Genomics in medicine: From promise to practice

The culmination of the Human Genome Project, with the publication of two 'reference' genomes, introduced the genomic era.[1,2]

Before this, human genetics concerned itself with techniques to analyse the chromosomes (cytogenetics) and to detect the genes causing Mendelian diseases such as cystic fibrosis or sickle cell anaemia (molecular genetics), and was largely the preserve of human geneticists. Genetic testing was accurate, but slow and costly. Chromosome analysis was limited by its low resolution (a DNA deletion of 5 million base pairs of DNA may be undetectable), and molecular genetics by the inability to sequence more than a few hundred DNA bases at a time.

The growth of genomics has been facilitated by the development of much more rapid and large-scale methods to analyse genetic information, including microarray and next-generation sequencing (NGS) technologies.

Available from the early 2000s, microarrays permitted testing for several million preselected items of genetic information in a single test. This allowed the development of approaches such as 'molecular karyotyping' and genome-wide association studies (GWASs).

The arrival of NGS in 2007 took this further, allowing sequencing of millions of segments of DNA in a single experiment. NGS may be used to test preselected genetic sequences, but may also be used for open-ended testing of the entire genome or large subsets thereof. This has been facilitated by great reductions in cost: since 2014 it has been possible to sequence a whole human genome for less than USD1 000.

Since each haploid genome comprises three billion base pairs of DNA, and any two human genomes are expected to have at least three million points of difference (genetic variants), the development of computational 'bioinformatic' methods has also been crucial. Computational algorithms using a range of public access databases provide the potential for clinically meaningful interpretation of genomic information. Comparison of a genomic test with the 'reference sequence' and with databases of normal and pathogenic variants allows for classification of each piece of genomic information as 'normal', 'pathogenic' or a 'variant of unknown significance' (VOUS).

Cytogenetic disorders and microarray

Since microarray has been the forerunner to other genomic methods, it is instructive to track its role in cytogenetics. Molecular

karyotyping or array competitive genomic hybrid (array CGH) was introduced as a research technique for childhood intellectual disability over a decade ago. Compared with conventional karyotyping, array CGH can detect much smaller deletions or duplications of chromosome material (known as 'copy number variants'). Evidence has accumulated that the cause of intellectual disability is detected in 15 - 20% of cases by array CGH compared with 3% by karyotyping. Over several years, array CGH transitioned into diagnostic practice as laboratories became more familiar with the methods involved, companies produced more user-friendly array platforms, and databases defined the normal and pathogenic genetic variants more clearly.

Array CGH is now rapidly supplanting chromosome analysis for investigation of intellectual disability, autism and multiple dysmorphic features of unknown cause,[3] and other indications.

Multifactorial disease and GWASs

Multifactorial diseases, resulting from the cumulative effect of multiple genetic and environmental predispositions, are a major contributor to the burden of disease in South Africa.[4] The GWAS research design allowed specific genetic loci to be linked to specific multifactorial diseases, and to date over 4 000 such loci have been detected.[5] Detection of a relevant locus permits further research to detect the specific genes and biochemical pathways involved, and potentially allows for new treatments to be developed.

Mendelian disorders and NGS

The arrival of NGS greatly accelerated *inter alia* the discovery of the genetic causes of Mendelian (single-gene) disorders. The genetic causes of over 3 600 single-gene disorders have been described to date.[5] Making a genetic diagnosis has many practical benefits. It clarifies the cause of the condition and ends the 'diagnostic odyssey'. It allows for genetic counselling relating to options for preventing recurrence of the condition, including carrier testing, prenatal diagnosis, or possibly even preimplantation genetic diagnosis. In some instances the genetic test may alter treatment (e.g. a genetic diagnosis of Dravet's syndrome indicates that certain antiepileptic drugs should be avoided). In a few instances it may allow for gene therapy that, although not a cure, significantly improves function. This is the case with RPE65 gene therapy for individuals with a particular cause of blindness, Leber's congenital amaurosis.[6]

The laboratory method most often used to detect new or unidentified Mendelian disorders has been whole-exome sequencing (WES), since most mutations causing these disorders are within the 1% of the genome that codes for proteins (i.e. the exome). WES is no longer just a research tool, and has gained an accepted diagnostic role for rare probably genetic disorders, with a 25% detection rate described by Yang *et al*.[7] In some instances the result has significantly changed clinical management – in one case prompting a curative bone marrow transplant for a child with intractable inflammatory bowel disease.[8]

Mendelian disorders that may be caused by mutations in any one of a number of known genes, such as familial cancers, visual loss, epilepsies or immunodeficiencies, can increasingly be diagnosed by means of sequencing a panel of genes known to cause that condition. Prior to the advent of NGS, such gene panels were impractical. Compared with WES, gene panels have the advantage of giving greater accuracy and less excess information.

Cell-free DNA and non-invasive prenatal testing (NIPT)

The detection of cell-free fetal DNA in the maternal plasma[9] allowed the development of NIPT. Early NIPT tested DNA sequences unique to the fetus, e.g. the presence of Y-chromosome DNA identified the fetus as male. More recently it has been possible to test for aneuploidies such as Down syndrome. The high sensitivity and specificity of NIPT for Down syndrome led to its rapid acceptance as a first-line screening method for women at high risk of having a child with Down syndrome.[10] Uptake of NIPT in the USA has been very rapid, with over 800 000 tests performed in 2014, to the extent that there have been concerns that testing is proceeding ahead of the clinical evidence.[11]

Cancer genomics

Cancer is a fundamentally genomic disorder, with tumorigenesis resulting from multiple mutations involving a variety of genes involved in control of the cell cycle and DNA repair.

Historically, tumour prognosis and treatment decisions have almost exclusively been determined by clinical staging and histological grading of the tumour. It has since become evident that tumours that look clinically and pathologically similar may have a very different molecular basis, which may be associated with different clinical outcomes. An early example was the finding that breast cancers over-expressing the HER-2 receptor are sensitive to treatment with a monoclonal antibody (Herceptin).

More recently gene-expression profiles for multiple genes, using mRNA extracted from tumour tissue, have become available to refine the prognosis of early-stage breast cancers, thereby improving decision-making regarding the need for chemotherapy.[12] For a range of cancers there is increasing evidence that the 'molecular signature' has great practical potential for determining diagnosis, prognosis and treatment.[13]

Analogous to NIPT, it is possible to test for cell-free tumour DNA. This has a possible role as a biomarker for a variety of cancers, with potentially important implications for screening and monitoring.

Pharmacogenomics

Pharmacogenomics is the study of how genes affect a person's response to particular drugs, and it aims to improve the efficacy, safety and dosing of medications. The efficacy of drugs as currently used is limited. Across a range of disorders, the proportion of patients who respond to treatment varies from 25% to 80%.[14] It is anticipated that improved molecular knowledge will improve drug targeting and increase the range of available drugs.

Accurate dosing is particularly important for drugs with a narrow therapeutic index, such as warfarin. Incorporating genetic information into decision-making on the warfarin dose was found to reduce the need for hospitalisations for haemorrhage by 28% in the 6 months after initiating therapy.[15] As a result, the Food and Drug Administration in the USA revised the label on warfarin to recommend the use of genotype information when prescribing warfarin and, based on this information, provision of genotype-specific dose ranges.[16]

In some cases, severe adverse events have been linked to specific genetic variants (e.g. HLA types). The finding that the HLA-B*5701 allele has good predictive value for abacavir hypersensitivity, and that this allele has a 5% prevalence in Caucasians, led to widespread implementation of genetic testing prior to initiation of therapy.[17]

To date, the uptake of pharmacogenomic tests by clinicians has been relatively low internationally. Various reasons are cited, including uncertainty about the clinical utility of the test and the lack of simple clinical algorithms. A further complexity is that the prevalence of pharmacologically relevant variants is often not well characterised in local populations.[18]

Precision medicine

Precision medicine is set to become a paradigm for the medicine of the future. It builds upon several of the approaches described above, and involves more precise identification of a person's illness at a genetic and biochemical level, whether it be hypertension or prostate cancer. More precise diagnosis will facilitate more precise treatment, using a currently available drug or perhaps a drug designed to take advantage of new biological knowledge. In addition, precision medicine should facilitate earlier diagnosis and prevention.

The recent announcement of a Precision Medicine Initiative by President Barack Obama[13] has given a significant boost to the field. It includes funding for a cohort of up to 1 million individuals who will be receiving extensive genomic testing and long-term clinical follow-up, in order to correlate genotypes, phenotypes and responses to treatment.

The implications of the precision medicine paradigm are far-reaching in the long term. Everything, from the *International Classification of Diseases* to the use of electronic medical records to medical education to medical aid benefits, will change accordingly.[19]

Ethical and implementation challenges

Conventionally, diagnostic laboratory tests are assessed to ensure that they meet criteria for analytical validity (the assay should be accurate) and clinical validity (the test should be clinically meaningful).[20] Genomic testing challenges this paradigm because of the vast amount of information it produces. Genomic tests regularly detect VOUS, and it will take years to identify the clinical relevance of each variant. The need is for a flexible regulatory environment that stimulates rather than stifles research, while not condoning tests that are without clinical value. The Precision Medicine Initiative envisages an emphasis on developing collaborative public access databases to efficiently increase knowledge of the clinical implications of specific genetic variants.[13] A related challenge is how best to report the range of possible genetic variants detected, e.g. VOUS or 'actionable' incidental findings.[21]

As the ethical issues are solved and the evidence for clinical utility and cost-effectiveness becomes clearer, the use of genomic and precision medicine approaches will scale up. Given the complexities of implementation, it will initially require a multidisciplinary approach that includes both state-of-the-art molecular genomics and bioinformatic components, and insightful clinical and genetic counselling components.[22]

Conclusion

Genomics is a complex field that is taking an increasing role in many aspects of healthcare. Genomic medicine is expected to transition into the broader paradigm of precision medicine, with profound long-term implications for the practice of medicine and the training of future practitioners.

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