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Newer Genetics in Mental Retardation - II: Imprinting, Disomy, Prenatal Screening and Gene Therapy

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Abstract

In the last five years, there have been tremendous advances in the field of medical genetics. Relevant literature is reviewed with special reference to the genetics of mental retardation. Genomic imprinting has led to 'parent-of-origin' effects in the phenotypic expression of a disease. Uniparental disomy i.e., both chromosomes of a pair coming from the same parent, has important clinical and diagnostic implications. Advances in parental screening technqiues have improved our ability to detect genetic disease with greater accuracy. Preimplanation genetic diagnosis has been pioneered with success. Gene therapy for various diseases has yielded encouraging results with the possibility of its application to many other diseases hitherto considered untreatable.

Key words -

Mental retardation,
Genomic imprinting,
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Gene therapy

Genomic imprinting and uniparental disomy

The term genomic imprinting describes a situation in which differences in structure and function (i.e., the phenotype) reflect which parent was the source of the genetic material. Phenotypic differences have been demonstrated when certain genes or chromosomal regions are inherited from the father rather than from the mother [1].

Prader-Willi syndrome is a rare genetic condition characterized by obesity, mild to moderate mental

retardation, hypotonia, genital hypoplasia, characteristic facies, short stature, and behavioural problems. Angelman syndrome is phenotypically a different syndrome with severe MR, ataxia, hyperactivity, convulsions, paroxysms of laughter, characteristic facies and jerky movements. (Happy Puppet syndrome) [2]. It is difficult to believe that they are caused by the same deletion on the same chromosome, but they only differ with respect to the sex of the parent from which the defective chromosome is derived. In both the syndromes the deletion is in the band q 12 of chromosome 15. In Prader-Willi syndrome, this chromosomes is inherited from the father and in Angelman syndrome, from the mother.

Prader-Willi syndrome can be caused in several ways. Absence of a critical region of the paternally derived chromesome 15 which can result from a deletion or from translocation leading to the loss of critical region on chromosomes 15 derived from the father can cause Prader-Willi syndrome. In other cases, it may result from the absence of the entire paternal chromosomes 15 with the offspring receiving both chromosomes 15 from the mother. Angelman syndome results from similar processes involving maternal chromosomes 15 [3]. Smeets et al [2] have reported a case of Prader-Willi syndrome and a case of Angelman syndrome from the same family. Cytogenetic and molecylar studies revealed that both syndromes resulted from a familial translocation between chromosomes 6 and 15, leading to a deletion in the paternally derived chromosome 15 in Prader-Willi syndrome. In the patient with Angelman syndrome, both copies of chromosomes 15 were inherited from the father, i.e., maternal chromosome 15 was absent.

The situation in which both chromosomes of a pair come from the same parent is called uniparental disomy, which explains a number of unusual conditions. It is not as rare as was thought to be earlier [4]. But, why would both chromosomes of a pair come from a single parent? The most likely explanation is that conception begins as a trisomy because of some meiotic error. Most trisomic conceptions are lethal and the embryo is aborted unless a cell line with only two copies of the chromosome (a disomic cell line) arises and survives. In trisomy, by definition, there are three copies of a chromsome-two from one parent and one from the other. Hence, when one of the copies is lost during cell division to produce a viable disomic cell line, the result will be uniparental disomy in one-third of the cases, with both the chromosomes coming from one parent [5]. There can be several other mechanisms as proposed by Smeets et al [2]

Mascari et al [6] have studied the frequency of uniparental disomy in Prader-Willi syndrome in 30 patients and found that about 20 per cent of cases result from maternal uniparental disomy. In addition to chromosome 15, uniparental disomy has been observed for chromosomes 4, 6, 7, 11, 14, 16 and 21 [5].

There are certain interesting clinical implications of uniparental disomy. The abnormal trisomic cell lines are usually found only in the placenta (confined placental mosaicism) and this can be detected by chorionic villus sampling, while amniocentesis or cord blood sampling reveals only cells with normal number of chromosomes. If only amniocentesis is performed, the gestation is allowed to continue without further analysis. But in a third of cases an infant with a syndrome such as Prader-Willi syndrome may be born [5]. Secondly, if the two copies of the chromosome coming from the same parent are identical (isodisomy) and each carries an abnormal recessive disease in the child. There are reports of cystic fibrosis in children, who inherited both defective chromosomes 7 from the mother [7]. An autosomal recessive disease in which only one parent is a carrier!

When in the course of development would these maternal and patternal differences be produced? The

imprinted regions must be affected during meiosis, which is different in males and females. In males it occurs from puberty to old age, while in females, it begins during embryonic development and is completed during ovulation [5]. Thus, an event during the grandmother's pregnancyy with the mother affects the mother's developing eggs, which in turn affect the mother's offspring two to four decades later.

Consanguinity

Consanguinity refers to mating between individuals who have a recent ancestor in common i.e., in the preceding two or three generations. From 20 to 50 per cent of all unions in Asia and Africa are consanguineous unions. Consanguinity is associated with increased gross fertility due, at least in part, to younger maternal age at first livebirth. It also leads to an increase in the amount of homozygosity and increased expression of deleterious recessive genes [8].

With advances in medicine and public health and with control of infectious diseases, genetic disorders will account for an increased proportion of disease worldwide. As consanguineous unions are favoured in developing countries, this burden will fall more heavily on them. But studies conducted show that only a minority of families experience the adverse effects of inbreeding. With industrialisation, greater population movement, decrease in family size and high literacy rates, some reduction in certain types of consanguineous unions is inevitable [8].

Newer parental screening techniques

It is well-documented in literature that the risk of Down's syndrome in the foetus increases with increasing maternal age [9]. Prior to 1984, prental screening for Down's syndrome, in the absence of family history of the disorder, consisted of asking a pregnant woman her age and then offering amniocentesis and chromosomal analysis, if she was 35 years or older [9]. Later it was expanded to include women under 35, with reports that maternal serum alpha-fetoprotein concentration at midtrimester was approximately 25 per cent lower in pregnancies affected by Down's syndrome than in unaffected pregnancies [10]. In combination with the woman's age, the detection rate in women under 35 rose to 20 per cent at a screening cut-off value that initially identified 5 per cent of the women as being at high risk.

More recently, studies have found that the average maternal serum concentration of chorionic gonadotrophin is atleast two times higher than normal [11] and that of unconjugated oestriol 25 per cent lower than normal and that of unconjugated oestriol 25 per cent lower than normal in the presence of fetal Down's syndrome [12]. These findings were later confirmed by another study [13] in which maternal serum concentrations of alpha-fetoprotein, chorionic gonadotrophin and unconjugated oestriol were found to be largley independent predictors of Down's syndrome and all were independent of maternal age. The biological rationale for the altered concentrations has not been elucidated. The combined biochemical technique has a detection rate of 64 per cent and an initial positive rate of 64 per cent and an initial positve rate of 6.6 per cent. This technique has a favourable ratio of cases detected (1 per 38 amniocenteses) to the risk of fetal loss. It is concluded, that addition of these two

measurements can be readily incorporated into existing prenatal screening programmes [14].

Another recent advance in prenatal screening is the isolation of fetal cells, such as nucleated red blood cells, trophoblasts, lymphocytes etc. from maternal circulation for non-invasive prenatal cytogenetic diagnosis [15]. Polymerase chain reaction has been used to demonstrate the existence of fetal cells in maternal blood. Trisomy 21 and trisomy 18 have been detected by this technique. But, several unanswered question remain - the optimal cell type for isolation, their frequency in maternal blood, timing of maternal blood sampling and the likelihood of persistence of fetal cells after delivery [15].

Preimplantation genetic diagnosis

Prenatal diagnosis is now well known for detection of serious birth defects that are genetically determined and complicate and threaten the lives of 3 per cent of newborns [16] and account for one-fifth of neonatal deaths [17]. Amniocentesis, choronic villus biopsy, percutaneous umbilical blood sampling, fetal biopsy and ultrasonography are some of the techniques for prenatal diagnosis [18], [19]. In the last few years, in vitro fertilization has offered an opportunity for genetic diagnosis even before implantation occurs. The advantage is obvious, in that, couples need not go through pregnancy, amniocentesis and if necessary, termination of pregnancy. There are three general approaches to preimplantation diagnosis-polar body biopsy, trophoectodermal biopsy and blastomere analysis [20]. The last technique - Blastomere Analysis Before Implantation (BABI) was pioneered by Handyside et al at the Hammersmith hospital in London in the case of cystic fibrosis [21]. Cystic fibrosis is a common, severe autosomal recessive disease caused by a three nucleotide deletion (Δ F 508) in the cystic fibrosis transmembrane regulator (CFTR) gene. BABI was attempted in three couples, both members of which carried the deletion. Three days after in vitro fertilization, at the 8-celled stage, biopsy was performed for removing one or two cells for DNA amplification by nested-primer polymerase chain reaction and analysis. When the normal homozygous embryo was implanted, one woman became pregnant and gave birth to a normal girl free of deletion in both chromosomes 7 [21]. Thus, pre-implantation genetic diagnosis is possible now and its application to other diseases is eagerly awaited.

Genetic counselling

Closely linked to prenatal diagnosis is genetic counselling. Genetic counseling usually begins with a question, raised by the advent of a child with a suspected genetic disorder. The diagnosis is reached after the assessments of clinicians, syndromologists, X-ray studies, laboratory tests etc. A probability is estimated using information from family history, cytogenetics, relevant literature. Mendelian principles and Bayesian calculations. Following informative and supportive counselling, the counselee may reach a decision either to refrain from reproduction or to go ahead. Both the decisions may need appropriate referral to other specialists. If the 'go ahead' decision results in a recurrence, further counselling may be needed. Follow-up of the counselee and the extended family may result in re-entry into the process of counselling [22].

Gene therapy

Human gene therapy is now a reality. The basic tenet of gene therapy is the introduction of a functional gene to replace or supplement the activity of a resident defective gene [23]. Admittedly, this is more plausible in well-defined, single gene defects such as those in inherited metabolic disorders, many of which are associated with MR, e.g., phenylketonuria, lysosomal storage disorders etc.

There are three general approaches to introduce a functional gene into cells - germ line cell gene therapy, somatic cell gene therapy and activation of endogenous genes to augment or circumvent the defective gene [23]. The first approach consists of introduction of the gene into the zygote or embryo so that it is passed on to the next generation. This is not applicable to human disease. The basic principle of somatic cell gene therapy is the use of vectors to introduce the gene sequence of interest into targeted host cells. Retroviral vectors provide viral practicles that encode foreign nucleic acid and contain mechanisms for chromosomal integration. One such strategy consists in the insertion of a cDNA construction between two viral long terminal repeats in a plasmid vector. This construction can be introduced into cultured cell lines that provide viral proteins necessary to package the pseudovirus. Target cells can be removed, purified and infected with these viruses and the target organ repopulated with these cells. Delivery to the bone marrow is easier, than to other tissues such as liver. But, a major hurdle in this type of therapy is the long-term high level expression of the gene, which is difficult [23].

The first delivery systems were retroviral vectors. The first clinical marker protocol was the introduction of adenosine deaminase gene into the marrow of a patient with adenosine deaminase deficiency, a form of severe combined immunodeficiency [24], [25]. Retroviral infected infusion of marrow precursors over ten months significantly increased the adenosine deaminase level in circulating cells from less than 2 per cent to 20 per cent [26]. After this initial success, several other gene therapy protocols were approved for various diseases. Gaucher's disease is one of the diseases associated with MR and is currently the target of gene therapy. Recently, delivery of genes to other organs such as liver, muscle, respiratory cells is being studied [26]. With the discovery of the cystic fibrosis gene in 1989, the prospects for its gene therapy are great [27], [28].

In conclusion, genetics is moving today in directions which are close to the long-cherished ambition of medical science - to conquer nature. Umpteen new directions for further research are being opened up and it is becoming difficult to keep pace with the explosion of knowledge in this area.

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