Important points

- DNA genetic testing and genetic screening involve the same testing processes to confirm or refute a suspected DNA change
- Tissues tested include blood, skin, saliva and hair follicles and, prenatally, embryo, placental tissue and amniotic fluid; DNA can be tested using blood
- ***Genetic screening*** is done for a particular condition in individuals, groups or populations without family history of the condition
- ***Genetic testing*** is done for a particular condition where an individual is suspected of being at increased risk due to their family history or the result of a genetic screening test
- **Direct gene testing** looks at the presence or absence of a known gene mutation by examining the sequence of letters in the information in the gene
- The test is very accurate and used for diagnosis and screening including prenatal, genetic carrier testing and screening, presymptomatic and predictive testing
- Limitations include:
  - Interpretation of the test result eg. finding that a person has a faulty gene does not always relate to how a person is, or will be, affected by that condition
  - The testing may be time-consuming and expensive for the health service if not for the patient
  - For some complex conditions eg. cancer, the testing may have to be done on a family member with the condition to identify a family-specific mutation in the gene (mutation searching) before unaffected family members can be offered predictive testing
- **Indirect gene tracking (linkage)** relies on comparing DNA markers from family members with the condition to markers in unaffected relatives
- Used in situations where the gene itself has not been precisely located or where mutation(s) in a gene have not yet been defined; the test is not as accurate as direct gene testing but can be used in diagnosis including prenatal and presymptomatic and predictive testing
- It may not always be possible to find DNA markers that enable the scientists to tell the difference between the faulty gene copy and the working gene copy

Both genetic testing and genetic screening involve the same testing processes to examine an individual's chromosomes, DNA or the biochemical product of a gene, typically a protein to confirm or refute a suspected chromosomal, DNA or gene product change. See Genetics Fact Sheets 1, 4, 5 & 6 for an explanation of genes, chromosomes, mutations and chromosomal changes.

The difference between genetic testing and genetic screening is the target group for the testing.

- ***Genetic screening*** is done for a particular condition in individuals, groups or populations without family history of the condition
- ***Genetic testing*** is done for a particular condition where an individual is suspected of being at increased risk due to their family history or the result of a genetic screening test

How is the testing done?

Different types of genetic tests are used depending on whether an individual's chromosomes, the protein-product of a gene, or the DNA itself are examined.

Body tissues used in testing depend on the particular test:

- The examination of chromosomes (called cytogenetic testing) is usually carried out on blood or, in the case of testing in pre-pregnancy or pregnancy, on the embryo, amniotic fluid or chorionic villus material (see Genetics Fact Sheets 17C and 18)
- Blood is usually also the source for determining if a protein is either absent, present in abnormal amounts or has a changed structure. Occasionally other body fluids or tissues need to be examined
- DNA to be tested can be extracted from the cells of a variety of body fluids or tissues.

  While the majority of tests are carried out using DNA from blood cells, cells obtained from the lining of the cheek using a mouth-wash or the cells in the roots of an individual's hair may also be sources of DNA

Testing the DNA

Figures 21.1 and 21.2 show the basic principles underlying how DNA testing is done.

Step 1:

In the laboratory, using enzymes that are chemical 'scissors', the DNA is cut into hundreds of small pieces (*Figure 21.1*) at sites where there are specific sequences of the DNA letters (usually 4-6 letters in length).

As everyone's DNA has some small differences, the sites may be at different places in people's non-coding DNA and so the enzymes will cut the DNA into different sizes in different people.

Step 2:

The cut DNA is placed into a slab of 'jelly' (a gel matrix) and an electrical current is applied so that the 'jelly' becomes electrified and has a 'positive' (+) end at the top and a negative (-) end at the bottom - just like the positive and negative ends of a battery. As the DNA is a chemical which has a negative charge, the DNA moves towards the positive end of the gel or from the top to the bottom.

The pieces of DNA separate on the gel according to size: the biggest pieces move the slowest and so will be closest to the top of the gel. The gel now contains all of the individual's DNA spread from the top to the bottom of the gel.

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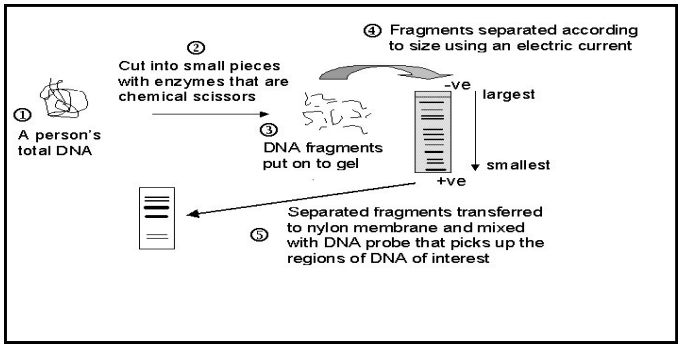

Figure 21.1: *When testing the DNA, it is extracted from the tissue, cut into pieces by chemical 'scissors' and then the pieces are separated on a gel. The piece of DNA of interest that contains a particular gene can be selected from the 20,000 or so genes in an individual's DNA using a special chemical 'probe'.*

Step 3:

To select out the pieces of DNA that need to be analysed, the pieces of DNA that have spread through the gel are covered with special DNA 'probes'. The probes have been made in the laboratory and contain a match for the DNA sequence that the test is designed to identify. The probes in fact have the opposite letters in the genetic code sequence to the sequence in the gene or DNA segment that needs to be isolated. The two sequences match up because of the ability of the letters A and T, and C and G to pair with each other as shown in *Figure 21.2.*

The development of the probes used is critical. They can be expensive to develop and the process may take some time.

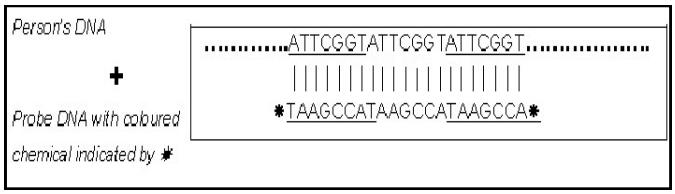

Figure 21.2: *The sequence of 'letters' in the DNA probe is opposite to that in the genetic code in the gene so that the probe hybridises with the gene.*

Next generation sequencing

The development of automated systems began in the 1990s and is now the usual method of DNA testing in most laboratories. The number of DNA sequences that could be analysed at the same time dramatically increased and so the technique was initially called massively parallel sequencing but is now generally called Next Generation (NG) DNA Sequencing. Each year, the technology developments has meant that DNA sequencing is now faster and cheaper than ever before.

The results are also automated and computerised so that the sequence is generated graphically as shown in *Figure 21.3*. Four colours are used for each nucleotide: guanine (black); thymine (red); adenine (green) and cytosine (blue).

The aim to develop a system to produce the sequence in an individual's whole genome for under $1,000 has been achieved in 2012. To put this into context, the first sequence that was produced in 2000 cost about $3 billion.

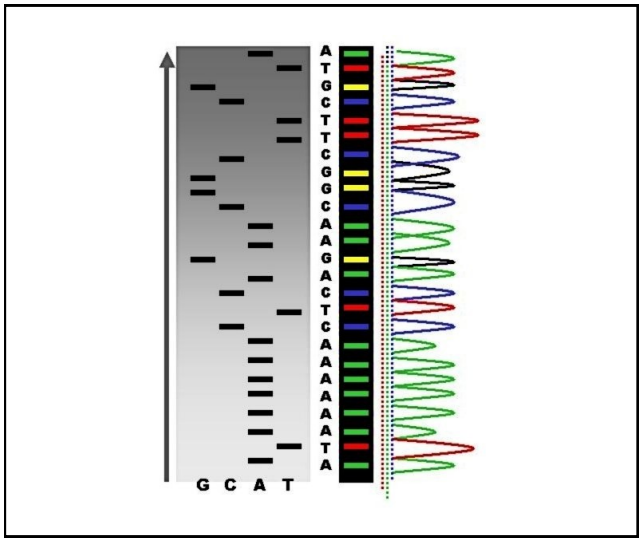

Figure 21.3 *Comparison of DNA sequencing results generated using the traditional method (left) and the computer generated sequence. Source: http://en.wikipedia.org/wiki/:Radioactive_Fluorescent_Seq.jpg Attribution: Abizar at en.wikipedia*

While whole genome sequencing is increasingly possible and affordable, many laboratories are using more targeted techniques.

Targeted DNA testing

In this technique, DNA from the person being tested is applied to a very small unit on which many different 'probes' representing the genes of interest have been placed.

- One example of this is, for about the same cost, all of the genes known to be associated with breast cancer (about 20 in 2011) can be examined at once instead of looking at just the two most common genes (*BRCA1* and *BRCA2*—see Genetics Fact Sheet 48).

Exome sequencing

Analysis of of the expressed parts of the protein-coding region (the **exome**) that makes up 1% of an individual's entire genome, using a technique that allows multiple strands of DNA to be sequenced simultaneously. The exome is thought to contain the majority of disease-causing mutations. Analysing only 1% of the genome greatly reduces the amount of information to be filtered and the cost of the process.

This technique has enable the discovery of new gene involved in conditions that follow the traditional (Mendelian) pattern of inheritance described in Genetics Fact Sheets 8, 9 and 10).

Microarray testing

The same principles described above are used in microarray testing except that the DNA from the person being tested is applied to a very small unit on which thousands of different 'probes' representing thousands of regions of DNA have been placed.

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Comparative Genomic Hybridisation (CGH) is a genetic testing technique that detects whether there is less or more genetic material (DNA) present than normally expected. DNA is found in every cell in our body and it is important that we have the right amount so that our body's systems can run correctly. Having more or less DNA than is required may cause health or developmental problems. This testing is also called a chromosomal microarray or molecular karyotype.

CGH testing may be suggested if a person has a pattern of development or health concerns that are suspected to be caused by extra or missing sections of DNA. CGH is usually used when a doctor has ruled out well-known genetic conditions and a broader more exploratory test is needed.

A CGH test requires a sample of an individual's DNA. DNA can be extracted from blood, skin tissue or even from the fluid surrounding a developing baby during pregnancy. Once the DNA is at the testing laboratory, the CGH test is used to compare the patient's DNA against a common sample (control sample). This specialised technique involves using a computer program that can then read the results and compare the DNA samples to detect any differences in the amount of genetic material. This is referred to as ***copy number variation***.

CGH testing may establish if particular patterns of health or developmental concerns are caused by extra or missing pieces of DNA. This information may be useful in diagnosing a genetic condition and giving information about a how symptoms may develop over time. This information may also be helpful in giving couples information about the possibility of a genetic condition affecting future children.

CGH testing looks at thousands of different pieces of the genetic code, some of which we do not fully understand. Therefore, there is a chance that this test may find a change in the code that is not understood and may or may not be the cause of the developmental or health concerns being tested for. This type of finding is *called a* ***variant of unknown significance***.

As the use of CGH testing becomes more common, information may become available as we learn more about the role of these pieces of DNA code in health and development.

However, interpreting the results of CGH testing is a very specialised skill. It is recommended that if any loss or gain of DNA is found, that the results be discussed with a genetic specialist.

Genetic testing and genetic screening

a) Diagnosis

Genetic testing can be used to diagnose conditions at all stages of life, from conception to the very end of life.

b) Genetic carrier testing

People can also be 'carriers' of changes in genes without showing any signs or symptoms of a genetic condition. Sometimes, this is because these changes make no difference to the gene product. genes with no effect on how the body works.
In other cases, the change makes the gene faulty and the gene product is changed, but as we usually have a 'back-up' system that sends the right message to the cell, we can 'carry' faulty genes with no effect on how the body works.

We all carry a number of faulty genes without showing any effects. When, however, both parents are 'carriers' of the same faulty gene, there is a chance that their children will inherit both faulty genes from them and will be affected by a condition.

In this case the condition follows a pattern of 'autosomal recessive inheritance' (see Genetics Fact Sheet 8).

Genetic carrier testing may be available for people who have a family history of an inherited condition to determine if they are carriers of the faulty gene involved. This information may be useful in planning pregnancies.

c) Genetic carrier screening

The term 'genetic carrier screening' is used to describe direct gene testing applied to a whole population or to a defined group. For example, genetic carrier screening may be available for people in the population who have no personal or family history of a condition but who have a greater than average chance of carrying a particular faulty gene due to their ancestry.

In Australia, these groups include people with ancestry:

- From Northern Europe and the United Kingdom who have a 1 in 25 chance of being an unaffected carrier of the faulty gene involved in cystic fibrosis (CF) (see Genetics Fact Sheet 33)
- From the Southern European region, the Middle East, the Indian Sub-continent, Africa or Asian countries who have a greater chance of carrying the faulty gene involved in thalassaemia or sickle cell disease (see Genetics Fact Sheet 34)
- Of Ashkenazi Jewish origin who have a 1 in 25 chance of being unaffected carriers of the faulty gene involved in Tay-Sachs disease or several other genetic conditions (see Genetics Fact Sheet 35)

d) Newborn screening

Genetic screening is done on all newborn babies in Australia and New Zealand by a simple blood test to detect a few rare genetic or metabolic conditions.
The blood sample is taken by a heel-prick before the baby leaves hospital, or for home births, on about day 4, and is sent to a special laboratory (see Genetics Fact Sheet 20).

e) Presymptomatic genetic testing

Direct gene testing is now being used to determine if a person will develop certain inherited conditions later in life. This type of genetic testing is referred to as presymptomatic testing where the detection of a faulty gene in a person with a family history of a particular condition, but who currently has no symptoms of that condition, means that that person will certainly develop the condition in later life.

Presymptomatic testing is available for a number of neurodegenerative diseases such as Huntington disease (see Genetics Fact Sheet 44) and some forms of bowel cancer (see Genetics Fact Sheet 49).

f) Predictive Genetic Testing

Sometimes the detection of the faulty gene provides the person with an increased risk estimate, rather than certainty, that they will develop a particular condition later in life. This type of direct gene testing is called predictive testing.

Predictive testing for some families is available for inherited conditions such as an inherited predisposition to haemochromatosis or breast cancer (see Genetics Fact Sheets 36 and 48).

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Limitations of genetic testing

- Finding that a person has a variation in a gene involved in a particular condition does not always relate to how a person is, or will be, affected by that condition. There may be modifying factors (other genes, environmental factors) that can affect the expression of the message from the gene. This may explain the variability of expression between the affected members of one family
- Despite the recent advances in DNA examination, identifying and understanding the meaning of variations in the DNA sequences in genes is not always easy.
  - Many of the genes in which variations lead to a condition 'code' for very large messages: changes can occur anywhere along the length of the DNA segment making up the gene
  - A single gene may have many possible variations – some make the gene faulty (mutations); others have no effect on how the gene works and others are of unknown significance. For example, there are over 1500 mutations that have been detected to date, at different places along the length of the gene involved in cystic fibrosis (CF) (see Genetics Fact Sheet 33). It is also likely that there are other mutations that have not yet been identified
  - Laboratories often test for only some of the more commonly known mutations in a gene and not for the presence of all of the mutations that occur much more rarely.
- For some complex conditions that develop as a result of the interaction between the person's genetic make-up and other environmental or genetic factors, e.g. cancer, the testing may have to be done on a family member with the condition to identify the family-specific mutation in the gene (mutation searching) before unaffected family members can be offered predictive testing. The testing may be time-consuming and expensive for the health service if not for the patient.

A case study of DNA genetic testing

In the family represented diagrammatically in *Figure 21.4*, neither Jan nor Bill knew that they each carried the faulty gene for cystic fibrosis (CF) until they had Sue who was born with CF ie. there were no other family members with the condition. They now know that James has 2 chances out of 3 of being a faulty gene carrier for CF (Genetics Fact Sheet 8).

Jan and Bill are concerned about whether any future children will have CF. Jan is currently 8 weeks pregnant and they wish to have testing.

Jan and Bill's brothers and sisters are also concerned and would like to see whether they are carriers of the faulty gene for CF. They each have a 1 in 2 chance of carrying the faulty gene.

- Sue will have two copies of the faulty gene: one inherited from Jan and one from Bill. Sue may have the same mutation in both her gene copies or she may have different mutations in each copy: in either case, both gene copies will be faulty
- The DNA from Sue is examined using gene testing to see if the mutation in each copy of the gene involved can be identified
- If the mutations are detected, testing can be offered to Jan and Bill in this or future pregnancies. If the mutations causing the gene to be faulty are relatively common, it will be possible to examine the baby's DNA for these common mutations

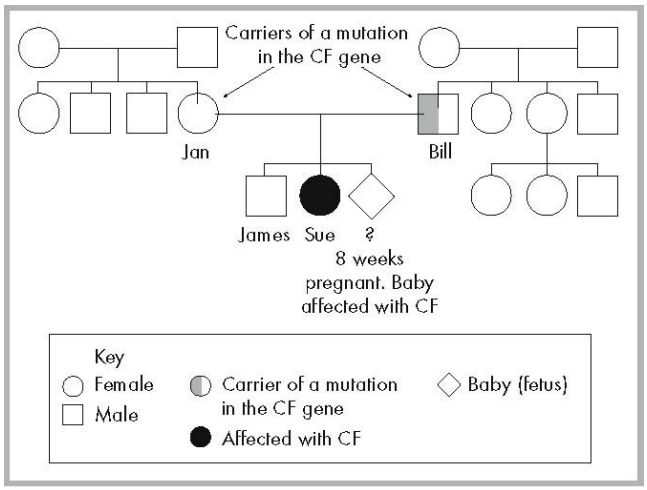

Figure 21.4: An example of genetic testing when a family member is affected by cystic fibrosis. The family is represented by 'pedigree' symbols.

If the mutation (s) making the gene faulty in Sue can be identified, Jan's and Bill's brothers and sisters who are planning to have children, can be offered direct gene testing to see if they carry a faulty copy of the gene for CF.

- If any of them is a genetic carrier, it may be possible to check their partners to determine if they are also carriers of the faulty gene
- In the future, when James is planning to have children, he will also be able to choose to have carrier testing if he wishes

If however, it is not possible to identify the particular mutation in the two gene copies that Sue inherited, Jan and Bill can be offered **indirect gene tracking (linkage testing)** for prenatal testing during this and further pregnancies.

- DNA markers from Sue, Jan and Bill will be compared to those from the developing baby
- The test will show if the baby's gene sequence is like Sue's and therefore will have CF *(Figure 21.5)*
- If the pattern is the same as Jan's or Bill's, the baby will be a genetic carrier of CF just like the parents
- Jan and Bill's brothers and sisters may not be able to use this information for prenatal diagnosis in their pregnancies as the markers that are examined are special to Jan and Bill and their children. The markers in the partners of Jan's and Bill's brothers and sisters and in Jan's and Bill's nieces and nephews may also be different

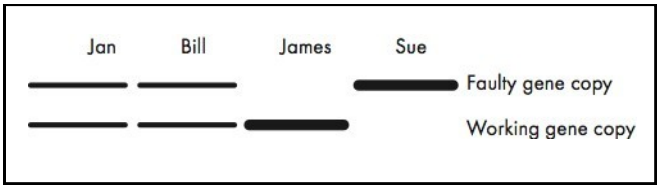

Figure 21.5: *The genetic test will show that both Jan and Bill have two bands, as they both have a faulty gene copy and a working gene copy. Sue has two copies of the faulty gene and so has cystic fibrosis (CF). James has inherited both working copies from his parents. The baby's DNA pattern will indicate if she or he has CF, is an unaffected faulty gene carrier for CF or is unaffected with both gene copies having the right information.*

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Ethical issues

There are advantages and disadvantages to genetic testing. Genetic testing should only be used after all the benefits, costs and implications have been considered.

Genetic counselling is recommended both before and after testing (see Genetics Fact Sheet 3). Ethical issues arising from genetic testing are discussed further in Genetics Fact Sheet 23.

Other Genetics Fact Sheets referred to in this Fact Sheet: 1, 3, 4, 5, 6, 8, 9, 10, 17C, 18, 20, 23, 24, 33, 34, 35, 36, 44, 48, 49

Information in this Fact Sheet is sourced from:

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