ARCHIVES OF DISEASE IN CHILDHOOD

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Annotations

Genomic imprinting

Over the last few years a compelling body of evidence has accumulated indicating that genomic imprinting must be occurring in parts of the human genome.[1] The concept of genomic imprinting is difficult for people who have grown up with the concepts of mendelian inheritance. In general, the question has not been asked whether a particular gene has a different outcome when it is inherited from the parent of one sex as compared with when the same gene is inherited from a parent of the opposite sex. The concept of genomic imprinting implies there are differential modifications of genetic material, either chromosomal or single gene, depending on whether the genetic material is passed on from the male or the female parent. This kind of modification of the nuclear DNA in the somatic cells is thought to lead to differential expression of the genetic material and therefore different phenotypes. However, this kind of modification is not permanent, not a mutation of the gene itself, not an allele of the particular gene, but rather a temporary change in the function of the genetic material, albeit perhaps for the lifetime of the individual.

The evidence which has been accumulated from many different areas of research suggests very strongly that imprinting effects must occur in some parts of the human genome. At the present time, there are seven different kinds of observations that suggests the presence of genomic imprinting. Some come from studies in mice and some from studies in humans. These studies include the observations of nuclear transplantation—for example, parthenogenetic work in mice by Solter[2] and Surani,[3] in which zygotes are constructed so that all the nuclear genes (that is, both sets of haploid chromosomes) are entirely derived either from the male or female parent. These reconstituted zygotes each have a complete set of chromosomes, but a set of chromosomes that has been derived entirely from only one type of parent. When these zygotes are allowed to develop they have strikingly different phenotypes suggesting that maternally and paternally derived genetic information has different roles.

A second piece of evidence comes from human triploid work by McFadden and Kalousek (D McFadden, D Kalousek, personal communication) in which a triploid with two paternal and one maternal complement is compared with a triploid with two maternal and one paternal complement. They have quite different phenotypes with the paternal complement contributing more to the placenta and the maternal complement to the embryo.

The third type of evidence comes from uniparental disomy work in mice by Searle and Beechey,[4] Lyon and Glenister,[5] Cattanach and Kirk,[6] and others in which mice are constructed using translocation chromosomes such that both copies of a specific chromosome segment have come from one parent. Thus these mice have a balanced set of chromosomes but both copies of a particular segment will have come from only one parent. In the case of eight (and maybe nine) mouse chromosome segments there are appreciable phenotypic differences on growth behaviour and survival when both copies of a specific mouse chromosome segment are derived from one parent. There are at least two situations in humans in which there is uniparental disomy (that is, both copies of a chromosome have been shown to come from only one parent) for a specific chromosome and both have been associated with changes in growth and behaviour. One of these situations is found in cases of maternal uniparental disomy with the patient having cystic fibrosis.[7 8] Both reported cases had intrauterine and postnatal growth retardation. There are many alternative explanations, but the homologous chromosomal disomy in mouse also has intrauterine growth retardation. The second situation in humans is found in the reported case of Prader-Willi syndrome in which there were two chromosomes 15 from the mother and no deletion, but no paternal 15.[9] It appears that the absence of the paternally derived chromosome 15 produces typical Prader-Willi phenotype.

The fourth type of disorder that indicates that there are differences between maternally compared with paternally derived segments of chromosomes are the chromosome deletion syndromes such as Prader-Willi and Angelman's syndromes where there appear to be dramatic differences in the parental derivation of the deleted chromosome.[10 11] There are similar if not identical chromosome deletions of chromosome 15 in both conditions; however, in Prader-Willi syndrome there has always been a deletion or absence of the paternal chromosome 15. Most cases of sporadic Angelman's syndrome are associated with a deletion of chromosome 15 and it is always the maternally derived 15. These findings imply that part of the chromosome 15 that is inherited from the father is treated differently than the same part of chromosome 15 when inherited from the mother.

Work on congenital and infantile tumours suggests that a similar kind of phenomenon is occurring. These tumours are associated with loss of heterozygosity—that is, loss of predictable chromosome segment.[11] However, Wilms' tumour, retinoblastoma, and some sarcomas have dramatic differences in the parent of origin for the chromosome

involved in the loss of heterozygosity. In cases of sporadic Wilms' tumour when there is loss of chromosome 11, it is almost always loss of the maternal chromosome 11.[12] Interestingly, however, familial Wilms' tumour is usually transmitted from the father and is specifically not linked to chromosome 11.[13] Thus there must be two or more genes with different roles involved in the production of Wilms' tumour, at least one of which has parental derivation effects.

With regard to the retinoblastoma gene, sporadic sarcomas (not those in the retinoblastoma patients) are frequently associated with chromosome 13 loss. Again, it is almost always the maternal chromosome 13 which is lost.[14] By contrast, in new unilateral and some sporadic bilateral retinoblastoma when there are mutations of chromosome 13 there does not seem to have been any particular parent of origin selection. In new germ line mutations for retinoblastoma, however, there is preferentially involvement of the paternal chromosome 13. These findings imply that tissue specific modifications of the retinoblastoma gene allow tumour development to occur first in a paternally derived gene, and then there is secondary loss of the tumour suppressing maternal gene (loss of heterozygosity).[15] Sporadic sarcomas act differently from sporadic retinoblastomas, however, suggesting that there may be tissue differences in the imprinting of the same gene.

The sixth kind of observation which suggests that there is differential modification of genes depending on the parent of origin are those made on transgenic mice in which something like 20% of transgenes expressed preferentially when inherited from one parent compared with the other.[16] Non-expression is associated with methylation. In transgenic mice there is incorporation of the transgene DNA into the chromosome. The DNA does not change from generation to generation but the ability to express the gene does. Thus there will be different phenotypic effects depending on whether the gene is transmitted from the mother mouse or from the father mouse.

The last kind of observation which suggests that imprinting occurs is that made of specific genes. In humans the classic examples are Huntington's disease and myotonic dystrophy. In 5–10% of families with Huntington's disease, when the Huntington gene is transmitted from the father, there may be a severe rigid juvenile onset form. By contrast in 10–15% of families affected with myotonic dystrophy a severe congenital form may occur when the gene is transmitted from the mother. In addition, there are a number of other disorders in which more severe or earlier an onset or specific manifestations are observed when the gene is inherited from one parent compared with the other.[1]

Taken together these seven types of observations strongly suggest that genomic imprinting—that is, differential expression of maternally and paternally derived DNA—does occur in some parts of the human genome and therefore would be expected to play a part in human disease.

How can we identify these diseases? The first way is to be suspicious in the various classes of disorders, to ask the question whether there is earlier onset, more severe affliction, and multiple manifestations of disease when inherited from mother versus from father. In chromosomal abnormalities with deletions, duplications, and translocations we must ask the same kind of question.

From the mouse transgenic work and in human diseases observed thus far we would expect that an imprinting effect might *not* be absolutely constant. In other words, it may be something that only shows up in 10%, 15%, or 20% of families with a specific disorder. In mice, we call these strain differences in expression of imprinting, in humans we might expect there to be ethnic differences.

Another clue to finding imprinting in humans would be to anticipate what a pedigree in which imprinting was occurring would look like. Again, the term imprinting is used to describe a modification which does not allow expression. Thus in maternal imprinting there will be phenotypic expression or more noticeable expression when a gene is transmitted from the father but not when transmitted from the mother.[1] Paternal imprinting is the term used to imply that there is a phenotypic expression or more noticeable expression of the gene when transmitted from the mother. Because there will be phenotypic effects only when the gene or chromosome segment in question is transmitted from one or the other parents, there will be a number of non-manifesting transmitters. It is important to point out that there are equal numbers of males and females affected or non-affected phenotypically in each generation. A non-manifesting transmitter gives a clue to the sex of the parent who passes on the genetic information that could be expressed. In other words, in maternal imprinting there will be 'skipped' male non-manifesting individuals and in paternal imprinting there will be 'skipped' female non-manifesting individuals.

It is important as well to point out that in these pedigrees depending on what part of the pedigree is being examined, the pedigree can look like that of a dominantly inherited trait or a recessively inherited trait, or as a multifactorial disorder. Thus we need to re-examine pedigrees from multifactorial disorders and ask the question whether the expression has to do with the parent of origin. Similarly, there is a wealth of data to reanalyse from the clinics for common disorders such as sickle cell and Tay-Sachs diseases and phenylketonuria.

Another clue to potentially imprinted disorders are the homologous areas of human and mouse genome. Using the Oxford grid it is possible to define, with some accuracy, in humans where the homologous areas of DNA are mapped when compared with the mouse.[1 10] Using the areas from the translocation disomy of mice that are involved in imprinting and transferring those areas to homologous areas of the human chromosomes, one can ask whether or not human disorders suspected of having imprinting phenomenon are located in the areas homologous to mouse 'imprinted' areas. Indeed we find that myotonic dystrophy, cystic fibrosis, Beckwith-Wiedemann syndrome, and many other human disorders are in mouse imprinted homologous areas. Angelman's and Prader-Willi syndromes are close to the mouse areas where very similar phenotypes are seen in the mouse disomies.[1]

Thus it behoves us all to re-examine specific parts of the human genome. One can look for a particular gene in a particular area or ask about genes related to a particular disease type process, for example one can examine the growth factors and endocrine genes in mouse homologous imprinted areas.[1] A number of very interesting diseases which have been described as having variable expressivity are present in those areas. Specific family trees need to be re-examined to see whether or not a pattern of expression is related to the sex of the parent of origin.

In summary, compelling evidence has been accumulated that some areas of the genome function differently depending on from which sex parent they are inherited. This process appears to be normally reversible and involves a temporary DNA modification. The differential function depends on which parent transmits the gene. This may be a ubiquitous phenomenon explaining a variety of observations that hitherto have been considered to be atypical, decreased penetrance, or to have inconsistent inheritance patterns. Heterogeneity, variable expression, and decreased penetrance are terms that have been developed to explain empiric observations. Reconsideration of family history, expression patterns, and disease processes may reveal that imprinting effects are much more common than we

expected. It is clear that mammalian development requires the functional and complementary presence of at least parts of both maternal and paternal genome. Affects on embryonic and fetal growth and behaviour have been observed. The challenge now is to determine how many childhood and adult disorders also are associated with genomic imprinting.

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Neonatal infections with coagulase negative staphylococci

Staphylococci are members of the family of bacteria Micrococcaceae. They are Gram positive, catalase positive cocci that form grape like clusters (from the Greek staphyle: bunch of grapes, coccus: grain or berry). The classification of staphylococci is extremely complex and has been revised repeatedly over the last 30 years. More and more new species have been recognised, so that there are now 19 distinct species recognised by Bergey's *Manual of Systematic Bacteriology* (1986). The coagulase test is one of several tests used by clinical laboratories to distinguish *Staphylococcus aureus* from other staphylococci. 'Coagulase negative staphylococci' is a term used to describe species that do not coagulate plasma under the defined conditions of the coagulase test.

Coagulase negative staphylococci are frequent blood culture isolates from neonates in many intensive care units.[1 2] Neonatal infections with coagulase negative staphylococci are hospital acquired and are usually diagnosed after the first week of postnatal life.[3] Quantitative blood culture techniques have shown that the numbers of coagulase negative staphylococci in the blood of premature neonates with bacteraemia may exceed 1000 colony forming units/ml.[4 5] Defective opsonisation and phagocytosis may allow these large numbers of coagulase negative staphylococci to circulate.[6] *Staphylococcus epidermis* is the species most frequently associated with neonatal infection,[7] although other species such as *Staphylococcus hominis*, *Staphylococcus warneri*, and *Staphylococcus haemolyticus* have also been implicated. The strains involved in neonatal infection are usually resistant to a wide range of antibiotics.[8] Antibiotic resistant strains are spread from neonate to neonate on the hands of medical and nursing staff leading to colonisation of the skin of premature neonates in intensive care units within the first week of life.[9] Apart from the skin, another major reservoir of antibiotic resistant coagulase negative staphylococci is the bowel of neonates, where numbers may exceed 10^{10} colony forming units/g dry weight.

Many strains of coagulase negative staphylococci secrete a complex mucopolysaccharide,[10] which has been termed 'extracellular slime substance'. Electron microscopy studies suggest that it stabilises the attachment of coagulase negative staphylococci to the surfaces of foreign bodies such as intravascular catheters.[11] The influence of extracellular slime substance on the adherence of coagulase negative staphylococci to the immature skin of premature neonates is not known. Extracellular slime substance has been reported to have a number of immunomodulating effects such as inhibiting antibody binding to the staphylococcal cell wall, reducing the chemotactic response of neutrophils, and interfering with T and B cell function.[12] Although extracellular slime substance is probably important in stabilising the attachment of bacteria on surfaces, its importance in determining pathogenicity is controversial.[13] It is not produced by all clinically significant isolates of coagulase negative staphylococci and other bacterial surface characteristics such as bacterial cell surface hydrophobicity may be more important determinants of pathogenicity.[7 14]

It is not possible to sterilise the skin and therefore a proportion of percutaneously collected samples will be contaminated with cutaneous flora. Use of quantitative blood culture techniques may help to differentiate blood culture contamination from bacteraemia.[4 5] The proportion of blood cultures collected from neonates and children that are contaminated with coagulase negative staphylococci has been estimated at 7–10%.[5 15] This proportion is similar to that reported for blood cultures collected from neonates with necrotising enterocolitis.[16]

There is no evidence that coagulase negative staphylococci can cross intact skin. Intravascular catheters frequently become colonised with antibiotic resistant strains,[17] and neonatal bacteraemia with coagulase negative staphylococci is frequently associated with the use of intravascular catheters.[4 5 18] It is likely that infected catheters are the most common source of neonatal infection with coagulase negative staphylococci. The mechanisms by which bacteria