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The future of genetics

Zornitza Stark and Kathryn North

It took over 10 years and US$3 billion to sequence the first human genome. Next generation sequencing (NGS) technologies have made it possible to replicate this feat in a matter of days and at a fraction of the cost, and have led to an explosion in our understanding of the genetic determinants of health. Genomic testing is set to transform healthcare, from disease diagnosis to personalised risk assessments, tailored treatments and preventative interventions. Where are we going to be in 2050, and what challenges lie ahead?

What is next generation sequencing (NGS)?

The genome is the complete set of genetic information of an individual. The genes are the unique instructions that determine growth, development and physical characteristics such as hair colour, eye colour, height and intellect, to name a few. Genes are made up of a chemical molecule called deoxyribonucleic acid (DNA). DNA contains four nucleotides, adenine (A), thymine (T), cytosine (C) and guanine (G), the sequence of which makes up the genetic code. The genes are arranged on larger structures called the chromosomes. Every cell in the body contains a complete set of genetic instructions; we have around 23,000 genes and our genome contains 3.2 billion nucleotides.

Traditional genetic testing employs a variety of techniques to detect disease-causing genetic changes called mutations, including chromosomal microarray to identify gene deletions and duplications, polymerase chain reaction (PCR) to detect triplet nucleotide expansions, and Sanger sequencing to identify single nucleotide changes (point mutations). Sanger sequencing is typically performed on single genes of interest based on clinical presentation. It is expensive and time consuming, although it affords a high degree of accuracy as a complete sequence of the nucleotides in a gene is obtained, and is thus considered the 'gold standard'.

NGS techniques allow the simultaneous sequencing of many or all genes using massively parallel sequencing assays. Whole exome sequencing (WES) refers to the capture of most of the coding regions of the genome, the gene exons. These account for 2% of the genome but are thought to contain the majority of disease-causing mutations. Whole genome sequencing (WGS) evaluates almost all of the genome, including both coding and non-coding regions, and in addition is able to detect structural variations such as gene deletions (missing genetic material) and duplications (extra genetic material). NGS tests generate vast amounts of data, typically many thousands of variants per person undergoing WES, thus posing challenges for data storage and especially for data analysis and interpretation. There are also concerns regarding incomplete and uneven gene coverage using current NGS methods, with the possibility of disease-causing mutations being missed altogether. The choice between WES and WGS is currently mostly determined by cost. As the cost of WGS decreases, its accuracy increases and we develop solutions to deal with the huge amounts of data generated, it is expected that WGS will supersede both WES and chromosomal microarray in the coming years.

NGS approaches are fast becoming available in the clinical setting, and it is expected that in the next 10 years, WES and WGS will become routinely available tests. The two main areas of clinical care that are likely to benefit the most from this development in the short to medium term are rare disease diagnosis and cancer.

Rare disease diagnosis

Rare diseases are estimated to affect millions of individuals worldwide. Accurate and timely diagnosis is critical to our ability to provide prognostic information, targeted surveillance for complications, and interventions directed at ameliorating the natural course of the condition. Traditional diagnostic pathways are aptly described as 'odysseys' and are incredibly burdensome for patients, families and healthcare systems. All too often, multiple specialist assessments, hospital admissions, imaging, biochemical studies and invasive tissue biopsies merely serve to narrow down the diagnostic possibilities. As many of the patients are infants and young children, some of these investigations require general anaesthesia. Traditionally, when a genetic condition is suspected, testing of single genes was undertaken in a sequential manner, with the results of one test awaited before another was initiated, and the number of genes tested in any one individual was frequently limited due to cost considerations. Of the estimated 7,000 rare single-gene disorders, the genes responsible for just over half of these are currently known, further hampering diagnostic efforts.

NGS technologies have accelerated the pace of gene discovery, with new genes being reported in the medical literature on a weekly basis, and predictions that most of the remainder will be identified in the next 5–10 years (Boycott et al., 2013). At the same time, WES and WGS have become available as clinical tests, with diagnostic yields ranging from 25–73%, depending

on the patient cohort studied (Farwell et al., 2015; Lee et al., 2014; Neveling et al., 2013; Saunders et al., 2012; Soden et al., 2014; Yang et al., 2013). While these tests are currently often reserved for patients at the end of the diagnostic trajectory, there are indications that early use will optimise opportunities to influence clinical management and decrease the cost of investigation. In an Australian study comparing WES with standard diagnostic care in infants suspected of having rare genetic conditions, a diagnosis was achieved through WES in three times as many patients at a quarter of the cost (Stark et al., 2016; Stark et al., 2017). These diagnoses led to a change in clinical management in a third of patients and have had a major impact in restoring the reproductive confidence of affected families.

Case study

Joshua was admitted to hospital at the age of 8 months with low muscle tone, developmental delay, failure to thrive and deafness. He underwent extensive investigation, including blood and urine tests, a lumbar puncture and a MRI scan of the brain under a general anaesthetic. A diagnosis of a likely mitochondrial disorder was made. A mitochondrial disorder is one where the mitochondria are impaired. Mitochondria are tiny structures in cells that provide the cells with energy. There are 1000s of mitochondria in cells.

Mitochondrial disorders affect the body's ability to produce energy and can affect brain, muscle, heart, liver, vision and hearing. They can be due to mutations in over 100 genes. Narrowing down a more specific diagnosis usually requires biopsies of muscle, liver and skin, which are obtained under a general anaesthetic. General anaesthetics can precipitate a deterioration in children with a mitochondrial condition, and

liver biopsies can result in bleeding that can sometimes be life threatening.

Joshua underwent WES, and mutations in a gene called *RMND1* were identified. This causes a mitochondrial disorder called 'combined oxidative phosphorylation deficiency 11'. Children with this particular mitochondrial disorder are at risk of kidney dysfunction. With this information in mind, testing of kidney function at the follow-up appointment was undertaken, and indeed indicated kidney impairment and a dangerously high level of potassium in the blood. Joshua was urgently admitted to the Paediatric Intensive Care Unit for treatment. The potassium level was normalised, and he is now under the care of a kidney specialist.

Exome sequencing in Joshua enabled a specific diagnosis to be made in a timely manner without the need for invasive and costly biopsies. Having a specific diagnosis directed his management towards identifying and treating the complications he is at risk for and was life saving.

In addition to improved diagnostic yield and accuracy, NGS technologies have the potential to provide an answer within a very short time frame. One proof-of-principle study has reported a 26-hour time to a result using WGS (Miller et al., 2015). The ability to consistently deliver rapid results for selected patients will be key to realising the full benefits of NGS in the acute care setting and will transform the place of clinical genetics consultation and genetic testing in the diagnostic pathway.

Accurate delineation and diagnosis of rare genetic conditions holds the broader promise of increased understanding of disease biology and development of targeted therapies. Very few rare diseases have treatments available at present, and these are generally either costly enzyme replacement therapies

for metabolic conditions, or repurposed drugs that were originally developed for other conditions, such as the use of beta-blockers in the management of Marfan syndrome, a condition that can lead to distension and sometimes rupture of the aorta. Improved understanding of how different mutations cause disease has led to the introduction of specific therapies such as the drug Ivacaftor in cystic fibrosis (CF). CF is one of the most common genetic conditions, affecting 1 in 2,500 Caucasian newborns. Mutations affecting the CFTR chloride channel result in the production of abnormally thick secretions, which in turn impair lung and gastrointestinal function, resulting in the need for daily treatment, frequent hospitilisations and shortened lifespan. Traditional therapies focus on improving secretion clearance by physiotherapy, treatment of lung infections, replacement of pancreatic enzymes at mealtimes and treatment of nutritional deficiencies. While some mutations such as the common p.F508del result in misfolded proteins that are not delivered to the cell surface, with other mutations such as p.G551D, the channel reaches the cell surface but has an impaired ability to transport chloride. Ivacaftor is a channel potentiator that works by binding to the faulty CFTR and increasing the probability of the channel opening (Ramsey et al., 2011). It is the first drug that treats the underlying disease rather than the symptoms of CF. This mutation-specific approach has been shown to result in improvements in lung function and reduced need for hospitalisation.

After decades of research effort, gene therapy approaches that address the primary gene defect are also finally coming of age. Clinical trials are in progress for Duchenne muscular dystrophy (DMD) and for drugs that both overcome specific types of gene faults, such as Ataluren or the longwinded-named 'RNaseH-independent antisense oligonucleotides (AONs)' (Fairclough et

al., 2013). It is hoped that these types of approaches will be effective in other neuromuscular conditions such as spinal muscular atrophy (SMA) and myotonic dystrophy.

Cancer

Advances in genomic technologies are revolutionising our approach to the diagnosis and treatment of cancer. Cancer arises as a consequence of the accumulation of genetic changes, collectively termed 'somatic mutations', which comprise changes to single nucleotides, chromosomal rearrangements and epigenetic changes. Some of these, the so-called 'driver mutations' confer growth advantage and result in cells being positively selected during the evolution of cancer. That is, these cells grow in an uncontrolled manner. Another important group of driver mutations are those that arise or are selected as a result of treatment and confer resistance to therapy.

The treatment of cancer has traditionally relied on a series of steps: surgical removal (where possible), classification of severity based on examination of the tumour under the microscope, and then application of broad-acting agents such as chemotherapy and radiotherapy, which are more likely to damage rapidly growing cells than normal tissue.

Understanding cancer drivers at the genetic level holds the promise of improved risk stratification, the development of specific therapies leading to reduced toxicity and improved outcomes. Large-scale cancer genome studies such as the International Cancer Genome Consortium (http://icgc.org) and the Cancer Genome Atlas (https://cancergenome.nih.gov) aim to define these drivers across multiple cancer types. In the clinical setting, performing WES and/or WGS on tumour samples and comparing this with a healthy tissue sample from the same patient allows the identification of patient-specific tumour mutations and the provision of personalised cancer

care. The use of genetic testing to define disease subtypes, inform prognosis and target treatment is already a reality for some common cancer types such as acute myeloid leukaemia and breast cancer. A new generation of drugs targeting the products of specific driver genes and mutations are emerging, such as vemurafenib, a so-called BRAF inhibitor aimed at a particular mutation called p.V600E. Metastatic melanoma has a very poor prognosis, with median survival of around 8–18 months. BRAF is a component of a chemical pathway, which is an important driver in melanoma, with more than 50% of patients having the activating p.V600E mutation. Administration of vemurafenib leads to a marked improvement in both overall survival, and progression-free survival in patients with unresectable and metastatic melanoma (Chapman et al., 2011), offering hope for the development of new treatment paradigms for advanced malignancies based on genetic profiling.

What next?

Population level screening

Over the next few years, the price of sequencing is expected to plummet to a level where it will become feasible for individuals to have their genome sequenced before they develop health problems. Individuals are currently screened at birth in many countries for a range of potentially treatable rare paediatric-onset conditions such as metabolic disorders (e.g. phenylketonuria), low thyroid hormone levels and CF, through testing a blood sample taken on about day 3 of life. Newborn screening using NGS would enable screening for thousands of potentially treatable disorders, with the individual's genomic sequence becoming part of their medical record so that it is available for

future interrogation in a clinical setting (http://www.genomes2people.org/babyseqproject/).

Genomic data will gradually become integrated with electronic medical records (EMRs). EMRs offer an unparalleled opportunity to link genetic data to a range of other data — clinical signs and symptoms, results of laboratory and imaging investigations, prescribing information and clinical outcomes, further driving our understanding of genetic variation and its effect on health. At a patient level, EMRs will provide a platform through which to interrogate genomic data for different indications over the lifetime of an individual and in real time, while integrating this with dynamic consent.

Pharmacogenomics

Certain genetic variants are known to influence drug efficacy and toxicity. Data regarding personal response to particular drugs and risk of complications can become routinely available as part of the EMR at the time of prescribing rather than having to be requested as a separate test once an adverse reaction has occurred, or a patient has failed to respond to treatment. Similar to systems that currently provide computerised drug interaction warnings, doctors will be alerted to optimise their prescribing based on patient genetic information.

Genetic risk factors for common disorders

Genomic data will offer insights into complex diseases and common disorders with a genetic component such as autism spectrum disorder, diabetes, cancer and autoimmune conditions. Improved understanding of the contribution of multiple gene variants towards risk of developing certain conditions will allow information to be provided regarding individual risk and how environmental factors can be altered to achieve better health outcomes. The current prevailing medical model is that

of 'reactive' medicine, where individuals seek medical care with signs and symptoms of disease, and are then given lifestyle advice and/or treatment to try and ameliorate symptoms and delay disease progression. Understanding individual risks based on genomic information will enable the focus to shift to preventative medicine and early intervention.

Reproductive carrier screening and prenatal testing

At reproductive age, information on carrier status for rare recessive genetic conditions can be assessed for a couple to provide accurate information regarding the risk of having an affected child.

As NGS approaches increase the rate of gene discovery and mutation detection for early childhood onset disorders that result in severe disability (e.g. severe intellectual impairment), it has become apparent that a large proportion of these are caused by de novo new dominant mutations (i.e. not inherited from a parent but a mutation that occurs in the egg or sperm) (Veltman & Brunner, 2012), and thus would not be detected on reproductive carrier screening. Prenatal diagnosis is currently routinely available for a limited number of conditions (e.g. chromosomal abnormalities such as Down syndrome) or when there is a known risk of a genetic disorder in the family. NGS approaches will eventually be possible as part of prenatal diagnosis, potentially in combination with non-invasive testing methods through a blood test on the mother. However, interpretation of whether a specific de novo genetic variant will cause any problem for the baby, in the absence of ability to assess the clinical symptoms of the unborn child, is far from simple. There is a wide variability in the severity of disease symptoms associated with specific genetic variants. Accurate estimation of the risk associated with a rare variant will rely on

the ability to access genomic data on millions of individuals, which will only be possible if data sharing occurs on an international scale.

Challenges to realising the potential of genomics

It is clear that genomics has the potential to transform the delivery of healthcare, but many challenges lie ahead. In rare disease diagnostics, it will be essential to maintain and accelerate the pace of disease gene discovery. Many of the initial gene discovery success stories have relied on clinicians identifying groups of patients with the same recognisable rare disorder and then comparing their genomic data to identify the causative gene (Ng et al., 2010). However, as gene discovery moves to ultra-rare conditions, there is an increasing need for the sharing of clinical and genomic data, and the development of computational tools that integrate these data on a large scale. Matchmaker Exchange (http://www.matchmakerexchange.org) is an example of a collaborative platform that connects multiple rare disease consortia and data depositories such as Gene Matcher, Phenome Central and DECIPHER. Clinical data from rare disease patients who remain undiagnosed following genomic testing can be deposited without personal identifiers using a set of standardised terms (the Human Phenotype Ontology; HPO), together with the genomic data and/or a set of candidate genes. The exchange then queries all the participating databases, allowing clinicians to make contact if a match is made. There are already many examples of successful rare disease gene discoveries that have been facilitated through this process (Philippakis et al., 2015), and this is only set to grow as more data are deposited.

Data-sharing initiatives are also crucial in facilitating data interpretation. Databases such ExAC from the Broad Institute

(http://exac.broadinstitute.org) that contain genomic data from tens of thousands of individuals without rare genetic diseases improve our understanding of normal variation, while well curated mutational databases such as the BRCA exchange (http://brcaexchange.org) facilitate accurate disease variant interpretation. Overarching collaborative public-private partnerships such as the Global Alliance for Genomics and Health (GA4GH), which includes over 450 organisations from 47 countries, aim to provide a framework to catalyse the development and implementation of common technical and ethical standards to facilitate clinical and genomic data sharing on an unprecedented scale, while breaking down structural, technological and cultural barriers (Global Alliance for Genomics and Health, 2016). Data entry and sharing remain resource-intensive, and it is hoped that these initiatives will benefit from crowd-sourcing approaches and direct patient engagement in the future. In addition to the power of data sharing, there is also an increasing need to develop rapid functional genomics tests, such as animal models (e.g. fish), with the same alteration in a gene that was identified in a person, which can be generated very quickly, and cell-based tests to identify whether likely disease-causing variants are a mutation and thus the cause of the problem or a benign alteration unrelated to the problem in the individual.

While we now have the ability to diagnose ultra-rare disorders that have only been seen in a handful of other patients worldwide or have only been described in the medical literature a few weeks previously, this increased diagnostic ability needs to be coupled with increased understanding of the natural history of rare diseases to be able to provide meaningful information to patients and families. The internet and social media are playing an increasing role in connecting families

with ultra-rare conditions and establishing family-driven natural history studies.

The successful implementation and adoption of genomic medicine in routine practice is dependent on generating an evidence base demonstrating diagnostic and clinical utility, and cost-effectiveness. In particular, making the economic case for personalised medicine will be crucial in funding sustainable service delivery models and ensuring equity of access. Keeping pace with technological developments and delivering them in the healthcare context will require a vast expansion of the clinical and laboratory genetics workforce, as well as improvement in the level of genomic literacy of a range of healthcare professionals and of the general population. Such knowledge and skills will be key in empowering individuals to use their genomic data to manage their health, as well as to prevent data misuse or misinterpretation. The potential clinical and personal utility of genomic information will need to be balanced with the rights of individuals to privacy and autonomy, and with protection against discrimination through developing models for appropriate informed consent and data safety.

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