Development 1990 Supplement, 131-139
Printed in Great Britain © The Company of Biologists Limited 1990



# Genetic imprinting in clinical genetics

ANGUS CLARKE

*Institute of Medical Genetics for Wales, University Hospital of Wales, Heath Park, Cardiff CF4 4XW, UK*

## Summary

**Genetic, and indeed genomic, imprinting does occur in humans. This is manifest at the level of the genome, the individual chromosome, subchromosomal region or fragile site, or the single locus. The best evidence at the single gene level comes from a consideration of familial tumour syndromes. Chromosomal imprinting effects are revealed when uniparental disomy occurs, as in the Prader-Willi syndrome and doubtless other sporadic, congenital anomaly syndromes. Genomic imprinting is manifest in the developmental defects of hydatidiform mole, teratoma and triploidy. Fragile (X) mental retardation shows an unusual pattern of inheritance, and imprinting can account for these effects. Future work in clinical genetics may identify congenital anomalies and growth disorders caused by imprinting: the identification of imprinting effects for specific chromosomal regions in mice will allow the examination of the homologous chromosomal region in humans.**

Key words: human imprinting, genome effects, chromosome effects, Huntington's Chorea famial cancer disorders, fragile (X) mental retardation.

## Introduction

It is known that there is a functional difference between maternally and paternally derived chromosomes in a number of organisms, including the laboratory mouse and other mammals. Good evidence that this genomic imprinting occurs in man exists at multiple levels – those of the diploid and haploid genomes, the single chromosome, the subchromosomal region and the single locus. Furthermore, the phenomenon of imprinting could account for some otherwise unexplained genetic disorders, notably the fragile X mental retardation syndrome. This paper will briefly review the evidence that imprinting occurs in man, and will then discuss the importance of imprinting in the context of human genetic disease. Further aspects of this are covered in the contribution of Judith Hall in this volume.

## Genome effects

Conceptions of normal karyotype are known to occur, in which all chromosomes are of paternal origin: they result in (complete) hydatidiform moles. Such molar pregnancies are therefore androgenetic (Kajii and Ohama, 1977), usually arising by the duplication of one haploid chromosome set (Jacobs *et al.* 1980; Lawler *et al.* 1982), but sometimes being the result of dispermy and elimination of the maternal genomic contribution (Fisher *et al.* 1989). The mitochondrial DNA is of exclusively maternal origin (Wallace *et al.* 1982; Edwards *et al.* 1984). Such androgenetic conceptions result in the proliferation of extraembryonic tissues, with characteristic degenerative changes in the placenta and with little development of the embryo. The chorionic villi show no fetal vascularization, and are distended with multiple cysts. Such findings suggest that the paternal genome is particularly important for the growth of extraembryonic tissues.

Maternally derived diploid 'conceptions', with no paternal genome component, also may be recognised. These are ovarian teratomata, which consist of differentiated structures derived from ectoderm, mesoderm and endoderm: there is no placental component. It was shown by Linder *et al.* (1975) that such teratomata are derived parthenogenetically, being of chromosomal constitution 46,XX but containing only maternal chromosomes. These tumours are frequently homozygous for centromeric chromosomal polymorphisms for which the host individual is heterozygous, suggesting they are derived following the first meiotic division. Teratomata support the supposition that the maternal genome is essential for fetal development, but is unable to generate the extraembryonic membranes.

In the mouse, similar conclusions have been reached by consideration of experimental manipulations rather than the analysis of nature's own perturbations. Thus the transplantation of pronuclei between fertilised eggs has demonstrated that the maternal and paternal contributions to the genome are not equivalent. Successful embryogenesis requires a complete set of chromosomes from both parents (McGrath and Solter, 1984; Mann and Lovell-Badge, 1984), even after activation of the embryonic genome (Surani *et al.* 1986). The paternal genome appears to be indispens-

able for development of the extraembryonic tissues, and the maternal genome for development of the embryo itself (Barton *et al.* 1984). A parthenogenetic embryo will develop apparently adequately until implantation, but then fails: this was shown to be related to the absence of nuclear factors rather than the lack of cytoplasmic factors from the sperm (Mann and Lovell-Badge, 1984).

This scheme is further supported by a consideration of human triploid conceptions: diandric (usually dispermic) conceptions result in molar changes in the placenta and a high probability of the early cessation of fetal development (Vejerslev *et al.* 1987), whereas digynic conceptions develop a normal placenta and have a greater chance of fetal survival and the achievement of a live birth (Jacobs *et al.* 1982).

Such abnormalities demonstrate gross anomalies as a consequence of genomic imprinting when the organisation of the genome has been disrupted. While the general trend is clear, that paternal chromosomes are essential for the extraembryonic tissues, and maternal chromosomes for embryonic and fetal development, the level of our understanding is still crude. We know that the X chromosome fails to conform to this model in some species, with the preferential inactivation of the paternal X chromosome in the extraembryonic membranes of marsupials (Takagi and Sasaki, 1975). We do not know whether the effects of autosomal imprinting are the result of the cumulative, independent effects of imprinting at many loci, or whether the control of imprinting is achieved at a higher order of biological complexity.

## Chromosome effects

Uniparental disomy (UD) is the inheritance of two copies of one chromosome type from one parent, with no homologue from the other parent. This can cause problems if the individual has uniparental isodisomy for a chromosome carrying a recessive gene defect, or in the presence of imprinting at one or more loci on the chromosome. Evidence from the mouse that demonstrated the existence of imprinting at the level of the chromosome and the subchromosomal region in this way, has come from the work of several scientists, including Cattanach and Kirk (1985). Through the mating of mouse pairs in which both carried the same balanced translocation, offspring that demonstrated uniparental disomy for a whole chromosome (Robertsonian translocations) or for part of one chromosome arm (reciprocal translocations) were generated. These offspring were identified by means of tail or coat marker genes. UD for some chromosomes produces no apparent effects. Where an abnormal phenotype develops it is specific for the chromosomal region involved and for the parental source of this chromosomal material. Uniparental disomy for certain regions will produce embryonic lethality, or marked differences in growth and body size, or in the level of bodily activity. For example, it was found for mouse chromosome 11 and for the distal part of chromosome 2 that the phenotype of mice exhibiting uniparental disomy was abnormal, and differed from normal in opposite directions (overgrowth or growth retardation; over- or under-activity) depending upon the parent of origin of the UD.

Human phenomena that may correspond to these mouse examples of UD are now described. Thus, two children with cystic fibrosis have been identified, who have maternal isodisomy for chromosome 7 (Spence *et al.* 1988; Voss *et al.* 1989): they both have severe short stature, out of proportion to the severity of their cystic fibrosis, and this may be the result of their UD.

Imprinting may also account for the phenotypic differences between the Angelman and Prader-Willi syndromes, which appear to have similar deletions (Fraccaro *et al.* 1983; Pembrey *et al.* 1989) but distinct clinical features. The Prader-Willi syndrome is characterised by marked hypotonia in infancy, moderate developmental delay, and by subsequent hyperphagia, obesity and hypogonadism; Angelman syndrome results in a characteristic appearance of face and skull, moderate–severe intellectual retardation, a jerky ataxia and seizures. These two distinct conditions are often associated with cytogenetically indistinguishable deletions of chromosome 15q11–13. It has now been shown that molecular deletions (Donlon, 1988) or other rearrangements (Gregory *et al.* 1990) of the proximal long arm of chromosome 15 are frequently found in cases where no cytogenetic deletion is visible. It is of great interest that these two distinct phenotypes are associated with a 15q deletion on chromosomes derived from the parents of the opposite sex (Knoll *et al.* 1989). This strongly suggests that imprinting occurs in one or several genes in 15q11–13, and that both maternal and paternal copies of this region are required. Furthermore, maternal heterodisomy for chromosome 15 has recently been reported in a number of cases of Prader-Willi syndrome, and this strongly supports the importance of imprinting in this disease (Nicholls *et al.* 1989). It is expected that non-deletion cases of Angelman syndrome will arise as a result of paternal disomy for chromosome 15 (Hall, 1990) (see Table 1).

It may be that uniparental disomy of other chromosomes will be detected in patients with other, sporadically occurring syndromes with disturbances of growth and development, and possibly with malformation. Such cases are being actively sought among those with suitable 'candidate' disorders, such as Rubinstein-Taybi, de Lange, Sotos, Weaver and Floating Harbor syndromes. Useful clues may be obtained as to which

**Table 1.** *Probable aetiological relationship of Prader-Willi and Angelman syndromes*

| | PWS | AS |
|---|---|---|
| 15q11-13 deletion | Paternal | Maternal |
| Uniparental disomy 15 | Maternal | Paternal |
| Contrasting features | Lethargic | Active |
| | Dull | Lively |

areas of the human genome are subject to imprinting, by a consideration of man–mouse homologies. Thus, those mouse chromosomes for which uniparental disomy results in developmental disturbances are likely to contain imprinted regions, or at least imprinted genes. From a consideration of the maps of man–mouse gene-mapping homologies, corresponding regions of the human genome can be identified (Searle *et al.* 1989) and then investigated in human disorders possibly associated with imprinting.

If uniparental disomy could only arise by the chance encounter of a gamete disomic for one chromosome with another gamete nullisomic for the same chromosome, then it would have to be a rare event indeed. Making the conservative assumptions of Warburton (1988), uniparental disomy for all chromosomes considered together would occur at a frequency of 1 in 9600, and isodisomy (two *identical* copies of the same chromosome from the same parent) at a frequency of 1 in 30 000. In fact, a much more common cause is likely to be the early loss of one chromosome from a trisomic zygote/embryo. We know that many trisomic conceptions abort spontaneously, but how many 'self-correct' is quite unknown.

This scheme is lent credence by the high rates of chromosomal anomaly in sperm and egg cells. Abnormal karyotypes are found in nearly 50 % of oocytes recovered by laparoscopy after clomiphene stimulation (Wramsby *et al.* 1987), and this may well be typical of normal healthy females. Structural chromosomal anomalies are found in 6–8 % of sperm, with numerical anomalies in up to 4 % (Brandriff *et al.* 1985; Martin *et al.* 1987). Such high rates of aneuploidy make UD a phenomenon that is potentially fairly frequent. There will be enormous cell-selective pressure to eject one of the trio of chromosomes, especially where the trisomy is always lethal. That such selective pressures can operate during intrauterine life, to ensure fetal survival, is known from studies of placental mosaicism in trisomy 13 and 18 syndromes (Kalousek *et al.* 1989). It is of interest that paternal disomy for the sex chromosomes, X and Y, has been proven in a case of father-to-son transmission of haemophilia A, although this case does not relate directly to the question of imprinting in human genetic disease (Vidaud *et al.* 1989).

Imprinted regions of the human genome can also be identified by chromosomal translocations in which the phenotype of the unbalanced offspring differs according to the parent who has transmitted the anomaly. Large pedigrees demonstrating the transmission of such translocations (e.g. Wolf-Hirschorn syndrome, 4p-) will need to be studied clinically in a search for such effects. Where such families are unavailable, the parental source of the deleted or duplicated chromosomal segment in de novo cases can be determined, but differing breakpoints will then be a complicating consideration.

A similar opportunity of discerning imprinting of the human genome arises with the recognised, autosomal microdeletion, 'contiguous gene' entities, such as Miller-Dieker (17p-), Langer-Giedion (8q-) and DiGeorge (22q-) syndromes. If there are clinical distinctions between those cases in which the deletion arises in a paternal chromosome, and those in which it arises in a maternal chromosome, then imprinting may account for these differences. On the other hand, while the finding of a preponderance of paternal or maternal origins for the deleted chromosomes would be of interest, it would be open to several interpretations. While imprinting could be important, either protecting the deletion carrier from the adverse effects experienced in others, or possibly resulting in early embryonic lethality, other explanations are possible. The simplest explanation would be that the mutational event in gametogenesis that results in the deletion is more frequent in the parent of one sex than of the other. To date, the parent of origin of the deleted chromosome has been determined in too few reported cases of such disorders, for any firm conclusions to be drawn.

## Single gene imprinting and disease

Evidence that imprinting also operates at the level of the individual gene locus has come from studies on transgenic mice. Reik *et al.* (1987), Sapienza *et al.* (1987) have demonstrated that the methylation pattern around an inserted transgene, and its transcriptional activity, may differ in the fetal mouse depending upon the sex of the parent of origin. These differential patterns of methylation are reversed upon each germline transmission by the opposite sex, and may be tissue-specific (Swain *et al.* 1987). It is essential to study the patterns of methylation and of gene activity in a tissue- and locus-specific manner, rather than at the level of the whole genome or the whole organism in order to gain precise understanding of the imprinting process (Sanford *et al.* 1985). In some studies, methylation is observed to be stably inherited through human pedigrees without any apparent influence of parental sex (Silva and White, 1988). Certainly, different regions of the genome are associated with different patterns of methylation and different degrees of reversibility with respect to some transgenic loci (Hadchouel *et al.* 1987).

### *Huntington's Chorea*

In a number of autosomal dominant disorders, there appears to be a difference in severity and/or age of onset of symptoms depending upon the gender of the affected parent. Thus, the age of onset of Huntington's Chorea (HC) is lower in those who inherit the HC gene from their father rather than their mother (Myers *et al.* 1982; Newcombe *et al.* 1981). This results in 'anticipation' – the tendency for the age of onset of an autosomal dominant disorder to be younger in the proband than in the parent. If early-onset cases had fewer offspring, this could account for a degree of anticipation; however, there is no such evidence of a reproductive advantage for the later-onset individuals (Myers *et al.* 1985). Furthermore, the anticipation is confined to the children of affected males, amongst whom there is a minority in whom the age at onset of

the disorder is on average 24 years younger than in their fathers (Ridley *et al.* 1988). Corresponding to this, there is a very considerable excess (at least 80%) of early-onset cases of HC born to affected fathers (Merritt *et al.* 1969; Went *et al.* 1984), late-onset cases have more frequently derived the HC gene from their mothers (Myers *et al.* 1983), and the mean age of onset for the offspring of affected men is 33 years, as opposed to 42 for the offspring of affected women (Myers *et al.* 1985). This effect may be accounted for by imprinting, which could reduce the activity of the 'normal' maternally derived HC allele by means of altering the methylation status around the HC locus, leaving only the defective paternal allele to function (Ridley *et al.* 1988; Reik, 1988). The suggestion that those inheriting the HC gene through their father from their paternal grandfather might be particularly susceptible to early-onset disease (Newcombe *et al.* 1981), which effect would weigh against a simple imprinting model, has received no further support (Myers *et al.* 1985). If such an observation were confirmed, it would suggest that imprinting could be cumulative, rather than reversed with each passage through the germline.

Although imprinting could account for these observations, there is no direct evidence. One other possible explanation for this 'imprinting' would be the involvement of a locus on the X chromosome or in the mitochondrial genome (Boehnke *et al.* 1983; Myers *et al.* 1983). However, there is no evidence of a multigenerational maternal lineage effect (Myers *et al.* 1985). Neither do suggestions of other, modifying nuclear genes have any direct evidence in their favour (Boehnke *et al.* 1983). In fact, Myers *et al.* (1985) showed that the modifying effect of the sex of the affected parent occurs in a single parental generation. The proposal of Farrer and Conneally (1985) that the age at onset is determined by independent ageing genes, and that there is an additional maternal protective factor, claims that age at onset varies with familial longevity trends; however, this is not supported by other studies.

One other model of HC has been proposed, which could account for the clinical observations: the model of Laird (1989). He proposes that the HC mutation is a chromosomal alteration close to the HC gene locus, rather than a point mutation or deletion in the gene. By analogy with studies of dominant position-effect variegation in *Drosophila*, this model would account for the completely penetrant and fully dominant nature of the mutation. Heterochromatin inactivation of the HC gene would be expected to result in a recessive phenotype, but a few examples of dominant position-effect variegation have been discovered in *Drosophila*. In particular, dominant-brown variegation achieves *trans*-inactivation of the intact homologous chromosome at the transcriptional level. It is thought that heterochromatic proteins spread between the two homologous loci by virtue of somatic pairing between the two chromosomes, resulting in *trans*- as well as the usual *cis*-inactivation.

By invoking a sex-linked recessive modifier of the HC gene, Laird's model can account for the effects of the sex of the affected parent upon the age at onset in their offspring. While this may seem to be a cumbersome model, it does account for the differences in age of onset among the offspring of affected fathers. Thus, affected children who inherit HC from parents who are hemi- or homo-zygous for an infrequent, enhancing allele at an X-chromosome HC-modifier gene, will tend to develop juvenile-onset disease. This would account for the existence of juvenile-onset disease among the offspring of some women. The enhancer gene would increase the probability that the HC gene from that parent would be inactivated in the offspring.

*Myotonic dystrophy*

Myotonic dystrophy is another disorder in which anticipation may occur, with older gene carriers appearing to have milder disease than their offspring (Harper, 1989). In this disorder, there is generally a progressive wasting of the muscles, which also show myotonia; there can be a variety of other, extra-muscular effects of the condition. Much of the apparent anticipation may be a consequence of ascertainment biases that systematically distort the observed clinical picture: more severe cases reproduce less and are diagnosed earlier. Nevertheless, there may be a genuine anticipation as well (Howeler *et al.* 1989). The most striking feature to be accounted for, is the existence of a severe, congenital form of myotonic dystrophy. In this, an affected child born to an affected mother will sometimes be affected by a severe myopathy resulting in weakness even during intra-uterine life; the affected fetus is hypoactive and may die *in utero*. Those cases born alive will often succumb to severe respiratory problems; those who survive in the long term usually make slow but steady motor progress for at least their first decade. In contrast, most carriers of the myotonic dystrophy mutation have a much milder clinical course, with the onset of slowly progressive weakness and wasting in the second, third or fourth decades. While genomic imprinting could provide an explanation for the occurrence of the congenital form only in the affected children of affected mothers, this is not the only possible explanation. The true explanation could concern some aspect of the intrauterine environment, and be physiological rather than genetic. That the surviving congenitally affected infants improve in muscle strength and motor performance for many years after birth, is weighty evidence in favour of this explanation and counts against the role of imprinting in this context.

The existence of anticipation in myotonic dystrophy is a possibility but is not established with certainty: after allowance is made for ascertainment bias, and after excluding the cases of congenital myotonic dystrophy, some doubt remains. Imprinting at the myotonic dystrophy locus could contribute to the phenomenon, if indeed it is genuine.

Neurofibromatosis is another dominant disorder in which the gender of the affected parent has been claimed to be significant for the clinical effects in the

next generation: this has been shown to be most unlikely for NF1 – von Recklinghausen's disease – (Riccardi and Wald, 1987). There is simply insufficient information to reach a conclusion for NF2, where a similar effect has been claimed (Kanter *et al.* 1980).

*Familial cancer disorders*

The best evidence in favour of clinically important effects of imprinting at the level of the single gene is found in the field of inherited cancer predispositions. Hereditary glomus tumours provide a good example (van der May *et al.* 1989). While the susceptibility gene for this uncommon, benign tumour is transmitted as an autosomal dominant trait, it is only manifest in those who have received the gene from their father. This pattern of inheritance can be explained if the maternal locus is inactivated during oogenesis, and can only be reactivated during spermatogenesis. Furthermore, the apparent excess of non-familial cases among females would also be accounted for, because the proband's offspring would not be at risk.

Imprinting has also been suggested as the explanation of clinically important effects concerning the biology of Wilms' tumours. In common with other embryonal cell tumours, Knudson's two-hit model of oncogenesis is here generally accepted as valid (Knudson, 1971), in the form developed by Comings (1973). The finding of 11p deletions in the somatic cells of some children with Wilms' tumours, sometimes in association with aniridia and developmental disturbances (the WAGR microdeletion/contiguous gene deletion syndrome), indicated that a Wilms' regulatory gene lay at 11p13 (Turleau *et al.* 1984). It has more recently been observed that the maternal chromosome 11 is preferentially lost from sporadic Wilms' tumours (Schroeder *et al.* 1987; Williams *et al.* 1989). Normally, it would be expected that the 'first hit' in Wilms' tumorigenesis can be of either allele of the Wilms' regulatory gene on 11p; defects in this gene functioning as a recessive tumour suppressor gene by regulating one or more transforming genes. A second hit to the other Wilms' regulatory gene will then result in malignancy. Since chromosome loss is likely to be the second hit rather than the first, the observations suggest that the first hit is usually of the paternal allele of the Wilms' regulatory gene. There is no evidence to suggest a higher germline mutation rate in the paternal than the maternal copy of this gene.

An alternative explanation has been proposed by Wilkins (1988). In this model, the Wilms' regulatory gene (tumour-suppressing) is located on 11p, and there is a Wilms' transforming gene (TG) located elsewhere on chromosome 11. If the maternal copy of the Wilms' transforming gene (TG) is inactivated by imprinting, while both copies of the Wilms' regulatory gene are usually active, then the observations made are those to be expected (see Fig. 1). The first hit in a sporadic Wilms' tumour could be to either copy of the regulatory gene, but the consequences will differ depending upon the nature of the second hit. If this is a point mutation in the other regulatory gene, then malignancy will be expected. However, if the second hit is the loss of one

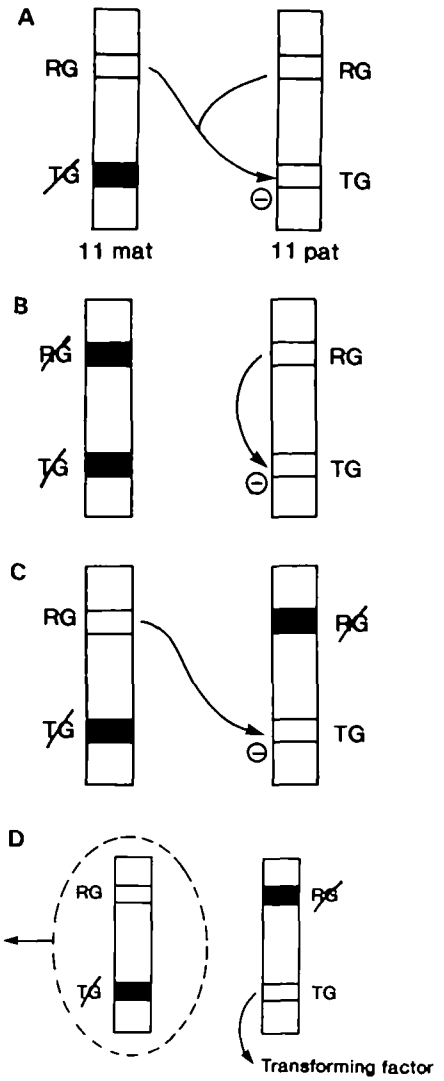


**Fig. 1.** Wilkin's model of Wilms' tumorigenesis. (A) Normal. RG=Wilms' regulatory gene; TG=Wilms' transforming gene. (B) Maternal RG mutation: loss of neither chromosome 11 will result in malignancy. (C) Paternal RG mutation and loss of paternal 11 fails to result in malignancy. (D) Paternal RG mutation and loss of maternal 11 does result in malignancy.

chromosome 11, then malignancy will only occur if the maternal chromosome 11 is lost: if the paternal 11 is lost, then the persisting, maternal copy of the transforming gene (TG) will be inactive because it is imprinted, and malignancy will not occur. The finding that the familial Wilms' tumour predisposition gene does not map to 11p13 spares this hypothesis the need to account for maternally inherited cases of familial Wilms' tumour. It should be noted that, in this model, the assumption has been made that unilateral cases of Wilms' tumour arise as somatic mutations.

In familial retinoblastoma, the two copies of the retinoblastoma (Rb) gene (located on chromosome 13) must be mutated before transformation occurs. One hit can be inherited through the germline, and cytogenetically visible germline deletions may be found, usually in

the paternal chromosome 13. This gene (RB1) is also frequently defective in sporadic cases of osteosarcoma, and survivors of familial Rb are highly susceptible to this tumour too. It has recently been shown that a mutation of the paternal copy of RB1 is frequently the initial event in sporadic osteosarcoma (Toguchida *et al.* 1989) but not in sporadic cases of Rb. However, new familial germline mutations of RB1 preferentially affect the paternal allele (Dryja *et al.* 1989; Zhu *et al.* 1989). Differences in mutation rate during gametogenesis could account for these observations on germline mutations, but not on the somatic mutations. In those cases, a possible difference in susceptibility to mutation must be present, depending upon the parent of origin of the gene. This effect must also be tissue-specific to account for the difference between bone and retina in the origin of sporadic tumours. This difference in somatic mutation rates could be a result of an imprinting phenomenon. In these studies, it has been generally assumed that cases of osteosarcoma without Rb, and isolated cases of unilateral Rb, have suffered a somatic mutation of one of their RB1 genes.

In summary, the familial glomus tumour phenomenon is a good example of genetic imprinting at a single locus. The retinoblastoma/osteosarcoma observations suggest that the susceptibility to somatic mutation at the RB1 locus varies with parental origin of the gene and also with the type of tissue. Wilkins' model of Wilms' tumorigenesis suggests that there is inactivation by imprinting of the maternal copy of a hypothetical Wilms' transforming gene on chromosome 11, a gene that is quite distinct from the Wilms' regulatory gene at 11p13.

*Fragile (X) mental retardation*

This disorder is now known to be the commonest inherited cause of mental retardation. However, it has been widely recognised medically for less than ten years. It was first reported by Martin and Bell (1943), long before Lubs described the fragile site at Xq27. It was not until 1977 that Sutherland described the folate-dependent nature of expression of the fragile site and subsequently its association with many cases of X-linked mental retardation (Sutherland, 1977; Sutherland and Ashforth, 1979).

The pattern of inheritance of fra(X) is remarkable for several features. First, it is so common – affecting as many as 1 in 1000 children (Webb *et al.* 1986). Second, about 30% of the female carriers manifest mental impairment, while at least 20% of male carriers of the gene appear to be unaffected by it and are mentally normal (Sherman *et al.* 1984). Furthermore, the daughters of normal transmitting males are themselves mentally normal. Third, there is a rough correlation between the presence of mental impairment and the finding of the fra(X) chromosome in cytogenetic analysis. Fourth, the observed penetrance of fra(X) in the brothers of normal transmitting males is lower than expected (0.18 vs 0.5) while the penetrance among the brothers of affected males is higher (0.74 vs 0.5) than expected (Sherman *et al.* 1985).

Several models of fra(X) inheritance have been proposed to account for these features, but none has yet been generally accepted. Pembrey *et al.* (1985) suggested that transmitting males carried only a premutation, and that the full mutation could appear after a recombination event in female meiosis; however, observed molecular recombination events do not support this model (Oberle, 1986; Brown *et al.* 1987). Steinbach (1986) and Israel (1987) have proposed the existence of autosomal modifier genes to account for the unusual inheritance pattern, but such models do not conform well to the data, and appear somewhat contrived. Hoegerman and Rary (1986) suggested that transposable elements (TEs) might account for what is known of fra(X), but their model predicts father-to-son transmission of such TEs, and these have not been observed.

Laird's model (Laird, 1987) sets out to account for these observations with a hypothesis based upon imprinting. He suggests that the fragile X mutation acts as a local (*cis*-acting) block to the reactivation of the inactivated X, a process that occurs in the female just prior to oogenesis. An X chromosome carrying the mutation, if inactivated, will then be transmitted to children in an inactive, imprinted form because it fails to be reactivated appropriately. The fra(X) phenotype will result from the inhibition of transcription of gene loci around or distal to the fra(X) site. Normal transmitting males will result when a male inherits the mutated, but not imprinted, fra(X) chromosome. Females will manifest the disorder to a variable extent, depending upon random X-inactivation: there is an inverse correlation between IQ score in a female carrier of fra(X), and the proportion (above 50%) of early-replicating fra(X) chromosomes. A woman inheriting the imprinted fra(X) chromosome may escape intellectual impairment if she inactivates the fra(X) chromosome in more than 50% of her cells.

The crucial distinction here is between the mutated (but not imprinted) chromosome and the mutated (and imprinted) chromosome. A carrier female whose fra(X) chromosome is imprinted can only transmit a normal or an imprinted chromosome: all fra(X) sons will be affected, all her carrier daughters will be 'imprinted', but not all will be retarded. Of those daughters who inherit an imprinted chromosome, the pattern of X inactivation will determine who is retarded (approx. 50%). A female carrier who has a mutated, non-imprinted fra(X) chromosome, will have only half as many retarded offspring – reflecting the X-inactivation process in her own embryogenesis, a prerequisite for imprinting to occur (see Table 2).

The observed frequencies of mutated (<6% fragile site expression) and of imprinted (>10% expression) males and females do not fit Laird's hypothesis particularly well, until allowance is made for ascertainment bias. Once correction is made for the systematic failure to ascertain transmitting males until they have affected grandchildren, the observations outlined above are fully accounted for.

Interestingly, there is a marked clustering of affected

**Table 2.** *Laird's model of fragile-X syndrome: expected outcome for the fragile-X-carrying offspring of fragile-X-carrying parents*

| | | | Offspring inheriting fragile-X chromosome | | | | |
|---|---|---|---|---|---|---|---|
| | | | Male | | Female | | |
| Parent | Fragile X imprinted? | Retarded? | Affected males (imprinted) | Transmitting males (not imprinted) | Normal non-imprinted females | Normal but imprinted females | Affected imprinted females |
| Male | No | No | – | – | 100% | – | – |
| | Yes | Yes | – | – | Probably all | ? | ? |
| Female | No | No | 50% | 50% | 50% | 25% | 25% |
| | Yes | 50% | 100% | 0% | 0% | 50% | 50% |

males and females within sibships, such that the empiric risk of a carrier female having an affected child is much higher if she has already had one such infant. This is essentially the same observation as that of the difference in penetrance between the brothers of a transmitting male and the brothers of an affected male, although ascertainment bias is a complicating factor. Laird's model accounts for the clustering of retarded offspring within a sibship by supposing a very small pool of oogonial progenitor cells at the time of X-inactivation: the data agree with the model on the assumption that there are only two such progenitor cells at the time (Laird *et al.* 1990). Thus, 0, 50 or 100% of the fra(X) children of a female carrying a mutated, not-imprinted fra(X) mutation will be imprinted: this agrees well with clinical observations. There will be as many sibships with clusters of normal, transmitting fra(X) males or females as there are those with clusters of retarded children, but the former are not ascertained. Laird's model is elegant in its simplicity, and in its capacity to explain the confusing events surrounding fra(X) gene segregation. No model, however, has yet been proven.

## Concluding remarks

It is clear that genetic imprinting occurs in humans, as in other mammals. The male and female parental contributions to the nuclear genome are not fully equivalent. Cases of imprinting are revealed by genetic disease. Genomic imprinting is revealed in the developmental defects of hydatidiform mole, teratoma and triploidy. Imprinting is revealed at the chromosomal level when uniparental disomy results in the Prader-Willi phenotype, and possibly in other sporadic congenital anomaly syndromes. Single locus imprinting is important in the aetiology of some tumours, and may contribute to the phenotypic effects seen in certain Mendelian inherited disorders. Imprinting may also be important in the aetiology of the fragile X mental retardation syndrome.

Imprinting, therefore, is a phenomenon of great interest in the study of human genetic disease, but its importance is still not fully understood. Much remains unclear in genetic disease, and I believe that imprinting will soon prove to be highly relevant to the explanation of 'awkward' clinical and genetic observations, as in fragile X mental retardation.

## References


Barton, S. C., Surani, M. A. H. and Norris, M. L. (1984). Role of paternal and maternal genomes in mouse development. *Nature* **311**, 374–376.

Boehnke, M., Conneally, P. M. and Lange, K. (1983). Two models for a maternal factor in the inheritance of Huntington disease. *Am. J. hum. Genet.* **35**, 845–860.

Brandriff, B., Gordon, L., Ashworth, L., Watchmaker, G., Moore, D., Wyrobek, A. J. and Carrano, A. V. (1985). Chromosomes of human sperm: variability among normal individuals. *Hum. Genet.* **70**, 18–24.

Brown, W. T., Sherman, S. L. and Dobkin, C. S. (1987). Hypothesis regarding the nature of the fragile X mutation. *Hum. Genet.* **75**, 294–295.

Cattanach, B. M. and Kirk, M. (1985). Differential activity of maternally and paternally derived chromosome regions in mice. *Nature* **311**, 496–498.

Comings, D E. (1973). A general theory of carcinogenesis. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3324–3328.

Donlon, T. A. (1988). Similar molecular deletions on chromosome 15q11.2 are encountered in both the Prader-Willi and Angelman syndromes. *Hum. Genet.* **80**, 322–328.

Dryja, T. P., Mukai, S., Petersen, R., Rapaport, J. M., Walton, D. and Yandell, D. W. (1989). Parental origin of mutations of the retinoblastoma gene. *Nature* **339**, 556–558.

Edwards, Y. H., Jeremiah, S. J., McMillan, S. L., Povey, S., Fisher, R. A. and Lawler, S. D. (1984). Complete hydatidiform moles combine maternal mitochondria with a paternal nuclear genome. *Ann. hum. Genet.* **48**, 119–127.

Farrer, L. A. and Conneally, P. M. (1985). A genetic model for age at onset in Huntington disease. *Am. J. hum. Genet.* **37**, 350–357.

Fisher, R. A., Povey, S., Jeffreys, A. J., Martin, C. A., Patel, I. and Lawler, S. D. (1989). Frequency of heterozygous complete hydatidiform moles, estimated by locus-specific minisatellite and Y-chromosome-specific probes. *Hum. Genet.* **82**, 259–263.

Fraccaro, M., Zuffardi, O., Buhler, E., Schinzel, A., Simoni, G., Witkowski, R., Bonifaci, E., Caufin, D., Cignacco, G., Delendi, N., Gargantini, L., Losanowa, T , Marca, L., Ullrich, E. and Vigi, V. (1983). Deficiency, transposition and duplication of one 15q region may be alternatively associated with Prader-Willi (or a similar) syndrome. Analysis of seven cases after varying ascertainment. *Hum Genet.* **64**, 388–394.

Gregory, C. A., Kirkilionis, A. J., Greenberg, C. R., Chudley, A. E. and Hamerton, J. L. (1990). Detection of molecular rearrangements in Prader-Willi syndrome patients by using genomic probes recognizing four loci within the PWCR. *Am. J. med. Genet.* **35**, 536–545.

Hadchouel, M., Farza, H., Simon, D., Tiollais, P. and Pourcel, C. (1987). Maternal inhibition of hepatitis B surface

antigen gene expression in transgenic mice correlates with *de novo* methylation *Nature* **329**, 454–456.

HALL, J. G. (1990). Angelman's syndrome, abnormality of 15q11-13, and imprinting. *J. med. Genet* **27**, 141–144.

HARPER, P. S. (1989). *Myotonic Dystrophy*, 2nd edition. Philadelphia, W. B. Saunders.

HOEGERMAN, S. F. AND RARY, J. M. (1986) Speculations on the role of transposable elements in human genetic disease with particular attention to achondroplasia and the fragile X syndrome. *Am. J. med. Genet.* **23**, 685–699.

HOWELER, C J., BUSCH, H. F M., GERAEDTS, J P. M., NIERMEIJER, M. F. AND STAAL, A. (1989). Anticipation in myotonic dystrophy: Fact or fiction? *Brain* **112**, 779–797.

ISRAEL, M. H. (1987). Autosomal suppressor gene for fragile-X: An hypothesis. *Am J. med Genet.* **26**, 19–31.

JACOBS, P. A., WILSON, C. M., SPRENKLE, J. A , ROSENHEIM, B. AND MIGEON, B. (1980). Mechanism of origin of complete hydatidiform moles. *Nature* **286**, 714–716.

JACOBS, P. A., SZULMAN, A. E., FUNKHOUSER, J., MATSUURA, J S. AND WILSON, C. C. (1982). Human triploidy: reltionship between parental origin of the additional haploid component and development of partial hydatidiform mole. *Ann. hum. Genet.* **46**, 223–231.

KAJII, T. AND OHAMA, K. (1977). Androgenetic origin of hydatidiform mole. *Nature* **268**, 633–634.

KALOUSEK, D K., BARRETT, I. J. AND MCGILLIVRAY, B. C. (1989). Placental mosaicism and intrauterine survival of trisomies 13 and 18. *Am. J. hum. Genet.* **44**, 338–343.

KANTER, W. R., ELDRIDGE, R., FABRICANT, R., ALLEN, J. C. AND KOERBER, T. (1980). Central neurofibromatosis with bilateral acoustic neuroma genetic, clinical and biochemical distinctions from peripheral neurofibromatosis. *Neurology* **30**, 851–859.

KNOLL, J. H. M., NICHOLLS, R. D., MAGENIS, R. E., GRAHAM, J. M., LALANDE, M AND LATT, S. A (1989). Angelman and Prader-Willi syndromes share a common chromosome 15 deletion but differ in parental origin of the deletion. *Am J. med Genet.* **32**, 285–290.

KNUDSON, A. G. (1971). Mutation and cancer: Statistical study of retinoblastoma *Proc. natn. Acad Sci. U.S.A.* **68**, 820–823.

LAIRD, C D. (1987). Proposed mechanism of inheritance and expression of the human fragile-X syndrome of mental retardation. *Genetics* **117**, 587–599.

LAIRD, C. D. (1989) Huntington's disease proposed to result from dominant position-effect variegation. *First World Congress on Psychiatric Genetics*, Cambridge, England. August 1989.

LAIRD, C. D., LAMB, M. M. AND THORNE, J. L. (1990) Two progenitor cells for human oogonia inferred from pedigree data and the X-inactivation imprinting model of the fragile-X syndrome. *Am J hum Genet.* **46**, 696–719.

LAWLER, S. D , POVEY, S., FISHER, R. A. AND PICKTHALL, V J. (1982). Genetic studies on hydatidiform moles II: the origin of complete moles. *Ann hum. Genet.* **46**, 209–222.

LINDER, D , MCCAW, B., KAISER, X. AND HECHT, F. (1975) Parthenogenetic origin of benign ovarian teratomas. *New Engl. J Med.* **292**, 63–66.

MANN, J. R. AND LOVELL-BADGE, R. H. (1984). Inviability of parthenogenones is determined by pronuclei, not egg cytoplasm. *Nature* **310**, 66–67.

MARTIN, J. P. AND BELL, J. (1943). A pedigree of mental defect showing sex-linkage. *J. Neurol. Psychiatr* **6**, 154–157.

MARTIN, R. H., RADEMAKER, A. W., HILDEBRAND, K., LONG-SIMPSON, L., PETERSON, D. AND YAMAMOTO, J. (1987). Variation in the frequency and type of sperm chromosomal abnormalities among normal men. *Hum. Genet.* **77**, 108–114

MCGRATH, J. AND SOLTER, D. (1984). Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell* **37**, 179–183.

MERRITT, A. D., CONNEALLY, P M., RAHNAM, N F. AND DREW, A. L. (1969). Juvenile Huntington's chorea. In *Progress in Neurogenetics*, pp. 645–650. Amsterdam: Excerpta Medica.

MEY, A. G. L. VAN DER, MAASWINKEL-MOOY, P. D., CORNELISSE, C. J., SCHMIDT, P. H. AND KAMP, J. J P. VAN DE (1989). Genomic imprinting in hereditary glomus tumours: Evidence for new genetic theory *Lancet* **ii**, 1291–1294.

MYERS, R. H , CUPPLES, L A., SCHOENFELD, M , D'AGOSTINO, R. B., TERRIN, N. C., GOLDMAKHER, N. AND WOLF, P. A. (1985). Maternal factors in onset of Huntington Disease. *Am J. hum. Genet.* **37**, 511–523.

MYERS, R. H., GOLDMAN, D , BIRD, E. D., SAX, D. S , MERRIL, C. R., SCHOENFELD, M. AND WOLF, P A. (1983). Maternal transmission in Huntington's disease. *Lancet* **i**, 208–210.

MYERS, R. H., MADDEN, J. J., TEAGUE, J. L. AND FALEK, A. (1982). Factors related to onset age of Huntington disease *Am. J. hum. Genet.* **34**, 481–488.

NEWCOMBE, R. G., WALKER, D. A. AND HARPER, P. S (1981). Factors influencing age at onset and duration of survival in Huntington's chorea. *Ann. hum. Genet* **45**, 387–396.

NICHOLLS, R. D., KNOLL, J. H M., BUTLER, M. G., KARAM, S. AND LELANDE, M. (1989) Genetic imprinting suggested by maternal heterodisomy in non-deletion Prader-Willi syndrome *Nature* **342**, 281–285

OBERLE, I., HEILIG, R., MOISAN, J. P., KLOEPFER, C , MATTEI, M. G., MATTEI, J. F., BOUE, J., FROSTER-ISKENIUS, U., JACOBS, P., LATHROP, G. M., LALOUEL, J M. AND MANDEL, J. L. (1986). Genetic analysis of the fragile-X mental retardation syndrome with two flanking polymorphic DNA markers. *Proc natn. Acad Sci. U S.A.* **83**, 1016–1020.

PEMBREY, M., FENNELL, S J., BERGHE, J. VAN DEN, FITCHETT, M., SUMMERS, D., BUTLER, L., CLARKE, C., GRIFFITHS, M., THOMPSON, E., SUPER, M. AND BARAITSER, M. (1989). The association of Angelman's syndrome with deletions within 15q11-13. *J med. Genet.* **26**, 73–77.

REIK, W. (1988). Genomic imprinting: a possible mechanism for the parental origin effect in Huntington's chorea. *J med. Genet* **25**, 805–808.

REIK, W., COLLICK, A , NORRIS, M. L., BARTON, S. C. AND SURANI, M. A. (1987). Genomic imprinting determines methylation of parental alleles in transgenic mice. *Nature* **328**, 248–251

RICCARDI, V. M. AND WALD, J. S (1987). Discounting an adverse maternal effect on severity of neurofibromatosis. *Pediatrics* **79**, 386–393.

RIDLEY, R. M , FRITH, C. D., CROW, T. J. AND CONNEALLY, P M. (1988). Anticipation in Huntington's disease is inherited through the male line but may originate in the female. *J. med. Genet.* **25**, 589–595.

SANFORD, J. P., CHAPMAN, V. M. AND ROSSANT, J. (1985). DNA methylation in extra-embryonic lineages of mammals. *Trends in Genetics* **1**, 89–93.

SAPIENZA, C., PETERSON, A C., ROSSANT, J. AND BALLING, R. (1987). Degree of methylation of transgenes is dependent on gamete of origin. *Nature* **328**, 251–254.

SCHROEDER, W. T., CHAO, L.-Y , DAO, D. D., STRONG, L. C , PATHAK, S., RICCARDI, V., LEWIS, W. H. AND SAUNDERS, G. F (1987). Nonrandom loss of maternal chromosome 11 alleles in Wilms tumors. *Am J hum. Genet.* **40**, 413–420.

SEARLE, A G., PETERS, J., LYON, M. F., HALL, J. G., EVANS, E P , EDWARDS, J. H. AND BUCKLE, V. J. (1989). Chromosome maps of man and mouse IV. *Ann. hum. Genet.* **53**, 89–140

SHERMAN, S. L., MORTON, N. E , JACOBS, P. A AND TURNER, G (1984). The marker (X) syndrome: a cytogenetic and genetic analysis. *Ann. hum. Genet.* **48**, 21–37.

SHERMAN, S L., JACOBS, P. A., MORTON, N. E., PROSTER-ISKENIUS, U., HOWARD-PEEBLES, P. N., NIELSEN, K. B., PARTINGTON, M. W., SUTHERLAND, G. R., TORNER, G. AND WATSON, M. (1985). Further segregation analysis of the fragile X syndrome with special reference to transmitting males. *Hum. Genet.* **69**, 289–299.

SILVA, A. J. AND WHITE, R. (1988). Inheritance of allelic blueprints for methylation patterns. *Cell* **54**, 145–152.

SPENCE, J. E., PERCIACCANTE, R. G., GREIG, G M., WILLARD, H W., LEDBETTER, D. H., HEJTMANCIK, J. F., POLLACK, M. S., O'BRIEN, W. E. AND BEAUDET, A. L. (1988). Uniparental disomy as a mechanism for human disease. *Am. J hum Genet.* **42**, 217–226

STEINBACH, P. (1986). Mental impairment in Martin-Bell syndrome is probably determined by interaction of several genes: simple explanation of phenotypic differences between unaffected and

affected males with the same X chromosome. *Hum. Genet.* **72**, 248–252.

SURANI, M. A. H., BARTON, S. C. AND NORRIS, M. L. (1986). Nuclear transplantation in the mouse: Heritable differences between parental genomes after activation of the embryonic genome. *Cell* **45**, 127–136.

SUTHERLAND, G. R. (1977). Fragile sites on human chromosomes: demonstration of their dependence on the type of tissue culture medium. *Science* **197**, 265–266.

SUTHERLAND, G. R. AND ASHFORTH, P. L. C. (1979). X-linked mental retardation with macroorchidism and the fragile site at Xq27 or 28. *Hum. Genet.* **48**, 117–120.

SWAIN, J. L., STEWART, T. A. AND LEDER, P. (1987). Parental legacy determines methylation and expression of an autosomal transgene: a molecular mechanism for parental imprinting. *Cell* **50**, 719–727.

TAKAGI, N. AND SASAKI, M. (1975). Preferential inactivation of the paternally derived X chromosome in the extraembryonic membranes of the mouse. *Nature* **256**, 640–642.

TOGUCHIDA, J., ISHIZAKI, K., SASAKI, M. S., NAKAMURA, Y., IKENAGA, M., KATO, M., SUGIMOT, M., KOTOURA, Y. AND YAMAMURO, T. (1989). Preferential mutation of paternally derived RB gene as the initial event in sporadic osteosarcoma. *Nature* **338**, 156–158.

TURLEAU, C., DEGROUCHY, J., TOURNADE, M. F., GAGNADOUX, M. F. AND JUNIEN, C. (1984). Del 11p/aniridia complex: report of three patients and review of 37 observations from the literature. *Clin. Genet.* **26**, 356–362.

VEJERSLEV, L. O., DISSING, J., HANSEN, H. E. AND POULSEN, H. (1987). Hydatidiform mole: genetic origin in polyploid conceptuses. *Hum. Genet.* **76**, 11–19.

VIDAUD, D., VIDAUD, M., PLASSA, F., GAZENGEL, C., NOEL, B. AND GOOSSENS, M. (1989). Father-to-son transmission of haemophilia A due to uniparental disomy. *American Society of Human Genetics* **1989**, Abstract 0889.

VOSS, R., BEN-SIMON, E., AVITAL, A., GODFREY, S., ZLOTOGORA, J., DAGAN, J., TIKOCHINSKI, Y. AND HILLEL, J. (1989). Isodisomy of chromosome 7 in a patient with cystic fibrosis: could uniparental disomy be common in humans? *Am. J hum. Genet.* **45**, 373–380.

WALLACE, D. C., SURTI, U., ADAMS, C. W. AND SZULMAN, A. E. (1982). Complete moles have paternal chromosomes but maternal mitochondrial DNA. *Hum. Genet.* **61**, 145–147.

WARBURTON, D. (1988). Uniparental disomy. a rare consequence of the high rate of aneuploidy in human gametes (editorial). *Am. J. hum. Genet.* **42**, 215–216.

WEBB, T. P., BUNDEY, S., THAKE, A. AND TODD, J. (1986). The frequency of the fragile X chromosome among schoolchildren in Coventrey. *J. med. Genet.* **23**, 396–399.

WENT, L. N., VEGTYER-VAN DER VLIS, M. AND BRUYN, G. W. (1984) Parental transmission in Huntington's disease. *Lancet* **i**, 1100–1102.

WILKINS, R. J. (1988). Genomic imprinting and carcinogenesis. *Lancet* **i**, 329–331.

WILLIAMS, J. C., BROWN, K. W., MOTT, M. G. AND MAITLAND, N. J. (1989). Maternal allele loss in Wilm's tumour. *Lancet* **i**, 283–284.

WRAMSBY, H., FREDGA, K. AND LIEDHOLM, P. (1987). Chromosome analysis of human oocytes recovered from preovulatory follicles in stimulated cycles. *New Engl. J. Med.* **316**, 121–124.

ZHU, X., DUNN, J. M., PHILLIPS, R. A., GODDARD, A. D., PATON, K. E., BECKER, A. AND GALLIE, B. L. (1989). Preferential germline mutation of the paternal allele in retinoblastoma. *Nature* **340**, 312–313.