Recent Advances

Medical genetics

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If reports in the media are anything to go by, genome research in 1995 was mostly about genes for criminality, obesity, or "wolfmen" (the term coined by one newspaper to describe the members of a unique Mexican family affected by X linked generalised hypertrichosis). This is a far cry from the clinic and families seeking advice about cystic fibrosis, myotonic dystrophy, fragile X mental retardation, or other serious inherited disorders. For these patients the availability of reliable molecular genetic testing is the tangible benefit of the human genome project.

The volume of data generated by genome research is hard to envisage. Short segments of DNA derived from genes (so called expressed sequence tags—ESTs) are currently being identified at the rate of several thousand a week. Most of these are fragments of novel genes. The remarkable progress that has been made in gene mapping and isolation has recently been reviewed by Collins.¹ The potential for the analysis of human disease is enormous and encompasses single gene disorders and multifactorial (polygenic) diseases including cancer.

Single gene disorders

The genes for most common single gene disorders have now been identified and characterised. The extent to which this has been exploited in clinical practice varies widely and depends on the spectrum of mutations and their ease of detection. For diseases due to a single consistent mutation, molecular genetic diagnosis is usually straightforward. However, many disease genes are large and complex, with a diverse spectrum of mutations. In such cases mutation detection as a routine service is not feasible with current technology. The situation is exacerbated if the disease can be caused by faults in more than one gene (locus heterogeneity). The current status of some of the commoner single gene disorders is summarised in box 1.

Over 60 disease genes were isolated during 1995, and the commoner of these are listed in box 2.²⁻³⁸ Of the two novel genes found to cause Alzheimer's disease,^{2,4} presenilin-1 (PS-1) is the more important, accounting for an estimated 70% of early onset familial Alzheimer's disease (about 10% of all cases of Alzheimer's disease). The closely related gene PS-2 and the previously identified β A4 amyloid protein precursor gene are responsible for most but not all the remaining early onset familial cases. It will be interesting to see whether the presenilins play a role in sporadic Alzheimer's disease. Retinitis pigmentosa provides a striking example of heterogeneity, with the two newly discovered genes (rod cGMP gated cation channel α subunit²⁸ and rod cGMP phosphodiesterase α

Summary points

Single gene disorders:

- Over 60 disease genes were isolated in 1995
- The genes for most common single gene disorders have been isolated and characterised

Multifactorial diseases:

- Work on insulin dependent diabetes shows the complexity of genetic susceptibility to multifactorial disease

Cancer genetics:

- Mutations in the familial breast-ovarian cancer gene, BRCA1, are an important cause of early onset cases, particularly in Ashkenazi Jews
- A second breast cancer susceptibility gene, BRCA2, has been isolated

Laboratory advances:

- Fluorescence in situ hybridisation (FISH) is being used increasingly in diagnostic cytogenetics
- Techniques for detecting gene mutations are improving, but better methods are needed

subunit²⁹) bringing the total isolated to six (excluding genes for syndromes with retinitis pigmentosa), but these still only account for 20-25% of all cases.³⁹ Six other autosomal and at least two X linked genes have been mapped but not yet isolated, and there are certainly more to find.³⁹ It is a sobering thought that in the fruit fly the number of genes known to cause photoreceptor degeneration or dysfunction is over 70.

Disease genes are increasingly being identified by the so-called positional candidate approach. This is well illustrated by the discovery of the gene for achondroplasia, the commonest cause of restricted growth and characterised by proximal limb shortening and a large skull. The gene was mapped to the tip of the short arm of chromosome 4 by family linkage studies. The fibroblast growth factor receptor 3 gene (FGFR3) had already been mapped to the same location, being one of a family of fibroblast growth factor receptors known to have important roles in signal transduction. It was a good candidate for achondroplasia because research on mouse embryos had shown that the gene was expressed in the developing skeleton, particularly in the long bones. DNA analysis in patients soon led to the identification of mutations in FGFR3 and showed that only two mutations occur, both of which result in the same single amino acid substitution in the trans-

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Box 1—Availability of molecular genetic testing for common single gene disorders

Testing is also available for many rarer single gene disorders. New or improved testing for some of the diseases listed is likely to become available during 1996-7.

Autosomal dominant

Adult polycystic kidney disease—Mutations in PKD1 gene account for approximately 85% of families. Mutation detection is difficult (research basis only). In suitable families linkage analysis can augment renal ultrasound for exclusion of the diagnosis in relatives. PKD2 gene is associated with milder disease and has not yet been isolated

Familial adenomatous polyposis—Approximately 50% of mutations can be readily detected. Presymptomatic diagnosis in relatives is usually possible by mutation analysis or linkage

Familial breast-ovarian cancer—Mutation detection in BRCA1 gene is currently on research basis but likely to become a service in due course

Familial hypertrophic cardiomyopathy—Four genes isolated, accounting for perhaps two thirds of families. Mutation detection on research basis only

Charcot-Marie-Tooth disease (hereditary motor and sensory neuropathy type 1)—There is genetic heterogeneity but most families have a duplication of the peripheral myelin protein 22 gene, allowing molecular genetic diagnosis

Hereditary non-polyposis colon cancer—80% of families have mutations in the DNA repair genes MSH2 or MLH1. Mutation detection can facilitate presymptomatic diagnosis in relatives but is not yet generally available as a service

Huntington's disease—Disease due to abnormally large number of copies of a triplet repeat sequence. Identification of triplet expansion permits symptomatic and presymptomatic diagnosis

Marfan syndrome—Mutation detection in fibrillin gene is difficult and currently available only on research basis. Prenatal diagnosis sometimes possible by linkage

Myotonic dystrophy—Disease is due to abnormally large number of copies of a triplet repeat sequence. Identification of triplet expansion permits symptomatic, presymptomatic, and prenatal diagnosis

Neurofibromatosis type 1—Mutation detection is difficult and currently only available on research basis

Neurofibromatosis type 2—Mutation detection or linkage can facilitate presymptomatic diagnosis in relatives but is not available as a service

Tuberous sclerosis—Mutations in the TSC2 gene account for half of families. Mutation detection is difficult (research basis only). The TSC1 gene has not yet been isolated

Von Hippel-Lindau disease—Mutation detection or linkage permits presymptomatic diagnosis in most families

Autosomal recessive

Cystic fibrosis—Carrier detection and prenatal diagnosis is possible in most families with cystic fibrosis by mutation detection or linkage. Testing for the common mutations permits DNA diagnosis in about 65% of patients with cystic fibrosis and identification of approximately 85% of carriers in the general population (the mutation spectrum in cystic fibrosis depends on ethnic background and varies within populations; these figures are representative of the British population)

Spinal muscular atrophy—Homozygous deletion in survival motor neurone gene in 98% of childhood onset disease provides confirmation of diagnosis and permits prenatal diagnosis. Carrier detection in relatives may be possible by linkage; carrier detection in general population is not possible

Sickle cell disease—Prenatal diagnosis by mutation detection

Thalassaemia (α and β)—Prenatal diagnosis by mutation detection or linkage, or both

X linked recessive

Duchenne muscular dystrophy—Dystrophin gene deletions are present in 60% of cases and permit DNA diagnosis. Carrier detection and prenatal diagnosis usually possible by mutation detection or linkage. Mutations in the same gene cause milder Becker muscular dystrophy

Fragile X mental retardation—Disease due to abnormally large number of copies of a triplet repeat sequence. Identification of triplet expansion permits diagnosis, carrier detection, and prenatal diagnosis

Haemophilia (A and B)—Carrier detection and prenatal diagnosis usually possible by mutation detection or linkage

membrane domain of the protein.⁴⁰⁻⁴² Subsequent research has shown that mutations elsewhere in the same gene cause a similar but milder skeletal dysplasia called hypochondroplasia¹¹ and also the congenitally lethal condition thanatophoric dysplasia,¹⁹ which has radiological similarities to achondroplasia.

The discovery that fibroblast growth factor receptor genes cause skeletal dysplasias has had a remarkable knock on effect. During the past year mutations in fibroblast growth factor receptors have been shown to be the cause of several syndromes characterised by deformities of the skull due to premature fusion of the cranial sutures (craniostenosis). These include Apert syndrome⁵ (mutations in FGFR2), Crouzon syndrome⁷ (FGFR2), and Pfeiffer syndrome¹⁵⁻¹⁸ (FGFR1 or FGFR2). The progress made in unravelling the molecular genetics of these rare conditions will have useful benefits in terms of DNA diagnosis, but has much wider significance. Identification and study of the genes causing these and other developmental disorders will provide valuable information about normal and abnormal human development.

Common multifactorial diseases

Many diseases result from interactions between genes and the environment. These include major

causes of morbidity such as diabetes, coronary artery disease, hypertension, venous thrombosis, arthritis, and some common cancers. In most cases it is not clear how many genes determine genetic susceptibility, but progress in genome analysis now makes it possible to address this issue.

Type I diabetes has become an important model for researchers attempting to understand multifactorial disease. The predominant role of genes within the major histocompatibility locus has long been known; some 95% of people with insulin dependent diabetes have either HLA DR3 or DR4, compared with 50% in the general population. Subsequent research has identified another five potential sites for susceptibility genes, the most important being in the vicinity of the insulin gene itself on chromosome 11.⁴³⁻⁴⁶ Recent work has shown that disease susceptibility is determined by variation in the copy number of a tandemly repeated DNA sequence upstream of the insulin gene. Furthermore, these sequences seem to influence the transcriptional activity of the gene, susceptible haplotypes being associated with higher levels of messenger RNA in pancreatic islets.^{47,48} How this predisposes to autoimmune destruction of the β cells has yet to be explained. If further work confirms that the VNTR (variable number of tandem repeats) at the insulin locus does indeed have a regulatory function, the same

could be true for other VNTRs, of which there are several thousand in the human genome. Work on type I diabetes shows the remarkable complexity of genetic susceptibility to multifactorial disease, but it also provides encouragement that useful progress is now possible.

Cancer genetics

Several cancer susceptibility genes have been identified from studies of rare families showing autosomal dominant inheritance of a predisposition to one or more types of cancer, such as breast, ovary, or colon. An important issue is the extent to which these genes contribute to cancer in the general population.

BREAST CANCER

For breast cancer, approximately 10% of patients have a family history of the disease in a close relative. Much rarer are families showing autosomal dominant inheritance of breast and ovarian cancer, due in most cases to mutations in the recently isolated BRCA1 gene⁴⁹; in these families women inheriting a BRCA1 mutation have a greater than 70% lifetime risk of breast cancer and 30% or higher lifetime risk of ovarian cancer.⁵⁰ Unfortunately, screening for mutations is not straightforward—more than 100 different mutations have already been identified, scattered throughout the gene.^{49 51-53} The end of 1995 brought the discovery of a second breast cancer susceptibility gene, BRCA2.⁶ Mutations in BRCA2 confer a risk of breast cancer which is similar to BRCA1, but the risk of ovarian cancer is lower. Together, these genes account for at least 80% of families showing autosomal dominant inheritance of breast cancer, with BRCA1 being the more common.⁵⁴ What is the contribution of these genes to the overall incidence of breast and ovarian cancer? By combining penetrance data with the results of genetic epidemiological studies, Ford *et al* esti-

mated the carrier frequency of BRCA1 mutations in the general population at approximately 1 in 800.⁵⁵ This implies that BRCA1 accounts for about 2% of breast cancer and 3% of ovarian cancer diagnosed below 70 years of age; it is more significant in early onset cases, accounting for 7.5% of breast cancer and 6% of ovarian cancer diagnosed below 30 years of age. These indirect estimates agree closely with two recent studies of mutation screening in women with early onset breast cancer (under age 30 and age 35), in whom the frequency of BRCA1 mutations was 7.5% (combined data for non-Jewish subjects).^{56 57}

The analysis by Ford *et al* suggests that a substantial proportion of small familial clusters (less than four cases) of breast cancer are attributable to commonly occurring susceptibility genes of low penetrance rather than rare highly penetrant genes such as BRCA1 or BRCA2.⁵⁵ There is the intriguing possibility that carriers for ataxia telangiectasia may be at increased risk of breast cancer, perhaps accounting for up to 4% of all cases.³⁸ This hypothesis can be tested now that the gene for ataxia telangiectasia has been isolated.²⁰

It is becoming clear that BRCA1 mutation screening could have a useful role in familial and early onset cases, but it is unlikely to have a major impact on breast and ovarian cancer in the general population, with one possible exception. A recent study in Ashkenazi Jews has shown that approximately 1% of the population carries a specific mutation in BRCA1 which could account for 16% of breast cancer and 39% of ovarian cancer in Jewish women diagnosed before 50 years of age.^{57 59} But what can be offered to women shown to be at high risk apart from prophylactic surgery? Screening for breast cancer is of uncertain value in young women, and there is no proved method for the early detection of ovarian cancer. Extensive clinical trials will be needed to address these issues before the benefits of BRCA1 mutation testing can be determined.

Box 2—Some disease genes isolated during 1995

Autosomal dominant

Alzheimer's disease—Two more genes for early onset familial disease isolated (presenilin-1 and presenilin-2), bringing the total to three²⁴

Apert syndrome—Fibroblast growth factor receptor 2⁵

Breast cancer susceptibility (BRCA2)—Second breast cancer susceptibility gene to be isolated⁶

Crouzon syndrome—Fibroblast growth factor receptor 2⁷

Familial hypertrophic cardiomyopathy—Cardiac myosin binding protein C is the fourth gene to be isolated⁹

Hereditary multiple exostoses—Putative tumour suppressor gene which accounts for most families.¹⁰ At least two other genes mapped but not yet isolated

Hypochondroplasia—Fibroblast growth factor receptor 3¹¹

Long QT syndrome (Romano-Ward syndrome)—Genetic heterogeneity with mutations identified in the cardiac sodium channel gene SCN5A and also in a putative potassium channel gene HERG.^{12 13} A third gene has been mapped but not yet isolated

Multiple epiphyseal dysplasia (MED)—Mutations identified in cartilage oligomeric matrix protein gene.¹⁴ Other genes not yet isolated can cause the same condition

Pfeiffer syndrome—Fibroblast growth factor receptor 1 and 2¹⁵⁻¹⁸

Thanatophoric dysplasia—Fibroblast growth factor receptor 3¹⁹

Autosomal recessive

*Ataxia telangiectasia*²⁰

*Batten disease*²¹

*Bloom syndrome*²²

Congenital muscular dystrophy—Mutations in the laminin $\alpha 2$ chain gene confirmed in merosin deficient congenital muscular dystrophy²³

Limb-girdle muscular dystrophy—Mutations in β and γ sarcoglycan (SG) shown to cause severe childhood disease²⁴⁻²⁶ (as does α sarcoglycan). Muscle specific calpain (CANP3) shown to cause less severe disease²⁷ (as can α and β sarcoglycan). At least one other gene is still to be isolated

Retinitis pigmentosa—Two genes isolated, bringing the total for autosomal retinitis pigmentosa to six.^{28 29} Many more to find

Sanfillipo A syndrome (mucopolysaccharidosis type IIIA)—Sulphamidase gene cloned and mutations identified³⁰

Spinal muscular atrophy—Homozygous deletions of the survival motor neurone gene are found in almost all cases of childhood onset and adult onset disease.³¹⁻³³ Some patients also have deletions of the adjacent neuronal apoptosis inhibitor protein gene. The exact role of these genes remains to be clarified

Usher syndrome, type I—Mutations identified in myosin VIIA gene.³⁴ At least two other genes for Usher syndrome type I have been mapped but not yet isolated

X linked recessive

*Hypophosphataemic rickets*³⁵

*Mental retardation with α thalassaemia (ATR-X syndrome)*³⁶—One of many rare X linked mental retardation syndromes, most of which have been approximately mapped but the genes not yet isolated

Ocular albinism—Only a third of families have mutations in the coding sequence of the newly isolated gene^{37 38}

Colon cancer also has a significant genetic component; mutations in DNA mismatch repair genes, particularly MSH2 and MLH1, are responsible for most families affected by hereditary nonpolyposis colon cancer. From recent data it appears that patients with early onset colorectal cancer but no family history of the condition may also have mutations in these genes.⁶⁰

Laboratory advances

Fluorescence in situ hybridisation (FISH) is a new technology that is becoming part of the routine service in most cytogenetics laboratories.⁶¹ Fluorescently labelled pieces of DNA permit the identification of tiny deletions not visible on Giemsa banded metaphase chromosome spreads. This facilitates the diagnosis of several microdeletion syndromes, such as the deletion of the elastin gene at 7q11.23 found in most cases of Williams syndrome (infantile hypercalcaemia, supra-valvular aortic stenosis, characteristic facies, learning disabilities) and the 22q11.2 deletion associated with the Di George syndrome (conotruncal cardiac defects, thymic and parathyroid gland hypoplasia or aplasia, dysmorphic facies) and the velocardiofacial syndrome (cleft palate, congenital heart defect, characteristic facies, learning disabilities). Similar microdeletions of

Fig 1—Fluorescence in situ hybridisation (FISH) used to show a microdeletion of 22q11.2 in a child with tetralogy of Fallot. In a normal chromosome 22 (left) the fluorescently labelled probe hybridises to sequences within the critical region to give a signal midway down the chromosome; it also incorporates sequences that hybridise lower down the chromosome as a control. In the deleted chromosome (right) the upper signal is missing. The microdeletion is not visible in the routine Giemsa banded preparation.

Fig 2—Fluorescently labelled chromosome 5 specific DNA (chromosome paint) used to show a subtle t(5;14) reciprocal translocation in a patient investigated for recurrent miscarriages. In routine Giemsa banded preparations a possible abnormality was noted at the tip of the short arm of chromosome 5 (larger chromosome, centre pair) when compared to normal chromosomes (left hand pair). Chromosome 5 paint (right) identifies the translocated portion of chromosome 5 attached to the long arm of chromosome 14. The portion of chromosome 14 attached to the short arm of chromosome 5 is unpainted.

22q are present in about 30% of children born with isolated conotruncal cardiac defects such as interrupted aortic arch, truncus arteriosus, and tetralogy of Fallot.⁶¹ Figure 1 shows the use of fluorescence in situ hybridisation.

Another powerful molecular cytogenetic technique uses fluorescently labelled chromosome specific DNA as "chromosome paint."⁶¹ This can be invaluable in determining the composition of familial or de novo chromosomal rearrangement when this is not apparent from routine Giemsa banded preparations. The technique is illustrated in figure 2.

The detection of mutations is the key step in the identification of new disease genes. It is also central to the application of molecular genetics to clinical practice. Ten years after the discovery that mutations in the dystrophin gene are the cause of Duchenne muscular dystrophy there is still no simple means of pinpointing mutations in the third of cases that do not have a sizeable deletion. Mutation detection is equally difficult in several other diseases, such as Marfan syndrome and neurofibromatosis type 1 (von Recklinghausen disease). Not surprisingly, better technology for mutation detection continues to be the focus of much research and was the subject of a recent conference reported by Forrest *et al.*⁶³ Several improvements to existing techniques are being developed, notably to the "chemical mismatch cleavage method" by the use of bacteriophage resolvase enzymes to recognise and cut DNA at the site of a single base mismatch. Another promising technique currently being evaluated by service laboratories is protein truncation testing (PTT), a functional assay that identifies mutations causing premature termination of protein synthesis.⁶⁴ Promising results using this technique have already been reported for Duchenne muscular dystrophy,^{64,65} familial adenomatous polyposis,⁶⁶ and the breast-ovarian cancer susceptibility gene BRCA1.⁵² More speculative research on mutation detection is aimed at developing technologies for high volume automated analysis, which would be essential for any form of large scale population screening programme based on DNA testing.

Conclusion

For the most part, the emphasis in this review has been on recent advances which have relevance to clinical practice. Equally exciting is the progress being made in understanding some of the basic mechanisms in molecular genetics. This is having an impact on almost every area of research in biological science.

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