THE GENETIC BASIS OF HUMAN DISEASE

BIOCHEMISTRY ACROSS THE SCHOOL CURRICULUM
GUIDANCE NOTES FOR ADVANCED BIOLOGY No. 10
The Genetic Basis of Human Disease

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Introduction

It has been known for centuries that individuals differ, that children tend to resemble their parents and that certain diseases tend to run in families. Progress towards understanding the genetic basis for these observations has been made at an ever-increasing rate during the past 150 years. Mendel’s laws of heredity, the discovery of the chemical basis of inheritance, the elucidation of the structure and function of DNA, as well as the development of methods for manipulating DNA in vitro (genetic engineering), have all paved the way towards the precise understanding of the molecular basis of many of the human disorders that result from single-gene defects. This understanding of ‘simple’ Mendelian disorders has formed a basis for the even more challenging investigation of complex disorders. It is now known, for example, that certain forms of cancer arise from an accumulation of genetic mutations and that many common disorders, such as diabetes and obesity, involve the interaction of many genes as well as environmental factors. With these discoveries, genetic research is no longer confined to the study of rare events or individual tragedies but has an impact on virtually every aspect of medicine and society, as is clearly evident from the increasing number of newspaper, radio and television reports of genetic discoveries and their implications.

The aim of this booklet is to provide an insight into how, at the molecular level, such a diversity of genetic disorders may arise and a perspective on the potential consequences such knowledge may have in terms of the treatment and management of affected individuals. In order to understand the text, a basic knowledge of the principles of heredity (mitosis and meiosis), the structure and function of DNA and recombinant DNA technology is required. Such topics have been covered in previous BASC booklets and in articles in the Biological Sciences Review, and are listed in the Background reading section at the end of
the booklet. In the Additional reading section at the end of the booklet is a list of articles that have appeared in the Biological Sciences Review that complement or cover in greater depth the topics of this text. These articles are referred to numerically within the text.
Chromosomal disorders

Introduction

Chromosomes are present in all nucleated cells and contain DNA and proteins (histones) in a compact structure. Each species has a chromosome complement that is characteristic in number and form. This is referred to as the species karyotype and a karyogram is the name given to the photographic representation of stained chromosomes arranged in

Figure 1. Normal human male karyogram, 46,XY (Giemsa banding). The normal human female karyogram would differ in that there would be two X chromosomes and no Y chromosome. Reproduced courtesy of William Newman; ©1999, The Biochemical Society.
order of decreasing length (Figure 1). In 1956, the normal human chromosome number was shown to be 46 in all cells of the body with the exception of the egg cells, spermatozoa and mature red blood cells (erythrocytes). Half of these chromosomes are derived from the mother’s egg cell whereas the other 23 come from the father’s spermatozoon. Since the eggs and spermatozoa each contain only 23 chromosomes, they are said to be haploid (Figure 2), whereas those cells containing 46 chromosomes are said to be diploid. These 46 chromosomes in diploid cells comprise 22 matching or homologous pairs of autosomes (non-sex chromosomes)

**Figure 2.** Fusion of a haploid \((n)\) sperm and an egg generates a diploid \((2n)\) zygote. Successive mitotic cleavages generate the diploid somatic cells during development and cell turnover. Reproduced from Strachen, T. and Read, A. (1996) Human Molecular Genetics, 1st edn., Bios Scientific Publishers, Oxford with permission; ©, Bios Scientific Publishers.
and a pair of sex chromosomes, X X in females and X Y in males. The shorthand for the normal female karyotype is 46,XX and for the male 46,XY.

Human chromosomes can be studied in any growing tissue where cells are dividing rapidly, such as white blood cells (lymphocytes) in blood samples. Cell division (mitosis) can be arrested in metaphase and the chromosomes stained in different ways to allow their precise identification when viewed under a microscope. This microscopic study of chromosomes is called cytogenetics. For routine karyotyping, Giesma (G) staining is usually preferred and this produces alternating dark and light bands (chromosomal banding), which reflect differential chromosomal structures that are characteristic for each chromosomal pair. Each chromosome has a narrow waist, called the centromere, which separates the short arm of the chromosome, called p, from the long arm, called q. Most arms are divided into two or more regions by prominent bands and each region is further subdivided into sub-bands. Thus, band Xp21.2 is to be found on the p arm of the X chromosome in region 2, band 1, sub-band 2 (Figure 3).

When a patient’s chromosomes are examined under the microscope, it is possible to identify aberrations in chromosome number and structure. The resulting phenotype (the observable characteristics) of a person with a chromosomal disorder depends on the nature of their chromosomal defect. In 1959, the first human chromosomal disorder, trisomy 21, which causes Down syndrome, was discovered. The development of chromosomal banding in 1970 markedly increased the ability to resolve small chromosomal aberrations. Chromosomal disorders can arise from alterations in chromosome number and structure and the phenotype can even be altered depending on whether the chromosomal abnormality has occurred on the paternally or maternally derived chromosomes. The resolution of chromosomal structure is continually improving and, currently, missing or additional DNA of 4000 kb [1 kb (kilobase)=1000 bp (base pairs)] or greater can be visualized on routine chromosomal analysis. In addition, more advanced techniques such as fluorescence in situ hybridization (FISH) [1] allow very precise identification and localization of chromosomal aberrations.
Numerical aberrations

Any abnormality in chromosome number where the chromosome number is an exact multiple of the haploid number \((n=23)\) but exceeds the diploid number \((n=46)\) is called polyploidy. Polyploidy usually arises from fertilization of the egg by two sperm (which will increase the total number of chromosomes to 69) or the failure of one of the maturation divisions of either the egg or the sperm so that a diploid gamete is produced. Polyploidy usually causes early spontaneous abortion and survival of the fetus to the full term of the pregnancy is very rare. If the chromosome number is not an exact multiple of the haploid number then this is called aneuploidy. Aneuploidy usually arises from failure of paired chromosomes (at first meiosis) or sister chromatids (at second meiosis) to disjoin at anaphase (non-disjunction) or delayed movement of a chromosome at anaphase. Two cells are then produced — one with a missing copy of that chromosome and one with an extra copy of a chromosome (Figure 4).

Figure 3. Diagrammatic representation of the X chromosome showing the p and q arms, bands and sub-bands.
Examples of syndromes caused by aneuploidy (see also Table 1)

**Down syndrome**

Down syndrome is a relatively common genetic condition [2]. On average, three babies are born with Down syndrome every day in the U.K. (that is about one baby with Down syndrome for every 1000 babies born in the U.K.). In 95% of cases a person with Down syndrome is born with an extra chromosome 21 in each cell of his or her body (i.e. they have the karyotype 47,XX\(^{+}/\) or 47,XY\(^{+}/\); Figure 5). Most cases

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**Table 1. Examples of numerical chromosomal aberrations**

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>92,XXYY</td>
<td>Tetraploidy</td>
</tr>
<tr>
<td>69,XXY</td>
<td>Triploidy</td>
</tr>
<tr>
<td>47,XX +21</td>
<td>Trisomy 21 (Down syndrome)</td>
</tr>
<tr>
<td>47,XY +18</td>
<td>Trisomy 18 (Edward’s syndrome)</td>
</tr>
<tr>
<td>47,XX +13</td>
<td>Trisomy 13 (Patau syndrome)</td>
</tr>
<tr>
<td>47,XXY</td>
<td>Klinefelter syndrome</td>
</tr>
<tr>
<td>47,XXX</td>
<td>Trisomy X</td>
</tr>
<tr>
<td>45,X</td>
<td>Turner syndrome</td>
</tr>
</tbody>
</table>
arise from non-disjunction and, in 80% of cases, the mother contributes the extra chromosome. The chances of having a baby with Down syndrome are strongly linked with the age of the mother and are 1 in 1400 if the mother is 25 years old, rising to 1 in 110 if the mother is 40 years old and to 1 in 30 if she is 45 years old.

In about 4% of cases of Down syndrome, only an extra part of chromosome 21, the 21q22 region, is present (partial trisomy), rather than the whole of chromosome 21. This happens as a result of an unbalanced translocation (see below). Identification of the genes that are within the extra portion of chromosome 21 that cause Down syndrome has allowed detailed investigation of the molecular and biochemical basis of the condition (see [2]).

Figure 5. Chromosomes from a female with Down syndrome. This is an example of trisomy where the individual has three copies of chromosome 21 instead of two. Reproduced courtesy of William Newman; © 1999 The Biochemical Society.
People with Down syndrome all have some degree of mental retardation. Many children with Down syndrome learn to walk, read and write but some may have more severe learning difficulties. Some of the physical features that you might see in a person with Down syndrome include eyes which appear to slant slightly upwards, a flatterish face, rather short fingers and a small stature. It is fairly common for people with Down syndrome to be born with heart defects, and with sight and hearing problems.

**Klinefelter syndrome**

A male with Klinefelter syndrome is born with an extra X chromosome in each cell of his body (i.e. they have the karyotype 47,XXY). On average, one baby is born with Klinefelter syndrome for every 1000 boys born, and the risk of having a son with Klinefelter syndrome increases with an increase in the age of the mother. The extra chromosome is from the mother in 60% of cases and from the father in 40% of cases and arises by non-disjunction during maternal or paternal meiotic division (Figure 6). Affected males are infertile and have mild mental retardation.

**Turner syndrome**

A female with Turner syndrome is born with a single X chromosome (i.e. 45,X). The birth incidence is 1 per 5000 female births. Monosomy X may arise from non-disjunction in either parent, but in 75% of girls with Turner syndrome only the maternal X is present, indicating that the problem occurs most often during the formation of the father’s sperm (Figure 6). Females with Turner syndrome are short in stature and rarely undergo secondary sexual development and so are mostly infertile. Their intelligence and life span are normal.

**Structural aberrations**

Structural aberrations all result from chromosomal breakage. When a chromosome breaks, two unstable sticky ends are produced. Generally, repair mechanisms rejoin these two ends without delay. However, if more than one breakage has occurred then the repair mechanisms cannot distinguish one sticky end from another and there is the possibility of rejoining the wrong ends. This can lead to many different chromosomal
structures that involve, for example, the transfer of chromosomal material between non-homologous chromosomes (translocation) or loss of parts of a chromosome (deletion).

**Translocations**

If the exchange of chromosomal material that occurs during a translocation results in no loss of DNA, then the individual will be clinically normal and is said to have a balanced translocation (Figure 7). Such a balanced translocation is, however, of medical significance for future generations, as a balanced translocation carrier is at risk of producing chromosomally unbalanced (i.e. where there is loss of DNA) offspring (Figure 8). Most fetuses with unbalanced translocations will abort spontaneously. Consequently, persons with balanced translocations may only come to the attention of medical practitioners when they and their
partner are investigated because of repeated miscarriage. Infants with unbalanced translocations that are liveborn show multiple dysmorphic features and mental retardation. Occasionally, specific genes may be damaged at the translocation breakpoints and, depending on the nature of the genes that are damaged, very specific disorders can result. Translocations can also cause two genes to be fused or can place a gene in a chromosomal region that is more transcriptionally active. These kinds of translocations can cause forms of cancer (see Chapter 5).

**Deletions**

Deletion of part of a chromosome can arise between two breakpoints as a result of unequal crossing over during meiosis or as a result of a parental translocation. Any microscopically visible chromosomal deletion almost invariably produces a phenotype with multiple abnormal features and mental retardation because of the absence of expression from the deleted genes.
Examples of two syndromes that result from a 15q11-13 deletion

Two well-characterized syndromes that result from deletions are Prader-Willi syndrome (frequency 1 in 25000) and Angelman syndrome (exact frequency not known). Both of these syndromes result from the ‘microdeletion’ of the band 15q11-13. However, these syndromes have different phenotypes. People with Prader-Willi are mentally retarded, have small external genitalia and characteristic facial features, and overeating with obesity occurs. In contrast, in Angelman syndrome, there is developmental delay, absent speech, jerky movements, paroxysms of inappropriate laughter and characteristic facial features that differ from those of Prader-Willi. The reason that these two different syndromes can arise from the same microdeletion lies in the parental origin of the chromosome on which the deletion has occurred.
Figure 9. Diagram showing the alternate outcomes of a deletion in the region of q11–13 when it occurs on either the paternally or maternally derived homologues of chromosome 15.
For Prader–Willi syndrome the deletion occurs invariably on the chromosome 15 inherited from the father, whereas in Angelman syndrome the deletion occurs almost exclusively on the chromosome 15 inherited from the mother (Figure 9). Thus it appears that inheritance of both the paternal and the maternal copies of this region of chromosome 15 is important for normal development. This phenomenon is known as genetic imprinting and affects many regions of the genome other than that on chromosome 15. The precise mechanisms whereby individual cells express either the paternal or the maternal copy of a gene are not yet fully understood but appear to involve the modification of specific sequences of DNA by, for example, the addition of methyl groups that alter the transcriptional activity of that gene.
Single-gene disorders

Introduction

The entire DNA content of the chromosomes of the nucleus of a cell is called the genome. Somatic cells contain two copies of the genome whereas gametes contain a single copy of the genome. It is estimated that there are about 65,000–80,000 genes in a single copy of the human genome and there is currently a co-ordinated research programme — the Human Genome Project (HGP) — aimed at mapping all of these genes to specific regions within the genome [3]. Tremendous progress has been made in the past 10–20 years in matching many of the over 5000 known human single-gene disorders to genes in the genome and in identifying precisely the nature of the causative mutations. This progress, although made more rapid by the HGP, has built on the expertise of clinical geneticists who over the centuries have classified disorders into groups and subgroups on the basis of phenotype and mode of inheritance. Although not always accurate, such classification systems have provided a framework on which molecular geneticists have based their hunt for mutated genes within the genome.

Modes of inheritance

For most simple single-gene disorders, inheritance of a mutated copy or copies of a gene causes a characteristic phenotype; and inheritance of that phenotype follows a Mendelian segregation pattern. The pattern of inheritance can predict whether the mutated gene is on an autosome (chromosomes 1–22) or is sex-linked (on the X or Y chromosomes) and whether the disorder is dominant (in which case a single copy of the
Figure 1. Basic Mendelian pedigree patterns: (A) autosomal dominant; (B) autosomal recessive; (C) X-linked recessive; (D) X-linked dominant; (E) Y-linked. Key shows main symbols used in pedigrees. Reproduced from Strachan, T., and Read, A. (1996) Human Molecular Genetics, Bios Scientific Publishers, Oxford, with permission; ©1996, Bios Scientific Publishers.
mutated gene is sufficient to cause the disorder) or recessive (both copies of a gene need to be mutated to cause the disorder) (Figure 1).

**Autosomal inheritance**

**Autosomal dominant inheritance**

In autosomal dominant inheritance an affected person usually has at least one affected parent; the disorder affects either sex and can be transmitted by either sex, and an affected person has a 50% chance of passing the defect on to their children. Examples of autosomal dominant conditions are achondroplasia and Huntington disease.

**Autosomal recessive inheritance**

In autosomal recessive inheritance, affected persons are usually born to unaffected parents. Parents of affected people are usually asymptomatic (i.e. do not have symptoms of the disease) but carry a single copy of the mutated gene. There is an increased incidence of autosomal recessive disorders in families where parents are related (consanguineous). Children of parents who are both heterozygous for the mutated gene have a 25% chance of inheriting the disorder and the disorder affects either sex. Examples of autosomal recessive disorders are cystic fibrosis [4] and sickle cell anaemia [5].

**X-linked inheritance**

There are two forms of X-linked inheritance: X-linked dominant inheritance and X-linked recessive inheritance. A female has two X chromosomes, one of paternal and one of maternal origin, whereas a male has only one X chromosome, which is of maternal origin. To ensure that the levels of X-linked gene products produced by non-sex cells of males and females are equal, in the female, one of the X chromosomes (with the exception of some genes near the tip of the short arm) is inactivated in each cell. This process is random such that the percentage of cells with an active paternal X or with an active maternal X is about equal. X-linked recessive inheritance (Figure 2) affects males almost exclusively. Affected males are usually born to unaffected parents as the mother is usually an asymptomatic carrier, although she may have affected male relatives.
There is no male-to-male transmission in the pedigree. Examples of X-linked recessive conditions are Duchenne muscular dystrophy and haemophilia [5,6] (Figure 2). X-linked dominant inheritance, however, affects either sex. Females are often more mildly and variably affected than males and the child of an affected female has a 50% chance of being affected, regardless of sex. For an affected male, all of his daughters but none of his sons will be affected. An example of an X-linked dominant disorder is Vitamin-D-resistant rickets.

Figure 2. The X-linked recessive inheritance of haemophilia. (A) Inheritance through the mother. Daughters are either unaffected or carriers of the disorder. Sons are either affected or unaffected. (B) Inheritance through the father. Daughters are all carriers of the disorder. Sons are unaffected. $X^H$, normal X chromosome; $X^h$, X chromosome carrying the defect causing haemophilia; $Y$, Y chromosome; $\sigma^+$, male; $\sigma^-$, female.
Y-linked inheritance

Y-linked inheritance would only affect males, the affected males would always have an affected father and all sons of an affected man would be affected. One would expect then that Y-linked inheritance of a disorder would be clearly identifiable. However, no Y-linked diseases have ever been discovered and so presumably do not exist. As females are perfectly normal without any Y-linked genes, these genes must code for either non-essential characters or for male-specific functions. Defects in these genes are therefore unlikely to cause diseases apart from male infertility.

Locating a mutated gene within the genome

Most genetic methods to identify disease-causing mutated genes first require the identification of families where defects can be clearly traced from generation to generation. The next step is to locate on which chromosome and in what region of that chromosome the mutated gene resides. If a disorder has a sex-linked mode of inheritance then it is possible to assign the disorder to one of the sex chromosomes and, in rare instances, chromosomal aberrations such as translocations and deletions can provide clues as to the chromosomal location (or locus) of a mutated gene. For most disorders, however, techniques such as genetic linkage analysis need to be employed. Linkage analysis involves tracing the pattern of inheritance within families of DNA sequence changes present at specific sites on chromosomes. These DNA sequence changes (or DNA markers) do not necessarily cause an altered phenotype but allow the chromosomes in a chromosomal pair to be distinguished such that one can determine which of the mother’s and father’s chromosomes have been inherited by their offspring. It is then possible to examine whether a DNA marker for a particular region of a chromosome is co-inherited with a disorder in a family. Independent segregation (or a lack of linkage) of the DNA marker and the defect implies that the marker and the mutated gene are either on different chromosomes or on different regions of the same chromosome, such that they are separated by independent assortment or recombination during prophase I of meiosis. Co-inheritance (or linkage) of the marker and the defect in a family implies that the mutated gene and the marker are close enough together on a chromosome not to be separated by recombination during meiosis (Figure 3) [3,7,8]. Such analyses, in the last few years, have pinpointed
Once a chromosomal locus is identified for a specific disorder, the next task is to find the gene responsible within that region. This involves complex molecular biological techniques that have been developed during the past 10 years and are continually being refined. Once a candidate gene is identified within that region, analysis of the gene is undertaken to determine whether it contains any mutations that could affect gene expression or function. The chromosomal loci of many autosomal and sex-linked disorders (Table 1).

Figure 3. An example of genetic linkage analysis. (A) Linkage of the marker (M) locus to the disease (D) locus in an autosomal dominant pedigree. The marker locus has two forms, either M1 or M2, which allows the chromosomes in a chromosomal pair to be distinguished and their pattern of inheritance to be followed. The pattern of inheritance of M2 shows that it is consistently inherited with the disease. The reason for this is that the M locus is close to the D locus and so they are not separated by recombination. (B) Lack of linkage between the marker (S) locus and the disease (D) locus in an autosomal dominant pedigree. Neither S1 nor S2 are consistently inherited with the disease, as the S locus is not close to the D locus and so they become separated by recombination. The marker status of each individual is indicated below the symbols of the pedigree. M1/M2 denotes an individual who is heterozygous at the M locus and M1/M1 denotes an individual who is homozygous at the M locus (similarly for the S locus). (See Figure 1 in this chapter for key to symbols used.)
cause the disorder. The polymerase chain reaction (PCR) is a technique that has greatly enhanced the speed whereby mutations in gene sequences can be identified, as it generates multiple copies of a gene sequence that can be tested for the presence of mutations [9]. Sequence analysis of the PCR fragments from patients and normal (unaffected) individuals allows the precise identification of disease-causing mutations.

**When is a mutation a mutation?**

A simple definition of a mutation is any DNA sequence change that leads to a clinical phenotype. In contrast, a polymorphism is a sequence change that does not lead to any 'obvious' clinical phenotype. The word 'obvious' is important as some sequence changes that previously would have been considered neutral polymorphisms and which occur in the general population are now known to have subtle effects that could contribute to more complex disorders (see Chapter 7 in this booklet). Using the simple definition of a mutation given above one would then expect a mutation to have a significant effect on the production or function of the gene product. The effect of a mutation depends on the nature of the DNA sequence change, where within a gene that sequence change has occurred and which gene(s) that sequence change has affected. Mutations can occur in coding regions of the gene (i.e. the part of the gene that specifies the amino acid sequence of the protein); in non-coding regions of the gene that control the rate of transcription or translation of the gene; or in introns, non-coding sequences of genes that interrupt the coding sequence and are removed during mRNA processing (Figure 4). Depending on the nature of the gene mutated, some mutations may never be found because they lead to early embryo lethality, whereas some mutations may have very mild effects if they are

### Table 1. Examples of the chromosomal loci for disorders that have been mapped by linkage analysis

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Chromosomal locus</th>
</tr>
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<tbody>
<tr>
<td>Achondroplasia</td>
<td>4p16.3</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>7q31.2</td>
</tr>
<tr>
<td>Duchenne muscular dystrophy</td>
<td>X p21.2</td>
</tr>
<tr>
<td>Huntington disease</td>
<td>4p16.3</td>
</tr>
</tbody>
</table>
in genes encoding proteins with less crucial functions. Different types of mutations and their effects are summarized below and four examples of single-gene disorders have been chosen to highlight some of the consequences of specific mutations.

**Types of mutations and their effects**

**Point mutations**

Point mutations replace a single nucleotide base with a different nucleotide base. The following are examples of the possible consequences of point mutations.

**Amino acid changes**

If a point mutation occurs within the coding region of a gene it may alter the amino acid sequence of the gene product (Figure 5). For example, the
A mutation of a G to a C of the first base of one of the four codons for the amino acid glycine (GGT, GGC, GGA or GGG) would alter the codon for glycine to the codon for the amino acid arginine (CGT, CGC, CGA or CGG). Such amino acid changes can dramatically alter the structure and function of the encoded protein if they occur in, for example, the active site of an enzyme or the recognition site of a receptor (see achondroplasia and cystic fibrosis).

Premature stop codons

Point mutations can change the codon for an amino acid to a stop codon (i.e. the codon that signals the end of translation) (Figure 5). For example, the mutation of a C to a T of the first base of one of the codons for arginine (e.g. CGA) would convert it to a stop codon (TGA). The introduction of a stop codon into a coding sequence leads to premature

**Figure 5** Examples of the consequences of point mutations: amino acid change; premature stop codon; and deletion of an exon. N, normal sequence; M, mutant sequence; *, site of mutation.

mutation of a G to a C of the first base of one of the four codons for the amino acid glycine (GGT, GGC, GGA or GGG) would alter the codon for glycine to the codon for the amino acid arginine (CGT, CGC, CGA or CGG). Such amino acid changes can dramatically alter the structure and function of the encoded protein if they occur in, for example, the active site of an enzyme or the recognition site of a receptor (see achondroplasia and cystic fibrosis).

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termination of the translation of a protein and, consequently, a shorter protein or no protein at all is produced. Equally, a point mutation could alter a stop codon into a codon for an amino acid such that a longer protein is produced.

**Splice-site mutations**

Point mutations can occur in non-coding regions of the gene, such as introns, with serious consequences. At the junctions between introns and exons (the coding regions of the gene) are specific sequences which lead to the excision of the intronic sequences from the transcribed mRNA, a process called splicing (Figure 4). A single-base-pair change in these specific sequences can lead to incorrect processing of the mRNA such that exons are deleted from the mature mRNA (Figure 5). Such deletions in the mRNA lead to deletions in the translated protein (see below and Duchenne muscular dystrophy).

**Deletions**

Deletions can be as small as a single nucleotide or as large as an entire gene. Deletions can occur in the genomic DNA or in the mRNA (as a consequence of splice-site mutations — see above) but in both instances the protein product is either absent or reduced in size. Deletions of a single base pair can, in certain circumstances, have a more dramatic effect on the protein product than large deletions (Figure 6). For example, in the coding region of a gene each amino acid is encoded by a codon of three base pairs and these codons are arranged in sequence from one end of the mRNA to the other. The first codon that is translated in the mRNA is the AUG codon for methionine and this codon specifies the frame of the subsequent codons. If there is a single-base-pair deletion within the coding region, all subsequent codons will be thrown out of frame by a single base pair, such that if a protein is produced from the mRNA the protein will be altered from the site of the mutation until a stop codon is reached. Such mutations are termed frameshift mutations and can alter a protein sequence to a lesser or greater degree. In general, deletions where the number of base pairs deleted is not divisible by three appear to have more serious effects than deletions where the number of base pairs is divisible by three (see Duchenne muscular dystrophy).
As with deletions, insertion mutations can range from a single base pair to many thousands of base pairs in length. If these insertions are within a coding region and do not alter the frame of the protein, then they will lead to additional amino acids being inserted into the resultant protein, which may abolish or alter its function. Insertions, as with deletions, can also lead to a shift in the reading frame of the mRNA such that an altered protein is produced. One class of insertions that has been found to be the cause of a number of genetic disorders is due to an increase in the number (n) of trinucleotide repeat sequences (usually CAG\textsubscript{n}, CGG\textsubscript{n} or CTG\textsubscript{n}) that occur in the coding or non-coding regions of genes. If these expanded trinucleotide repeats occur in non-coding regions of genes they can cause altered transcription of the mRNA from that gene [10]. Expanded trinucleotide repeats that are in frame within the coding

**Figure 6.** Deletion mutations can cause frameshifts in the resultant protein. (A) Deletion of a single base pair results in a frameshift and the generation of a premature stop codon; (B) deletion of two base pairs results in a frameshift and the generation of a premature stop codon; (C) deletion of three base pairs leads to the deletion of an amino acid but no frameshift. N, normal sequence; M, mutant sequence. The deleted base pair(s) are underlined in the normal sequences.

**Insertions**

As with deletions, insertion mutations can range from a single base pair to many thousands of base pairs in length. If these insertions are within a coding region and do not alter the frame of the protein, then they will lead to additional amino acids being inserted into the resultant protein, which may abolish or alter its function. Insertions, as with deletions, can also lead to a shift in the reading frame of the mRNA such that an altered protein is produced. One class of insertions that has been found to be the cause of a number of genetic disorders is due to an increase in the number (n) of trinucleotide repeat sequences (usually CAG\textsubscript{n}, CGG\textsubscript{n} or CTG\textsubscript{n}) that occur in the coding or non-coding regions of genes. If these expanded trinucleotide repeats occur in non-coding regions of genes they can cause altered transcription of the mRNA from that gene [10]. Expanded trinucleotide repeats that are in frame within the coding
A region of a gene can lead to the insertion of a string of amino acids, for example, glutamine (see Huntington disease).

Examples of single-gene disorders

Achondroplasia

People with achondroplasia are very short in stature (Figure 7). Their restricted growth is disproportionate (i.e. unequal) because growth is most restricted in the long bones of the legs and the arms whereas the trunk is near to average size. The head is also slightly enlarged with a flattened nose and a prominent forehead and the fingers may be short and stubby. People with achondroplasia have the same range of intelligence as the general population. The pattern of inheritance of achondroplasia is autosomal dominant. However, most people with achondroplasia have no family history of the disorder as mutations causing it often occur spontaneously in the formation of the egg or the sperm before fertilization. The gene for achondroplasia was found by linkage analysis in 1994 on the short arm of chromosome 4. Within the linked region on chromosome 4, a gene encoding a cell-surface receptor

![Figure 7. Achondroplasia in a father and son. Apart from their shortness in stature, they have prominent foreheads and depressed nasal bridges. Reproduced from Beighton, P. (1988) Inherited Disorders of the Skeleton, 2nd edn., Churchill-Livingstone, Edinburgh, with permission; ©1998, Churchill Livingstone, Edinburgh.](image-url)
for a growth factor, the fibroblast growth factor receptor 3 (FGFR3), was known to be located. This receptor is made by the cells in cartilage during skeletal development and so was an excellent candidate for the gene for achondroplasia. Mutations causing achondroplasia have since been found in the FGFR3 gene and it is now known that 99.9% of people with achondroplasia have one of two mutations in the same codon, both of which lead to the glycine at codon 380 of the FGFR3 gene being replaced by arginine. The mutations are either GGG to AGG or GGG to CGG. These mutations alter the function of the receptor in a very specific way such that cartilage and bone formation does not occur normally and the phenotype of achondroplasia results.

Cystic fibrosis

Cystic fibrosis (CF) affects mainly the lungs and the pancreas. Thick, sticky mucus builds up in these organs causing infections and damage to the lungs, and the non-functional exocrine pancreas makes the digestion of food difficult. The male reproductive system can be affected. Affected children may be small for their age and puberty may be delayed. CF is an autosomal recessive condition. In 1985, the gene for CF was mapped to chromosome 7q31–32. There were no obvious candidate genes within the linked region and the gene was isolated only after extensive genetic mapping and exhaustive molecular characterization of the linked region. The CF transmembrane regulator (CFTR) gene was eventually found in 1989. The CFTR gene encodes an ion channel and the CF phenotype is caused by defective regulation of chloride ion transport across membranes. Some 500 different mutations within the CFTR gene have now been shown to cause CF. Many are private mutations detected in just one family. In most of northern Europe, however, one single mutation accounts for more than half of all mutations. This mutation is a trinucleotide deletion of TTT, which leads to the deletion of the amino acid phenylalanine (F), amino acid number 508 in the CFTR protein. This mutation is therefore called deltaF508. This trinucleotide deletion leaves the rest of the codons in frame and the protein is still made. However, the deletion of this amino acid means that the CFTR is not correctly located in the cell membrane and so does not function properly.
Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is a severe condition that produces progressive muscle weakness and massive elevations of muscle enzymes in the serum. The onset of DMD is in early childhood and most children are chairbound by the age of 10 years and die of infection by the age of 20 years. DMD is an X-linked recessive disorder. The incidence of DMD is 1 in 3000 male births. The DMD gene was the first to be mapped using linkage analysis and was assigned to Xp21 as far back as 1982. The DMD gene was cloned from this region and found to encode a muscle protein, which is now known as dystrophin. The dystrophin gene is the largest known gene in humans, covering a genomic distance of 2.3 million base pairs. It consists of 79 small exons and the mRNA produced is 14000 base pairs in length. Two-thirds of the mutations that cause DMD are deletions of one or more exons. Some of these deletions cause the lethal condition DMD whereas other deletions in the same gene cause the milder condition, Becker muscular dystrophy (BMD). In BMD, affected boys are often chairbound by about 25 years from onset but their lifespan may be normal. The reason why BMD is a milder condition than DMD is that most deletions that cause DMD result in frameshifts whereas most BMD deletions do not (see Figure 6). This occurs as the number of base pairs of the exons deleted in DMD is not divisible by three, whereas the number of base pairs of the exons deleted in BMD is divisible by three.

Figure 8. Schematic representation of the HD mRNA. The trinucleotide repeat, CAG, is unstable and expands such that an extended polyglutamine tract is present in the HD protein, huntingtin. The presence of the extended polyglutamine tract causes HD.
**Huntington disease (HD)**

Huntington disease (HD) affects people in middle age. It attacks nerve cells in the brain and as these cells slowly degenerate, a person with HD loses all control of his or her mental and physical abilities. The disease usually begins with mild symptoms such as forgetfulness, clumsiness and personality changes. As the disease progresses, physical and mental control steadily deteriorates. The affected person develops jerky movements and walking, talking and eating become difficult. The affected person loses the ability to think clearly, plan, solve problems and remember. People with HD can live for 10–20 years from the onset of the disease. Death is not usually due to HD itself but to another physical cause such as heart failure or pneumonia. The pattern of inheritance of HD is autosomal dominant. The gene for HD was found on 4p16.3 in 1983 by linkage analysis. It took a further 10 years for the HD gene to be identified by the Huntington Disease Collaborative Research Group. Within the coding region of the HD gene is a polymorphic trinucleotide (CAG)$_n$ repeat which was found to be expanded and unstable on HD chromosomes. For normal individuals the CAG was repeated 9–35 times, whereas, for people with HD the CAG was repeated 37–100 times (Figure 8). The expansion of the CAG tract in the HD gene does not alter the synthesis of the protein but the protein has an expanded polyglutamine tract. This polyglutamine tract presumably alters the normal properties of the protein and its function within the brain.
Mitochondrial disorders

Introduction

Mitochondria are present in virtually all eukaryotic cells. They are membrane-bound, cytoplasmic organelles and are primarily involved in oxidative energy metabolism (Figure 1). Mitochondria have their own, self-replicating chromosomes and it was discovered in 1988 that faults in the mitochondrial genes can cause human diseases. Since these initial

Figure 1. Electron micrograph of a mitochondrion magnified 140,000 times.
observations, a large number of defects in mitochondrial DNA (mtDNA) have been reported in association with a broad spectrum of multi-system disorders, usually with prominent neuromuscular disease.

**The mitochondrial genome**

The human mitochondrial genome consists of a single, circular double-stranded DNA molecule of 16569 base pairs, which has been completely sequenced. It is present in thousands of copies in most cells and in multiple copies per mitochondrion. The genome contains 37 genes: 28 are encoded on one of the strands of DNA and 9 on the other. These genes encode 22 transfer RNAs and two types of ribosomal RNA required for mitochondrial protein synthesis (see Bullied, 1992, in the Background reading section) in addition to 13 proteins, which are involved in cellular oxidative phosphorylation [11]. The mitochondrial genome thus encodes only a small proportion of the proteins required for its specific functions; the bulk of the mitochondrial polypeptides are encoded by nuclear genes and are synthesized on cytoplasmic ribosomes before being imported into the mitochondria. The mitochondrial genome resembles that of a bacterium in that genes have no introns, and that there is a very high percentage of coding DNA (about 93% of the genome is transcribed as opposed to about 3% of the nuclear genome) and a lack of repeated DNA sequences. Consequently, mitochondria are believed to have originated as aerobic bacteria living within bigger cells that could not themselves carry out oxidative phosphorylation [11].

**Inheritance of the mitochondrial genome**

Human cells usually contain thousands of copies of the double-stranded mtDNA molecule. During zygote formation a sperm cell contributes its nuclear genome but not its mitochondrial genome to the egg cell. Consequently, the fertilized zygote contains only the mitochondria that were present in the unfertilized egg and are maternal in origin. Thus the mitochondrial genome is maternally inherited: males and females both inherit their mitochondria from their mother, and males cannot transmit their mitochondria to subsequent generations (Figure 2). Thus a typical mitochondrially inherited condition can affect both sexes but is passed on only by affected mothers.
Mitochondrial disorders

In comparison with the nuclear genome, the mitochondrial genome is a small target for mutation (about 1/200 000 of the size of the nuclear genome). The proportion of clinical disease due to mutations in the mitochondrial genome might therefore be expected to be extremely low. However, due to the large amounts of non-coding DNA in the nuclear genome, most mutations in the nuclear genome do not cause diseases. In contrast, the bulk of the mitochondrial genome is composed of coding sequence and mutation rates in mitochondrial genes are thought to be about 10 times higher than those in the nuclear genome (probably

Figure 2. Pedigrees of mitochondrial diseases. (A) A typical pedigree pattern for a mitochondrial disorder; (B) the atypical pattern of Leber’s hereditary optic neuropathy, which affects mainly males. Reproduced from Strachan, T. and Read, A. (1996) Human Molecular Genetics, Bios Scientific Publishers, Oxford, with permission; ©1996, Bios Scientific Publishers. (See Figure 1 in Chapter 3 for key to symbols used.)

Mitochondrial diseases

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because mtDNA replication is more error-prone and the number of replications is much higher). Accordingly, mutation in the mitochondrial genome is a significant contributor to human disease.

Mitochondrial diseases can be caused by the same range of mutations as cause disorders of the nuclear genome. An important aspect of the molecular pathology of mtDNA disorders, however, is whether every mtDNA molecule carries the causative mutation (homoplasy) or whether the cell contains a mixed population of normal and mutant mitochondria (heteroplasm). Where heteroplasm occurs, the disease phenotype may therefore depend on the proportion of abnormal mtDNA in some critical tissue. Also, this proportion can be very different in mother and child because of the random segregation of mtDNA molecules at cell division.

The idea that defects in mitochondrial respiratory chain function might be the basis of disease has been considered for some time but it was not until 1988 that molecular analysis of mtDNA provided the first direct evidence for mtDNA mutations in neurological disorders, notably Leber’s hereditary optic neuropathy.

**Leber's hereditary optic neuropathy**

Leber's hereditary optic neuropathy (LHON) is an inherited form of blindness that presents in mid-life and is characterized by rapid bilateral central vision loss due to atrophy of the optic nerve. A major focus of early studies of LHON was the elucidation of the mode of inheritance of the disorder. Perplexing features of the disease were its maternal inheritance pattern and that more males than females were affected (Figure 2). In 1988 it was demonstrated that LHON was caused by mtDNA mutations and it is now known that three primary mutations are present in at least 90% of affected families. These mutations all cause substitutions of highly conserved amino acids. Thus the mtDNA mutations explain the maternal transmission of LHON but the reasons why more males are affected than females remain unknown. Hypotheses for the male bias have included that expression of the phenotype may require the co-inheritance of the mtDNA mutation plus an X-linked recessive mutation, that LHON could be hormonally influenced by androgens or that environmental factors may contribute. In terms of environmental effects, heavy tobacco smoking has been proposed and is still being con-
sidered as one possible factor influencing the penetrance of the condition.

**mtDNA mutations in aging**

In the course of investigating mtDNA deletions in disease it became apparent that normal individuals can also be heteroplasmic for deleted mtDNA and that the fraction of deleted DNA increases exponentially with age. These observations raised interest in the role played by mtDNA mutations in aging. One hypothesis is that continuous oxidative damage to mtDNA is responsible for an age-related decline in oxidative phosphorylation capacity. Whether a causal relationship exists between mtDNA mutations and aging, however, remains to be established.
Somatic mutations and cancer

Introduction

In some families an inherited condition can be traced back for generations, whereas in other families a disorder known to be heritable may appear ‘out of the blue’ or sporadically as the result of a new mutation. The effect of that new mutation depends, in part, on when during development it has occurred. If the new mutation is present in the fertilized egg (i.e. the mutation has occurred during the formation of the sperm or the egg) then this mutation will be transmitted to all daughter cells. If, however, a mutation arises after the first cell division, then this mutation will be found only in a proportion of cells (the cells that derive from the cell carrying the mutation) and the remaining cells will not carry the mutation. Individuals whose cells do not all contain the same genetic material are termed mosaic. A sporadic mutation may be confined to the gonadal cells (a gonadal mosaic mutation) or to the somatic cells (a somatic mosaic mutation) or may occur in a proportion of both. If the mutation has occurred in the gonadal cells then there is a chance that the mutation will be passed on to successive generations, whereas if a mutation occurs only in somatic cells it cannot be inherited. Mutations that occur in certain somatic cells and their descendants are known to play a key role in the cause of many common cancers and may also be involved in disorders of the immune system and the aging process.
Somatic mutations in cancerous diseases

Cancer affects about 1 in 4 adults at some stage of their lives and is caused by the uncontrolled division of a cell to form a tumour. Each tumour results from one or more mutations of the cellular DNA. In a small proportion of cancers the first mutation is inherited and is present in all cells but, for most cancers, the faults occur after birth in a restricted number of somatic cells. Recent studies have identified three groups of genes that are frequently mutated in cancer: oncogenes, tumour-suppressor (TS) genes and mutator genes.

TS genes

TS gene products are thought to inhibit cell proliferation and their absence in cells causes tumour formation. The TS genes were discovered as a result of studies of rare inherited forms of cancer, particularly retinoblastoma. Retinoblastoma is a malignant eye tumour of childhood. All of the cases where both eyes are affected have an autosomal dominant mode of inheritance. Genetic linkage analyses of families with this condition localized the gene to 13q14. A autosomal dominant inheritance implies that only one mutant copy of a gene is sufficient to cause the disorder. However, for retinoblastoma, as for other disorders caused by TS genes, this is not the case. By comparing DNA isolated from tumours with DNA isolated from blood cells using markers for chromosome 13, it was discovered that in a number of cases where the blood DNA was heterozygous for a chromosome 13 marker, the tumour DNA from the same person was homozygous for that marker. This led to the ‘two hit’ hypothesis for the inheritance of TS genes. The first ‘hit’ is a mutation (most often a point mutation) in one of the copies of the TS gene and this mutation is inherited. However, in order for a tumour to develop, a second hit has to occur. This second hit usually involves loss of all or part of the chromosome that contains the normal counterpart of the TS gene and is evident in tumour tissue because of loss of heterozygosity (LOH) for specific chromosomal markers. The combination of linkage analysis and LOH studies that was used in the study of retinoblastoma has since been used to locate a number of TS genes found in other forms of cancer, e.g. genetic breast cancer.
Breast cancer

About 1 in 12 women in the U.K. will develop breast cancer at some time in their life and about 34,000 new cases are recorded each year. A large-scale study of over 1500 families that was completed in 1988 found that about 4–5% of cases of breast cancer might be attributable to genetic factors and that the evidence for genetic factors was strongest in those families where there was early onset of breast cancer. Families where there appeared to have a Mendelian pattern of inheritance for breast cancer were then collected for linkage analysis. In a proportion of families the breast cancer was found to segregate with markers on chromosome 17 and in other families with markers on chromosome 13. The chromosome 17 gene, called BRCA1, was found in 1994 and the chromosome 13 gene, BRCA2, was found a year later. Both genes have properties of TS genes in that a mutated copy of either gene is inherited and a second mutation (usually LOH in breast tissue) has to occur in order for the breast cancer to develop. This means that, in families affected by genetic breast cancer, each child has a 50% chance of inheriting the faulty gene and it is estimated that 80–90% of women who inherit the BRCA1 or BRCA2 genes will develop breast cancer. It is very unusual for men to get breast cancer (although not impossible) but men can carry a copy of the mutated gene and pass it on to their children. So, genetic breast cancer could occur in a family after a couple of unaffected generations because it has been passed down through the male line.

Oncogenes

Oncogenes are genes whose action positively promotes cell proliferation and the mutant versions are excessively or inappropriately active. Oncogenes were first discovered by the analysis of the genomes of retroviruses (viruses that have a genome of RNA and replicate via a DNA intermediate in a host cell) that cause cancer in mice, cats and monkeys [12]. It was found that, in addition to the viral genes, other genes (oncogenes) had been inserted into the viral genome and that it was the expression of these genes in the host cell that caused the cell to become cancerous. Analysis of the sequences of the retroviral oncogenes revealed that normal cells had counterparts of all the retroviral oncogenes and that oncogenes were in fact mutated versions of genes involved in a variety of normal cellular functions (Figure 1). The viral
genes are sometimes designated v-src, v-myc, etc., and their mammalian cellular counterparts c-src, c-myc, etc. The forms of the c-oncogenic genes in normal cells are properly termed proto-oncogenes but nowadays the term oncogene is used for these normal genes and the abnormal versions are described as activated oncogenes. The oncogene products include growth factors, cell-surface receptors, transcription factors and factors that govern progress through the cell cycle which, if overexpressed in an uncontrolled manner, cause cellular hyperproliferation (Figure 1). The causes of the ‘inappropriate’ activation of oncogenes are usually somatic events and there are a

Figure 1. The proteins encoded by different oncogenes act at different sites in the chain of controls regulating cell division. Reproduced from [12] with permission, ©1989, Philip Allan Publishers, Ltd.
number of ways in which an oncogene can become activated, two examples of which are given below.

**Activation by point mutations**

Comparison of the DNA sequence of oncogenes in tumour tissue with other somatic tissues from the same individual has revealed that specific point mutations can lead to different tumour types. For example, in the HRAS1 gene (from the viral disease Harvey rat sarcoma, where the v-oncogene is v-ras and the c-oncogene HRAS1), a glycine residue is normally present at position 12, but in some patients with bladder cancers, lung cancer or melanoma the tumour tissue shows a point mutation (GGG → GTC), leading to a substitution of valine at position 12. This change is not inherited but is a somatic mutation within the cells that originate the cancer. Specific point mutations have also been identified at other critical positions (e.g. 13, 61 and 119). In normal cells, the Ras protein is involved in conveying the signal received from specific cell-surface receptors to sites in the cell such that specific cellular functions can be performed. Mutations in the HRAS1 gene produce a mutated form of the Ras protein, which causes excessive cellular response to the signal from the receptor.

**Activation by chromosomal translocations**

Tumour cells typically have grossly abnormal karyotypes with multiple extra and missing chromosomes, many translocations, etc. Most of these changes are random and reflect a general genomic instability. However, tumour-specific changes have been recognized on this background of random changes and over 150 different tumour-specific breakpoints have now been recognized. The best-known tumour-specific rearrangement produces a smaller-than-normal chromosome 22, known as the Philadelphia (Ph) chromosome, which is found in patients with chronic myeloid leukaemia (CML). The majority of affected patients show this chromosome within the malignant bone marrow but not in the unaffected somatic tissues. The Ph chromosome is actually caused by a reciprocal translocation between chromosomes 9 and 22. As a result of this translocation, the ABL oncogene is translocated from its normal site in 9p34 to 22q11, where it rearranges with a specific sequence called the breakpoint cluster region (BCR), creating a novel fusion gene. The
hybrid gene produces a novel protein in the CML cells that is responsible for their becoming cancerous (Figure 2).

**Mutator genes**

The products of mutator genes are responsible for repairing the genome when it becomes damaged (see Bryce, 1998, in the Background reading section). Mutations in these genes lead to inefficient replication or repair of DNA and therefore to an increase in the mutation rate. This increase
in the mutation rate means that cancer-causing mutations are more likely to occur. Studies of different forms of cancer have led to the identification of genes responsible for maintaining DNA integrity. For example, genetic linkage analysis of the autosomal dominant disorder hereditary nonpolyposis colon cancer (HNPCC) have mapped genes to two locations, 2p15–22 and 3p21.3. The genes at both of these loci have been found to have a role in checking DNA for mismatched base pairs after DNA replication.

**Somatic mutations in non-cancerous diseases**

Every one of us carries somatic mutations, but these generally do not cause an obvious phenotype. The somatic mutations that most often cause problems are those in cancer-causing genes. However, somatic mutations can also occur in genes that do not cause cancer. If these mutations occur early enough in embryonic life or in a stem cell then these embryos can give rise to clinically abnormal people. Mosaics for Down syndrome, Klinefelter syndrome and Turner syndrome [2] are commonly discovered when abnormal patients are referred for cytogenetic investigation. Their phenotype lies somewhere between normal and the full-blown phenotype. People can also be mosaic for single-gene mutations. The new mutation may appear in a mosaic form in a clinically normal person or in a person with only minor manifestations of the disorder. If the mutation occurs in the germline, then children of the mosaic individual are at risk of inheriting the mutated gene and developing the full-blown phenotype.
Polygenic disorders

Introduction

In Chapter 3 in this booklet, examples of ‘monogenic (or single-gene) disorders’ are described that have Mendelian patterns of inheritance and are caused by mutations in single genes. Such disorders often show extreme phenotypes. However, the major burden of genetic ill health lies in the more common ‘polygenic disorders’, such as diabetes, hypertension and multiple sclerosis. Rather than being caused by the action of a single gene, polygenic disorders are caused by the interaction of many genes as well as by factors in the environment. The mutations in the genes that underlie polygenic disorders are often subtle and occur in the normal population. It is the combination of a number of these subtle mutations, plus factors in the environment, that cause the disorder. Consequently, for most polygenic disorders a person’s genetic background is not sufficient to cause the disorder but can make that person more susceptible to the disorder. Such polygenic disorders do not have simple patterns of inheritance but affect more people in some families than in other families (i.e. there is a clustering of the disorder in certain families, more so than one would expect by chance alone). Identifying the genes that are responsible for genetic susceptibility to polygenic disorders has become feasible because of the increased efficiency of genetic mapping. Major progress has been made in the last 5 years in the mapping and identification of susceptibility genes underlying polygenic disorders such as, for example, type 1 diabetes.
**Genes versus environment**

Before embarking on a long-term, expensive and complex study to identify susceptibility genes that underlie a common disorder, one would want to be reasonably confident that there is a strong genetic basis to the condition. One of the first steps in the investigation of a polygenic disorder therefore involves trying to estimate the relative contributions of the environment and the genetic background towards the risk of developing the disorder. Distinguishing the roles of genes versus the environment is not always as easy as it sounds, however, as individuals in families share a proportion of their genes as well as their environment. Further, even for clearly defined genetic and infectious diseases there is an interaction between genetic background and the environment. For example, although phenylketonuria (see Chapter 7 in this booklet) is clearly an inherited condition, if an affected individual follows a diet that provides just sufficient phenylalanine to supply the need for making protein, it ceases to be a disease. Conversely, for example, signs of the infectious disease leprosy, which is caused by the bacterium *Mycobacterium leprae*, will only develop in 10–20% of people exposed to the bacterium and only 50% of those will go on to develop the full-blown condition. The responses of an individual to infection by this bacterium are likely to be caused by that individual's genetic background, which is clearly evident in some ethnic groups, where leprosy appears to be inherited as a Mendelian trait.

Twin and adoption studies have played a major role in distinguishing which disorders are primarily caused by a shared environment versus a shared genetic background. Such studies have been carried out to determine, for example, whether the tendency to gain weight, a trait that tends to run in families, has a genetic basis. Monozygotic (or ‘identical’) twins are derived from the same zygote and have the same genetic background, whereas dizygotic (or ‘non-identical’) twins are derived from different zygotes and share the same proportion of genes as brothers and sisters. Studies of obesity have compared the body mass indices (a measure of a person’s weight relative to their height) of monozygotic twins with those of dizygotic twins. These studies have indicated that the degree of concordance of body mass indices for monozygotic twins is higher than for dizygotic twins, indicating that there is an underlying genetic component. Such studies have, however, been criticized as identical twins may share more of their
environment than dizygotic twins. Therefore, studies have compared the body mass indices of monozygotic and dizygotic twins where the twins have been reared apart because of adoption. A doption studies have also been carried out where the body mass indices have been compared between the adoptee and their natural and adopted family. All of these studies have revealed that genetic factors do control a large part of one's body mass index. Just which genes influence our eating, metabolism and physical activity and how they exert their power are subjects of intense research as, although obesity in itself cannot be considered a disease, it is a major contributor to disease.

**Identifying susceptibility genes**

Once it has been determined that a trait or a disease has a significant genetic contribution, the next phase is to try to identify those susceptibility genes. Such studies have been carried out in mice and humans but are more difficult to perform than finding a gene for a monogenic disorder. For a monogenic disorder one can trace the inheritance of a genetic marker and a disorder in a family. Co-inheritance means that the marker and the mutated gene are physically linked on the chromosome. Such studies rely on families where there are clear inheritance patterns. For most polygenic disorders, however, the disorders cluster in families but do not conform to simple Mendelian inheritance patterns. Also, as is known from the degree of concordance of disorders in monozygotic twins, even if individuals have inherited the same combination of genes, whether they develop the disease or not will depend on their environmental exposure. Thus if a marker close to a susceptibility gene is inherited in a family there may not be an exact correlation between inheritance of that marker and manifestations of the disease.

Some researchers have made headway in identifying susceptibility genes in mice. For example, in order to find genes that may be involved in obesity, strains of mice that fatten dramatically on high-fat diets have been crossed with closely related strains of mice that remain relatively lean on the same diet. By tracking the way the trait is passed from one generation to the next, and correlating the inheritance pattern with chromosomal markers, it has been demonstrated that, for these mice, fat sensitivity is carried by 1–4 dominant genes that reside in specific chromosomal regions. For the ob/ob (genetically obese) mice the gene causing their obesity was found to produce a protein called leptin, which
is now known to signal the brain to reduce food intake (Figure 1) [13]. Similar mice-breeding experiments have also been used to identify genes that could be responsible for a mouse form of diabetes, which mimics human type I (insulin-dependent) diabetes mellitus. Breeding experiments have mapped at least 15 chromosomal loci that could be responsible for this condition.

In humans, the breeding experiments that have been conducted for mice are not feasible and so linkage analysis methods have been developed that examine the degree of sharing of regions of the genome by relatives that are affected by the same disorder. Usually, affected sibling pairs (affected sister/sister, brother/sister or brother/brother pairs) are used. The first step in such an analysis is to examine a range of chromosomal markers that are evenly spaced throughout the genome in the DNA from the affected sibling pair and, where possible, their parents. If the marker is not near a susceptibility gene one would expect that there would be random segregation of the marker, and so it would not be shared more often by the siblings than one would expect by chance. If the marker is close to a susceptibility gene, however, the region of the genome containing that susceptibility gene, and therefore

Figure 1  A n ob/ob (genetically obese) mouse compared with lean litter mates. The designation ob/ob means the possession of an obesity gene inherited from each parent. Reproduced from [13] with permission; courtesy of John Sholtis.
the region of the genome carrying the marker, would be more often shared or in common between the affected sibling pairs. Many hundreds of sibling pairs need to be analysed in this way in order for the results to be statistically significant. Such linkage analyses have been carried out for disorders such as diabetes and multiple sclerosis, and regions of the genome are currently being investigated to identify the susceptible genes within linked regions.

The future

Many polygenic disorders are currently being examined using genetic linkage analysis approaches to identify the underlying susceptible genes. Within the next 5–10 years numerous reports will appear in the medical and public literature about the identification of potentially linked regions of the genome and potential susceptible genes. Pursuit of the genes that underlie common polygenic disorders is of enormous interest to health-care professionals, pharmaceutical industries, ethicists and to the many affected members of the public, as such discoveries will potentially have a major impact on our society. Investigations of exactly how susceptibility genes contribute to disease will, however, be challenging, as will the possibility of altering a person’s susceptibility to a condition by altering the function of the susceptibility gene product.
Prevention and treatment of genetic disorders

Introduction

The discoveries that have been made regarding the molecular basis of heritable disorders have led to the development of a battery of tests that allow the accurate prenatal and postnatal diagnoses of genetic conditions and have paved the way for the development of effective therapies for genetic conditions. These advances should, however, be seen in perspective, as there is to date no effective and long-term cure available for any genetic condition. Thus parents of affected children, parents at risk of having affected children and affected individuals themselves often have difficult choices to make regarding whether they should take advantage of genetic testing and, equally, how they should proceed in the event of a positive test result. Specially trained clinical geneticists and genetic counsellors can provide information regarding the complications and potential treatment regimes for specific disorders and the sensitivity and specificity of diagnostic tests [14]. On the basis of this information the consultant is then free to make a choice as to whether to take a genetic test or how to proceed if a positive test result is obtained. A person’s social, religious or moral background or their own personal experiences of the disorder may well influence such decisions. Against this background, this chapter provides an overview of some of the available approaches to the prevention and treatment of genetic disorders.
Prevention

Prevention for the purposes of this chapter is defined as the prevention of the birth of an affected fetus. Two major possibilities are available to parents in terms of prevention: reproductive choice or prenatal diagnosis followed by selective termination of an affected fetus.

Reproductive choice

Knowledge of the patterns of inheritance and incidence of genetic diseases allows the calculation of the risk of a fetus being affected by a genetic disorder. Such calculations of risk were all that was available to genetic counsellors before it was possible to perform genetic tests. So, for example, for a couple with no previous history of cystic fibrosis (CF) in either of their families, their risk of having a baby with CF would be the same as that of the general population (about 1 in 1000–1200). However, if the couple have previously had a child with CF then both parents would be proven carriers of the mutant gene, as CF is an autosomal recessive disorder. Their risk of having another affected child would then be 1 in 4, which is the risk for an autosomal recessive condition. This risk may be unacceptably high for the parents and on this basis they may choose not to have further children. Even for disorders without clear Mendelian patterns of inheritance, calculations can be made as to the risk of a couple having an affected baby. For example, the risk of Down syndrome is known to increase with increasing maternal age [2]. Depending on the age of the mother, a couple may choose not to have further children because of their risk of having a child with Down syndrome.

Prenatal diagnosis

Two forms of prenatal diagnosis are used routinely on all pregnant women: ultrasound scanning and maternal blood sampling. Ultrasound scans are performed on most pregnant women between 16 and 19 weeks of pregnancy. Abnormalities in the development of fetus can be detected at this stage; for example, growth retardation, and heart and kidney defects. On the basis of such findings further prenatal testing may be recommended to provide a more precise diagnosis. Between 15 and 19 weeks of pregnancy women attending antenatal clinics are offered a
blood test. The blood is analysed to assess the levels of β human chorionic gonadotrophin, oestriol and α fetoprotein. The three levels in combination with the mother's age can be used to estimate the risk of the baby being affected by a chromosomal problem, especially Down syndrome. If the result indicates a high level of risk then the mother will be offered further tests to assess the status of her baby.

For pregnancies where there is a risk of the fetus having a genetic abnormality, tissue for genetic testing can be obtained either by amniocentesis or chorionic villus biopsy (Figure 1). Amniocentesis is performed between 15 and 20 weeks of pregnancy and involves removing a sample of amniotic fluid from the mother’s abdomen. The amniotic fluid contains fetal cells that can be cultured for karyotype analysis or from which DNA can be extracted directly and tested for the presence of a specific mutation in a gene. Chorionic villus biopsy can be performed at about 10 weeks of gestation, which is an advantage over amniocentesis, but has the disadvantage of a higher miscarriage risk than amniocentesis. The chorionic villi are part of the developing placenta and are fetally derived. A sample of this tissue can be removed under ultrasound guidance using a fine needle that is passed either through the mother’s abdomen or through the cervix. Both chromosomal and DNA analyses can be performed on this tissue.

**Treatment**

**Prophylactic therapy**

Prophylactic therapy involves the prevention of symptoms before they occur. In order for such therapy to be effective presymptomatic diagnosis needs to be performed. This means that a test has to be performed on an individual to determine whether they have a genetic condition before they develop any symptoms. One of the best known forms of prophylactic therapy for a genetic condition is for phenylketonuria (PKU). PKU is an autosomal recessive condition caused by mutations in the gene for phenylalanine hydroxylase, which is the enzyme that converts phenylalanine to tyrosine. Few physical signs of PKU are evident in a newborn baby but with the ever-increasing levels of phenylalanine accumulating in the body and with the absence of tyrosine severe mental retardation results. A baby with PKU will, however, develop normally and have a normal lifespan if fed a diet very early on that is low
Figure 1. (A) Amniocentesis — the removal of amniotic fluid by transabdominal sampling. (B) Chorionic villus biopsy — an ultrasound scan is used to locate the villi, which are removed through the vagina and the cervix. Reproduced from [2] with permission; ©1998, Philip Allan Publishers, Ltd.
in phenylalanine and supplemented with tyrosine. Thus both early diagnosis and therapy of PKU are mandatory if normal development is to occur. All babies in the U.K. are tested a few days after birth for PKU. A blood spot from a heel-prick is collected on a card (the Guthrie card) during a home visit and sent to a central laboratory where the phenylalanine level in the blood is measured. Babies whose levels are above a threshold are called in for further tests to confirm the finding. If the result is positive the baby can be put on the correct diet immediately. Presymptomatic diagnosis is now also possible for some inherited forms of cancer, such as breast cancer and colon cancer. For genetic breast cancer, it is possible to test individuals with a family history of early-onset breast cancer to determine whether they have inherited one of the predisposing mutations. If they have inherited one of these mutations then their risk of developing breast cancer is about 80%. In the event of a positive result some women have opted to undergo a double mastectomy to ensure that they will not develop breast cancer. Likewise, individuals who have tested positive for mutations making them susceptible to colon cancer have elected to undergo a colectomy at the first sign of symptoms.

Replacement therapy

Replacement therapy involves replacing the gene product (protein) that is missing or defective in an affected person with a functioning gene product. For many years such therapy has been available to individuals with, for example, diabetes (where insulin is replaced) or growth hormone deficiency (where growth hormone is replaced). Originally, the insulin and the growth hormone were extracted from animal tissues but nowadays they are synthesized by bacteria that contain the cloned human gene sequences. These disorders have been relatively easy to treat because the normal gene products are transported around the body in the bloodstream and so it is possible simply to inject the synthetic gene product. However, even for these disorders, the treatment has the disadvantage of having to be continually applied. Further, for most disorders, the proteins that are defective are not blood borne or in easily accessible tissues or subcellular locations. For example, replacing the defective CF transmembrane regulator (CFTR) protein in the treatment of CF by injecting the protein into the bloodstream or even into the lungs would
not be effective, as in order for the protein to function it needs to be specifically located in the membranes of cells in the mucosal tissues.

Gene therapy offers an alternative approach to replacement therapy. For genetic disorders, gene therapy offers the potential to introduce and express cloned genes in the cells of a patient in order to overcome the disease. Germ-line gene therapy (i.e. the replacing of the defective gene in either the sperm or the ova) offers a means of ensuring that all the cells in the resultant person contain the corrected gene and would be of benefit to subsequent generations. However, this form of gene therapy is not considered at this time to be technically feasible or ethically acceptable for human use. An alternative approach is somatic-cell gene therapy, which targets the non-reproductive cells of the body (e.g. skin, muscle, bone, brain, cells of the blood, etc.). Two major approaches are used in the transfer of genes for gene therapy (Figure 2).

**Gene transfer ex vivo**

This initially involves transfer of cloned genes into cells grown in culture. Those cells that have been transformed successfully are selected, expanded by cell culture in vitro and then introduced into the patient.

**Figure 2.** In vivo and ex vivo gene therapy. In vivo gene therapy (white arrow) involves the genetic modification of cells by introduction of the cloned gene directly into the patient. Ex vivo gene therapy (shaded arrows) means that the cells are modified outside the body before being implanted into the patient. Reproduced from Strachen, T. and Read, A. (1996) Human Molecular Genetics, Bios Scientific Publishers, Oxford, with permission; ©1996, Bios Scientific Publishers.
This approach is only applicable to tissues that can be removed from the body, altered genetically and returned to the patient where they will engraft and survive for long periods of time (e.g. bone marrow cells, skin cells, etc.).

**Gene transfer in vivo**

Here, the cloned genes are transferred directly into tissues of the patient. This may be the only possible option in tissues where individual cells cannot be cultured in vitro in sufficient numbers (e.g. brain cells). As there is no way of selecting and amplifying cells that have taken up and expressed the foreign gene, the success of this approach is crucially dependent on the general efficiency of gene transfer and expression.

**Gene transfer methods**

One of the major requirements of gene therapy is to ensure that the cloned genes are transferred efficiently into disease cells and that the introduced genes are expressed at suitably high levels. Although a number of methods have been developed, most are too inefficient for clinical use. Available gene transfer methods include the use of chemical, physical and fusion methods (involving liposomes), receptor-mediated endocytosis and recombinant virus vectors. Mammalian viral vectors are particularly attractive as viruses have evolved to infect cells with high efficiency and molecular biologists have exploited this property to introduce cloned genes into human cells.

**Gene therapy trials**

The first apparently successful human gene therapy experiment was performed in 1990 in the treatment of a 4-year-old girl who had the rare recessively inherited disorder, adenosine deaminase (ADA) deficiency. ADA is an enzyme which, if deficient, has a particularly severe effect on the T-lymphocytes, which are one of the major classes of immune system cells (Figure 3). Patients without this enzyme suffer from severe combined immunodeficiency, meaning that they are not able to mount an immune response to fight infection. The ADA gene therapy approach involved four steps (Figure 4). First, a normal copy of the ADA gene was cloned into a viral vector. Second, T-lymphocytes were purified from the
girl’s blood and were grown in the laboratory. Third, the ADA recombinant virus was then used to infect the cells. Those T-cells that had taken up the virus were selected and grown to high numbers in culture. Fourth, these normalized T-cells were then infused back into the patient’s bloodstream. Following treatment, the little girl showed evidence of improved immune function. Precisely whether this improvement was due to the gene therapy was uncertain, as complementary treatments involving replacement of the purified enzyme were ongoing during the gene therapy trial.

Since this first attempt at gene therapy, trials have been initiated for a few other inherited disorders such as CF. In CF, the primary expression of the defect is in the lungs but there are no methods to culture lung cells routinely in the laboratory, so in vivo gene therapy approaches have been adopted. A smaller version of the CFTR gene has been inserted into a viral vector and transferred into the lungs of patients with CF. However, some problems have been encountered, as in order to have

![Diagram of ADA pathway](image-url)

**Figure 3.** The biochemical pathway of ADA, an intracellular enzyme that functions in the purine salvage pathway of nucleic acid degradation. A lack of ADA activity results in the build-up of deoxyadenosine and other toxic products that leads to T-lymphocyte cell death. Other cell types have alternative metabolic pathways to degrade deoxyadenosine that are not cytopathic.
enough of the product expressed in the lung cells, high doses of the recombinant virus were administered and this led to reactions in the patients. Clearly such protocols need to be fine-tuned for CF but such gene therapy trials show enormous potential for the treatment for genetic disorders.

**Treatment of symptoms**

For most genetic disorders the main prospect for affected individuals is treatment of the symptoms of the disorders. Such treatments include surgery, the administration of drugs and the control of a patient’s environment. The following are just a few examples. For achondroplasia, a controversial form of treatment is the surgical lengthening of the limbs so that affected persons can have near normal height. It is common for people with Down syndrome to be born with heart defects, which can be corrected surgically. To stay well, people with CF require daily physiotherapy and breathing exercises to help clear the mucus from their lungs.
They also need to take pancreatic enzyme supplements to help them digest their food and antibiotics to combat infections. For women with Turner syndrome, hormone-replacement therapy is given to prevent early-onset osteoporosis that occurs because of the absence of oestrogen. For males with Klinefelter syndrome, testosterone is used to combat lethargy and promote muscle development. The excessive eating and weight gain of patients with Prader–Willi has to be managed with strict diet control. These forms of treatment for genetic disorders are likely to continue to expand with the ever-increasing knowledge of their pathogenesis and progression.
Bibliography

Background reading

Article describes transcription and translation and the cellular machinery required for protein synthesis.
Article covers basic techniques of gene cloning and how such cloning can be used to produce proteins, for example, insulin, for medical use.
Article describes the structure and function of DNA and the genetic code.

Additional reading

Article includes a description of the technique FISH (fluorescence in situ hybridisation) and its use in detecting chromosomal abnormalities.
A comprehensive review of Down syndrome, including investigations of the molecular basis of the condition.
Article describes the world-wide research project aimed at mapping and sequencing the entire human genome.
Article covers the calculation of risk of a genetic disease, using cystic fibrosis as an example, the cloning of the CFTR gene and the testing for causative mutations.
Article describes haemoglobin structure and synthesis, globin gene structure, sickle cell anaemia and the thalassaemias.
Article explains the biology behind the inheritance of haemophilia and the consequences for European history.

Additional resources

Educational videos: Educational Broadcasting Services (EBS) Trust is running a project to produce video resources for institutions of Higher Education. Forthcoming titles include: Diabetes, Cystic fibrosis, Sickle cell anaemia, Phenylketonuria, Galactosaemia and G6PD deficiency.

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