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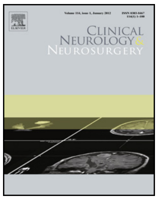

Next generation sequencing for neurological diseases: New hope or new hype?

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ABSTRACT

Over the past year huge advances have been made in our ability to determine the genetic aetiology of many neurological diseases through the utilisation of next generation sequencing platforms. This technology is, on a daily basis, providing new breakthroughs in neurological disease. The aim of this article is to clearly describe the technological platforms, methods of data analysis, established breakthroughs, and potential future clinical and research applications of this innovative and exciting technique which has relevance to all those working within clinical neuroscience.

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1. Introduction

Progress in our understanding of the genetic basis of neurological disease has expanded rapidly over the past 20 years. Whilst it is now clear that there is an extensive spectrum of genetic involvement in the aetiology of neurological disease, the common paradigm employed in most studies has been to essentially divide the disorders into two dichotomous groups. Firstly, ‘common’ neurological diseases with complex phenotypes and a probable multigenic component to their aetiology (such as Parkinson’s disease, multiple sclerosis and epilepsy), and secondly ‘rare’ neurological diseases which are perceived to have a more narrow phenotype and obey strict Mendelian laws of inheritance.

For the investigation of many common, complex diseases, candidate gene and subsequent genome-wide association studies (GWAS) have been widely adopted identifying an extensive list of loci associated with neurological disease [1–6]. Such studies have assisted in determining mechanisms of disease, however, most identified alleles are predicted to have a very small effect size [7], and infrequently lead to the discovery of causal polymorphisms [8].

Approaches to identify the genetic basis of rare Mendelian disorders have advanced slowly but surely over the past decade, being largely based on well-established techniques such as positional cloning and linkage analysis followed by targeted candidate gene screening. Cloning genes were critically dependent on identifying large well-characterised pedigrees, groups of pedigrees with a presumed identical genetic aetiology, or consanguineous families with a disease likely due to homozygous mutations. To some extent, finding the gene was a matter of “luck”, based on the pedigree structure, compounded by the cost and physical time taken to sequence a large number of genes, which severely limited progress. As a result the genetic basis of less than 50% of all Mendelian disorders has been determined [9], and this has particular relevance in neurology. For example, in the USA over half of patients recently recruited to a national clinical programme tasked with determining the genetic basis of rare diseases have neurological disorders; by far and away the more prevalent specialty [10]. Our own observations echo this estimation, with approximately half of the patients in our neurogenetic clinic having no confirmed molecular diagnosis (*unpublished observations*).

However, this last year has seen a paradigm shift in the investigation of these rare Mendelian disorders, largely based on the technical advance of a new DNA sequencing technology termed ‘next generation sequencing’ (NGS), also known as deep resequencing, or massively parallel sequencing, which is revolutionising the investigation of rare disorders [11]. NGS may also enable the elucidation of the contribution of rare alleles in common disorders, potentially offering significant breakthroughs in our understanding.

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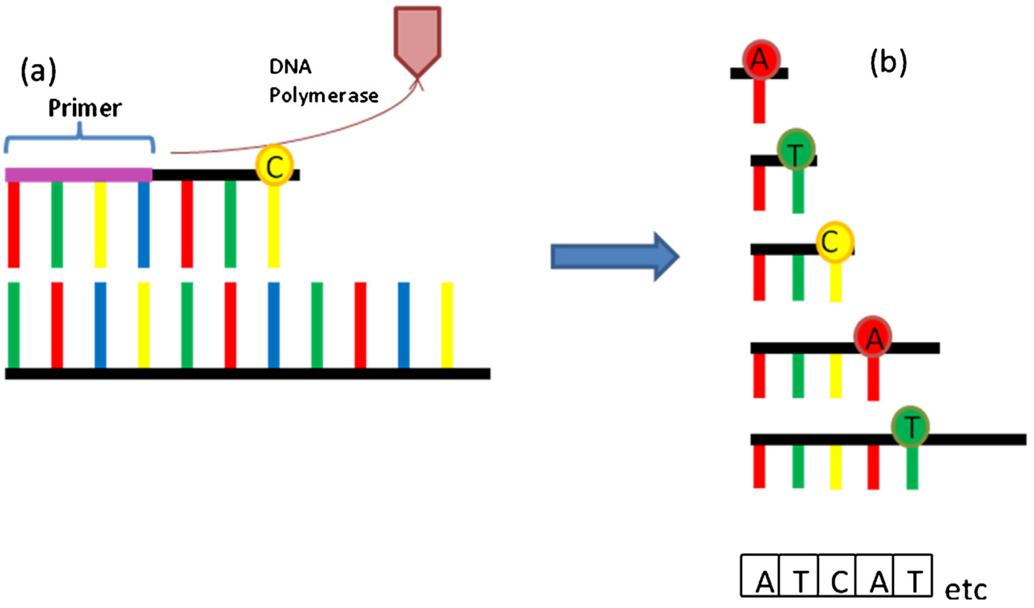

Fig. 1. (a) A DNA primer anneals to the template sequence of DNA. DNA polymerase binds incorporating free nucleotides into the DNA strand. The strand elongates until randomly a fluorescent labelled nucleotide in incorporated, and its chemical alteration results in termination of the DNA strand. (b) DNA strands are read in order of chain length (top to bottom in diagram) and the sequence is therefore determined (ATCAT).

The aim of this article is to review the most widely used approach, its role for rare Mendelian neurological disorders, and its potential for wider use across the continuum of neurological disease.

2. A new technique

Next generation sequencing has been evolving since 2005 [12] and provides the ability to dramatically increase the speed at which DNA can be sequenced at a fraction of the cost of older sequencing technology. To illustrate this, in 2001 the Human Genome Project used first generation Sanger sequencing technology to sequence the human genome, taking 13 years and $2.7 billion to achieve its goal [13–15]. Next generation sequencing can now sequence an individual genome in under 2 weeks for approximately US$ 4000, representing remarkable progress.

However, based on current thinking, the whole genome does not need to be sequenced to identify most human disease genes. Eighty-five percent of pathogenic mutations causing Mendelian disorders are found within the segments coding for proteins (exons) [16], which collectively are referred to as the “human exome” [17]. This dramatically cuts down the region that needs to be sequenced in patients and families with undiagnosed neurogenetic disorders, reducing the cost and time to approximately $1500 and 48 h

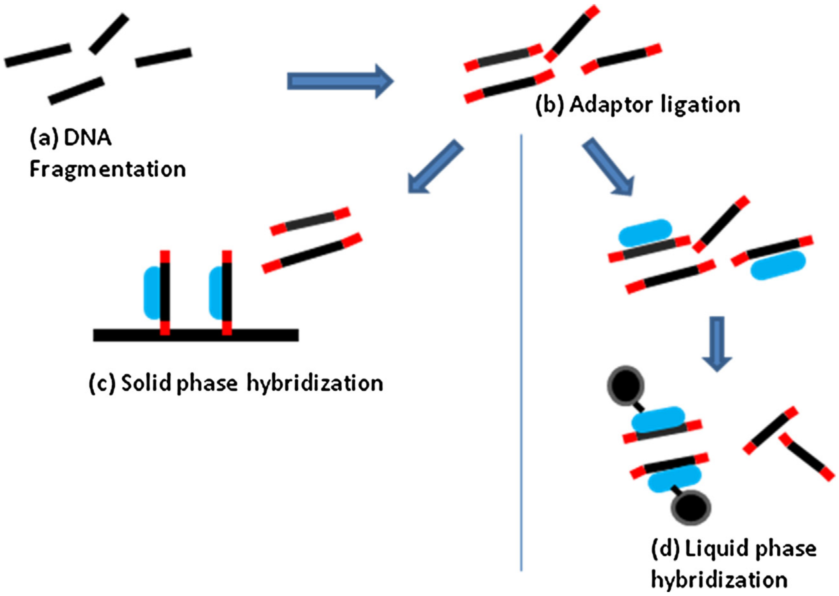

Fig. 2. An example of two different DNA enrichment approaches though numerous methods are available (a) DNA is randomly fragmented (black lines). (b) Adaptors are ligated to each end of the DNA (red lines). (c) Solid phase hybridisation occurs on a DNA microarray. A collection of DNA spots/bait probes (blue line) bind to DNA regions from the exome but not intronic fragments of DNA which are washed away. Thereafter the exomic DNA is eluted. (d) Liquid phase hybridisation. DNA probes (blue lines) attach to DNA fragments from the exome. Thereafter, streptavidin beads (black and grey circles) are added to allow physical separation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

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