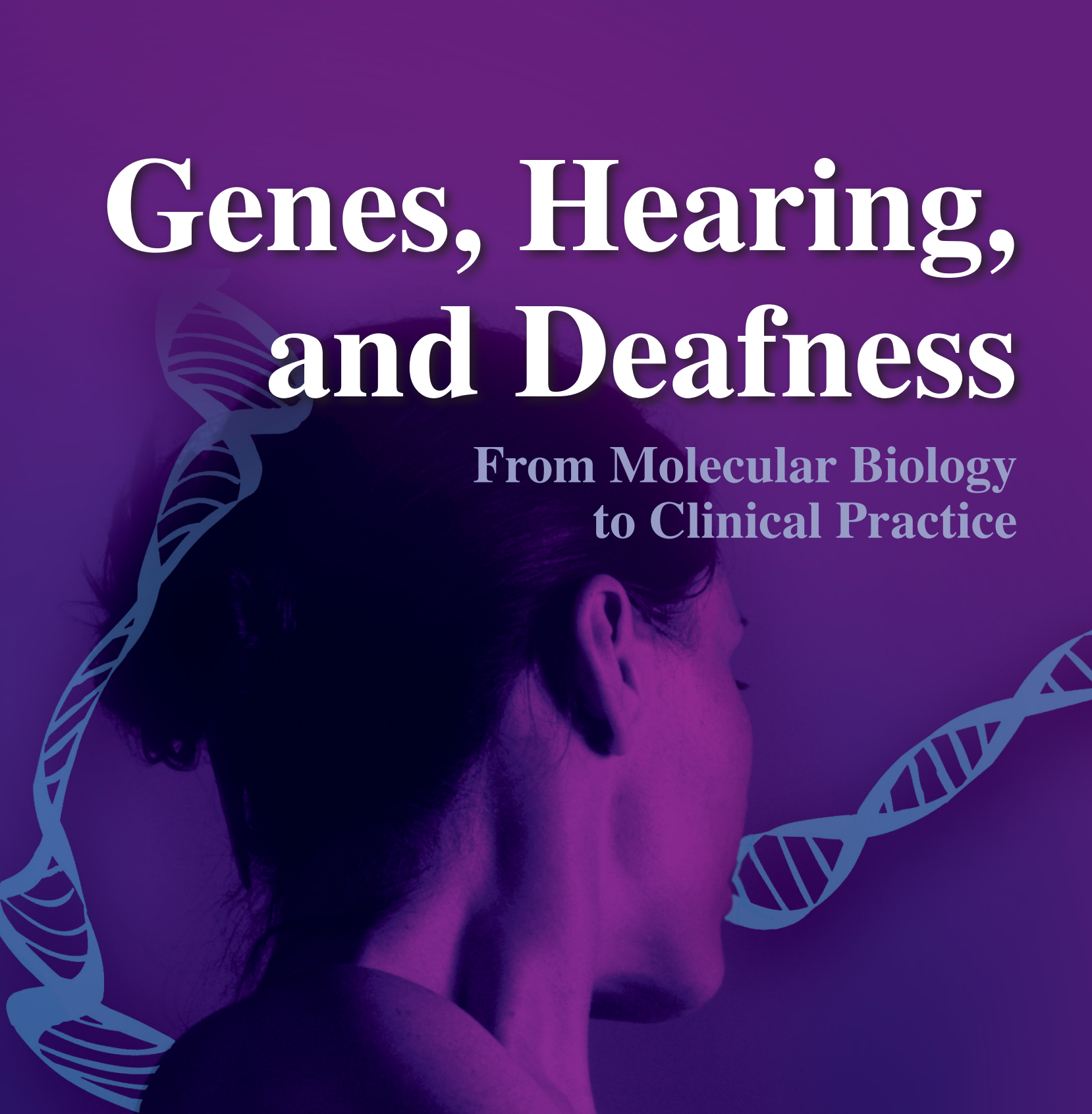
Genes, Hearing, and Deafness

From Molecular Biology to Clinical Practice



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Preface

Frequently, hearing impairment has been considered to require no more than the provision of a hearing aid, with little understanding of the need for thorough aetiological investigation to ensure prevention and remediation where possible and structured rehabilitation programmes, if the distressing personal and social consequences of hearing impairment are to be avoided.

It is worth pointing out that one in every 1,000 new-born babies suffers from congenital severe or profound hearing impairment. Furthermore, epidemiological studies demonstrate that the percentage of the population who have a hearing impairment that exceeds 45 dB HL and 65 dB HL are about 1.3% and 0.3% between the ages of 30 and 50 years, and 7.4% and 2.3% between the ages of 60 and 70 years, respectively (Davis, 1989). Hearing loss has for some time, been considered a permanent effect and consequence of factors such as infections, ototoxicity, trauma and ageing. In recent years, molecular biology and molecular genetics have made a key contribution to the understanding of the normal and defective inner ear, not only in congenital profound hearing impairment but also in late onset/progressive hearing impairment.

The HEAR and GENDEAF projects

In September 1994, when a Preparatory Workshop for the Constitution of a European study group on genetic deafness was held in Milan, only four loci of non-syndromal hearing impairment and only three genes responsible for syndromal hearing impairment had been discovered, whereas at the time of writing, some 45 genes which can cause non-syndromal hearing impairment have been identifies and over 110 loci found.

The importance of establishing common terminology and definitions and co-ordinating the multi-disciplinary approach was the core aim of HEAR project-European Concerted Action HEAR (Hereditary Deafness: Epidemiology and Clinical Research 1996–1999). The idea was to deal with the problem of combining clinical in-depth family and phenotype studies with basic molecular genetics and gene mapping methods in a more standardized way, with the aim of establishing a stable international collaboration. The initiative also wanted to create a bank of updated information on these disorders that would be useful not only to experts but to the entire scientific community in identifying sources of information and specialized centres to which specific cases may be referred. This project stimulated a considerable amount of work in this field leading to developments in molecular genetics and the mapping of human loci associated with hearing disorders. The numerous and scattered loci mapped reflect a heterogeneous set of genes and mechanisms responsible for human hearing and suggest a complicated interaction between these genes (Lalwani and Castelein, 1999).

GENDEAF European Union Thematic Network Project 2001–2005 has helped to further open and widen the analysis of genotype/phenotype correlations, the effects of deafness on the family and the psychosocial aspects (also involving patient associations).

This book is aimed as a follow up of these two projects. It endeavours to provide a broad and up to date overview of genetic hearing impairment for audiologists, otolaryngologists, paediatricians and clinical geneticists to improve the quality of care for the large group of patients with suspected genetic hearing impairment. It does not set out to be a comprehensive description of syndromes such as the excellent and complete text of Toriello, Reardon, and Gorlin (2004), but to provide an easily read sourcebook for those students and clinicians with an interest in this field.

The book is divided into three parts:

The first part reports the important elements of current knowledge of the various situations in which genes have an influence on inner ear dysfunction. Chapters 1 and 2 provide the reader with an appropriate background, presenting an introduction to auditory function, basic genetics and genetic techniques significant to this field. Chapter 3 does not list the various syndromes, but intends to discuss and help clinicians to interpret the signs in order to better understand how molecular genetics can be informative. Chapter 4 tackles the complex genetic aspect of deaf/blindness. Chapter 5 analyses the role of the various genes as a causative of non-syndromal hearing loss. Chapters 6 to 9 analyse the responsibility of genetic factors in certain complex situations such as ageing, noise exposure, ototoxic drugs and otosclerosis.

Part II discusses current approaches to and management of hearing impairment in different ways. Thus Chapters 10 and 11 review the psychosocial impact of genetic hearing impairment and how culturally Deaf people react to genetic interventions. Chapter 12 looks at the related area of genetic factors in speech and language while Chapters 13 to 15 provide guidance on the identification of specific genotypes from phenotypic information, steps which should be taken in this respect in deaf children and how geneticists approach such a challenge. Developments in the pharmacological approach to hearing impairment and tinnitus are covered in Chapters 16 and 20, while Chapters 17 to 19 discuss the medical and surgical management of specific genetic disorders affecting the outer/middle ear, the cochlea and the cochlear nerve respectively.

Finally, the third part delves into our future and is an update of various lines of research covering a range of therapeutic strategies. These include the use of stem cells, tissue transplantation into the inner ear, gene therapy and finishes with an overview of the important process of apoptosis and how it can be prevented.

The contributing experts are all authoritative in their fields and have been asked to present up to date, concise and brief reviews of their particular subject matter; the reader should find this book follows the rapid pace of change in medical science.

Alessandro Martini
Dafydd Stephens
Andrew P Read
Editors

Part I
Genetics and hearing impairment

1 Understanding the genotype: basic concepts

Andrew P Read

Introduction

This chapter is for readers who feel threatened by genetics, who are apt to see genetics as a malignant growth, taking over familiar areas of medicine and rendering them strange and incomprehensible. It is a survival kit but also an entry ticket to this most intellectually exciting area of biomedical science. Genetics is not taking over medicine; it is burrowing under it and rearranging the foundations. Genetics is relevant to hearing and deafness at two levels. In everyday clinical practice, effective diagnosis and management of patients require some familiarity with common patterns of inheritance and with the availability, use, and limitations of genetic tests. More fundamentally, to understand the causes and pathology of hearing impairments, we need to understand the molecular pathology of the genes that program cells in the inner ear. What follows is a review of the concepts and vocabulary of genetics as it applies to both these levels. Italicised words are defined in the Glossary at the end of this chapter. For readers who would like more detail, references are given below to the relevant sections of Strachan & Read Human Molecular Genetics; the text of the second edition ("S&R2") is freely available on the NCBI Bookshelf website (1).

Genes, DNA, and chromosomes

These are the three most basic elements in genetics. "Genes," like elephants, are easier to recognize than to define. Unlike elephants, genes are recognised in two fundamentally different ways:

- As determinants of characters that segregate in pedigrees according to Mendel's laws
- As functional units of DNA

Genes recognised in the first way are rather formal, abstract entities. In retrospect, their connection to physical objects began early, with the recognition of chromosomes and crystallised with Avery's 1943 demonstration that the genetic substance of bacteria was DNA. However, it was not until the 1970s that physical investigation of genes acquired any clinical relevance. Developments in molecular genetics in no way make formal mendelian genetics obsolete. The ability to recognize mendelian pedigree patterns and calculate genetic risks remains an essential clinical skill, while understanding the relation between the DNA sequence and an observable character is a central intellectual challenge of genetics.

DNA is the molecule that carries genetic information. For understanding most of genetics, it is sufficient to view DNA as a long chain of four types of unit called A, G, C, and T. Organic chemists define the structure of A, G, C, and T as nucleotides (nts), each composed of a base (adenine, guanine, cytosine, or thymine) linked to a sugar, deoxyribose, and a phosphate. Watson and Crick in 1953 showed how DNA consists of two polynucleotide chains wrapped round one another in a double helix. The two strands fit together like the two halves of a zip, with A on one chain always next to T on the other, and G always opposite C. As Watson and Crick famously remarked, "it has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." Note, however, that in itself the Watson-Crick structure sheds no light on how the sequence of nucleotides along a DNA chain might control the characteristics of an organism—understanding of that process only began to dawn in the 1960s. Chapter 1 of S&R2 provides rather more detail on DNA structure and function.

Geneticists use some conventions and shortcuts in describing DNA that can confuse the unwary.

- The terms base, nucleotide, and base pair (bp) are normally used interchangeably to describe the A, G, C, and T units

of a DNA chain, although strictly they mean different things. A double helix with 100 units in each chain is 100 bases, 100 nt, or 100 bp long (not 200).

- Looking at the detailed chemical structure shows that DNA chains are not symmetrical. The two ends are different and so the sequence AGTC is not the same as the sequence CTGA. The ends are labelled 5′ ("5 prime") and 3′, and it is a universal convention that sequences are always written in the 5′→3′ direction. It is just as wrong and unnatural to write a sequence in the 3′→5′ direction as it is to write an English word from right to left. This may seem a trivial and pedantic point, but its importance comes from the fact that the two chains in a Watson–Crick double helix run in opposite directions (the structure is described as antiparallel). Thus the strand complementary to AGTC is not TCAG but (5′→3′) GACT.
- Geneticists are no happier than anybody else about the way this makes a sequence and its complement look very different, and they get round it by a convention that makes the relation between the sequence of a gene, a messenger RNA (mRNA), and (via a table of the genetic code) a protein all immediately obvious. So for most purposes you can forget about 5′ and 3′, but the convention needs mentioning because otherwise readers with enquiring minds will run up against seemingly baffling inconsistencies. (see section 3.2 for the detail.)

"Chromosomes" (Fig. 1.1) are seen in cells when they divide. These visible chromosomes represent the DNA packaged into a set of compact bundles so that it can be divided up between the daughter cells. The 46 human chromosomes (23 pairs) each contain between 45 and 280 million base pairs (Mb) of DNA in the form of a single immensely long double helix. Before a cell divides, it replicates all its DNA. When the chromosomes become visible, each consists of two identical sister chromatids, each containing a complete copy of the DNA of that chromosome. Cell division separates the two chromatids, sending one into each daughter cell, and in their normal state each chromosome consists of a single chromatid but with the DNA somewhat decondensed and fluffed out so that it is not visible under the microscope. Even in this state, the DNA is still quite highly structured. It exists as chromatin, a complex of DNA, and various proteins, particularly histones. Chapter 2 of S&R2 describes and illustrates the structure and function of chromosomes.

Back in the 1880s, biologists recognised that there were two types of cell division. The usual form is mitosis. This precisely divides the replicated genetic material between the two daughter cells so that each is genetically identical. All the normal cells of a person are derived by repeated mitosis from the original fertilised egg. That is why you can use a blood, skin, or any other sample to study somebody's DNA; it is the same in every cell (more or less). Gametes (sperm and egg) are formed by a special process, meiosis, which has two purposes. It halves the number of chromosomes so that a 23-chromosome sperm fertilizes a 23-chromosome egg to produce a 46-chromosome zygote. It also shuffles genes so that every sperm or egg that a person produces contains a novel combination of the genes he or she inherited from his or her mother and father. Mendelian pedigree patterns are a consequence of the events of meiosis. Linkage analysis, which maps a gene to a specific chromosomal region, also depends on features of meiosis, as detailed below.

Note the disparity between chromosomes and DNA. The smallest chromosome abnormality visible under the microscope involves around 5 Mb of DNA. Molecular genetic techniques are most efficient when dealing with no more than 1000 bp (1 kb) of DNA. New techniques that fill the gap between these two scales ("molecular cytogenetics") have been important recent drivers of genetic discovery.

Patterns of inheritance

Humans have around 25,000 genes and every human genetic character must depend on the action of very many genes, together with environmental factors. However, for some variable characters, presence or absence of the character depends, in most people and in most circumstances, on variation in a single gene. These are the mendelian or single-gene characters that are by far the easiest genetic characters to analyse. When following the segregation of alternative forms of a gene (alleles)

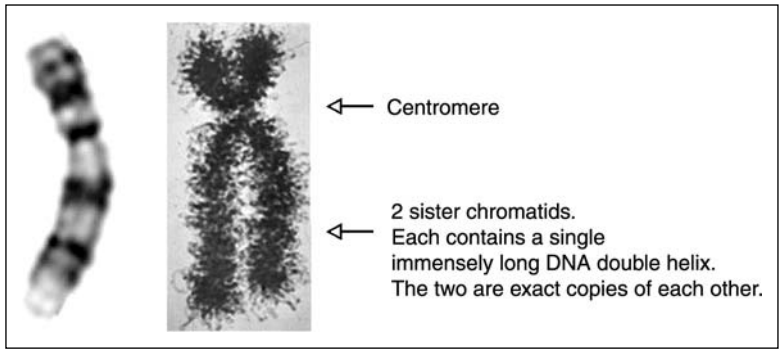

Figure 1.1 The structure of a chromosome as seen in a cell dividing by mitosis. (*Left image*) chromosome 9 as seen in a conventional cytogenetic preparation. The two sister chromatids are tightly pressed together. The banding pattern (G-banding) is produced by partial digestion with trypsin before staining with Giemsa stain. It helps the cytogeneticist to recognize chromosome abnormalities. (*Right image*) chromosome 9 as seen under the electron microscope. Threads of chromatin of diameter 30 nm can be seen, which form loops attached to a central protein scaffold (not visible). DNA is highly packaged even within the 30 nm thread. Overall, chromosome 9 is about 10 μm long and contains about 5 cm of DNA.

through a pedigree, the alleles are conventionally designated by upper and lower case forms of the same letter, e.g., "A" and "a."

The art of human pedigree interpretation is to make a judgment of the most likely mode of inheritance. The two main questions are the following questions:

- Is the character dominant or recessive?
- Is the gene autosomal, X-linked, or mitochondrial?

An initial hypothesis is formed by asking the following questions:

- Does each affected person have an affected parent? If so, the condition is probably dominant; if not, either it is recessive or something more complex is going on.
- Are there any sex effects? If not (affects both sexes, can be transmitted from father to son, from father to daughter, from mother to son, or from mother to daughter), the character is probably autosomal. If yes, it may be X-linked. Y-linked pedigrees are possible in theory but are unlikely in human diseases. Characters that are transmitted only by the mother, but affect both sexes, may be mitochondrial.

The pedigree is then tested for consistency with the initial hypothesis by writing in presumed genotypes. If this process requires special coincidences (an unrelated person marrying in, who happens to carry the same disease allele; a new mutation), alternative hypotheses are tested. The most likely interpretation is the one that requires the fewest coincidences. It is important to stress that for most pedigrees these interpretations are provisional because families are too small to be sure. Sometimes past experience tells us that a particular condition is always inherited in a particular way, but this is often not the case and particularly not with nonsyndromic hearing impairment.

Pedigree description of autosomal dominant inheritance. Both males and females can be affected. The disorder is transmitted from generation to generation and can be transmitted in all possible ways—female to female, female to male, male to female, or male to male. With human autosomal dominant diseases, affected people are almost always heterozygotes; when married to an unaffected person each offspring has a 50:50 chance of inheriting the mutant allele. In small families, the mode of inheritance can be difficult to determine, but transmission of a rare condition across three generations is good evidence for dominant inheritance. Many dominant conditions are variable (even within families) and may skip generations (nonpenetrance, see below).

Pedigree description of autosomal recessive inheritance. Both males and females can be affected. Both parents are usually unaffected heterozygous carriers, and the risk for any given child is 1 in 4. Recessive inheritance is likely when unaffected parents have more than one affected child, especially if the parents are consanguineous. In most cases, there is only one affected individual in the family, making the pedigree pattern hard to identify, but in large multiply inbred kindreds, affected individuals may be seen in several branches of the family.

Pedigree description of X-linked inheritance. Many X-linked diseases are seen only or almost only in males; where females are affected, they may be more mildly or more variably affected. The X chromosome is transmitted to a male from his mother and never from his father, so male-to-male transmission rules out X-linked inheritance. The line of inheritance in a pedigree must go exclusively through females (or affected males). All daughters of an affected male are carriers.

Having the wrong number of chromosomes is usually fatal; yet males and females manage to be healthy despite having different numbers of X chromosomes. This is because of a special mechanism, X inactivation or Lyonisation (named after its discoverer, Mary Lyon). In each early embryo, each cell somehow counts the number of X chromosomes it contains. If there are two, each cell picks one at random and permanently inactivates it. The chromosome is still there, but the genes on it are permanently switched off. If there are more than two X chromosomes, all except one are inactivated. Thus every cell, male or female, has only one active X chromosome.

X inactivation happens only once in the early embryo, but the decision as to which X to inactivate is remembered. As the few cells of the early XX embryo divide and divide, whichever X was inactivated in the mother cell is inactivated in both daughter cells. Thus, an adult woman is a mosaic of clones, some derived from cells that inactivated her father's X and others that inactivated her mother's X. If the woman is a heterozygous carrier of an X-linked disease, some of her cells will be using just the good X and others just the bad X. Depending on the nature of the disease, this may be evident as a patchy phenotype, as in some skin conditions, or there may just be an averaging effect, as in hemophilia. Either way, the distinction between dominant and recessive is not as obvious in X-linked as in autosomal conditions. For males, of course, there is no question of dominance or recessiveness because here are no heterozygotes.

Pedigree description of mitochondrial inheritance. The mitochondria in cells have their own little piece of DNA, probably a leftover from their origin as endosymbiotic bacteria. It is tiny compared to the nuclear genome (16.5 kb and 37 genes, compared to 3.2 million kb and around 24,000 genes; see S&R2 section 7.1.1), but mutations in the mitochondrial DNA are important causes of hearing loss (and other problems). A person's mitochondria come exclusively from the egg; the sperm contributes none. Thus, mitochondrial conditions are passed on only by the mother (matrilineal inheritance). An affected mother transmits the condition to her children of either sex. The resulting pedigrees can look very like autosomal dominant pedigrees unless they are large enough for the exclusively maternal transmission to be obvious.

Cells contain many mitochondria, and it often happens that these are a mixture of normal and mutant versions (heteroplasmy). Heteroplasmy, unlike nuclear genetic mosaicism (see below), can be passed from mother to child, because the egg

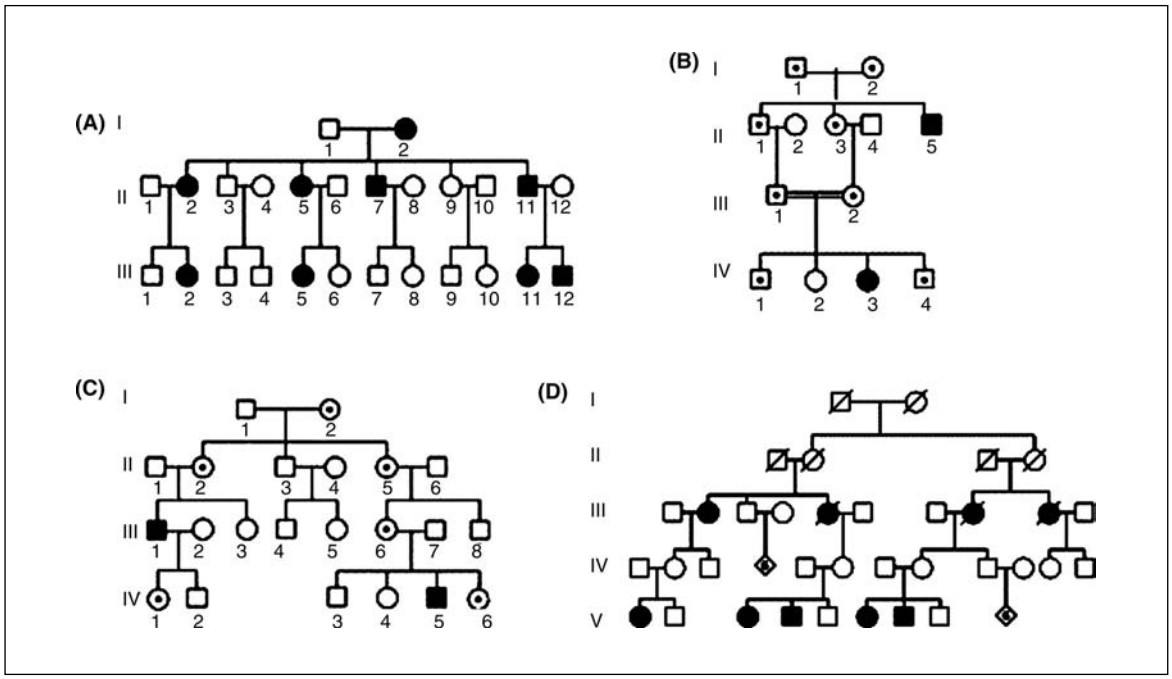

Figure 1.2 Autosomal dominant (A), autosomal recessive (B), X-linked (C), and mitochondrial (D) pedigree patterns. Squares represent males; circles, females. Blacked-in symbols are individuals affected by the condition. Dots in a symbol indicate a phenotypically normal carrier. An unshaded diamond-shaped symbol containing a number, e.g., 6 means 6 unaffected offspring, sexes not specified. Consanguineous marriages can be highlighted by a double marriage line. Generations are numbered in Roman and individuals are numbered across each generation in Arabic numerals. These are ideal pedigrees; those encountered in the clinic are rarely so clear-cut.

contains many mitochondria. Mitochondrial mutations show a particularly poor correlation between genotype and phenotype—for example, the A3243G mutation has been identified as the cause of nonsyndromal hearing loss in some people but diabetes in others (2).

Figure 1.2 shows ideal pedigrees for the main modes of inheritance. Note the conventions used in drawing pedigrees.

Several factors commonly complicate pedigree interpretation:

- *Nonpenetrance*. This describes the situation where a person carries a gene that would normally cause them to have a condition but does not show the condition. Evidence for this can come from the pedigree (an unaffected person who has an affected parent and an affected child) or from DNA testing. The cause is straightforward: a rare lucky combination of other genes or environmental factors may occasionally rescue the person from the condition. Penetrance can be age related, as in late onset hearing loss. Mitochondrial conditions are especially likely to show reduced penetrance. Nonpenetrance is a serious pitfall in genetic counselling.
- *New mutations*. For dominant or X-linked conditions that seriously diminish reproductive prospects, many new cases are caused by fresh mutations. This is not normally the case for recessive conditions.
- *Mosaicism*. A person carrying a new mutation may have a mixture of mutant and nonmutant cells if the mutation happened in one cell of the early embryo. This can directly affect their phenotype and can also produce an unusual pedigree pattern if their gonads contain some mix of normal and mutant cells. Such germinal mosaicism explains why occasionally a phenotypically normal person with no family history produces two or more offspring affected by a dominant condition.
- *Phenocopies*. People who clinically have the condition, but for a nongenetic reason. Obviously, this is a major problem in interpreting pedigrees of hearing loss.
- *Deaf–deaf marriages*. These can make it impossible to work out who inherited what from whom.

Genes as functional units of DNA

Overview

Back in the 1940s, Beadle and Tatum recognised that the primary function of a gene is to direct the synthesis of a protein. In modern terms, the sequence of A, G, C, and T nucleotides in the DNA is used to specify the sequence of amino acids in the polypeptide chain of a protein. In essence, the process consists of two steps:

1. An RNA copy is made of the gene sequence (transcription).
2. The nucleotide sequence in the RNA is used to specify the sequence in which amino acids are assembled into a protein, via the genetic code (translation).

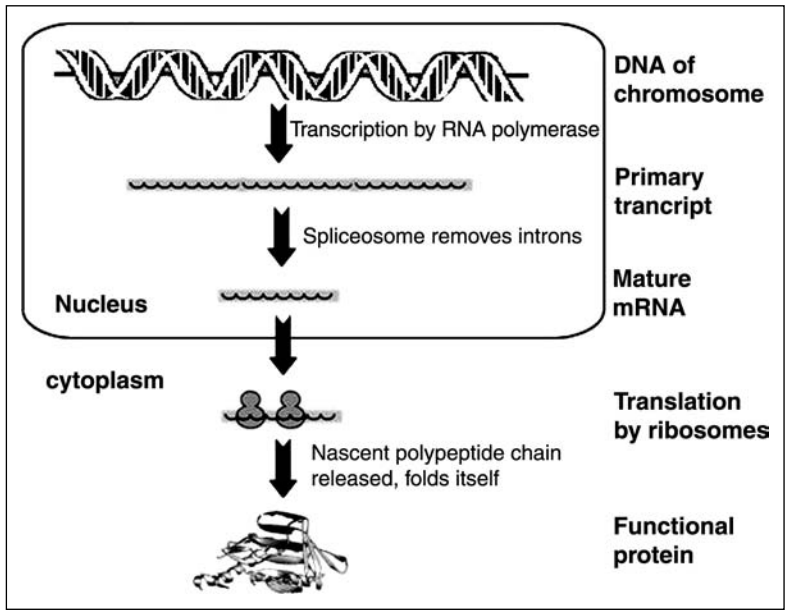

Figure 1.3 Essentials of gene expression.

In slightly more detail (Fig. 1.3):

1. A decision is made to transcribe a particular small segment of the continuous DNA strand.
2. An RNA copy of the whole gene sequence (the primary transcript) is made by the enzyme RNA polymerase.
3. The primary transcript is processed, mainly by cutting out introns (see below) and splicing together exons, to produce the mature mRNA.
4. The mRNA moves out from the nucleus to the cytoplasm, where a complicated machinery comprising ribosomes and transfer RNAs (tRNAs) translates it. In the genetic code, (Fig. 1.4) three consecutive nts form a codon, coding for one amino acid. Since there are 64 possible codons to encode only 20 amino acids, most amino acids are encoded by more than one codon. When the ribosome encounters any one of three stop codons (UGA, UAA, or UAG), it detaches from the mRNA and releases the newly synthesised polypeptide chain.

1st base in codon	2nd base in codon: U	C	A	G	3rd base in codon
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Figure 1.4 The genetic code. This code is almost universal in all living organisms, although the small protein synthesis apparatus within mitochondria uses a slightly modified version.

Transcription

Transcription starts when a large multiprotein complex including RNA polymerase is assembled at a particular point on the DNA. DNA sequences (promoters) that are able to bind the complex mark the genes, which are quite thinly scattered along either strand of the double helix. The DNA-binding proteins of the complex (transcription factors) include some that are universal and others that are present only in specific cells or tissues, or in response to specific signals. Other proteins stabilize or destabilize the complex purely through protein–protein interactions (co-activators and co-repressors). This specific and variable activation or repression of transcription is the major way in which cells establish their identity (muscle cells, neurons, and lymphocytes all contain the same genes, but activate them differentially) and control their activity. Not surprisingly, mutations in the genes encoding transcription factors are a major cause of genetic disease, including hereditary deafness.

The primary transcript is an RNA molecule corresponding precisely to the DNA sequence of the gene. RNA is chemically virtually identical to DNA, with small differences:

- RNA has ribose where DNA has deoxyribose
- RNA has uracil (U) wherever the corresponding DNA has thymine.

These small differences allow the cell to target different enzymes to RNA and DNA so that they perform different functions in the cell. DNA is the central archive of genetic information; RNA molecules have quite a variety of different roles (Table 1.1). RNA is single stranded simply because cells do not contain enzymes to synthesise a complementary strand. Splicing, ribosomes, and transfer RNA are described below.

If the DNA sequence at some point reads AGTC, the RNA transcribed from this strand would read GACU, writing the sequences, as always, in the 5′→3′ direction. This is where the convention mentioned above is brought in to make life simpler. Rather than give the sequence of the DNA strand that was used as a template in transcription, we give the sequence of the opposite strand (the "sense strand"). This is GACT, and so, immediately relates to the RNA sequence. Gene sequences are always written in this way.

Processing the primary transcript: exons and introns

In 1977, a wholly unexpected and baffling feature of our genes was discovered. At that time, the broad outlines of transcription and translation had been identified through work on the bacterium *Escherichia coli*. But in 1977, researchers discovered that in humans and chickens, the coding sequence of a gene was split into several noncontiguous segments (exons) separated by noncoding introns. This exon–intron organisation turned out to be typical of the great majority of genes in all eukaryotes (organisms higher than bacteria). There is no seeming logic in the number or size of introns. Over the whole human genome, the average number is eight, but for different genes, it varies from 0 to over 100. Their size also varies enormously, from a dozen nts up to over 100 kb. Exons also vary in size from a few nucleotides up to several kb, though the distribution is more clustered around 100 to 150 bp. Introns are usually bigger than exons—the average in humans is 1300 bp—and so the majority of most genes consists of noncoding sequence. Table 1.2 shows some typical examples. Arguably, this exon–intron organisation allows novel proteins to evolve by shuffling exons that encode functional modules; but it is fair to say that nobody predicted that our genes would be organised in this way, and it still remains one of the most remarkable aspects of the human genome.

Overall, the average human gene is 27 kb long, has nine exons averaging 145 bp, and the introns average 3365 bp—but as the table shows, the range is very wide. Note that the "size in genome" figure refers to the transcribed sequence (exons + introns) and does not include the promoter or other regulatory sequences.

Within the cell nucleus, the primary transcript is processed by physically cutting out the introns that are degraded and splicing together the exons. This is done by a complex machine, the spliceosome, which consists of numerous proteins plus some small RNA molecules. Spliceosomes recognize introns in the primary transcript through details of the nucleotide sequence. Introns nearly always start with GU and end with AG. In themselves those signals would not be sufficient—most GU or AG dinucleotides do not function as splice sites. Splice sites are recognised when the invariant GU or AG is embedded in a broader consensus sequence.

For many genes—at least 40% of all human genes, probably the majority—primary transcripts can be spliced in more than one way, so that several isoforms are produced. There may also be alternative start points for transcription. Thus, a single gene often encodes more than one protein. Much of this alternative

Table 1.1 A partial list of the types of RNA in a cell

RNA species	Typical size	Role in cell
Messenger RNA (>100,000 types)	500–15,000 nt	Mediators of gene expression
Ribosomal RNA (2 species)	4700, 1900 nt	Structural and functional components of the ribosome
Transfer RNA (ca. 50 species)	Ca. 80 nt	Each ferries a specific amino acid to the ribosome and base pairs with the appropriate codon in the mRNA
Small nuclear and nucleolar RNAs (many types)	Typically ca. 100 nt	Control mRNA splicing and other RNA processing
Micro-RNA (several hundred types)	20–22 nt	A recently discovered class believed to be important regulators of gene expression

Abbreviation: nt, nucleotide.

Table 1.2 Structures of some human genes

Gene	Size in genome (kb)	No. of exons	Average exon size (bp)	Average intron size (bp)	Exons as % of primary transcript
Interferon A6 (IFNA6)	0.57	1	570	–	100
Insulin (INS)	1.4	3	154	483	32
Class 1 HLA (HLA-A)	2.7	7	160	269	41
Collagen VII (COL7A1)	51	118	78	358	18
Phenylalanine hydroxylase (PAH)	78	13	206	6264	3.4
Cystic fibrosis (CFTR)	189	27	227	7022	3.2
Dystrophin (DMD)	2090	79	178	26615	0.7

splicing is functional and may be controlled according to the needs of the cell, though in some cases it may just reflect inefficiencies in the machinery. Figure 1.5 shows a typical human gene, with large introns and several alternative splice isoforms.

Mutations that alter splice signals are a major cause of malfunction of genes. Researchers have found it difficult to predict when a sequence change near an intron–exon boundary will affect splicing, and probably this class of mutations often goes unrecognised when it does not alter the invariable GU…AG signal. The only sure way to identify splicing patterns is to study mRNA extracted from appropriate cells.

To complete processing of the primary transcript, a specific nonstandard nt (the "cap") is added to the 5′ end and a string of around 200 A nts is added to the 3′ end [the poly(A) tail]. The mature mRNA is now ready to move to the cytoplasm to be translated.

Translation

In the cytoplasm, ribosomes engage the 5′ end of mRNA molecules. Ribosomes are huge multimolecular complexes comprising two large RNA molecules (ribosomal RNA) and around 100 proteins. The ribosomes physically slide along the mRNA until they encounter a start signal. This is the codon AUG embedded in a consensus ("Kozak") sequence. At this signal, they start assembling amino acids into a polypeptide chain, moving along the mRNA and incorporating the appropriate amino acid in response to each triplet codon, according to the genetic code (Fig. 1.4). Amino acids are brought to the ribosome by yet another class of small RNA molecule, tRNAs. Ultimately, which amino acid is incorporated in response to which codon depends on which tRNA carries that amino acid, which in turn is determined by the specificity of the enzymes that join amino acids onto tRNA molecules. There is no evident logic in the code. It is a sort of frozen accident; it could perfectly well have been different, but once arrived at, any mutation that changed it in an organism would cause such chaos as to be lethal.

Translation continues until a stop codon (UAG, UAA, or UGA) is encountered, at which point the ribosomes detach from the mRNA and release the newly synthesised polypeptide chain. This may then undergo a whole series of specific enzyme-catalysed modifications—cleaving off parts, attaching sugars or other residues—before being transported to the location inside or outside the cell where it is required.

Note that only part of the mature mRNA carries the code for protein. Although the introns have all been removed within the nucleus, the remaining exonic sequence includes the two untranslated ends of the mRNA—the 5′ untranslated sequence (5′UT) between the cap and the start codon, and the 3′UT between the stop codon and the poly(A) tail. Both these untranslated sequences are important for correct control of translation and mRNA stability, so changes in them can have consequences for gene function. However, we understand very little of the detail; so, it is usually impossible to predict whether a mutation in either of these sequences will be pathogenic.

Overview of the human genome

Our genome (one copy of chromosome 1, one copy of chromosome 2, etc.) comprises about 3.2×10^9 bp of DNA. There are about 24,000 protein-coding genes on current estimates; this figure is provisional because there is no sure-fire way of recognising genes. Genes are scattered quite thinly and apparently randomly along the chromosomes, with no evident reason why a gene is in one place rather than another. Figure 1.6 shows an example. This figure also illustrates one of the main computer programs (genome browsers) used to make sense of the raw human-genome sequence in the public databases.

About 1.5% of our genome codes for protein. So what does the other 98.5% do?

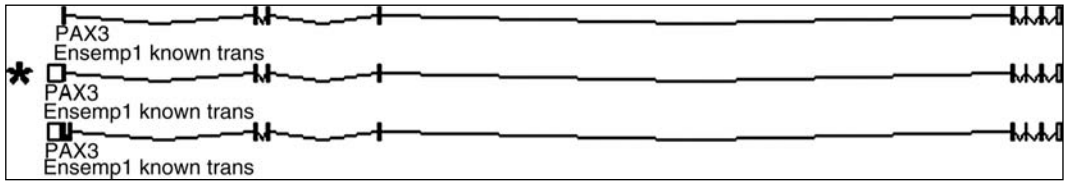

Figure 1.5 A typical human gene. The diagram shows a 100 kb section of chromosome 2 containing the *PAX3* gene, which encodes three isoforms. The horizontal lines represent the primary transcripts. Vertical bars represent exons; the lines linking each set of exons represent the introns. Solid bars are coding sequence; open bars are the 5′ and 3′ untranslated sequences. The transcript marked by an asterisk has eight exons that in total make up 3% of the primary transcript. *Source*: From Ref. 3.

- The transcribed gene sequences, as explained above, include the 5′ and 3′ untranslated sequences and also all the introns. Genes, including their introns, account for about 20% of our genome.
- Gene expression is regulated by nontranscribed sequences. Most obviously, this includes the promoter, which lies immediately upstream of the gene, but other regulatory elements ("enhancers" and "locus control regions") may be situated anything up to 1Mb either side of the transcribed sequence. These poorly understood elements bind activating or repressing proteins, and the DNA may loop round so that physically it lies close to the promoter of the gene it regulates. Alternatively, the regulatory proteins may trigger chemical modification of the histone proteins in chromatin, causing a structural change in the chromatin. Chromatin configuration ("open" vs. "closed") is a key determinant of gene activity. Much current interest attaches to identifying and investigating noncoding sequences that are highly conserved in evolution (i.e., are little changed between humans, mice, and, maybe, other organisms), on the assumption that evolutionary conservation implies an important function. Such conserved noncoding sequences make up around 3% of our genome.
- Exploring our DNA reveals many nonfunctional copies of active genes. These pseudogenes are believed to have arisen through accidental duplication of a gene. Once there are two copies, there is no pressure of natural selection to prevent mutated versions of one copy being transmitted to the offspring.
- As well as the 24,000 protein-coding genes, we have other genes whose product is a functional RNA. These include all the classes of non-mRNA shown in Table 1.1. The computer programs that are used to identify genes in the raw genome sequence are very poor at identifying genes that do not encode proteins; so, we have little idea how many such genes we have. A large fraction of our genome is at least occasionally transcribed, but it is not known how much of this is functional and how much is just mistakes by the transcription machinery. Micro-RNAs (miRNAs), in particular, are a very hot topic in research. Some workers believe miRNAs will turn out to control much of the way our genome functions.
- Some DNA sequences control chromosome structure and function. These include centromeres, telomeres (the ends of chromosomes, which are marked by special structures), and scaffold attachment regions that bind the DNA in large (20–100kb) loops to the central protein core of the chromosome.

Some 50% of our DNA consists of repetitive sequences. That is, the same sequence is present several times in the genome. A small proportion of this represents genes that are present in many copies, particularly the genes that encode the various functional RNA molecules shown in Table 1.1. The rest fall into two categories:

- *Tandem repeats*: The same sequence is repeated a few to several thousand times one after another at a particular location in the DNA. Tandem repeats are important for the structure of centromeres and telomeres; other tandem

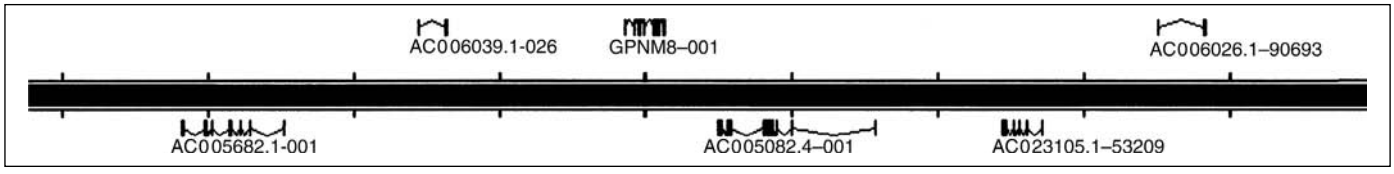

Figure 1.6 Genes in a 0.5 Mb stretch of the short arm of human chromosome 7. The chromosome is shown as a thick black line. Genes are shown as exons (*vertical lines*) linked together to show how they are spliced. Note the small proportion of the total sequence that is occupied by exons. Genes shown above the line are transcribed from left to right, using the upper DNA strand as the sense strand; those below the line use the lower strand and so are transcribed in the opposite direction (remember that the two strands of the double helix are antiparallel). *Source*: From Ref. 3.

repeats are thought to arise from mistakes in DNA replication ("stuttering") and are not functional but are important research tools for gene mapping (microsatellites, see below).

- *Interspersed repeats*: The same sequence is present at many different locations in the genome. The great majority of all repetitive DNA, and about 45% of the entire human genome, is made up of families of repeats that have, or had in the past, the ability to replicate themselves within the genome, almost like viruses. Scientists argue about whether these "transposon-derived repeats" are useless "junk DNA" or whether they have some beneficial function. Studying these repeats reveals much about the evolution of mammalian genomes. We have about 1,200,000 copies of one family, the 280bp Alu sequence, and about 600,000 copies (mostly incomplete) of the 6.5kb LINE1 sequence.

One cannot fail to be struck by the contrast between, on the one hand, our anatomy and physiology, where we constantly encounter marvels of natural engineering, elegant functional adaptation, and beautiful fitness for purpose, and, on the other hand, our genome, which seems disorganised and chaotic. Maybe there is some deep organising principle of genomes that we do not understand, but more probably, it is because natural selection has no interest in a tidy genome, just as long as it works.

Mapping and identifying genes

Two ways of identifying genes

At the start of this chapter, I described the two ways genes are recognised, as functional units of DNA or as determinants of mendelian characters. These two views underlie the two broad strategies for identifying genes.

Genes as functional DNA units are identified by careful study of the genome sequence ("annotating the sequence"). Computer programs scan the sequence for open reading frames—stretches of the DNA that can be read as protein code without hitting a stop codon. Figure 1.7 shows a hypothetical example.

This sort of analysis is fairly straightforward in bacteria, but in higher organisms, the open reading frames are fragmented by introns. Programs must try to identify fragments of coding sequence flanked by plausible splice sites and thinly scattered through much longer regions of noncoding DNA. As mentioned above, even this route is not available for genes that encode functional RNAs rather than proteins. As a result, gene predictions are uncertain and provisional until supported by laboratory identification of the predicted mRNA.

In the laboratory, for technical reasons, it is convenient to study mRNA in the form of synthetic DNA copies [complementary DNA (cDNA)]. Because cDNAs represent only a small fraction of our genome (maybe 2%) but contain all the protein-coding information, much human genome research has focused on cDNAs. Databases compiled by industrial-scale sequencing of small segments of cDNAs (expressed sequence tags) prepared from different tissues are important resources for identifying genes and for seeing which genes are expressed in a given tissue.

Genes as determinants of mendelian characters cannot be picked out in this way. No amount of analysis of the DNA sequence databases, or sequencing of cDNAs, could produce anything labeled "Late-onset hearing loss" or "Pendred syndrome." Genes defined in this way can only be found by studying families where the condition is segregating.

Genetic mapping

The principle of genetic mapping of a mendelian character is to find a chromosomal segment whose segregation in a family or series of families exactly parallels the segregation of the character being investigated. Figure 1.8 shows the principle.

Chromosomal segments are followed through pedigrees by using genetic markers. A genetic marker can be any character that is variable in a population and is inherited in a mendelian fashion. In practice, DNA polymorphisms are invariably used. Two types of common DNA variants are the main tools for current genetic mapping:

- *Single nucleotide polymorphisms (SNPs)*: The history of our species has endowed us with a rather counterintuitive pattern of variability in our DNA. Most nucleotides are the same in all of us, with occasional rare variants, but about 1 nucleotide in every 300 is polymorphic, with two alternatives being reasonably common in populations worldwide. Around 10 million SNPs have been identified. Almost all are in the 98% of our DNA that does not code for protein, and they have no phenotypic effect.
- *Microsatellites*: These are a subgroup of the tandem repetitive DNA in which the repeating unit is a two-, three-, or four-nt sequence. Often, the number of units in the repeated block varies from person to person. For example, everybody might have a run of CACACACA… at a particular location on chromosome 3, but in some people there might be 10 CA units, in others 11, 12, 13, etc.

```
5′ CCTATGGCATGGTCTCGCTAAACATTCCACATCGTGCATAGCGGC 3′
3′ GGATACCGTACCAGAGCGATTTGTAAGGTGTAGCACGTATCGCCG 5′
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Figure 1.7 Looking for an open reading frame. Both strands of the DNA are shown. Any ATG triplet (reading 5′⟶3′ as always) could mark the start of an open reading frame (AUG in a mRNA). But each of the underlined ATGs leads quickly to a stop codon, TGA, TAA, or TAG when the sequence is read 5′⟶3′ in triplets. Only the double-underlined ATG starts an open reading frame, suggesting it might mark the translation start of a gene. A real gene should have an open reading frame of 100 amino acids or more.

Either type of marker can be easily scored by standard laboratory methods (see S&R2 section 17.1.3 for details).

The protocol for mapping a mendelian condition consists, in principle, of the following:

1. The starting point is a large family, or more often a collection of families, in which the condition of interest is segregating. DNA samples must be obtained from all family members, and the diagnoses carefully confirmed by an experienced clinician.
2. All the DNA samples are typed for a genetic marker.
3. The results are checked to see whether segregation of the marker follows segregation of the condition. The test statistic is the lod score, calculated by computer. This is the logarithm of the odds of linkage versus no linkage. A lod score of 3.0 corresponds to the conventional $p < 0.05$ threshold. (See S&R2 section 11.3 for an explanation of lod scores.)
4. Assuming the lod score falls short of 3.0, try another marker and keep trying marker after marker until you find evidence of linkage. In a typical family collection, about 300 microsatellites or 1000 SNPs would be required to test every chromosomal segment.
5. When convincing linkage is found, the chromosomal location of the relevant DNA polymorphism (which can be looked up in public databases) identifies the approximate location of the disease gene. If the marker tracks nearly but not quite always with the disease, other markers from nearby on the chromosome can be used to define the minimal chromosomal segment that tracks completely with the disease. This defines the candidate region that must contain the disease gene.

Positional cloning

Once a candidate region has been defined by genetic mapping, we need to find which gene within that region is mutated to cause the condition. In years past, this endeavour, called positional cloning, was a massive undertaking that often involved years of intensive toil by small armies of postdoctoral scientists. Now that we have the human genome sequence, it is very much easier. We can search the public databases to draw up a list of the genes within the candidate region. Hopefully, the list will be not more than a few dozen. These are then prioritised for investigation based on any available knowledge about their function, domain of expression, etc. A gene causing nonsyndromal hearing loss should be expressed in the inner ear, and ideally it should encode an ion channel, motor protein, or gap junction protein, since these are the commonest genes involved in hearing loss. A gene causing syndromal hearing loss should be expressed during the development of the ear and the other organs involved, and ideally, it should encode a transcription factor.

Given a candidate gene, its sequence is then examined in a panel of unrelated individuals who have the condition being investigated. The correct gene is one that is mutated in those people but not in unaffected controls. The techniques used to do this are the same as those used in genetic testing (see below).

How genes go wrong

The mechanics of mutations

As we have seen, the route from genotype (the DNA sequence) to phenotype (an observable character) is long and complex.

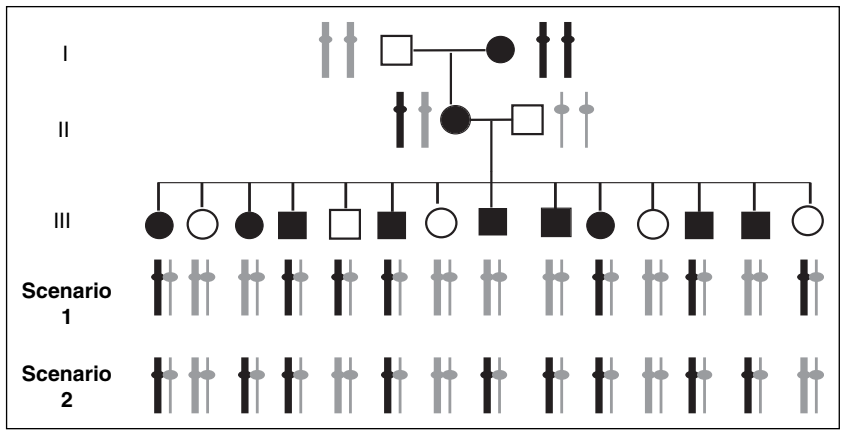

Figure 1.8 The principle of genetic mapping. The diagram shows two possible ways a specific chromosome might segregate in a family in which hearing loss is being transmitted as an autosomal dominant trait. The mother in generation II (II-1) inherited her hearing loss from her father. The chromosome that she inherited from her father is shown in bold. In Scenario 1, there is no relation between whether an offspring in generation III inherits hearing loss and whether they inherit the marked chromosome. This suggests that the gene responsible for the hearing loss is not on that particular chromosome but on one of the other 22 that II-1 received from her father. In Scenario 2, inheritance of the bold chromosome exactly parallels inheritance of hearing loss. If this happens sufficiently often, it would suggest that the hearing-loss gene is carried on that chromosome. This is the principle of linkage analysis. However, in real life, pairs of chromosomes swap segments during each meiosis, so what we have to follow through the pedigree is a chromosomal segment rather than a whole chromosome.

Inevitably, it can go wrong in many different ways. Table 1.3 lists the main things that can go wrong.

Frameshifts are best explained by an example. Consider a string of letters that is to be read as a series of three-letter words:

- The big bad boy hit the cat. . . .

If we add or delete one letter, from then on the whole message is corrupted:

- The bix gba dbo yhi tth eca t
- The bib adb oyh itt hec at. . . .

When the ribosomes translate an mRNA, the reading frame is fixed by the AUG start codon, and there is no further check. So just as in the above examples, it can be thrown out by insertions or deletions. Frameshifts result not only from insertion or from deletion of any number of nucleotides that is not a multiple of three but also from splicing mutations or exon deletions that remove a nonintegral number of codons. Since 5% (3/64) of random codons are stop codons, when ribosomes read an mRNA out of frame, it is usually not long before they encounter a stop codon. Unexpectedly, premature stop codons (whether due to frameshifts or nonsense mutations) usually do not result in production of a truncated protein. Instead, in most cases, the mRNA is broken down and the result is no product. This "nonsense mediated decay" probably functions to protect the cell against deleterious effects of partially functional proteins.

A major distinction is between mutations that totally abolish gene expression or totally wreck the product and those that lead to an abnormal degree of expression or to a recognizable but abnormal product. As indicated in the table, this is not always easy to predict just by looking at the sequence change and may need to be checked experimentally. Many missense mutations have no effect on the function of the gene product, but this is virtually impossible to predict—as genetic diagnostic laboratories have learned to their cost.

Table 1.3 How genes go wrong

Type of change	Likely effect	Whether function should be totally abolished
Delete all of the gene	Total absence of product	Yes
Delete one or more exons	Variable	Generally yes, but missing exon(s) may not be necessary for gene function or may be used in only one splice isoform
Mutation in promoter	May change level of expression of the gene	Generally no, but hard to predict
Missense mutation in coding sequence	Replace one amino acid with another	Not unless that amino acid is vital to the function
Synonymous change in coding sequence	Replaces one codon for an amino acid with another for the same amino acid	Usually no effect, but sometimes the change may affect splicing
Nonsense mutation	Mutate an amino acid codon to a stop codon	Usually yes
Frameshift mutation	Insert or delete 1, 2, or any number of nts that is not a multiple of 3, so as to change the reading frame	Usually yes
Change invariant GT … AG splice signal	Exon may be skipped, or intronic material retained in the mature mRNA	Usually yes, but it may just alter balance of splice isoforms
Mutations in 5′UT or 3′UT	Might affect stability of mRNA	Unlikely, but hard to predict
Mutations in introns	None, unless they affect splicing	Usually no, but effects on splicing are hard to predict

Abbreviations: bp, base pair; nt, nucleotide.
Source: From Ref. 3.

The effects of mutations

In attempting to think through the likely effect of a mutation, the first question to ask is whether it causes a loss of function or a gain of function.

- *Loss of function* results from complete gene deletions, most frameshift, nonsense, and splice site mutations, and from some missense mutations. All mutations that cause complete loss of function of a gene would be expected to have the same phenotypic effect. What this effect is depends on how vital the function is and the other allele. Assuming the other allele functions normally, cells of a heterozygous person have 50% of the normal amount of the gene product. For many genes, this is sufficient for normal function; the person is normal and the condition is recessive. The common frameshifting mutation in *connexin 26*, 35delG, is an example. In some cases 50% is not sufficient ("haploinsufficiency"), a heterozygote will be affected and the condition is dominant. Loss of function mutations in the *PAX3* gene causing Type 1 Waardenburg syndrome are an example of haploinsufficiency. Mutations causing a partial loss of function might be expected to have similar but milder effects, though much depends on the details.
- *Gain of function* does not usually mean gain of an entirely novel function—this happens in tumours when chromosomal rearrangements may combine exons of two genes, but it is almost unknown among inherited mutations. Rather, it means a gene being expressed inappropriately—at the wrong level, in the wrong cell, in response to the wrong signal, etc. Alternatively, it can mean that the product of the mutated gene is toxic or interferes with the working of the cell. For example, some missense mutations in connexin 26 cause dominant hearing loss because the abnormal protein causes gap junctions between cells to behave abnormally. This is called a dominant negative effect. Gain-of-function mutations are likely to produce dominant effects because the gain of function is present even in a heterozygous person. Since the effect depends on the presence of the gene product, these are normally missense mutations.

Genotype–phenotype correlations are the Holy Grail of clinical molecular genetics. We would like to be able to see a change in the DNA sequence and predict what effect that would have on the person carrying it. Very seldom is that possible. Although we know many genes that, when mutated, can cause hearing loss, it is unrealistic to expect that a given mutation will always cause a specific degree of loss, a specific audiogram configuration, or a specific age of onset in every mutation carrier. Even mendelian conditions do not really depend on just a single gene, and the innumerable genetic and environmental differences between people are likely to have some effect on the phenotype. Thus, although it is always sensible to look for genotype–phenotype correlations, we should not hold exaggerated hopes of what we might find.

Genetic testing

The central problem in genetic testing is to see the one particular piece of DNA of interest against a background of the 6×10^9 bp of irrelevant DNA in every cell. There are two general solutions to this:

- Selectively amplify the sequence of interest to such an extent that the sample consists largely of copies of that sequence.
- Pick out the sequence of interest by hybridising it to a matching sequence that is labeled, e.g., with a fluorescent dye.

In the past, selective amplification was achieved by cloning the sequence into a bacterium, but nowadays the polymerase chain reaction (PCR) is universally used. For details of this technique see S&R2 section 6.1; for present purposes, it suffices to know that PCR allows the investigator to amplify any chosen sequence of up to a few kilobases to any desired degree in a few hours. All that is necessary is to know a few details of the actual nucleotide sequence that is to be amplified and to order some specific reagents (PCR primers) from one of the firms that custom-produce these. Almost all genetic testing involves PCR, although some companies make kits based on alternative methods, mainly to avoid the royalty payments required of users of the patented PCR process. The big limitation of PCR is that it can only be used to amplify sequences of, at most, a few kilobases. It is not possible to PCR-amplify a whole gene (average size 27 kb), still less a whole chromosome (average size 100 Mb).

Hybridisation depends on the fact that the two strands of the DNA double helix can be separated ("denatured") by brief boiling, and when the resulting single-stranded DNA solution is cooled, each Watson strand will try to find a matching Crick strand. If a dye-labeled single strand corresponding to the sequence of interest (a "probe") is added, some of the test DNA will stick to the probe and can be isolated, followed, or characterised by using the label. Hybridisation was important in the now largely obsolete technique of Southern blotting, and it has regained importance as the principle behind microarrays ("gene chips").

Various applications of PCR and/or hybridisation make it relatively straightforward to check any predetermined short stretch of a person's DNA—but the key word is "short." In general, each exon of a gene must be the subject of a separate test, and when DNA is sequenced, a maximum of around 500 to 700 bp can be sequenced in a single test. Details of how these methods work are given in S&R2 sections 6.3 and 17.1, but the key point to appreciate is that our ability to answer questions about a person's DNA depends crucially on the precision with which the question is posed.

Consider three possible questions:

1. Does this patient have any genetic cause for her hearing loss?

2. Does this patient have any mutation in her *connexin 26* genes that could explain her hearing loss?
3. Does this patient have the 35delG mutation in her *connexin 26* genes?

Question 1 is unanswerable in any diagnostic setting—it might well be too challenging even for a PhD project. Question 3, on the other hand, can be answered cheaply and in an afternoon. Question 2 lies somewhere in between. To answer it, it would be necessary to examine the entire gene. For *connexin 26*, this is fairly simple because it is a small gene with only two exons. The same question in Type 1 Usher syndrome is a very different proposition. Several different genes can cause Type 1 Usher syndrome, and they are large—*MYO7A* for example has 50 exons. Most diagnostic laboratories would not be willing to devote so much effort to a single case, and even if they were willing, the cost would be high. Gene chips and/or developments in laboratory automation may, in the near future, make such problems much more tractable—but it remains true that the key to successful genetic testing is to pose a precise question.

DNA technology is developing very fast. Sequencing and genotyping become cheaper every year and new technologies allow both to be done on scales that were unthinkable a few years ago. Some companies claim to be developing methods that would allow a person's entire genome to be sequenced in a few days for a few thousand dollars. Optimists and pessimists alike dream of the day when everybody's complete genome sequence will be stored in vast databases; they differ only in their reaction to this prospect. Among all this heady talk, it is important to remember that DNA analysis can reveal only those things about us that are genetically determined.

References

1. Strachan T, Read AP. Human Molecular Genetics. 2nd ed. Oxford: Bios Scientific Publishers, 1999 on the NCBI Bookshelf: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db = Books (accessed 31st May 2005).
2. Mitomap www.mitomap.org (accessed 31st May 2005).
3. Ensembl genome browser www.ensembl.org/Homo_sapiens/ (accessed 23rd February 2005).

Glossary

Allele: One or several possible forms of a particular gene, which may or may not be pathological.

Autosome: Any chromosome other than the X or Y sex chromosomes.

Autosomal Dominant: The pedigree pattern seen when an allele at an autosomal locus causes a dominant character.

Autosomal Recessive: The pedigree pattern seen when an allele at an autosomal locus causes a recessive character.

Base: The heterocyclic rings of atoms that form part of nucleotides. Chemically, adenine and guanine are purines, cytosine, thymine, and uracil are pyrimidines.

Base Pair: The A-T and G-C pairs in the DNA double helix. They are held together by hydrogen bonds.

Carrier: An unaffected person with one pathogenic and one normal allele at a locus. Best restricted to heterozygotes for recessive conditions, but the word is sometimes applied to unaffected people with a gene for an incompletely penetrant or late-onset dominant condition.

cDNA: A DNA copy of a mRNA, made in the laboratory. DNA is more stable than RNA and can be cloned, sequenced, and manipulated in ways that RNA cannot.

Chromatin: A nonspecific term for the DNA-protein complex in which the DNA of eukaryotic cells is packaged. Heterochromatin is a highly condensed genetically inert form of chromatin, characteristic of the centromeres of chromosomes; the alternative is euchromatin.

Coactivator: A protein that helps to assemble the various protein components needed to initiate transcription by binding to several components of the complex.

Corepressor: A protein that works in the same way as a co-activator, but to opposite effect.

Codon: The trio of nucleotides in a gene or mRNA that encodes one amino acid. Codons in the mRNA base pair with anticodons in the tRNA.

Consanguineous: Parents are consanguineous if they are blood relatives. Since ultimately everybody is related, a practical working definition is that the parents are second cousins or closer relatives. Second cousins are children of first cousins; first cousins are children of sibs.

Dominant: A character that is manifest in a heterozygote.

Dominant Negative Effect: An inhibitory effect, seen in a heterozygote when a mutant protein prevents the normal version from functioning by sequestering it in nonfunctional dimers or multimers.

Eukaryote: Any organism higher than bacteria. Characterised by cells with a membrane-bound nucleus, internal organelles, DNA in the form of chromatin and genes with introns.

Exon: The parts of the DNA or primary transcript of a gene that are retained in the mature mRNA.

Expressed Sequence Tag (EST): A partial sequence, typically around 300 nt, of a cDNA—incomplete but sufficient to recognise it uniquely.

Genetic Marker: Any character used to follow a segment of a chromosome through a pedigree. SNPs and microsatellites are the genetic markers of choice.

Genotype: The genetic constitution of a person. One can talk of the genotype at a single locus, or the overall genotype. Cf. Phenotype.

Germinal Mosaicism: Mosaicism affecting the gonads, so that a person can produce sperm or eggs representing each genotype present in the mosaic.

Haploinsufficiency: The situation where a 50% level of function of a locus is not sufficient to produce a fully normal phenotype. It causes loss-of-function mutations to produce dominant conditions.

Haplotype: A series of alleles at linked loci on the same physical chromosome.

Heterozygous: Having two different alleles at a locus.

Homozygous: Having two identical alleles at a locus.

Hybridisation: The process where two single strands of DNA or RNA that have complementary sequences stick together to form a double helix.

Intron: The parts of a primary transcript of a gene that are removed and degraded during splicing. It is sometimes called intervening sequences (IVS).

Isoforms: Different forms of the protein product or mature mRNA of a single gene produced by alternative splicing of exons or the use of alternative start sites—a normal feature of gene expression.

Locus: The position that a gene occupies on a chromosome. Since people have a pair of each autosome, a person has two alleles (identical or different) at each autosomal locus.

Locus Heterogeneity: Locus heterogeneity is seen when indistinguishable mendelian disorders can be caused by mutations at more than one locus. This is a common finding in genetics, e.g., Usher syndrome Type 1 can be caused by mutations at loci on the long arm of chromosome 14 (14q31), the long arm of chromosome 11 (11q13) or the short arm of chromosome 11 (11p13).

Lod Score: The statistical outcome of linkage analysis. It is the logarithm of the odds of linkage versus no linkage. A lod score above +3 gives significant evidence for linkage, and a score below −2 gives significant evidence against linkage.

Lyonisation: An alternative name for X inactivation, a phenomenon discovered by Mary Lyon.

Marker: See Genetic marker.

Meiosis: The specialised cell division that produces sperm and eggs. It consists of two successive cell divisions that ensure each gamete contains 23 chromosomes with a novel combination of genes.

Mendelian: A character or pedigree pattern that follows Mendel's laws because it is determined at a single chromosomal location. Characters determined by combinations of many genes are called multifactorial, complex, or nonmendelian.

Microsatellite: A small run of tandem repeats of a very simple DNA sequence, usually 1 to 4 bp, for example $(CA)_n$.

Microarray: A postage-stamp size wafer of silicon or glass carrying a large arrayed set of single-stranded oligonucleotides corresponding to parts of the sequence of one or more genes. When fluorescently labelled PCR-amplified genomic DNA or cDNA is hybridised to the array, the pattern of hybridisation can be used to read off the sequence, to check which genes are expressed in a tissue, or to genotype a sample for a large number of SNPs in parallel.

Mitosis: The normal process of cell division by which each daughter cell receives an exact and complete copy of all the DNAA in the mother cell.

Mosaic: An individual who has two or more genetically different cell lines derived from a single zygote (because of a fresh mutation or chromosomal mishap).

Nonpenetrance: It describes the situation when a person carrying a gene for a dominant character does not manifest the character. This is because of the effects of other genes or of environmental factors.

Nucleotide: The units out of which DNA and RNA chains are constructed. It consists of a base linked to a sugar (deoxyribose in DNA, ribose in RNA) linked to a phosphate group. A nucleoside is the same but without the phosphate.

Obligate Carrier: A person who is necessarily a carrier by virtue of the pedigree structure. For autosomal recessive conditions, this normally means the parents of an affected person, for X-linked recessive conditions, a woman who has affected or carrier offspring and also affected brothers or maternal uncles. A woman who has only affected offspring is not an obligate carrier of an X-linked condition, because new mutations are frequent in X-linked (but not autosomal recessive) pedigrees.

Open Reading Frame: A stretch of genomic DNA that could be translated into protein without encountering a stop codon.

Penetrance: The probability that a phenotype will be seen with a given genotype.

Phenocopy: An individual who has the same phenotype as a genetic condition under study, but for a nongenetic reason, e.g., somebody with nongenetic deafness in a family where genetic deafness is segregating. Phenocopies can be a major problem in genetic mapping.

Phenotype: The observed characteristics of a person (including the result of clinical examination). Compare with Genotype.

Poly(A) Tail: The string of around 200 consecutive A nucleotides that is added on to the 3′ end of most mRNAs. It is important for the stability of mRNA.

Polymerase Chain Reaction (PCR): A method for selectively copying a defined short (no more than a few kilobases) segment of a large or complex DNA molecule. The basis of most genetic testing.

Primary Transcript: The initial result of transcribing a gene: an RNA molecule corresponding to the complete gene sequence, introns as well as exons.

Probe: A labelled piece of DNA that is used in a hybridisation assay to identify complementary fragments. Depending on the application, probes may be pieces of cloned natural DNA around 1 kb long, or much shorter (20–30 nt) pieces of synthetic DNA.

Promoter: The DNA sequence immediately upstream of a gene that binds RNA polymerase and transcription factors, so that the gene can be transcribed.

Pseudogene: A nonfunctional copy of a working gene. Pseudogenes are quite common in our genome and represent the failed results of abortive evolutionary experiments.

Recessive: A character that is manifest only in the homozygous state and not in heterozygotes.

Sibs (Siblings): Brothers and sisters, regardless of sex. A sibship is a set of sibs.

SNP (Single Nucleotide Polymorphism): The main class of genetic marker used for very high-throughput genotyping. About 1 nucleotide in every 300 is polymorphic. Most SNPs have no phenotypic effect, but some may contribute to susceptibility to common complex diseases.

Southern Blotting: A method of studying DNA based on separating fragments by size and hybridising them to a labelled probe. It is largely superseded by PCR, which entails much less work but still used for some special applications. It is named after its inventor; Northern blotting and Western blotting are similar techniques used on RNA and proteins, respectively—the names are jokes.

Transcription Factor: A protein that binds the promoters of genes so as to activate transcription. Basal transcription factors are involved in transcription of all genes; tissue-specific transcription factors cause different cells to express different subsets of their genes.

X–Inactivation: The mysterious process by which every human cell has only a single working X chromosome, regardless of how many X chromosomes are present.

X-Linked Inheritance: X-linked inheritance is seen when a condition is caused by an allele located on the X chromosome.

2 Understanding the phenotype: basic concepts in audiology

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Introduction

Knowledge in audiology, as in many other medical fields, advances discontinuously, paralleling developments in technology applied to scientific research. After the eras of psychoacoustics, tympanic measurements, electrophysiological responses, and otoacoustic emissions, it is apparent today that molecular biochemistry will play an important role in the exploration of auditory function. From a clinical point of view, it will transform the classification of hearing impairment and the possibilities for new therapeutic approaches.

Studies in molecular genetics are accumulating an impressive quantity of knowledge on the aetiopathology of hearing loss, as the mapping and cloning of genes reveal their functions in the inner ear, its structural organisation, and its homeostasis. Currently, several hundred chromosomal loci have been identified and associated with syndromal and nonsyndromal hearing impairments. This number has been estimated to represent about half of the genetic changes resulting in hearing impairments. Thus, genetic factors have to be considered in diagnostic audiology much more frequently than in the past. At present, clinical audiology has to meet two requirements. First, there is the need for deeper knowledge of the pathophysiological changes that gene mutations induce in the auditory system; second, there is a need for new audiological diagnostic tools sensitive enough to elucidate these changes. This could help to better define the phenotype and narrow, to within a reasonable range, the set of genetic investigations necessary.

Pure-tone hearing-threshold measurements

The principal audiometric test entails measuring the auditory thresholds for pure tones. Results indicate the minimum sound pressure levels (dB SPL) that evoke the minimal auditory sensation within the frequency range between 125 and 8000 Hz. International standards define the SPL threshold values for normal hearing, and, after normalisation, relate them to 0 dB hearing loss (HL). Threshold increments up to 25 dB HL, although irrelevant for medicolegal purposes, may be valuable for diagnostic purposes. Two separate measures of the hearing threshold, respectively air-conducted (through an earphone or an insert) or bone-conducted (a vibrator on the forehead or the mastoid process) stimuli, permit the distinction between two main kinds of hearing losses: conductive and sensorineural. The first show a normal bone-conducted and an elevated air-conducted hearing threshold. The second show equal values of the two thresholds. There are also mixed hearing losses, which have elements of both conductive and sensorineural losses. When a marked difference exists between the hearing thresholds of the two ears, noise masking is needed for the better ear, in order to ensure that a sensation evoked in the better ear does not interfere with the sensation elicited in the worse ear.

A diagnosis of conductive hearing loss made by pure-tone audiometry indicates a dysfunction of the external or middle ear, but its origin cannot be pinpointed without otoscopic examination and admittance measurement. A diagnosis of sensorineural hearing loss indicates dysfunction in either the cochlea or the auditory pathway: other investigations are needed to confirm the site of the lesions. Clinical pure-tone audiometry, such as the psychoacoustical tests described below, is based on a stimulus-response behavioural model, which requires active cooperation and attentive attitude by the subject being tested. Simulators, individuals with low levels of vigilance and reduced attention may give unreliable results, i.e., a hearing threshold poorer than the actual threshold or one excessively variable at retest. Three- to five-year-old children can reliably perform pure-tone audiometry: Younger children can be examined by special conditioning procedures.

Table 2.1 Relevant terms and definitions

Hearing threshold level

It means the threshold value averaged over frequencies 0.5, 1, 2, and 4 kHz in the better ear

Hearing threshold levels (0.5–4 kHz)	Frequency ranges
Mild: over 20 and <40	Low: up to and equal to 500 Hz
Moderate: over 40 and <70 dB	Mid: over 500 up to and equal to 2000 Hz
Severe: over 70 and <95 dB	High: over 2000 up to and equal to 8000 Hz
Profound: equal to and over 95 dB	Extended high: over 8000 Hz

Types of hearing impairment

Unilateral: one ear has either >20 dB pure-tone average or one frequency exceeding 50 dB, with the other ear better than or equal to 20 dB

Asymmetrical: >10 dB difference between the ears in at least two frequencies, with the pure-tone average in the better ear worse than 20 dB

Progressive: a deterioration of >15 dB in the pure-tone average within a 10-year period. Results in those aged over 50 years should be treated with some caution. In all cases the time-scale and patient age should be specified

Conductive: related to disease or deformity of the outer/middle ears. Audiometrically, there are normal bone-conduction thresholds (<20 dB) and an air-bone gap >15 dB averaged over 0.5-1-2 kHz

Mixed: related to combined involvement of the outer/middle ears and inner ear/cochlear nerve. Audiometrically >20 dB HL in the bone-conduction threshold together with >15 dB air-bone gap averaged over 0.5-1-2 kHz

Sensorineural: related to disease/deformity of the inner ear/cochlear nerve with an air-bone gap <15 dB averaged over 0.5–1-2 kHz

Sensory: a subdivision of sensorineural related to disease or deformity in the cochlea

Neural: a subdivision of sensorineural related to a disease or deformity in the cochlear nerve

A relative contraindication to pure-tone audiometry may be the presence of occluding wax in the ear canal, since this may be responsible for a conductive loss of 20 to 30 dB HL. By examining patients suspected of having noise-induced hearing loss, an unexposed interval of 16 hours is needed to avoid false results due to "temporary threshold shift" phenomena.

Commonly, the hearing threshold is measured at frequencies separated by octave intervals, from 0.125 to 8 kHz. The addition of intermediate frequencies (1.5, 3, 6, 10, and 12 kHz) may improve the overall threshold estimate. Indeed, as the threshold values of contiguous frequencies are correlated, the more the frequencies recorded, the less the probability of the errors associated with a single-frequency threshold measurement. The measurement error for air-conduction testing is usually estimated within ± 5 dB, and it is about twice that figure for bone-conduction testing. These errors mainly originate from the transducers' incorrect positioning as well as subject-related factors.

The accuracy of the pure-tone hearing threshold is crucial in defining any progression of the hearing impairment (1). Some genetic hearing impairments show this characteristic. Hence, the first pure-tone threshold has to be measured with high precision, since it will then be the reference for successive threshold comparisons.

Table 2.1 gives relevant terms and definitions, derived from Stephens (2), on the basis of recommendations of the HEAR European project.

Relationship between pure-tone hearing thresholds and auditory damage

External and middle ear

A variety of genetic syndromes can affect the anatomy of these structures. By altering the sound transmission to the cochlea, they present as a conductive hearing impairment. Such anomalies range from simple stenosis of the external meatus to total lack of the tympano-ossicular complex, with intermediate conditions including an atretic external canal, an absence of the tympanic bone, and a lack or fusion of the ossicles, stapes

fixation, and atretic Eustachian tube. [See Van de Heyning (3) for an otosurgical classification.] Even in young children, the consequences of these anomalies can be measured by means of auditory-evoked potentials presented by air and bone conduction. Two extreme pathological pictures may be taken as a reference to predict the pure-tone threshold: (*i*) Simple atresia of the external meatus causes a 30 to 35 dB conductive hearing impairment due to the attenuation of the sounds directed to the tympanic membrane. (*ii*) A complete lack of the tympanic function causes a 60 to 70 dB conductive hearing loss, essentially due to the attenuation of the acoustical energy directed to the cochlea. Between these two extremes, the hearing loss may vary in respect to the anatomical structures involved and their consequence on auditory function (Fig. 2.1).

Inner ear

Inner ear lesions resulting in a sensorineural hearing loss show a moderate relationship with the pure-tone threshold. An elevated threshold at high frequencies indicates damage to the basal portion of the cochlea. An elevated threshold for low frequencies suggests damage of the apical portion. Schuknecht's (4) studies on the comparison of audiograms to cochlear histology ("cochleograms") corroborates such a relationship. A further distinction involves the degree of hearing loss. Based on the role of outer and inner hair cells, we can assume that a total loss of outer cells causes a hearing impairment of 55 and 65 dB for low- and high-frequency ranges, respectively. A complete loss of inner hair cells should cause a profound hearing impairment (95 dB HL to total hearing loss). In practice, the lesions usually involve both the outer and the inner hair cells, with the proportions depending on the causative factor. Apart from these observations, other conditions have to be considered, in which the audiogram–histology relationship may break down.

One of these is that the cochlea may appear anatomically normal in its microscopic structure, but the biochemical–metabolic processes responsible for its function and homeostasis are altered. In addition, there are several other cochlear sites of damage than those examined in the traditional cochlear histological studies, locations that molecular genetics has demonstrated. These include the gap-junction system, the ionic-transport channels, the synaptic organisation, as well as some components of extracellular matrix (5,6). Such alterations may affect auditory function in different ways, independently of the anatomical loss of hair cells. A second exception to the audiogram–cochlear damage correspondence is represented by the possible existence of cochlear dead regions. A dead region is a section of the cochlear partition where inner hair cells are totally lacking. This condition is not reflected in the audiogram, since frequencies that should be processed by the dead zone are made audible by contiguous zones when the stimulus intensity is high enough to generate a mechanical pattern spreading towards them (off-frequency listening). Finally, another limitation of pure-tone measurements is that the typical "auditory residue" observable at low frequencies in profound hearing loss is difficult to attribute unquestionably to an auditory rather than a tactile sensation (Fig. 2.2) (7).

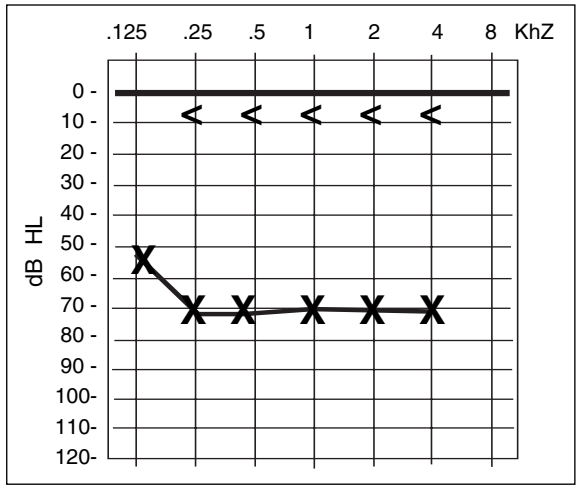

Figure 2.1 An example of conductive hearing loss (normal bone-conduction threshold) due to a severe malformation of external-middle ear.

Cochlear nerve

Pure-tone thresholds are relatively resistant to lesions involving the cochlear nerve. Schuknecht et al. (8) demonstrated in animal studies that only a lesion involving over 75% of the nerve fibres causes effects evident in the pure-tone threshold. In humans, the vestibular Schwannomas represent the most common clinical cause of cochlear nerve lesions. Among patients with this pathology, about 80% show a hearing impairment, although the amount of hearing loss correlates only poorly with the tumour size. For those cases with a high-frequency hearing loss, a mechanism was suggested in which the most external nerve fibres coming from the basal cochlea would be the most vulnerable to compression by the tumour. There are, however, many exceptions to this picture. In fact, the hearing may be variously compromised in relation to the complex effects resulting from tumour growth: for instance, demyelinisation (9), neural ischaemia, indirect cochlear damage due to a reduced blood supply from the compressed labyrinthine artery, and retrograde degeneration (10).

Automated procedures for self-recording hearing thresholds

Modern audiometers often incorporate automated modalities to record the hearing threshold. The most popular, based on an adaptive procedure, is known as Békésy audiometry. This technique requires the subject to control the stimulus intensity according to his responses. Pure tones are either continuous or interrupted (2.5/sec) at continuously changing frequencies or at the discrete frequencies of the classical audiometry, whereas intensity changes in steps of 2.5 or 5 dB.

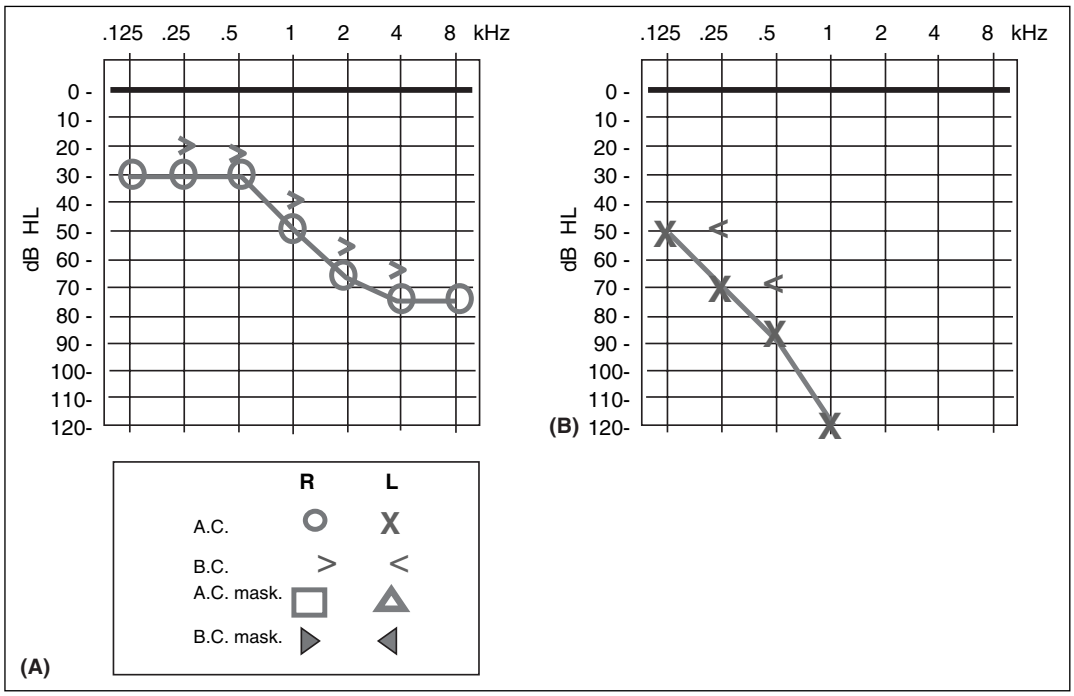

Figure 2.2 (A) An example of sensorineural hearing loss (bone-conduction equal to air-conduction threshold) due to an inner ear disorder. This common threshold configuration may approximately suggest the type and distribution of the lesion within the cochlea: at the basal end (high frequencies), probable involvement of both outer and inner hair cells; at the apex (low frequencies), probable involvement of half of the population of outer hair cells. (B) This threshold profile is common in profound hearing loss and could indicate residual function in hair cells at the apex. However, the thresholds recorded in response to low frequency and very intense tones could be due to vibrotactile perception.

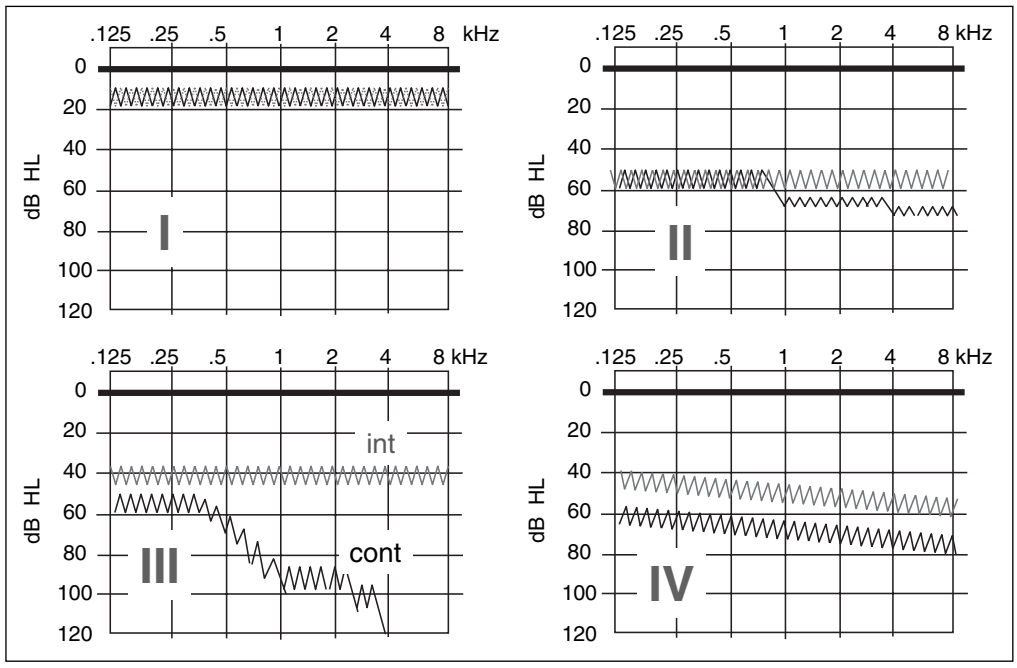

Figure 2.3 Classification of Békésy audiometry tracings. Type I (overlapping of thresholds for continuous and interrupted stimuli) is observable in normal hearing and conductive hearing loss. Type II (small excursions with a continuous stimulus) is observable in cochlear lesions. Type III (progressively diverging thresholds) can be found in eighth nerve disorders. Type IV can be observed in cochlear and retrocochlear lesions. Type V, not shown here, display a threshold change in interrupted tones worse than that for continuous tones; it is associated with nonorganic hearing loss.