Diagnosis and treatment using DNA technology

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Contents

Abstract

Genetic conditions are caused by defects in ones genes. These can be in the form of tiny point mutations such as SNPs to large insertions and deletions of from a gene, which effects the resultant protein. Much progress has been made in the area of recognising which mutations cause or lead to which disease and as this knowledge grows we can begin to diagnose more and more using DNA technology. Powerful tools have been created, and are forever improving, to detect these mutations. Such techniques include micro-arrays, FISH, shotgun sequencing, PCR and multiplex PCR, all of which perform the complex task of finding the sequence of DNA in ones cells. Gene technology is also being used to treat genetic disease by altering or replacing defective genes with functioning ones. Gene therapy to treat leukaemia, which involves inserting DNA to fight the offending cells, and gene therapy to treat Cystic Fibrosis, which involves inserting a normal gene are explored here.

Introduction

The use of DNA technology is fast becoming one of the most important tools in the diagnosis and treatment of genetic disease. Its use as diagnostic tool is increasing all the time and is seen as a vital tool for early, late and even pre-disease diagnosis. As genetic diseases are caused by defective genes, simply analysing ones DNA we can find mutations that cause specific diseases. We need to know what we are looking for, however, so many hours of scientific research has been spent to determine and catalogue these genetic defects. Treatment using DNA technology such as gene therapy, however, is not as widely used yet but with many clinical trials proving successful it won't be long until this powerful tool will become part of mainstream treatment.

Disease causing defects

There are several different problems that can occur in a DNA sequence, causing incorrect protein formation which ultimately leads to disease.

SNP

Single Nucleotide Polymorphism (SNP) = A SNP is the replacement of a single nucleotide. (See figure 1). These are the thought to make up 90% of all mutations. As we will see later, SNP's are also involved in the treatment of certain diseases.

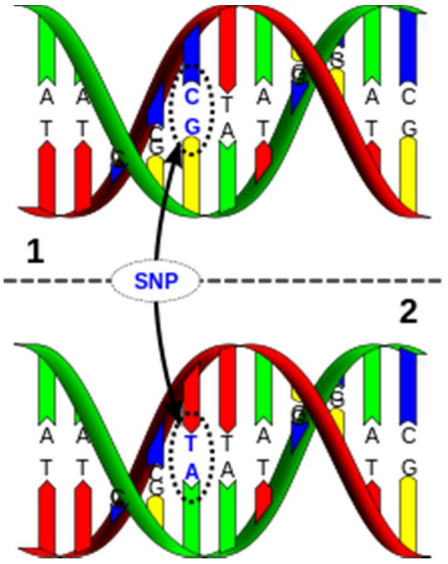

Figure 1 A SNP is the difference of one DNA molecule (1)

Deletions

A deletion is when a part of the chromosome or DNA sequence is missing. This can be anything from a single base pair right up to a whole piece of chromosome. An example of this is Angelman syndrome, which is caused by a deletion of approximately 90K base pairs from chromosome 15.

Insertions

Insertions are the opposite of deletions in that some extra base pairs are added into a DNA sequence. Like deletions these two can be large or small sections.

Substitution

One (SNP) or more bases being incorrectly represented in a sequence or "substituted", forming a defective gene.

Frameshift

Frameshift is caused when the number of insertions or deletions is not divisible by three. This causes the codons to group differently and thus affects the resultant protein. As we can see in figure 2, the insertion of the codon uracil produces two different amino acids in the resultant protein.

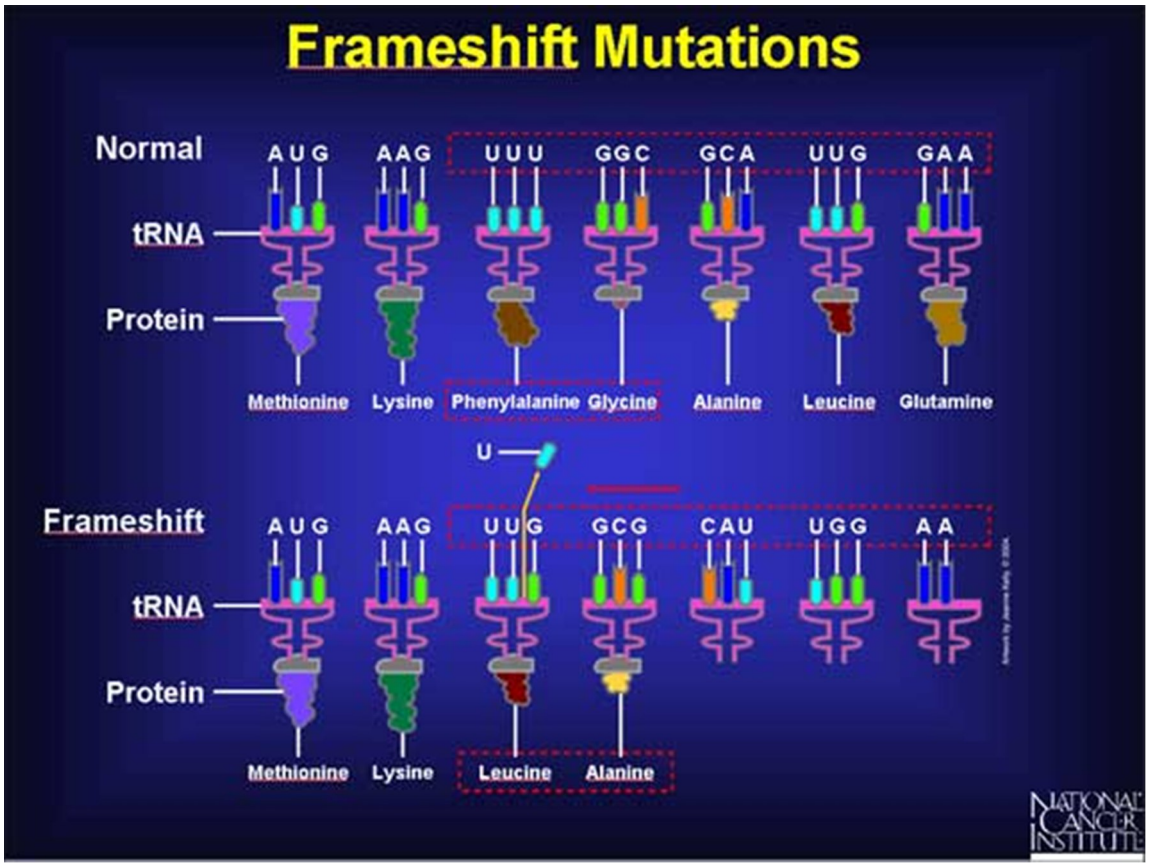

Figure 2 how a single insertion can affect the resultant protein (3)

DNA techniques used for diagnosis

DNA technology is being used to help diagnose a range of genetic diseases.

SNP analysis

SNP detection through molecular beacons makes use of specially designed probes. The molecule is designed so that only the probe's sequence is complementary to the genomic DNA to be used in the analysis.

If the probe sequence comes across its target DNA during the study, it will fluoresce, or shine brightly alerting the scientist to the presence of a SNP.

Micro-array

Even though every cell contains the same genetic material it is not always expressed in the same way. Micro-arrays produce a map of large sections of the genome for a cell which can be analysed for deletions and/or mutations. By applying normal and tumorous probes we can see the level that these cancer causing genetic defects exist with the subjects DNA. In the case of figure 3 if the spot is red, it signifies a target gene is more expressed in the tumour sample, meaning cancerous cDNA sequences. If a spot is Green, the gene is more expressed in the Normal tissue. Yellow indicates equal expression of each.

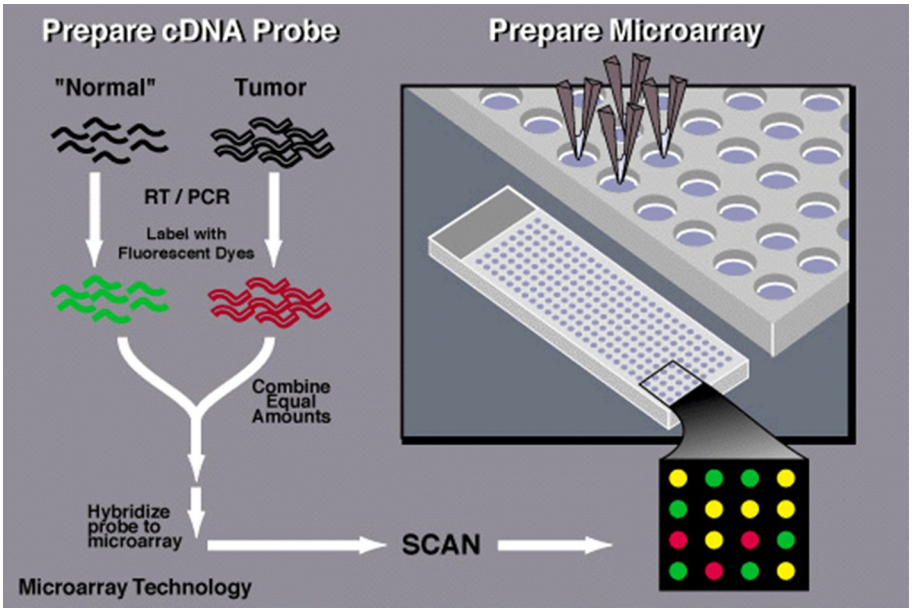

Figure 3 Micro-arrays to diagnose cancer

Micro-arrays are also used in treatment see Micro-arrays in treatment.

FISH

This involves using a florescent DNA or RNA copy of the area (sequence) to find the desired area so it can be analysed. This technique is more suited to large mutations rather than SNP's or short sections.

PCR

This is essentially creating copies of sections of DNA for use in analysis. This is especially useful when very little genetic material is available. Primers (short DNA sequences which are complimentary to the target area) can be used to copy an offending area and analyse it. For example, as a cystic fibrosis gene has 3 missing base pairs the resultant copy will be 151bp as opposed to the normal 154bp.

Sequencing

DNA sequencing is a method for mapping/finding the exact order of a strand of DNA. This is done to find mutations, such as SNP's or insertions. It is not done over the whole genome as are micro-arrays but is done when a specific target is sought. Next generation methods are replacing the older dye termination methods and are capable of sequencing millions of strands concurrently. Sequencing is can be used as a diagnostic tool for any disease caused by a defect in the DNA.

Multiplex ligation-dependent probe amplification (MLPA)

This involves using a multiplex PCR method to detect abnormal "copy numbers". These are areas of the DNA that have been incorrectly copied multiple times. A microarray may see this as a correct length gene even though it still has large deletions.

It is also particularly good at detecting small changes in DNA such as SNP's and very short insertions, whereas microarrays are better for detecting large mutations and deletions.

Treatment using DNA technologies

DNA technology is also being used to treat disease.

Gene therapy

Gene therapy is the pharmaceutical application of DNA to treat diseases. This is usually using a man-made gene, which is functional, to replace a mutated (defective) gene. This can be done in vivo, new DNA inserted directly into the tissue or ex vivo, where cells are removed from the body and cultured with the DNA (see figure 4. It can also be inactivating (switching off) genes that are incorrectly expressed.in a cell, which is causing disease. Vectors are used to bind these strands to the existing DNA of the cell, similar to the way a virus binds its genetic material to a cell. The main viruses currently used are Retroviruses, Adenoviruses, Adeno-associated viruses and Herpes simplex viruses. Some study has been done with non-viral vectors but these have been less successful. Certain vectors have been developed to deposit "good DNA" into a cell and thus prevent or reverse disease causing mutations or destroy cancerous cells. Most gene therapies are still in clinical trials, the main disease currently being cancer. Other areas of use are in respiratory and neurological disease.

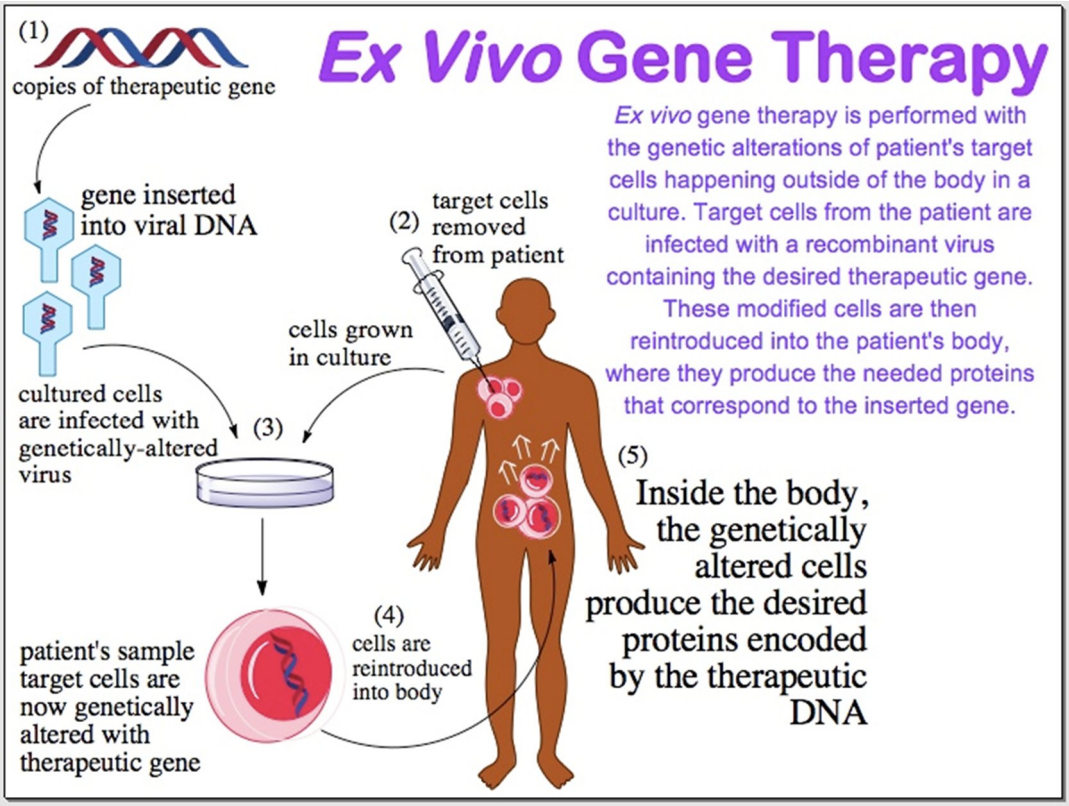

Figure 4 The Ex Vivo gene therapy process

An example of gene therapy development – Cystic Fibrosis (CF)

The first question to as ask is, is the disease caused by a genetic defect and how?

In the case of CF this is caused by a small deletion in the CFTR gene. Since CF is an inherited, recessive disorder two defective genes are required. This means we only need to add a normal copy of the CTFR gene rather than fix the mutated area.

Is it feasible to treat the affected area? Yes the lungs are particularly accessible so the genes could be delivered by an inhaler.

CF seems to be a good candidate for gene therapy, now we need to select a vector.

Since the CFTR gene is 4,443 base pairs it is small enough to fit onto any of the common viral vectors[1]. Of these viruses we can exclude viruses that don't:

- infect the airways
- that divide regularly
- enter a high percentage of cells
- cause an immune response

[1] Retroviruses, Adenoviruses, Adeno-associated viruses and Herpes simplex viruses

So we are left with only one, an adeno-associated virus, to use as our vector to deliver normal functioning CFTR genes to the lungs cells.

Gene Therapy to treat leukaemia

A new therapy, currently being trialled, involves identifying a molecule that is unique to a cancer cell, then modifying some genes in the immune system to create proteins that will attack this molecule. In acute lymphoblastic leukaemia - B-cells, which are a type of immune cell, become malignant. For this particular disease a surface molecule, only present on B-cells, called CD19 was identified. The process goes as follows:

1. Identify a molecule that is unique to the malignant cells
2. Design a gene that will attack any cells housing CD19 molecules
3. Select a cell type suitable for fighting B-cells. In this case it is T-cells, which are also immune cells.
4. Select a vector, such as a virus, suitable for inserting the gene into a cell, thus changing the DNA of that cell.
5. Install the new genes into these T-cells
6. The T-cells will now kill off all B-cells and with it the cancer

This method is currently being developed to treat other cancers with effectively this same process. The obvious limitation of this treatment is the cancer cells must have some unique identifiable trait for the new genetic creation to target.

Micro-arrays in treatment.

As we have seen it is possible to analyse a cells expression quickly and cheaply. We can then compare this to another cells expression for example treated/untreated or cancerous/non-cancerous. Using these methods clinicians can monitor the expression of cancerous cells while under treatment, which will allow them to modify the therapy based on the changes in expression.

Recombinant DNA

As the name suggests this process involves combining two or more strands of DNA, usually from different organisms. This is then attached to an appropriate vector as seen in gene therapy. For example recombinant DNA has been used to create erythropoietin (EPO), which controls red blood cell production, to successfully treat sickle cell anaemia.

DNA technology in the Future

Human Genetic Variation - 1,000 Genomes Project

There is a currently a project under way called the **1,000 Genomes Project**. It was initially aimed at sequencing 1000 people, hence the name, but is now aiming to sequence 2,500

people, which should cover approximately 98% of human genome variation. It is currently too expensive to “deep sequence” the entire genome, which is to sequence it 28 times (28x). This project plans to “light sequence” which is only 4 times (4x). They are looking for SNP’s and mutations that can be appear across a population and cataloguing this data. The data to come out of this will be used by scientists to localize more specifically the root cause of genetic diseases. It will also provide better SNP and probe choices for disease diagnostics.

There are thought to be two kinds of genetic variation, these are:

1. Simple genetic defects that cause diseases i.e. deletions causing cystic fibrosis
2. More complex defects that cause more complex problems such as Diabetes and heart disease.

It is specifically this second area that is not well understood, this is another of the goals of the 1000 genome project.

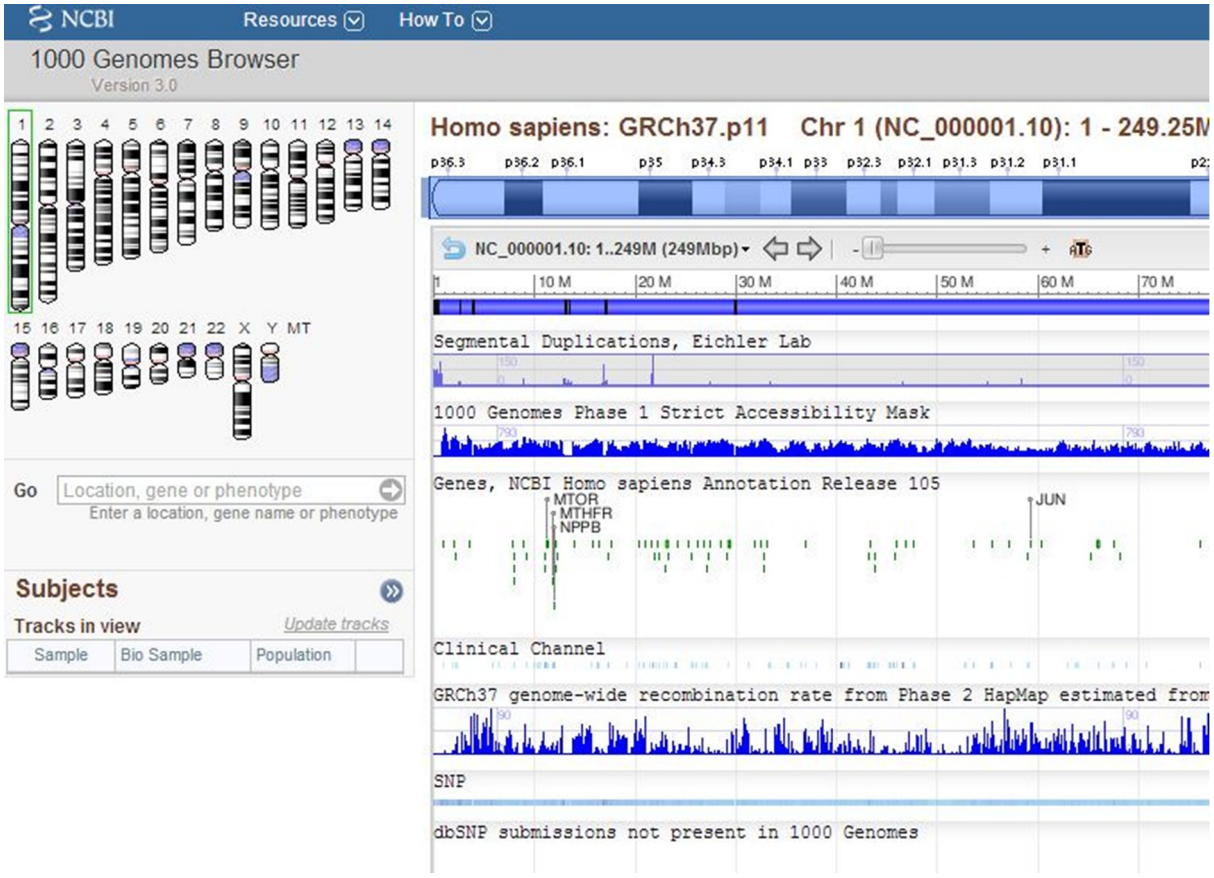

Figure 5 1000 Genomes browser (6)

Conclusion

Whilst there are many techniques used for diagnosis there are still a large number of genetic diseases that we cannot yet detect. Sometimes because they are rare, so very little research is being done, just too complex to detect with current equipment or the results are

not accepted to be accurate enough. We need to continue to advance this area as it is capable of early detection and/or prevention which go a long way to decreasing the severity and occurrence of such diseases. Gene therapy, whilst still largely used in clinical trials, is proving to be the next tool in the fight against diseases caused by defective genes.

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