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Genetics in Medicine

1. Conception and Early Life

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Front cover image

Array analysis at the Genomic Diagnostic Laboratory, Manchester Centre for Genomic Medicine

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Genetics in Medicine 1. Conception and Early Life

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Genetics – the basis of 21st century medicine?

Virtually every human disease and ailment has at least some genetic aspects, and restoring health always involves biological systems - metabolism, cells or organs - that are under genetic control. Our increasing understanding of genetics, and our ability to manipulate genetic systems have led to some far-reaching visions of the role of genetics in 21st century medicine.

Single gene defects directly cause a large number of disorders that are individually rare but collectively account for a substantial fraction of all reproductive problems and paediatric hospital admissions. For some of these, the so-called inborn errors of metabolism such as phenylketonuria, where the basis of the disorder was discovered by biochemical tests of blood and urine long before it was possible to test genes, effective newborn screening programmes (the heel prick test) have for many years identified affected babies allowing the introduction of special diets to prevent serious effects of the disorder (page 32). For other conditions, the journey from disorder recognition to precise diagnosis and treatment has been much longer. The following narrative illustrates how far we have come in one disorder – but also how far we still have to go.

> *Duchenne muscular dystrophy (DMD) is an X-linked condition (page 16). Affected boys suffer progressive muscle weakness, leading inevitably to death in their twenties or thirties. Families want two things: a cure, of course, but also reliable carrier testing and prenatal diagnosis to enable carrier women to have healthy children.*
>
> *Forty years ago the only option for a woman at risk was fetal sexing and, if she wished, termination of male fetuses – in the full knowledge that she might well not be a carrier at all, and even if she were, half of the males would have been unaffected. Then in the mid-1980s genetic markers were developed that allowed the still unknown DMD gene to be tracked through pedigrees. In Manchester, as in other genetics centres, we set out to apply the procedure systematically to many at-risk women. We were able to show that many were actually not carriers, enabling*

them to embark with confidence on pregnancy. Shortly thereafter, brilliant research by Lou Kunkel's group in Boston identified the DMD gene, and at last we were able to test directly for a mutation. This lifted some of the burden from family members, but did nothing for the affected boys.

*However, now we know what the gene is and how the various mutations prevent it from working, we can begin to think about ways to counter the effects. One way is to rewrite the **RNA** transcript of the faulty gene by changing the way the **exons** are spliced together (page 34). Drugs to do this are now in clinical trials and the first results are encouraging. There is still a long way to go in finding a true cure, but thanks to these advances and others in the clinical care of affected boys, the outlook for patients is much brighter than forty years ago.*

What is true of Duchenne muscular dystrophy is true of many other genetic conditions; advances in genetics are already transforming the lives and prospects of many families. This booklet sketches some of the progress to date and hopes for the future.

Further to our growing understanding of these single gene conditions, we now know that almost every aspect of health and disease is affected by genetic factors; differential genetic susceptibility plays a role in virtually every clinical problem.

Clinicians in all branches of medicine have to deal with the diagnosis and management of genetically-influenced disease. Rare, complex and difficult cases come to regional genetics centres, which offer specialised clinical and laboratory diagnosis and genetic counselling and collaborate in the management of patients with genetic disorders.

Technical advances in **DNA** sequencing are rapidly expanding the scope of genetic testing. Soon it may be routine to sequence every gene of a patient. There is much current exploration of the best ways to use the resulting avalanche of data for the benefit of the patient and family.

Introduction – the burden of genetic disease

All our inherited characteristics are governed by our genes, but in this booklet on Genetics in Medicine, our focus is on the role of genetics in disease. These come in three main categories:

- Some conditions are caused by a variant in the **DNA** code of a single gene (the unit of inheritance). Examples are cystic fibrosis, Huntington disease and Duchenne muscular dystrophy. These are the so-called **mendelian** or **monogenic** disorders. They follow (more or less closely) the inheritance patterns shown on page 14. Individually, mendelian conditions are mostly rare – cystic fibrosis, one of the commonest, affects one child in 2,500 in the UK – but several thousand such conditions have been described and collectively they account for up to 40% of paediatric hospital admissions and 50% of childhood deafness and blindness. The picture is somewhat complicated by the fact that, for some inherited conditions, the exact same clinical picture can be caused by different individual genes; this is known as genetic heterogeneity. The Orphanet database is a source of information on rare disorders, including most genetic conditions. OMIM (Online Mendelian Inheritance in Man) provides further information.
- Some disorders are caused by having large amounts of extra or missing genetic material that may encompass dozens of genes. Genes are packaged into structures called chromosomes, and sometimes there is the wrong number of chromosomes because of an error in cell division, as in most cases of Down **syndrome** (**trisomy** 21, the presence of three copies of chromosome 21 instead of the normal two). In other cases the chromosome number is correct (46) but one or more chromosomes have an abnormal structure, with extra, missing or misplaced material. Chromosome abnormalities occur in about 1% of all births.
- Many other conditions are not caused by changes in a single gene and do not show the classic mendelian pedigree patterns, but nevertheless are at least partly genetically determined. Combinations of genetic variants, each

of which may on its own be fairly innocuous, create a genetic susceptibility, so that some environmental insult which would not harm a genetically resistant individual triggers disease in a susceptible person. Almost every major medical condition (eg: heart disease; cancer; diabetes) shows at least some degree of genetic susceptibility of this type, and identifying the components of susceptibility factors has been a major strand of genetic research over the past two decades.

Genetic conditions are not necessarily congenital (present at birth) – for example, Huntington disease typically manifests in middle age – and congenital conditions are not necessarily genetic: the **teratogenic** effects of the drug Thalidomide, which caused limb abnormalities and other defects in babies of mothers who were prescribed the drug in the late 1950s and early 1960s to treat morning sickness in pregnancy. Equally, genetic conditions are not necessarily familial: many individual cases of severe intellectual disability are the result of *de novo* mutations (genetic variants that occur spontaneously for the first time in the egg, sperm or early embryo), and the same may be true of many other sporadic conditions. Nor are familial conditions necessarily genetic; we give our children their environment as well as their genes.

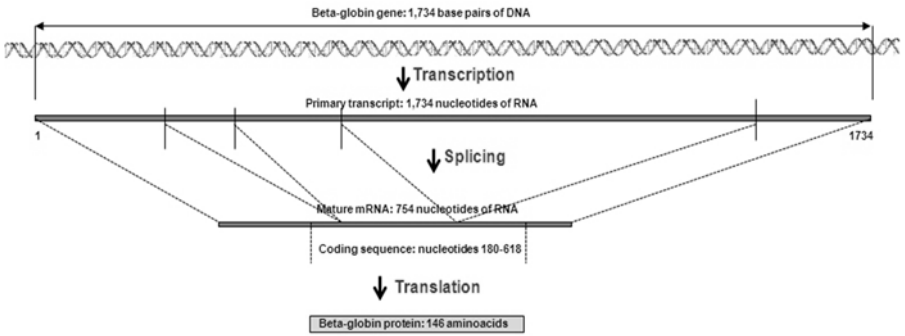
Over many generations the **genomes** of a population are in a state of flux. The average newborn carries around 60 new mutations caused by errors in DNA replication and damage to existing DNA molecules. Most of these new variants are harmless but some confer either a selective advantage or selective disadvantage.

DNA – The code of life

The human genome consists of about three thousand million (3×10^9) letters of DNA code. The molecular units of the code are **nucleotides** (represented by the letters A, C, G and T), each comprising a base (adenine, cytosine, guanine or thymine) combined with sugar and phosphate subunits, forming a long string wrapped into the famous double helix. Each of the four bases in the strand is paired with a base on the opposite strand (A with T, and G with C) such that the sequence of one strand predicts the sequence of the complementary strand.

The size of a piece of DNA is measured in base-pairs (bp), **kilobases** (kb, 1,000 bp) and **megabases** (Mb, 1 million bp). The best-understood function of DNA is that of the genes, most of which specify the structure of proteins, as summarised in the 'Central Dogma' of molecular biology: **DNA -> RNA -> Protein**.

Example: the β-globin gene. This small gene comprises 1,734 base pairs of DNA. It is active ('expressed') in the precursors of red blood cells. First an RNA copy of the gene is made (transcription). Like DNA, RNA is a string of four nucleotides, but contains uracil (U) in place of thymine (T). As with most mammalian genes, the parts of the β-globin gene that code for the amino acids are split. Three **coding** segments (**exons**) are separated by stretches of **non-coding** DNA (**introns**). The number of exons in a gene varies widely, from 1 to over 100. Within the cell nucleus the exons of the primary RNA transcript are cut out and spliced together to make the mature messenger RNA (mRNA). For the β-globin gene the 1,734 nucleotide primary transcript is cut and spliced, joining exons 1, 2 and 3. Introns 1 and 2 are discarded. The mRNA is 754 nucleotides long and is exported to the cytoplasm of the cell where a set of large protein-RNA machines (ribosomes) use the mRNA sequence to direct assembly of amino acids into the β-globin protein, following the genetic code (see opposite). This process (translation) ignores the first 179 nucleotides of the mRNA (which carries signals to set the translation process up), and the last 136, so the coding sequence is nucleotides 180-618, which is read in triplets to produce a protein of 146 amino acids.



The Genetic Code

UUU Phenylalanine	CUU Leucine	AUU Isoleucine	GUU Valine
UUC "	CUC "	AUC "	GUC "
UUA Leucine	CUA "	AUA "	GUA "
UUG "	CUG "	AUG Methionine	GUG "
UCU Serine	CCU Proline	ACU Threonine	GCU Alanine
UCC "	CCC "	ACC "	GCC "
UCA "	CCA "	ACA "	GCA "
UCG "	CCG "	ACG "	GCG "
UAU Tyrosine	CAU Histidine	AAU Asparagine	GAU Aspartic acid
UAC "	CAC "	AAC "	GAC "
UAA STOP	CAA Glutamine	AAA Lysine	GAA Glutamic acid
UAG STOP	CAG "	AAG "	GAG "
UGU Cysteine	CGU Arginine	AGU Serine	GGU Glycine
UGC "	CGC "	AGC "	GGC "
UGA STOP	CGA "	AGA Arginine	GGA "
UGG Tryptophan	CGG "	AGG "	GGG "

Each amino acid is encoded by a triplet of nucleotides. Since there are 64 different triplets, but only 20 amino acids in the genetic code, often more than one triplet encodes a given amino acid. Three triplets (UAA, UAG, UGA) are signals that mark the end of a coding sequence. So, a base change (mutation) might cause early termination of the protein chain, change an amino acid, or have no effect at all.

Coding sequences occupy only ~ 1.2% of the genome. What does the rest do?

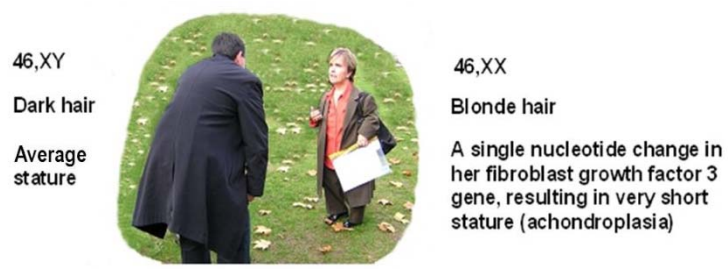
- Some forms the introns in genes, as described above.
- In addition to 20,848 protein coding genes, there are 22,486 genes that specify a **functional RNA** molecule – that is, an RNA that is not just a messenger RNA but has some function of its own in a cell (statistics from www.ensembl.org, accessed 28/03/2013).

The non-coding DNA between genes contains many control elements that determine where and when a gene is expressed. The ENCODE project is identifying these. These control elements work by binding different sets of tissue-specific proteins. These affect the way the DNA is packaged and control access to it by the transcription machinery and other proteins. The different cells of the body all share the same two genomes – except for any occasional variants that arise due to errors in DNA replication as cells divide – but run different genetic programs because of this differential control.

Genetic mutation and variation

Comparing the genomes of two unrelated individuals, we would typically see around 3-4 million differences.

- A few of these determine a distinct phenotype (a measurable or identifiable characteristic or set of characteristics).



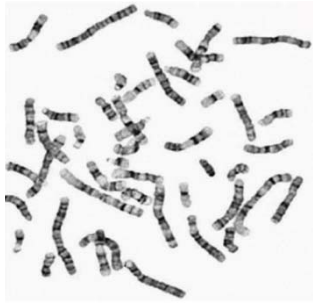
- Many act with other genes and environmental factors to contribute towards an overt characteristic, but do not alone determine it. For example, normal variation in height is determined by a number of different genes *and* by a person's nutrition. A further example is the particular genetic variants that may slightly increase or decrease a person's chances of developing diabetes; however, the biggest risk factor is obesity, which is mostly determined by diet and lifestyle.
- The great majority have no apparent biological effect – as most occur in non-coding DNA – but can be useful tools for identifying individuals (eg: in DNA profiling) and tracking ancestry.

Epigenetics is the study of a further layer of variation: the function of a DNA sequence is influenced by chemical modifications – such as methylation – that affect its packaging and activity. Much current research is focussing on the role of epigenetic changes in disease, particularly cancer.

The differences between humans and other organisms have more to do with differences in mechanisms of genetic control than having different genes – it turns out that despite their obvious differences, most mammals have very similar repertoires of genes.

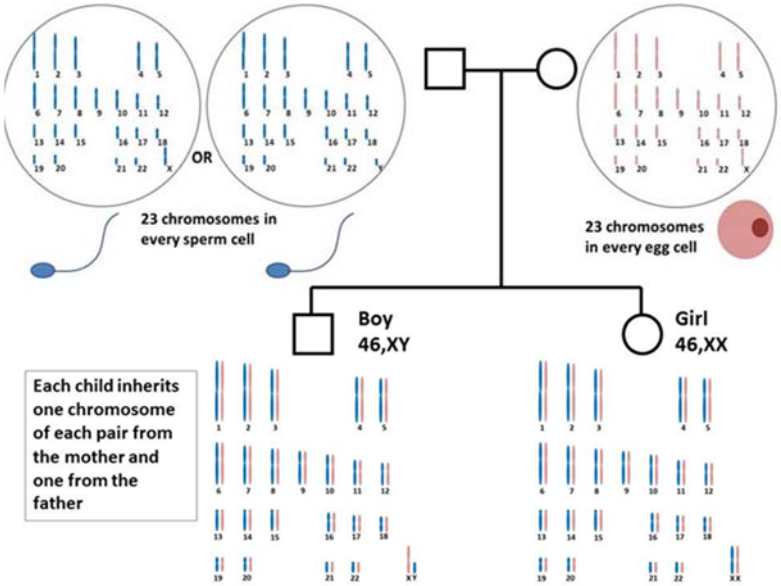
Chromosomes – DNA packaging

The human genome of 3×10^9 base pairs of DNA is organised into 23 packages, called chromosomes, which sit in the nucleus of nearly every cell.



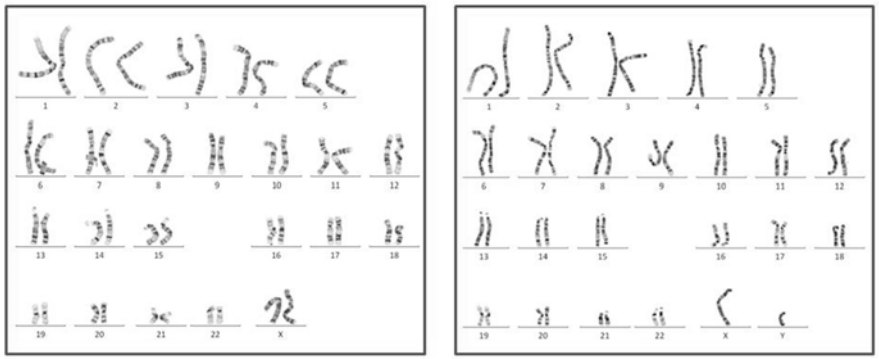
Human chromosomes

Every cell contains two genomes (in 23 pairs of chromosomes, giving in total 46), one from the mother and one from the father. So every cell has two copies of very nearly every gene.



Females and males both have 22 pairs of **autosomes** and one pair of sex chromosomes, which determine the sex of an individual. A female has two **X chromosomes**, and a male has one X and one **Y chromosome**, which contains only a very small number of genes. These are shown in the **karyotypes** overleaf.

Chromosomes – DNA packaging *(continued)*



The **gametes** – the egg and sperm cells – are produced by a special form of cell division (**meiosis**) that produces cells with just 23 chromosomes each: 22 autosomes and one sex chromosome. The process of fertilisation brings the chromosomes of the egg and sperm together to give a cell containing the full complement of 46 chromosomes.

Each time a fertilised egg cell divides by mitosis, the DNA is copied to produce the 10^{13} cells of the adult body, each containing 6 x 10^{9} bp DNA. At each cell division new mutations are introduced into the DNA; these entirely normal events give rise to genetic variation within the body – **somatic** variation – and few have any functional effect. Should a somatic mutation have any deleterious effect, mostly it will be weeded out as it will be selected against in the body.

X-inactivation – an epigenetic story

Having the wrong number of chromosomes has a dramatic effect. An extra copy of even the tiny chromosome 21 brings all the characteristics of Down syndrome, while having an extra copy of any of the larger autosomes, or a missing copy of any of them, is generally incompatible with survival. Yet we cope perfectly well with having or not having a Y chromosome, and with having one or two X chromosomes. How is this done?

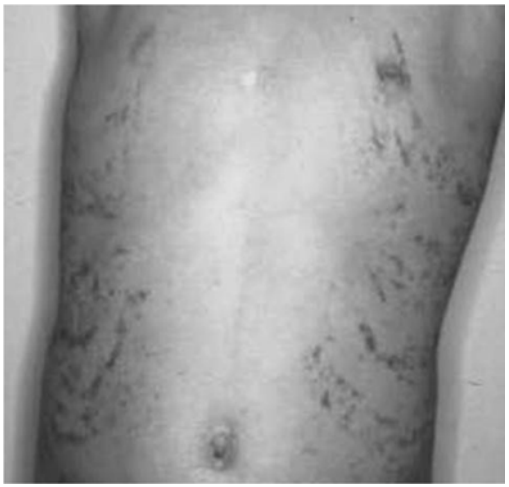
In the case of the Y-chromosome, the answer is that it carries very few genes, and most of those few are dispensable in females. But the X chromosome has over 1,000 genes, many of which play essential roles in both sexes. So how do

46,XX females and 46,XY males both function normally?

The answer is dosage *compensation*. A special mechanism ensures that, regardless of the number of X chromosomes in a cell, females use only one copy. All X chromosomes except one are permanently inactivated. This is a prime example of an *epigenetic change*: the genes are still there, but their expression is silenced in all but the 'active' X chromosome.

X-inactivation takes place in the early embryo, at a stage when it consists of only a few hundred cells. In each cell of a 46,XX embryo, one of its X chromosomes is individually selected at random and inactivated. In some cells the X that came from the mother is inactivated, in others the X that came from the father. As the embryo develops, individual cells give rise to **clones** of daughter cells, and each daughter cell 'remembers' which X was inactivated in the parent cell . As a result, an adult female is a *mosaic* of cell clones, some expressing only her mother's X-chromosome genes, others only her father's. If the woman has inherited an X-linked mutation from one parent, this can have important consequences.

Figure: Female with the X-linked condition incontinentia pigmenti showing streaked appearance of the skin, characteristic of cells with the active X chromosome carrying the variant gene (darker pigmented skin) and cells with the active X chromosome carrying the normal gene (lighter skin).

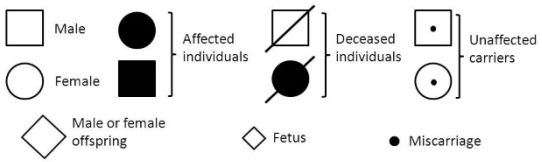


Genetic conditions and their inheritance

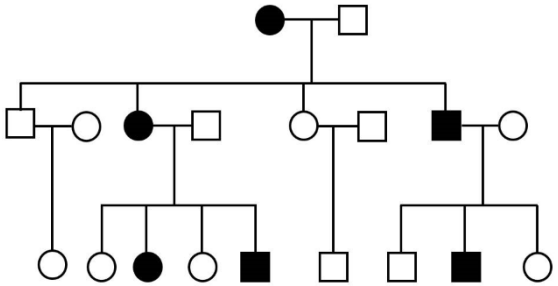
When one specific genetic variant is both necessary and sufficient to produce a certain phenotype, a characteristic inheritance pattern can be seen.

Pedigree symbols

Pedigrees are drawn like family trees, with people in each generation arranged in birth order along a horizontal line. Squares denote males, circles females. Filled-in symbols are used to indicate persons having the characteristic being illustrated. Special symbols can be used to include further information (see Case 2).

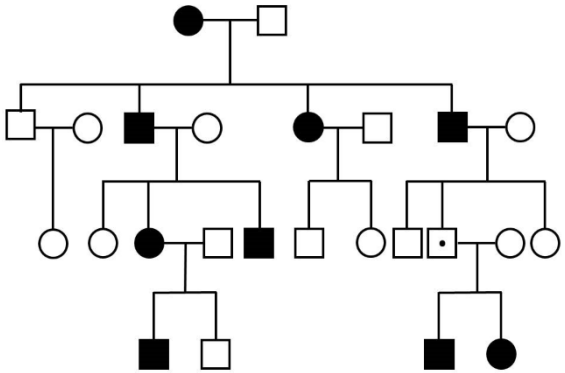


Autosomal dominant inheritance is seen when one of the pair of genes on an autosome (a non-sex chromosome) carries a variant that determines the phenotype. Affected cases usually have an affected parent (but fresh mutations are frequent with dominant conditions), the sexes are usually equally affected, and for an affected person, the risk of an affected child is 1 in 2.

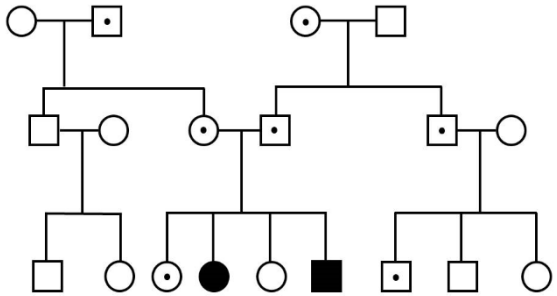


Sometimes, other genes, environmental factors and simple chance can complicate the simple mendelian pedigree pattern. If the pattern is nevertheless fairly close to the mendelian ideal, the exceptions are explained by the concept of **reduced penetrance**. In the case of autosomal dominant conditions, for example, a condition can appear to 'skip' generations, and some individuals who, by the pattern of inheritance, *must* carry a gene for the

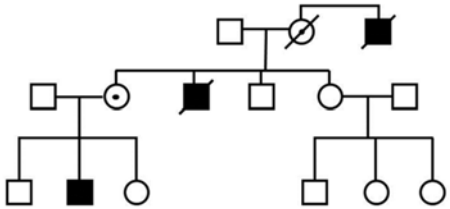
condition, show none of its symptoms. The **penetrance** is the probability that a genetic variant will cause a particular phenotype. An example of a pedigree showing an autosomal dominant inheritance pattern with ~90% penetrance is shown below.



Autosomal recessive inheritance is seen when both of the pair of copies of the relevant gene must carry the variant in order for the character to manifest. Affected persons usually have unaffected parents, who each carry one copy of the variant (they are called **heterozygotes**). Each child of such a couple has a 1 in 4 risk of being affected.



X-linked inheritance is seen when the gene variant is located on the X chromosome. Males (46,XY) have only one X, and so are either affected or unaffected. Females (46,XX) can be heterozygotes, and for most X-linked conditions are either unaffected or mildly affected. A female carrier transmits the variant to 50% of her offspring, of whom only the males usually are affected (25% of all children).



There are rare cases of X-linked *dominant* conditions, where only one copy of the variant gene is sufficient to cause the disorder in a female. Examples include Rett syndrome and incontinentia pigmenti (IP) (page 13). Rett syndrome is a severe condition which usually arises as a new mutation with no family history but IP can be mild in some women and so can be passed on to a daughter. However, most such X-linked dominant conditions cause early miscarriage of male fetuses, which have only a single X with the mutated gene.

These simple pedigree patterns are seen where genetic conditions are caused by variants in single genes; these conditions are called mendelian or monogenic, and whilst most are individually rare, collectively they affect one in every 100 births.

Often mendelian conditions diverge from these idealised patterns because of new mutations, variable expression (when people carrying the same variant are affected to differing degrees), and non-penetrance. Thus expert genetic counselling is important, even if a condition is said in the textbooks to be monogenic. Furthermore, most common conditions such as diabetes and asthma have a genetic component, but will not follow the simple pedigree patterns shown above, since multiple genes and/or environmental factors are involved.

Some common mendelian and chromosomal conditions		
Condition	**Incidence in UK**	**Characteristics/phenotype**
Autosomal dominant conditions		
Huntington disease	1 in 10,000	Onset usually in middle age; involuntary movements; eventual dementia; death ~15 years after symptom onset.
Familial hypercholesterolaemia	1 in 500	Raised cholesterol resulting in early vascular disease and risk of heart attack.
Autosomal recessive conditions		
Cystic fibrosis	1 in 2,500 births	Chronic disorder affecting lung and digestive tract; failure to thrive.
Tay-Sachs disease	1 in 3,600 births in Ashkenazi Jewish population	Progressive neurodegeneration and developmental delay; babies affected with infantile form die by third year.
Phenylketonuria (PKU)	1 in 10,000 births	Inability to metabolise phenylalanine. Treated by giving special diet; if not serious intellectual disability (ID) results.
Deafness (most cases)	1 in 1,250 births	Profound deafness from birth in otherwise healthy babies.
X-linked conditions		
Duchenne muscular dystrophy	I in 3,500 male births	Progressive muscle weakness; unable to walk by 12 years; life limiting in third or fourth decade.
Fragile-X syndrome	1 in 3,000 male births	ID; behavioural problems; autism; female carriers sometimes are affected.
Chromosomal conditions		
Down syndrome (trisomy 21)	1 in 650 live births	Characteristic facial appearance; mild to moderate ID.
Translocations (involvement of any chromosome)	1 in 170 live births	Balanced translocation – usually normal; unbalanced translocation – variable multiple abnormalities (page 23).
22q11 (**deletion** of part of chromosome 22)	1 in 4,000 live births	Variable, but include heart disease; ID; cleft palate; characteristic facial features.
Turner syndrome (female with only one X and no Y chromosome)	1 in 2,500 live births	Short stature; webbed neck; underdeveloped ovaries (infertile).

The consequences of consanguinity

Marrying a blood relative (a consanguineous marriage) increases the risk of producing offspring with an autosomal recessive condition. Studies have suggested that the average healthy person carries at least three genes for a recessive condition which would be harmful in homozygous form.

Suppose one of these is present in one person in 100 in the general population. If one person carries it, the chance of an unrelated partner carrying the same variant is 1 in 100, and the risk that a child of the couple would be homozygous (ie: would inherit two copies of the gene variant) is 1 in 400. If, however, a carrier marries his/her first cousin, the chance s/he also carries the variant is 1 in 8, and the risk of a homozygous (affected) child is 1 in 32.

Similar calculations show that consanguinity greatly increases the risk of rare recessive conditions, but has relatively little effect on the risk of more common recessive conditions. Despite these worrying-looking calculations, and contrary to folklore, in practice the offspring of cousin marriages are usually perfectly healthy. The risk of a serious congenital condition is only increased from 2% (the background risk for any newborn) to 4% - in other words, the chance of a healthy baby is only reduced from 98% to 96%.

In some populations in the Middle East and the Indian subcontinent, consanguineous marriage is common. The deleterious genetic consequences of marrying within the family may be balanced by the social and cultural advantages. In many communities now, after the birth of a child with a genetic condition, the extended family will often request carrier screening, with prenatal diagnosis where requested and appropriate.

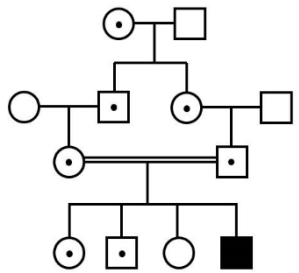


Figure: A homozygous affected child (filled symbol) born to first cousins whose grandmother is a carrier of an autosomal recessive condition. Dots mark heterozygous carriers.

Genetics in medical practice

Overview of roles and services

Genetic services are not an isolated branch of medicine. Clinicians in every speciality encounter patients with genetic problems. However, the great diversity of rare genetic conditions and the rapidly increasing base of specialist knowledge and techniques mean that patients often need referral to a clinical genetics service.

In the UK clinical genetics services are in regional centres, each delivering services to a population of typically 2-5 million, plus more specialised services on a wider (national or international) basis. Centres are usually headed by medically trained consultant clinical geneticists and include specialised genetic counsellors and clinical scientists. Most centres are affiliated to universities and actively pursue research. The centres' laboratories offer tests of DNA, chromosomes and biochemistry. Many tests results are delivered directly to outside clinicians, but patients with complicated family histories, rare dysmorphic syndromes or more complicated laboratory results will be seen by the clinical geneticists.

The primary aims of a genetic consultation are to achieve a diagnosis, assess risk, and plan management. Clinical geneticists are unusual among hospital clinicians in that they see patients of all ages, with problems affecting all body systems, and also unaffected (but possibly at risk) family members. Diagnosis is based on clinical examination (including appropriate investigations) and/or laboratory tests. A successful diagnosis allows accurate genetic counselling and may indicate appropriate management of affected individuals. Even if there is no definite diagnosis, the genetic counsellors can help the family cope, as described below.

Genetic counselling

Peter Harper (2010) defined genetic counselling as "the process by which patients or relatives at risk of a disorder that may be hereditary are advised of the consequences of the disorder, the probability of developing or transmitting it and the ways in which this may be prevented, avoided or ameliorated".

Crucially, genetic counselling should always aim to be *non-directive*; this means that the counsellor should set out options to help the patient explore the implications of the various possible courses of action, but should not *recommend* any particular option. This may be difficult because patients, faced with difficult decisions, may well ask "what would *you* do in these circumstances?".

Genetic counselling may be carried out by a clinical geneticist or by a counsellor who may have a nursing background, but increasingly will have specific postgraduate training in genetic counselling.

Figure: A genetic counsellor with a patient

Three case histories: 1. A case of sickle cell disease

Aduke is the baby of unrelated parents of Nigerian ancestry. At three months of age she developed swelling of her fingers which seemed painful and she was referred to a paediatrician, who noticed that Aduke seemed pale, had a yellow tinge to the whites of her eyes and had an enlarged spleen. Haemoglobin levels in her blood were low, the level of newly forming red blood cells was higher than normal, as was the bilirubin level, indicating she had anaemia with mild jaundice. The diagnosis of sickle cell disease was made on examination of a blood film which revealed red blood cells with a sickled shape.

Over the next five years Aduke was admitted to hospital four times with severe bone pain or abdominal pain and fever. These episodes, known as 'crises', were associated with a throat infection on one occasion, and on another with diarrhoea and dehydration. In addition her mother reported that she had periods when she seemed tired and listless. The paediatrician prescribed regular penicillin treatment because she knew that Aduke's spleen may well have been damaged during a crisis making her more vulnerable to further infections. She was followed up regularly for other complications of sickle cell disease such as kidney damage and retinal problems, and her parents were advised how to keep her well hydrated, how to cope with the pain when it occurred and they were put in touch with the local sickle cell centre (http://www.sicklecellsociety.org/).

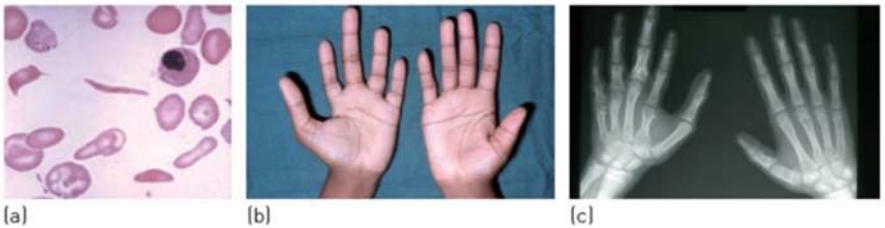


Figure: Sickle cell disease. (a) Blood film showing a sickled cell, abnormally shaped red cells and a nucleated red cell. (b,c) Blocked blood vessels to the bones of the hands can result in unequal finger length.

A single nucleotide change in the β-globin gene on chromosome 11 results in production of β-globin in which the code for amino acid 6 is changed from GAG (encoding glutamic acid) to GTG (GUG in the corresponding RNA) which encodes valine (page 9).

Normal gene: ..CTG ACT CCT G**A**G GAG AAG TCT

Sickle gene: ..CTG ACT CCT G**T**G GAG AAG TCT

This makes the β-globin molecule more sticky and liable to aggregate. In heterozygotes this happens only under conditions of abnormally low oxygen tension; these individuals have sickling trait, but are generally healthy. In **homozygotes** frequent aggregation leads to distorted (sickled) red cells, which are liable to block capillaries, causing crises of serious bone and abdominal pain necessitating hospital admission. Patients may need transfusions as well as lifelong penicillin.

Because sickle cell disease is seen only in homozygotes it is a recessive condition. The recurrence risk for future children is 1 in 4. Sickle cell disease is frequent among people from many parts of Africa because heterozygotes have an increased resistance to falciparum malaria, and so are favoured by natural selection.

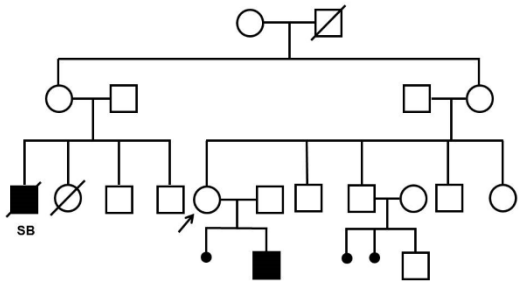
Figure: Genetic Alliance UK is the national charity of 150 patient organisations, supporting all those affected by genetic conditions. http://www.geneticalliance.org.uk/

Three case histories: 2. A chromosome abnormality

Martha (arrowed) had one miscarriage and in her next pregnancy had a baby with multiple congenital abnormalities (many different medical and developmental problems). She asked the doctor what could have gone wrong, and she was referred to her local genetics centre.

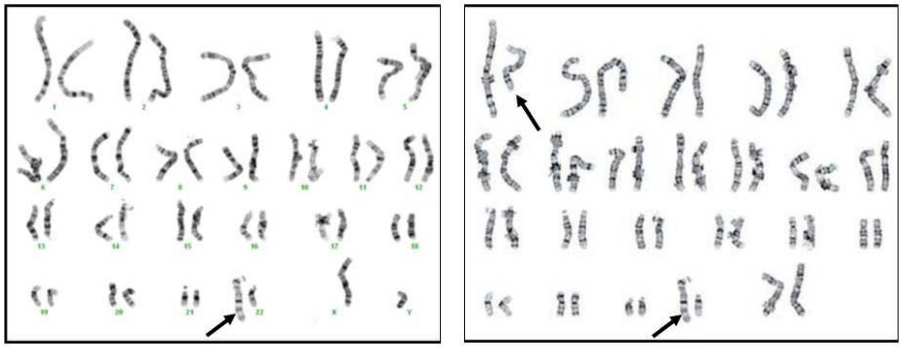
The geneticist took a pedigree and was struck by the many reproductive problems in this family (see Figure below): three miscarriages (small closed circles) and the birth of two infants with different sets of multiple congenital abnormalities, one stillborn (SB; filled symbols).

Such events can have many causes, but the combination of multiple reproductive failures and live born infants with multiple congenital abnormalities raised the suspicion that a chromosomal abnormality might be segregating in the family.



With the parents' consent, 2 ml of blood was taken from the baby to check his chromosome pattern. Today this would probably be done using microarrays (described later), but for illustrative purposes we show conventional chromosome preparations (karyotypes). To create these, white blood cells were cultured in the laboratory to produce many dividing cells (chromosomes can only be seen in dividing cells). The cells were processed to create a chromosome 'spread' on a microscope slide and stained in a procedure (G-banding) that shows each chromosome with a characteristic pattern of dark and light bands. Using the banding patterns as a guide, the scientist produced a karyotype showing the chromosomes paired up and in order of size.

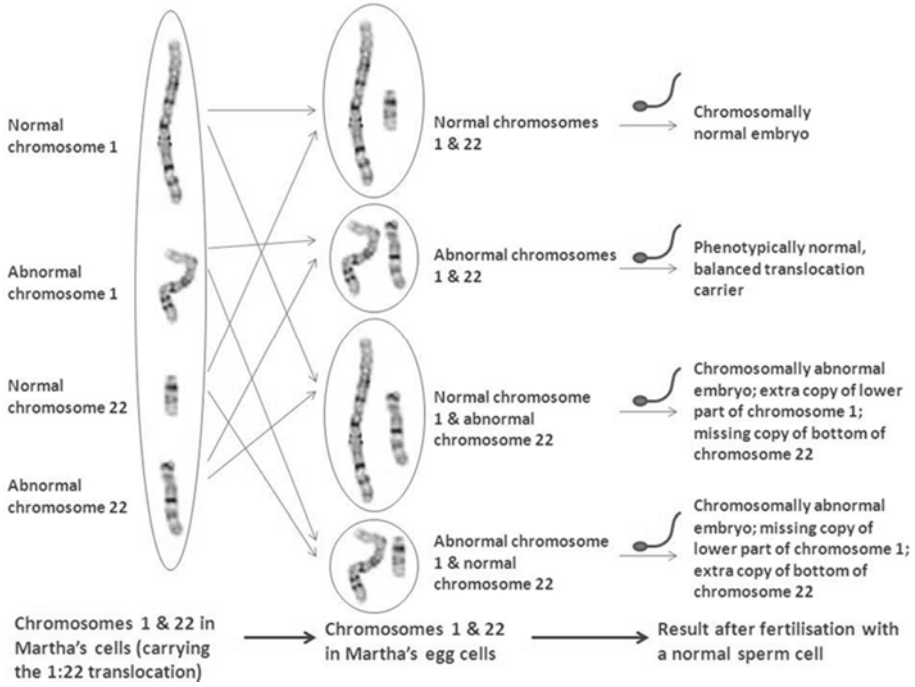
The karyotype (Figure below, left hand image) showed that there was a large amount of extra material on one of the chromosome 22 pair (arrow). Having extra or missing chromosome material (an unbalanced karyotype) will usually cause serious problems, as in this case.



Analysis of Martha's chromosomes (Figure, right hand image) showed that a block of material had been transferred from chromosome 1 to chromosome 22. She has the correct amount of material, but it is wrongly packaged into chromosomes (a balanced **translocation**). People with balanced abnormalities are usually physically normal, but they are at risk of producing offspring with an unbalanced chromosome pattern. The Figure (opposite) shows the main possible complements of chromosomes 1 and 22 in an egg of Martha's and the outcome if the egg is fertilised by a normal sperm, which carries one normal copy of each chromosome.

Quantifying the risk of each abnormal outcome can be difficult, and it is rarely possible to predict with confidence whether the result would be a miscarriage, stillbirth or a live born baby with multiple congenital abnormalities.

Other family members who were worried about their reproductive risks were invited to attend the clinic and have their blood tested. This would identify individuals who carried the translocation and, importantly, reassure those who did not. People identified as carrying the translocation would be offered prenatal diagnosis to check the chromosome pattern of any fetus.



The abnormality in this family is a translocation: two chromosomes have exchanged segments. One cell in the **germ line** of a forebear suffered an error during DNA replication or misrepair of DNA damage. Many other types of chromosome abnormality can be found, including **inversions**, **deletions** and **duplications**. Depending on the chromosome preparation, an abnormality will normally need to involve at least three million base pairs (3 Mb) of DNA to be detectable under the microscope. Other techniques must be used to detect smaller changes, as described below.

Three case histories: 3. A child with cleft lip and palate

Joshua was the first child born to his parents. There were no complications in early pregnancy but an ultrasound scan at 24 weeks of pregnancy detected a cleft lip and palate. Detailed scans of his heart and the rest of his body did not reveal any other problems; an amniocentesis showed he had a normal chromosome pattern and his measurements were well within normal limits all of which reassured his parents. As soon as he was born, members of the specialist cleft team visited the ward and gave the parents help and advice about feeding and arranged for early assessment by the team to develop a plan for surgery, to plan investigations and, later, to arrange orthodontic treatment. One of the team was a clinical geneticist and she examined Joshua to confirm he had no evidence of the presence of a more complex clinical syndrome.

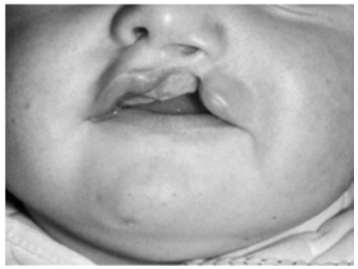
Cleft lip and palate are due to failure of normal developmental events. They can occur together or separately. At around seven weeks of gestation the facial structures (frontonasal, maxillary and mandibular processes) which will form the lips and jaws, come together and eventually fuse. If this does not happen then cleft lip results. Cleft palate can also occur when the lack of fusion of the facial processes prevents the palatal shelves coming together and fusing. Cleft palate can sometimes occur without cleft lip, in association with a small lower jaw which pushes the tongue upwards, thus preventing palatal closure. A whole range of genetic and environmental factors influence the rate at which facial structures and the palatal shelves grow together

The cause (aetiology) of cleft lip and/or palate is thus highly heterogeneous, meaning there are many different factors which can contribute to the condition, and this varies from case to case. Such heterogeneity is typical of many common birth defects. There are forms where defects in known single genes are responsible, often seen as part of a wider clinical syndrome, but in most cases no specific cause can be assigned (complex condition).

Counselling about recurrence risks is straightforward in the single gene cases, but for the majority all that can be offered is an empiric (survey-based) risk. If

past experience has shown that 5% of couples in similar circumstances had another affected baby, a 5% recurrence risk would be quoted. In reality, probably some of the 5% had unknown genetic factors that made a recurrence highly likely, while in many of the 95% the cause was an unfortunate coincidence that was very unlikely to recur. Hopefully the current rapid accumulation of genome sequence data will eventually allow us to distinguish those cases, in cleft lip +/-palate and in many other common complex conditions.

Figure: An infant with cleft lip, which was later successfully repaired



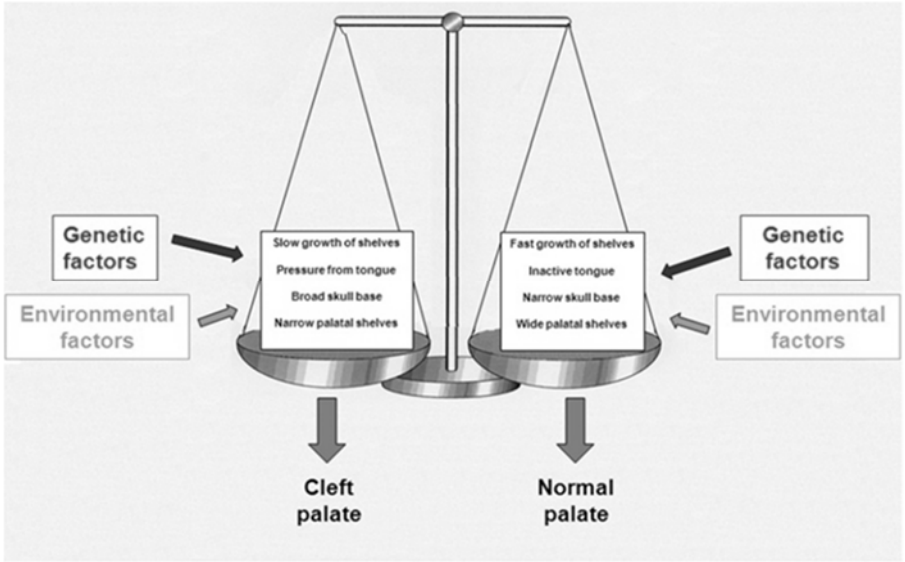


Figure: Varying combinations of genetic and environmental factors can tip the balance of development, resulting in cleft lip and/or palate.

Diagnosis and management of genetic conditions: 1. Technologies for identifying genetic changes

A useful way of categorising the many possible techniques that a diagnostic laboratory might use is to distinguish between *targeted* and *global* technologies.

Targeted methods look at a predefined gene or small DNA sequence, which has been chosen for examination because of some prior hypothesis or evidence. The two main methods are:

- **FISH (Fluorescence in situ hybridisation) uses a fluorescently** labelled piece of cloned DNA to stick (hybridise) to the matching sequence in a spread of the patient's chromosomes on a microscope slide. The result shows the presence or absence of the matching sequence in the patient and its chromosomal location.

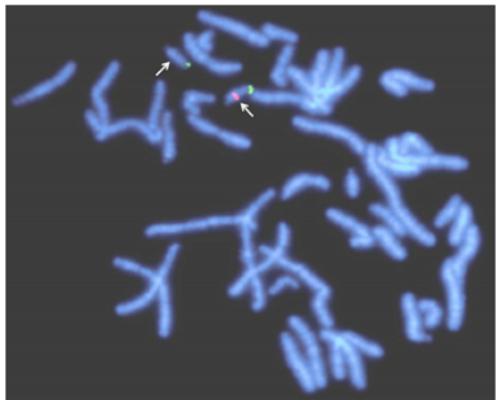


Figure: FISH showing a microdeletion on chromosome 22. The green labelled DNA hybridises to both copies of chromosome 22, identifying it. The pink labelled DNA hybridises to only one copy (white arrows). On the other copy the matching sequence must be missing.

- **PCR** (Polymerase chain reaction) is a method of producing millions of copies of a short predefined segment of the patient's DNA, which can then be sequenced to reveal any changes from the normal sequence.

Global methods avoid the need to decide in advance which gene or DNA segment to check as they examine the whole genome. In the past few years new technologies have greatly increased the efficiency and reduced the costs of these global methods, so that their use in diagnosis is now routinely possible.

- **Array-CGH** (array-comparative genomic hybridisation) has largely superseded karyotyping (described above) for routine analysis. DNA from the patient is labelled with a fluorescent dye (say, a red dye) and mixed with an equal quantity of a control DNA (DNA from an individual without known medical problems) that has been labelled with a different dye, say green. The DNA molecules in the mix compete to hybridise to their complementary sequence on an array of known DNA fragments set out on a small chip. The analysis looks at the overall colour of the DNA that hybridises to each of the arrayed fragments. If a given fragment shows more green than red, the patient's DNA must be missing one or both copies of the sequence. Conversely, if more red than green hybridises to one of the arrayed fragments, that sequence must be present in a higher copy number (ie: larger quantities) in the patient than in the control 'normal' DNA. Array-CGH is a powerful and flexible technique that can detect deletions or duplications that are far too small to be seen by conventional karyotyping. Note, however, that it cannot detect balanced abnormalities, such as the translocation described earlier, where there is no extra or missing material.

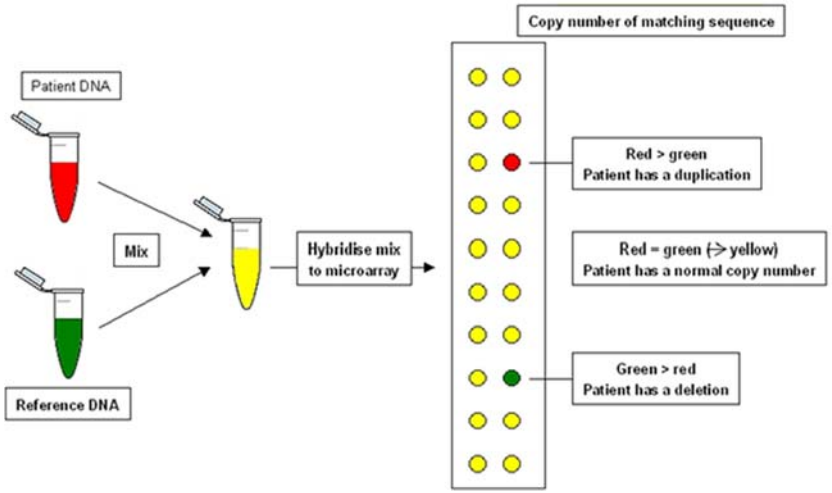


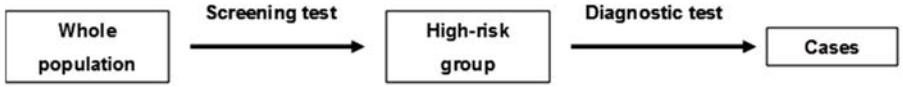
Figure: Principle of array-CGH

- **DNA Sequencing** is increasingly the method of choice for most purposes. Until around 2005 sequencing was based on a method invented by Fred Sanger in 1977, for which he shared the Nobel Prize for Chemistry in 1980. It is accurate, but limited to sequencing no more than one thousand bp (1 kb) of DNA at a time, and so had to be targeted. Starting in 2005 new technologies ('next generation sequencing') emerged that vastly extended the capacity of routine sequencing by sequencing millions of DNA fragments in parallel (ie: at the same time). This means large panels of genes, or indeed every gene in the genome, can be sequenced in a single operation for an acceptable cost. A semi-targeted approach might sequence 100 genes known to be involved in hereditary blindness in order to identify the mutation that caused a patient's loss of vision. A global approach would sequence every exon of every gene in the genome (known as whole **exome** sequencing). With continuing technical development it is likely that it will soon become routine to sequence a patient's entire genome, rather than just the 1.2% of coding sequence. Exactly how the resulting avalanche of data will be handled is an interesting question.
- **DNA profiling** is a technique used by forensic scientists to check whether DNA recovered from a crime scene matches that of a suspect. One type of DNA profiling involves measuring a standard set of a dozen or so highly variable DNA segments. Like conventional fingerprints, this type of DNA profile is unique to the individual, but does not provide any general information about them (although special sets of variants can be used to make inferences about a person’s likely ancestry or paternity).

Diagnosis and management of genetic conditions: 2. Prenatal screening

Prenatal screening tests are offered to all pregnant women (who are free to opt out of the programme). For example, in the UK, women are normally offered screening tests for Down syndrome at 10-14 weeks of pregnancy, consisting of an ultrasound scan to measure the baby and test for excess fluid at the back of the baby's neck (nuchal translucency), and blood tests. These results are combined with the mother's age to calculate whether she falls into the high risk group. Women are also offered an ultrasound anomaly scan at 18-20 weeks gestation and further blood tests to check for diabetes and other problems.

If the screening test – or the family history – indicates an elevated risk of a genetic condition, women may be offered an invasive prenatal test.



Two tests are widely used for genetic conditions:

Chorion villus biopsy (CVB) takes a sample of the placenta (a fetal tissue) at 10 -12 weeks of gestation.

Amniocentesis samples amniotic fluid (containing fetal cells) at 14-20 weeks.

Both procedures carry a risk of about 1% of provoking a miscarriage, and so are only used when a screening test or the family history justify it. CVB and amniotic samples can be used for chromosome, DNA or biochemical analysis.

Free DNA is present in the blood of a pregnant woman and about 5% of this is of fetal origin. There is great interest in using this for genetic screening and diagnosis. Technically this is difficult because of the small proportion and fragmented state of the fetal DNA. Fetal sex can be detected reliably (looking for Y-chromosome DNA), and there have been trials for detecting chromosomal trisomies, single gene disorders and even reconstructing the entire fetal genome sequence. Free fetal DNA testing has the advantage of being non-invasive, and technical development is likely to move it into practice over the next few years.

Diagnosis and management of genetic conditions: 3. Newborn screening

Newborn babies are screened for a range of conditions including structural birth defects, enzyme deficiencies and deafness, many of which have a genetic cause. Screening is only practicable for conditions that are reasonably common, where testing is easy and where early intervention improves the outlook.

In the UK the screening is divided into three programmes; 1) NHS Newborn and Infant Physical Examination Screening Programme – examination within 72 hours of birth 2) NHS Newborn Hearing Screening Programme – hearing screen within 2 weeks of birth 3) NHS Newborn Blood Spot Screening Programme – sample taken 5-8 days after birth.

Genetic screening uses a blood spot obtained by pricking the baby's heel. This can be used to detect a range of genetic disorders. The conditions checked vary quite widely between different countries and regions, but in the UK consist of phenylketonuria, congenital hypothyroidism, sickle cell disease, cystic fibrosis and medium-chain acyl-CoA dehydrogenase deficiency (MCADD) where appropriate treatment (dietary and/or drugs) is initiated, rather than leaving diagnosis until serious damage has already been done.

Screening for phenylketonuria (PKU) is almost universal. This autosomal recessive condition affects about 1 baby in 10,000 in the UK. Affected individuals lack the enzyme phenylalanine hydroxylase, which converts the amino acid phenylalanine into tyrosine. Phenylalanine accumulates from dietary protein, and eventually causes brain damage and serious intellectual impairment. If the blood spot shows an elevated level of phenylalanine the baby is referred for an urgent diagnostic test. By stringently controlling the amount of phenylalanine in the diet from the very earliest age, the brain damage can be avoided and the individual grows up to have normal intelligence (refuting the commonly held idea that if a condition is genetic it is untreatable).

Cystic fibrosis is screened by checking for an elevated level of trypsin in the blood, which triggers further investigations, including DNA analysis to look for mutations. Again, early treatment improves outcomes for affected individuals.

Diagnosis and management of genetic conditions: 4. Clinical management and treatment

Although genetic disorders are often regarded as untreatable, that is far from the truth for many conditions today. Understanding in detail the symptoms, the clinical course, and the genetic basis of various conditions has enabled doctors to anticipate and treat complications and to develop treatments for a wide range of disorders.

Examples of clinical management strategies to prevent complications of genetic disorders include: 1) regular scans for early detection of kidney cancer in children with Beckwith-Wiedemann syndrome, a rare disorder associated with overgrowth, a large tongue, umbilical hernia (a gap in the muscle wall near the navel) and increased risk of tumours; 2) early diagnosis and, if needed, laser treatment to prevent retinal detachment in people with Stickler syndrome, a dominantly inherited disorder with vision, hearing and skeletal problems. 3) For many rare syndromes – in which multiple body systems are affected – management guidelines and patient-held records have been developed to optimise clinical care and ensure all professionals are appropriately informed.

Some of the earliest treatments for genetic disorders involved so-called inborn errors of metabolism (IEM). For example, once the underlying cause of phenylketonuria (the inability to make the enzyme phenylalanine hydroxylase) was discovered, scientists and dieticians developed a special diet low in phenylalanine which prevents the build up of this amino acid and its adverse effects on brain development. A few IEMs have been shown to be responsive to specific vitamin therapies, and more recently enzyme replacement therapies have been developed for children with other rare IEMs.

If a genetic disorder involves a missing protein such as in the cases of haemophilia (which involves a missing blood clotting factor), or growth hormone deficiency, this can sometimes be corrected through replacement by a regular injection of a synthetic protein.

There is much current interest in treating rare genetic disorders with drugs

which have already been developed and are on the market for common conditions such as high blood pressure or kidney disease. This interest is based on recent discoveries of genes mutated in rare disorders, coupled with the knowledge that the genetic pathways involved are known to be influenced by existing drugs. Already there are trials with such drugs to prevent complications of the conditions tuberous sclerosis (associated with non-malignant tumours in various organs of the body, most commonly brain, eyes, heart, kidney, skin and lungs) and Marfan syndrome (associated with, amongst other symptoms, tall stature and the potential fatal rupture of the aorta).

As mentioned in the introduction, there are current clinical trials in patients with Duchenne muscular dystrophy which utilise knowledge of the specific mutation in an affected boy to 'overcome' the block in transcription of the gene with a compound that results in a functional protein being produced.

Finally, gene therapy is, at last, developed or in clinical trials for many conditions. Gene therapy is the process whereby functioning genes are introduced into the part of the body where the absent or non-functional gene/protein result in the symptoms of the disorder. These conditions include immune deficiency disorders of childhood, in which affected children would usually succumb to overwhelming infection, retinal dystrophies (inherited eye conditions), which cause blindness, and lipoprotein lipase deficiency (a loss of an enzyme responsible for breakdown of certain fats in the body).

Glossary

Autosome – any chromosome except the sex chromosomes (X and Y).

Clone – a DNA sequence, cell or whole organism that is an exact genetic copy of another.

Coding – DNA containing the genetic code for a protein.

Deletion – a missing segment of a gene or chromosome.

DNA – deoxyribonucleic acid, the ultimate repository of genetic information.

Duplication – a repeated segment of a gene or chromosome.

Exon – a section of the DNA of a gene of which a copy is present in the mature messenger RNA (**cf intron**).

Exome – the totality of exons in the genome.

Functional RNA – an RNA molecule that itself does some job in the cell, rather than simply carrying the genetic code for a protein.

Gametes – sex cells (sperm and egg).

Genome – the totality of genes or genetic material of an individual.

Genotype – the genetic constitution of an individual (at one or more loci, or over the whole genome) Cf**. phenotype**.

Germ line – the line of cells that produce the **gametes**, aspects of whose genotype may therefore be transmitted to the next generation.

Heterozygote – of an individual, having different variants of a gene at a specific locus.

Homozygote – of an individual, having identical versions of a gene at a specific locus.

Intron – a section of the DNA of a gene that is present in the primary transcript, but which is removed during processing of the messenger RNA (cf. exon).

Inversion – a chromosomal abnormality in which part of a chromosome is the wrong way round.

Karyotype – the chromosomal constitution of an individual, e.g. 46,XY. Karyotype is also used to describe the standard format for displaying the chromosomes of an individual, set out in pairs and in descending order of size (more correctly a karyogram).

Kilobase (kb) – 1,000 base pairs of DNA.

Megabase (Mb) – 1 million base pairs of DNA.

Meiosis – the specialised type of cell division that produces gametes. During meiosis the chromosome number is halved and maternal and paternal copies of genes are shuffled so that each gamete is genetically unique.

Mendelian – of a character, determined by a single gene locus (= Monogenic).

Methylation – a chemical modification of a molecule, consisting of adding one or more methyl ($-CH_3$) groups. Methylation of specific bases in DNA is used by cells as a signal to change the way the DNA is packaged and genes are expressed.

Mitosis – the normal process of cell division, which creates two genetically identical daughter cells (cf. **meiosis).**

Monogenic – of a character, determined by a single gene locus.

Non-coding – DNA that does not code for protein. It may however still have important functions, encoding functional RNAs or controlling gene expression.

Nucleotide – the basic unit of DNA or RNA, consisting of a base (normally adenine, guanine, cytosine or thymine in DNA; adenine, guanine, cytosine or uracil in RNA), a sugar (deoxyribose in DNA, ribose in RNA) and a phosphate.

Penetrance – the probability that a particular genetic variant will produce a certain phenotype that is normally associated with it. Reduced penetrance can cause a dominant condition to skip a generation.

Phenotype – the observable properties or behaviour of an organism (as distinct from the **genotype).**

Reduced penetrance – the case where a gene does not always manifest itself by conferring an associated phenotype.

RNA – ribonucleic acid. RNA molecules are very heterogeneous and have many different functions in the cell.

Somatic cell – a body cell, as distinct from a **germ-line** cell. The genotype of somatic cells is not transmitted to the next generation.

Syndrome – a combination of clinical features which occur together and are due to the same underlying defect or factors

Teratogen – a substance that causes birth defects.

Translocation – a chromosomal rearrangement in which two chromosomes swap segments.

Trisomy – having three copies of a particular chromosome.

X chromosome, Y chromosome – the sex chromosomes. Males have one X and one Y, females have two Xs.

Further information

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Genetics in Medicine - 1. Conception and Early Life

Virtually every human disease and ailment has at least some genetic aspects, and restoring health always involves biological systems – metabolism, cells or organs – that are under genetic control. Our increasing understanding of genetics, and our ability to manipulate genetic systems have led to some far-reaching visions of the role of genetics in 21st century medicine.

nowgen

engagement • education • training