Section 1 Genetics and antenatal screening

Chapter 1

Genetics of fetal anomalies

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The counseling of families with known or possible genetic disorders is discussed in Chapter 2. In this chapter, two important preliminary questions are addressed:

- what types of genetic disorders are there and how do they arise?
- what methods are available for diagnosing them?

Types and classification of genetic disorders

The unit of inheritance is the gene and there are an estimated 21,000 protein-encoding genes in the human genome. These genes are arranged on 23 pairs of chromosomes (22 autosomes and one pair of sex chromosomes) in the nucleus of the cell. In addition, there are 37 genes in each of the thousands of mitochondria in each cell. These genes are arranged in a circular structure and their products all have a function within mitochondria in oxidative phosphorylation. The replication of mitochondrial genes is controlled by the products of nuclear genes.

Whilst each somatic cell contains the same genes, within each tissue some genes will be expressed and others will be silenced. The control of gene expression is a complex process and errors in this process can result in cellular malfunction. Alterations in gene expression may be caused by various factors that are not yet fully understood but are not (in many situations) due to alterations in the structure of the genes themselves, and hence are termed "epigenetic" changes.

Disorders due to alterations (mutations) in chromosomes and genes can thus be classified as follows.

Constitutional chromosome disorders

Numerical abnormalities

In these cases, instead of 46 chromosomes per cell, the actual number of chromosomes may be reduced or increased in number. This numerical imbalance may be present in all cells examined or just in a proportion ("mosaicism"). Where mosaicism is present, the percentage of abnormal cells may vary from tissue to tissue.

Structural abnormalities

In this situation, there may be the correct number of chromosomes, but one or more chromosomes may be structurally abnormal and have segments deleted or duplicated, or there may be complex inter- or intrachromosomal rearrangements, such as translocations, inversions, insertions, etc. These complex rearrangements may lead to the disruption of the function of genes and hence be "unbalanced," or alternatively they may not alter function and be "balanced" rearrangements.

Single-gene disorders

Mutations in individual genes within the nucleus (which may lead to loss or gain of function) can be of sufficient effect as to result in phenotypes that follow Mendelian patterns of inheritance. Mendelian disorders may be classified by their pattern of inheritance:

- Autosomal dominant conditions, e.g. Huntington's disease (HD), neurofibromatosis types 1 and 2, tuberous sclerosis, adult polycystic kidney disease, etc.
- Autosomal recessive conditions, e.g. cystic fibrosis (CF), hemoglobinopathies, spinal muscular atrophy, congenital adrenal hyperplasia, etc.

Fetal Medicine, ed. Bidyut Kumar and Zarko Alfirevic. Published by Cambridge University Press.

- X-linked disorders (usually X-linked recessive), e.g. Duchenne muscular dystrophy (DMD), hemophilia A and B, Fragile X syndrome, etc.

Some disorders may follow more than one pattern of inheritance. For example, there are autosomal dominant, autosomal recessive and X-linked forms of retinitis pigmentosa, the most common inherited form of visual impairment. There are also a few examples of disorders that in some families result from mutations in two separate genes ("digenic inheritance"); examples include some cases of Bardet–Biedl syndrome and retinitis pigmentosa. In these cases of digenic inheritance, it is likely that the genes involved encode proteins that act in the same cellular pathway. Counseling families with nuclear gene disorders is discussed in detail in Chapter 2.

In addition to the nuclear genes, there are 37 mitochondrial genes. Mitochondrial DNA (mtDNA) gene mutations, such as those that account for Leber's optic neuropathy and some mitochondrial myopathy syndromes e.g. mitochondrial disease-encephalopathy (MELAS), lactic acidosis and stroke-like episodes, myoclonic epilepsy-ragged red fibres (MERRF) and neurogenic weakness, ataxia, retinitis pigmentosa (NARP), do not follow Mendelian patterns as mitochondria are exclusively inherited via the oocyte. Therefore, a woman who carries a mitochondrial gene mutation will theoretically transmit that mutation to all of her offspring, whereas a man carrying a mitochondrial gene mutation will not transmit the disorder to any of his offspring. It must also be noted that each human cell contains thousands of copies of mtDNA. A mutation may be present in only some of the mitochondrial genomes. This situation is termed "heteroplasmy." If the mutation is present in all of the mitochondrial genomes in the cell, then there is "homoplasmy." Prenatal genetic testing and interpretation of test results for mtDNA disorders are difficult because of mtDNA heteroplasmy. The percentage level of mutant mtDNA in a chorionic villus sampling (CVS) biopsy may not reflect the percentage level of mutant mtDNA in other fetal tissues, and the percentage level may change during development and throughout life; therefore, for most heteroplasmic mtDNA mutations, prenatal diagnosis is not recommended. However, the mutations m.8993T>G and m.8993T>C (typically but not always associated with a NARP phenotype) show a more even tissue distribution and successful prenatal diagnosis has been achieved. In the situation of homoplasmy, the mutation will be transmitted to all offspring.

In addition to mtDNA point mutations, mtDNA deletions and duplications occur, which usually arise de novo. The risk of transmission of an mtDNA deletion from an affected woman is low and estimated at 4%[1].

Multifactorial disease

Many conditions, including many congenital abnormalities, are more common among family members but the pattern in families does not follow Mendelian inheritance. The familial pattern is due to the combined effect of a number of gene variants interacting with each other and with the environment. The number of predisposing gene alterations may be few (oligogenic) or many (polygenic) and risk of occurrence of a disease cannot be calculated accurately from simple principles and as such, recurrence risk counseling is based on empiric data.

Somatic genetic diseases

Many disorders (such as all cancers) are due to mutations or an accumulation of mutations in somatic cells. In this situation, gonadal cells are not involved and the disorders are not heritable.

Epigenetic disorders

Epigenetic factors are noninherited changes in the DNA (e.g., methylation of certain DNA bases) or in the folding or position of the chromatin within the nucleus, which do not affect the sequence of bases in a gene but influence its expression (i.e., whether it is switched on or off). A small percentage of genes (probably less than 1%) are only expressed on either the paternally derived or the maternally derived chromosome – these are said to carry a paternal or maternal "imprint." In Prader–Willi syndrome (PWS), for example, the genes responsible on chromosome 15 are only expressed on their paternal copies (the copies on the chromosome inherited from the child's mother are "switched off"). If a mutation (usually a deletion) affects the paternally derived genes, then no copies of these genes will be expressed and an abnormal phenotype results. This same result can occur if the child has two maternal copies of chromosome 15 and no paternal copy (a situation known as "maternal uniparental disomy"). For the large majority of the genome, uniparental disomy (two copies present) of paternal or maternal chromosomes or chromosome regions has no phenotypic effect, but if the chromosome region contains "imprinted" genes, a phenotype may result. Other well-known

imprinting disorders are Angelman syndrome and Beckwith–Wiedemann syndrome (BWS). In the above example of PWS, the phenotype results from silencing of genes that should be expressed. In other disorders, the phenotype may result from expression of genes that are normally silenced. Altered methylation of DNA underpins many of these conditions. In BWS, the cause of the loss or gain of methylation is usually unknown but not heritable.

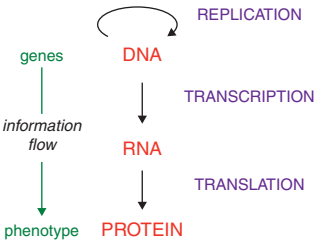

Figure 1.1 The classical paradigm of Mendelian genetics – genes encode proteins.

Structure of the genome

Chromosome structure

Genetic material consists of DNA that is packaged into 46 chromosomes in humans. This packaging is achieved by complexing the DNA with DNA-binding proteins called histones. This complex of DNA and histones is termed "chromatin." For each autosome, there is disomy in each DNA-containing cell apart from the gametes, which should contain one copy of each autosome and one sex chromosome. Normally in each somatic cell, one copy of each autosomal gene has come from the father ("paternal allele") and one from the mother ("maternal allele"), with the term "allele" simply meaning an alternative form of the same gene. There are about 21,000 protein-encoding genes located on these 46 chromosomes. Other genes encode various RNA species, and mutations in these genes can cause genetic disease, but for the purposes of this chapter we shall only consider the protein-encoding genes.

DNA is a double-stranded molecule made of four nucleotides or bases: adenine (a purine base that pairs with thymine on the opposite strand), thymine (a pyrimidine base that pairs with adenine), guanine (a purine base that pairs with cytosine) and cytosine (a pyrimidine base that pairs with guanine), which are usually referred to by their initial letters – A, T, G and C. These bases are bonded to each other in each DNA strand by strong phosphodiester bonds, whereas the bonds that hold together the double helix are hydrogen bonds, which are weak electrostatic bonds.

Gene structure and function

Figure 1.1 illustrates three important processes in molecular biology, i.e., DNA *replication*, *transcription* of the DNA code into RNA and the *translation* of the RNA code into a polypeptide/protein molecule. When cells divide, the DNA has to replicate itself (a process requiring substrate and DNA polymerases), and this

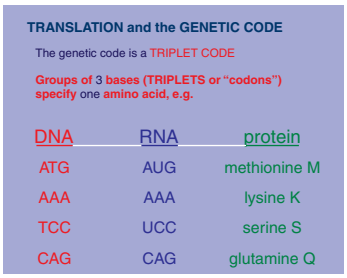

Figure 1.2 Translation and the genetic code:
The diagram shows four codons with their DNA and RNA sequences and the amino acid that they encode; each amino acid is represented by a letter. The genetic code is "degenerate" with, on average, each amino acid specified by about three different codons.

process results in copy errors or mutations. We shall return to this later in the chapter. One of the two DNA strands (the template strand) serves as a template for RNA synthesis; the RNA strand produced (requiring an RNA polymerase enzyme) is complementary to the template strand and has the same base sequence (except that uracil replaces thymine) as the opposite, nontemplate strand.

With regard to protein synthesis, the genetic code is a triplet code: many triplets (or groups of three bases or "codons") encode amino acids (examples given in Figure 1.2) but one triplet (AUG) is a start codon and there are three stop codons (UAA, UAG and UGA).

The sequence of bases in the DNA strands does not form one continuous "read-out." The sections of the gene that will encode the protein molecule reside within "exons" and the intervening DNA segments are termed "introns." During transcription, all the exons and introns are transcribed, but then the RNA molecule is modified so that the intron sequences are "spliced out" and the molecule has other modifications made to it (a methyl cap – a methylated guanosine molecule – on the 5' end and poly-A tail on 3' end) before a mature messenger RNA (mRNA) molecule is produced as illustrated in Figure 1.3.

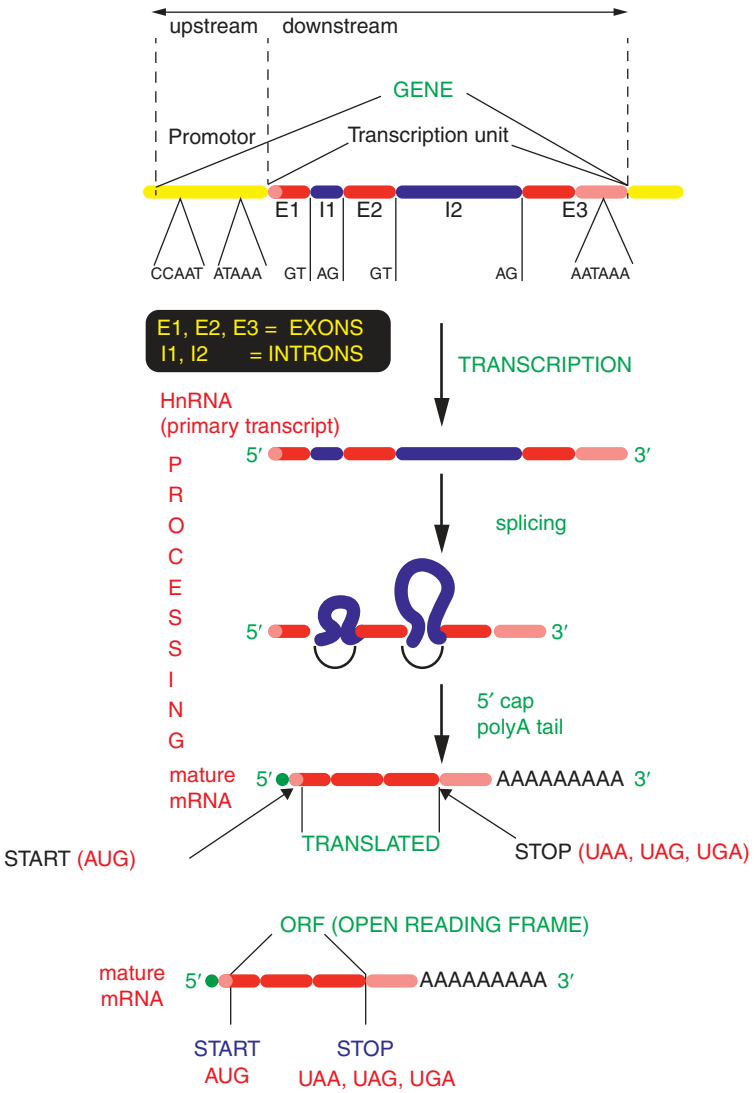

Figure 1.3 (a) The stylized gene template strand has three exons (E1, 2 and 3) and two introns (I1 and I2). Exon 1 contains the 5′ untranslated region (UTR) and exon 3 contains the 3′ UTR. Transciption produces an RNA molecule that is then modified to produce mRNA.
(b) A mature mRNA molecule. The open reading frame lies between the start and stop codons. Exon sequences also contain the 5′ and 3′ UTRs.

Translation takes place on the ribosomes in the cytoplasm, with transfer RNA acting as an adaptor between each amino acid and its codon.

Genome organization

Only approximately 1% of our DNA is comprised of protein-encoding exons (some exons/parts of exons encode untranslated regions). However, we now recognize that an additional (approximate) 15% of the human genome is functional, i.e., controls the expression of protein-encoding genes in different cells and at different developmental stages. These regulatory elements include promoters, enhancers, insulators and silencers etc., which provide exquisite control of gene expression. A pseudogene is a DNA sequence that is very similar to that of a functional gene but is itself nonfunctional. Long interspersed nuclear elements are a class of repetitive DNA sequences, and long terminal repeats are low copy number repeats. Figure 1.4 summarizes the complexity of the genome.

As is clear from Figure 1.4, there is an abundance of repetitive DNA within the genome. Throughout the genome, large deletions or duplications often seem to result from nonhomologous recombination between direct repeat sequences (unequal exchange between repeats on homologous chromosomes or sister chromatids), as illustrated in Figure 1.5.

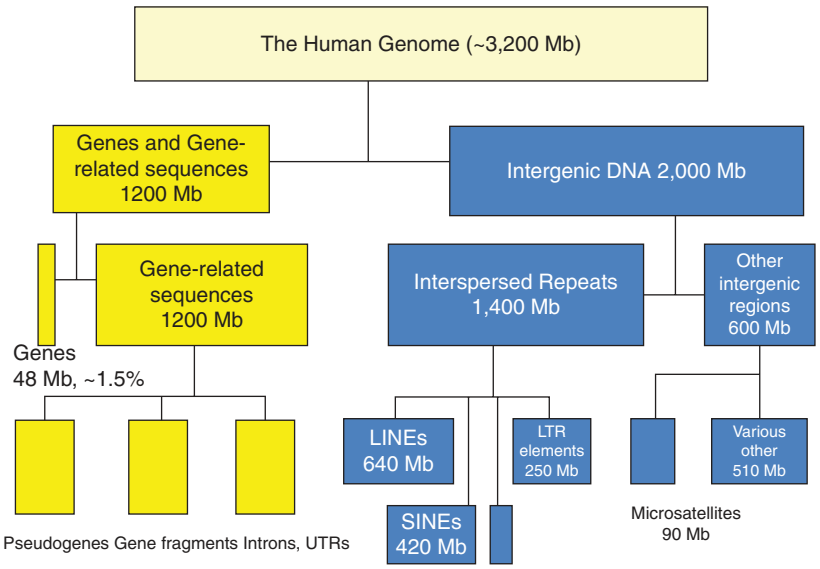

Figure 1.4 The structure of the genome.

The exon sequences or "exome" (labeled "genes" in the diagram) account for only 1.5% of the total cellular DNA. Over 80% of the genes in the exome currently have no known role in disease. LINEs, long interspersed nuclear element; LTR, long terminal repeats; SINEs, short interspersed nuclear element; UTRs, untranslated regions.

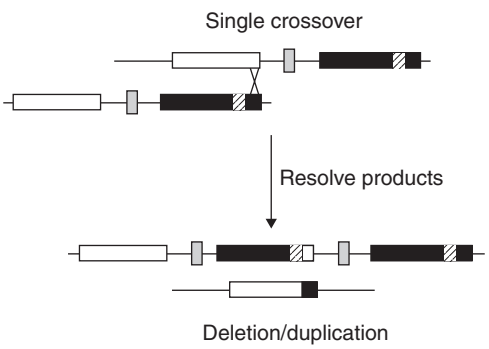

Figure 1.5 Misalignment and recombination between homologous chromosomes leading to deletion/duplication. The white and black boxes have very similar sequences and there is a gene in between (grey box). If the white and black boxes mispair at meiosis so that the white box pairs with the black box and a recombination (crossover) event occurs as shown, the resultant chromosome will contain either a deletion of the gene sequence (no grey box) or a duplication (two grey boxes).

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Types of mutation

Large-scale rearrangements

- Deletions and duplications
- Inversions and insertions

For some disorders, these types of mutations are common mutational mechanisms. Deletions and duplications may involve part of, or the whole of, a gene. For example, in DMD and Becker muscular dystrophy, approximately 66% of cases are due to deletions of one or more exons of the dystrophin gene and in type 1 spinal muscular atrophy, 95% of cases have a homozygous deletion of the *SMN1* gene. Large deletions of several genes are often mediated by repeated sequences and result in several well-known microdeletion syndromes, e.g. DiGeorge syndrome (22q11 deletion) and Williams' syndrome. One of the best-known duplication syndromes is hereditary motor and sensory neuropathy type 1A, which is due to a duplication of the *PMP22* gene on chromosome 17. One of the best examples of an inversion mutation is in hemophilia A, where 50% of severe cases are due to an intron 22 inversion (Figure 1.6).

Point mutations

- Base substitutions
- Small insertions /deletions

These point mutations can result in a variety of possible consequences, as illustrated in Figure 1.7. A nonsense mutation results in the generation of a stop codon, which would terminate protein translation. In the example in Figure 1.7, the conversion of C to T results in production of TGA that is transcribed into UGA in mRNA, which encodes a stop codon. Such mutations are therefore usually pathogenic. Similarly, the frameshift mutation illustrated by the deletion of one cytosine residue results in the production of a downstream stop codon (UAA). Mutations in splice sites can

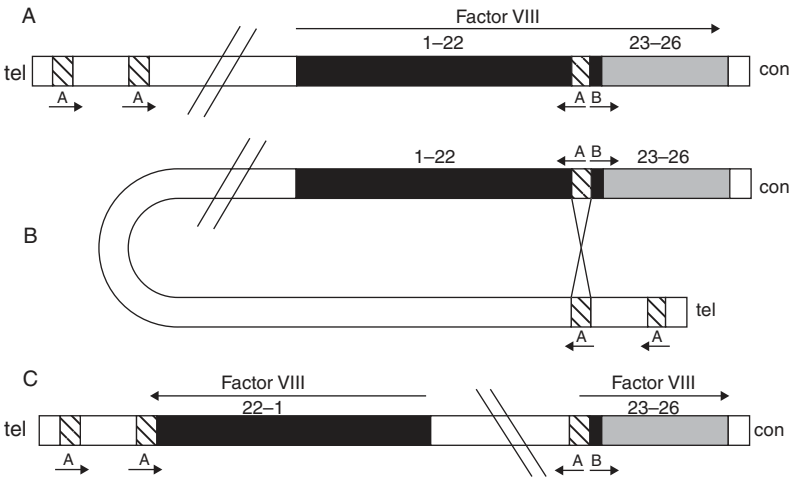


Figure 1.6 Intron 22 inversion in the factor VIII gene as a major cause of severe hemophilia A.
(a) The factor VIII gene is represented as a black box (exons and introns 1–22) and a grey box (exons and introns 23–26). A repetitive sequence "A" (hatched box) in intron 22 of the factor VIII gene is present in two additional copies 360 kb and 435 kb "upstream" of the gene. The arrows represent the relative orientations of the three copies of "A."
(b) During male meiosis, this part of the X chromosome has no pairing partner and the repetitive sequences "A" may pair, forming a loop. The repetitive segments are orientated in the same direction. A crossover can occur as indicated.
(c) A crossover has occurred. This causes an inversion of the black box segment (exons 1–22) and totally disrupts the gene structure.

Figure reproduced from Purandare SM, Patel PI. Recombination hot spots and human disease. *Genome Res* 1997; 7: 773–86. With kind permission of CSH Press.

Nonsense mutation

ATG CCC TCA **C**GA GCT CGG AAG CTA
Met Pro Ser Arg Ala Arg Lys Leu

↓

ATG CCC TCA **T**GA GCT CGG AAG CTA
Met Pro Ser STOP

Mutation = c. 10C>T (p.R4X or p. Arg4Ter)

Frameshift mutation

ATG CCC TCA **C**GA GCT CGG AAG CTA
Met Pro Ser Arg Ala Arg Lys Leu

↓

ATG CCC TCA GAG CTC GGA AGC TAA
Met Pro Ser Glu Leu Gly Ser STOP

Mutation = c. 10delC

Splice site mutation

a) disruption of existing splice sites

Intron Exon Intron
ttcacag**G**CCCATGGATTCAGTCG**g**tctatacc

↓

ttcacat**G**CCCATGGATTCAGTCG**g**tctatacc

Leads to exon skipping or translation of intonic sequence

b) creation of novel splice site (deep intronic changes)

Missense mutation

ATG CCC TCA **C**GA GCT CGG AAG CTA
Met Pro Ser Arg Ala Arg Lys Leu

↓

ATG CCC TCA **G**GA GCT CGG AAG CTA
Met Pro Ser Gly Ala Arg Lys Leu

Mutation = c. 10C>G (p. R4G or p. Arg4Gly)

Figure 1.7 Some types of point mutation.
cDNA is the DNA sequence complementary to the mRNA sequence. Thus, c.10C>G means that at the 10th nucleotide in the cDNA sequence, cytosine has been replaced by guanine. c, complementary; del, deletion.

result in failure to splice out the intron sequences or failure to splice in the next exon. Missense mutations that result in a change of amino acid (e.g., in Figure 1.7, arginine is converted to glycine) are the most difficult to interpret in terms of determining pathogenicity. Interpretation may involve a variety of steps including searching databases to see if the mutation has been reported previously in patient cohorts (in which case it may be pathogenic) or in the general population (in which case it may be a benign variant or "polymorphism"), and asking whether it changes the nature of the amino acid (e.g., polar to nonpolar), if it potentially affects splicing, if it is in an evolutionarily conserved region of the gene (in which case it may be affecting a region of great functional significance), if it affects a region of known functional significance, if mutations have been reported in the same codon, and whether it segregates with the disease in the family (if there are other affected family members available for testing).

Expanding trinucleotide repeats

This is an important mutational mechanism that is illustrated in Figure 1.8, along with a number of disorders where this is the sole or main mutational mechanism.

Organization of genetic services

In the UK, genetic services are currently organized in regions serving populations of between 2 and 5 million. The clinical genetic service is staffed by consultants in clinical genetics and genetic counselors. There is usually an allied genetic laboratory that provides chromosome and gene analysis using a variety of techniques. There are close links between the genetic laboratories in each region and biochemical genetics and specialist hematology laboratories for the prenatal diagnosis of inborn errors of metabolism and hematologic disorders. Samples that are sent to the laboratory for prenatal testing include amniotic fluid, chorionic villus samples, fetal blood and increasingly maternal blood (noninvasive prenatal testing (NIPT)).

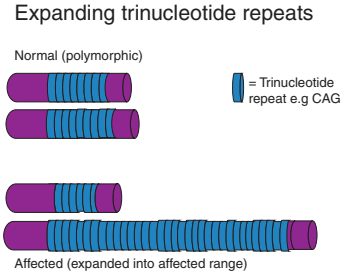
Detecting chromosome abnormalities: methods

Karyotyping

Karyotyping involves assessing the chromosomes using a light microscope and requires dividing cells, so the tissue has to be cultured to stimulate cell division (mitosis); the cell cycle is arrested at metaphase, cells harvested, slides made and stained, and then analysed. The cells to be examined can be obtained from amniocentesis, CVS or fetal blood sampling. Long-term culturing takes 7–10 days and in the UK, professional best practice guidelines indicate that a prenatal report should be issued within 14 days in 95% of cases.

Culture failure is rare and if it occurs it is usually if the sample is small or heavily blood-stained or contaminated. Best practice guidelines indicate that a report should be issued successfully in 99% of amniocentesis and chorionic villus samples. Some authors have suggested that failure of amniotic fluid cell growth may be related to fetal aneuploidy, but this has not been observed by others.

With an amniocentesis, cytogenetic results are highly reliable – sources of error could be maternal contamination or failure to detect fetal mosaicism. The risk of a false-negative result for a chromosome abnormality due to maternal cell contamination has been estimated at between 1 in 4,000 and 1 in 8,000. When mosaicism is detected, the laboratory has to decide whether the mosaicism is likely to be "true" or whether it may have arisen during the culturing process in the laboratory ("pseudomosaicism"). Diagnostic and counseling issues arise when true mosaicism is detected (0.3% of amniocenteses), particularly when it involves rare trisomies.



Expanding trinucleotide repeats

- CAG = HD / SCA's / SBMA (Affected range 40–120 repeats)
- CTG = Myotonic dystrophy (Affected range 50–3000 repeats)
- CGG = Fragile X (Frax A) (Affected range 200–3000 repeats)
- GAA = Friedreich Ataxia (Affected range 100–2000 repeats)

Figure 1.8 Trinucleotide repeat expansion mutations. The left upper diagram shows two alleles, one with eight CAG repeats and another with nine repeats. The left lower diagram shows an expansion in one allele to 36 repeats. The right-hand list contains important disorders for which trinucleotide repeat expansions are the sole or main mutational mechanism. HD, Huntington's disease; SBMA, spinobulbar muscular atrophy; SCA, spinocerebellar ataxia.

With CVS, chorionic villi can be examined directly or after short-term culture (when the source of cells is trophoblast) or after long-term culture (when it is the mesenchymal core of the villus that is the source of cells). Analysis of direct CVS cultures has largely been replaced by quantitative fluorescent polymerase chain reaction (QF-PCR) or fluorescent in situ hybridization (FISH) for rapid identification of the common aneuploidies. With CVS, false-negative results are very rare following long-term culture. There is, however, the potential for false positives or diagnostic difficulty due to "confined placental mosaicism" (CPM), i.e. the abnormal cell line is present in the placenta but not in the fetus. The incidence of true mosaicism identified in chorionic villus tissue is about 2.1%, with 1.9% being CPM and 0.2% being true fetal mosaicism. If mosaicism is detected a follow-up amniocentesis is usually recommended. If the amniocentesis suggests that there was CPM, one may still need to consider the possibility of an abnormal phenotype in the fetus due to trisomy rescue, resulting in uniparental disomy if the chromosome involved contains an imprinted gene (e.g., chromosomes 7, 14 or 15).

The resolution of chromosome analysis by karyotyping is in the region of 5–10 megabases (Mb) on a postnatal blood sample, but at amniocentesis or CVS the resolution tends to be in the region of 10–20 Mb, and is therefore only sufficient to exclude aneuploidy and large structural rearrangements.

Fluorescent in situ hybridization

FISH can be performed on cultured or uncultured cells. Unlike karyotyping, this is a targeted approach to identifying specific chromosome abnormalities using fluorescently labelled DNA probes (single-stranded DNA sequences that will hybridize to complementary sequences in the target region). It can be used for rapid aneuploidy screening, detecting specific deletions or additional material (e.g., 22q11 deletion in a fetus with a congenital heart defect detected on a scan or the presence of an additional 12p isochromosome seen in Pallister–Killian syndrome in the fetus with a diaphragmatic hernia) or detecting specific balanced and unbalanced familial translocations. The recommended reporting time for a rapid result on uncultured cells is 3 working days – occasionally cultured cells are required, but this can only be determined on a case-by-case basis. FISH can also be used to confirm and/or characterize a possible karyotype anomaly, and can help give positional information when an imbalance has been identified by microarray.

Quantitative fluorescent polymerase chain reaction (PCR-based copy number analysis)

QF-PCR analyses DNA sequences along chromosomes 21, 13, 18 and the sex chromosomes that are specific for those chromosomes in DNA extracted from CVS or amniotic cells. It is a test designed for the rapid detection of trisomies of these chromosomes. The target turnaround time is 3 working days but in most cases a result is achieved within 24 h. Abnormal results are confirmed by karyotyping and/or FISH. In the case of Down's syndrome (DS), karyotyping is essential to exclude a translocation form of DS, such as a Robertsonian translocation. QF-PCR may not detect mosaicism or structural rearrangements of the chromosomes.

Microarray-comparative genome hybridization

Microarray-comparative genome hybridization (microarray-CGH) is a hybridization method whereby single-stranded DNA in the patient sample is compared with a reference DNA sample when hybridized to complementary sequences that are spotted on to a slide. It is a method that has a huge capacity for miniaturization and automation. The surfaces used are microscope slides or nitrocellulose-coated glass surfaces onto which individual oligonucleotides are spotted in individual locations. Thus the "array" of thousands of oligonucleotides fixed to the slide becomes the set of probes to which the patient's DNA is added after being labeled with a fluorophore (usually Cy3 (green) and Cy5 (red)). Two DNA samples – one from the patient and one control sample (made from mixing DNA from several individuals) – are "denatured" (made single-stranded), and each is labeled with a different fluorophore and then hybridized together onto the "microarray" (the slide onto which the oligonucleotides are spotted) (Figure 1.9). Following hybridization, bound label is detected using a high-resolution laser scanner; the signal intensity obtained is analyzed with digital imaging software.

Possible results include:

- No copy number imbalance detected – a normal report with a standard rider is issued.
- Copy number variant (CNV) detected that is a known benign polymorphism – a normal report is issued.
- Known pathogenic CNV detected.
- Pathogenic CNV that has been found to be more prevalent in cohorts of patients with learning

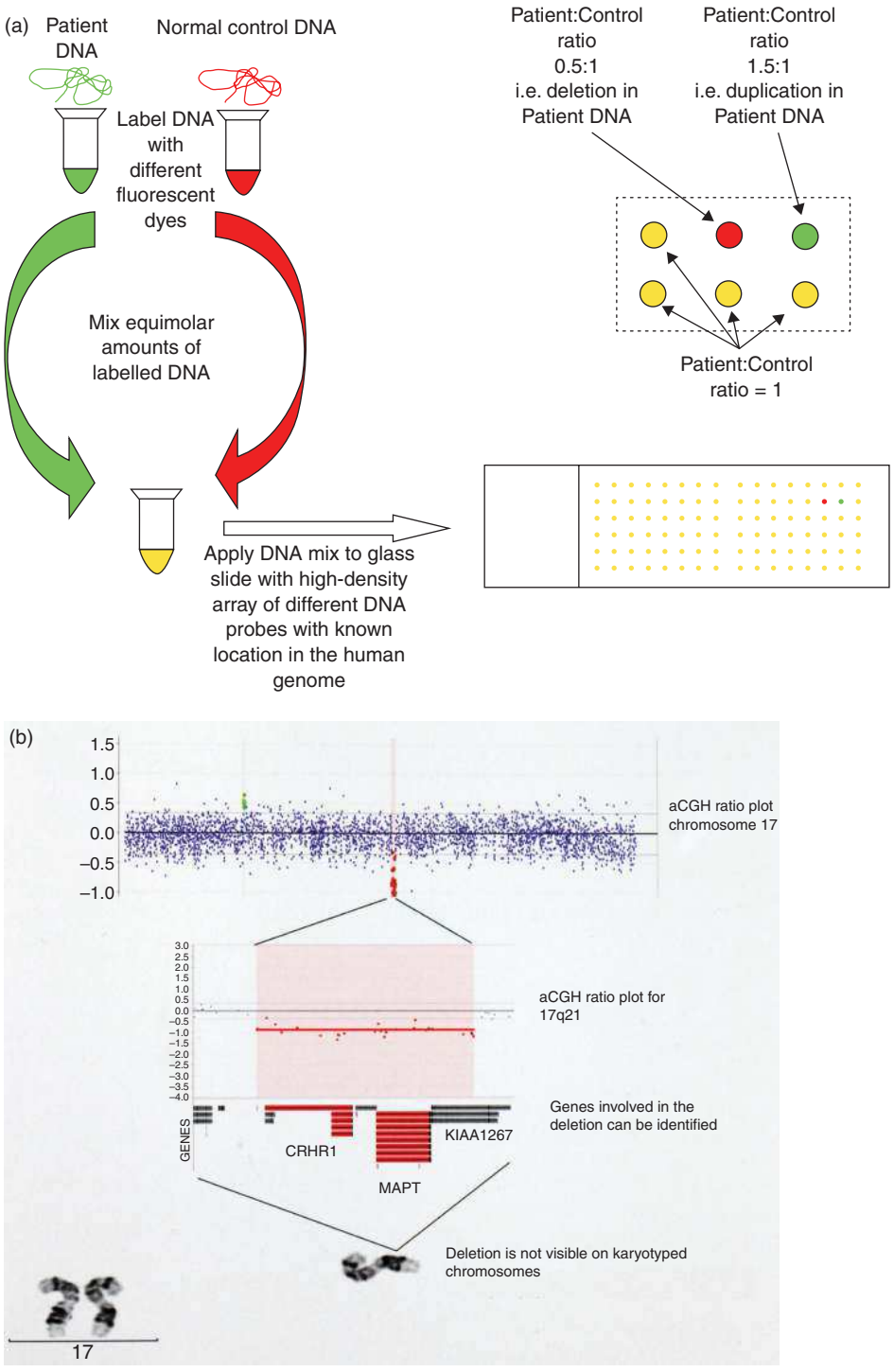


Figure 1.9 Microarray-comparative genome hybridization:
(a) The diagram illustrates the patient sample labeled with green dye being mixed with the control (reference sample) labeled red. The mixture is then hybridized to the array onto which is spotted thousands of unique oligonucleotides. The green- and red-labeled DNA should hybridize equally to complementary sequences on the array; a deletion in the patient DNA will result in more red than green hybridizing (resulting in an orange colour) and a duplication in the patient sample results in excess green.
(b) The diagram illustrates the result when an array has been exposed to a high-resolution laser scanner and the signal intensity obtained analyzed with digital imaging software. The computer analysis shows a deviation from a linear output with an excess of red indicating a deletion; in this example a very small deletion at 17q21.31 that would not be visible down a microscope. The array result also gives information about the genes that are in the deleted region.
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disability or neurodevelopmental problems but may be carried by apparently neurologically normal individuals ("neurosusceptibility loci"). These CNVs thus show reduced penetrance – penetrance being the probability that a person carrying this variant will manifest an abnormal phenotype. Some of these neurosusceptibility CNVs can also be associated with birth defects.

- Copy number imbalance detected but is neither a known pathogenic CNV nor a known benign polymorphism. The imbalance is validated using another method, e.g. FISH. If confirmed, parental bloods are requested. If imbalance is de novo, it may be reported as likely to be pathogenic depending on the gene content. If it is found to be familial, it is reported as "significance uncertain."

Where a variant of uncertain significance (VOUS) is found, several factors are considered as well as whether it is familial or de novo. Databases of CNVs known to occur in healthy individuals and in known patient cohorts are consulted. Other considerations are: does the variant overlap a "known syndrome"?; does it contain morbid genes?; and is it a gene-rich or a gene-poor area?

The advantage of microarray-CGH is primarily that it is more sensitive and accurate than conventional karyotyping, i.e., there is a higher abnormality detection rate (DR) and it can reveal specific genes that have been deleted or duplicated. It can also detect mosaicism down to a level of around 20%. The typical arrays currently used in postnatal analysis have an average resolution of 60 kb with increased clustering of probes in known microdeletion/duplication regions. A wider range of array designs and formats are now available and higher resolution arrays that detect deletions and duplications, even down to the single exon level, are becoming increasingly used in a clinical setting.

The possible disadvantages are that arrays will not detect balanced rearrangements and that they will detect variants of uncertain significance or reduced penetrance, and may detect (very rarely) an "unexpected" finding of clinical significance unrelated to the reason for the test (e.g., deletion of a breast cancer gene). Best practice guidelines suggest a target reporting time of 28 calendar days or 56 days where parental follow-up is required for postnatal samples.

Microarray-CGH has revolutionized the postnatal detection of chromosome abnormalities and is being introduced into prenatal testing. There is a significant body of literature that shows that the use of arrays when applied to fetuses with a major structural ultrasound abnormality results in an increased diagnostic yield of 4–6% pathogenic CNVs in the presence of a normal karyotype[2]. Following the results of the National Institute of Health study in the USA[3], the American College of Obstetricians and Gynecologists issued a recommendation that "in patients with a fetus with one or more major structural abnormalities identified on ultrasonographic examination, and who are undergoing invasive prenatal diagnosis, chromosomal microarray replaces the need for karyotyping"[4]. In the UK, there is also support for the use of prenatal arrays (after the common trisomies have been excluded by QF-PCR) in the context of:

- one or more structural anomalies identified on an ultrasound scan
- isolated nuchal translucency ≥3.5 mm when crown–rump length measures from 45 mm to 84 mm (at approximately 11 weeks 0 days to 13 weeks 6 days
- fetuses with a sex chromosome aneuploidy that is unlikely to explain the ultrasound anomaly (e.g., XXX, XXY and XYY).

Most laboratories introducing prenatal arrays are using the same array platform (with the same sensitivity) that they currently have in place for postnatal arrays. It has been recommended that any variant that will potentially inform the management of the pregnancy *or* of the family, in the clinical context in which the array was done *or* in the future should be reported, regardless of size of imbalance. This obviously includes pathogenic variants related to the indication for the array but can also include:

- high-penetrance neurosusceptibility loci that are associated with a risk of a severe phenotype to enable discussion about the overall likely phenotype of the child
- neurosusceptibility loci associated with an increased incidence of anomalies detectable on a scan, as reporting these may help direct further scanning
- unsolicited pathogenic findings fulfilling the above criteria, such as deletion of a known cancer predisposition gene, e.g., BRCA1 or a female fetus carrying a deletion in the dystrophin gene (responsible for the X-linked condition DMD).

It has been recommended that incidental findings that should not be reported include any finding that is not linked to potential phenotypes for the future child in