

# **Essential Revision Notes in Paediatrics for the MRCPCH**

## **Third edition**

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# Chapter 11

## Genetics

Natalie Canham

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# Genetics

## 1. CHROMOSOMES

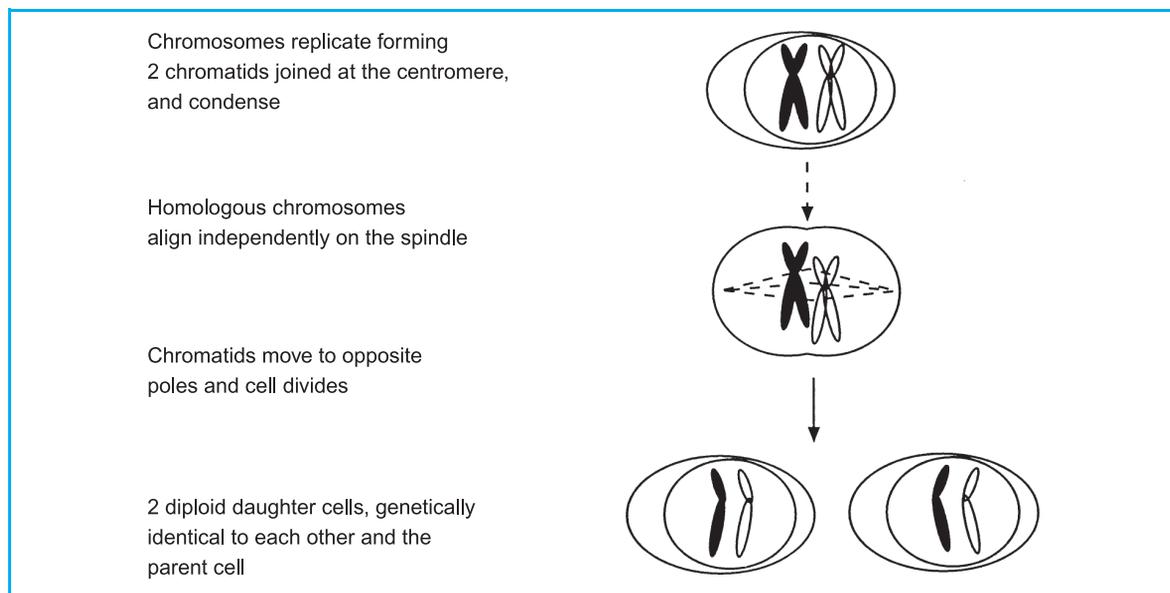
### Background

Within the nucleus of somatic cells there are 22 pairs of autosomes and one pair of sex chromosomes. Normal male and female karyotypes are 46,XY and 46,XX respectively. The normal chromosome complement of 46 chromosomes is known as **diploid**. Genomes with only a single copy of every chromosome or with three copies of each are known respectively as **haploid** and **triploid**. A karyotype with too many or too few chromosomes, where the total is not a multiple of 23, is called **aneuploid**. Three copies of a single chromosome in a cell are referred to as **trisomy**, whereas a single copy is **monosomy**.

Chromosomes are divided by the centromere into a short 'p' arm ('petit') and a long 'q' arm. **Acrocentric** chromosomes (13, 14, 15, 21, 22) have the centromere at one end and only a q arm.

**Lyonization** is the process in which, in a cell containing more than one X chromosome, only one is active. Selection of the active X chromosome is usually random and each inactivated X chromosome can be seen as a Barr body on microscopy. Genes are expressed only from the active X chromosome.

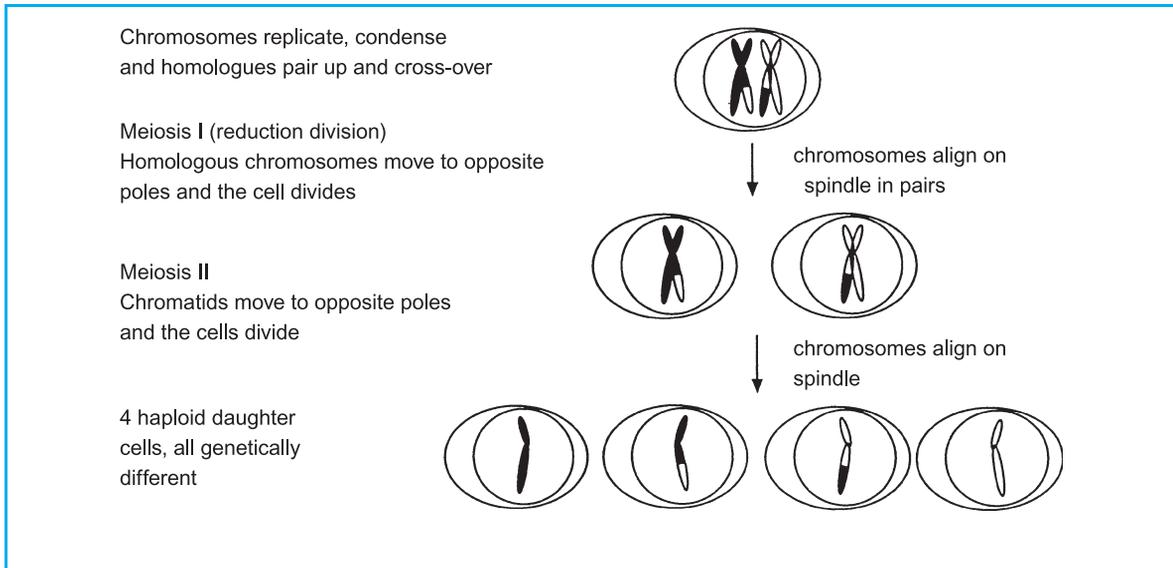
**Mitosis** occurs in somatic cells and results in two **diploid** daughter cells with nuclear chromosomes which are genetically identical both to each other and the original parent cell.



Mitosis

**Meiosis** occurs in the germ cells of the gonads and is also known as '**reduction division**' because it results in four **haploid** daughter cells, each containing just one member (**homologue**) of each chromosome pair, all genetically different. Meiosis involves two divisions (**meiosis I** and **II**). The reduction in chromosome number occurs during meiosis I and is

preceded by exchange of chromosome segments between homologous chromosomes called **crossing over**. In males the onset of meiosis and spermatogenesis is at puberty. In females, replication of the chromosomes and crossing over begins during fetal life but the oocytes remain suspended before the first cell division until just before ovulation.



Meiosis

## Translocations

- **Reciprocal** – exchange of genetic material between non-homologous chromosomes
- **Robertsonian** – fusion of two acrocentric chromosomes at their centromeres, e.g. (14;21)
- **Unbalanced** – if chromosomal material has been lost or gained overall
- **Balanced** – if no chromosomal material has been lost or gained overall

Carriers of balanced translocations are usually phenotypically normal but are at increased risk for having offspring with a chromosomal imbalance. There is also commonly an increased risk of miscarriage and of reduced fertility.

Carriers of a robertsonian translocation involving chromosome 21 are at increased risk of having offspring with translocation Down syndrome. For female and male (14;21) translocation carriers the observed offspring risks for Down syndrome are approximately 15% and 5%, respectively. This may be due to a selective disadvantage to spermatozoa carrying an extra chromosome. Remember, translocation carriers can also have offspring with normal chromosomes or offspring who are balanced translocation carriers like themselves.

## 1.1 Common sex chromosome aneuploidies

### Turner syndrome (karyotype 45,X)

This affects 1 in 2500 live-born girls but it is a frequent finding among early miscarriages. Patients are usually of normal intelligence. They have streak ovaries that result in failure of menstruation, low oestrogen with high gonadotrophins and infertility. Normal secondary sexual characteristics may develop spontaneously or can be induced with oestrogens. If puberty is achieved, the uterus is usually normal and pregnancy is possible with the use of donated ova. Short stature throughout childhood with failure of the pubertal growth spurt is typical. Final height can be increased by early treatment with growth hormone. Other features may include:

- Webbed or short neck
- Low hairline
- Shield chest with widely spaced nipples
- Cubitus valgus (wide carrying angle)
- Cardiovascular abnormalities (particularly aortic coarctation in 10–15%)
- Renal anomalies (e.g. horseshoe kidney, duplicated ureters, renal aplasia) in a third
- Non-pitting lymphoedema in a third

### Triple X syndrome (karyotype 47,XXX)

This affects 1 in 1000 live-born girls. These patients show little phenotypic abnormality but tend to be of tall stature. Although intelligence is typically reduced compared with siblings it usually falls within normal or low–normal limits. However, mild developmental and behavioural difficulties are more common. Fertility is normal but the incidence of early menopause is increased.

### Klinefelter syndrome (karyotype 47,XXY)

This affects 1 in 600 live-born boys. Phenotypic abnormalities are rare prepubertally other than a tendency to tall stature. At puberty, spontaneous expression of secondary sexual characteristics is variable but poor growth of facial and body hair is common. The testes are small and associated with azoospermia, testosterone production is around 50% of normal and gonadotrophins are raised. Gynaecomastia occurs in 30% and there is an increased risk of male breast cancer. Female distribution of fat and hair and a high-pitched voice may occur but are not typical. Intelligence is generally reduced compared with siblings but usually falls within normal or low–normal limits. Mild developmental and behavioural problems are more common.

### 47,XYY males

This affects 1 in 1000 live-born boys. These males are phenotypically normal but tend to be tall. Intelligence is usually within normal limits but there is an increased incidence of behavioural abnormalities. Previous studies suggesting an increase in criminality have been disproved.

## 1.2 Common autosomal chromosome aneuploidies

### Down syndrome (trisomy 21)

Down syndrome affects 1 in 700 live births overall and is usually secondary to meiotic non-disjunction during oogenesis, which is more common with increasing maternal age. Around 5% of patients have an underlying robertsonian translocation, most commonly between chromosomes 14 and 21. Around 3% have detectable **mosaicism** (a mixture of trisomy 21 and karyotypically normal cells) usually resulting in a milder phenotype.

Phenotypic features include:

- Brachycephaly
- Upslanting palpebral fissures, epicanthic folds, Brushfield spots on the iris
- Protruding tongue
- Single palmar crease, fifth finger clinodactyly, wide sandal gap between first and second toes
- Hypotonia and moderate learning disability

The following are more common in patients with Down syndrome:

- Cardiovascular malformations in 40%, particularly atrioventricular septal defects
- Gastrointestinal abnormalities in 6%, particularly duodenal atresia and Hirschsprung disease
- Haematological abnormalities, particularly acute lymphoblastic, acute myeloid and transient leukaemias
- Hypothyroidism
- Cataracts in 3%
- Alzheimer disease in the majority by 40 years of age

### Edward syndrome (trisomy 18)

This typically causes intrauterine growth retardation, a characteristic facies, prominent occiput, overlapping fingers (second and fifth overlap third and fourth), rockerbottom feet (vertical talus) and short dorsiflexed great toes. Malformations, particularly congenital heart disease, diaphragmatic hernias, renal abnormalities and dislocated hips, are more

common. Survival beyond early infancy is rare but associated with profound learning disability.

### Patau syndrome (trisomy 13)

Affected infants usually have multiple malformations including holoprosencephaly and other central nervous system abnormalities, scalp defects, microphthalmia, mid-line cleft lip and cleft palate, post-axial polydactyly, rockerbottom feet, renal anomalies and congenital heart disease. Survival beyond early infancy is rare and associated with profound learning disability.

## 1.3 CGH microarray

CGH (**comparative genomic hybridization**) microarray is a method of more detailed chromosome analysis than that provided by karyotyping. Patient genomic DNA and control genomic DNA are differentially labelled with different fluorescent probes and then hybridized together. The ratio of fluorescent intensity between patient and control DNA is then compared which detects areas of copy number difference. This can detect microdeletions and microduplications as well as anomalies that would have been visible on karyotype. The sensitivity of the test, and thus the size of the imbalances detected, are determined by the distances between and number of the fluorescent probes. High-resolution arrays can detect imbalances as small as 200 base-pairs, but those in current diagnostic use typically detect anomalies above 100 kilobases (kb). Arrays are not able to detect balanced rearrangements, so the karyotype is still appropriate in cases such as recurrent miscarriage. Many small anomalies detected are inherited from a normal parent, and thus are probably not significant in the pathogenesis of developmental problems.

## 1.4 MLPA

MLPA (**multiplex ligation-dependent probe amplification**) is a multiplex PCR (polymerase chain reaction) method able to detect abnormal copy numbers of multiple genomic DNA sequences. This can be used at a gene level, detecting exon deletions or

duplications, or at a chromosomal microdeletion level. Typically kits are generated with a set of probes such as all the telomeres, or the common microdeletion syndromes.

## 1.5 Qf-PCR

Qf-PCR (**quantitative fluorescence polymerase chain reaction**) is a technique allowing fast assessment of copy numbers of whole chromosomes on small samples. Small sections of DNA from the sample are amplified, labelled with fluorescent tags and the amounts measured by electrophoresis. This is most commonly used for identification of aneuploidy on prenatal samples. Typically only chromosomes 13, 18 and 21, and perhaps the sex chromosomes, are tested because no other whole chromosome aneuploidy is survivable to term. Results are available in 24–48 hours.

## 1.6 FISH testing

FISH (**fluorescent in situ hybridization**) is a technique used to assess the copy number of specific DNA sequences in the genome. Fluorescently labelled probes are designed that are complementary to the DNA sequences being assessed, and they are allowed to hybridize to the chromosome spread. The number of copies can then be visualized as fluorescent spots using confocal microscopes. FISH can be performed much more rapidly than formal karyotyping. However, the use of MLPA, Qf-PCR and CGH microarray has largely superseded this process, except in testing other members of a family for a known chromosomal anomaly.

## 1.7 Microdeletion syndromes

These are caused by chromosomal deletions that are too small to see on standard microscopy but involve two or more adjacent (contiguous) genes. They can be detected using specific FISH testing, MLPA or CGH microarray.

Examples of microdeletion syndromes:

- **22q11 microdeletion** (parathyroid gland hypoplasia with hypocalcaemia, thymus

hypoplasia with T-lymphocyte deficiency, congenital cardiac malformations, particularly interrupted aortic arch and truncus arteriosus, cleft palate, learning disability) also previously called by many names including DiGeorge syndrome. There appears to be an increased incidence of psychiatric disorders, particularly within the schizophrenic spectrum

- **Williams syndrome** (supravalvular aortic stenosis, hypercalcaemia, stellate irides, characteristic facial appearance, learning disability) due to microdeletions involving the elastin gene on chromosome 7
- **16p11.2 microdeletion syndrome** (autism, seizures, learning disability) no real diagnostic phenotypic features meant that this was not previously identified, but with the widespread use of CGH microarray it is now apparent that this is the most common microdeletion syndrome, found in 1 in every 100 on the autistic spectrum. Frequently also found in a normal parent, giving a high recurrence risk.

## 1.8 Genetic counselling in chromosomal disorders

As a general rule the following apply.

### For parents of a child with trisomy 21

Recurrence risks will be around 1% above the maternal age-related risks for which there are tables. At age 36 years the background risk for Down syndrome is 0.5%. Parents with a Robertsonian translocation involving chromosome 21 have a much higher recurrence risk.

### For parents of a child with any other trisomy

Recurrence risks in future pregnancies for that specific trisomy will be <1%. However, couples are generally counselled that there is a 1% risk for any chromosome abnormality in future offspring, which takes into account the small risks that one parent may be mosaic or may have an increased risk of chromosome mis-segregation at meiosis.

### For parents of a child with a microdeletion

Parental chromosomes should be checked. If they are normal, recurrence risks will be <1%. If one parent carries the microdeletion then recurrence risks will be 50%.

### For parents of a child with any other chromosome abnormality

Parental chromosomes should be checked. If they are normal then recurrence risks are usually small (<1%). If one parent carries a predisposing translocation then recurrence risks will be higher, depending on the nature of the translocation.

Prenatal karyotyping is available for any couple who have had a previous child with a chromosome abnormality.

## 2. MENDELIAN INHERITANCE

### 2.1 Autosomal dominant (AD) conditions

These result from mutation of one copy of a pair of genes carried on an autosome. All offspring of an affected person have a 50% chance of inheriting the mutation. Within a family the severity may vary (**variable expression**) and known mutation carriers may appear clinically normal (**reduced penetrance**). Some conditions, such as achondroplasia and neurofibromatosis type 1, frequently start anew through new mutations arising in the egg or (more commonly) sperm.

#### Examples of autosomal dominant conditions

Achondroplasia  
Alagille syndrome  
Ehlers–Danlos syndrome (most)  
Faciocapulohumeral muscular dystrophy  
Familial adenomatous polyposis  
Familial hypercholesterolaemia  
Gilbert syndrome  
Huntington disease

Marfan syndrome  
Myotonic dystrophy  
Neurofibromatosis types 1 and 2  
Noonan syndrome  
Porphyrias (except congenital erythropoietic which is AR)  
Tuberous sclerosis  
Von Willebrand disease

Conditions pre-fixed 'hereditary' or 'familial' are usually autosomal dominant.

### 2.2 Autosomal recessive [AR] conditions

These result from mutations in both copies of an autosomal gene. Where both parents are **carriers** (with only one mutation and a normal copy), each of their offspring has a 1 in 4 (25%) risk of being affected and a 2 in 4 (50%) chance of being a carrier. Carriers are usually indistinguishable from normal other than by DNA analysis.

#### Examples of autosomal recessive conditions

Alkaptonuria  
Ataxia telangiectasia  
β-Thalassaemia  
Congenital adrenal hyperplasias  
Crigler–Najjar syndrome (severe form)  
Cystic fibrosis  
Dubin–Johnson syndrome  
Fanconi anaemia  
Galactosaemia  
Glucose-6-phosphatase deficiency (von Gierke disease)<sup>a</sup>  
Glycogen storage diseases  
Homocystinuria  
Haemochromatosis  
Mucopolysaccharidoses (all except Hunter syndrome)  
Oculocutaneous albinism  
Phenylketonuria  
Rotor (usually)  
Sickle cell anaemia

Spinal muscular atrophies  
Wilson disease  
Xeroderma pigmentosa

<sup>a</sup>Do not confuse with glucose-6-phosphate dehydrogenase deficiency (favism) which is X-linked recessive.

Most metabolic disorders are autosomal recessive – remember the exceptions.

### Risk calculations for AR disorders

Remember:

- People who have no family history of an autosomal recessive disorder have the background population carrier risk
- The parents of a child with an autosomal recessive disorder are assumed to be carriers
- Where both parents are known to be carriers for an autosomal recessive disorder, any of their children who are known to be unaffected are left with a two-thirds carrier risk (because if the possibility that they are affected is discounted, only three possibilities remain).

### Autosomal recessive inheritance and consanguinity

It is believed that everybody carries a few deleterious autosomal recessive genes. First cousins share on average one-eighth of their genes because they share one set of grandparents. As a result, they are more likely to be carrying the same autosomal recessive disorders. For consanguineous couples in a family with a known AR disorder, specific risks should be calculated and appropriate testing should be arranged. For first-cousin parents who have no known family history of any autosomal recessive disorder, their offspring have around a 3% increased risk above the general background risk of any genetic abnormality of 2% (i.e. a 5% overall risk). Screening should be offered for any autosomal recessive disorder that is available and known to be common in their ancestral ethnic group, e.g.:

- White people – cystic fibrosis
- African/African–Caribbean people – sickle cell anaemia
- Mediterranean/Asian people – thalassaemia
- Jewish people – Tay–Sachs disease and multiple other recessive disorders

Although consanguinity is regarded as taboo in many societies, around 20% of all marriages are consanguineous (second cousin or closer). There are sound financial and societal reasons for consanguineous marriages in societies where these relationships are common, and the majority of offspring are healthy. Geneticists would never advise against consanguineous marriage (or indicate that a child's recessive disorder is the fault of the marriage), but families affected with recessive disorders have been known to employ carrier testing to assist in marriage planning.

### 2.3 X-linked recessive (XLR) conditions

These result from a mutation in a gene carried on the X chromosome and affect males because they have just one gene copy. Females are usually unaffected but may have mild manifestations as a result of lyonization. New mutations are common in many XLR disorders which means that the mother of an affected boy, with no preceding family history, is not necessarily a carrier. XLR inheritance is characterized by the following:

- No male-to-male transmission – an affected father passes his Y chromosome to all his sons
- All daughters of an affected male are carriers – an affected father passes his X chromosome to all his daughters
- Sons of a female carrier have a 50% chance of being affected and daughters have a 50% chance of being carriers

**Examples of X-linked recessive conditions**

- Alport syndrome (usually XLR; some AR forms)
- Becker muscular dystrophy
- Duchenne muscular dystrophy
- Fabry disease
- Fragile X syndrome
- Glucose-6-phosphate dehydrogenase deficiency (favism)
- Haemophilias A and B (Christmas disease)
- Hunter syndrome (MPS II)
- Lesch–Nyhan disease
- Ocular albinism
- Red–green colour blindness
- Testicular feminization syndrome
- Wiskott–Aldrich syndrome

males. New mutations are common. For the reasons outlined above:

- There is no male-to-male transmission
- All daughters of an affected male would be affected
- All offspring of an affected female have a 50% chance of being affected

**Examples of X-linked dominant conditions include:**

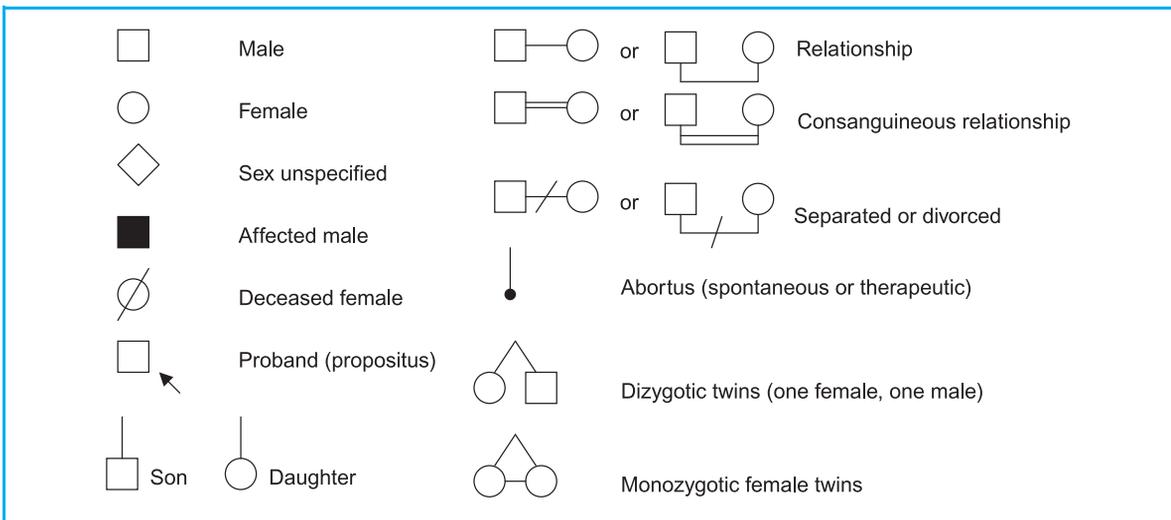
- Goltz syndrome
- Incontinentia pigmenti
- Rett syndrome
- Hypophosphataemic (vitamin D-resistant) rickets

**2.4 X-linked dominant (XLD) conditions**

These are caused by a mutation in one copy of a gene on the X chromosome but both male and female mutation carriers are affected. As a result of lyonization, females are usually more mildly affected and these disorders are frequently lethal in

**2.5 Constructing a pedigree diagram (family tree)**

The basic symbols in common usage are shown in the figure below. Occasionally symbols may be half shaded or quarter shaded. This generally means that the individual manifests a specified phenotypic feature denoted in an accompanying explanatory key, e.g. lens dislocation in a family with Marfan syndrome.



Basic symbols used in pedigree diagrams

### 3. MOLECULAR GENETICS

#### 3.1 DNA (deoxyribonucleic acid)

DNA is a **double-stranded** molecule composed of purine (adenine + guanine) and pyrimidine (cytosine and thymine) bases linked by a backbone of covalently bonded **deoxyribose sugar** phosphate residues. The two anti-parallel strands are held together by hydrogen bonds which can be disrupted by heating and reform on cooling:

- **Adenine (A) pairs with thymine (T)** by two hydrogen bonds
- **Guanine (G) pairs with cytosine (C)** by three hydrogen bonds

#### 3.2 RNA (ribonucleic acid)

DNA is **transcribed** in the nucleus into messenger RNA (mRNA) which is **translated** by ribosomes in the cytoplasm into a polypeptide chain. RNA differs from DNA in that:

- It is **single-stranded**
- Thymine is replaced by **uracil (U)**
- The sugar backbone is **ribose**

#### 3.3 Polymerase chain reaction (PCR)

This is a widely used method for generating large amounts of the DNA of interest from very small samples. PCR can be adapted for use with RNA provided that the RNA is first converted to DNA.

PCR is a method by which a small amount of target DNA (the template) is selectively amplified to produce enough to perform an analysis. This might be the detection of a particular DNA sequence such as that belonging to a pathogenic microorganism or an oncogene, or the detection of differences in genes such as mutations causing inherited disease. Therefore the template DNA might consist of DNA

derived from peripheral blood lymphocytes, a tumour biopsy or a biological fluid from a patient with an infection.

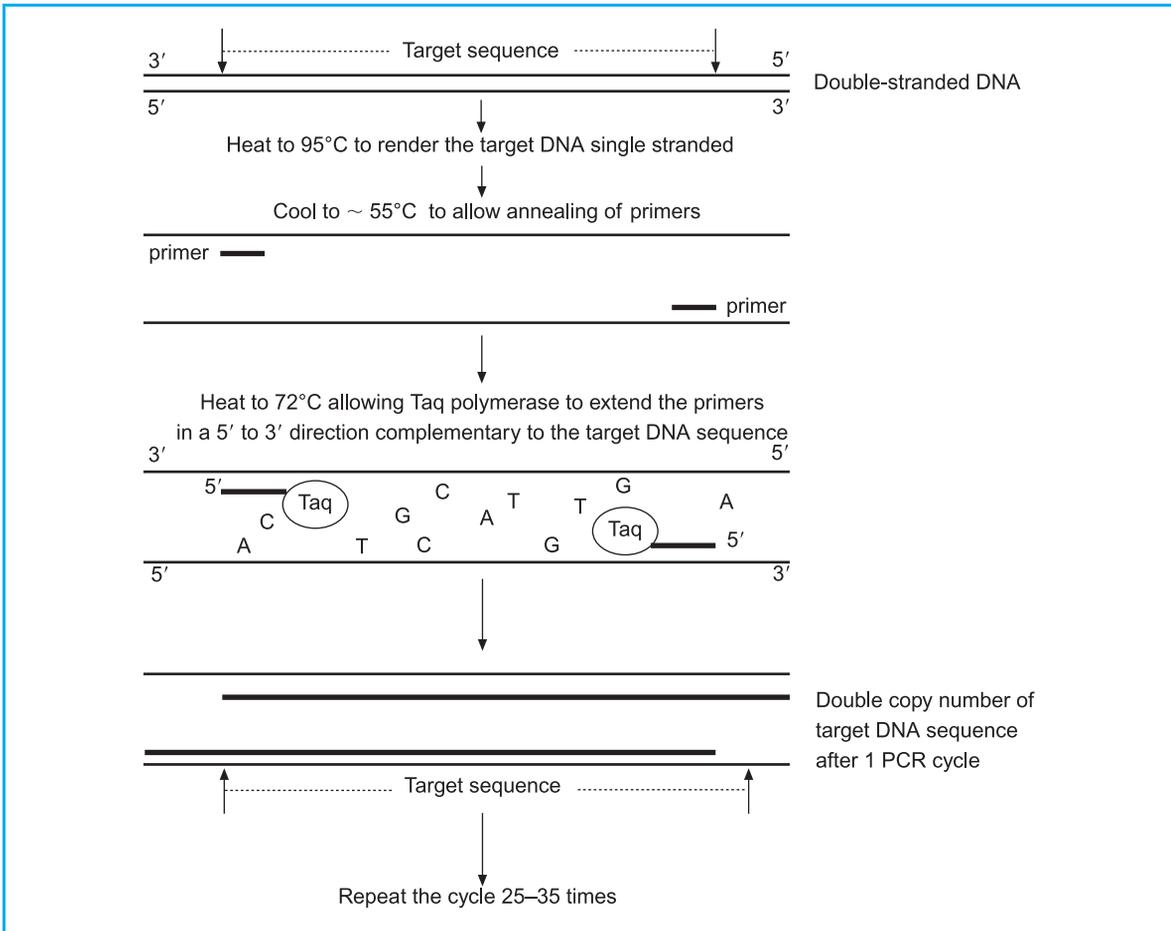
In order to perform PCR, the sequence flanking the target DNA must usually be known so that specific complementary oligonucleotide sequences, known as primers, can be designed. The two unique primers are then mixed together with the DNA template, deoxyribonucleotides (dATP, dCTP, dGTP, dTTP) and a thermostable DNA polymerase (Taq polymerase, derived from an organism that inhabits thermal springs):

- In the initial stage of the reaction the DNA template is heated (typically for about 30 seconds) to make it single stranded. As the reaction cools the primers will anneal to the template if the appropriate sequence is present.
- The reaction is then heated to 72°C (for about a minute) during which time the Taq DNA polymerase synthesises new DNA between the two primer sequences, doubling the copy number of the target sequence.
- The reaction is heated again and the cycle is repeated. After 30 or so cycles (each typically lasting a few minutes) the target sequence will have been amplified exponentially.

The crucial feature of PCR is that to detect a given sequence of DNA it only needs to be present in one copy (i.e. one molecule of DNA): this makes it extremely powerful.

#### Clinical applications of PCR

- Mutation detection
- Single cell PCR of in vitro fertilized embryo to diagnose genetic disease before implantation
- Detection of viral and bacterial sequences in tissue (herpes simplex virus in CSF, hepatitis C, HIV in peripheral blood, meningococcal strains)



Polymerase chain reaction

### 3.4 Reverse transcription PCT (rt-PCR)

This is a modification of conventional PCR used to amplify messenger RNA (mRNA) sequence in order to look at the expression of particular genes within a tissue. mRNA is single stranded, unstable and not a substrate for Taq DNA polymerase. For that reason it must be converted to complementary DNA (cDNA) using reverse transcriptase, a retroviral enzyme, which results in a double-stranded DNA copy of the original RNA sequence. PCR can then be performed in the normal way.

### 3.5 Next generation sequencing

DNA sequencing is used to identify point mutations, or small deletions/duplications, in a specific gene. Typically a small number of individuals' DNA is tested for mutations in one gene. This is expensive in terms of time and substrates. Next generation sequencing allows multiple parallel analyses to be performed at the same time. This can be used to test a single individual's DNA for mutations in multiple genes, or to test large numbers of individuals at the same time. Chips are being developed for specific related conditions caused by multiple genes, such

as aortic dissection, Noonan syndrome, cardiomyopathies and cardiac arrhythmias. These will allow rapid genetic diagnosis of individuals with a clinical diagnosis. Next generation technology is also the basis of exome sequencing.

### 3.6 Exome sequencing

Whole genome sequencing is expensive and time-consuming. The exome consists of only the coding sequences in the genome, i.e. the parts of the genome that are translated into protein. This only represents around 5% of the total genome, but is estimated to contain 85% of all disease-causing mutations. Exome sequencing is a method of analysing the entire exome for mutations. This is primarily a research tool used to identify unknown genes responsible for mendelian disorders, but has also been used to identify functional variation associated with more common conditions such as Alzheimer disease.

## 4. TRINUCLEOTIDE REPEAT DISORDERS

These conditions are associated with genes containing stretches of repeating units of three nucleotides and include:

- Fragile X syndrome – X-linked
- Myotonic dystrophy – AD
- Huntington disease – AD
- Friedreich ataxia – AR
- Spinocerebellar ataxias – AD

In normal individuals the number of repeats varies slightly but remains below a defined threshold. Affected patients have an increased number of repeats, called an **expansion**, above the disease-causing threshold. The expansions may be unstable and enlarge further in successive generations causing increased disease severity (**'anticipation'**) and earlier onset, e.g. **myotonic dystrophy**, particularly congenital myotonic dystrophy after transmission by an affected mother. Between the normal range and

the affected range, there are two other expansion sizes. **Premutation** sizes are smaller than the lowest copy number to cause disease and are not associated with a risk of the condition, but have a high risk of increasing into the disease range during gametogenesis, generating an affected child. This risk can be gender dependent in some conditions. **Intermediate alleles** are smaller than the premutation range, but larger than normal. They have a risk of increasing into the premutation range during gametogenesis.

### 4.1 Fragile X syndrome

This causes learning disability, macro-orchidism, autism and seizures, and was historically associated with a cytogenetically visible constriction ('fragile site') on the X chromosome. The inheritance is X linked but complex. Among controls there are between 6 and 45 stably inherited trinucleotide repeats in the *FMR1* gene. The intermediate allele size is 50–58 repeats, and people with between 58 and 230 repeats are premutation carriers but are unaffected. Only female gametogenesis carries a risk of expansion into the disease-causing range (230 to >1000 repeats) known as a full mutation which is methylated, effectively inactivating the gene. All males and around 50% of females with the full mutation are affected, though females are typically less severely affected. The premutation does not expand to a full mutation when passed on by a male. Male premutation carriers are known as normal transmitting males and will pass the premutation to all their daughters (remember that they pass their Y chromosome to all their sons). Although premutation carrier status is not associated with learning disability, female carriers have a high risk (around 50%) of premature ovarian failure or early menopause. There is also a condition called **fragile X-associated tremor and ataxia syndrome (FXTAS)**, which predominantly affects male premutation carriers over the age of 50. Parkinsonism and cognitive decline are also features. The lifetime male risk of developing FXTAS is 30–40% though 75% of men older than 80 show signs.

## 5. MITOCHONDRIAL DISORDERS

Mitochondria are **exclusively maternally inherited**, deriving from those present in the cytoplasm of the ovum. They contain copies of their own **circular 16.5-kilobase chromosome** carrying genes for several respiratory chain enzyme subunits and transfer RNAs. Mitochondrial genes differ from nuclear genes in having no introns and using some different amino acid codons. Within a tissue or even a cell there may be a mixed population of normal and abnormal mitochondria known as **heteroplasmy**. Different proportions of abnormal mitochondria may be required to cause disease in different tissues, known as a **threshold effect**. Disorders caused by mitochondrial gene mutations include:

- **MELAS** (mitochondrial encephalopathy, lactic acidosis, stroke-like episodes)
- **MERRF** (myoclonic epilepsy, ragged red fibres)
- Mitochondrially inherited diabetes mellitus and deafness (typically caused by the same mutation as seen in MELAS but at lower levels)
- Leber hereditary optic neuropathy (note that other factors also contribute)

## 6. GENOMIC IMPRINTING

For most genes both copies are expressed but for some genes, either the maternally or paternally derived copy is preferentially used, a phenomenon known as genomic imprinting. The unused copy is frequently methylated, which inactivates the gene. These genes tend to aggregate together in **imprinted regions** on chromosomes. Abnormalities of inheritance or methylation of imprinted genes can therefore cause disease even in the presence of two apparently normal copies. The best examples are the Prader–Willi and Angelman syndromes, both caused by cytogenetic deletions of the same region of chromosome 15q, **uniparental disomy** of chromosome 15 (where both copies of chromosome 15 are derived from one parent with no copy of chromosome 15 from the other parent), or abnormalities of methylation, which labels both chromosomes as deriving from one parent. The disease condition is caused by the absence of one parent's copy of genes in the region, rather than by excessive numbers of copies of the other.

### Prader-Willi syndrome

#### Clinical

Neonatal hypotonia and poor feeding  
Moderate learning disability  
Hyperphagia + obesity in later childhood  
Small genitalia

#### Genetics

70% deletion on paternal chromosome 15  
30% maternal uniparental disomy 15  
(i.e. no paternal contribution)

### Angelman syndrome

Unprovoked laughter/clapping  
Microcephaly, severe learning disability  
Ataxia, broad-based gait  
Seizures, characteristic EEG

80% deletion on maternal chromosome 15  
2–3% paternal uniparental disomy 15  
(i.e. no maternal contribution);  
remainder due to subtle mutations

## Other imprinting disorders

### Silver–Russell syndrome

Prenatal onset growth retardation, relative macrocephaly, triangular facies, asymmetry, fifth finger clinodactyly and frequently normal IQ. Around 35% are caused by abnormal methylation of genes on chromosome 11p15, whereas 10% are associated with maternal uniparental disomy of chromosome 7. The cause in the remainder is not yet known.

### Beckwith–Wiedemann syndrome

Prenatal-onset macrosomia, facial naevus flammeus, macroglossia, ear lobe creases, pits on the ear helix, hemihypertrophy, nephromegaly, exomphalos (omphalocele) and neonatal hypoglycaemia. There is an increased risk of Wilms tumour, adrenocortical and hepatic tumours in childhood. Similar to Silver–Russell syndrome, the condition results from abnormalities of inheritance or methylation of chromosome 11p15 which contains several imprinted genes, including the *IGF-2* (insulin-like growth factor 2) gene. The results in BWS tend to be directly opposite to those in Silver–Russell syndrome.

## 7. GENETIC TESTING

Genetic tests can be thought of as diagnostic, predictive or for carrier status. Informed verbal, and increasingly written, consent (or assent) should be obtained before genetic testing.

### Diagnostic tests

These are chromosomal investigations such as karyotype and CGH microarray, or mutation analysis of specific genes. The latter is frequently used where the diagnosis is already suspected on clinical grounds but genetic testing is useful for confirmation, or for counselling or predictive testing in the wider family.

### Predictive tests

When an individual is clinically normal but is at risk for developing a familial disorder, such as Huntington disease, myotonic dystrophy or a famil-

ial cancer syndrome. Predictive testing is not usually offered without a formal process of genetic counselling over more than one consultation with time built in for reflection. Where there are intervening relatives whose genetic status may be indirectly revealed, there are additional issues that must be taken into consideration. Written consent for predictive testing is required by most laboratories. Nationally agreed guidance is that predictive testing in children for disorders that have no implications in childhood should not be undertaken until the child is old enough to make an informed choice.

### Carrier tests

These are usually undertaken in autosomal recessive or X-linked recessive disorders where the result has no direct implications for the health of the individual, but is helpful in determining the risks to their offspring. Carrier status may be generated as a by-product of diagnostic or prenatal testing. National guidance is that specific testing for carrier status should be avoided in children until they are old enough to make an informed choice.

### Genetics in children

Diagnostic tests are obviously necessary and useful, as are predictive tests for disorders that may manifest in childhood, and have a screening programme or treatment, such as the multiple endocrine neoplasias (MEN1, MEN2) and familial adenomatous polyposis. Predictive testing for adult onset disorders such as *BRCA-1/-2* or Huntington disease are not appropriate in children, because they are unable to give informed consent, and a diagnosis can never be removed once it has been made. Many adults opt not to have predictive tests for untreatable disorders such as Huntington disease, and an at-risk child should be allowed to make the same decision. Equally, carrier status for AR or X-linked disorders will impact only on a child's reproductive decisions, not childhood health, and thus is only tested when the child is able to participate in the process and give proper informed consent. Parents do occasionally request such testing, and a clinical geneticist would meet them in clinic to discuss their reasons for testing and the reasons for a reluctance to offer it.

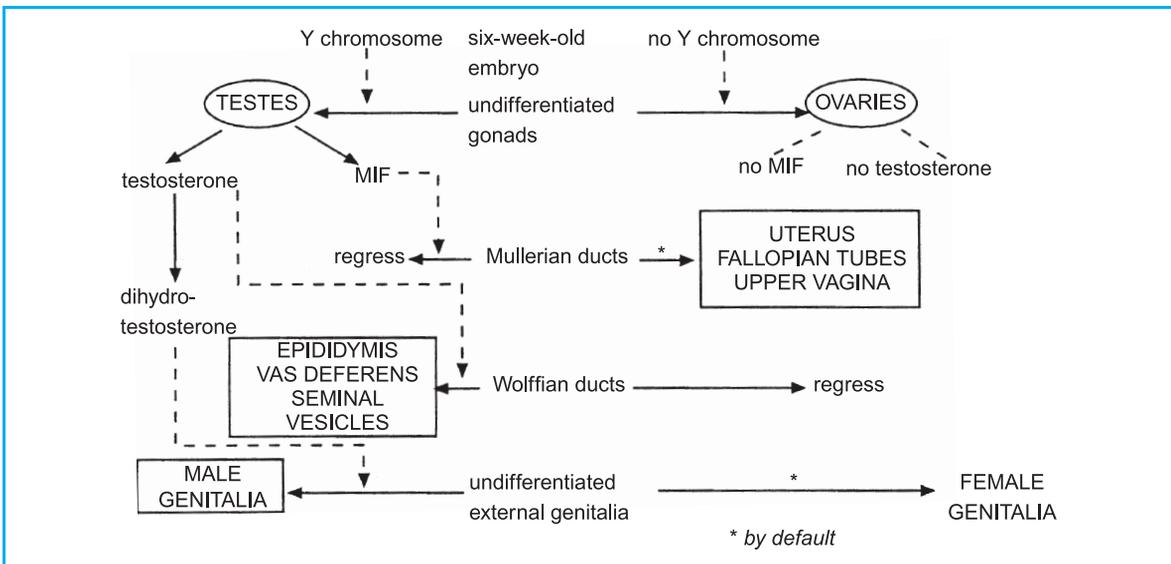
## 8. IMPORTANT GENETIC TOPICS

This section includes short notes on conditions that form popular examination topics.

### 8.1 Ambiguous genitalia

#### Normal development of the reproductive tract and external genitalia

A simplified outline is shown below.



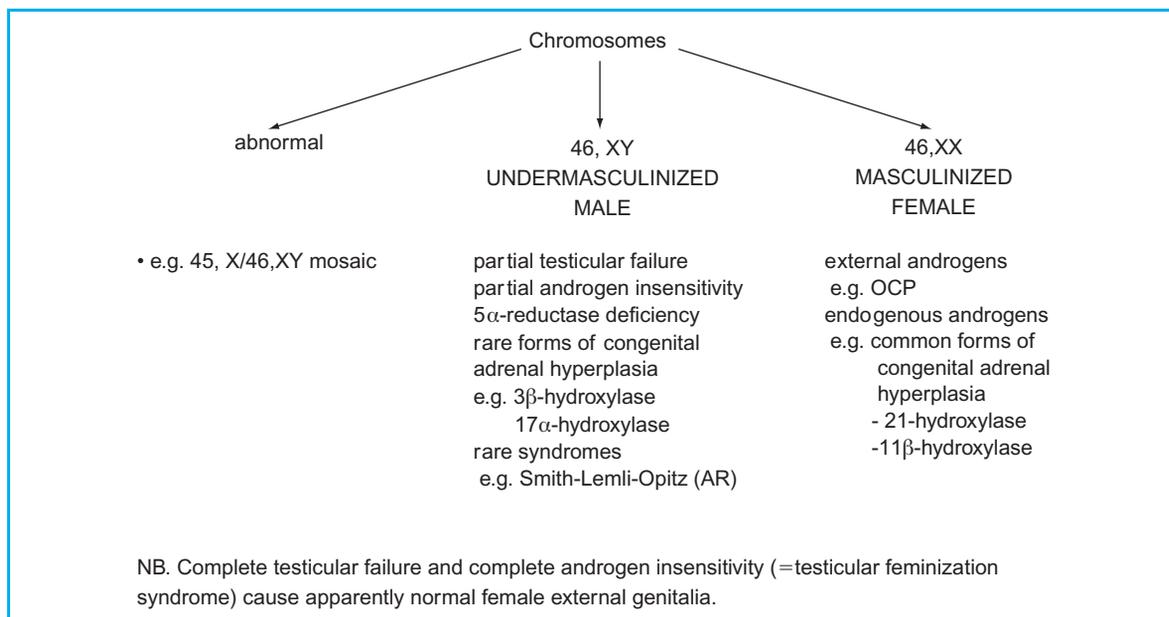
Outline of the normal development of the reproductive tract and external genitalia

The 6-week embryo has undifferentiated gonads, müllerian ducts (capable of developing into the uterus, fallopian tubes and upper vagina), wolffian ducts (capable of forming the epididymis, vas deferens and seminal vesicles) and undifferentiated external genitalia.

In the presence of a Y chromosome the gonads become testes that produce testosterone and müllerian inhibiting factor (MIF). Testosterone causes the wolffian ducts to persist and differentiate and, after conversion to dihydrotestosterone (by 5 $\alpha$ -reductase), masculinization of the external genitalia. MIF causes the müllerian ducts to regress.

In the absence of a Y chromosome the gonads become ovaries which secrete neither testosterone nor MIF and, in the absence of testosterone, the wolffian ducts regress and the external genitalia feminize. In the absence of MIF, the müllerian ducts persist and differentiate.

The causes of **ambiguous genitalia** divide broadly into those resulting in undermasculinization of a male fetus, those causing masculinization of a female fetus, and those resulting from mosaicism for a cell line containing a Y chromosome and another that does not. They are summarized in the diagram opposite.



Ambiguous genitalia – outline of causes

## 8.2 Cystic fibrosis

This results from mutations in the *CFTR* (cystic fibrosis transmembrane regulator) gene. The  $\Delta F508$  mutation (deletion of three nucleotides coding for a phenylalanine residue at amino acid position 508) accounts for 75% of mutations in white people. Most laboratories now screen for 32 common mutations including  $\Delta F508$ . Such testing identifies 90% of cystic fibrosis mutations in white people, but a

much smaller proportion in many other ethnic groups. Therefore, negative molecular testing cannot exclude a diagnosis of cystic fibrosis.

## 8.3 Duchenne and Becker muscular dystrophies

These result from different mutations within the dystrophin gene on chromosome Xp21.

### Important distinguishing features of Duchenne and Becker muscular dystrophies

	<b>Duchenne muscular dystrophy</b>	<b>Becker muscular dystrophy</b>
Immunofluorescent dystrophin on muscle biopsy	Undetectable	Reduced/abnormal
Wheelchair dependence	95% at <12 years	5% at <12 years
Learning disability	20%	Rare

In around a third of boys with Duchenne muscular dystrophy, the condition has arisen as a new mutation, whereas a further third are the result of a new mutation in the mother. Mutation analysis in the affected boy can often identify mothers who are carriers, but a normal result does not exclude germline mosaicism, where mutated cells are present in the ovaries but not the blood. A woman proven to be a carrier has a 25% (1 in 4) recurrence risk, but a woman without the mutation in her blood still has up to a 20% recurrence risk, and prenatal diagnosis is offered in all circumstances.

Given the high new mutation rate, both in the affected child and in the mother, calculation of risks to other family members can be challenging. The risk that the mother of an isolated case is a carrier is two in three. The maternal grandmother's risk is one in three, due to the chance of a new mutation in the mother. Thus, the sister of the isolated affected boy has a one in three risk of being a carrier, but the maternal aunt has a one in six risk, and so on.

In practical terms, most families will have an identifiable mutation, and thus carrier identification will be relatively easy. In the absence of a mutation, e.g. the affected individual has died with no DNA stored, or no mutation is identified (a small proportion), then the above risks can be modified using linkage to the X chromosome and Bayes theorem to take into account the number of unaffected males in the family, and the creatine kinase (CK) levels in the at-risk females. Carrier females can have elevated CK levels, although a normal result does not exclude carrier status because they follow a normal distribution. A woman known to be at high risk, but with no identifiable mutation, may only be able to opt to terminate male pregnancies if she wishes to avoid having an affected child.

## 8.4 Neurofibromatosis

There are two forms of neurofibromatosis (NF) that are clinically and genetically distinct:

	<b>NF1</b>	<b>NF2</b>
<b>Major features</b>	<ul style="list-style-type: none"> <li>≥6 Café-au-lait patches</li> <li>Axillary/inguinal freckling</li> <li>Lisch nodules on the iris</li> <li>Peripheral neurofibromas</li> </ul>	<ul style="list-style-type: none"> <li>Bilateral acoustic neuromas (vestibular schwannomas)</li> <li>Other cranial and spinal tumours</li> </ul>
<b>Minor features</b>	<ul style="list-style-type: none"> <li>Macrocephaly</li> <li>Short stature</li> </ul>	<ul style="list-style-type: none"> <li>Café-au-lait patches (usually &lt;6)</li> <li>Peripheral schwannomas</li> <li>Peripheral neurofibromas</li> </ul>
<b>Complications</b>	<ul style="list-style-type: none"> <li>Plexiform neuromas</li> <li>Optic glioma (2%)</li> <li>Other cranial and spinal tumours</li> <li>Pseudarthrosis (especially tibial)</li> <li>Renal artery stenosis</li> <li>Phaeochromocytoma</li> <li>Learning difficulties</li> <li>Scoliosis</li> <li>Spinal cord and nerve compressions</li> <li>Malignant change/sarcomas</li> </ul>	<ul style="list-style-type: none"> <li>Deafness/tinnitus/vertigo</li> <li>Lens opacities/cataracts</li> <li>Spinal cord and nerve compressions</li> <li>Malignant change/sarcomas</li> </ul>
<b>Gene</b>	Chromosome 17	Chromosome 22
<b>Protein</b>	Neurofibromin	Schwannomin

## 8.5 Tuberos sclerosis

There are at least two separate genes that cause tuberous sclerosis (TS), on chromosomes 9 (*TSC1*; hamartin) and 16 (*TSC2*; tuberin).

### Clinical features of tuberous sclerosis

#### Skin/nails

- Ash-leaf macules
- Shagreen patches (especially over the lumbosacral area)
- Adenoma sebaceum (facial area)
- Subungual/periungual fibromas

#### Eyes

- Retinal hamartomas

#### Heart

- Cardiac rhabdomyomas, detectable antenatally, usually regressing during childhood

#### Kidneys

- Angiomyolipomas
- Renal cysts

#### Neurological

- Seizures
- Learning disability

#### Neuroimaging

- Intracranial calcification (periventricular)
- Subependymal nodules
- Neuronal migration defects

## 8.6 Marfan syndrome

This results from mutations in the fibrillin 1 (*FBN1*) gene on chromosome 15. Intelligence is usually normal. New diagnostic criteria do not include joint

laxity or hyperextensibility, and this alone in a tall individual is not sufficient to suspect the diagnosis of Marfan.

### Clinical features of Marfan syndrome

#### Musculoskeletal

- Tall stature with disproportionately long limbs (dolichostenomelia)
- Characteristic facial appearance
- Arachnodactyly
- Pectus carinatum or excavatum
- Scoliosis
- High, narrow arched palate with dental overcrowding
- Pes planus

#### Heart

- Aortic root dilatation and dissection
- Mitral valve prolapse

#### Eyes

- Lens dislocation (typically up)
- Myopia

#### Skin

- Striae

#### Lungs

- Spontaneous pneumothorax
- Apical bullae

## 8.7 Homocystinuria

(see also Chapter 16)

This is most commonly the result of cystathione- $\beta$ -synthase deficiency and causes a Marfan syndrome-like body habitus, lens dislocation (usually down), learning disability, thrombotic tendency and osteoporosis. Treatment includes a low methionine diet  $\pm$  pyridoxine.

## 8.8 Noonan syndrome

This is an autosomal dominant condition. Around 50% of individuals with Noonan syndrome have mutations in the *PTPN11* (protein-tyrosine phosphatase, non-receptor-type 11) gene on chromosome 12. A further 10–15% are caused by *SOS1* (son of sevenless homologue 1 (*Drosophila*), on chromosome 2) and *RAF1* (v-raf-1 murine leukaemia viral oncogene homologue 1 on chromosome 3) causes another 5–10%. Multiple other genes on the RAS-MAPK pathway have also been implicated in small proportions of cases. The karyotype is usually normal.

### Clinical features of Noonan syndrome

#### Cardiac

- Pulmonary valve stenosis
- Hypertrophic cardiomyopathy
- Septal defects (atrial and ventricular septal defects)
- Branch pulmonary artery stenosis

#### Musculoskeletal

- Webbed or short neck
- Pectus excavatum or carinatum
- Wide-spaced nipples
- Wide carrying angle (cubitus valgus)
- Short stature in 80%

#### Other features

- Ptosis
- Low-set and/or posteriorly rotated ears
- Small genitalia and undescended testes in boys
- Coagulation defects in 30% (partial factor XI:C, XIIC and VIIC deficiencies, von Willebrand disease, thrombocytopenia)
- Mild learning disability in 30%

## 8.9 Achondroplasia

A short-limb skeletal dysplasia resulting from specific autosomal dominant mutations in the *FGFR3* (fibroblast growth factor receptor 3) gene on

chromosome 4. There is a high new mutation rate. Important complications are hydrocephalus, brain-stem or cervical cord compression resulting from a small foramen magnum, spinal canal stenosis, kyphosis and sleep apnoea. Intelligence is usually normal.

## 8.10 Alagille syndrome

A variable autosomal dominant disorder resulting from deletions of or mutations in the *JAG1* (jagged) gene on chromosome 20. Major features of the syndrome include:

- Cardiac – peripheral pulmonary artery stenosis ± complex malformations
- Eye – posterior embryotoxon, abnormalities of the anterior chamber
- Vertebral – butterfly vertebrae, hemivertebrae, rib anomalies
- Hepatic – cholestatic jaundice, paucity of intrahepatic bile ducts

## 8.11 CHARGE syndrome

A malformation syndrome including:

- Colobomas
- Heart malformations
- Atresia of the choanae
- Retardation of growth and development (learning disability)
- Genital hypoplasia (in males)
- Ear abnormalities (abnormalities of the ear pinna, deafness)
- Cleft lip/palate and renal abnormalities are also common

The majority of patients with CHARGE syndrome have new mutations or deletions of the *CHD7* (chromodomain helicase DNA-binding protein 7) gene on chromosome 8.

## 8.12 VATER (VACTERL) association

A sporadic malformation syndrome including:

- Vertebral abnormalities
- Anal atresia ± fistula

- Cardiac malformations
- Tracheo-oesophageal fistula
- Renal anomalies, radial ray defects
- Limb anomalies, especially radial ray defects

The cause is not yet known.

### 8.13 Goldenhar syndrome

Also known as oculo-auriculovertebral spectrum, or first and second and branchial arch syndrome. It is mainly sporadic and the cause is unknown. Major features include:

- Craniofacial – asymmetry, hemifacial microsomia, micrognathia
- Ears – malformed pinnas, deafness, preauricular tags
- Eyes – epibulbar (scleral) dermoid cysts, microphthalmia
- Oral – macrostomia, cleft lip/palate
- Vertebral – hemivertebrae
- Cardiac – cardiac malformations
- Renal – renal malformations

### 8.14 Pierre Robin sequence

An association of micrognathia and cleft palate which may occur alone, but a proportion will have 22q11 deletions or Stickler syndrome.

### 8.15 Potter sequence

Oligohydramnios as a result of renal abnormalities, urinary tract obstruction or amniotic fluid leakage may lead to secondary fetal compression with joint contractures (arthrogryposis), pulmonary hypoplasia and squashed facies known as the Potter sequence.

## 9. FETAL TERATOGENS

### 9.1 Maternal illness

#### Maternal diabetes

Maternal diabetes is associated with fetal macrosomia, neonatal hypoglycaemia and increased risk

of a wide variety of malformations, particularly cardiac (transposition of the great arteries, aortic coarctation, septal defects, cardiomyopathy), vertebral (sacral abnormalities, hemivertebrae), renal (agenesis, duplex collecting systems), intestinal (imperforate anus, other atresias) and limb abnormalities (short femurs, radial ray abnormalities).

#### Maternal myasthenia gravis

This is associated with fetal arthrogryposis.

#### Maternal phenylketonuria

Although the fetus is unlikely to be affected by phenylketonuria (PKU: which is autosomal recessive), if an affected mother has relaxed her low phenylalanine diet, the fetus is at risk of microcephaly, cardiac defects and learning disability secondary to exposure to the raised maternal phenylalanine levels.

#### Maternal systemic lupus erythematosus

Maternal systemic lupus erythematosus (SLE) with anti-Ro and anti-La antibodies is associated with an increased risk of fetal bradycardia and congenital heart block for which pacing may be required. A self-limiting neonatal cutaneous lupus may also occur.

### 9.2 Infectious agents

The following agents are associated with increased fetal loss in the first trimester; hepatosplenomegaly, jaundice and thrombocytopenia in the neonate; and abnormalities particularly those affecting the central nervous system, vision and hearing.

#### Fetal cytomegalovirus

Infection may be associated with microcephaly, intracranial calcification, chorioretinopathy, deafness and learning disability.

#### Fetal toxoplasmosis

Infection with *Toxoplasma* species, a protozoan, may be associated with microcephaly, hydrocephala-

lus, intracranial calcification, chorioretinopathy and learning disability.

### **Fetal rubella**

Infection with rubella virus is most often associated with deafness particularly in the first and early second trimesters, but cardiac abnormalities (persistent ductus arteriosus, peripheral pulmonary stenosis, septal defects), microcephaly, chorioretinopathy, cataract and learning disability are also associated.

### **Congenital syphilis, herpes and varicella**

See Chapter 15, Section 11.1.

## **9.3 Other teratogens**

### **Fetal alcohol syndrome**

Pre- and postnatal growth retardation, neonatal irritability, microcephaly, learning disability, hyperactivity in childhood, cardiac defects (particularly ventricular and atrial septal defects), small nails on fifth fingers and toes, facial anomalies (short palpebral fissures, ptosis, smooth philtrum, thin upper lip) and a variety of less common, often midline, malformations. It is likely that the effects on any one fetus are determined by the degree, timing and duration of exposure as well as the susceptibility of the fetus which is probably genetically determined.

### **Illicit drugs in pregnancy**

Opiate drugs in pregnancy have a high risk of dependency in the newborn, intrauterine growth retardation and still birth, but do not appear to be associated with significant risk of structural anomalies. There are behavioural issues during childhood. Fetal cocaine has a higher risk of defects, apparently associated with vascular disruption, such as limb reduction defects and pencephaly. Survivors of this do not appear to have long-term intellectual deficit once their home circumstance has been taken into account, though there is evidence of some attention and behavioural problems.

### **Fetal retinoic acid**

Exposure to retinoic acid (which is used in the treatment of acne) is associated with structural brain abnormalities, neuronal migration defects, microtia and complex cardiac malformations.

### **Fetal valproate syndrome**

Fetuses exposed to valproate have an increased risk of cleft lip and palate, neural tube defects, cardiac defects, radial ray defects, learning disability and facial anomalies (frontal narrowing including metopic craniosynostosis, thin eyebrows, infraorbital skin grooves, long philtrum, thin upper lip). These effects appear to be dose dependent.

### **Fetal warfarin syndrome**

Fetuses exposed to warfarin typically have nasal hypoplasia, stippled epiphyses and are at risk of learning disability and brain, eye, cardiac and skeletal malformations.

## **10. PRENATAL TESTING**

- **Chorionic villous sampling or biopsy** (CVS or CVB) – a small piece of placenta is taken either transabdominally or transvaginally. CVS testing can be safely performed from 11 weeks' gestation
- **Amniocentesis** – amniotic fluid is taken, containing cells derived from the surfaces of the fetus and amniotic membranes. Amniocentesis is usually performed from 15 weeks' gestation
- **Cordocentesis** – a method of obtaining fetal blood that can be performed from 18 weeks' gestation

Chromosome and DNA testing can be performed on any of the above types of sample, and biochemical analyses can often also be performed if necessary. Each method carries a small risk of miscarriage. As a result, most couples opt for prenatal testing only if they wish to terminate an affected pregnancy. Although chromosome analysis can be performed on any pregnancy, DNA analysis

can be used only in families where known mutations have already been identified, and the family is at significant risk.

It is possible to identify the sex of an unborn fetus by prenatal testing and, in the case of X-linked conditions where no specific mutation has been identified, this is often the only available prenatal test. However, it is illegal in the UK to terminate a pregnancy on the basis of gender alone unless the child is at risk of a genetic condition due to its gender.

### 11. NON-INVASIVE PRENATAL TESTING

Cell-free fetal DNA can be detected in the mother's circulating blood from 4 weeks' gestation. The vast majority of the cell-free DNA is maternal, however, so testing is currently limited to the identification or exclusion of genetic material not present in the mother, such as Y chromosome, or rhesus D in RhD-negative women. In those at risk of an X-linked disorder in sons, this process will remove the necessity for invasive testing in 50% of pregnancies. Currently it is not possible to test for trisomy 21 or other chromosomal anomalies by this method.

### 12. PREIMPLANTATION GENETIC DIAGNOSIS

This technique is an in vitro fertilization (IVF)-based process. At the 8- to 16-cell stage a single cell is removed from each embryo for testing. Only embryos predicted to be unaffected are reimplanted into the mother. Preimplantation genetic testing (PGD) is technically difficult and has a similar

viable pregnancy rate to IVF (25%). It is available in the UK for a limited, although increasing, number of conditions and virtually all inherited chromosome anomalies. For funding purposes it is frequently regarded as fertility treatment, so families can find it hard to get NHS treatment. Overseas centres have a wider range of conditions, but it is very expensive.

### 13. GENETIC COUNSELLING

This is the process of assisting families or individuals affected by genetic disease to understand the cause of their condition, the risk of recurrence and the options available to them. It is entirely non-directive and the aim is to deliver all available information to allow the family to make the appropriate decisions. Some families will opt for prenatal diagnosis and termination, although this will not be acceptable for others. Equally, with predictive testing, not everyone at significant risk of a condition chooses to have testing to clarify this risk further. Genetic counselling will be offered to all, with no obligation to pursue testing.

### 14. FURTHER READING

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