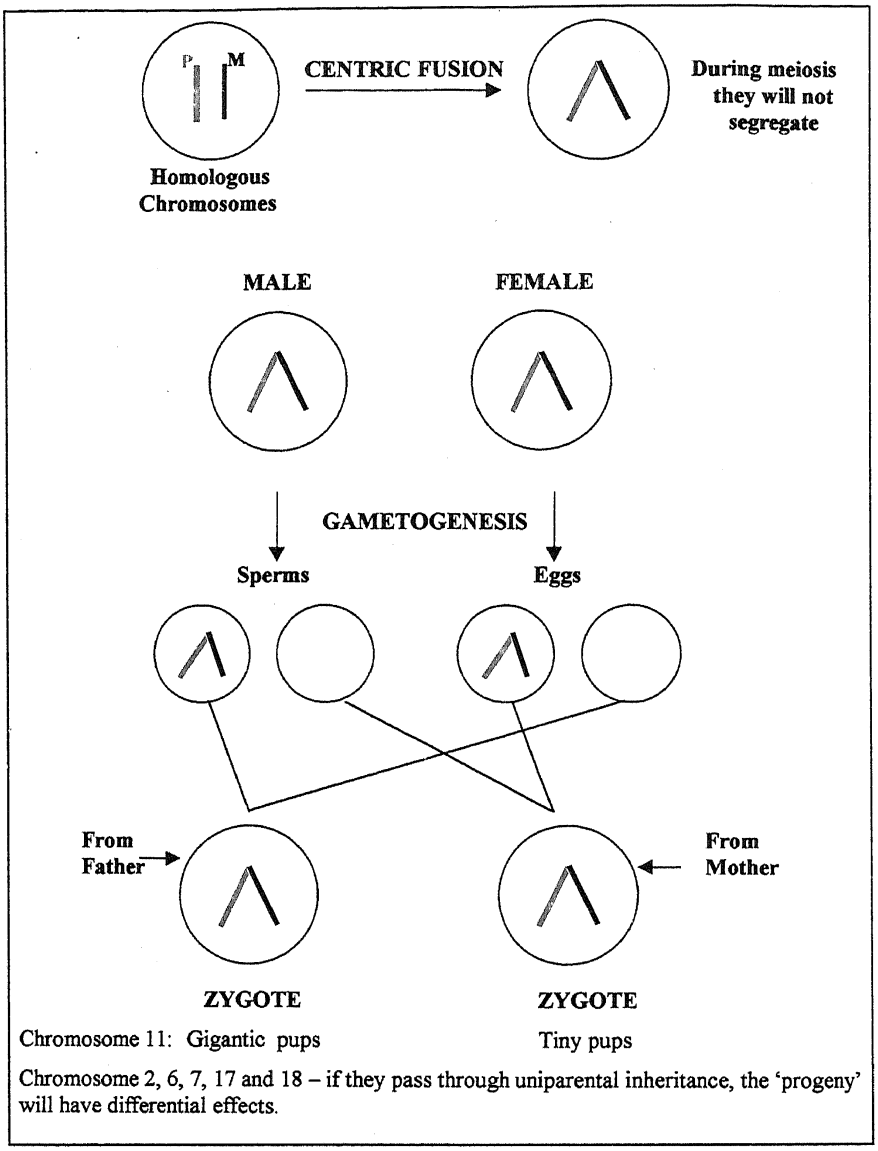


Figure 2. Uniparental disomies in mouse. Because of centric fusion between acrocentric chromosomes, both the copies of the homologous chromosomes are held together and both copies are inherited either from the mother or the father.

mother or father are different. In humans, the naturally occurring pathological condition cystic fibrosis is due to inheritance of both copies of chromosome 7 from the mother. Similarly, Prader–Willi syndrome in humans is due to the inheritance of both copies of chromosome 15 from the mother.

Human Triploids

The triploid (i.e. 3 copies of the haploid genome are present instead of the normal 2 copies) phenotypes in humans are quite different, depending on whether the extra haploid set of chromosomes came from the father or the mother. For instance, a

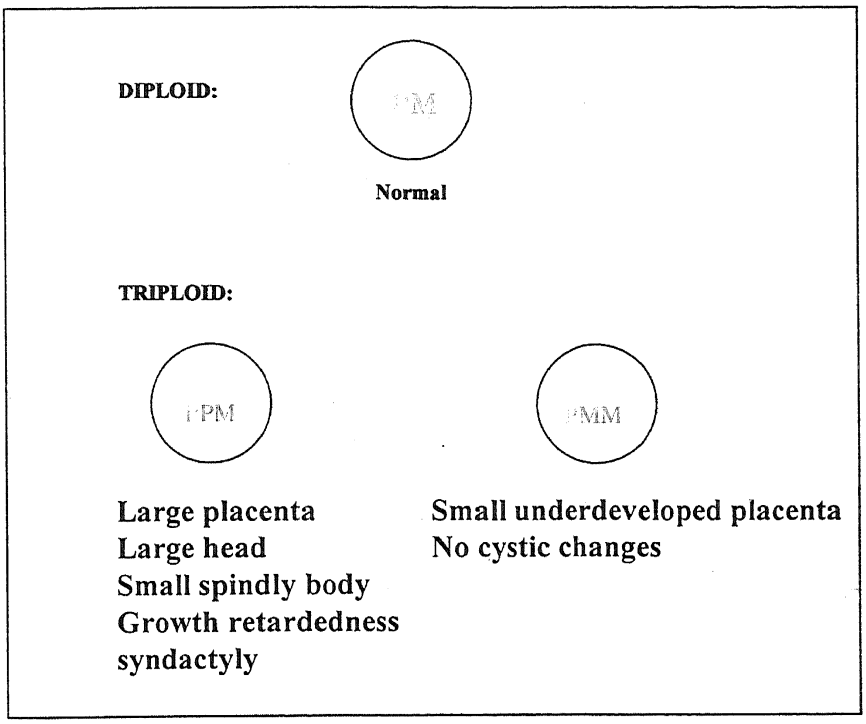


Figure 3. Human triploids have different types of abnormalities and the nature of defects depends on which of the two (maternal or paternal) genomes is represented twice.

human triploid tissue having two paternal and one maternal chromosome complements is observed as a large cystic placenta and, if the foetus survives, it will have a large head, small spindly body, severe intrauterine growth retardedness and syndactyly. On the other hand, a triploid with two maternal and one paternal chromosome complements exhibits a small underdeveloped placenta without cystic changes.

Analysis of Transgene Expression

Several techniques are now routinely used to incorporate a specific foreign gene into the genome of a given organism and such individuals are called 'transgenics'. The expression of a foreign gene (Troponin gene) in different tissues and in different generations of transgenic mice has been studied and it is seen that expression of the transgene depends on the sex of the parent that transmits it. When the transgene is inherited from a transgenic male mouse, it is expressed in both the sons and the daughters. However, when a transgenic female expressing the transgene transmits the same gene to her offspring, the transgene is not expressed.

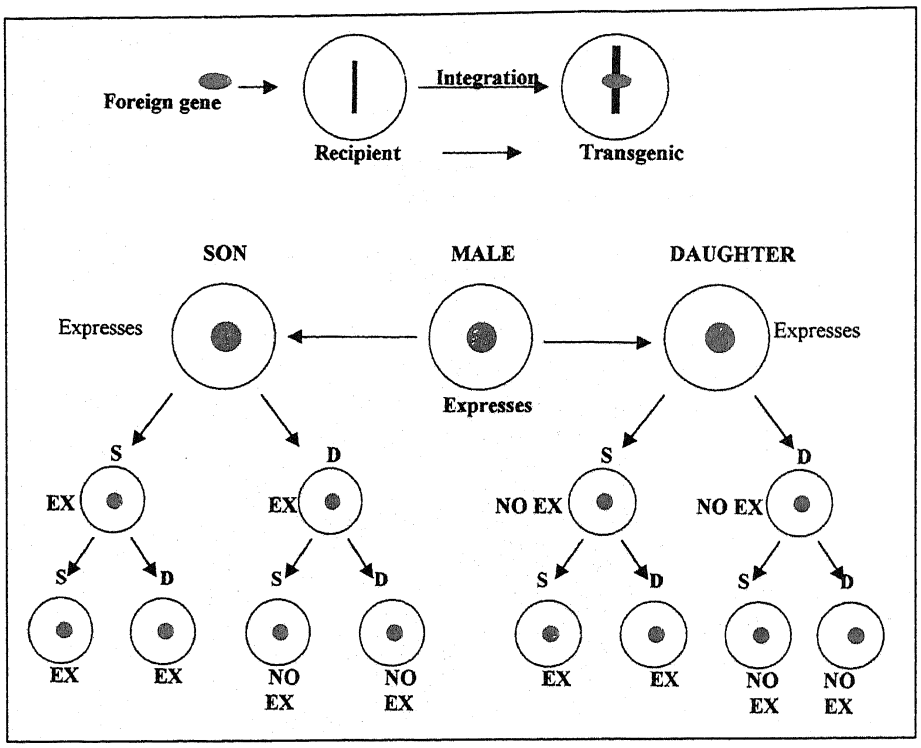


Figure 4. Analysis of transgene (Troponin gene) expression in mouse. The transgene is expressed in the progeny (sons and daughters) only when it is inherited from the father. (S=sons; D=daughters; EX=expression)

Sex Determination in Coccids

In several families of coccid insects there are no sex chromosomes and the sex of an embryo is determined by the number of genetically active genomes present (one set = males; two sets = females). Both males and females develop from fertilized eggs that have both the paternally and maternally derived genomes. However, in the eggs destined to develop into males, the set of chromosomes (genome) of paternal origin becomes heterochromatic and genetically inactive and is not transmitted to offspring. On the other hand, both the paternally and the maternally derived genomes remain active (euchromatic) in females, and they are transmitted to offspring.

In a soft scale insect, *Pulvinaria hydrangeae*, embryos develop parthenogenetically from unfertilized eggs. In females, the oogenesis results in the production of a haploid female pronucleus. This pronucleus mitotically divides once and products are fused to form a diploid zygote. In most of the embryos, both genomes remain euchromatic and these embryos develop into females. In about 5% of the eggs however, half of the chromosomes become

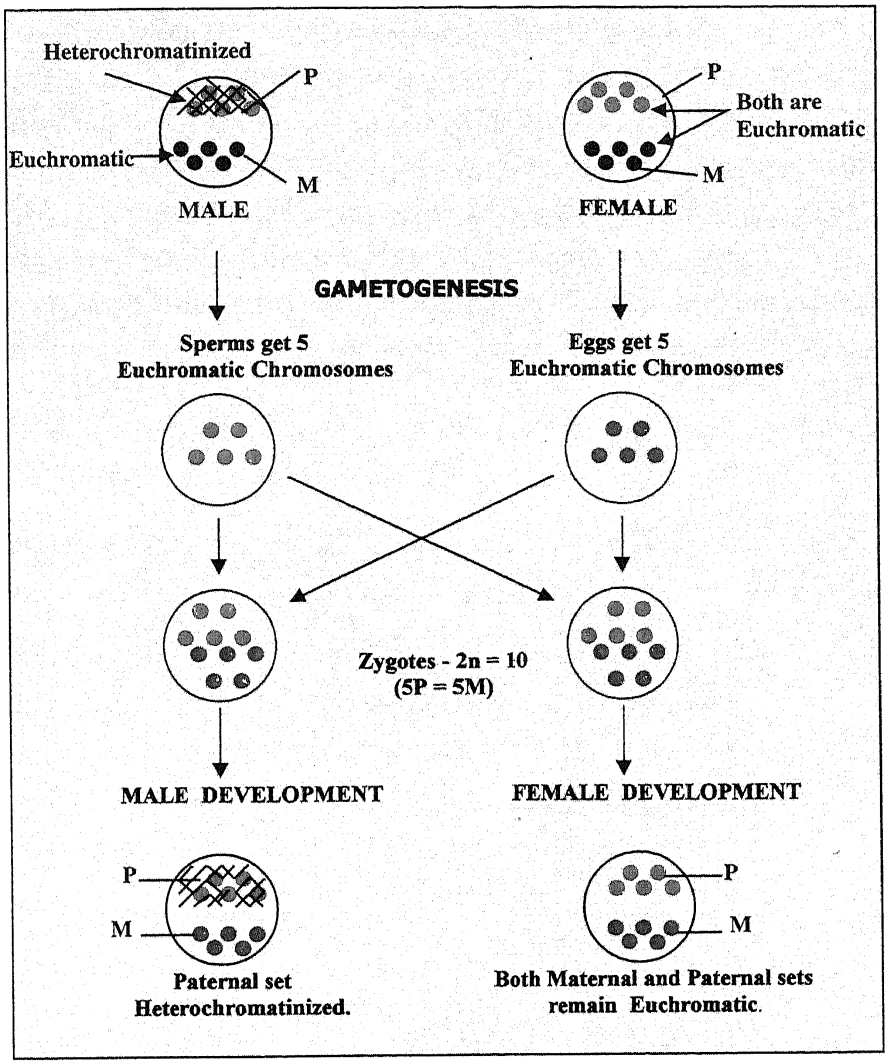


Figure 5. Sex determination in Coccids: in males the chromosome set inherited from the father will be heterochromatinised while in females the chromosomes inherited from both the paternal and maternal sides remain euchromatic. (P=paternal; M=maternal)

heterochromatic and these embryos either fail to develop or develop into non-functional adult males. This is an example for the heterochromatinization of maternally derived genomes.

On the other hand, in *Sciara*, the paternally derived chromosomes in embryos destined to develop into males are eliminated, while the females retain both the maternal and paternal sets of chromosomes.

Heterochromatinization of X-Chromosome in Mammals

In all female mammals, one or the other of the two X-chromosomes is inactivated in somatic cells. One of the first examples of imprinting observed in mammals was the preferential inactiva-

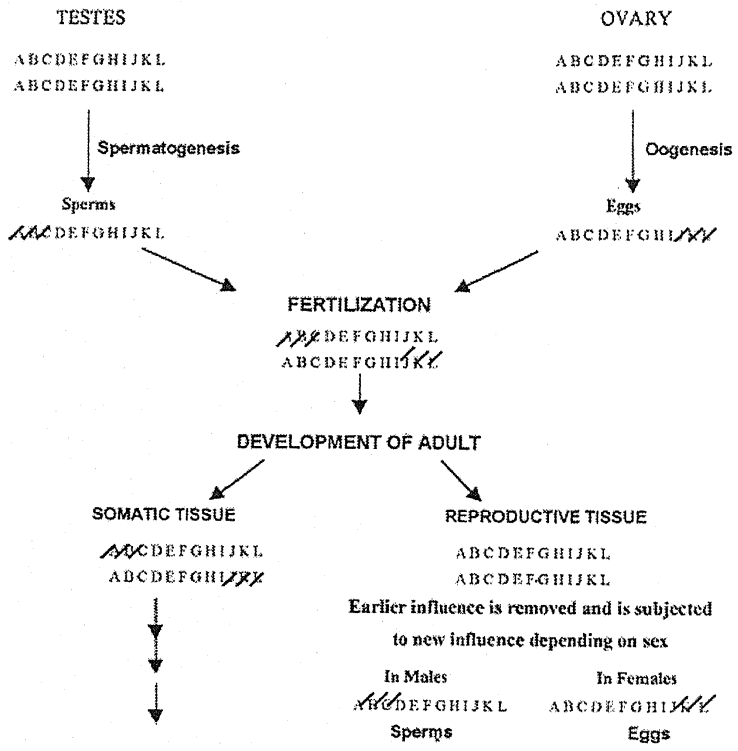
tion of the paternally inherited X-chromosome in female marsupials. Similarly, in the extra embryonic tissues of developing female rodents the paternal X-chromosome is inactivated. In all other cases studied, there is random inactivation of either the paternal or the maternal X-chromosomes. This means that only one X-chromosome is active in female mammals. Therefore, the females are equivalent to the hemizygous males with respect to X-linked gene products and thus dosage compensation is achieved.

Conclusion

The above discussed examples clearly illustrate that there are at least a few genes whose phenotypic expression depends upon

Box 1.

MODEL: A to L are the components of a genome. During spermatogenesis only ABC units are specifically marked, while during oogenesis only JKL are imprinted. Fertilization between these sperms and eggs results in a zygote where ABC inherited from the mother and JKL inherited from the father remain active, while their counterparts remain inactive. The same status continues in all the cells of the somatic tissues of the adult. On the other hand, in the reproductive tissue, during gametogenesis, these earlier markings of ABC and JKL units are erased and these components are now subjected to new imprinting depending on the sex of the individual.



In this example, in an androgenetic embryo both the copies of ABC genes would be inactive. Similarly, in the gynogenetic embryo both the copies of JKL units would be inactive. Therefore, both these types of embryos would be abnormal and lethal. Thus, for normal development the embryo must receive equal contributions from both the parents.

the parent from which they have been inherited. Thus, the differential manifestation of a few of the paternal or maternal genes/chromosomes in the progeny has shown yet another dimension to our understanding of the mechanisms of inheritance by annulling the principle of equivalence of reciprocal hybrids. In short, sometimes it does matter from which parent a gene is inherited.

Imprinting is not a permanent change or a mutation in the DNA, but rather a temporary alteration in the functional status of the DNA. Imprinting is defined as an epigenetic, gamete-of-origin dependent modification of the genome. This is also referred to as reversible silencing of genes (*Box* 1). The mechanism of imprinting involves (a) Wiping off of any previous imprint; (b) New modifications of the paternal genome in germ cells of each sex; (c) New imprinting or tagging of the chromosome as maternal or paternal and (d) Differential tissue specific phenotypic expression of the new imprinted genes in the offspring.

There is now strong evidence that the genome imprint could be the result of a direct modification of DNA by a process of DNA methylation. Experiments have often shown that particular genes are methylated differently depending on whether they are maternally or paternally inherited, and that these modifications are erasable.

Evidence is also accumulating from various lines of research that genomic imprinting is a common occurrence in many organisms including humans. Parent of origin differences in transmission have been observed for many pathological conditions in humans such as cerebral ataxia, spinocerebellar ataxia, non-specific seizures, Goldenhar syndrome, Adams–Oliver syndrome, familial glomus tumors, psoriasis, neural tube defects, congenital heart disease and narcolepsy. Since genome imprinting is implicated in the inheritance of several complex human disorders, it has become important in the area of clinical genetics.

Suggested Reading

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