Next generation sequencing for clinical diagnostics and personalised medicine: implications for the next generation cardiologist

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ABSTRACT
The fast moving field of genomic medicine is already impacting on clinical care and cardiologists are fortunate to be in a position to benefit early from the transformative advances in genomics. However, the challenges associated with genomics in the clinic in general, and with next generation sequencing technologies in particular, are significant and cardiologists need to be prepared if they wish to surf the wave of genomic opportunity. This paper presents an overview of the implications of next generation sequencing for clinical diagnostics and personalised medicine in the cardiology clinic.

INTRODUCTION
Clinical cardiology is constantly evolving and at an ever-increasing pace. More than 200 years passed between Hales' first equine cardiac catheterisation and the routine clinical application of the technique in humans.1 By contrast, just 24 years separated the development of DNA sequencing technology2 and the publication of the draft human genome.3 Four years later, commercially available next generation sequencing (NGS) was available4 and now, 6 years on, NGS is already used in clinical practice. High-throughput genomics has great potential for the diagnosis and treatment of patients with rare and common forms of cardiovascular disease. However, before we can realise these goals for our patients, the next generation of clinical cardiologists must train in genomics and understand the potentials and pitfalls of genomic medicine, and there must be a coordinated investment in technology and informatics at a local and national level.

What is next generation sequencing and why is it important for the next generation of clinical cardiologists?
Since the 1970s the principal technique used for DNA sequencing was the Sanger method,5 and this remains the gold standard for sequencing accuracy today. The current generation of automated high-throughput Sanger sequencing machines read ~2 million bases of sequence per day and were used to sequence the first human genome.6 This throughput is very low compared with the size of the human genome (one strand of the human genome is 3000 000 000 base pairs), so NGS technologies parallelise the sequencing process, thereby massively increasing throughput (up to ~50 billion bases per day) at a reduced cost per base (figure 1). Clinical laboratories face major challenges as a consequence of this high throughput, such as optimising the use of capacity, processing and storing the large volumes of data and analysing the results, requiring a team of informaticians, statisticians, clinical scientists and clinicians.

While NGS per base sequencing costs are relatively low, the cost of sequencing entire human genomes remains high and whole genome sequencing is unnecessary for many diagnostic and research applications. It is estimated that the protein-coding regions of genes (exons) constitute approximately 1% of the human genome but harbour 85% of the mutations with large effects on disease.7 Hence, targeted sequencing of the exons of all genes (the 'exome') or a subset of genes of interest is an attractive approach. Such specificity can be achieved by preparing a library of DNA fragments for sequencing, typically either by pooling many PCR amplicons or by fragmenting genomic DNA and using sequence-specific hybridisation to select genomic regions of interest (figure 2). Neither approach is straightforward on the scales appropriate for NGS applications, although off-the-shelf target enrichment products that integrate well with NGS workflows are now available.

In the short term, Sanger sequencing will continue to be used as a complementary strategy to sequence genomic regions that are difficult to target in these ways (eg, regions with high GC content or repetitive sequence) and also to validate NGS findings. As the accuracy and efficiency of targeted sequencing improves, we anticipate that NGS will increasingly be used as a stand-alone technique.

Sequencing capacity can also be shared between several samples by tagging each sample with a unique DNA 'barcode'. In this way it is possible to use a single NGS run to sequence either a handful of genes from many patients or many genes from fewer patients.

The UK House of Lords Genomic Medicine report8 and the Life Sciences Blueprint9 highlighted the urgent need to apply genomic technologies for patient benefit and identified investment in new technologies (including NGS), bioinformatic and genomic databases as strategic priorities for the NHS.
Both the Human Genetics Commission and the Foundation for Genomics and Population Health identified inherited cardiac conditions (ICCs) as a key area to help focus the development of an overall NHS strategy for NGS-based research and diagnostics. Within this context, we will look at diagnostics and personalised medicine as two areas of direct relevance to clinical cardiology where NGS approaches will have an important and increasing role.

**DIAGNOSIS**

Conventional sequencing is already available for diagnostic use by cardiovascular clinicians in the UK, primarily for familial conditions. However, although genetic testing of probands and cascade screening of families with ICCs is recommended by the National Service Framework for Coronary Heart Disease and the National Institute for Health and Clinical Excellence, these tests are often not used. Commissioned funding for genetic testing in cardiology is needed to address this and is currently under discussion. NGS diagnostics for ICCs will most likely considerably reduce costs and increase availability if implemented in high-volume centres (table 1). For example, accredited NGS of all coding regions of the breast cancer genes **BRCA1** and **BRCA2** is available in the UK for £600 compared with £350 for conventional testing. Although accredited NGS analysis of ICC genes is not yet routinely accessible in the UK, it is available in other countries (eg, genedx.com and http://www.sistemagenomicos.com both offer a panel of 12 genes for long QT syndrome (LQT) (KCNQ1, KCNH2, SCN5A, ANK2, KCNJ2, KCNE2, KCNE1, CACNA1C, CAV3, SCN5A, AKAP9, SNTA1); see also http://pcpgm.partners.org and http://www.correlagen.com/). The benefits of NGS in terms of a more comprehensive genetic analysis at reduced cost are likely to be striking for genetically heterogeneous conditions such as LQT, hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) (table 2).

**Mendelian disease**

Disease aetiology lies on a continuous spectrum from genetic to environmental. A purely genetic disease arises when a fully penetrant mutation in a single gene is sufficient to produce disease and is inherited in a Mendelian fashion. Mutations are typically in protein-coding regions of the genome and cause substantial changes in protein function by altering splicing, truncating the protein or substituting amino acids within critical functional domains. Although variation in a single gene may cause disease, many ICCs are genetically heterogeneous: autosomal dominant HCM may be caused by variants in at least nine genes, LQT by variants in at least 13 genes, and DCM by variants in more than 20 genes.

Determining whether a particular variant causes disease is one of the greatest challenges of contemporary clinical genetics, largely due to our limited understanding of the spectrum of rare but non-pathogenic genetic variation. This will become an increasing challenge as we are inundated with NGS data from disease-causing genes from research and diagnostics laboratories. Geneticists have traditionally relied on identifying co-segregation of a candidate variant with disease phenotype in a sufficiently large family, but at the population level this is not possible and we will increasingly need large and well-annotated databases to ascribe probabilistic disease scores to variants. Assigning causality to a variant is further complicated as many ICCs are not completely penetrant (ie, not all individuals carrying a mutation manifest the disease), and variants in several genes may act together to cause disease or modify expressivity. NGS analysis of comprehensive panels of genes associated with a phenotype represents a timely advance, providing the tools to address these shortcomings, but also presenting major informatic challenges (table 2).

**Cascade screening**

Alongside guiding treatment of the proband, a benefit of identifying a causative mutation in a patient is the ability to perform cascade screening in relatives. Many ICCs are inherited in an autosomal dominant fashion, so that many relatives are at risk and should be screened. However, the variable penetrance of these conditions means that screening based on phenotype alone is unreliable. Once a causative variant has been identified, genetic testing allows accurate diagnosis of family members. Importantly, family members who do not carry the causative mutation may be reassured and confidently discharged from follow-up. As cascade screening only requires confirmation of the presence or absence of the known causative mutation, it remains amenable to conventional sequencing or genotyping at low cost, although NGS may be needed to make the initial diagnosis.

Cascade screening can pose clinically challenging decisions for asymptomatic and phenotypically normal relatives carrying a potentially disease-causing variant. A recent study showed that relatives of patients with LQT who carry the causative variant but who have a normal QT interval are at increased risk of sudden cardiac death (SCD). At present, if a relative of a patient with an ICC is found to carry the same genetic variant as the proband but is clinically unaffected, they are usually followed clinically for an indefinite period but treatment would seldom be advised in the absence of any phenotypic features. In the NGS era we will gather much more longitudinal data on these types of individuals that, together with an increased understanding of the role of disease-modifying genes, may allow us to better advise them.

**Molecular autopsy and sudden unexplained death**

In many instances post-mortem examination of SCD victims reveals a morphologically normal heart and relatives are risk-stratified on the basis of a clinical assessment of limited sensitivity. Molecular autopsy can be used to identify occult causes of SCD and may prove a valuable adjunct to clinical assessment. A recent literature review concluded that about 50% of autopsy-negative cases of sudden unexplained death and 10% of sudden infant deaths may stem from mutations in genes associated with susceptibility to LQT or catecholaminergic polymorphic ventricular tachycardia. Given the marked decrease in cost of sequencing using NGS, it is not difficult to envisage that all cases of sudden death will be screened for causative mutations, which would have very important implications for the families.

**Prenatal diagnosis of structural cardiovascular disease and ICCs**

Recent studies have reported non-invasive prenatal diagnosis (NIFD) by genetic analysis of free fetal DNA isolated from maternal plasma. This avoids the increased risk of miscarriage associated with conventional invasive prenatal testing but, without NGS, the application is largely limited to screening
for specific variants inherited from the father or arising de novo in the fetus, as discrimination of fetal and maternal alleles and aneuploidy detection are extremely challenging. NGS has already broadened the application by enabling detection of trisomy 21, suggesting that in the future NGS may permit screening for multiple conditions using NIPS. Although in its infancy, the ease and safety of sample collection for NIPD could lead to an increased uptake of prenatal diagnosis with wide-ranging implications.

If the fetus carries a known pathogenic mutation, the parents face the difficult decision of whether or not to continue with the pregnancy. Preimplantation genetic diagnosis (PGD) in the context of in vitro fertilisation (IVF) circumvents the issue of termination of pregnancy following prenatal diagnosis as embryos derived by IVF are tested for a specified genetic disorder prior to transfer to the uterus. The Human Fertilisation and Embryology Authority maintains a list of conditions for which PGD is licensed, which includes several conditions involving the heart such as Fabry disease, Barth syndrome, Ehlers-Danlos type IV and cardiac valvular dysplasia. Hence, for the next generation cardiologist, the use of NGS for NIPD and PGD may become increasingly important in the context of ICC and grown-up congenital heart disease clinics.

PERSONALISED MEDICINE

Molecular diagnostics and stratified medicine

Many diseases are heterogeneous and can be usefully classified into subtypes on the basis of features that predict disease progression and treatment response—for example, type 1 versus type 2 diabetes or ST elevation versus non-ST elevation myocardial infarction. Identification of the specific molecular mechanism underlying an inherited disease provides another level of information for stratified medicine approaches. Molecular classification has enabled subtype-specific treatment for the three commonest LQT syndromes—genotype-specific SCD risk prediction in HCM and the identification of rapidly progressing subtypes of DCM requiring early transplant assessment. As we gather more phenotype-genotype profiles in rare diseases (eg, LQT 4–15), genotype-specific risk calculators may be developed. Treatment may also be targeted to correct a specific molecular defect, as is already the case for some cardiovascular diseases including monogenic hypertension and monogenic type 2 diabetes.

Stratifying risk in common diseases

In common diseases, unlike Mendelian disease, carrying a specific genetic variant is not sufficient to cause the disease. However, the variant may contribute to the total disease risk, for example, by altering gene expression levels or modifying the impact of environmental risk factors. Recent genetic studies have identified a number of genetic variants that are associated with common cardiovascular diseases, including coronary artery disease, hypertension, diabetes and arrhythmia. A number of studies have assessed the utility of genetic risk stratification by comparing the predictive value of conventional risk factors against risk factors plus genotype, but the additional power from current genetic markers is marginal. This is because the effect size associated with individual genetic variants is typically very small. Conventional risk factors often also assess family history, and a family history effectively interrogates many more genetic loci than can be meaningfully tested in a laboratory. In the near future, deep sequencing with NGS will probably identify variants of larger effect size than those currently known, which may be more informative of risk. In situations where conventional risk stratification is limited, such as assessing risk of SCD, NGS assessment of risk may be particularly informative.

Pharmacogenetics

Of particular interest to clinical cardiologists are genotype-specific drug responses for commonly used drugs such as clopidogrel, warfarin and statins. Common genetic variants in CYP2C19 reduce conversion of clopidogrel to its active metabolite, and studies have suggested that poor metabolisers treated with clopidogrel after acute myocardial infarction have higher rates of subsequent cardiovascular events than normal metabolisers, leading the FDA to issue a black box warning advising physicians of the availability of CYP2C19 genotype testing. While subsequent studies have led to divergent opinions on the interpretation of these results, it remains a powerful illustration of the application of genomic information in routine clinical practice. In the case of warfarin therapy, it is clear that genotype can predict the dose required to achieve target INR, but there is not yet evidence of associated improved clinical outcome. For statins, the genotype identifies a subset at increased risk of specific statin-induced myopathy for whom an alternative lipid-lowering therapy may be more appropriate. It is clear that the next generation cardiologist will increasingly rely on pharmacogenetics to guide therapy, often at the point of care.

IMPLICATIONS FOR TRAINING AND ETHICAL ISSUES

An increase in the availability of genetic testing for clinical diagnostics and personalised medicine requires genetic literacy among cardiologists, together with increased support from clinical geneticists and genetic counsellors, most likely embedded within cardiology services. Speciality training will have to ensure that trainees are able to interpret genetic information in the context of rare and common diseases. For more applied uses, trainees will need to understand the implications of variable penetrance, genetic heterogeneity, compound and digenic heterozygosity and the fundamentally probabilistic nature of the risk conferred by variants. This will require a large investment in training schemes and development of a teaching base of experts.

Genetic testing plays an important role in the management of inherited diseases, but there are many caveats. One area of concern is genetic testing in children. A negative test may free a child from follow-up or invasive investigation but a positive result may have complex ramifications. For instance, many ICCs have low penetrance, so a positive result could result in lifestyle restrictions and anxiety for a child who may never become symptomatic. On the other hand, delayed testing for childhood-onset conditions may lead to a delay in appropriate management. These potential benefits and burdens must be considered carefully.

Another concern is that genetic testing could produce unexpected information that may not be directly relevant to the original clinical question. Extreme examples include unexpected non-paternity or consanguinity. As NGS costs fall, we may
move towards whole exome or whole genome sequencing and this could lead to the possibility of collateral findings such as a mutation in a cancer-causing gene during investigation for HCM. How will we report this result to the patient and do they need counselling about this eventuality? Perhaps consent for results relating only to the disease genes of interest should be obtained prior to whole exome or genome sequencing?

There are also potential epigenic connotations as seen following the widespread use of fetal ultrasound in south east Asia where there has been a significant decrease in the female birth rate.58 With the advent of NIPT and the potential for screening panels of many genes, there are obvious concerns of ‘designer babies’ and stringent ethical frameworks for the reporting of results will need to be developed and implemented. Finally, study results may only be applicable to individuals of the same genetic background as the study population. It has been suggested that knowledge of the prevalence of variants in different ethnic groups could in theory lead to differential drug development for alleles associated with wealthy populations.

CONCLUSIONS

NGS is a mature technology with immediate implications for clinical genetics and should yield significant improvements in patient care. NGS will not be a stand-alone technology for advancing genomic medicine, and rapid genotyping assays will be increasingly important to guide drug choice and dosage. It would not be an overstatement to say that there are huge challenges ahead, and substantial investment in informatics, infrastructure and training are needed if we are to realise the enormous potential of NGS technologies and genomics for improving patient care.

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Competing interests

None.

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