The growth of molecular diagnostics: Stratified Medicine Programme, the 100,000 Genomes Project and the future

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Abstract

There is a steady increase in the application of molecular diagnostics to supplement histological diagnosis. In the UK, recent large-scale genomic programmes are paving the way for more consistent and global genomic assessment of cancer samples as standard of care. The aim of this review is to provide a brief overview of the development and breadth of genomic technologies and the challenges encountered in their application. It will describe the key genomic alterations detected, their relevance and the challenges involved in interpretation.

It will review several large scale sequencing initiatives that have generated a body of data across different cancer types allowing a better understanding of the complexity of genomic aberrations in tumours and how these changes relate to tumour behaviour and potential treatment strategies.

In the UK, molecular diagnostics is being embedded in the NHS, first through the Cancer Research UK Stratified Medicine Programme and now through the 100,000 Genomes Project. The opportunities, challenges and lessons learnt from these ambitious programmes will be discussed.

The wider application of genomic sequencing is changing the face of clinical trials and paving the way for precision medicine; some of the major trials are reviewed and their potential impact for future studies.

Finally, we will discuss the challenges of implementing more comprehensive molecular diagnostics in the NHS and what this might look like.

Keywords 100,000 Genomes Project; basket trials; Stratified Medicine Programme; umbrella trials; whole exome sequencing; whole genome sequencing

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The development of genomic technologies

There have been huge advances in molecular technology over the last 50 years (Figure 1). Sanger sequencing, also known as the chain termination method, was developed by Frederick Sanger and colleagues during the 1970s and described in a seminal paper in 1977.[1] During Sanger sequencing, which uses four separate reactions (one for each base), DNA polymerases are used to copy single-stranded DNA templates by adding base-specific deoxyribonucleotide triphosphates (dATP, dTTP, dCTP, dGTP) to the growing chain from the 3′ end of a labelled oligonucleotide primer that anneals to the template. DNA polymerase can also incorporate analogues of nucleotide bases and this characteristic is exploited by the inclusion of dye labelled dideoxyribonucleotide triphosphates (ddNTPs) into the reaction mixtures. ddNTPs are chemically modified nucleotides in which the 3′ hydroxyl (OH) has been removed from the deoxyribose. When ddNTPs are incorporated at the 3′ end of the growing chain, the absence of a functional OH group prevents formation of a phosphodiester bond with the incoming dNTP, selectively terminating DNA replication at either A, C, T or G. This results in a collection of DNA fragments, varying in length, each with the base specific ddNTP at the 3′ end.[2] Resulting fragments are separated on the basis of size using gel electrophoresis and visualised using autoradiography or UV light, allowing the DNA sequence to be read directly from the X-ray film or gel image.[2] Despite having the capacity to sequence single genes (single-plex) and allow highly accurate analysis of relatively long stretches of DNA (each run can sequence up to 1000 bp) there are limitations with this method: it is low-throughput and therefore unable to deal with large sequence output. It is generally expensive and, despite its simplicity, is laborious, therefore limiting clinical sequencing tests to single genes or small regions of the genome.

Chemical sequencing method: Maxam and Gilbert

Maxam–Gilbert sequencing is a non-enzymatic, chemical degradation DNA sequencing method developed by Allan Maxam and Walter Gilbert in 1977.[3] In this method (also involving four different reactions, one for each base), P^{32} radio-labelled DNA fragments are chemically cleaved by base specific purine and pyrimidine attacking reagents (piperidine which selectively attacks A and G, and hydrazine which selectively attacks C and T). The DNA fragments are then separated using gel electrophoresis and visualised using autoradiography allowing the DNA sequence to be deduced in a similar way to Sanger sequencing.[4] The main disadvantages to Maxam–Gilbert DNA sequencing are technical complexity, the use of toxic chemicals piperidine and hydrazine to cleave the DNA at consecutive bases, and use of radioactive labelling of the DNA fragment.

The Human Genome Project

The Human Genome Project (HGP) was launched in 1990 with the aim of establishing the order of the human genome's 3 billion base pairs, creating a detailed annotation and thus a 'reference sequence' within 15 years. The International Human Genome Sequencing Consortium (IHGSC) published the first draft of the human genome in *Nature* in early 2001 with the full sequence completed and published in April 2003.[5,6]

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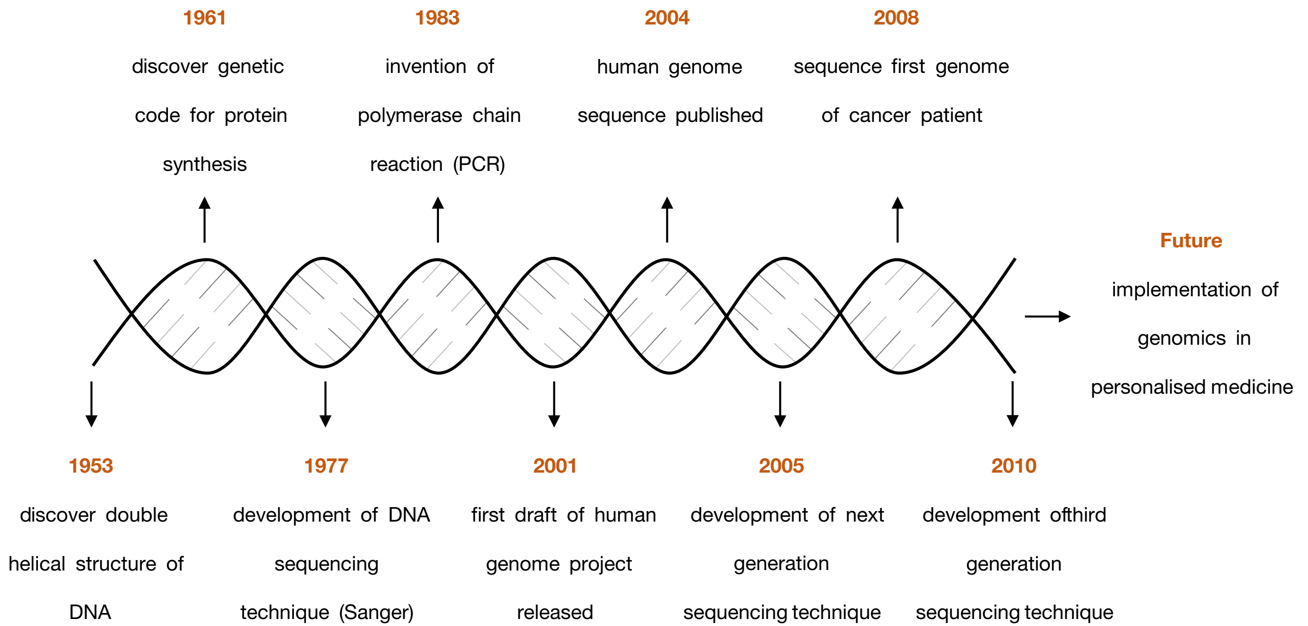

Figure 1 The History and Future of DNA Sequencing. Plots some of the key landmarks in the development of genomic technologies.

The HGP used an almost identical sequencing method introduced by Sanger in 1977. However, the length of time and high cost it took to complete the project (13 years and an estimated $3 billion) clearly illustrated that there was an urgent need to move away from 'first-generation' single gene Sanger sequencing and move towards the development of more cost effective, efficient and higher-throughput sequencing methods capable of sequencing a large number of individual genomes. These newer methods are termed next-generation sequencing (NGS), or massive parallel sequencing and offer a major advantage over the traditional Sanger method in terms of the sheer volume of data that can be produced per instrument in a relatively short space of time for significantly less in terms of cost, so allowing the rapid sequencing of large regions of the human genome in multiple samples at any one time.

Next generation sequencing technologies (Figure 2)

Next generation sequencing (NGS) is based on massive parallel sequencing of DNA followed by processing of the data and sequence alignment to a reference genome. NGS allows the

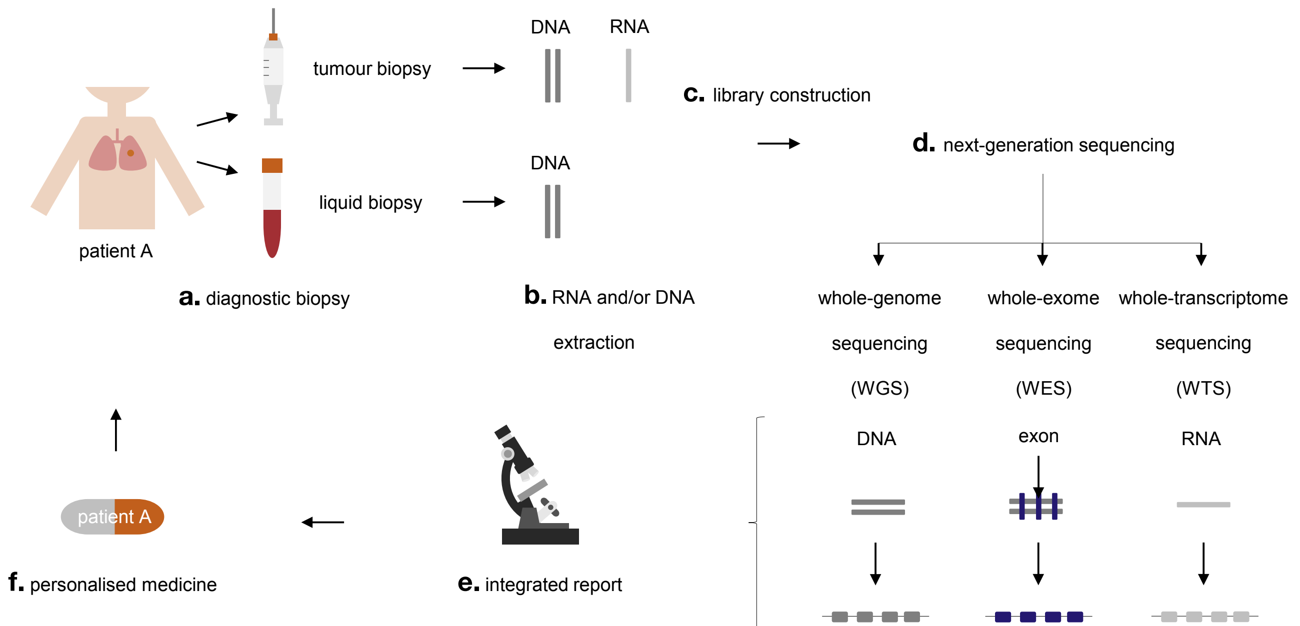

Figure 2 Implementation of Next-Generation Sequencing in Personalised Medicine. Isolated tumour DNA and RNA, and DNA from blood is used to construct libraries for sequencing using NGS technologies. NGS can be used to look at the entire genome (WGS), protein-coding regions (WES) or the transcriptome (RNA-Seq). Information gained from the molecular profile of the patients tumour can help guide clinicians in treatment decision making and a more personalised approach to cancer treatment.

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