Unravelling the Tangle of Genetic Testing

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In the past 60 years the identification of the genetic basis of various diseases has steadily increased. Whilst great strides have been made in sequencing the whole human genome project, the identification of disease causing mutations especially for multifactorial conditions is still in its infancy. On the other hand, in the majority of genetic disorders where the causative gene is known, molecular genetics tests are available with which to identify the causative mutations.

'Do you know of anyone else in your family that has a similar condition?' This question has become almost a routine one in clinical practice. It reflects the growing awareness that practically all pathological conditions have some genetic background. Though most probably the inheritance of physical characteristics must have been known since time immemorial, the major breakthrough in the identification of the units of inheritance (genes) came about in the mid 19th century, when the Augustinian monk Gregor Mendel carried out his noted pea-breeding experiments. Through his observational and experimentation work, Mendel set the basic principle of one gene one trait.

Though the concept of the gene was established at around this time, it took almost 100 years before the first experimental evidence that DNA transmits genetic information was published by Avery et al[1] of the New York's Rockefeller Institute followed by Franklin and Gosling[2] and Wilkins et al[3], of King's College in London, who through x-ray diffraction patterns of DNA showed that it had a regular and helical structure. In 1953, armed with this information and knowledge of the chemistry of DNA, James Watson and Francis Crick[4], then at the Medical Research Council laboratories in Cambridge, England, proposed a model composed of two helically twisted strands connected to each other by a number of molecular rungs. These rungs were made up of either an adenine (A)-thymine (T) or guanine (G)-cytosine (C) base pair. Thus the theory that it is the order of these G, A, T and C bases on the DNA strand that determined the genetic make up of every living organism was born. This arrangement could also account for the way DNA strands and thus the genes, were copied and transmitted to the offspring. The next piece of the jigsaw puzzle was put in place in 1966 with the identification of the triplet of bases (e.g. CTG) that code for particular amino acids (in this case leucine) or else for the start (usually ATG) or stop (TGA, TAG, TAA) of protein coding regions.[5]

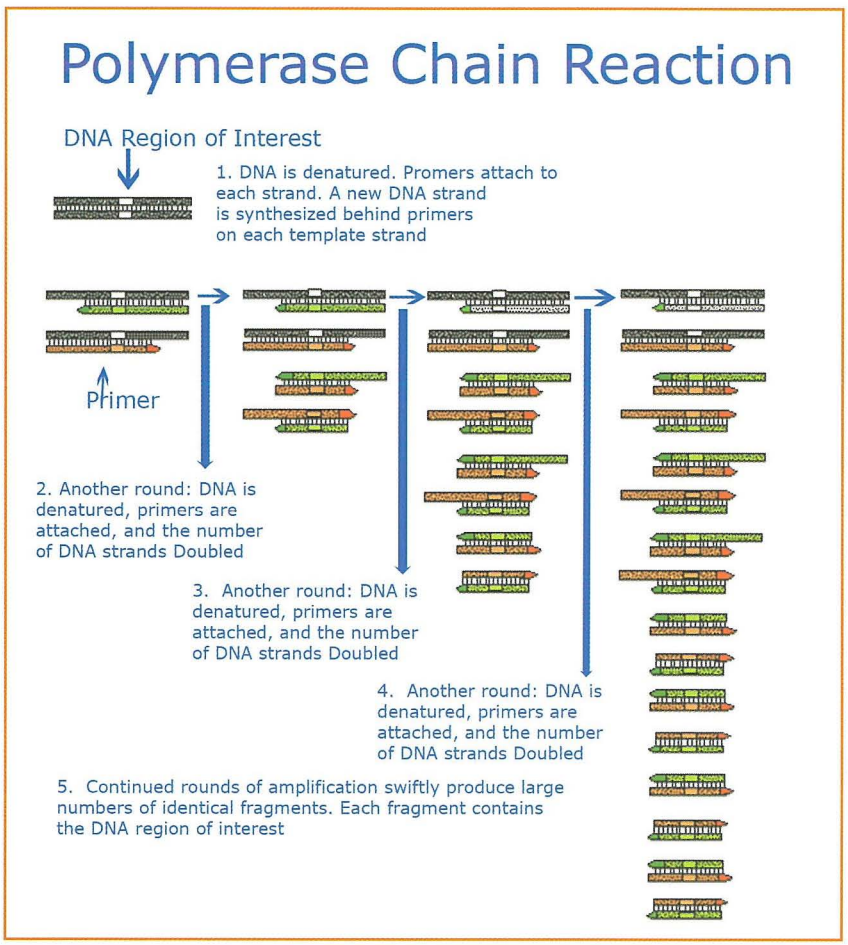

Figure 1. *The steps and reactions in the Polymerase Chain Reaction (adapted*

The scene was thus set for the hunt for disease-causing genes. Initially the tools that the molecular geneticist could use consisted of enzymes called restriction enzymes (RE), ligase and heat labile DNA polymerase. RE are enzymes present in eukaryotic cells that protect them against invading phages (bacterial viruses). These enzymes recognise specific sequences and cut the DNA strand. The ligase could then ligate the fragment with other fragments and the polymerase could replicate the fragments. These tools enabled researchers to digest genomic DNA and then separate the fragments by size through gel electrophoresis as well as introduce DNA fragments into bacteria for amplification followed by sequencing. With these primitive tools, researchers started to recognise background patterns of RE sites (haplotypes) that were linked to certain genetic disorders. In the late 1970's and early 1980's these techniques enabled researchers to identify the first molecular causes of genetic disease.

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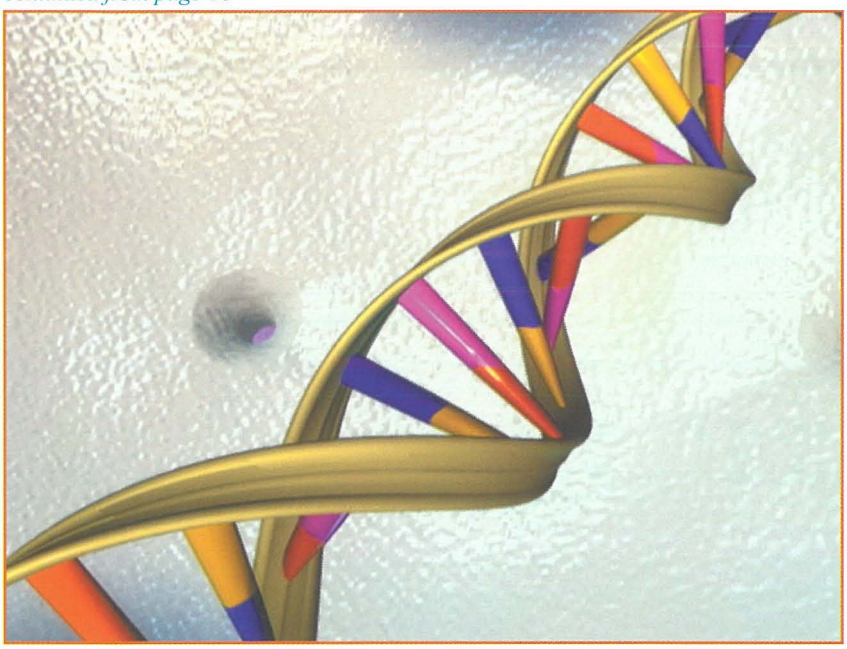
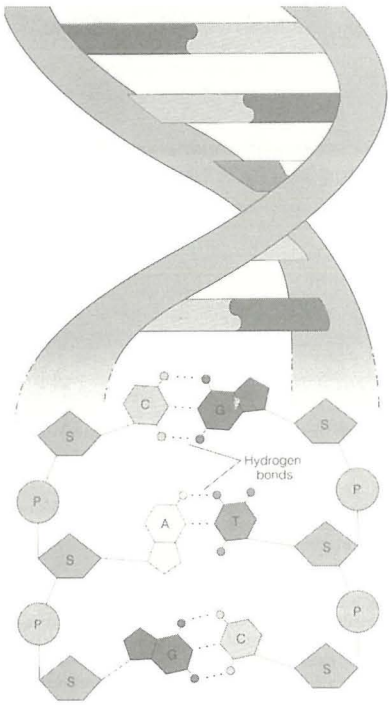


Since then, it was a rapid progression both in techniques and identification of possible mutations that could cause genetic disorders. The pivotal discovery was the principle of the Polymerase Chain Reaction (PCR) in 1986 by Mullis et al.[6] This technique utilises four components - the template DNA (usually full genomic DNA), two short DNA fragments (primers) that are synthesised in the lab, a buffer solution containing the nucleotides, and heat stable DNA polymerase. The genomic DNA can be obtained from any cell (apart red blood cells – they contain no nucleus) of the body and even from one single cell. The primers are two short (15 to 30 nucleotide-long) fragments of DNA that are artificially synthesised. The sequences of these fragments are chosen so as to be complimentary to the 3' region upstream of the gene of interest in both strands.



The sequence of events in PCR (figure 1) involves a cycle (25 to 30) of heating and cooling steps. The initial step is that of heating the mixture of genomic DNA, primers, nucleotides and polymerase enzyme to a temperature, high enough to obtain complete separating of the two complimentary strands of DNA (this step is called denaturing and usually takes place between 95°C and 99°C). The mixture is then cooled to a temperature that is optimal for the two primers to anneal to their respective complimentary sequence (usually between 63°C and 68°C). The last step brings the mixture to the ideal temperature for the polymerase to build a new complimentary strand starting from the annealed primers (depends of the enzyme which is used but the ideal temperature of the most commonly used enzyme – Thermus aquaticus (TAQ) polymerase – is 72°C). With every cycle, the number of fragments of interest doubles up and thus after 25 cycles and starting from the genomic DNA from one single cell, one would, theoretically, end up with 2^{25} (33,554,432) copies of the fragment of interest within 3 to 4 hours. This technique opened the doors for various important research and diagnostic techniques, including large scale, rapid sequencing as well as rapid methods to identify mutations.

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