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# Human genetic research

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## Introductory remarks

The latter part of this century has witnessed a markedly rapid development in the area of molecular biology, and in the following collection of papers we shall try to document some of the major advances made in several key areas of this exciting discipline.

The review opens with H.J. Evans' Introduction which provides a very comprehensive overview of recent developments in the different areas of human genetics. It is obvious from this Introduction that one technique has revolutionized genetics as no other method before: recombinant DNA. There is practically no paper in which these new techniques are not at least mentioned as a major breakthrough in the respective field. Human genetics has long been confined to the observation of pedigrees and populations with respect to phenotypic traits, as experimentation with humans was not possible. In the last few decades, however, advances in cell biology, biochemistry, cytogenetics, and immunology have enabled geneticists to study the human genome more directly.

In the following selection of papers, we shall try to review these developments without going into excessive detail. The papers have been designed to give the interested reader working in science an impression of what has been achieved in the 20th century. We have tried to get capacities in their respective fields from all over the world to participate in this review and I would like to thank all contributors for their support and cooperation.

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## New trends in human genetic research – An introduction and overview

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*Key words.* Mapping; linkages; mutant alleles; antenatal diagnosis; genetic therapy; genetics and cancer; mutagenesis.

A history of advances in human genetics would recall a long list of enterprising individuals who made remarkable discoveries through astute observation and penetrating analysis of nature's experiments in human variation. Advances in modern human genetics depend to no less an extent on individual enterprise and ingenuity in studying such variation, but the rapid pace in the extension of our knowledge of the genetics of man has been stimulated by developments in two areas. First, the introduction of new approaches and new techniques in cellular and molecular biology and in biochemistry and cytogenetics, which have opened up new ways of analysing, dissecting, probing and indeed modifying the human genome. Second, the evergrowing realisation outside the genetics laboratory, or clinic, that a considerable burden of human disease is attributable to our genetic inheritance and that modern advances enable us to detect an increasing variety of such diseases early in fœtal development and in some instances provide a basis for successful therapy.

Looking at the state of human genetics at the beginning of the 1960s it was evident that the techniques of biochemistry that were available at that time had contributed greatly to our understanding of the nature of some of the mutations that were then known to be involved in inherited disease, as well as to knowledge of the frequencies and distribution of allelic forms of a range of

genes in populations; serology and protein chemistry had provided powerful tools to unravel some of the complexities and patterns of inheritance of a range of blood groups; and family pedigree studies on the inheritance of genes exhibiting allelic variation had defined a number of linkages between genes and was beginning to provide a framework for a map of the human genome. At around that time, major advances had been made in developing and applying cytogenetic techniques to the study of human chromosomes and these, allied with the very important development of methods for culturing human lymphocytes and stimulating them to undergo mitosis in vitro, heralded the birth of the science of human cytogenetics. The ability to study the chromosome constitution of any individual was a major development which led, and continues to lead, to a string of important advances in human genetics.

Also in that decade came the application of somatic cell genetic techniques to cultured human cells; the introduction of methods to make interspecific cell hybrids; and the later discovery that preferential loss of human chromosomes occurred in many human-rodent hybrid cells. Studies on gene assignment and linkage in man had previously been only possible from family pedigree studies and on genes with known allelic variants. The segregation of human chromosomes in proliferating hybrid cells now enabled the use of the powerful and rapid techniques of somatic cell genetics to identify, locate, and study under laboratory conditions, a whole range of human genes. The fruits of the combined approaches of cytogenetics and somatic cell genetics are well illustrated by the enormous increase in our knowledge of the human genome that took place throughout the 1970s. At the First Human Gene Mapping Conference that took place in Yale in 1973, human geneticists had assigned some 46 genes to the autosomes in the complement and at least three chromosomes (numbers 3, 8 and 9) appeared to be devoid of genes at that time. The rate at which new genes were being discovered and mapped, however, began to increase exponentially and ten years later, in 1983, at the Seventh Human Gene Mapping Conference held in Los Angeles, the numbers of loci allocated to the X chromosome had risen to over 200 and the number of genes assigned to the autosomes in the human genome had risen to over 500.

This increasing knowledge of the components of the human genome, and of their organisation and function, has laid the foundations for very considerable recent advances in the application of human genetics to many areas of medicine and in contributing to our overall understanding of growth, development, health and behaviour of man. Moreover, the overall pace of acquisition of knowledge has increased, particularly following the introduction and exploitation of ideas and techniques in the fields of molecular biology, cell biology, immunology and cytogenetics. Technologies involving recombinant DNA, monoclonal antibodies, hybrid cells, sophisticated protein chemistry and prophase chromosome banding are all being brought to bear on a variety of problems in human genetics, so what then are the trends in the 1980s? The field is large, but there are a number of well-defined growing points which I shall try to summarise under a number of headings.

*Genome architecture and organisation*

The application of various techniques of molecular biology and cytogenetics have brought about many advances in our understanding of the organisation and arrangement of the approximately $3 \times 10^9$ base pairs of DNA in the 23 chromosomes of the haploid complement. At the cytological level the power and resolution of a variety of chromosome staining and banding techniques has been increased by their application to prophase chromosomes and the cytogenetic map now locates over $10^3$ bands. This is getting towards the limit of practical usefulness and indeed of optical resolution, with each band representing an average of $3 \times 10^6$ base pairs of DNA. In addition to the standard banded structures, much attention has recently been devoted to secondary constricted regions referred to as fragile sites (these are useful markers on chromosomes although their nature, structure and consequences are yet to be elucidated) and to amplified regions expressed as Homogeneously Stained Regions within the chromosome, or as free double minutes. Amplified regions develop under conditions of intense selection, but are also common in neoplastic cells and indeed could well turn out to be a not uncommon feature of normal development and differentiation. At the nucleosomal level the association of the DNA with histone proteins is reasonably well understood, but our knowledge of higher order structure and the nature of the associations between DNA and the acidic structural scaffold, or core, proteins of the chromosome is still sketchy. Molecular techniques are beginning to tell us something about the sequences and structures involved in organising telomeres and centromeres and in situ immunological and autoradiographical methods are revealing more of the gross arrangement of DNA in the chromosome.

In considering the DNA of the genome, a great deal has been learnt concerning the types, quantities and distribution of various kinds of repetitive sequences and the powerful techniques of gene cloning and DNA sequencing have laid bare the basic structures of a number of human genes. There have been a number of surprises, including initially the discovery of the split nature of the gene with its intervening introns and the later findings of non-functioning gene copies, or pseudogenes, and particularly of scattered processed pseudogenes representing DNA copies of processed mRNAs which had become incorporated into the genome. Large and clinically important gene clusters, such as those of the major histocompatibility complex, beta-globins and the immunoglobulins, have been, and will continue to be, the subject of much study. The genetic basis of the antibody repertoire, coded for by the immunoglobulin loci and estimated to exceed $10^6$ different immunoglobulin molecules, is still not completely understood and a great deal is yet to be learnt concerning the recombination and elimination of DNA sequences of the immunoglobulin genes during B cell development. Similar diversities are associated with the somewhat related antigenic protein molecules coded for by the MHC complex and the combination of genetic, immunological and biochemical approaches will continue to be required to understand the structures and functions involved in these large gene complexes.

The mechanisms involved in gene activation and inactivation are major problems in biology so that transient, or

indeed permanent, structural changes associated with such phenomena will continue to attract much attention. There is now evidence for changes in chromatin structure at chromosome sites prior to their becoming transcriptionally active and nuclease sensitive sites, enhancers and promoters have been identified at various loci. Many mutations associated with inherited disease in man are a consequence of gene inactivation, e.g. some betathalassaemias, and in addition to stop codons in controlling sequences, rearrangements many kilobases removed from an intact structural gene have been shown to result in its inactivation. These and the phenomena of X-inactivation in the female, the involvement of methylation and Z-DNA formation in gene activation/inactivation are all problems that will continue to demand solution if we are to understand the regulatory mechanisms underlying normal and abnormal development and differentiation.

*Mapping the genome and defining linkages*

Defining the location and association of genes and gene clusters in the genome is essential for the understanding of genome organisation and for the application of genetic techniques in identifying and understanding inherited disease. Family studies give us a measure of distance between linked loci by determining the recombination fraction between them in family offspring. The limitations of this approach have already been referred to and many are circumvented by somatic cell studies which give a physical somatic metaphase map of loci revealing the same gene order, but not necessarily the same intergenic distances, as that provided from family mapping studies. Both family (meiotic) and somatic (mitotic) approaches have now been dramatically extended by the introduction of recombinant DNA technology and in particular through the use of restriction fragment length polymorphisms and the isolation and cloning of DNA sequences of known and unknown function.

Restriction fragment length polymorphisms (RFLPs) are a consequence of frequent and detectable variations in DNA sequence that result in the generation, or eradication, of restriction endonuclease cutting sites in the DNA. A base change affecting an endonuclease site in a given DNA region of one, but not the other, homologous chromosome in a complement will result in the production of two different sized DNA fragments following digestion with that site specific enzyme. These fragments will be separable on agarose gel electrophoresis and visualised by autoradiography following hybridisation with a labelled DNA probe that recognises the sequences within the fragments. RFLPs can therefore be used as markers for any locus of known or unknown function within the genome and, depending upon the degree of linkage, may be used as signposts for the presence of an abnormal allele within members of a family.

Chromosome-specific single-copy DNA probes for linkage analysis have been isolated from all of the chromosomes in the human complement using either DNAs extracted from human-murine hybrid cells containing single human chromosomes, or from human chromosomes separated and accumulated using fluorescence activated flow sorters. Libraries of single copy DNAs will shortly be available for all the chromosomes in the complement and the approach using RFLPs has already been used to provide identifiable closely linked markers to abnormal genes of known function and location, e.g. beta-globin, as well as to a member of important abnormal alleles responsible for genetic defects of unknown biochemistry, e.g. Huntington's chorea, X-linked retinitis pigmentosa, Duchenne and Becker muscular dystrophies, etc. A few hundred RFLPs should be sufficient to provide markers in a family study to show linkage to any particular disease gene, but there are a number of limitations with this approach, in addition to the fact that an abnormal gene may only be associated with a particular polymorphism in a number of related individuals (families) within a population and not to others in that population.

The increased information that is already accruing on linkage in the human genome underlines the fact that recombination between two adjacent markers may be related not only to the physical distance separating them, but also to other factors including position on the chromosome. Cytogenetic studies of meiosis point to a higher frequency of crossing-over – and hence recombination – near the ends of human chromosomes and linkage studies on genes located near the tips of chromosome 1p and Xq show a higher concentration of genes in terms of physical distance than is implied from meiotic recombinational data. Over short distances a non-random association of genes reflecting close linkage, or disequilibrium, is common, but linkage equilibrium, 'hot spots', with random association between markers has been reported in a number of instances between markers but 12 Kbases apart. Genetic map distance is most certainly not directly proportional to metaphase cytological length, and further knowledge on the relationship between these two matrices at the various regions throughout the genome is an important quest.

*Identifying and cloning mutant alleles and the nature of genetic disease*

Several dozen genes coding for proteins recognised as being involved in disease processes have been isolated and cloned and it is already evident that in the next few years many hundreds of such genes will have been identified, isolated, cloned and characterised. The isolation of mRNAs or the specific proteins, using monoclonal antibodies, provide routes to produce probes to pull out the appropriate genomic DNA sequences coding for the RNAs or proteins of interest. Knowledge of the amino acid sequence of only a few peptides in a protein chain is sufficient information for the synthesis of short oligonucleotide probes complementary to the coding mRNA or relevant genomic DNA coding for that protein. The synthesis of such oligonucleotide probes enables the isolation of the gene in question – and such an approach was successfully used for example to isolate the gene responsible for heamophilia B (factor IX deficiency). However, there are a large number of human diseases which family pedigree studies show to be inherited as single gene defects, but where the nature of the gene product, or even its function, or indeed whether it is present or absent, is completely unknown. The classical examples here include the autosomal dominant Huntington's chorea and recessive cystic fibrosis and X-linked recessives such as macro-

orchidism associated with mental retardation, and Duchenne muscular dystrophy. Here various approaches are possible to attempt to isolate the gene in question.

A direct comparison between genomic DNAs of individuals with and without a specific inherited disease is not possible because of the large size of the genome and because of the large numbers of random base changes in non-coding DNA. If the disease is a consequence of lack of expression of a given gene in a specific tissue, then tissue-specific cDNA libraries can be made from mRNAs from the tissues of normal and affected individuals and the libraries compared by cross hybridisation to identify a missing sequence. A similar kind of approach can be used to identify a missing protein by comparing 2-D gel electrophoresis patterns of proteins from diseased and normal tissue. Neither of these approaches have proved particularly rewarding to date and it should be emphasised that cross library hybridisation would in any case not be applicable if the mutation is in a control sequence and not the structural locus, or if an mRNA from the mutated gene is present in low abundance, or if the mutation affects protein function, but not the level of hybridisation of the relevant mRNA.

As referred to above, pinpointing a gene by linkage to RFLPs will certainly enable us to locate its position to within $10^6$ bases (1 cM) or so, but homing in on the gene of interest is not a simple matter. Single base polymorphisms are very frequent in the human genome, of the order of 1 in every 1–200 nucleotides, so that in a 100 Kb stretch of DNA associated with an abnormal allele there may be more than 500 or so possible differences in DNA sequence between a normal and a diseased individual, with perhaps just one (or none) of these differences being responsible for the disease. With current techniques, 'walking the genome' will be an almost impossible task if linked markers are more than 100 kilobases removed from the gene of interest. If this gene is mutated as a consequence of a chromosome rearrangement, as is the case of certain females with Duchenne muscular dystrophy, then the juxtaposition of pieces of two different chromosomes may offer an approach to isolate the breakage point in the rearranged chromosome and hence the gene at that point. In the absence of this kind of approach we will need improved methods of handling relatively large DNA fragments and of homing in on the much smaller DNA segments of interest. A number of recently developed techniques may help in this quest, including the electrophoretic separation of large DNA molecules of the order of $10^6$ base pairs in length and the mechanical isolation and subsequent cloning of very small DNA fragments from metaphase chromosomes. Moreover we can define reading frames, TATA boxes and sequences for poly A tails, but improved techniques will certainly be necessary to identify abnormal genes defined by their gross phenotypic consequences and located by linkage analyses.

### *Antenatal diagnosis*

Diagnosis, including that undertaken in early antenatal stages, is an important practical aspect of human genetics. In the early 1960s the only techniques available for antenatal diagnosis were palpation and radiology. In the 1970s ultrasound and second trimester amniocentesis arrived on the scene and enabled the application of the techniques of cytogenetics to detect constitutional chromosome anomalies in the fœtus and, using a variety of biochemical assays on amniotic fluid (as well as the use of maternal serum for AFP estimation), to detect inherited metabolic defects. In the 1980s we are moving into the use of chorionic biopsies taken at 8–10 weeks gestation as a source of diagnostic material (long before the neighbours and sometimes even the husband knows of the pregnancy!). Chorionic villus material is suitable for chromosome analysis, but is of course not informative for detecting inherited metabolic defects that are not expressed in chorionic tissue. However, small samples of villus are sufficient to provide enough material for the application of various powerful techniques of DNA analysis. Fœtal cells (lymphocytes) can also be obtained from the maternal circulation and although their presence may be detected by appropriate antibodies and the cells collected by flow sorters, their numbers are small and this approach is some way from offering a practical avenue for fetal diagnosis.

Chromosome-specific DNA probes exist for a number of chromosomes so that fœtal sex, sex chromosome anomalies, trisomies and other aneuploidies will all shortly be diagnosable using simple DNA blotting techniques on chorionic DNA. These methods, however, will not distinguish translocation carriers or fœtuses with smaller, but cytologically demonstrable, duplications or deletions. Diagnosis of haemoglobinopathies by fœtal blood sampling is already superceded by DNA analysis using RFLPs in informative families and synthesised oligonucleotide probes for mutated genes in others. Probes now exist for an increasing number of mutated genes responsible for human disease and recombinant DNA technology is obviously going to play a major role in antenatal diagnosis.

### *Genetic therapy*

Genetic therapy is to be clearly distinguished from, but nevertheless embraces, gene therapy and despite uninformed comment, is already successfully applied in a number of inherited diseases. The most notable here are of course insulin dependent diabetes, certain forms of growth retardation, and haemophilia A and B where the missing gene products, insulin, growth hormone and factors VIII and IX can be, and are, supplied from external sources. It is not surprising that each of these four genes have been cloned and recombinant DNA technology already provides synthetic human insulin and growth hormone and will shortly provide the blood clotting factors. Another important disease, phenylketonuria, can be successfully treated by adherence to a low phenylalanine diet in early life (and in pregnancy in PKU mothers) and the gene for phenylalanine hydroxylase has now been cloned and prenatal detection and the identification of carriers of the abnormal gene is now possible for a large proportion of the population.

For many inherited diseases therapy is limited or non-existent and in many cases the underlying biochemical cause of the abnormality is not known. In those instances where the presence of a defective gene has been demon-

strated, much interest has centered on the possibility of 'gene replacement therapy', or more correctly 'gene supplementation therapy'. A variety of methods exist for gene transfer into cells usually using DNAs parcelled into various efficient vectors. Successful gene transfers using engineered non-infective retroviruses are beginning to be reported for various genes introduced into mouse bone-marrow cells which have then been reintroduced into mice, but the problems associated with control of expression of the inserted gene, and its site of insertion, have yet to be overcome. The use of tissue specific enhancers would ensure gene expression in the appropriate cell type such as liver, bone-marrow etc., and the prospects here are exciting. However, although it is a relatively simple matter to cure in the laboratory a cell line from a patient with Lesch-Nyhan syndrome, and lacking a functional gene for the enzyme hypoxanthine-guanine-phosphoribosyl-transferase, curing the patient where the deficiency of HPRT in brain may be the important problem is rather a different matter. Nevertheless the prospects for gene supplementation in certain deficiency diseases are very real. Techniques for the introduction of DNAs into embryonal cells of the mouse are now becoming fairly well established, but are also associated with the problems of sites of insertion and expression in inappropriate tissues. Despite much loose talk, the use of gene therapy in relation to human embryos, as opposed to children, is surely a non-starter. Half of the embryos produced by a couple in which one parent carries a dominant disease gene and three-quarters of the embryos produced by a couple who are both carriers for a recessive mutant allele, are normal embryos. Any society which will allow of tinkering with the genetic make-up of a genetically abnormal human embryo will surely allow, and prefer to allow, selective abortions and let nature undertake the engineering of normal conceptuses.

*Genetics and cancer*

One of the very exciting recent developments in human genetics has been the convergence of three areas of research in human cytogenetics, virology and molecular biology, which has resulted in the identification of DNA sequences that appear to be specifically involved in the genesis of some human neoplasms.

It has been evident for some years following the original discovery of the $Ph^1$ chromosome associated with malignant cells in chronic myeloid leukemia, that a substantial proportion of the chromsome structural rearrangements seen in malignant cells were not randomly distributed throughout the complement. The increased resolution afforded by chromosome banding techniques have more recently led to the identification of a number of chromosomes and chromosome sites specifically involved in deletion or rearrangement in certain malignancies and high resolution prophase analysis will doubtlessly reveal further specific associations in the future. These associations are confined not only to acquired malignancies of the reticulo endothelial system, or to solid malignancies of other tissues, but also to a number of inherited specific cancer predispositions.

Although most human cancer is an acquired disease, all types of human cancer occur in heritable as well as in non-heritable forms and heritability may be associated with a dominant (e.g. medullary carcinoma of the thyroid – MEN II) or recessive (e.g. ataxia telangiectasia) expression at a single locus, or with a constitutional chromosome anomaly. The deletion of part of the long arm of chromosome 13 that is specifically associated with inherited retinoblastoma and that of the short arm of chromosome 11 that is associated with inherited Wilms' tumour with aniridia, are the two major examples of an association between an inherited constitutional chromosome anomaly and a specific cancer predisposition. Doubtless other such associations must occur in relation to other inherited cancer predispositions and this is an area which is actively being pursued.

The constitutional chromosomal changes associated with inherited predisposition to cancer must involve genetic alterations or mutational events at the sites of chromosome anomaly and there is now good evidence for this in the case of retinoblastoma. In the case of acquired malignancies there is also evidence for oncogene activation in association with chromosomal rearrangement, as for example exhibited by the *c-myc* gene on chromosome 8 in Burkitt's lymphoma. Some of these acquired chromosomal changes may be involved in the early stages of malignant transformation, whereas others may be generated during early tumour growth and appear as mutations which are selected as favouring the continued growth of transformed cells.

The discovery that transfection by DNAs from malignant rodent and human cells could effect a malignant transformation of NIH3T3 mouse cells in culture, and the later demonstration that the active DNA involved in this process was virtually identical with a DNA sequence present in a retrovirus responsible for producing sarcomas in rats, has opened up a whole new approach to our understanding of human cancer and the involvement of DNA changes in the process of malignant transformation. Studies on tumours induced in birds and rodents by the acutely transforming retroviruses had led to the identification of around 20 such viruses which, it transpires, have at one time or another (and as a consequence of a recombinational event) picked up a single mammalian DNA sequence – or proto-oncogene – which in man and other animals is almost certainly involved in the normal processes of growth and development. These virally acquired mammalian genes are responsible for the oncogenicity of the virus that contains them, and they are referred to as *v-onc* genes to distinguish them from their normal counterparts in man, or *p-onc* genes, and from the activated oncogenes, or *c-onc* genes, found in human tumours. At the time of writing (April 1984), some 18 different proto-oncogenes have been assigned to 18 different sites on 13 chromosomes in the human complement. Some of these, and in particular, *c-myc* on chromosome 8q24 and *c-abl* on chromosome 9q34, are located at chromosomal sites that undergo specific rearrangements in specific malignancies, whereas others do not, as yet, appear to show such an association. Some of these oncogenes are activated in malignant cells; some have their sequences amplified; and some have specific mutations; but the exact processes whereby these genes play a role in malignant transformation is by no means understood.

A number of the oncogenes encode proteins with ty-

rosine-specific kinase activity and are integral membrane proteins which are tightly bound to lipid, others have their product located in the cell nucleus or the golgi apparatus and cytoskeleton. The identification, isolation, and sequencing of some human oncogenes has already led to the finding that one, *c-sis*, is structurally related to human platelet derived growth factor and another, *c-erb B*, is closely related to epidermal growth factor receptor. Considerably less, but nevertheless some, homology exists also between sequences of other oncogenes and peptide sequences homologous to proteins in the transferrin family and to transferrin receptor. Studies on transcription of proto-oncogenes in the early development of the mouse has shown that some are actively transcribed in certain tissues at certain stages of development and a better understanding of the role played by these genes in normal cell proliferation is certainly required. In vitro studies indicate that in terms of neoplastic transformation one may distinguish between oncogenes that may confer in vitro immortality on cells and oncogenes associated with anchorage independent proliferation and tumorigenicity.

There is already evidence that the co-operation of two or more oncogenes acting in concert, or in sequence, may be necessary for transformation to a malignant state, but further studies in this area will be necessary to reveal a clearer picture of the events involved in this process.

Oncogenes as defined by their role in in vitro transformation, or sequence homology to acutely transforming C-type retroviruses, are most certainly not the only genes involved in oncogenesis in man. In the case of retinoblastoma for instance, it is now clear that tumorigenesis may result from the development of homozygosity for the presence of a mutant allele at the Rb locus on chromosome 13. In a number of cases this homozygosity follows from mitotic non-disjunction resulting in a loss of the homologous wild-type chromosome and in others from mitotic recombination events. It is not improbable that the expression of recessive alleles of this sort may also be important in other cases of inherited tumour predispositions, and further information on this score will shortly become available.

*Mutagenesis*

The induction of germ cell mutations in human populations and their contribution to inherited disease, and the induction of somatic cell mutations and their contribution to acquired ill health, and in particular to the genesis of cancer, have long been subjects of some interest and importance. The realisation that most agents that are mutagenic to mammalian cells are also carcinogens and the increasing use, and misuse, of physical and chemical mutagens in society are clearly of concern and have stimulated various lines of research into human mutagenesis. An appreciable proportion of inherited disease is a consequence of new mutation. Indeed, the bulk of constitutional chromosome anomalies in the new born (which have an incidence of around 1 in every 170 consecutive live births) are new mutations and spontaneous mutation rates for single genes range from $\sim 10^{-4}$/gamete/generation for a few very highly mutable loci to non-measurable rates for the most stable. Exposure to additional mutagens would be expected to increase these rates, but although human populations are being continually exposed to mutagens, and often at high levels, there are few, if any, instances where associated and clearly significant increases in inherited disease have been noted. This is almost certainly because of inadequacies of ascertainment of a diverse collection of individually rare conditions of rather variable frequency. Attempts to improve our current methods of detecting induced inherited mutations will necessitate much improvement of ascertainment of inherited disease in the new born. An additional approach, which is also being pursued, is to analyse, by automated methods, a large number of gene products present in serum so that a range of mutations at a large number of loci, many of which may not adversely affect the phenotype, may also be screened.

In contrast to germ cell mutations, cytogenetic studies on somatic cells of individuals exposed to mutagens clearly show increased frequencies of chromosome aberrations whose incidence is dose related. Lymphocyte chromsome analysis is indeed being used to monitor exposure of individuals to mutagens and the future trends here are to develop and implement automated methods for scoring large numbers of metaphase cells rapidly and accurately. In addition, preparations of chromosomes can now be obtained from human sperm and it is possible that spermatozoa could also be utilised to monitor for induced chromosomal damage. Point mutations may also be detected in blood lymphocytes of mutagen exposed individuals and somatic cell genetic techniques, and in some cases the use of specific antibodies, are being developed to detect such mutants (or in some cases to detect DNA adducts) again with the use of slide scanning or flow sorting machines.

The techniques of human cytogenetics and somatic cell genetics are being increasingly used to identify individuals, and families, who show a heritable hypersensitivity to mutation induction. Such hypersensitivity, and associated genomic instability, is linked with increased risk of early, and often specific, cancers and certain other phenotypic traits, and is an expression of underlying genetic defects involving one or more of a whole range of cellular processes. Studies on cells from these mutant individuals, in addition to identifying individuals at risk, are yielding basic information on the genes and mechanisms responsible for DNA replication and repair, radical inactivation and other cellular housekeeping systems, all of which are important in maintaining genome integrity.

*Moral and ethical issues*

The new genetics raises formidable moral and ethical problems ranging from the ability to determine, or predetermine, fœtal sex; in vitro genetic manipulation of the early embryo; the use of cloned genes in gene therapy; and the dilemmas which may be associated with genetic counselling and elective abortion. Consider for example, the problems, and consequences, associated with genetic counselling in a case of Huntington's chorea. Some members of a family in which the Huntington's gene is segregating may not wish to know whether they carry the mutated gene that is destined to confer upon them a virtually untreatable and dreadful illness leading event-

ually to an early death. Others in the family will certainly want to know; and those who are wishing to produce a family of their own may want to take advantage of the possibility of detecting the presence of the abnormal gene in the early fœtus and electing to abort fetuses carrying the gene. Selective abortion of conception products carrying an autosomal dominant disease gene with a low spontaneous mutation frequency, such as Huntington's chorea, will, of course, result in the virtual elimination of familial disease of this sort; but the problems associated with screening, detection and implementing selective abortion in the absence of any treatment are formidable ones.

Genetic counselling is now being undertaken on a wide spectrum of inherited disease, not only in the case of inherited familial or age-related chromosomal disorders and single gene disorders such as Tay-Sachs syndrome, but also for other conditions which have a complex genetic or which have a major environmental component, e.g. certain neural tube defects. What stance should individuals and society take towards progressive genetic counselling in these cases? At the present time it is evident that programmes which are aimed at detecting heterozygotes for the gene that causes beta thalassaemia are successful in that in many countries there is a significant decrease in the birth rate of homozygous children suffering from thalassaemia major. In most countries the screening that is undertaken is on a voluntary basis, but in Cyprus, for example, where 20–30% of the population are carriers and 1–2% of children are homozygous and die an early death from thalassaemia, there is an obligatory screening programme at marriage, with the results only being divulged to the couple and with no pressure being brought to bear on whether the couple should or should not marry. Should screening for severe inherited diseases which are an emotional and financial burden on parents, the family and the community, be obligatory and required by law? If so, where does society draw the line, which diseases? Where does the law stand if a couple produces a child with thalassaemia major? Is it entirely their problem, or does their doctor, the obstetrician and the hospital involved in prenatal care have some responsibility, and can they be sued? In the USA there have been a number of cases of litigation against obstetricians and other physicians for not having informed Jewish parents of their risk of producing children with Tay-Sachs disease, most of which were won by the parents. There are other successful actions against physicians for incorrectly informing, or for not informing, carrier parents of their risks for producing children with other inherited diseases and for not screening for chromosomal anomalies in women of high maternal age who eventually produced children with Down's syndrome. In these cases the parents claim for what is called a 'wrongful birth', but there are also reports of successful claims for 'wrongful life' where, in California, but not New York State or the UK, individuals with a genetic disease essentially and successfully undertook litigation against their doctors for allowing them to be born!

From the viewpoint of society, rather than the individual, it is clear that in the future we have to pay more attention to the cost-effectiveness of screening programmes to detect genetic disease. It is already evident that the financial cost of looking after patients with various inherited conditions, e.g. thalassaemia major, Down's syndrome, etc., are far more than the financial cost of the screening programmes that aim to prevent the birth of such individuals. In the financial sense these screening programmes more than pay for themselves, but how do we weigh financial cost against the value of a life, or a potential life, and how do we evaluate quality of life in this context? These are major societal problems which we have hardly yet come to grips with, but which are going to assume ever increasing importance, and will require some answers, during this present decade.

*Postscript*

This introductory overview was written in April, 1984. Much has occurred since that time as perhaps exemplified by the fact that the number of cloned human genes reported at the Human Gene Mapping meeting in Helsinki held in August 1985 (249) was more than double the number (104) reported at the previous meeting in 1983. At the present time well over 1500 genes and DNA markers have been mapped to the human genome: an increasing number of important disease genes have been mapped, e.g. cystic fibrosis, or cloned, e.g. chronic granulomatous disease, and probes of these and other similarly inherited conditions are now being used in antenatal diagnosis. Rapid advances are being made in genetic aspects of normal and abnormal growth and development, as for example with the isolation of homeobox genes involved in the laying down of patterns of development, and the further isolation and characterisation of genes involved in normal growth and in neoplasia. Research in the overall field of the genetics of man is probably at its most exciting phase in its history, and is unquestionably proceeding at an enormous pace.

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## Biochemical diagnosis of genetic disease

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*Key words.* Genetic disease; early diagnosis; abnormal metabolites; enzyme defects; gene mutation; prenatal prevention.