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LESSONS FROM A SYMPOSIUM ON THE PAEDIATRIC IMPLICATIONS OF THE NEW GENETICS HELD IN THE COLLEGE ON 3 MAY 1996*

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New technology

The Human Genome Project which was established in 1988 is aimed at unravelling the entire human genome, and is driven by the need to learn about genetic diseases. Human DNA consists mostly of repetitive sequences and only around 3% of it codes for proteins. This 3% corresponds to somewhere between 50,000 and 100,000 genes. A useful analogy is that of an audio-cassette: this contains approximately 135m of tape, and if this represented the human genome, then one gene would correspond to around 2.7cm of tape.

Novel investigative techniques have allowed rapid advances in the field of genetics in recent years. The polymerase chain reaction (PCR) allows an exponential replication of DNA strands, and thus simplifies their detection and analysis. The use of restriction enzymes allows DNA to be cut up at particular sites, generating different sized DNA fragments which can be followed through families with inherited disease and accurately located in the human genome.

Studies of gene expression and tissue distribution, as well as the chromosomal location of mutations in diseased patients, allow identification and confirmation of candidate genes, for example abnormalities on chromosome 6 can be mapped in consanguineous families through HLA typing. Genetic mapping can be approached by cutting candidate DNA into multiple fragments, and then analysing overlapping sequences with markers. Viral reverse transcriptase can be used to synthesise complementary DNA (cDNA) from RNA allowing abnormally expressed genes to be localised quickly through these 'expressed sequence tags'. Fluorescent *in situ* hybridisation (FISH) is a technique that allows genes to be identified microscopically by attaching fluorescent labels to them. This can be useful in detecting microdeletions such as that seen on chromosome 22q in DiGeorge's syndrome.

An area of controversy arising from the pace of progress in this field is the issue of patenting genes. This is as yet unresolved and is complicated by the fact that information on a large number of genes is being made publicly available over the Internet.

Some mutations have now been demonstrated to change from generation to generation. The 'Fragile X syndrome' is caused by a base sequence repeat that expands in successive generations causing a worsening of the abnormal phenotype. Normal individuals may have 6-54 copies of the repeat. An expansion to between 52 and 200 repeats is a premutation stage, and once more than 200 repeats are seen, the phenotype is observed. This phenomenon is also observed in myotonic dystrophy.

The effect of single gene defects can be greater if they code for proteins which are only active when they complex as multimers. This can perhaps be better

* A list of speakers and the titles of their papers presented at this symposium is recorded in *Proceedings* Vol. 26 p. 518.

understood by the following analogy. If a bicycle is considered as a dimer formed by the random combination of two wheels from a pool of wheels, then if 50% of the wheels are abnormal, 75% of the bicycles will be non-functional as 25% of bicycles will have two normal wheels, 25% two abnormal wheels, and 50% one of each. This problem gets worse as trimers and tetramers are considered. This phenomenon is observed with abnormalities in the genes coding for mannose-binding proteins which provide non-specific protection against infection.

Developmental disorders and dysmorphology

Developmental abnormalities may arise from faults in genes whose function is to regulate the expression of other nearby genes. This is observed in the WAGR association; this acronym stands for Wilm's tumour, Aniridia, Genitourinary abnormalities and mental retardation. It is now recognised that these malformations are seen in association because the genes responsible for them are contiguous on chromosome 11. This discovery was made as a result of a series of observations. One per cent of Wilm's tumours are associated with a cytogenetically visible deletion on chromosome 11 (WT1). A candidate cDNA was found for this region in a DNA library. RNA *in situ* hybridisation in tissue sections demonstrated that this gene was expressed widely in renal, gonadal and mesodermal tissues. An abnormality in the same gene was demonstrated in the Denys Drash syndrome which includes early-onset, bilateral Wilm's tumour where there is a subsequent loss of the normal gene in the tumour itself. A gene 'knock-out' experiment in a mouse model resulted in a phenotype that was lethal in the embryonic stage of development with no kidneys or gonads and abnormal mesothelial tissues. Human FISH studies have demonstrated a deletion in the WAGR region of chromosome 11 in people with aniridia, and in a naturally occurring small-eye mouse phenotype which has a malformation in the same region.

Similar problems are seen during limb bud development; as limb buds grow and develop, events are controlled by local chemical messengers such as retinoic acid. This process is controlled by 'homeobox genes' which are strung out along the chromosome and are expressed sequentially during embryological development. Abnormalities of the expression of these genes at different time points result in recognised human malformation syndromes. Multisystem problems arising from faulty expression of groups of genes may be important in other human developmental disorders and can be studied in animal models.

Two per cent of all individuals have a congenital abnormality. Nearly all of these are multifactorial in origin with only a minority representing single gene defects. Nevertheless, the London Dysmorphology Database contains over 2,750 entries. More than 1,500 represent single gene defects, around 500 are sporadic syndromes, 40 are related to environmental exposure and 700 are either chance associations or presently unknown microdeletions. Of the single gene defects 102 genes have been specifically identified and a further 115 mapped. Over 1,300 have yet to be identified. Of the 217 genes identified or mapped, multiple sources of information contributed to their identification including mapping, cytogenetic clues, candidate gene identification, biochemical clues and animal models.

Molecular genetics of skeletal dysplasia and connective tissue disorders

Connective tissue disorders and skeletal dysplasias may result from abnormalities in the production of the structural proteins collagen and elastin or from abnormal

matrix proteins, which in turn may result from abnormalities in various regulatory molecules or their receptor proteins. Examples of abnormal structural proteins include osteogenesis imperfecta (type I collagen), epidermolysis bullosa (Type II collagen), Marfan's (fibrillin) and William's (elastin) syndromes. Abnormal fibroblast growth factor receptor molecules (FGFRs) cause Alport's and Crouzon's syndromes.

FGFRs have widespread roles in development. There are four different receptors and nine ligands currently known. Achondroplasia is an autosomal dominant condition that results from an abnormality in FGFR3, as does hypochondroplasia which has a similar but milder phenotype. The abnormal gene in achondroplasia has been located at the tip of chromosome four and two mutations in this gene, one sense, one anti-sense explain almost every case. This is probably the most mutable nucleotide in the entire human genome. The homozygous form of this disease is usually lethal. Thanatophoric dysplasia is also the result of an FGFR3 abnormality. Some syndromes, e.g. osteogenesis imperfecta type I, result from simple haploinsufficiency (production of half as much of a protein as normal), others e.g. Marfan's syndrome and osteogenesis imperfecta type II, from dominant negative effects. Dominant gain of function is the underlying mechanism in achondroplasia where there is an upregulation of FGFR3. A similar mechanism is responsible for Huntington's chorea.

Prediction of insulin dependent diabetes mellitus

Diabetes is a condition which readily lends itself to genetic investigation. The diagnosis is usually certain, large families are available for study and good animal models also exist. Identifying individuals who will later develop insulin-dependent diabetes is made difficult by the contribution of many gene loci, and by the interaction between genetic susceptibility and environmental factors in the eventual evolution of this condition. Around 10% of diabetes could be said to be familial. A background population risk of 0.3% increases to 6% when a sibling is affected, 18% between HLA identical individuals and 30% for identical twins. HLA DQ linkage is the strongest, but a number of other MHC genes are involved. Predictive models can be generated on the basis of the presence or absence of various genetic markers but these generally perform poorly, identifying far too many individuals as being at risk of developing diabetes for each true case. The importance of genetic predisposition to insulin-dependent diabetes lessens with age. Up to 15 years of age, males and females are equally affected but after this age, a male predominance emerges in new cases.

Alternative predictive models using multiple auto-antibodies and biochemical markers can be developed. These clearly outperform genetic models in individuals with a positive family history and show great promise as a screening tool for the general population. They are currently being evaluated in a population of 30,000 schoolchildren.

Inborn errors of metabolism

The rate of progress in the field of inborn errors of metabolism is illustrated by the fact that a core text on the subject, *The Metabolic Basis of Human Disease*, has had seven revised editions since 1961, and is now a 5,000 page text describing almost 1,000 metabolic abnormalities. There is often a poor relationship between genotype, biochemical phenotype and clinical disease entity because of the many different biochemical sources of various individual metabolites, and their multiple alternative

biochemical fates. This is further complicated in the case of some respiratory chain abnormalities by the involvement of the separate mitochondrial genome which is acquired solely from the maternal side. Because of the way that mitochondria segregate at cell divisions, mosaicism or heteroplasmy within individuals can result in considerable variation between tissues in the proportion of abnormal mitochondria present and this can result in multiple phenotypes for the same gene abnormality.

Treatment is now possible for a wide range of conditions and can involve the supply of missing factors, the activation of alternative biochemical pathways or even organ transplantation. Treatment has not been generally associated with good effects on clinical outcome, probably because diagnoses are so often made after a presentation in crisis at a time when irreversible damage has already occurred. Delay in diagnosis is a big problem and misdiagnosis is common. Good clinical results are only obtained at a very high price both financial and in terms of medical and family input. Transplantation, gene therapy and extended neonatal screening programs may all lead to improvements in the future.

Genetics and paediatric oncology

Childhood malignancy is generally more amenable to treatment than adult malignancy. Overall cure rates for children currently run at around 70%. Many factors contribute to these good outcomes, including the increased chemosensitivity of childhood malignancy, the centralisation of care and the evolution through careful studies of national and international treatment protocols. It is desirable to identify markers of disease severity and risk in order that innovative or more intensive treatment may be applied to poor prognosis tumours, whilst good prognosis tumours could be treated more simply with less toxic regimens, perhaps resulting in fewer late sequelae. Neuroblastoma accounts for 10% of all tumours and 30% of all childhood tumour deaths. Clinical features alone are not useful in prognostication. New insights into the genetics of this tumour rather than of the patient allow more accurate prognostication.

Many abnormalities with varying prognostic significance are recognised. Amongst the most important are N-myc gene amplification and allelic loss of chromosome 1p, but there are many others. Presence or absence of these markers can be detected with a variety of techniques including PCR, FISH and Southern blotting. Tumour genotype is now being used to determine treatment protocols along with other more traditional determinants of prognosis such as age and clinical stage at presentation. These principles apply to many other tumours including acute lymphoblastic leukaemia and embryonal rhabdomyosarcoma. It is increasingly recognised that treatment decisions based on tumour genetics will form the basis of future therapy.

New developments in cystic fibrosis

Cystic fibrosis is now known to be caused by abnormalities of a transmembrane chloride channel (CTFR) that is located on the apical membrane of epithelial cells. CTFR is a protein molecule containing 1,480 amino acids and is coded for by a large gene with 27 exons which is located on chromosome 7. There are now more than 400 known malformations of this gene although only a few are commonly encountered. The commonest is ΔF508 and this increases in prevalence from south-east to north-west Europe. Different genotypic abnormalities disrupt the function of the protein molecule to varying degrees with some resulting in a completely non-

functional ion channel and others in various degrees of hypofunction. The different genotypes are increasingly recognised to be associated with a spectrum of phenotypes, some resulting in milder disease.

The ability to identify the abnormal gene has led to the growth of antenatal and postnatal screening programmes as early diagnosis is associated with more successful treatment. Antenatal diagnosis also offers the option of termination of affected pregnancies. Sequential couple screening, offered in Edinburgh, has had 69% uptake and was associated with a cost per identified affected fetus of between 50 and 100 thousand pounds sterling. This compares favourably with the treatment costs of 10-15 thousand pounds per year for an adolescent with cystic fibrosis.

Gene therapy for cystic fibrosis is an attractive proposition because of the relative accessibility of the respiratory epithelium to direct topical application of treatment. Trials are underway both in animal models and in human subjects. Normal genes are transfected into cells by using modified adenovirus vectors or liposomes. It remains to be seen whether this treatment is safe or clinically effective, particularly in repeated application.

Many developments in cystic fibrosis care lie outside genetics. Transplant surgery is in wider use although this is limited by shortage of suitable donors. A new disease entity of fibrosing colonopathy, related to the use of certain high dose pancreatic enzyme supplement preparations, is likely to be due to the material used to enteric coat the tablets rather than to the enzymes themselves. Steroids and ibuprofen may alter the rate of lung disease progression and aerosolised recombinant human DNase may reduce the visco-elasticity of the sputum and result in improved lung function.