Molecular techniques: divide or share

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The second half of the 20th century was the era in which fundamental questions regarding the genetic basis of biological function were addressed. The emerging discipline of molecular genetics harnessed the newly evolving technologies and the result has been the dawning of a genetic revolution which will lead to an understanding of how genes direct biology. The application of this science to the study of human genetics has resulted in an unprecedented growth in our understanding of the basic mechanism of disease and the basis of the new clinical field of molecular medicine.

The genetic basis of an exponentially increasing number of diseases is being identified and this will have a major impact for diagnosis, prognosis, and informed management of patients. Virtually all the major clinical disciplines will find molecular diagnosis to be increasingly necessary in modern clinical management. A challenge for the pathology services in this new era is to support molecular medicine to ensure that technical innovations and novel developments can be delivered as routine service in a timely, efficient, cost–effective manner, for the benefit of the maximum number of patients.

Molecular genetic milestones

Current molecular genetic techniques have been developed, in only a few years, from basic academic research in many scientific fields. Some scientific milestones have been seminal.[1 2] The 1953 paper in *Nature* by Watson and Crick, which correctly proposed the double stranded structure of DNA, led to an understanding of gene function in molecular terms and marks the beginning of the molecular revolution. In 1975, Nathans and Smith[3] purified restriction enzymes, the ubiquitous tools of in vitro DNA manipulation. These were central to the isolation and amplification of specific DNA fragments by in vivo cloning, a technology developed from the recombinant DNA techniques of Berg in 1972.[4] In 1975, the novel method of Southern blotting could be used to investigate the location of genes, and this marked the beginning of the application of the technology to genetic disease. By 1977,[5] DNA sequencing techniques could identify individual, disease specific changes. The next major step forward was the polymerase chain reaction (PCR), an in vitro method of cloning developed by Kary Mullis in 1987.[6] This now ubiquitously used method of DNA isolation and amplification transformed the way biological problems were approached, in much the same way as Southern blotting had done 10 years earlier. The rapid advances in the field are shown by the fact that the codiscoverer of the structure of DNA, James Watson, was appointed the coordinator of the Human Genome Project in 1988. This project, which will be completed in the next few years, is the platform upon which all human genes will be identified. Southern, who developed the revolutionary blotting methods for the location of genes, is now developing chip based methods of DNA analysis with the potential to identify the complete polymorphic genotype of individuals.

Diagnostic service

The ground breaking research findings have subsequently been developed and refined, such that the analysis of DNA or of the chemical message RNA is now seminal to the work of a wide range of scientific disciplines. Within clinical care, the scope of DNA based diagnostics has led to a spiralling demand. Availability of rapid, automated means of DNA analysis such as that offered by chip based technology will lead to our ability not only to look for rare genetic events but also to produce genetic profiles of individuals. Genetic profiles will identify rare DNA changes or predisposition polymorphisms for common multifactorial diseases such as asthma or hypertension. Identification of acquired somatic mutations will be used in management of cancer patients. The inevitable result of the growing recognition that an individual's genetic variability has a profound affect on the efficacy, safety, and side effects of drugs will be genetic profile analysis before pharmacological intervention.

The polymerase chain reaction

The technique that has had the most profound effect on molecular analysis has been PCR. This comparatively simple technique has incredible sensitivity. Amplification of a single target molecule in a complex template can produce large amounts of specific DNA of sufficient abundance that the target can be analysed without interference from non-amplified background. PCR is the enzymatic catalysed extension of primers, where the primers are designed to specifically hybridise to a target DNA molecule. Twenty cycles of PCR yield about a million-fold copies; therefore target molecules can be a few human cells—for example, from cheek cell scrapes, a hair root, tissue containing a few bacterial or viral cells, archival paraffin embedded tissues, or a single cell removed from an in vitro developing embryo.

As PCR is apparently simple both in use and in the equipment required, it has been adopted by many clinical specialties; commercial kits make it even more attractive to run tests on a virtually ad hoc basis. This approach may succeed while demand is low and requests simple, but it does not lend itself to the requirements of a professional service.

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The extreme sensitivity of PCR is both its attractiveness and its difficulty. Procedures must be carried out carefully to avoid mistakes, but as PCR requires repetitive pipetting and tube transfers, human error can pose problems, particularly when large numbers of samples are processed. The major hazard is contamination of the sample, which can produce incorrect results. As one or only a few molecules can act as a template, any external material can be amplified, such as products from previous reactions or exogenous material from human, bacterial, or viral sources. The rigorous measures needed to combat this problem include the use of appropriate controls, careful laboratory procedures, and spatial separation of PCR and product analysis. Such procedures can be difficult to maintain and monitor, particularly in limited laboratory space and with inappropriately trained staff. Nonetheless, access to this basic technology should not be limited to those who have the time, funds, and expertise to establish their own specialised facilities.

Clinical molecular genetics services

The need for an organised molecular genetic diagnostic service has been recognised since the 1980s. Based on the rapid expansion of genetic information on human disease, clinical molecular genetics laboratories were established in the United Kingdom in 1985. Initially the techniques used were crude and mainly applicable to inherited disorders. The DNA sequence variation at restriction sites (RFLPs), demonstrated by Kan and Dozy in 1978,[7] was used to track mutant genes in affected families. Clinical geneticists, to support predictive diagnosis, used the results generated by this powerful tool. As new technologies developed, they were adopted by the laboratories, thus enabling increasingly sensitive testing to be carried out.

The visionaries who recognised the need for these laboratories could hardly have envisaged that within less than 15 years the demands on the service would have changed so dramatically. There is an exponential growth in the type and number of diseases that can be defined in precise molecular genetic terms, which means that the scope of DNA or RNA based diagnosis has expanded to encompass all the clinical disciplines, not only that of clinical genetics.

The inherited genetic disorders, although many in number, tend to be individually quite rare, and the present service is organised to deal with small numbers of specialised requests and not the handling of large numbers of samples. New developments are needed which recognise that the delivery of a cost-effective service requires innovative organisation. It needs to be recognised that manual methods of analysis will soon be obsolete and that investment must be made to make available the most appropriate technological advances for all of the clinical disciplines that require it.

In the United Kingdom, clinical molecular genetics is recognised as a specialty by the Royal College of Pathologists. Senior staff have the MRCPath and there are nationally accredited schemes for training to ensure a basic level of expertise. Quality assurance is maintained by national schemes, and laboratories are undergoing accreditation similar to schemes carried out in other countries. These laboratories and staff are a source of molecular genetic expertise and organisational ability, which could be used to seed the new developments. There are only 49 clinical genetic service laboratories in the United Kingdom, staffed by around 200 clinical scientists; therefore if the expertise of these laboratories is to be channelled to support new developments, innovative decisions regarding responsibility and management must be devised.

Pathology services

The aims of a pathology service are to support clinical care with respect to specific diagnosis and prognosis and to provide information for the informed clinical management of patients. To this end, various laboratory based disciplines have developed to both exploit available technology and specialise in specific forms of disease. When the technique has a universal use in clinical care it can be recognised as a discipline in its own right and developed as a central service. Clinical biochemistry laboratories are a model for this, but molecular analysis of germ line mutations is unique in that it is a lifetime diagnosis; therefore the delivery of the service need not always be within the hospital setting.

CORE FACILITIES

Core facilities are a common way of giving access to specialised technology. Local DNA sequencing facilities are common in research institutes and university departments, and national core facilities for highly specialised analysis have been established, such as the Centralised Synchroton Radiation Facility at Daresbury. Facilities such as these ensure the cost-effective availability of specialised analysis. Molecular analysis to support clinical care could also be best served by the establishment of core facilities.

If clinical molecular genetic core facilities are the way forward they need to be flexible, in order to provide a central service without inhibiting innovation. The models used should meet the needs of the local environment and it would be foolish to be too prescriptive in a field which is rapidly developing and where clinical expertise may be centred in specialist institutes.

LOCAL CORE FACILITIES

Local core facilities are already needed for PCR and DNA sequencing. Such local services may have to provide results rapidly—for example, when a definitive diagnosis is needed that will affect the immediate clinical care of the patient, such as analysis of viral and bacterial pathogens, diagnosis of cystic fibrosis, and detection of metabolic disorders in at-risk neonates. Specialist testing is also needed locally, as discussions between laboratory and clinician can lead to better care; examples include prenatal testing, analysis of rare disorders, family studies, and the confirmation of mutations identified by screening programmes.

A core facility would allow access to molecular techniques for specialties that do not wish to equip and train personnel when it is not an intrinsic part of their expertise. It could also allow those specialties that carry out analyses by labour intensive methods to have access to equipment which they could not justify purchasing for the number and range of tests they perform. The facility could provide specialised analysis, expert advice, and training and support for the needs of both clinical service and research. It could act to facilitate the central organisation, coordination, and planning of the service development of molecular testing within the hospital system. The advantages are of cost-effectiveness, saving of space, and a mechanism for transferring clinically relevant research findings into service in a planned rather than an ad hoc manner.

REGIONAL OR NATIONAL FACILITIES

The space available in hospitals is limited. Their main function is acute care; therefore they are not ideal sites for the development of large scale screening facilities. Testing for conditions where there is not a need for an immediate result but rather for assessment of future clinical management would most effectively be carried out in large regional or national facilities. This model could be appropriately managed to address the problems of quality assurance and accreditation, and it could purchase the most appropriate equipment for handling large numbers of samples—for example, robotics for the extraction and preparation of DNA. Mutation screening could be carried out by whatever state of the art equipment was available, using gel, chip, or novel based methods. All large scale screening could be handled by these facilities, including the future need for the genetic profiling of patients. The information technology needed to handle the large volume of data that would be generated could also be developed at these sites. Rather than the in-depth knowledge of clinical conditions needed by clinical molecular geneticists, staff would be trained to expert levels in technical and computer expertise.

These types of facilities could be lucrative commercial ventures. To avoid the ethical and practical problems of patient confidentiality and clinical support, it would be better if they were developed as part of the health care system, but collaboration with commercial companies may be the way forward.

STAFFING AND MANAGEMENT ISSUES

The management structure is the key element determining whether core facilities function well. A core unit needs a cohort of well trained staff with a wide range of expertise in specialist testing, data handling, and data analysis skills. Expertise will be needed in training and in recognising and bringing into service new developments. Senior staff will be needed who recognise the clinical implications of results and can liaise with appropriate clinical staff. Heads of units may appropriately be senior clinical molecular geneticists.

Conclusions

The effective delivery of DNA based testing within a health care system will be a persistent and growing problem. To justify the costs incurred in the purchase of equipment and the specialist training of staff there must be coordination and cooperation among clinical specialties. Clear guidance is needed with respect to the types of investigation that are appropriate for local testing and those that can be more effectively delivered from specialist centres. Centralisation is the best mechanism for the cost-effective use of scarce resources, for the purchase of expensive equipment, and for the development of specialist skills. An appropriate environment will enable the retention and development of staff and the monitoring of developments. Without access to such facilities, peripheral groups will waste time and resources in duplicating tasks carried out more effectively in a custom designed unit.

The revolution in the understanding of the central role of the genetic material of the cell has been a story of the second half of the 20th century. The continuing exponential growth in the understanding of the molecular genetic basis of human disease has been supported by technological developments which can replace the ingenious but labour intensive methods previously employed. To deliver an effective clinical service needs careful and innovative planning and management, without which the molecular medicine revolution may pass us by for many more years.

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