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Medical genetics

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Introduction

The 1980s have seen a number of exciting and important developments in our knowledge of human genetics. Instead of a rather empiric speciality based on clinical and pedigree information alone, the locations of many genes have now been discovered and the exact molecular configuration of the gene of some. Preclinical, even prenatal diagnosis becomes feasible for disorders where this was previously not possible. Hopefully genetics in the 90s will include the rolling back of the frontiers of gene therapy.

Chromosomes

Advances in chromosome analysis have come about with great improvements in techniques, first with prometaphase banding allowing for much longer chromosomes with very clear banding patterns. This has allowed a number of microdeletion disorders to be recognized including the interstitial deletion of chromosome 11 associated with the Wilm's tumour-aniridia syndrome, and of chromosome 15 with Prader-Willi and Angelman's syndromes.

The relationship of other chromosomal abnormalities with tumour predisposition, e.g. retinoblastoma and deletion of part of chromosome 13 (13q-), is better understood. Chromosomal rearrangement found in clones in specific tumours is slightly beyond the remit of this article and will not be discussed further.

An important chromosome disorder, the fragile X syndrome is now well recognized and requires special culture techniques for its demonstration.

Mosaic chromosome disorders, such as Killian-Pallister syndrome which associates mental handicap, hypotonia, a high anterior hairline and thin upper lip, reveal the abnormal chromosome only in

tissues with a slow turnover of cells, such as in cultured fibroblasts, while not being detectable in the blood.

A marriage of molecular genetic and cytogenetic techniques allows increasingly refined *in situ* hybridization. Unfortunately, this powerful diagnostic tool remains very much at a research level. Studies in Down syndrome, some of which have employed *in situ* hybridization have concentrated on the minimum amount of trisomic material necessary for the phenotype, with accurate mapping of the whole chromosome; localization of the gene for Alzheimer's disease to chromosome 21 and the occurrence of presenile fibrillary tangles in Down syndrome have helped to shed light on this.

There has been much interest lately in the parental origin of chromosome disorders, with imprinting recognized in man for the first time.

Single gene disorders

A great leap forward in our knowledge of single gene disorders has resulted from the somewhat unexpected success of reverse genetics – instead of starting off with a known protein or RNA, as was possible for disorders of haemoglobin, for instance, application of markers to families with more than one affected member has allowed accurate chromosomal localization of a number of single gene disorders, notably myotonic dystrophy, Huntington's disease, Duchenne and Becker muscular dystrophies, cystic fibrosis, Friedreich's ataxia, neurofibromatosis I, facio-scapulo-humeral muscular dystrophy and spinal muscular atrophy. More recently, Marfan syndrome has been provisionally located on chromosome 15q.

For some of these, notably Duchenne and Becker muscular dystrophies and cystic fibrosis, the actual responsible genes have been found, with the exact molecular abnormality identified in a high percentage. Duchenne and Becker's are allelic and the clinical picture can often be predicted from the type of mutation and whether it results in a

catastrophic effect on the dystrophin molecule.

For some disorders exact localization remains difficult or puzzling. Although there seems to be good evidence of a locus for adult type polycystic kidney disease linked to the alpha globin locus, on chromosome 16, in certain families an entirely different locus is suggested by linkage studies. Similarly, the gene for tuberous sclerosis is linked to a locus on chromosome 9 and British experience favours this localization of the gene. On the other hand a number of American families show linkage to a locus on chromosome 11 and the Americans cannot confirm the British linkage experience. Both groups have families which seem to show linkage to neither locus. One difficulty in such studies, on disorders very variable clinically, is assigning individuals to affected or not affected categories, within families.

New DNA analysis techniques

A major development in analysis is the polymerase chain reaction (PCR) which shortens the analysis time and dispenses with the need for radio-activity required for more formal Southern blot analysis.

PCR has extremely useful applications which have been quickly realized. These include analysis of tiny samples, theoretically possible on the DNA of a single cell. There are many applications of the technique, by no means confined to genetic disorders. There has been some success in amplifying DNA from single blastomeres, preimplantation, following *in vitro* fertilization as in ascertaining presence of the Y chromosome in sex-linked disorders. Similarly there are early attempts to study the paternal contribution to fetal cells in the mother's circulation. Such tests could avoid invasive prenatal diagnosis.

The discovery of great variability of the lengths of satellite DNA between functioning genes is a powerful tool in distinguishing between individuals by genetic 'fingerprinting'. This has already achieved widespread use in forensic and medico-legal work and by immigration authorities in checking parentage.

Diseases which involve abnormalities of DNA of the mitochondria have been discovered and the relationship of mitochondrial and nuclear DNA is better understood by studies in Kearns Sayers syndrome.

We still await clear elucidation of the genetic components of various multifactorial disorders, such as neural tube defect, coronary heart disease and hypertension.

Prenatal tests

Prenatal testing earlier than that feasible by amniocentesis is now well established, though still

not nearly as widely available. Chorionic biopsy performed at 9 weeks reflects the fetal status and allows chromosomal, DNA and enzyme analysis. Risks of miscarriage still exceed those of amniocentesis and new technicians have higher miscarriage rates, during their learning curve. Where the risk of the disorder is high, then chorionic villus sampling gives acceptably low miscarriage rates in experienced hands.

Screening for genetic disorders

Open neural tube defect screening by a combination of serum α fetoprotein (AFP) and ultrasound has been used successfully in a number of centres in many countries, notably in the United Kingdom and North America. Families need careful support when screening tests prove positive. There is growing enthusiasm for the introduction of pregnancy screening for Down syndrome, using combinations of tests including maternal age, serum AFP and serum human chorionic gonadotrophin (HCG). Such tests greatly increase the detection rates in young women to whom the majority of babies with Down syndrome are born. Currently about 60% of Down syndrome is detectable by such screening.

Cystic fibrosis (CF) carrier screening would allow a greater disease detection rate in pregnancy than screening for Down syndrome, but the condition is less well known by the public and lack of nursing and medical awareness of the disorder and of the medical advances makes it premature to introduce this on a wide scale now. Pilot cystic fibrosis carrier screening studies are in progress in a number of centres.

Tay-Sachs disease and thalassaemia carrier screening programmes have been very successful in some countries, whereas an early experience with sickle cell trait screening in New York is widely regarded as having been very unsatisfactory, with screened individuals feeling stigmatized and with results and the implications of the results being poorly understood by many.

The new genetics brings the emergence of a new set of ethical and moral problems and the enthusiasm to introduce new techniques must be made to keep time with medical, paramedical and lay education. New ways of treating genetic disorders are being developed. Organ and bone marrow transplantation have had some successes.

Specific gene therapy has the potential of being more effective with fewer side effects. Very early experiments in Duchenne muscular dystrophy and in cystic fibrosis bring the hope that we are on the threshold of a new era of treatment. Our capacity to recognize individuals at risk for certain disorders is likely to increase greatly at the same time. Pharmacogenetics will become more elegant and treatments more tailored to the individual's genetic

background.

Lastly, as described earlier, some of Mendel's laws of segregation and inheritance need further scrutiny. Experiments have shown undoubted examples of molecular imprinting in mice, and clinical instances in man are being recognized where inheritance of a maternal gene or portion of chromosome gives a different phenotype to inheritance from the father. A yet more dramatic occurrence is the existence of proven instances of uniparental disomy, with both chromosomes in the cell inherited from one parent and with no evidence of a contribution of that chromosome by the other parent. The phenomenon has been observed twice in cystic fibrosis with a phenotypic effect of short stature noted. Molecular analysis is certain to uncover more instances of this phenomenon.

This introduction has briefly touched on the majority of important growth areas in genetics of the past decade. The individual topics will now be expanded upon under the following headings: chromosomal advances – technical and clinical; advances in molecular biology: technical and clinical; screening for genetic disease; and ethical and moral issues.

Chromosomes

It was only with the introduction in the 1950s of colchicine into chromosome cultures that cells were 'frozen' in metaphase of mitosis and accurate counting became possible. Before this it was thought that there were 48 instead of 46 chromosomes per normal human cell. Numerical abnormalities of chromosome number were quickly discovered – Down syndrome¹ and later Klinefelter syndrome in 1959.¹ One rare disorder became disproportionately famous – the *cri du chat* syndrome associated with a significant deletion of the short arm of chromosome 5 (5p-),³ visible even in the rather short, dumpy chromosomes in karyotype spreads at that time. The incidence of major structural disorders of chromosomes in newborns was established in a large four centre study, with 56000 consecutive newborns analysed.^{4,5} The incidences of the various aneuploidies and of structural rearrangements, including balanced and unbalanced chromosomes, were provided by this valuable study. Parts of it have been repeated subsequently and other studies have defined the incidence of chromosome abnormality in spontaneous miscarriages and in stillbirths.⁶ A useful rough reminder of these incidences emerged, with each of the following being slight underestimates: chromosomal anomalies in spontaneous (recognized) miscarriage – 50%, in stillbirths – 5% and in live newborns – 0.5%. Phenotypes of specific chromosome abnormalities were described for the

first time – Edward's syndrome (Trisomy 18),⁷ Patau syndrome,⁸ to name but two. Klinefelter's⁹ and Turner's syndrome¹⁰ became better understood.

In the late 1960s Caspersson¹¹ introduced quinacrine banding which allowed differentiation of chromosomes of similar size from one another and Seabright's introduction of trypsin into cultures,¹² allowing partial digestion of the chromosomal skeleton, is still an integral part of most major banding techniques today. Quinacrine fluorescence which fades with time has been largely discarded in the standard chromosome laboratory. Caspersson was ahead of his time in the attempted computerization of chromosome analysis already in 1969. With the use of the fluorescent cell sorter, emissions from chromosome groups of various sizes do allow some subtle chromosomal abnormalities to be elucidated,¹³ though the technique does not form an important part of day-to-day practice. Younis added methotrexate to chromosome cultures,¹⁴ thus bringing cells into a uniform state of mitotic division and allowing for 'freezing' in prometaphase, giving much longer chromosomes, capable of more accurate analysis and with more subtle abnormalities noticeable, especially tiny deletions. Breakpoints in structural rearrangements can be defined more accurately and this has been of value in focusing DNA investigation when a gene site has been disrupted by a translocation, as occurred for instance in location of the gene for Duchenne muscular dystrophy.¹⁵ A certain amount of reinvestigation of people originally thought to be normal on chromosome analysis has proved necessary. This has applied especially to the fragile X syndrome, first noted by Lubs in 1969¹⁶ and then rediscovered by Gillian Turner in 1979.¹⁷ Cells need to be cultured in a folic acid-deficient medium to demonstrate the fragile site. Minor adjustments of the culture conditions¹⁸ allow for more regular detection.

Specific regions of the chromosome can be highlighted: for instance in C banding¹⁹ the centromeres show up while in telomeric staining²⁰ with silver stains, the ends of the chromosomes are highlighted. Beautiful photographs of chromosomes are possible on scanning electron microscopy and minute fragments can be accurately identified as chromosomal or otherwise.²¹

In situ hybridization

Combining the techniques of chromosome analysis and molecular genetics by *in situ* hybridization has played an important role in studies about the alpha and beta globin loci²² and was also helpful in identifying the site of male sex determinates on the X chromosome in XX males.²³ As techniques become more powerful and smaller and smaller

stretches of DNA are amplified to measurable proportions, these techniques will become increasingly important in the future and will help greatly in chromosome analysis.

Such techniques are useful in determining the chromosomal origin of additional material on a chromosome and carriers of small chromosome translocations can be detected with greater accuracy.

Chromosome nomenclature

An international agreement on chromosome nomenclature and description of defects exists.²⁴ The autosomes pairs are numbered according to size, from 1 to 22, with the sex chromosomes making up the normal complement of 46. The long arm, q, and the short arm, p, are divided into large mapping regions and two further subdivisions exist. Thus Xq27.3 denotes subregion 3 of subdivision 7 of major mapping region 2 on the long arm of the X chromosome, the site of the visible abnormality of the fragile X syndrome – in fact Fra Xq27.3 describes it completely.

Chromosome disorders are of number (aneuploidy), or of structure, with inversions, translocations both balanced and unbalanced, deletions, duplications and rings possible. They may arise by inheritance or mutation. The relationship of new mutations of apparently balanced translocations to phenotypic expression is currently under review. Theoretically, if there has been no gain or loss in the translocation event then there should be no phenotypic effect. However, empiric observation shows a finite increase in mental handicap.²⁵ Sampling bias is admitted²⁶ and a multicentre study by the Clinical Genetic Society is currently in progress and hopes to define the problem more accurately.

A valuable textbook on chromosome disorders is that by Schintzel,²⁷ and the interested reader is referred there for detailed clinical information on most described chromosome abnormalities. The whole of a recent supplement of the *American Journal of Medical Genetics* is devoted to Down syndrome.²⁹ An earlier monograph on Down's by Lubs is interesting.²⁸

Some specific recently described chromosomal syndromes

Fragile X, Killian Pallister syndrome, Prader-Willi and Angelman syndromes will be described in greater detail.

The fragile X syndrome (Fra Xq27.3) The name derives from a visible constriction in the body of the chromosome. 'Fragile' is a misnomer, for the chromosome very seldom breaks there. Though fragile sites are recognized on many

chromosomes,²⁹ only fragile X is known to be associated with clinical abnormality. The disorder is an important cause of mental handicap in males, affecting 1 in 1000.³⁰ About one third of carrier females are also mentally handicapped, though often to a lesser extent.³¹ The condition may arise by inheritance or new mutation – thought to be about 60%.³² Affected boys especially have increased head circumference, delay in the onset of speech, disruptive behaviour in childhood and become very shy as adults when they are usually gentle. True autism is not usually typical of fragile X. Macro-orchidism was an early described feature of the syndrome and is usually present in adulthood. On the other hand it proved a poor marker for the disorder in a study of males in mental institutions.³³ There is by now a very extensive literature on fragile X with whole issues of genetics journals devoted to it.^{34–36} Because of the special folic acid-poor culture conditions required to define the fragile site, it is important to *remember to request the investigation specifically* – it will usually be missed on standard culture. Folic acid therapy has been attempted but found to be of no benefit either to the degree of mental handicap or behaviour.^{37,38} The actual fragile site is very closely linked to the responsible gene in that families have been described with a number of affected males who show fragile sites, yet other males who are handicapped without abnormal chromosomes and males with the fragile site who are normal mentally. DNA analysis around the site has proved especially difficult because of the large amount of meiotic cross-over activity in that region of the X.³⁹ The very frequency of the disorder and high mutation rate are probably also reflections of the activity of the site. Definitive prenatal diagnosis of fragile X is possible but it needs cordocentesis with examination of fetal lymphocytes,⁴⁰ since chorionic villus examination seldom shows the site.

*Killian Pallister mosaic*⁴¹ This rare, though underdiagnosed, cause of severe mental handicap occurs on the basis of an isochromosome of the short arm of chromosome 12 occurring at an early stage of mitosis, giving rise to a mosaic situation, with the aneuploid cells containing, in effect, tetrasomy for 12p. The karyotype of an affected boy may be described as 46XY/47XYi12p. The tetrasomic cells in lymphocytes are selected against, so that even in the newborn the blood will be reported as simply 46XY. Skin biopsy and fibroblast culture are needed to demonstrate the mosaicism. The phenotype includes high birth weight, a high anterior hairline with thin hair anteriorly, a thin upper lip with long frenulum and thick lower lip (Figure 1). Features become coarse with age. The condition has been described in a 38 year old man.⁴²

Figure 1 Child with Killian-Pallister mosaic (Tetrasomy 12p in fibroblast culture). Note high hairline with fine temporal hair, thin upper lip, full lower lip. There is marked hypotonia and retardation. Permission has been received from the parents for this photograph to be published.

Other chromosome mosaics One relatively common somatic mosaic is trisomy 8 mosaic in which marfanoid body habitus may be associated with mental handicap and deep longitudinal creasing of the anterior sole.⁴³ One should also suspect mosaicism when mental handicap is associated with asymmetry or patchy hypopigmentation of the skin.⁴⁴ Sometimes the mosaic can be demonstrated in cultured lymphocytes but skin biopsy should be done where suspicion remains high following a normal karyotype report. Finding of somatic mosaicism allows for a definitive diagnosis and for very low recurrence risk figures to be given to parents, instead of an empiric intermediate or even high figure with no diagnosis and the suspicion of a genetic disorder.

Prader-Willi and Angelman's syndromes These are possibly examples of imprinting.⁴⁵ The same chromosome deletion, visible on good chromosome cultures, e.g. 46XX del 15q11, has been described in these two disorders with very little overlap in their clinical phenotype. In Prader-Willi syndrome the deletion is *de novo* and paternal in origin, or the paternal contribution is missing⁴⁶ and

thus there is maternal imprinting, while in Angelman's syndrome the opposite situation seems to apply.⁴⁷ Prader-Willi syndrome is a mentally handicapping disorder in which there is spectacular neonatal hypotonia with tube feeding needed at first too and later there is hypogenitalism and marked truncal obesity.⁴⁸

Angelman's syndrome⁴⁹ also shows hypotonia but the disorder is characterized by marked mental handicap, jerky movements, which earned it the unfortunate title of the 'Happy Puppet' syndrome, tongue thrusting and a characteristically abnormal sleep electroencephalogram. Early in childhood the condition may be difficult to tell from Rett syndrome,⁵⁰ a recently recognized condition of girls who become markedly autistic and develop hand-wringing after a seemingly normal start to life. The basis of Rett syndrome awaits elucidation.

Chromosome stress tests

Specific stress techniques may be employed in the study of certain disorders, particularly those of DNA repair. These include exposure to irradiation in ataxia telangiectasia⁵¹ and to U-V light in Cockayne syndrome,⁵² with an increase in breaks seen in both. Sister chromatid exchange shows an increase in spontaneous activity in Bloom syndrome⁵³ and the technique has been used as a monitor of subtle chromosome damage from environmental insult.⁵⁴

Down syndrome

Down syndrome has been the subject of intensive study which has become more and more sophisticated as new analytical techniques have been applied. The whole of a recent supplement of *American Journal of Medical Genetics* is devoted to the disorder.²⁸

Analysis of families in which partial translocation had occurred and careful molecular mapping has shown that the Down phenotype will occur when there is trisomy of band Xq22 and work is afoot to subdissect the bands still further.^{54,55} Similarly, the pathogenesis of the mental deficiency has been studied.⁵⁶ Prenatal and postnatal neurogenesis and synaptogenesis, with significant reduction in neurones and synapses and a quantitative deficiency of myelin, have been described.^{57,58} There are significant quantitative alterations of a number of neurotransmitters involving dopamine, serotonin and acetyl choline.⁵⁹ Alzheimer's disease changes occur very early in the brain in Down syndrome – 88.9% by the age 30 and 100% after 50⁶⁰ show the amyloid plaques and fibrillary tangles. The gene for Alzheimer's disease has been located to chromosome 21 as has the gene for the precursor amyloid protein.^{60,61}

Advances in molecular biology

Large tracts of DNA on the chromosomes consist of non-coding, often highly repetitive regions. Some of these may represent genes once active, now termed pseudogenes; others may act as spacers, perhaps playing a role in keeping the species apart. Active genes themselves consist of expressed regions or exons, and intervening sequences or introns which are not translated.

Abnormalities in the exons are far more likely to result in disease, than in the introns, though abnormalities at intron-exon boundaries may significantly alter characteristics of the RNA by increasing or decreasing the length of the exon. Alterations in the base pairs may alter the functional triplets or codons which code for the amino-acids of the final protein.

Thus a single base change or point mutation may have a profound effect if it affects a critical part of a functional molecule, as in sickle cell haemoglobin, where valine is substituted for glutamic acid because of a substitution of adenine for thymine, CAC for CTC in the relevant triplet from codon 6.⁶²

Deletions or duplications may or may not alter the normal triplet progression of the reading frame (depending simply on whether an exact multiple of three, counting from the reading end of the exon, is involved or not – when there is a frameshift, then there will generally be a more catastrophic effect on the final protein).

When a point mutation results in a prematurely occurring stop codon then a truncated protein results, whilst a mutation may alter a normally occurring stop sequence and result in a longer than normal protein, as in Haemoglobin Constance Spring.⁶³ The textbooks by Harry Harris *The Principles of Biochemical Genetics*⁶⁴ and by Sir David Weatherall,⁶⁵ *The New Genetics and Clinical Practice* give very useful introductions to the genetic code, its working and its relation to genes.

Although the genetic code was cracked in 1953,⁶⁶ it took a considerable length of time before the benefits to medical science became evident. Many laboratory technical advances combined to make this possible. Most of these make use of the complementary nature of matching DNA strands, where guanine is always matched with cytosine or vice versa and thymine always matched with adenine. Restriction enzymes, generally derived from micro-organisms, were discovered, capable of cutting DNA at particular sites according to the base pair composition into fragments of differing size and charge.^{67,68} Southern's blotting technique⁶⁹ was at first one of the few reliable methods of analysis, where DNA digested by restriction enzymes and separated by electrophoresis on agarose gel is blotted onto nitrocellulose paper or,

more recently, onto charged nylon membranes⁷⁰ and then incubated with radio-active probes comprising a stretch of (human) DNA grown up in a micro-organism, often phage or lambda. The DNA probe on incubation anneals to DNAs if present on the blot which contains the partnering complementary strand. This technique has many applications, including testing for the presence of a particular DNA fragment in somatic cell hybrids of mouse and human cells. Such hybrids shed human chromosomes during each passage and the technique has been very valuable in the mapping of genes to particular chromosomes.⁷¹ DNA digestion by restriction enzymes has allowed discovery of a great number of polymorphisms, i.e. normal differing forms which help to differentiate DNA from homologous chromosomes in regions of interest from one another – restriction fragment length polymorphisms (RFLP). Polymorphisms are necessary in mapping studies and in the tracking of disease genes within families.

Methods of obtaining very long fragments of DNA by a combination of the use of 'rare cutters', restriction enzymes for seldom encountered DNA sequences, combined with pulsed field gel electrophoresis (making DNA travel on a gel in which different current vectors are being applied),^{72,73} have proved of value in narrowing down searches by excluding or including large tracts of DNA at a time.

Reliable methods of DNA extraction from cellular material have been developed⁷⁴ with the product capable of long-term storage at – 70°C and with such DNA suitable for most experiments. Methods of RNA extraction require very fresh tissues before study by 'Northern' blotting⁷⁵ and certain laboratories have more technical success than others.

An important development in genetic analysis of DNA and RNA has been the introduction of the polymerase chain reaction (PCR),^{76,77} where primer sequences developed from complementary strands of DNA flanking a DNA region of interest are incubated with a sample for study. If the intervening sequences are present in the sample, then, under the influence of an enzyme capable of existing at very high temperatures – Taq polymerase (derived from the organism *Thermus aquaticus*), the intervening sequence is made good from bases added to the mixture and in a series of separation, polymerizing and annealing steps at differing temperatures, great amplification of the DNA takes place. The technique dispenses with the need for radio-activity because of the much greater quantities and specificity of the end product. A modification of PCR is artificial restriction mutation system (ARMS), where errors are built into one of the primers, allowing development of a positive-negative system of analysis. The technique

has been used successfully in studies of alpha-1-antitrypsin,⁷⁸ cystic fibrosis⁷⁹ and haemoglobinopathies.⁸⁰

Tiny samples are capable of analysis by PCR methods, including blood from Guthrie spots⁸¹ and single blastomeres following *in vitro* fertilization.⁸² There has been limited success in attempting to detect DNA from fetal cells in the maternal circulation, by searching for the paternal contribution.⁸³

Incorporation of human chromosomes into yeast, so called 'YAC' clones (yeast artificial chromosomes)⁸⁴ are likely to prove of great use in gene localizing experiments, being far less laborious than maintenance of somatic cell hybrids.

Tracts of DNA can be sequenced according to the order of occurrence of the bases adenosine, cytosine, guanine, thymine in vertical electrophoresis.⁸⁵ For some smaller genes with different abnormalities in different families being responsible for the disease state, direct sequencing may be the analytical method of choice – this applies for example to factor IX abnormalities.⁸⁶ Ways of incorporating normal or diseased genes into viral vectors and thence into human tissues, either in culture or *in vivo* will allow more detailed study of pathophysiology and will also presage gene treatment.⁸⁷

Mapping of the human genome is thought to be so worthwhile that there are now major North American and European initiatives. McKusick's invaluable catalogue of mendelian inheritance⁸⁸ is regularly updated and contains information on the known locations of human genes, either as C (confirmed), P (provisional) or L (limbo). An up-to-date human gene map is also regularly published in the *Proceedings of the National Academy of Sciences*.⁸⁹

Specific single gene disorders

The principles of DNA research and analysis by reverse genetics can best be understood by a detailed summary of the highlights of research in Duchenne and Becker's muscular dystrophies and in cystic fibrosis. In both of these the normal genes impaired in the disease states have now been defined. Readers are referred to Weatherall⁶⁵ for a description of classical genetic analysis applicable for example to the haemoglobinopathies and where the molecular configuration of the DNA was derived from a knowledge of the amino-acids comprising the globin chains.

Duchenne and Becker's muscular dystrophies (DMD and BMD)

Despite the 'head start' which the X-linked disorders enjoy over the autosomal disorders in

there being no doubt about the actual chromosomal localization, studies of those in which the gene product is not known have proved very laborious. In Duchenne and Becker's muscular dystrophies (also in fragile X syndrome) this has to do with the great deal of meiotic activity in the region of the gene and the high mutation rate.⁹⁰ Thus the search for the actual gene was bedevilled by high recombination rates of all the linked probes – one never seemed to be getting nearer to it in reverse genetic experiments and, without the gene product known, classical analysis was not possible.

The first clues as to the site were provided by the observation of DMD in a boy with an X-autosome translocation involving the site Xp21.¹⁵ The regions of DNA about this site were carefully mapped with the help of pulse-field gel experiments^{91,92} and eventually markers flanking the DMD-BMD site were discovered (DMD and BMD were once thought to be on different arms of the X, but analysis of family members with probes from the Xp21 site showed linkage of both disorders to this locus).⁹³ Detailed study of a boy with a combination of four diseases – DMD, chronic granulomatous disease, retinitis pigmentosa and MacLeod syndrome⁹⁴ – revealed a long deleted stretch of DNA, and Kunkel developed the so-called pERT probes.⁹⁵ About 10% of boys with DMD proved to have deletions with these probes and, at last a molecular way of making the diagnosis was available in some cases. Detailed study in the region of the deletion, searching for G-C (guanine-cytosine) rich regions, often present near functional genes, a search for open reading frames, i.e. stretches of non-repetitive DNA not containing stop codon sequences, and searches for stretches of DNA present in other species, allowed Kunkel and his colleagues to describe the gene for dystrophin,⁹⁶ the normal gene, disordered in DMD and BMD and named after the disease because, by their study, the discovery had been made possible.

The final confirmation of the accuracy of the finding was by demonstration of abnormalities of the gene product, dystrophin in muscle and some other tissues in diseased individuals. The gene proved to be extremely large with > 70 exons and > 70 introns. Deletions and duplications were discovered to be the cause of the majority of cases – in-frame deletions (i.e. which did not alter the normal triplet rhythm of the reading frame) were found to be associated with the milder Becker phenotype, by and large, while frame-shift mutations caused the more severe Duchenne MD.⁹⁷ Junctional mutations at exon-intron boundaries were discovered too. All in all a specific abnormality can now be detected in a male with DMD or BMD in about 70% of cases. However, because of the very high mutation rate at the site, individuals from different families seldom share exactly the

same mutation or deletion.

Analysis of some potential female carriers has proved difficult. It may be easy to classify a female at risk as being a carrier, on the basis of a family story with a significantly raised creatine kinase (with the tests done in a laboratory with its own bank of normal results for females). However, only 70% of obligatory carriers have a raised creatine kinase.⁹⁸ DNA based experiments which give a single band may indicate that the DNA from both X chromosomes is giving the same pattern (and the woman is not a carrier), or may in fact be giving the signal from one chromosome only, with a deletion for the probe on the other. Assessing one dose or two from the heaviness of the band is subject to experimental or reader error. Figure 2 provides a good example of the practical difficulties. It is difficult to know whether the mother has both X chromosomes giving the pattern or whether the signal is only coming from one. The apparent 'non-maternity' in her daughter provides the answer. This is because her mother is deleted within the dystrophin locus of one of her X chromosomes. The pattern which the daughter shows must be paternally derived, since her mother does not have this polymorphism at all. The daughter's male fetus was found to show the deletion like her brother and

she opted for termination. Creatine kinase in the same family some years earlier had been seriously misleading, suggesting a 5% carrier risk.

Very recently, by using a number of overlapping RNA probes for the dystrophin molecule, there has been success in telling female relatives of individuals with deletions apart from one another in terms of carrier status.⁹⁹

Staining of muscle biopsy tissue with antibodies to the dystrophin molecule has application in analysis of such tissues and may even be applicable to fetal muscle biopsy tissue in the 30% of families where no molecular abnormality has been discovered.¹⁰⁰

RNA for dystrophin has recently been successfully incorporated into the extensor digitorum brevis muscle of an individual with DMD.¹⁰¹ This was followed by an increase in the strength of the muscle and dystrophin could be demonstrated in the muscle after biopsy. Hopefully, this successful experiment will be followed by others on larger muscles, and a definitive treatment.

Cystic fibrosis

Siblings of people with CF, having been shown to be free of CF by standard sweat test, have a 2 in 3

Figure 2 The female II2 shows apparent 'non-maternity,' i.e. she has not inherited the 1 allele from her mother. Therefore her mother and she must be deleted for what she has inherited and passed on to her male fetus. Therefore the fetus is affected.

Results of Southern blot analysis with intragenic probe from dystrophin locus.

chance of being carriers, while siblings of the parents have a risk slightly greater than 1 in 2 (this takes account of the 50:50 chance of having inherited carrier status from the one grandparent of the CF individual and the 1 in 25 chance that the other grandparent is a carrier too.) Spouses of European origin, without a family history, have a 1 in 25 chance and thus odds for family members of their having affected offspring can be calculated (Table I).

For a long time these odds were all that one could offer in genetic counselling and there was no accurate prenatal test. An early marker for the CF gene, cystic fibrosis antigen,¹⁰² present in the serum of most people with CF and most of their obligate carrier parents, allowed a recalculation with more accurate carrier odds possible.¹⁰³ The technique of the test based on iso-electric focusing of serum proved technically difficult and subject to observer bias and thus was never introduced on a wide scale.

In 1982, Jalanko discovered reduced microvillar enzymes in the amniotic fluid in pregnancies in which the fetus was trisomic¹⁰⁴ and an extension of this work showed similar findings when the fetus had CF. Brock developed a microvillar enzyme test based on residual alkaline phosphatase (after inhibition by amino-acids), gammaglutamyl transpeptidase (GGT) and aminopeptidase M in amniotic fluid taken at 18 weeks.¹⁰⁵ This work did prove reproducible. However, error rates in both directions were found. Thus a diagnosis of CF in the fetus had a chance of being incorrect and reassurance of freedom from CF sometimes proved false. For a couple at a 1 in 4 risk of having offspring with CF, false positive error rates of 8% were quoted, with a 5% false negative rate.¹⁰⁶ For couples at a much lower prior risk of having CF offspring, e.g. siblings of CF subjects or of their parents, the prediction would be wrong more often than correct when an affected fetus was forecast. Negative tests, with the low prior carrier odds did allow further reassurance. The risk of becoming involved in a situation of wishing to ignore a positive test, because of its chances of being wrong, reduced the demand for tests in people at low prior risk. Even those at a high (1 in 4) risk were worried,

firstly, by the chances of a wrong prediction and, secondly, by the need for the amniocentesis to be performed well into the second trimester. Thus many such couples took decisions not to add to the family. Some of them felt it worthwhile to wait for a more accurate test which could be applied earlier in the pregnancy.

In 1985, Eiberg found linkage between a polymorphic enzyme, paraoxinase, and CF by studying nuclear families with more than one child with CF.¹⁰⁷ Whatever paraoxinase polymorphism was found in one child was very likely to be repeated in the other. Thus a lod score (logarithm of the odds) in excess of 4 of linkage between paraoxinase and CF became possible, i.e. odds of 10,000 to 1 that they were linked. From the number of affected sibling pairs showing differing polymorphisms, indicative of meiotic crossover between paraoxinase and CF in one or other parent, a recombination fraction, or theta, of 10% was calculated (i.e. the crossover 'distance' between the two markers is 10 centiMorgans). At this stage there was no idea of where the gene for paraoxinase was. A negative gene map for CF had been constructed from studies of families with more than one CF child with a host of polymorphic markers.¹⁰⁸ Lap Chee Tsui, using information from the negative gene map, found linkage between paraoxinase and an anonymous DNA marker, DOCR1-917,¹⁰⁹ which proved to be 5 centiMorgans from paraoxinase and 15 centiMorgans from CF. In a series of somatic cell mouse-human experiments his team rapidly showed that DOCR-917 was only present when human chromosome 7 was present in the culture.¹¹⁰ Research workers could then simply 'go to the fridge' to find other chromosome 7 markers. Two of these, discovered about 1 year earlier, proved to be extremely close to CF when tested in the families with more than 1 CF child. These were the Met oncogene¹¹¹ and an anonymous probe pJ3.11.¹¹² Distances of about 1.5 centiMorgans were quoted on the basis of 15 meiotic crossovers in 1000 chromosomes analysed.

In fact, when the study group was reinvestigated, the CF diagnosis proved wrong in a number and new distances of 0.3 cM between pJ3.11 and CF, and 0.4 cM between Met and CF were calculated. Virtually overnight a very accurate prenatal test had become available, with very low error rates, considered acceptable both by couples taking the tests up and by those offering them. The test was available on chorionic material and thus at 9 weeks. The first tests were described in early 1986.^{113,114} Between 1986 and 1990, a United Kingdom survey of prenatal diagnosis in CF showed 398 women who had undergone the test.¹¹⁵ Results of affecteds, carriers and those with no CF genes were found to obey the rules of mendelian recessive segregation. Accurate carrier detection tests became available

Table I Risk of having CF offspring where spouse has no family history of CF

	<i>Risk</i>
No family story in either parent	1 in 2500
Same but first cousin marriage	1 in 800
Sibling has child with CF	1 in 200
Sibling has CF	1 in 150
Divorced parent of CF child – remarried	1 in 100
Woman has CF	1 in 50

immediately for the healthy siblings. Interestingly, an analysis of findings in many laboratories worldwide showed that males found to be carriers were more often found to have inherited their carrier status from their fathers – thought to be an instance of meiotic drive and possibly linked to the heterozygote advantage thought to account for CF being so common.¹¹⁶

For prenatal tests to be applicable in a particular family one needed to be able to tell the polymorphisms of the linked probes apart in the parents and which each had passed on to the affected person. Since Met and pJ3.11 are normal polymorphic probes simply linked to CF, of course analysing them in a family in which CF had never occurred would not help in the slightest in predicting whether CF was likely to happen. The polymorphisms can *track* the CF gene in a family in which it is known to be occurring. One still had the problem in families then of not being able to tell whether the spouse marrying into a family with CF was a carrier.

Studies employing pulse-field gel electrophoresis suggested that Met and pJ3.11 were flanking the CF gene, with a probable distance of 1.5 Megabases between the two.¹¹⁷ The search for the gene between these two sites threw up a candidate gene CS7.¹¹⁸ Although the claim to have found the gene proved premature, strong linkage disequilibrium was found between CF and CS7, and between the disease locus and two nearby probes XV2c and Km19.¹¹⁹ Thus a *particular* polymorphism or restriction fragment size was found far more often with the CF bearing chromosome of the parent than with non-CF chromosome. Application of information using linkage disequilibrium proved of some value in increasing or decreasing the chances of there being a CF gene nearby in family tests where the child with CF had died, in the cases of spouses marrying into families with CF and in the testing of sperm donor panels where one could select a favourable haplotype in the face of a CF family history. Carrier odds of 1 in 4 could be given to a member of the general public in Britain with an unfavourable pattern on both chromosomes versus odds of 1 in 250 with favourable patterns on both.¹¹⁹

Interestingly, when eventually the CF gene was discovered, the majority of CF chromosomes showing the commonest mutation were found to show the unfavourable XV2c, Km19 haplotype, with the next commonest mutations also showing this haplotype.

Uniparental disomy in cystic fibrosis

Molecular analysis has allowed analysis of two children with cystic fibrosis and disproportionate

short stature.^{120–121} In both, analysis proved paternity, yet showed that there was a double representation of one of the maternal chromosome 7s, with no paternal contribution for this chromosome. This intriguing example of an imprinting effect must cause us to wonder how many unexplained unusual presentations of common diseases may be caused by the phenomenon. At last we have the tools to be able to realize the existence of so unexpected a finding and to search for it.

Discovery of the cystic fibrosis gene, CFTR

In September 1989 a series of three brilliant articles described the CF gene,^{123–125} termed cystic fibrosis transmembrane conductance regulator (CFTR). By a process of jumping over stretches of DNA situated between Met and pJ3.11 which gave technical difficulty in cloning experiments in the laboratory, a stretch was discovered rich in G-C fragments, with open reading frames and present in many different species. By the chemical composition of the protein which could be deduced from the DNA sequences of the exons, a protein with two nucleotide binding folds, thought capable of binding ATP and a highly charged R domain, could be predicted and hydropathy plots of the protein showed these regions to be hydrophilic (and intracytoplasmic) as opposed to the hydrophobic membrane portion of the protein. A deletion at position 508 of the 1480 amino-acid CFTR was discovered with an 'in-frame' three base-pairs AAG missing, resulting in a missing phenylalanine, thus Delta F508, in the first nucleotide binding fold and in exon 10 of the DNA in the gene. The majority of subjects showing this mutation also were found to be pancreatic insufficient (about 10% of patients with CF have normal pancreatic functions and are termed sufficient). In fact, while this finding described in the original articles remains substantially true, clinical variability and CF cannot be simply explained on DNA findings.¹²⁵

An international CF genetic analysis consortium has been set up and is a wonderful example of how rapid progress can be when there is free exchange. A map of the Delta F508 gene in Europe has been drawn,¹²⁶ showing an increase in incidence from south east to north west and with the highest proportion described in Denmark.

In the UK, a Delta F508 incidence of 80% in carriers has been described.¹²⁷ More than 60 further mutations have been found. One, G551D, accounts for about 5% of CF genes in people of Celtic origin and is common in the West of Britain and in Eire (glycine substituted for by aspartic acid at position 551 – a point mutation). Deletions, point mutations, 'nonsense' mutations, with a stop codon

occurring prematurely, frame-shift mutations and mutations at intron-exon boundaries have been described.¹²⁸⁻¹³³

Twelve separate mutations have been found in Britain and these account for more than 92% of CF chromosomes. Thus the carrier risk for someone without a family story of CF, negative for all 12 would be about 1 in 300 instead of 1 in 25. Odds like this make general carrier screening seem attractive, especially since those positive on testing are established as definite carriers. Two people from the general population, both negative on testing, would have a $1/300 \times 1/300 \times 1/4$ or a 1/360,000 chance of having offspring with CF and even an established carrier with someone negative for all 12 would only have a 1/1200 chance of affected offspring.¹³⁴ More importantly, a good carrier detection test exists now for people marrying a family where CF has occurred and for sperm or oocyte donor situations.

The CFTR has been shown to share characteristics with P-glycoproteins of multiple drug resistance and also to share similarities with a number of bacterial wall transport systems.¹²³ These have helped to derive hypotheses for the function of the protein. From its form it is likely to allow movement in only one direction, either into or out of the cell¹³⁵ and thus CFTR cannot itself be the chloride channel, since these channels allow bi-directional movement. Even if it is a secondary phenomenon, some blockage of chloride movement does occur in CF and manipulation of this, or of CFTR itself, could ameliorate conditions in the lung with clinical improvement. Amiloride has been used in this way¹³⁶ and is the subject now of a multicentre study in North America.

The final step in establishing that CFTR is the gene disordered in CF is contained in the experiments of Rich¹³⁹ and of Gregory¹⁴⁰ who have corrected the biochemical defect of CF tissues in culture by incorporating the normal gene. Experiments are afoot to incorporate the CF mRNA into modified adenovirus¹³⁹ or adeno-associated virus,¹⁴⁰ hopefully capable of safe administration by nebulizer on an intermittent basis, with a proportion of lung cells being returned to normal function. To make a significant (genetic) disorder something minor, as has been achieved in some bacterial infections, is an exciting but not impossible dream. Most people believe that genetic counselling, detection of those at risk, and termination of affected pregnancies, will continue to have a significant place in medical practice in CF for some time to come, to express a more sober view.

With more than 92% of CF genes now identifiable, testing can be very useful in confirming a CF diagnosis or in casting doubt where the subject proves negative on testing for all the discovered mutations.

Other genetic disorders

The genes for many of the known proteins or enzymes have been fully identified. The loci of many others have been derived from family studies. An ideogram gene map is shown in Figure 3 with the established gene placements of some of the better known genetic disorders. Applications of DNA technology to their study within families depend on the particular disorder and the accuracy of any predictive testing.

Screening and diagnostic 'screening' for genetic disease

A differentiation between screening and diagnostic tests is made, according to Wald and Cuckle's usage of the terms.¹⁴¹ They define screening as the identification in apparently healthy individuals of those who are sufficiently at risk of a specific disorder to justify a subsequent diagnostic test or procedure, or direct preventative action. They reserve the term *diagnostic* for those tests which would be followed by a therapeutic intervention. Wald and Cuckle outline a checklist of 29 questions applicable to *screening* and *diagnostic* tests. To these must be added an adequate general knowledge of the disorder tested for in the individual and in the medical and involved paramedical professions, i.e. there needs to be an adequate level of public awareness before any widespread tests are applied.

We can divide such tests into three categories, one diagnostic and two screening.

Diagnostic

Tests for phenylketonuria in the newborn have allowed countless thousands of people to achieve a normal range of intelligence on diets low in phenylalanine. In some centres other, rarer, inborn errors of metabolism are tested for automatically since they are detected by the paper chromatography test used. Nowadays, as part of 'Smack' tests, many adults have tests for hypercholesterolaemia. Diet and cholesterol lowering agents can delay the onset of coronary heart disease. One in 500 have the gene for Type 2 hypercholesterolaemia, an autosomal dominant. The responsible gene is on chromosome 19 and DNA markers can also be used in preclinical diagnosis.^{142,143}

There is a group of genetic disorders in which diagnostic tests in infancy would provide a pre-clinical diagnosis but where the case for improved management of the child, based on the diagnosis is not made. Included here is Duchenne muscular dystrophy – making the diagnosis would not improve management or prognosis and thus the only rationale for testing of all newborn males

Figure 3

copyright.

would be to allow accurate genetic counselling. In most parts of the United Kingdom it is thought that if direct treatment cannot be offered then tests should not be performed. A small study in Wales¹⁴⁴ showed that parents do not see things quite this way and certainly parents of affected children who have sometimes gone on to have a second and third affected child, before the diagnosis was made in the eldest, do not. Creatine kinase testing of newborn males would allow all DMD infants to be detected. It would also detect cases of Becker muscular dystrophy – this disorder may remain presymptomatic for up to 15 years and so one needs to weigh up the worry caused to parents and the boy soon after birth, with removal of the family from the 'normal' category.

Somewhat similar are feelings about newborn testing for cystic fibrosis using immune reactive trypsin.¹⁴⁵ It has proved extraordinarily difficult to prove that a child diagnosed after testing does better than one diagnosed after presenting ill, and therefore newborn testing has not become widespread. False positives are a great cause of anxiety to parents and paediatrician.¹⁴⁶ DNA tests done after a positive can clinch the diagnosis in up to 84% of cases in which a full molecular diagnosis will be made (see section on CF under single gene disorders). In a further 14.5% there will be some support for a possible CF diagnosis, with one CF gene identified. A problem in this group is that there appears to be an increase in benign hypertrypsinogenaemia (which would give a positive immunoreactive trypsin test) in CF carriers.¹⁴⁷

A drawback of newborn testing for CF with DNA probes would be the number of carriers detected. What would one do with this information? A similar ethical dilemma exists in the testing of newborns for sickle cell disease when carriers of the trait are detected. Here one can argue that anaesthetic or causes of potential hypoxia may pose a danger to carriers, thus testing is justified. Only recently has bronchorrhoea in adults been found more likely in CF carriers¹⁴⁸ – would this be a sufficient reason for widespread testing? Perhaps one of the strongest points in favour of routine widescale testing for these disorders is delay by the medical profession in making the diagnosis. The average age of diagnosis of Duchenne muscular dystrophy is in excess of 5 years, though the doctor had often been consulted from the age of 18 months with the story of delay in walking or generally delayed milestones. Creatine kinase should be measured whenever one has a story in a boy of delayed milestones. In CF there has been an average interval of about 13 months between onset of symptoms and making the diagnosis and the United Kingdom North-Western Region's CF register showed that 10% of diagnoses were made after the age of 5 years.¹⁴⁹

There is a category of tests which could be termed part-diagnostic since management may be affected by the results – an example would be the testing for sickle cell disease or trait prior to anaesthetic. Here we have a segment of a population being tested in particular circumstances. As our knowledge of genetics and pharmacogenetics increases there is likely to be a great and beneficial increase in this type of testing or screening.

Screening

(1) The detection of disorders in the fetus, generally with the option of termination of pregnancy being offered. (2) Screening for carriers of recessive disorders, to identify couples at high risk of having affected offspring.

In all these diagnostic and screening categories a positive family story may identify individuals at special risk and especially worthy of testing. When there is a disorder known of in an individual or family, then there is a medical responsibility to make individuals aware that they may have relatives at increased risk who should be offered the opportunity of further discussion or counselling and of being tested.

In general though we shall concentrate on screening in individuals or families with no family history.

(1) Tests in pregnancy

These include tests which indicate an increased risk of the disorder in the fetus, such as neural tube defect or Down syndrome. They also include general malformation screening by ultrasound, where the ultrasonographer examines the fetus in a systematic way searching for specific disorders or deviations from normal. In all of these the tests are directed at the fetus and obviously cannot be carried out in the absence of a pregnancy. A further category of testing in pregnancy includes tests on the parents for recessive disorder carrier status, which could have been performed before the pregnancy.

Neural tube defect screening Brock's findings of raised amniotic fluid alpha-feto-protein (AFP) in association with open neural tube defect¹⁵⁰ was followed by his finding of raised levels in maternal serum which provides a diagnostic window between 16 and 18 completed weeks of pregnancy. Before and after these dates there is no or insufficient separation between levels in women carrying unaffected versus affected fetuses. The test may be a general test of fetal well-being. Exomphalos, gastroschisis, congenital nephrosis (especially the Finnish type), and multiple pregnancy will give raised levels. Good ultrasound with

real time was introduced on a wide scale in the 80s and the test has proved a very valuable adjunct to serum AFP screening. False reassurance in instances where ultrasound alone has been used as the screening tool,¹⁵¹ lead most centres to wish to rely on both tests used together. The finding of a raised serum AFP between 16 and 18 weeks of pregnancy gives odds of about 1 in 8 that there will be an open neural tube defect. Amniocentesis and AFP measurement in the amniotic fluid will give odds of 20:1 in favour of the fetus having the defect. The finding of a band for acetylcholinesterase¹⁵² in amniotic fluid in someone in whom there was a raised serum AFP and raised amniotic AFP gives odds of 250:1 of an open neural tube defect in a region where there is a high background incidence of neural tube defect.¹⁵³

Where open neural tube defect has been detected, genetic counselling will need to include an explanation of the likely severity at birth. Many couples opt to end the pregnancy. The odds of a recurrence in a future pregnancy can be reduced by administration of periconceptual Pregnavite forte F, a multivitamin preparation,¹⁵⁴ though purists argue that the cause and beneficial effect is not proven.

Screening for specific disorders may have useful spin-off. Mentioned above is detection of gastro-schisis or infantile kidney disease following on AFP screening. Absence of the normal cholinesterase band in amniotic fluid may allow detection of atypical cholinesterase in the mother for whom succinyl choline, as given during anaesthetic, would pose dangers.¹⁵⁵ Cholinesterase in amniotic fluid is of maternal origin, while acetylcholinesterase is fetal in origin.

Screening for Down syndrome A further spin-off of AFP screening was the observation that most women carrying a fetus who turned out to have Down syndrome had had lower than normal serum AFP levels at 16–18 weeks.¹⁵⁶ It seems that the fetal liver, the origin of AFP, matures late in Down syndrome. Screening by using a combination of maternal age, serum AFP and serum HCG (human chorionic gonadotrophin, often raised in the presence of Down syndrome) allows recalculation of the odds of having an affected fetus – thus, if one allows for 5% of women to have amniocentesis then using specific cut-off points for serum AFP and HCG one can give odds greater or less than 1 in 250 of having an affected fetus, with amniocentesis,¹⁵⁷ the definitive test offered to those at higher risk. Sixty per cent of fetuses affected by Down syndrome could be detected in this way. Because the tests are independent of one another, risk figures derived for each of age, AFP and HCG are additive and more accurate than any of them alone.

The search continues for other tests which could result in an even higher pick-up rate and higher specificity, allowing fewer amniocenteses. Tests such as maternal serum oestriol (lowered in the presence of Down syndrome) and reduced fetal femur length could further refine the odds but add considerably to problems of organization and co-ordination.

All of these tests could be replaced if a reliable method of detecting trisomic fetal cells in maternal blood were to be developed by an advance in polymerase chain reaction, especially were reliable methods to measure gene dosage to be discovered. Down's screening is not generally available in the United Kingdom on the NHS but the tests are being offered increasingly on a payment basis.

Ultrasound screening Many ultrasound specialists now screen each fetus at least twice: once, as a measure of the length of gestation, at about 12 weeks, an adjunct to serum AFP screening, and a second time at about 18 weeks when specific anatomical details are studied carefully, especially the central nervous system, spine, bladder and kidneys, diaphragms and four-chamber view of the heart. Normal findings provide for reassurance and reduce greatly the chances of a significant malformation.¹⁵² However, false positive diagnosis of fetal abnormality is possible and so is false reassurance. The normal fetal cerebral ventricles and the fetal urinary collecting systems can contain generous amounts of fluid resulting in wrong diagnosis of hydrocephalus or of hydronephrosis. Sometimes pathological alteration may not yet be evident in the second trimester – an example is autosomal recessive microcephaly, when even specific searches in the face of a family story are unable to provide regular reassurance; in hypoplastic left heart syndrome, the hypoplastic process may start later than the scan date. With adequate counselling, the benefits of routine ultrasound scanning of every fetus should far outweigh the disadvantages of occasional wrong diagnosis. Spin-off includes early diagnosis of multiple pregnancy and accurate positioning of the placenta. New technology, including Doppler studies of placental blood flow, will bring new advantages. Those who talk against screening, stressing the mental harm which may result, should keep in mind the enormous increase in our knowledge which has followed from screening tests. 'If you don't look you can't find.'

(2) Carrier screening tests

Two carrier screening programmes, widely regarded as successful, involve Tay-Sachs disease in the United States and Canada¹⁵⁸ and thalassaemia in Sardinia and Cyprus.^{159,160} In each instance there

was an intensive education programme before screening started. Many couples and individuals were screened after publicity campaigns, attendant on the initial introduction of tests. Thereafter numbers tested fell off rather so that a recent publication on Tay-Sachs carrier screening in Toronto found that most people screened came from a population of pregnant Jewish women and their spouses.¹⁶¹ Thus, although pre-pregnancy, even pre-marriage screening is available, it is pregnancy which may concentrate the mind. A certain amount of premarital screening does occur at the request of orthodox Rabbis who see marriages arranged to avoid the risk of Tay-Sachs entirely as an infinitely better option than fetal testing and termination of pregnancy. Other religious groups where arranged marriage is common could see things in the same light.

Carrier testing is likely to become much more common and will include more than one disorder, thus helping to remove the stigma of being found to be a carrier. Studies demonstrating the great increase in autosomal recessive disorders, mental handicap and congenital malformations in the products of incest indicate that we all must carry four or five recessive genes capable of resulting in significant disease if our offspring inherits one of them from us and also from our partner who happens to have that one too. Children born as a result of incest are homozygous for at least one quarter of their genes; products of uncle-niece or double first cousin marriages are homozygous for 1 in 8 genes and first cousins for 1 in 16. This fraction is termed the coefficient of inbreeding.

Once an individual had taken a decision to wish to be screened, he or she would naturally be interested if carrier status for other potentially unpleasant disorders could be carried out on the same test specimen; diagnostic testing, for example, for hypercholesterolaemia or alpha 1 antitrypsin status could also be undertaken on the sample. Examples of disorders where there could be great interest in carrier detection screening programmes could be muscular dystrophy, cystic fibrosis, fragile X syndrome in females and severe forms of spinal muscular atrophy.

The debate on exactly when to introduce population screening for CF is well advanced. Ten Kate¹⁶² has argued that one needs to be able to leave a couple with as low a risk as when they opted to be screened, except for those in whom you find *both* partners carriers. Thus, he suggests that one wait until 96% of CF genes have been detected, when a proven carrier would only have a 1 in 2000 risk of having a child with CF if the partner is negative on all tests (this would imply testing for about 12 mutations). We have argued that where back-up facilities to educate and support families exist one need not wait quite that long¹⁶³ and feel that with

92% of mutations identified in our population we can get ready to introduce screening.

Ethics and genetics

Though there are deep personal differences about the acceptability of prenatal diagnosis leading to termination of pregnancy, such matters are ones of personal preference and opinion, rather than of ethics when one is dealing with termination following the diagnosis of a severe disabling disorder in the embryo or fetus.¹⁶⁴ The ethical issues surround the accuracy of advice given and the safety of untried prenatal diagnostic techniques for the mother and the unborn. We are dealing with a problem of degree in acceptability by couples, medical practitioners and society. It is the latter which makes the loudest disapproving noises, often wishing to impose a viewpoint on the individual. Allowing a child to die untreated may break the law. Under French law a termination of pregnancy is permitted right up until full-term when significant disability is certain; in Britain such termination is legal up to 24 weeks and some see a hypocrisy in this differentiation. Termination before 12 weeks is more acceptable to couple and practitioner and is safer for the mother than giving birth at full-term. Diagnosing a diseased zygote in the test tube with selective re-implantation is least unattractive but also the least available method of prenatal diagnosis.

How does one gauge whether a medical geneticist is doing his or her work well? Clarke has written warning those who would wish to see success measured in financial terms by the savings for society in terminations of pregnancies where disabling disability was forecast.¹⁶⁵

The recommendations of the Warnock enquiry in respect of experimentation which may be undertaken on the embryo up to 14 days after fertilization *in vitro* has become law. Experiments on genes and how they may work in this 14 day period may bring great benefit to mankind. The ethical problem is that the research does not benefit that particular early potential human being. It has been legislated that a genetic engineering experiment, such as the introduction of a normal gene into the early embryo before re-implantation, is not to be permitted. This would alter the basic genetic make-up of the individual and alter the genes passed on in procreation. Creation of a chimeric population of cells by introducing dystrophin into muscle or CFTR into lung cells is not seen as being unethical. Permission for the first genetic implantation experiments has been granted by the National Institutes of Health.

There is no obligation to reveal the results of preclinical diagnosis by means of predictive tests in

filling in an insurance form, though there would be an obligation to mention a positive family history of symptomatic individuals.

Though paternity tests are technically feasible, there could be some ethical issues involved in fathers wishing to check on their paternity of their children – one might need to consider the welfare of the child in offering such tests and marriage

guidance might be a better option. In any event there should be careful counselling before such tests are performed.

The 80s proved very exciting in terms of genetic discoveries and applications of the new-found knowledge. The 90s should prove even more exciting and the genetic text-book is likely to be rewritten by 2001.

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