Review

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Genetics and molecular biology in laboratory medicine, 1963–2013

Abstract

The past 50 years have seen many changes in laboratory medicine, either as causes or consequences of increases in productivity and expansion of the range of information which can be provided. The drivers and facilitators of change in relation to clinical applications of molecular biology included the need for diagnostic tools for genetic diseases and technical advances such as PCR and sequencing. However, molecular biology techniques have proved to have far wider applications, from detection of infectious agents to molecular characterization of tumors. Journals such as *Clinical Chemistry and Laboratory Medicine* play an important role in communication of these advances to the laboratory medicine community and in publishing evaluations of their practical value.

Keywords: genetics; laboratory medicine; molecular biology.

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Introduction

The substantial changes in clinical laboratories over the 50 years of *Clinical Chemistry and Laboratory Medicine's* (CCLM) publication are a result of both innovative and incremental improvements. Each contributes to our primary purpose, provision of information to assist in the diagnosis and management of patients' conditions and in the prevention of disease. The gradual improvement of existing methods and analyzers has led to increased reliability, better analytical quality, and faster test result delivery. These have been accompanied by increasing reliance on commercially-sourced methods, reagents and data management systems. In parallel with these trends we have experienced increased regulation and an emphasis on cost containment. However, it is the innovative and even revolutionary changes which are of greater interest for a research-based journal, and for those who contribute or make use of our published papers.

There will be varying views about which innovations have had the greatest impact on clinical laboratories over the past 50 years, but most lists would include the automation (or mechanization) of methods in the 1960s, immunoassays in the 1970s, and molecular biology techniques from the 1990s onwards. Automation allowed an immense increase in productivity. Immunoassays led to the measurement of a new range of diagnostic markers, initially in endocrinology and then for other protein biomarkers and for smaller molecules. Advances in genetics and in molecular biology techniques have had substantial impact on laboratory medicine, and are about to have more through improvements in sequencing technology and investigation of an expanding range of sample types. Having recently written an Editorial [1] on the prospects for clinical molecular biology, I will take a more historical approach to identifying important trends and the lessons we can derive from them. As in so many fields, a cycle of conceptual and technological developments can be seen and the expansion of our capabilities is built upon both of these. However, ideas and machines are not enough in themselves; the tests which become possible have to fulfill a clinical need and make a difference to outcomes.

Genetics and molecular biology up to 1963

Genetics, and clinical genetics, existed long before the development of molecular biology. In our own field the clinical and chemical studies by Garrod [2], and their integration with Mendel's theories of inheritance, have been hugely influential. However, Garrod's later work on 'diathesis' or genetic predisposition to common diseases [3] was undervalued because at that time there were few concrete examples to back up his idea and no practical

ways to test it. This relative neglect is not surprising; one or two patients investigated with ward side room tests could lead to recognition of an inborn error of metabolism but analysis of data from tens or hundreds of thousands of people may be necessary to show, e.g., that a specific gene variant affects plasma lipids and the risk of cardiovascular disease [4, 5].

A great expansion of the list of inborn errors took place about 50 years after Garrod's initial lectures, when semi-quantitative paper chromatography was applied to amino acids [6], but even this is before the period we are considering. The ingenious Guthrie test was described in 1963 [7] and, in combination with development of dietary treatments for phenylketonuria, made neonatal screening for genetic disease an effective public health measure. In the complementary area of chromosomal abnormalities, karyotyping became possible in the 1950s and the presence of an extra chromosome in Down syndrome was reported in 1959 [8].

By 1963, when the *Zeitschrift für klinische Chemie* first appeared, there was substantial knowledge about the molecular basis of inheritance but little application to the diagnosis of disease. This was pure rather than applied science; practical methods for genotyping human samples were 20 years away and a number of discoveries and technologies would have to be brought together to achieve it.

Technology, discovery and laboratory medicine

From a background of existing knowledge and methods, a novel technology permits new discoveries, which lead to novel clinical applications. Alternatively, a discovery suggests that if technology was available to exploit it then useful new investigations would be possible. The applications (in research or diagnostics) then drive mainly incremental improvements in reliability, cost and accessibility so that methods which were once restricted to highly specialized laboratories can be applied much more widely. One consequence is that the sphere of knowledge required to manage the service based on this technology, and to interpret the test results, keeps expanding. We have seen this in most areas of laboratory medicine, but it is well-illustrated by the molecular genetics area.

The important historical developments include, in approximate order of their initiation:

1. Cloning, in which a gene was introduced into a host organism to produce sufficient DNA for analysis or for testing its effects.
2. Techniques enabling genetic testing on genomic DNA without amplification. These included hybridization with sequence-specific and later allele-specific probes; Southern blotting [9]; and restriction digestion [10, 11].
3. Sequencing. Both Maxam-Gilbert and Sanger sequencing were developed in the mid-1970s and published in 1977 [12, 13]. Later developments, particularly automation of Sanger (dideoxy) sequencing, improved productivity for both research and clinical laboratories and led to a profusion of four color figures in journals, showing sequence as the familiar peaks with bases printed underneath. Alternative approaches have become available since completion of the human genome sequencing project, based on sequencing short fragments and assembly of the results by matching against a consensus sequence. Most of the commercial, highly automated, high-throughput systems [14] are based on this.
4. Amplification technologies, initially and particularly the polymerase chain reaction (PCR). The concept can be traced back to 1971, but the application of thermostable enzymes in 1985 made it practical and economical. The diagnostic usefulness of PCR when combined with restriction digestion was initially illustrated by analysis of sickle cell anemia [15] and then expanded to hundreds or thousands of other situations. It has been said that PCR democratized molecular biology; the advantages were not just an increased amount of DNA to work on and a specific fragment of DNA defined by the primers but a method which could be implemented by many laboratories.
5. Genetic association analysis. An important conceptual paper in 1996 [16] drew attention to the possibility that associations between genetic loci and multifactorial diseases could be discovered by typing very large numbers of genetic markers in large cohorts of cases and controls. Single nucleotide polymorphisms (SNPs) proved ideal for this but a great deal of work was required to translate this theoretical possibility into a practical approach. First the identification and location of hundreds of thousands of SNPs, then the development of chip genotyping (largely based on the technologies of the electronics industry), and finally the software to manage the data and test for associations. This genome-wide genotyping has had substantial research impact but so far there is little clinical application because the relative risk

associated with each locus is small. A number of companies have offered direct-to-consumer testing but the interpretation of results and the explanation of their meaning is highly challenging.

6. Expression analysis, based on similar technologies to the genotyping chips, has had more clinical impact. Research findings on molecular typing of cancers [17] are starting to have a clinical role and this is likely to develop, perhaps using sequencing on the somatic genome or transcriptome rather than arrays.
7. High resolution melting analysis [18, 19] for mutation discovery and genotyping has advantages for clinical laboratories because of the availability of suitable equipment, one tube analysis, and wide applicability to different polymorphisms or mutations.
8. The discovery that DNA circulates in the plasma [20] has led to array- or sequencing-based analysis of chromosomal abnormalities in birth defects [21] and this can potentially be exploited in cancers (including monitoring of recurrence) and through application to RNA. Circulating RNAs may be useable as tissue-specific biomarkers in a similar way to enzymes released from damaged tissues [22].

Genetics and molecular biology in *Clinical Chemistry and Laboratory Medicine*

CCLM has been an important vehicle for translation of scientific developments in this area into practical application. Review of the most highly-cited papers published in CCLM shows that many have been related to developments in genetic causes of disease or to technical advances in molecular biology and their clinical application. Out of the 300 most-cited papers over CCLM's 50-year history, 38 (13%) are in this category and they average 45 citations or a rate of 4.0 per year. This compares well with all papers in this top 300 (43 citations, 4.0 per year). The most highly-cited paper from CCLM [23] described and discussed approaches to quantitative PCR. In the past 5 years, the 100 most-cited papers include 12 on genetics or molecular biology and again their citation numbers and rates are comparable to those for other topics. The papers attracting attention cover a wide range; in addition to the paper on quantitative PCR there are others on genetic epidemiology [24, 25], quality control and standardization in PCR [26], and measurement of nucleosomes in plasma [27]. Many of the highly-cited papers are reviews and this emphasizes the importance of general overviews as well as focused research papers for the scientific community.

Genetic diagnostics, then and now

Initially, genetic diseases were recognized by their symptoms or natural history and their recurrence within families. Laboratory investigation then added a chemical dimension, such as the excretion of increased amounts of a chemical which would normally be metabolized, and such discoveries often pointed to the underlying enzymatic defect. Investigation of the DNA which codes for the identified enzyme would reveal a mutation or a set of mutations with similar effects, which led to clinical testing of variation in that gene or the protein it specifies. In time, the decreasing cost and increasing convenience of sequencing the entire exome [28] or genome will change the targeting of genes for investigation from an in vitro to an in silico process. The revolutionary steps in this example of disease investigation were metabolite measurement, enzyme assay, application of restriction enzymes to type polymorphisms in DNA, selective sequencing and most recently high-throughput sequencing.

The investigation of common polygenic diseases has not progressed so far. Although it is known that the risk for most diseases, including common ones such as cancers, cardiovascular disease and psychiatric conditions, is affected by genetic variation, we have not reached the stage of being able to make useful predictions about individual patients. There are exceptions, such as the less frequent but highly heritable familial cancers or early onset dementias, but these are not polygenic in their genetic architecture though they may be clinically similar to the sporadic forms. Research progress for common diseases has been impressive but incomplete; large studies based on genome-wide SNP marker typing have identified loci accounting for comparatively small differences in risk and it is not clear whether risk assessment can be improved by moving on to sequencing to detect uncommon SNPs or copy number variation. Pharmacogenomics has been much discussed but it seems likely that many treatment failures and side effects will be polygenic and their prediction will be subject to the same limitations as disease prediction.

Cancers, which are genetic diseases of a different kind, have generally been classified by their site of initial

occurrence and histological characteristics. Genetic characterization has to contend with the large number of possible abnormalities and heterogeneity within tumors, but current sequencing technologies for tumor genomes or transcriptomes allow a comprehensive analysis which may lead to a new approach to classification and a more effective guide to treatment. The likely scale of the sequencing effort required for each patient makes the clinical application of this approach highly dependent on supply of validated and reliable instrumentation and data analysis methods; and the cost implications are substantial.

Conclusions

It is too soon to assess the clinical value of recent advances in sequencing or the full implications of circulating nucleic acids. They seem likely, at least, to improve characterization of single gene diseases and chromosomal abnormalities in the neonatal period and during pregnancy, and to define the biology of individual patients' cancers. As in the past, technical advances will determine the ability of clinical laboratories to provide relevant, prompt and actionable information. There are important challenges in organizing and interpreting the information which molecular biology can provide, and overcoming these will require co-operation between researchers, bioinformaticians and clinical laboratory professionals. This continues the tradition of incorporating new concepts and skills which has sustained laboratory medicine, and made the scientific papers in our journals clinically relevant, over the past 50 years.

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References

1. Whitfield JB. Molecular biology and genetics in clinical chemistry and laboratory medicine. Clin Chem Lab Med 2010;48:431–4.
2. Garrod AE. Inborn errors of metabolism. Oxford: Oxford University Press, 1909.
3. Garrod AE. The inborn factors in disease: an essay. Oxford: Clarendon Press, 1931.
4. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature 2010;466:707–13.
5. Schunkert H, Konig IR, Kathiresan S, Reilly MP, Assimes TL, Holm H, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet 2011;43:333–8.
6. Dent CE. Detection of amino-acids in urine and other fluids. Lancet 1946;2:637–9.
7. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics 1963;32:338–43.
8. Lejeune J, Gautier M, Turpin R. Study of somatic chromosomes from 9 mongoloid children. C R Hebd Seances Acad Sci 1959;248:1721–2. [in French].
9. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 1975;98:503–17.
10. Coulondre C, Lucotte G. The molecular basis for the association between HLA-A3 and idiopathic haemochromatosis. Exp Clin Immunogenet 1984;1:25–30.
11. Lidsky AS, Ledley FD, DiLella AG, Kwok SC, Daiger SP, Robson KJ, et al. Extensive restriction site polymorphism at the human phenylalanine hydroxylase locus and application in prenatal diagnosis of phenylketonuria. Am J Hum Genet 1985;37:619–34.
12. Maxam AM, Gilbert W. A new method for sequencing DNA. Proc Natl Acad Sci USA 1977;74:560–4.
13. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 1977;74:5463–7.
14. Pareek CS, Smoczynski R, Tretyn A. Sequencing technologies and genome sequencing. J Appl Genet 2011;52:413–35.
15. Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, et al. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 1985;230:1350–4.
16. Risch N, Merikangas K. The future of genetic studies of complex human diseases. Science 1996;273:1516–7.
17. Ramaswamy S, Tamayo P, Rifkin R, Mukherjee S, Yeang CH, Angelo M, et al. Multiclass cancer diagnosis using tumor gene expression signatures. Proc Natl Acad Sci USA 2001;98:15149–54.
18. Wittwer CT, Reed GH, Gundry CN, Vandersteen JG, Pryor RJ. High-resolution genotyping by amplicon melting analysis using LCGgreen. Clin Chem 2003;49:853–60.
19. Erali M, Voelkerding KV, Wittwer CT. High resolution melting applications for clinical laboratory medicine. Exp Mol Pathol 2008;85:50–8.

20. Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485–7.
21. Bianchi DW. From prenatal genomic diagnosis to fetal personalized medicine: progress and challenges. Nat Med 2012;18:1041–51.
22. Chan RW, Wong J, Chan HL, Mok TS, Lo WY, Lee V, et al. Aberrant concentrations of liver-derived plasma albumin mRNA in liver pathologies. Clin Chem 2010;56:82–9.
23. Orlando C, Pinzani P, Pazzagli M. Developments in quantitative PCR. Clin Chem Lab Med 1998;36:255–69.
24. Siest G, Visvikis S, Herbeth B, Gueguen R, Vincent-Viry M, Sass C, et al. Objectives, design and recruitment of a familial and longitudinal cohort for studying gene-environment interactions in the field of cardiovascular risk: the Stanislas cohort. Clin Chem Lab Med 1998;36:35–42.
25. Gulcher J, Stefansson K. Population genomics: laying the groundwork for genetic disease modeling and targeting. Clin Chem Lab Med 1998;36:523–7.
26. Burkardt HJ. Standardization and quality control of PCR analyses. Clin Chem Lab Med 2000;38:87–91.
27. Holdenrieder S, Stieber P, Bodenmuller H, Fertig G, Furst H, Schmeller N, et al. Nucleosomes in serum as a marker for cell death. Clin Chem Lab Med 2001;39:596–605.
28. Kingsmore SF, Dinwiddie DL, Miller NA, Soden SE, Saunders CJ. Adopting orphans: comprehensive genetic testing of Mendelian diseases of childhood by next-generation sequencing. Expert Rev Mol Diagn 2011;11:855–68.

After training in Clinical Biochemistry in Birmingham and London, John B. Whitfield moved to Sydney where he worked as Principal Scientist in the Biochemistry Department of Royal Prince Alfred Hospital from 1973 to 2007. He has served as President of the Australasian Association of Clinical Biochemists (1991–1993) and Secretary of the IFCC (1997–1999). His research interests in the genetics of alcoholism and alcohol-related disease, and the use of biomarkers in studies of the genetics of common diseases, led to collaboration with the Genetic Epidemiology group at Queensland Institute of Medical Research and in 2007 he moved to Brisbane to continue this work. He is currently Associate Editor for genetics and molecular biology for *Clinical Chemistry and Laboratory Medicine*.