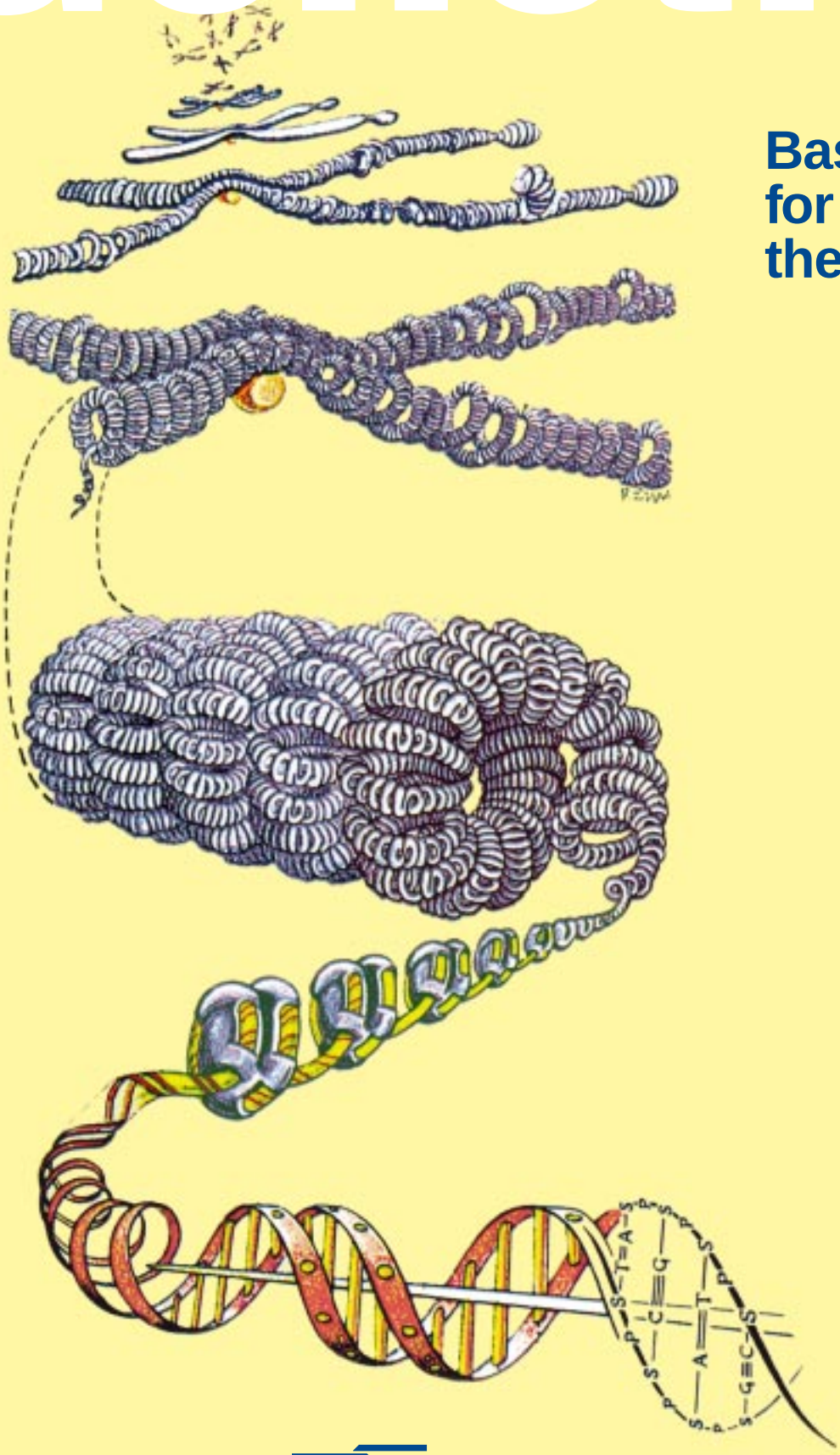
Genetics



Basis for medicine in the 21st century

Münchener Rück
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Genetics

Basis for medicine in the 21st century

An introduction to genes, diseases and genetic tests

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Contents

Introduction

Genome research in recent years has opened up new opportunities for diagnosis and has generated therapeutic progress which in its turn has created a new basis of knowledge in many areas of medicine. This development is already being commonly referred to today as the advent of the molecular age in medicine. Not only medical journals but also the mass media are treating human genetics and the opportunities it presents as a high-profile issue, with great attention being paid to the complex of topics of life insurance and genetic testing. Genome analysis, and genetic testing in particular, have political and social implications, although it could be said that public fears regarding the potential of genome research at the present time is vastly inflated.

Munich Re has been following these developments for some time now and has written “Genetics – Basis for medicine in the 21st century” in response to the growing demand for information on this field of medicine. In producing this publication, we have been assisted by a renowned human geneticist. In addition to supplying basic knowledge on genetics, it provides a resumé of the possibilities for diagnosis offered by genetic testing at the present time and undertakes a critical examination of their significance. It also takes a brief look at some of the future developments that are already emerging today.

“Genetics – Basis for medicine in the 21st century” is intended not only for underwriters and insurance medical officers but for all staff of companies in the insurance industry with an interest in medicine. Its structure and the glossary at the end will also make it useful as a reference work.

1 Basic principles of genetics

1.1 Chromosomes

The entire genetic information of an individual is contained in the chromosomes. The nucleus of every cell in the human body contains 46 chromosomes, which can be said to represent the library of hereditary information. These 46 chromosomes consist of 23 pairs, with each set of chromosomes deriving from one parent (mother/father). Chromosomes 1–22 are called **autosomes** and the 23rd chromosome pair is called the sex chromosome (**gonosome**), since it determines the human sexual characteristics. In women this chromosome pair consists of two X chromosomes whereas men have one X chromosome and one Y chromosome (see Fig. 1).

Apart from the cell nucleus, the mitochondria in the cytoplasm of the cell also possess an albeit negligibly small amount of hereditary information (< 0.003%).

Human chromosomes
(Karyotype)

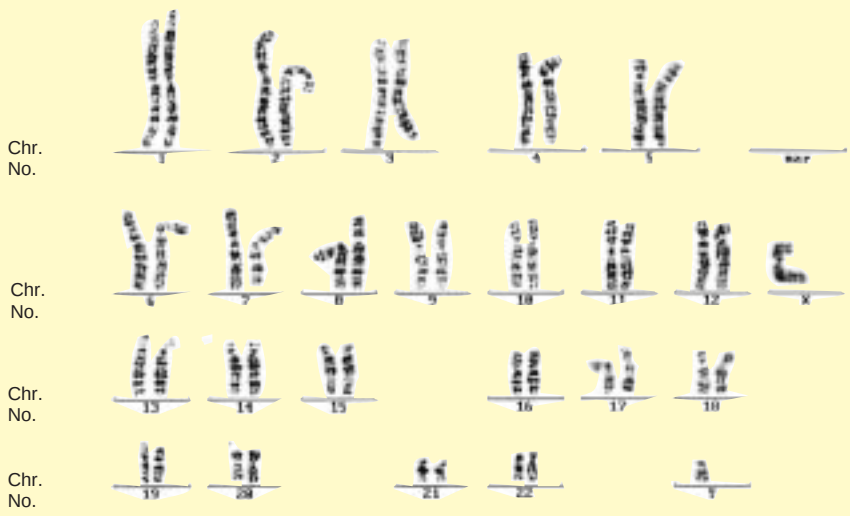


Figure 1

1.2 Genes, DNA

A single human chromosome is composed of 50 to 250 million building blocks, made of **deoxyribonucleic acid (DNA)**. The DNA consists of two long strands, which are twisted together in a double helix like a spiral staircase. The hand rail or the banister of the staircase is formed by a framework of sugar and phosphate molecules, while the individual steps are each formed by one base pair (see Fig. 2). Only four different building blocks (**nucleotide bases** or **nucleotides**) are involved in the formation of these steps: adenine (A), guanine (G), cytosine (C) and thymine (T). Only the nucleotide bases A and T on the one hand and G and C on the other hand fit together. The bases are complementary – they fit together like a key in a lock (i.e. as base “pairs”). This allows the DNA to reproduce itself with the help of certain enzymes: it splits into two single strands with each strand acting as the template for the reassembly of the missing strand (see Fig. 3).

The sequence of the bases within the DNA molecule (**DNA sequence**) contains the entire genetic information or “code” used by a cell in order to perform its functions. So nature utilizes a code with only four different “letters” – A, G, C and T – in order to determine such different traits as hair colour, blood type, etc.

The sequence of nucleotides (of which the base is the key coding component) used by a cell to manufacture an individual protein is called a **gene**. In the entire human genome it is thought that there are 70,000 – 100,000 different genes, which thus have the instructions for building every cell, organ and tissue of an individual. This information is contained in about 5% of the total DNA. This leaves 95% of DNA whose function is still unclear. It is believed, however, that these “extra” sections of DNA may govern how genes interact and are controlled (see also 1.7).

The DNA double helix

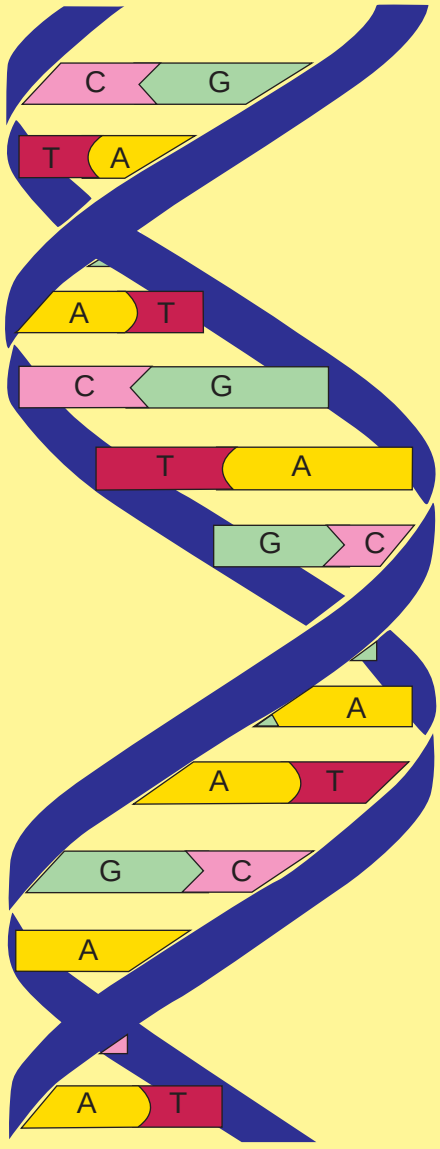


Figure 2

Duplication of DNA (replication)

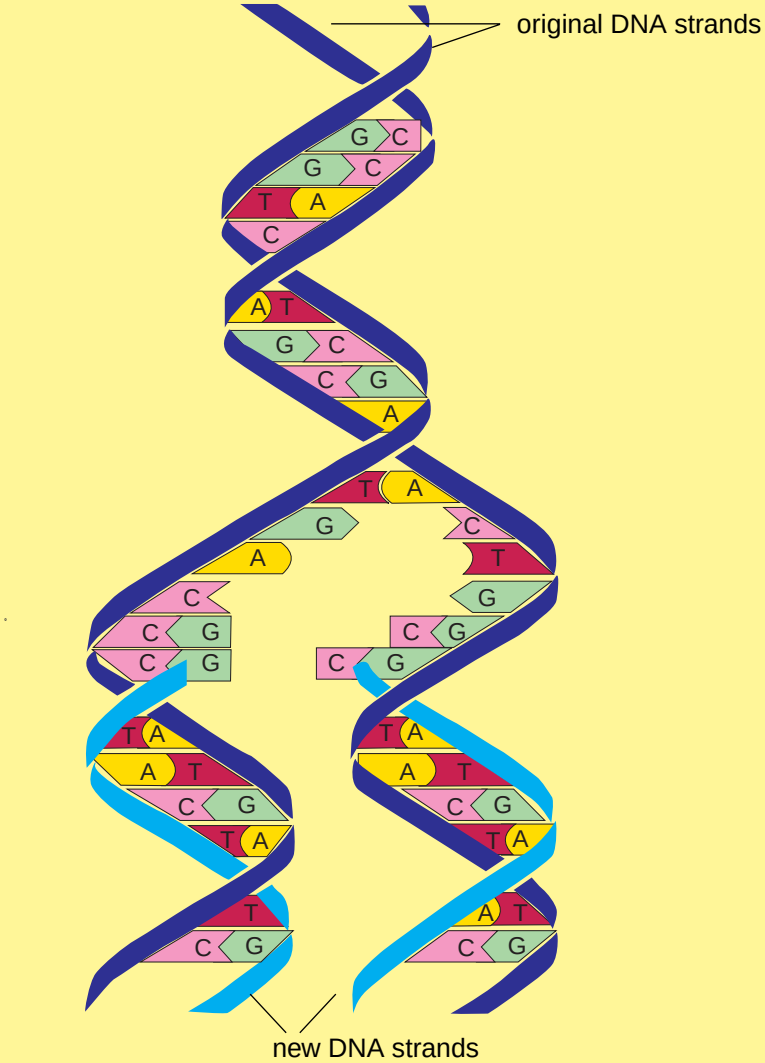


Figure 3

1.3 Messenger ribonucleic acid

For a certain cell (e.g. heart muscle cell, pancreas cell, stem cell in the bone marrow) to perform its pre-determined function (pumping action of the heart muscle, insulin secretion of the pancreas, formation of blood cells in the marrow), it must communicate its encoded information outside of the cell nucleus. This means that the information which is coded and stored in genes must be transported in a biologically active form. This process is called **gene expression** and takes place in several steps, which are shown in a simplified manner in Fig. 4.

Conversion of genetic information

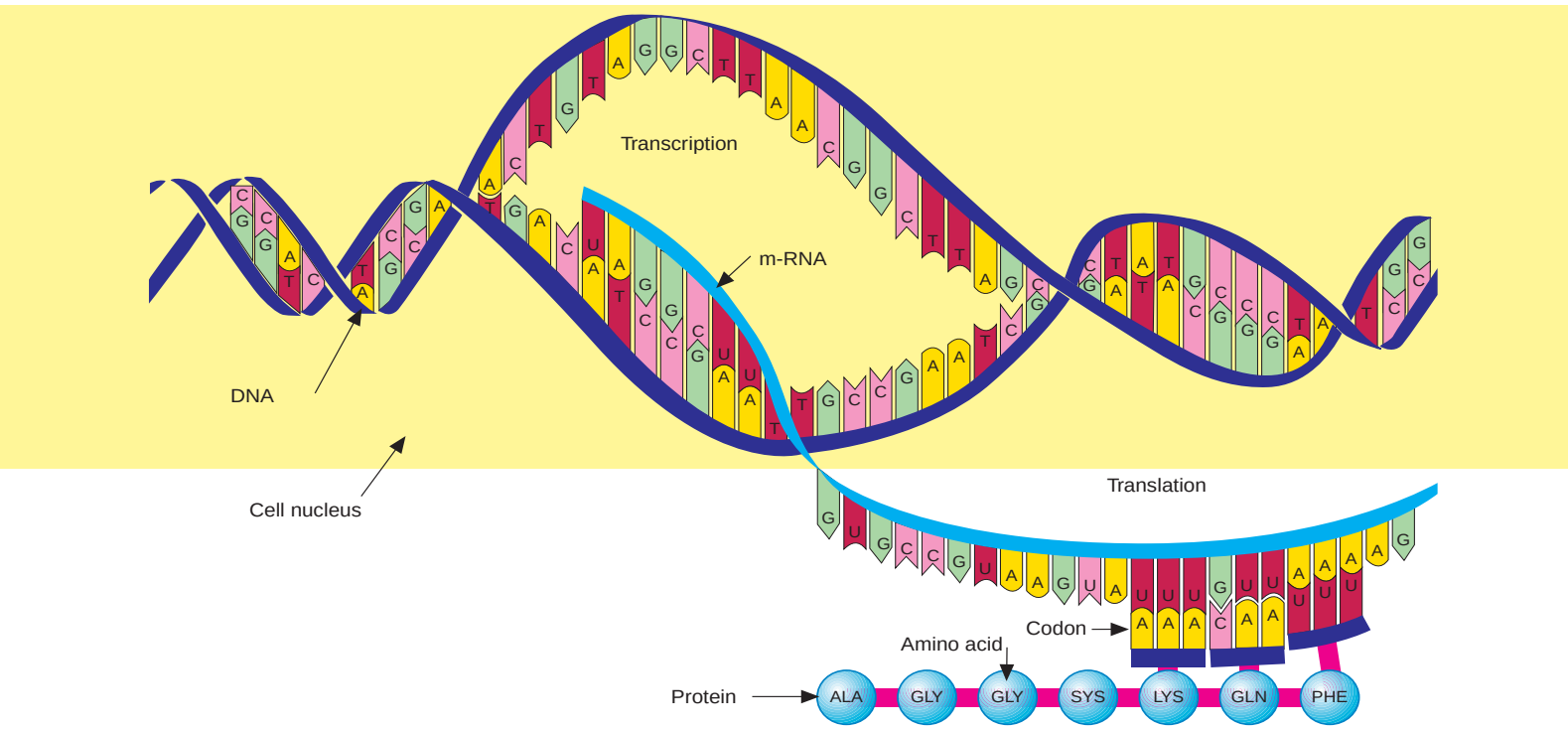


Figure 4

In order to transmit its information out of the cell nucleus to the periphery, the gene first makes use of a messenger. This messenger reads the information from the gene in the cell nucleus, as defined by the sequence of bases. In this way, a complementary nucleic acid chain may be produced using the DNA segment as a template. This newly made chain is called a **messenger ribonucleic acid (m-RNA)** and this process is called **transcription**. By using m-RNA as an intermediary, it is ensured that DNA as the source of information is not used up or destroyed.

Transcription is followed by so-called **translation**. This involves the messenger RNA being transported from the cell nucleus and then serving in the cytoplasm (part of the cell that contains cytosol, but excluding the cell nucleus) as the pattern for a specific amino acid to be incorporated in the protein chain. The genetic information of the m-RNA is transformed into an amino acid in the cell periphery on so-called **ribosomes**. Here three m-RNA nucleotides are “translated” to produce one amino acid, the primary component of all proteins.

*It is known from electron microscope images and biochemical investigations that the DNA of a gene and the corresponding m-RNA do not have the same length. What happens is that the m-RNA is further processed before it leaves the cell nucleus, with all DNA sequences which are not needed for protein synthesis stripped away. The DNA sequences responsible for protein synthesis are called coding **exons** and the RNA sections stripped away before translation are called non-coding **introns**. So a gene consists not only of information which lays down the composition of proteins but also of controlling and regulating sections (see also Fig. 5).*

The structure of a gene

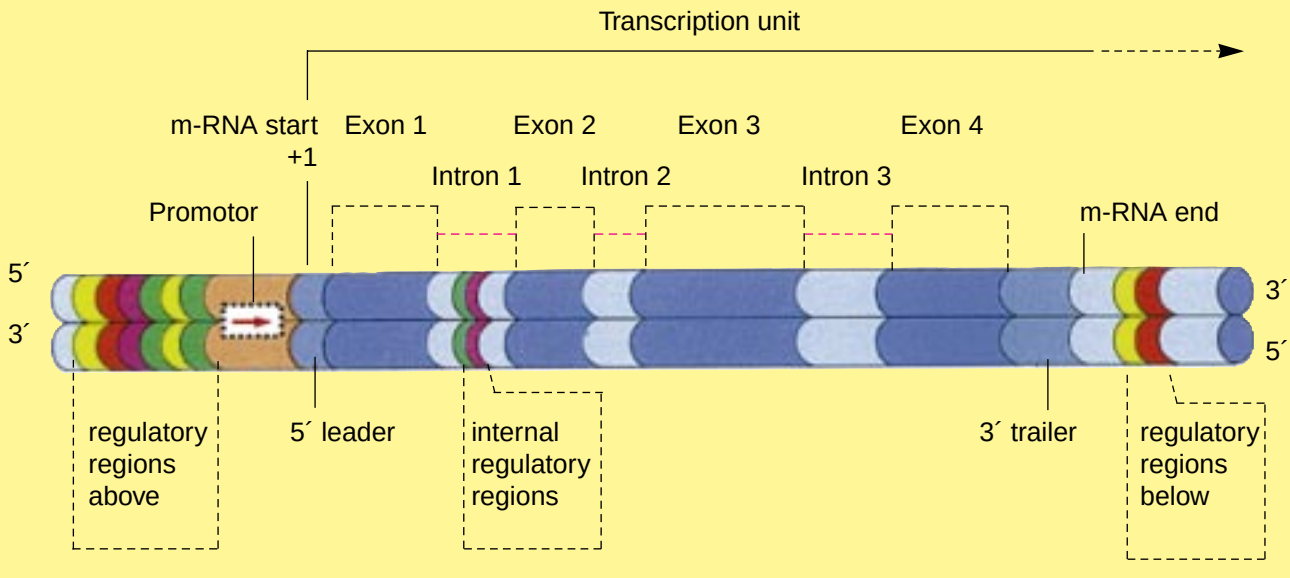


Figure 5

1.4 Proteins and their importance

Of all the organic compounds occurring in living forms, the carbohydrates (sugars), lipids (fats) and proteins are the most important substances. While the fats and carbohydrates mainly perform the function of energy carriers, proteins have a vast array of functions to fulfil. Proteins also form the majority of organic compounds, accounting for about 50% of these. Proteins are often called the building blocks of life since they represent the messengers and tools which are necessary for the processes of life in the organism. As enzymes they catalyse the metabolic process; as hormones, they control and regulate these processes; as receptors and messenger substances, they transmit important information to the inside of the cells; as antibodies, they form an essential part of the immune system; as plasma proteins, they transport important nutrients in the blood and as structural proteins, they form building blocks and mechanical supports for all cells, organs, bones and connective tissue.

Proteins consist of **amino acids**. Their function depends on which amino acids they are formed from and how many. In total, tens of thousands of different proteins are known in humans, with major differences in their function and size (see also Table 1).

Examples of human proteins and the corresponding responsible genes

Protein	Number of amino acids	Number of exons	Gene length
Insulin	51	3	1,400 nucleotides
Complement factor 3	1,663	29	41,000 nucleotides
Blood coagulation factor VIII	2,351	26	186,000 nucleotides
Dystrophin	3,685	79	2,400,000 nucleotides

Table 1

This wide variety of proteins is produced from only 20 different amino acids, which in turn are coded by only four different nucleotides (adenine [A], guanine [G], cytosine [C] and thymine [T]). Each of these 20 amino acids needs a combination of only three of the four nucleotides (**codon**; Fig. 6, see p. 14) to determine its identity. This genetic language (**genetic code**) applies in an identical manner to (almost) all living forms. In this way, a specific sequence of letters in the genes is always translated into the same protein.

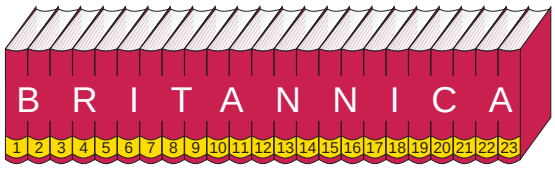
The genetic code

Nucleotide					
First	Second				Third
	Uracil (U)	Cytosine (C)	Adenine (A)	Guanine (G)	
Uracil (U)	F Phenylalanine (Phe) F Phenylalanin (Phe) L Leucine (Leu) L Leucine (Leu)	S Serine (Ser) S Serine (Ser) S Serine (Ser) S Serine (Ser)	Y Tyrosine (Tyr) Y Tyrosine (Tyr) Stop codon Stop codon	C Cysteine (Cys) C Cysteine (Cys) Stop codon W Tryptophan (Trp)	U C A G
Cytosine (C)	L Leucine (Leu) L Leucine (Leu) L Leucine (Leu) L Leucine (Leu)	P Proline (Pro) P Proline (Pro) P Proline (Pro) P Proline (Pro)	H Histidine (His) H Histidine (His) Q Glutamine (Gln) Q Glutamine (Gln)	R Arginine (Arg) R Arginine (Arg) R Arginine (Arg) R Arginine (Arg)	U C A G
Adenine (A)	I Isoleucine (Ile) I Isoleucine (Ile) I Isoleucine (Ile) Start (Methionine)	T Threonine (Thr) T Threonine (Thr) T Threonine (Thr) T Threonine (Thr)	N Asparagine (A sn) N Asparagine (Asn) K Lysine (Lys) K Lysine (Lys)	S Serine (Ser) S Serine (Ser) R Arginine (Arg) R Arginine (Arg)	U C A G
Guanine (G)	V Valine (Val) V Valine (Val) V Valine (Val) V Valine (Val)	A Alanine (Ala) A Alanine (Ala) A Alanine (Ala) A Alanine (Ala)	D Aspartic acid (Asp) D Aspartic acid (Asp) E Glutamic acid (Glu) E Glutamic acid (Glu)	G Glycine (Gly) G Glycine (Gly) G Glycine (Gly) G Glycine (Gly)	U C A G

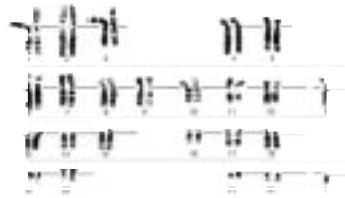
Figure 6

The **structural** relationship between chromosomes, genes, DNA and proteins is shown in Fig. 7, where the analogy with a library mentioned at the beginning of this paper is taken further. The **functional** relationship between chromosomes, genes, DNA and proteins on the other hand can be seen in Fig. 4 (see p. 11).

The structural connection between chromosome, gene, DNA and protein



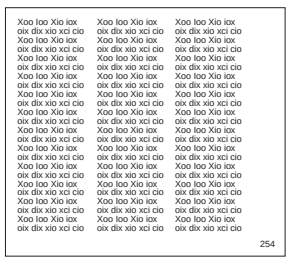
Encyclopaedia



Chromosome set

Volume

Chromosome



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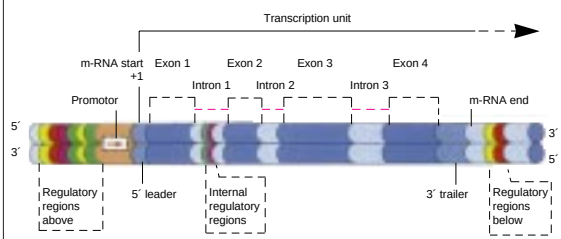


Gene

Sue

Word

Pairs of nucleotide bases (→ Amino acid)

Sue ate hot pie, but did not try the big, old eel, and she has pie for Max, too, who can see the sea.

Words

Protein

Figure 7

1.5 Reproduction of DNA (replication)

Most cells must divide all the time. They only have a limited lifespan. In order to maintain their function, they must pass on the hereditary information stored in their nuclei to daughter cells. The DNA strands therefore have to be copied. For this, the DNA double strands have to divide into single strands and a complementary strand is then produced (**DNA replication**; Fig. 3, p. 10). In this way the genome duplicates itself. Then the sets of chromosomes move to opposite cell poles and the cell divides. In this way, the cell has propagated itself. This process happens a million times every second in the human organism and ensures that the human body renews (regenerates) itself. In old age, the production rate of new cells no longer keeps up with the dying rate of old cells. This leads to atrophy and dying processes.

1.6 Importance of mutations

For perfect functioning of the human organism, stability and constancy of the genetic information is essential. On the other hand, such a complex process as the transfer of genetic information is very susceptible to error. If the hereditary information is altered in any way, this can have far-reaching consequences. Such alterations are called **mutations**. A mutation can occur spontaneously during the copying of the DNA (faulty replication) or through an external influence damaging the cell. Examples of external influences (mutagens) which can damage the hereditary information are chemicals, radiation and viruses. Sometimes mutations lead to an exchange of only a single DNA base (nucleotide). Such a mutation is called a point mutation. Other kinds of mutation are the deletion and the addition of several nucleotide sequences (see Fig. 8, p. 17). These lead to a change in the original nucleotide sequence with the likelihood that proteins will be improperly produced either quantitatively or qualitatively. This in its turn may have consequences with regard to the functions of the cell and the organism. Of course, mutations can affect the chromosomes as a whole, so that, for example, the number of chromosomes is increased or chromosome sections are deleted.

Types of mutation

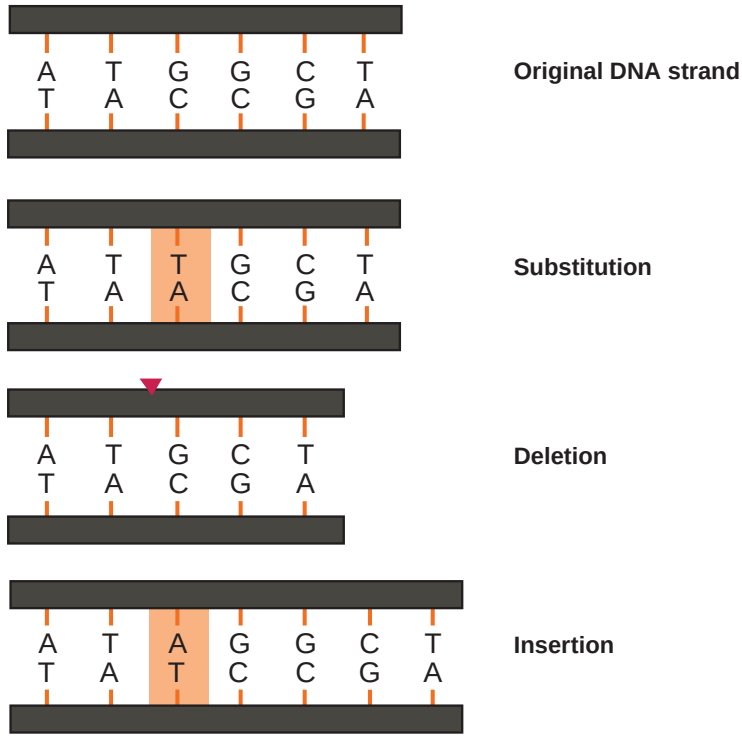


Figure 8

Mutations can occur in all types of cell: in a muscle cell, an intestinal cell or in a bone cell. Such mutations (**somatic mutations; mutations of body cells**) remain restricted to the corresponding body tissue of the individual and are not passed on to future progeny. On the other hand, if mutations occur in the **germ line** cells, these changes may be passed on to the next generation.

This distinction shows that genetic changes do not always have to be inherited. It is much more likely that during the innumerable cell divisions which take place in the organism during the course of a human life, (somatic) mutations will occur time and time again which may cause or contribute to an illness. This is the case, for example, in the majority of cancers. **A genetic disease is, therefore, not necessarily an inherited disease.** On the other hand, mutations in the germ line cells can cause inherited genetic illnesses to develop. Such a mutation may have happened several thousands of years ago and has since then been passed down from one generation to the next within certain families according to certain rules of inheritance (see also 2.2).

Not every nucleotide sequence departing from the norm within a gene (mutation) leads to illness. Our genes, with their innumerable mutations, exhibit a broad span of variation which gives every individual life form its own unique identity. In medicine this fact is used not only to provide evidence of current or future diseases. Genome analysis can also be used to identify persons (e.g. as part of a paternity test) by means of a process called genetic fingerprinting (**DNA fingerprinting**). DNA can also be recovered from the remains of the deceased even after long periods of time. For example, after a plane crash, DNA analysis enables casualties to be identified. Historical research also makes use of genome analysis. The most striking example of this is the recent identification of the remains of the last Russian Czar's family by comparing their DNA with that of living descendants.

1.7 Active and inactive genes

Human life starts when an egg cell is fertilized by a sperm cell. Each cell contributes half a set of its hereditary material. So 23 maternal chromosomes and 23 paternal chromosomes meet in a fertilized cell. This fertilized cell (**zygote**) doubles its set of chromosomes before its division and so then passes on the genetic information (the genome) to two daughter cells, which in their turn divide into four cells and so on until after 9 months a child is born with all its organs fully developed. Continuous cell division associated with DNA replication guarantees that the original genetic information in the starting cell is passed on to all other cells. And yet during the embryo phase, cells emerge which have to perform very different tasks and also "know" that they have to carry out different functions (**cell differentiation**). But why does a muscle cell look quite different and have a function different from say an intestinal cell although they both have the same hereditary material in their nucleus? In every cell, only a fraction of the entire genome is active. A large number of **regulatory and control sections** determine what part of the cell genome is inactivated at what time and what part actively synthesizes proteins and so also forms certain tissue structures. This is a highly complex process which is only beginning to be understood in the still relatively young branch of genome research.

2 Genes and diseases

The bases for hereditary diseases are the changes in the hereditary substances which are passed on from one generation to the next via the germ line cells. The change in the hereditary substance may take place at chromosome level or in the DNA itself. A distinction is made between monogenic and polygenic diseases. However, it is becoming increasingly apparent that the distinction between monogenic and polygenic diseases is an artificial one (see also 2.1.4). Many of the so-called monogenic diseases are not determined by a single gene but depend in their manifestation on the controlling and regulating influences of other genes. The term multifactorial illnesses refers to disorders which arise as a result of an interaction between heredity and environment that is not yet fully understood in the majority of cases.

It is thought that about 5% of all newborn babies come into the world with an inherited disorder. About 0.5% have a clinically relevant chromosome anomaly and about 1% have a monogenic inherited disorder. The remainder of disorders are multifactorial or due to external factors. Among those who die before their 65th year, inherited disorders are still the fifth most frequent cause of death. Most deaths result from inherited heart disorders followed by anomalies of the central nervous system (brain, spinal cord) as well as urogenital anomalies (urinary and reproductive organs) and gastrointestinal anomalies (digestive organs).

2.1 Genetic characteristics of inherited diseases

2.1.1 Chromosome aberrations

Changes in the chromosomes themselves are either expressed by a change in the number of chromosomes (e.g. in there being one chromosome too many or too few) or by changes in the chromosomal structure (e.g. **translocation**: transfer of chromosome segments to make a non-homologous chromosome). Advanced age in the mother is one risk factor for numerical chromosome aberrations. The degree of clinical severity can range from a high mortality risk on the one hand to asymptomatic status (absence of symptoms) on the other. If symptoms occur, however, they are often already present in early childhood.

2.1.2 Monogenic diseases

In **monogenic diseases**, only a single gene is altered (mutant) with the consequence that the pattern for a specific protein is flawed, which in turn leads to the manifestation (development) of a disease. Sickle cell disease is an example of this. Monogenic diseases are often rare and severe illnesses. At present, over 6,000 genes are known whose mutations lead to various monogenic disorders. Currently, a molecular genetics analysis can be made on 1,000 of these diseases.

The number of hereditary disorders amenable to genetic testing in European laboratories is not known, but it is certain that all of the more common disorders can be analysed.

Frequent monogenic anomalies

Disorder	Frequency	Genetic process	Characterization of the disease
Colour blindness (several forms)	1:12 (male)	X chr.	Colour vision disorders of differing extent
Alzheimer's disease (several familial forms)	1:100	a. dom.	Presenile dementia
Hereditary breast cancer	1:200 (female)	a. dom.	Breast cancer, often before the meno-pause, sometimes also ovarian cancer
Hereditary non-polyposis colon cancer	1:200	a. dom.	Cancer of the colon and other organs
Thrombophilia (Factor V Leiden deficiency)	1:200	a. dom.	Phlebothrombosis, thrombo-embolism
Ichthyosis vulgaris	1:300	a. dom.	Scaly skin
Diabetes, juvenile (MODY) (maturity onset diabetes of youth)	1:400	a. dom.	Diabetes due to faulty insulin secretion
Familial hypercholesterolaemia	1:500	a. dom.	Atherosclerosis, coronary heart disease
Other monogenic metabolic disorders together, without lipoprotein (a)	ca. 1:100	a. dom.	Increased risks of atherosclerosis and coronary heart disease
Isolated IgA deficiency	1:700	a. dom. a. rec.	Immune deficiency with a tendency to infection, autoimmune diseases
Polycystic kidney disease (adult form)	1:1,000	a. dom.	Formation of cysts in the kidneys (and liver), kidney failure in old age
Fragile X syndrome	1:2,500 (male) 1:2,000 (female)	X chr.	Mental retardation
Antithrombin deficiency	1:2,000 – 1:5,000	a. dom.	Deep vein thrombosis
Charcot-Marie-Tooth type disease (various forms together)	1:2,000	a. dom., X chr.	Muscle atrophy
Deafness, several inherited forms	1:2,000	a. rec., a. dom.	High-grade hearing difficulty up to deafness
Cystinuria	1:2,000 – 1:7,000	a. rec.	Kidney stones
Inherited pulmonary emphysema (alpha1-antitrypsin deficiency)	1:2,500 – 1:10,000	a. rec.	Pulmonary emphysema, liver cirrhosis in children

Frequent monogenic anomalies

Disorder	Frequency	Genetic process	Characterization of the disease
Cystic fibrosis	1:2,500	a. rec.	Formation of viscous mucus in the ung, pancreas, and other glands; functional failure of these organs
Ehlers-Danlos disease (different forms together)	1:3,000	a. dom. a. rec. X chr.	Faulty development of connective tissue
Achondroplasia	1:3,500	a. dom.	Cartilage growth disorder, dwarfism
Duchenne's muscular dystrophy	1:3,500 (male)	X chr.	Muscle atrophy
Neurofibromatosis type 1	1:3,500 – 1:10,000	a. dom.	Tumours of the nerve tissue and the skin
Alport syndrome	1:5,000	X chr. a. dom. a. rec.	Kidney insufficiency and hearing problems
Haemochromatosis	1:5,000	a. rec.	Iron deposits in the internal organs
Osteogenesis imperfecta	1:5,000 – 1:10,000	a. dom. a. rec.	Brittleness of the bones
Spherocytosis	1:5,000	a. dom. a. rec.	Round cells in the blood, haemolysis
Hypertrophic cardiomyopathy	1:5,000	a. dom.	Heart muscle disease
Familial polyposis coli	1:6,000	a. dom.	Colonic polyps, tendency to malignant tumours
Ichthyosis, X-linked	1:6,000 (male)	X chr.	Scaly skin
Myotonic dystrophy	1:7,000 – 1:8,000	a. dom.	Multiorgan disease; in particular, muscular weakness
von Willebrand's disease	1:8,000	a. dom.	Tendency to bleeding
Adrenogenital syndrome	1:10,000 – 1:18,000	a. rec.	Disturbance of the water-salt balance, abnormal signs of masculinity
Albinism (several forms)	1:10,000	a. rec. a. dom. X chr.	Deficient formation of pigment in the skin
Haemophilia A	1:10,000 (male)	X chr.	Tendency to bleeding

Frequent monogenic anomalies

Disorder	Frequency	Genetic process	Characterization of the disease
Marfan's syndrome	1:10,000 – 1:20,000	a. dom.	Faulty development of the connective tissue
Acute intermittent porphyria	1:10,000 – 1:20,000	a. dom.	Intestinal colic, paralysis, muscle weakness
Huntington's chorea	1:10,000 – 1:20,000	a. dom.	Involuntary movement disorders, personality breakdown
Kallmann's syndrome	1:10,000 – 1:15,000 (male)	X chr.	Sexual underdevelopment, absence of the sense of smell
Phenylketonuria	1:10,000 – 1:20,000	a. rec.	Mental retardation, tendency to cramps
Tuberous sclerosis	1:12,000	a. dom.	Malformation of the brain, formation of tumours in many organs
Retinoblastoma	1:14,000 – 1:20,000	a. dom.	Retinal tumour, bone tumours
Protein C deficiency	1:16,000	a. dom.	Tendency to thrombosis
MCAD (medium-chain acyl-CoA dehydrogenase) deficiency	1:20,000	a. rec.	Attacks of hypoglycaemia
Pyruvate kinase deficiency	1:20,000	a. rec.	Haemolytic anaemia
Stickler syndrome	1:20,000	a. dom.	Disease of the joints and the vitreous body
Vitamin D-resistant rickets	1:20,000	X chr.	Impairment of bone growth
Total (not including colour blindness)	**approx. 1:20 = 5%**		

Note: X chr. = X chromsome
a. dom. = autosomal dominant
a. rec. = autosomal recessive

Table 2 (taken from J. Schmidtke: "Vererbung und Ererbtes" – Inheritance and inherited)

2.1.3 Polygenic diseases

With **polygenic diseases** it is the interaction of several gene alterations (mutations) which leads to the development of an illness. So the mutation of a single gene has a much less serious effect than with a monogenic disease. Another important difference to monogenic diseases is that polygenic diseases are very common in the population. Apart from the mutation of several genes, one or more environmental factors usually contribute to the manifestation of these diseases (**multifactorial** diseases). Onset, severity and course of these illnesses are also significantly influenced by environmental factors (e.g. nutrition, exercise). For this group of illnesses, the contribution of the gene can be thought of as a “predisposition”. At present, there are only very few illnesses for which the contributory gene has been identified: e.g. in the case of thrombotic diseases (APC [Activated Protein C] resistance).

Examples of diseases for which a combination of several genes and environmental factors are thought to be responsible can be seen in the following Table 3.

Common multifactorial disorders

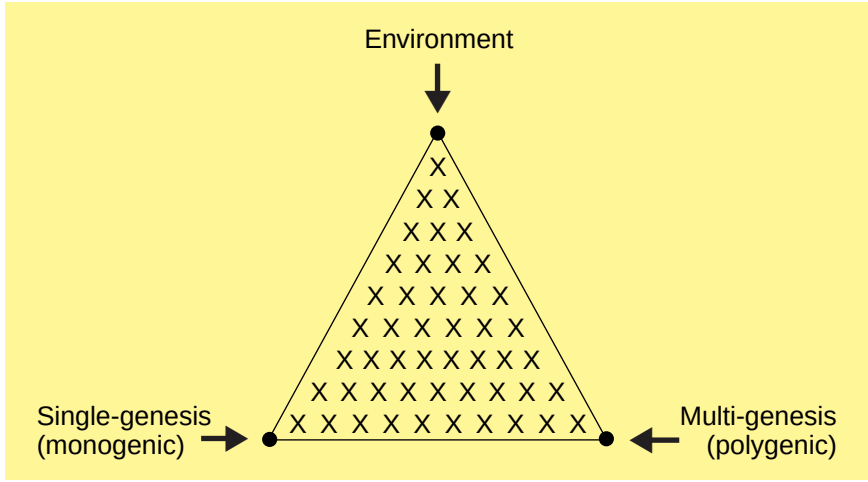
	Disease	Frequency
Congenital malformations	Heart defect	1:100 to 1:200
	Clubfoot	1:200 to 1:500
	Spina bifida	1:200 to 1:1,000
	Pyloric stenosis (constriction of the stomach exit)	1:300
	Cheilognatho-uranoschisis (cleft lip, palate or upper jaw)	1:500 to 1:1,000
Other disorders	Coronary heart disease (CHD)	1:3 to 1:5
	Stroke	1:3 to 1:5
	Short sightedness (myopia)	1:4
	Atopy (bronchial asthma, hay fever, neurodermatitis)	1:7
	Alcoholism	1:10 to 1:20
	Alzheimer's disease	1:10 (over 65s)
	High blood pressure (hypertension)	1:16
	Diabetes type II (adult-onset diabetes)	1:20
	Thrombotic diseases	1:20 to 1:50
	Mental retardation	
	– severe (IQ < 50)	1:300
	– moderate (IQ 50 to 70)	1:30
	Psoriasis	1:50 to 1:100
	Duodenal ulcer	1:50 to 1:100
	Schizophrenia	1:50 to 1:100
	Manic-depressive disease	1:100 to 1:200
	Rheumatoid arthritis	1:100
	Epilepsy	1:200
	Cataract	1:250
	Diabetes type I (juvenile diabetes)	1:400
	Gout	1:500

Table 3 (taken from J. Schmidtke: "Vererbung und Ererbtes" – Inheritance and inherited)

We can use coronary heart disease (CHD) as an example to illustrate the complex influence of several genes in causing disease and the heterogeneous (in the true sense of the word) composition of risk groups. Risk factors for CHD have been known for several decades: hypertension, hyperlipidaemia, diabetes mellitus, nicotine consumption, obesity and lack of exercise are the most important. Most risk factors can be attributed to an exogenous factor (behaviour of the individual) as well as an endogenous factor (genetic). This is the case with hyperlipidaemia. An increased cholesterol level in the blood is caused not only by eating food rich in cholesterol, but also by various genes which have a greater or lesser effect on fat metabolism. These genes code for the formation of proteins such as apolipoprotein E, apolipoprotein B, LDL receptors, lipoprotein (a), lipases (enzymes) among others. Mutations of the genes which control the formation of these proteins produce quite specific lipid metabolism disorders, which to a greater or lesser extent may contribute to premature atherosclerosis and therefore to coronary heart disease.

2.1.4 Genes and diseases

The boundaries between monogenic, polygenic and multifactorial diseases can be expressed by the following statement: genes can always be represented as more or less strongly predisposing factors for the development of an illness.



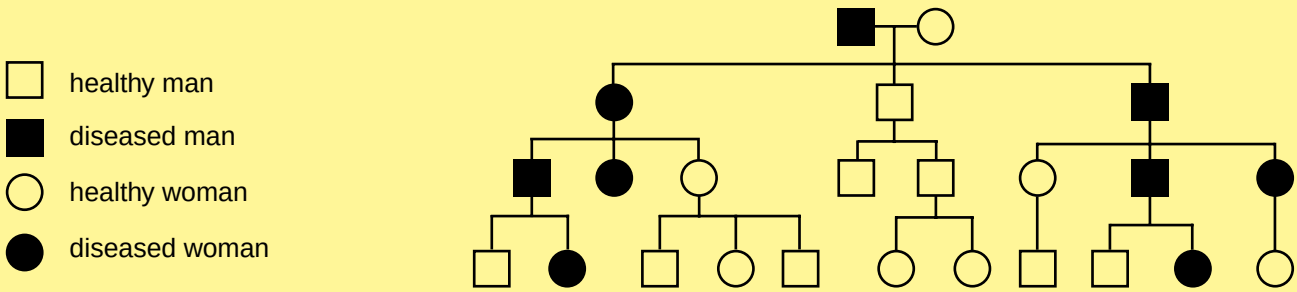
Every illness (X) lies within the control pattern triangle, none lie on an edge or a corner. Whether the environment, polygenic factors or monogenic factors predominate depends on where the illness is located inside the triangle.

2.2 Inheritance rules for genetic diseases

The following inheritance processes are known for monogenic diseases. In order to understand these rules of inheritance it is important to emphasize that every gene in the cell nucleus is expressed twice – in the same way as all chromosomes are expressed twice (one set from the maternal and one set from the paternal parental set). What is decisive for the onset of a genetic disease is whether a mutated gene can prevail in its pathological action against its “healthy” counterpart (**gene homologue**) or whether the other gene also has to be mutated. These inheritance patterns were already observed in the last century by Gregor Mendel in his famous cross-breeding experiments with peas, which form the basis for today's comprehensive family tree analyses.

2.2.1 Autosomal dominant inheritance process

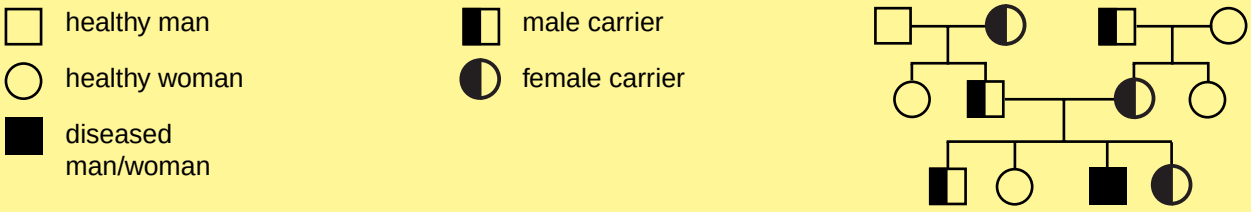
In this inheritance pattern only one of the two homologous genes is mutated and although another normal gene is present (**heterozygosity**), the illness still appears (**dominant gene effect**). If, therefore, one of the parents carries this gene, there is a 50% probability that it will be transmitted to each child. Both men and women can be affected by this. This inheritance pattern accounts for over 60% of monogenic diseases, representing by far the most common inheritance process.



This inheritance pattern is followed by many diseases which exhibit changes in the proteins forming the basis for connective and supporting tissue (e.g. Marfan’s syndrome, achondroplasia, hypertrophic cardiomyopathy). Obviously a mutated protein in just half the amount will have a pathological effect on the human organism in such cases.

2.2.2 Autosomal recessive inheritance

In this inheritance pattern, both homologous genes must be mutated (**homozygosity**) in order to produce an illness in the affected person. The individual must therefore have inherited a corresponding gene mutation from both the father and the mother. Individuals, who only receive one version of the mutated gene are called **carriers**. Both sexes can be affected. If, for example, both parents are carriers, there is a 25% chance that the child will receive both mutated genes and so develop the illness.

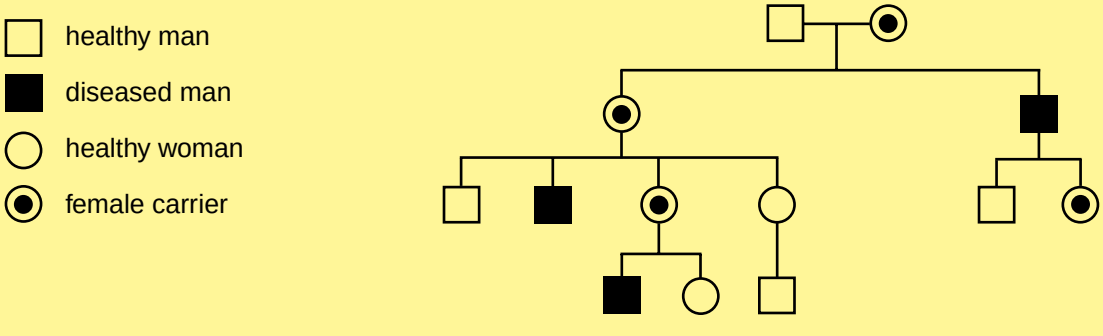


Many metabolic diseases fall into this category (e.g. cystic fibrosis, phenylketonuria, adrenogenital syndrome, haemochromatosis). In the heterozygote state (i.e. only one gene copy is mutant) it seems that the human organism can often compensate for this state and make do with half of the unchanged protein. Only when both gene copies are mutated does the amount of faulty enzymes have a pathological effect which can then no longer be compensated.

2.2.3 X chromosome inheritance (sex-linked inheritance)

Women have two X chromosomes. If they have a recessively acting mutated gene on one X chromosome, they are carriers for the corresponding illness. Men have only one X chromosome, since the other sex chromosome is a Y chromosome. If they have the mutated gene on the X chromosome, they will develop the illness as a rule.

If a woman is a carrier for the illness inherited by the X chromosome, there is a 50% chance that she will pass on this illness to her son. Her daughters have a 50% chance of becoming a carrier for this illness.



A few X-chromosomal genes have a **dominant** action; in such cases women can also develop the illness.

Examples of the three different patterns of inheritance which have been described can be seen in Table 2 (p. 20 ff.).

2.2.4 Inheritance process in polygenic diseases

Of course, in these cases too the individual genes take part in the above-mentioned inheritance processes. However, there are always several genes involved in causing polygenic diseases with different types of interaction possible. Sometimes the effect of several genes must be added together and at other times a certain threshold has first to be crossed before an illness becomes manifest. Often individual genes have a complex relationship with each other (**control and regulation genes** in a **gene network**). In addition, one or more environmental factors may contribute to the manifestation of the corresponding illness. Given the large number of causative factors and their interrelationships, it is easy to understand that there are also a large number of variations in the genesis of multifactorial diseases. These are expressed in different ages of onset of the illness, different courses of the illness and different degrees of severity. In general, there is a fluid transition from the healthy state to the pathological state in polygenic diseases if there is no threshold effect. This breadth in the variation of mutations leading to the same illness makes it impossible to make a prediction of symptoms and prognosis with current scientific knowledge.

2.3 Identification of inherited diseases

2.3.1 Conventional genetic tests

2.3.1.1 Phenotype analysis

The simplest and for centuries the most common type of genetic analysis is the analysis of the so-called phenotype. The analysis involves the end product of the genes which are translated into proteins by transcription and translation. These proteins determine the way an individual's body looks.

In this type of investigation, a certain body characteristic of the individual, in other words a phenotype, is examined and compared with members of a normal population. How the phenotype is defined depends considerably on the accuracy and methodology of the observations.

A phenotype analysis is facilitated by conventional clinical or clinical and chemical investigation methods, such as a physical examination to determine the red-green blindness phenotype, ultrasound or a blood analysis to determine the blood group.

The following points apply to phenotype analyses (here specifically: biochemical investigations):

- Genes are directly responsible for the production of hormones, enzymes and other proteins. To this extent, the analysis of these substances and metabolic products can also be classified as a genetic test in a wider sense.

- Indication: Especially in the case of metabolic diseases in newborn babies.

- Investigation procedure: Diagnostic measurement of altered or missing proteins using blood or urine analysis. This provides indirect evidence of a mutation of the gene responsible for this.

- Examples: Phenylketonuria, alpha1-antitrypsin deficiency

  There is clinical evidence of a connection between severe alpha1-antitrypsin deficiency and liver cirrhosis as well as lung emphysema. There are several different courses of the illness. In the early childhood form, in which liver cirrhosis and lung emphysema occur in infancy, the children usually die from the complications before their twelfth year. Another form primarily affects adults, in which emphysema with a rapidly progressive course occurs before their 40th year. Environmental factors (nicotine, dust) play an important part in promoting the manifestation of this illness.

2.3.1.2 Chromosome analysis (cytogenetic investigations)

- This includes microscope examinations to investigate chromosome alterations in terms of number (duplication or loss of individual chromosomes = **numeric chromosome aberration**) and in terms of structure

(wrong composition, chromosome breaking = **structural chromosome aberration**). There is no detailed investigation of individual genes in such cases.

- Indication: Anomalies in children (malformations, retarded development) in the context of prenatal diagnosis, tendency to miscarriages, infertility.

- Investigation procedure: Cells capable of division are isolated from the blood or skin biopsies and placed in a culture solution, where cell division is stimulated. After centrifuging and staining the cells, the chromosomes in a certain cell division stage (metaphase) are visible under a microscope (approximately 400 times magnification). Further staining techniques and higher magnification also make subsegments of the chromosomes visible under the microscope (**chromosome bands**).

- Examples of chromosome aberrations:
  Klinefelter's syndrome: 47, XXY
  Ulrich-Turner syndrome: 45, X
  Down's syndrome: 47, XY, +21; or 47, XX, +21

  Down's syndrome (earlier known as "mongolism") was described as early as 1866 and was first shown to be due to a defective chromosome distribution in 1959. This illness involves not just two copies of chromosome 21 in each cell but three (see Fig. 9). Down's syndrome is one of the most common genetic causes of mental retardation. The incidence in Europe is about 1:600 living births, with the incidence strongly dependent on the mother's age (35 years: 1:380, 40 years: 1:110, 45 years: 1:30). The disorder manifests as mental retardation, congenital heart disease (50% of all patients) and various striking anomalies in body structure. Life expectancy is reduced due to the heart disease and the relatively frequent leukemia occurring in adult life.

Chromosome set for trisomy 21 (male)

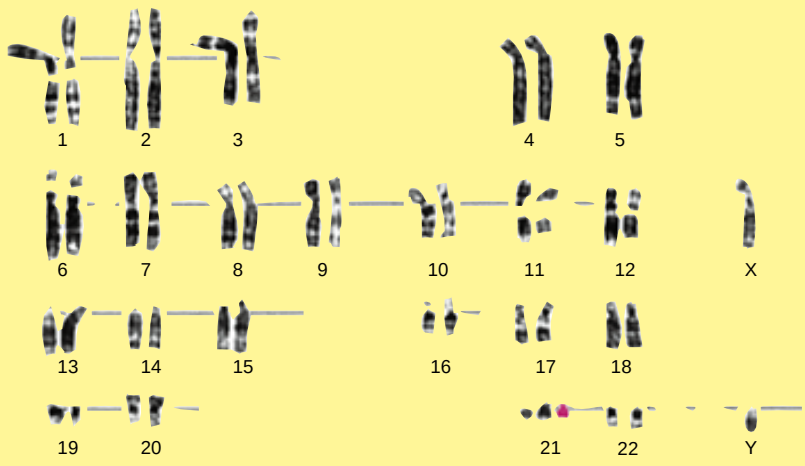


Figure 9

- A relatively new technique is **molecular cytogenetics**, which uses a combination of cytogenetic and molecular genetics methods. Fluorescence-labelled DNA sequences are often used as diagnostic “probes”, hence the designation “**FISH**” (**Fluorescence in situ hybridization**, see Fig. 10).

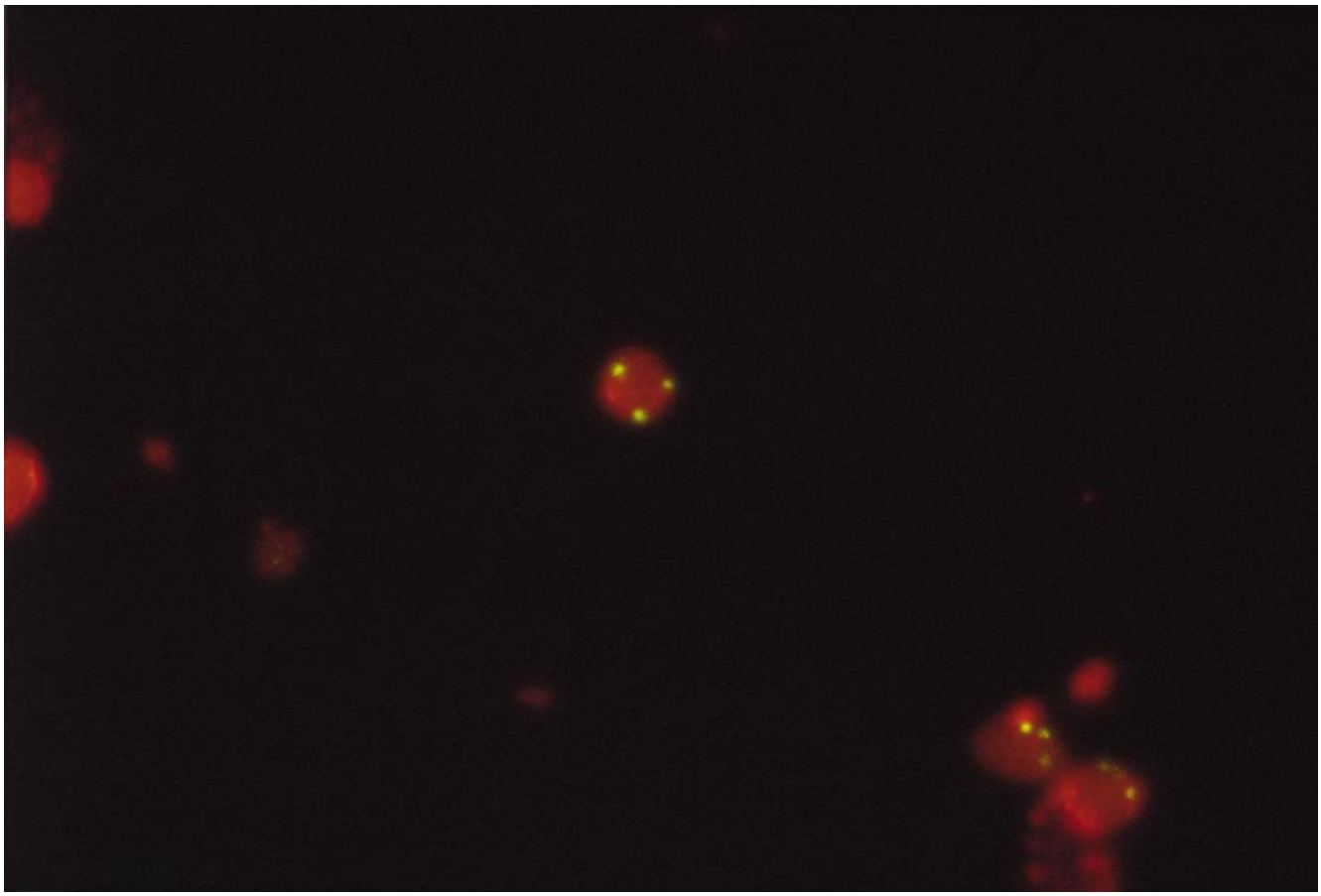


Figure 10

Fluorescence in situ hybridization (“FISH”) of an interphase cell nucleus using a DNA probe specific for chromosome 18. Normally two fluorescent blots are shown, since each diploid cell contains two copies of chromosome 18. However, since three signals are visible in the figure, trisomy for chromosome 18 must be concluded. This method is suited for quick prenatal screening tests (results within 24 hours) on uncultivated cells for demonstrating numerical chromosome aberrations.

2.3.2 Molecular genetics testing (DNA analysis, genome analysis DNA tests)

- This provides evidence of a gene mutation responsible for producing the illness. Here it is determined whether the sequence of the DNA bases (**nucleotide sequence**) has changed within the affected gene. A prerequisite for a "**direct genetic test**" is that the responsible gene must already have been located and its sequence worked out. In the case of an "**indirect genetic test**" (coupling analysis) only the position of the gene on the chromosome needs to be known.

- Indication: This is done as confirmation of preliminary clinical diagnoses, evidence of carriers or in predictive (including prenatal) investigations.

- Investigation procedure: Molecular genetics tests rely on revealing short segments of DNA sequence information and make use of the complementary nature of the strands of DNA in order to do this. The naturally occurring double-stranded DNA must always be dissolved in water first and then broken down into the individual strands. A **DNA probe** called ATCTGA then fishes out sequences with the letters TAGACT from a mixture of individual DNA strands.

  *There is a whole panoply of different techniques available for revealing specific DNA double-stranded molecules which are all variations of the same basic principle: In **DNA sequencing** the units of a specific sequence are read off one after the other; in the **polymerase chain reaction** (PCR) one or several identical starting sequences are used to generate so many copies that they can be seen with the naked eye; and in the **Southern blot procedure** a mixture of sequences is sorted electrophoretically according to size before a specific sequence is made visible in an autoradiograph using, for example, a radioactively labelled probe. With these techniques any deviation of a gene from the "normal state" can be revealed, whether it be a **deletion**, an **insertion** or an exchange of individual units (**point mutation**). The term **direct genetic test** is used if the alteration which is found is the direct cause of an illness.*

  *In the **indirect genetic test** (also known as **coupling or segregation analysis**), use is made of the fact that scattered all over the human genome there are sequence segments in which the two homologous chromosomes can be different. These differences in the sequence are in themselves in no way pathological, they are called **polymorphisms** and are passed on from generation to generation. Polymorphisms can lie inside or outside the genes, but they always occupy the same chromosomal position (locus). If it has been shown that a polymorphism lies close to a gene responsible for a disease then it can be used as a **disease marker**.*

- Examples: Cystic fibrosis, Huntington's chorea, polycystic kidney disease (see also Table 5, p. 39 f.).

2.3.3 Limitations of DNA analysis

It has long been known from research into identical twins that monogenic and also polygenic diseases sometimes do not occur in both twins, even though the genetic information is the same in identical twins. So it is not surprising either that in monogenic diseases in which only a single gene is altered, the phenotypic consequences are often difficult to predict even in the case of a positive test result. This is due to several factors:

- **Penetrance**: not every pathogenic mutation leads to the manifestation of a disease in the lifetime of a person. For example, the gene mutation for neurofibromatosis is manifested in almost all affected individuals. In inherited ovarian cancer this proportion is considerably reduced. This can be described as “reduced penetrance”.

- **Expressivity** on the other hand describes quantitative differences in the manifestation of the disease/symptoms. Sometimes, the two concepts are difficult to separate, when, for example, a disease is so weakly manifested that it can no longer be diagnosed. The expressivity can fluctuate strongly especially in dominant monogenic diseases (e.g. Marfan’s syndrome, neurofibromatosis).

- The age at which the disease manifests itself can vary strongly. An example of this is Huntington’s chorea. Differences in the onset of diseases are sometimes explained by so-called **dynamic mutations**. In passing on to the next generation, the disease-inducing mutation can lead to an earlier onset of the illness (**anticipation**) involving the extension of a mutated sequence of bases.

- In many cases, genetic information is manifested in a different way when it is inherited from the mother than when it is inherited from the father. Here one speaks of **imprinting**.

This list of examples of modifying factors is by no way exhaustive; it clearly shows that despite a proven pathological mutation (positive test result) for many inherited diseases, it is not possible to give an exact prognosis in terms of the disease and life expectancy.

3 Fields of use for genetic tests

3.1 Indications for genetic tests

3.1.1 Prenatal diagnosis

Even today, family planning taken in the broadest sense of the term is the most common reason for genetic counselling. Usually, one of the parents themselves, or a sibling or another person closely or distantly related is affected by an illness with a possible/likely genetic cause and the parents want to have a reasonable idea of the risk of an unborn child developing this illness as well. However, even in the case of family histories with no salient characteristics, parents planning pregnancies seek genetic counselling; for example, in the case of the advanced age of the mother, a history of miscarriages or when relatives marry. The most common reason at present is prenatal screening for the chromosome anomaly for Down's syndrome.

In the future, invasive prenatal diagnosis by chorion biopsy (removal of cells from the external membrane of the embryo), amniocentesis (puncture and analysis of fluid from the womb) and the puncture and analysis of umbilical cord blood will grow in importance, whereby cytogenetic and especially molecular genetics investigations of the hereditary material can be carried out. On the other hand, non-invasive procedures with greater potential for stimulating demand are also gaining in importance, whereby fetal cells (of the unborn child) can be extracted from the mother’s blood and used for molecular genetics or molecular-cytogenetic investigations.

3.1.2 Preclinical diagnosis

The second main reason for seeking genetic counselling involves investigation of the hereditary material in which it is not the unborn child but the person seeking the counselling that is the centre of attention. Here, as in prenatal diagnosis, it may happen that a certain inherited disease has manifested in the family or in close relatives and the person seeking advice now wishes to know what his/her own risk is of developing this illness in later life. Monogenic diseases are usually involved here and the cause can be shown to be from a specific mutation (**presymptomatic diagnosis**). In contrast to this, as a result of the rapid advances being made in genome analysis, it is possible to envisage genetic tests in the future which will be used to screen specific population groups for predisposition towards a multifactorial disease such as coronary heart disease, in order to find out how high their personal risk is (**predisposition diagnosis**, see Fig. 11).

3.1.3 Confirmation of preliminary clinical diagnoses

Diagnoses based on purely clinical evidence using chemical laboratory and immunological procedures are often uncertain. Genetic tests produce a final clarification here (e.g. cystic fibrosis, Huntington’s chorea).

3.1.4 Other indications

Other reasons for genetic tests can be: genealogical investigations, unfulfilled desire for children and marriage between relatives.

Figure 11 shows how a genetic test can suggest a greater or lesser risk.

Prognostic classification of a genetic test

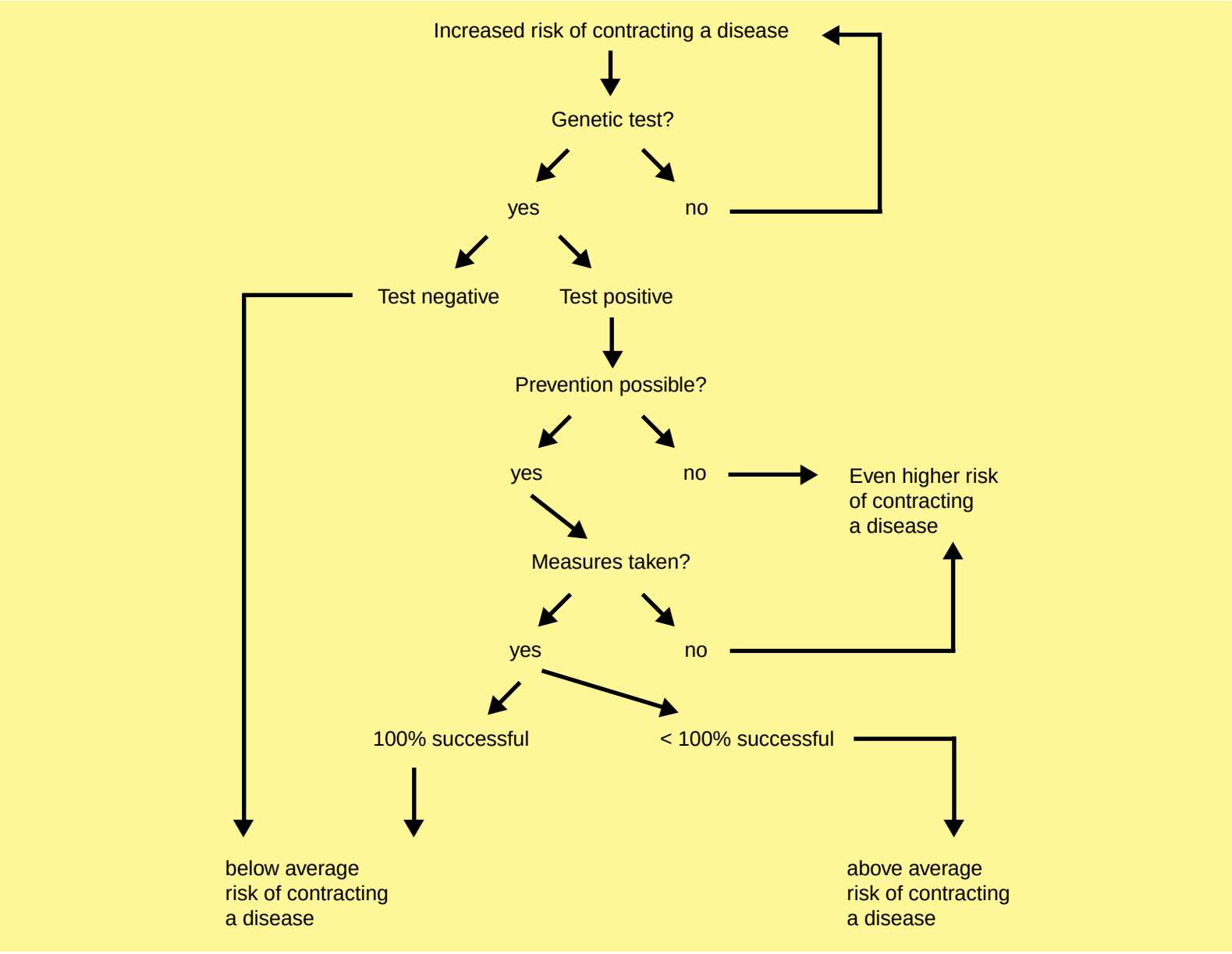


Figure 11

3.2 Molecular genetics laboratories in Europe

In many, but not all European countries, “Human Genetics”, or “Medical Genetics”, is an accredited medical speciality. Countries where this is the case include Austria, the Czech Republic, Denmark, Finland, France, Germany, Hungary, Italy, the Netherlands, Norway, Russia, Sweden and the UK.

In line with the generally growing role of genetics in medicine, human genetic techniques are of course being used in other specialist areas: in laboratory medicine and clinical chemistry, in legal medicine and also in clinical fields, especially in the in-patient departments of university clinics. It must, however, be stressed that a professional interpretation of the results of genetic tests (with all their implications for the individual and the family) requires human genetics expertise.

In many European countries human geneticists have their own scientific societies, including Austria, the Czech Republic, Germany and the UK. These societies, sometimes in cooperation with boards of accredited specialists, formulate guidelines and other policy statements, act as advisors to policy makers, and consult health authorities to work out treatment fees or design service purchasing, wherever applicable. Despite the vast differences in the provision of health services in European countries, the cost basis for genetic services and the amount of services provided in the countries of Central Europe are quite similar. In Germany, for instance, the cost of a genetic test ranges between £ 75 [approx. 100 euros] (screening for a specific mutation) and £ 250 [approx. 350 euros] (screening for ten mutations).

In Europe, there are many hundreds of different laboratories which offer genetic services. The vast majority are university-based, but, in many countries (such as the Czech Republic, Finland and Germany) genetic services are increasingly being provided by private practices. The number of samples tested genetically has not been counted systematically, but the data available suggest that Germany is typical in terms of the extent to which tests are provided in Central Europe (Table 4).

Number of DNA samples taken from patients for molecular genetics tests in Germany
(from Nippert et al., "Medizinische Genetik" 2/1997, p. 200)

Year	Investigated persons*	Annual growth in %
1991	5,792	(100.00)
1992	7,733	+33.51
1993	9,804	+26.78
1994	13,436	+37.05
....		
1997	32,000 (estimated)	

* investigated by members of the Professional Association for Medical Genetics
Source: Professional Association for Medical Genetics, 1997

Table 4

3.3 Quality assurance in molecular genetics diagnosis

Quality assurance in medicine is both an ethical issue and an economic necessity. The extremely sensitive area of human genetics deserves special attention in this respect. Individual, social and economic goals are often not compatible. It is in this context that the emphasis on the protection of the individual should be understood, an emphasis which has become one of the major principles of quality assurance. A central requirement is the combining of any human genetics diagnosis with individual genetic counselling. There is a wide variety of quality control measures. These include guidelines and directives, standardization of laboratory procedures, quality control groups and group experiments.

United Kingdom

The Human Genetics Advisory Committee on genetic testing has, as part of its terms of reference, to establish requirements, especially in respect of efficacy and product information, to be met by manufacturers and suppliers of genetic tests.

Undoubtedly this will lead to further reviews of genetic testing approaches in the UK. The HGAC has already stated that testing for some inherited diseases such as cystic fibrosis can be done without the permission of a doctor unless there is a family history of that disorder.

Germany

The work of developing guidelines and directives has been taken on by the Professional Association for Medical Genetics in consultation with the scientific and professional associations of human geneticists. The PAMG organizes regular cytogenetic group experiments and it supports several disease-specific quality control groups (molecular genetics diagnosis of cystic fibrosis, Huntington's chorea, muscular dystrophy, fragile X

syndrome among others). The relatively small number of service providers and their excellent cooperation have already led to a considerable degree of standardization. Harmonization of international standards deserves special attention and this is of growing importance in the face of globalization of test supply and demand. Interdisciplinary guidelines and directives (e.g. for prenatal diagnosis) are published by the Federal Medical Council in consultation with human genetics experts.

One of the most important quality criteria for medical genetics is the time required to carry out a test. Especially in prenatal diagnosis, waiting for the test result is experienced as particularly painful. For a classical chromosome analysis (including the necessary long-term cell cultivation) a processing time of 2–3 weeks has to be considered. As a rule, molecular genetics tests require 2–3 days, provided that the information required is not unusually complex. Otherwise several months of processing time may be necessary with the result that prenatal diagnosis may not be able to answer the questions in the available time span. Quality controls have not yet been introduced in a legally binding manner for medical geneticists in Germany although the majority of them voluntarily take part in group experiments and quality control groups.

Europe

Organized quality assurance in medical genetics comparable with the UK or Germany can be found in other parts of Europe too. Quite some time ago, Dutch human geneticists joined up with the molecular genetics quality control groups there. In addition, there is a consortium for the molecular genetics diagnosis of cystic fibrosis, the need for which has been underlined by the rather sobering initial results.

USA

While guidelines have existed for some years for cytogenetic investigations, the establishment of generally valid quality assurance methods for genetic tests is still in its infancy. A Task Force On Genetic Testing set up by the American Federal Government is endeavouring under the leadership of Professor N.A. Holtzman, from the John Hopkins University, Baltimore, to develop criteria for test reliability, efficacy and quality.

4 List of monogenic disorders amenable to DNA-based diagnosis

An overview of the range of molecular genetics diagnosis is given in Table 5. The list comprises a selection of monogenic diseases which can be diagnosed in various European laboratories.

Molecular genetics diagnosis of monogenic diseases in Europe (selection)

Achondroplasia
Adrenal cortex hypoplasia, congenital
Adrenogenital syndrome
Agammaglobulinaemia (Bruton type)
Alkaptonuria
Alpha1-antitrypsin deficiency
Alzheimer's disease, familial form
Amyloidpolyneuropathy, familial
Amyotrophic lateral sclerosis
Androgen resistance
Angelman's syndrome
Anhydrotic ectodermal dysplasia
Aniridia
Apolipoprotein B deficiency
Ataxia telangiectasia
Azoospermia
Breast cancer, familial form
Carcinoma of the colon, familial form (hereditary non-polypous colon cancer)
Creutzfeldt-Jakob disease, familial form
Cystic fibrosis
Diabetes (Maturity Onset Diabetes of the Young [MODY])
Ehlers-Danlos syndrome
Fanconi's Anaemia
Fragile X syndrome (X)
Friedreich's ataxia
Gardner's syndrome
Haemoglobinopathies
Haemophilia A
Haemophilia B
Huntington's chorea
Hydrocephalus (X)
Hyperkalemic periodic paralysis
Ichthyosis (X)
Kallmann's syndrome
Kearns-Sayre syndrome

Molecular genetics diagnosis of monogenic diseases

Langer-Giedion syndrome
Leber's atrophy of the optic nerve
Lesch-Nyhan syndrome (X)
Macular degeneration
Malignant hyperthermia
Mitochondrial encephalomyopathies
Multiple endocrine neoplasia
Myotonic dystrophy
Nephrogenic diabetes insipidus
Neurofibromatosis type 1
Neurofibromatosis type 2
Osteogenesis imperfecta
OTC deficiency (X)
Phenylketonuria
Polycystic kidney disease
Polyposis coli
Prader-Willi syndrome
Premature hereditary osteoarthrosis
Retinitis pigmentosa
Retinoblastoma
Spinal muscular atrophy
Spinobulbar muscular atrophy
Spinocerebellar ataxias
Testicular feminization
Thalassaemias
Thrombophilia (factor V Leiden deficiency)
Thyroid gland hormone resistance
Tuberous sclerosis
Waardenburg's syndrome type I/III
Wilms' tumour
Wilson's disease
Wiskott-Aldrich syndrome

(X) = X-chr.-linked

Table 5

5 Selection of molecular genetics tests

5.1 Breast and/or ovarian cancer (BRCA1, BRCA2)

5.1.1 Epidemiology, clinical testing

- In Europe about 0.5 –1% of women have a genetic predisposition (germ line cell mutation).
- 10% of all cases of breast cancer have a positive family history.
- The genetic form of breast cancer occurs earlier and more frequently on both sides than the sporadic form. The average age for manifestation of the genetic form is 45.

5.1.2 Genetics

- Mutations in the BRCA1 or BRCA2 gene can lead to breast or ovarian cancer.
- Autosomal dominant inheritance.
- In the familial occurrence of breast and/or ovarian cancer, 80–90% of cases have BRCA1 mutations.
- If BRCA1 is positive, there is a lifetime risk of up to 85% of developing a breast carcinoma, and of up to 60% of developing an ovarian carcinoma. In comparison to this, the lifetime risk of developing breast carcinoma is estimated to be 8 –12% in women.
- 45% of all familial breast carcinoma cases arise from mutations in chromosome 17 (BRCA1) and 35% from mutations in chromosome 13 (BRCA2). The remaining 20% is explained by genes which have not yet been discovered.
- At present a total of 250 different mutations (BRCA1 + BRCA2) are known. This genetic heterogeneity is probably one of the causes of the variable penetrance of the BRCA1 and BRCA2 mutations.
- The same mutation can lead to different probabilities of occurrence of breast carcinoma in different population groups.
- Still very unclear are the regulation processes inside the gene which when they interact with environmental factors (e.g. oestrogen parity, smoking, breast feeding) determine the risk of cancer.
- BRCA2 also predisposes for breast cancer in men.
- The above-mentioned figures refer to familial situations in which a large number of family members are ill. BRCA1 mutations have been found much less often in families with few cases of illness.

5.2 Huntington's chorea (HC)

5.2.1 Epidemiology, clinical testing

- Prevalence in the total population: 1:10,00–1:20,000.
- Classical symptoms: chorea, hyperkinesis, walking disorders, speech disorders, dementia.
- Death after approximately 10–20 years of illness by pneumonia, CHD, suicide.
- In 50% of patients, symptoms first appear between the ages of 30 and 50.

5.2.2 Genetics

- Autosomal dominant inheritance
- High penetrance:

up to the age of 20	about 2% affected
up to the age of 30	about 10% affected
up to the age of 50	about 60% affected
up to the age of 70	about 95% affected

- In subsequent generations there is a tendency for the illness to start earlier (anticipation). On average, the descendants of male patients are affected earlier than the descendants of female patients (imprinting).
- Mutation of the Huntington gene occurs on chromosome 4, involving the extension of a nucleotide triplet with the sequence CAG.
- CAG triplet repetitions: It has been observed that the length of the CAG triplet extension is negatively correlated to the age of manifestation, i.e. the longer the CAG triplet, the earlier the onset of the illness. An individual prediction does not seem possible, however. According to the latest research findings:

≤ 31 CAG triplets:	probably no manifestation of HC
32–38 CAG triplets:	grey zone with reduced penetrance
≥ 39 CAG triplets:	highly probable development of illness

5.3 Cystic fibrosis (CF)

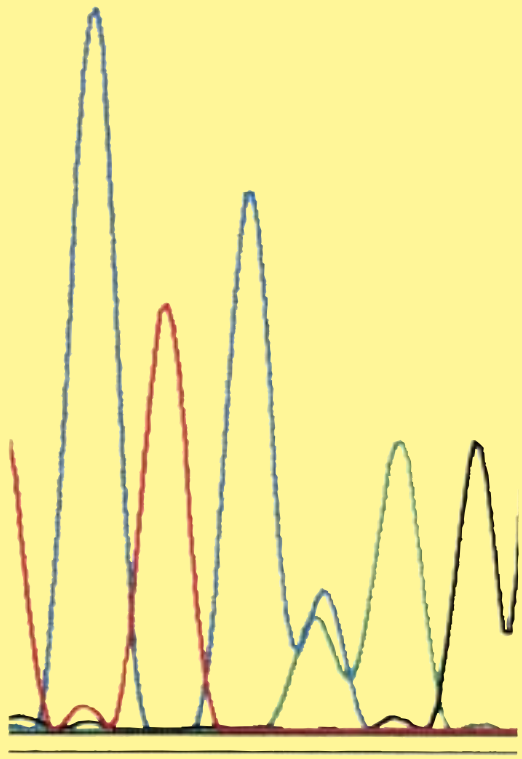
5.3.1 Epidemiology, clinical testing

- Prevalence in the total population: approximately 1:2,500 (homozygote); heterozygote carriers approximately 1:25. Thus it is one of the most frequent autosomal recessive inherited diseases in the European population.
- Heterozygote carriers are healthy.
- Cystic fibrosis shows a variable course of the illness, which can be partly explained by the marked genetic heterogeneity (> 700 known mutations leading to CF).

- Average survival time at present in men 30.6 years and in women 28.2 years.
- Clinical manifestation already in 10% of neonates; otherwise during infancy.

5.3.2 Genetics

- Mutations in the CFTR gene (cystic fibrosis transmembrane conductance regulator) occur on chromosome 7.
- There are at present over 700 known different mutations which in the homozygote state (the same CFTR on both chromosomes) and in the compound heterozygote state (different CFTR mutations on both chromosomes) can lead to cystic fibrosis (see Fig. 12).



Section from an automatically recorded DNA sequence of the CFTR gene of a patient with cystic fibrosis (CF). The sequence section in the normal sequence reads GAGGTC. In this patient the third G (corresponding to position 1756 in the CFTR gene) has mutated to T: thus the mutation reads 1756→T which, at the protein level, leads to the insertion of a translation stop codon where the amino acid glycine would normally be inserted (542Gly→Stop). The sequencing profile shows the patient to be heterozygote for the normal sequence and the mutated sequence at position 1756 because both G (blue spike) and T (green spike) are shown. The patient's CF must be due to the fact that he also bears a pathological mutation at another site on the CFTR gene (so-called compound heterozygotism).

Figure 12

- The extreme differences in the clinical manifestation of cystic fibrosis (pancreas sufficiency, pancreas insufficiency, limitation to aplasia of the seminal duct) can be partly explained by the genetic heterogeneity.

- The most common worldwide mutation is ΔF508. Its contribution to all CF mutations is 45–87% in European populations.

- Five other mutations reach proportions of over 1% (amounting to a total of 80.4%).

- With a test kit now coming on the market for 31 mutations (optimized for worldwide application) there is in Europe an 87% chance of detecting a CF mutation; with the inclusion of another four mutations a detection rate of almost 90% is achieved.

6 What makes genetic testing different from “conventional” testing?

The answer to this question assumes a clear definition of the concept of “genetic testing” which differentiates it from conventional tests. However, it has not been possible to agree on a uniform definition of a genetic test to date, especially because every medical test may reveal a certain degree of genetic influence.

The following terminology may be useful in this connection:

Genetic tests in the narrow sense: Testing for the presence of a gene alteration at the level of the genetic material (DNA).

- **Direct genetic test**: Detection or exclusion of the mutation itself.
- **Indirect genetic test**: Segregation analysis of a gene alteration in a family with the help of coupled gene markers.

Genetic tests in the broad sense include:

- analysis of quantitative or qualitative changes to the genetic products (m-RNA, proteins; essentially all routine biochemical tests);
- chromosome analysis (classical, molecular cytogenetics);
- cell function tests (e.g. microscopic investigation of sickle cell anemia);
- measurement of metabolic products (e.g. amino acids [phenylketonuria, etc.])
- clinical tests (e.g. using a colour sense table if colour blindness is suspected).

Using the definition of ‘genetic tests in the narrow sense’, the following special features are worth noting:

Genetic tests are extending the potential of predictive medicine. This means that as far as some mutations of human hereditary material are concerned, it is possible to examine the person seeking advice and to predict (with varying degrees of accuracy) whether that person is likely to contract a specific illness.

These predictions often embrace a long period of time and it is not unusual for them to cover several decades before the first symptoms (manifestation) can occur (**presymptomatic diagnosis**).

A distinction is often made between this type of test and those genetic tests which give an indication of whether there is an inherited disposition towards an illness which can only occur after a long period of time in interaction with one or more environmental factors (**diagnosis of a genetic predisposition**, see 2.1).

However, in terms of predicting the onset of the first symptoms, there is still no adequate certainty for many diseases. The understandable fear that the fate of a person's health is being foretold as in a "crystal ball" is therefore – at least given the current state of knowledge – unrealistic. The execution and application of such a test is usually not compulsory from the medical point of view since there is still no cure for most diseases. However, a new dimension emerges with certain diseases when the knowledge of the presence of a certain disease-causing mutation or mutations, such as in the case of Huntington's chorea, can also have an impact on unaffected relatives. This can cause a lot of stress to the carrier of the mutation and/or his/her family.

Predictive value of a test

The **validity of a test** is understood as its capacity to discriminate. This means, for example, its capacity to distinguish between persons with and persons without a predisposition for a certain illness. A distinction can be made between four different validity parameters:

– **Sensitivity**: Proportion of test positives out of all affected persons (true positives)

– **Specificity**: Proportion of test negatives out of all unaffected persons (true negatives)

– **Positive predictive value**: Proportion of affected persons out of test positives

– **Negative predictive value**: Proportion of unaffected persons out of test negatives

How good a test is can be determined by the following formulae:

Test validity

Illness / Test result	present	not present
positive	A	B
negative	C	D

A: True positive results
B: False positive results
C: False negative results
D: True negative results

Sensitivity: A/(A+C)
Specifity: D/(B+D)
Positive predictive value: A/(A+B)
Negative predictive value: D/(C+D)

Figure 13

Although sensitivity and specificity are dependent on each other, as distinguishing characteristics of the test procedure they are independent of the prevalence of the disease under test. On the other hand, the positive predictive value increases with the prevalence of a disease and the negative predictive value decreases. **If a disease is rare, the same test will therefore give more false positives than if it occurs more frequently**. Therefore a diagnostic test which is well-suited to correctly identify affected persons in a group of patients with possible indications of a certain disease (high positive predictive value) may turn out far worse as a screening test (low positive predictive value).

There is another problem that emerges in particular with regard to medical genetics in this connection. Genetic tests may have predictive force in families for which the tests were developed, but these families only represent a subgroup of all cases of the illness and they are often selected in terms of the unambiguous nature of the genetic material (high penetrance, see 2.3.3). However, in a larger-scale population study, this test could have considerably less sensitivity, namely when there is a high degree of genetic heterogeneity or the penetrance is reduced on average in the population.

Therefore the validity of genetic tests must often be empirically underpinned. Such confirmatory studies are all the more extensive the longer the lifetime period in which the illness can still manifest itself. The following possibilities are available: comparison of test results in certain affected and certain unaffected population groups; prospective studies (follow-up of test positives and test negatives); comparison of the proportion of test positives with the expected values from independent epidemiological studies.

A distinction must be made between the validity of the test and the **reliability** of a procedure. A test must measure what it has been set up to measure and it must produce essentially the same result when it is repeated on the same sample. Internal and external laboratory standards, internal and external quality controls, group experiments, voluntary quality control groups and other measures are required to achieve an adequate degree of reliability.

7 Public policy on genetic testing in the UK

There are many concerns about how genetic tests may be used or abused by the insurance industry, employers and society in general. These concerns are valid and have been expressed in a number of formal and informal ways.

The Association of British Insurers (ABI) initially stated that the industry had no desire to ask for any genetic tests to be carried out, but reserved the right to see the results of tests already performed. However, the House of Commons Select Committee suggested that this stance should be reappraised. This led to the ABI proposing a change whereby historical genetic test information would be ignored on mortgage-related business up to £ 100,000 [approx. 150,000 euros]. Some individual companies have even gone beyond this.

Since then the ABI has issued a draft Code of Practice. In addition the Human Genetics Advisory Commission has looked at the implications of genetic testing for insurance and indicated that it feels the industry has not gone far enough, suggesting that a moratorium on all genetic test information should be applied.

Important features of the ABI's code include:

– Insurance companies will not insist on genetic tests.
– Genetic test results will only affect insurance if they show a clearly increased risk of illness or death. A low increase in risk will not necessarily affect the premium.
– Insurance companies will always seek expert medical advice when assessing the impact of genetic results on insurance.
– Insurers may take account of a test result only when reliability and relevance have been established.
– Applicants for insurance will not be asked to take a genetic test, but existing test results should be given to the insurance company when it asks a relevant question, unless it has said this information is not required.
– An applicant will not be required to disclose the result of a genetic test undertaken by another person (such as a blood relative), and one person's test information will not affect another person's application.
– The reason for an increased premium or rejection of an insurance application will be provided to the applicant's doctor on request.
– Insurers will not offer lower than normal premiums on the basis of a genetic test result.
– An independent adjudication tribunal is being set up to consider complaints which are unresolved.

It is clear that further debate and discussion will continue.

8 The situation in Europe

Europe's human geneticists belong to a wide variety of organizations.

The European Society of Human Genetics (ESHG) has existed since 1967. It is an international scientific society which in its turn is a member of a worldwide association of all scientific societies belonging to this discipline. The Internet address of the ESHG is: http://www.infobiogen.fr/agora/eshg. The ESHG publishes the "European Journal of Human Genetics". The ESHG is not only an organization dedicated to pure science, it is also dedicated to contributing towards a European-wide health policy in the field of medical genetics among other things by producing policy statements with the force of consensus guidelines.

The Human Genome Organization (HUGO), a worldwide union of scientists cooperating within the framework of the Human Genome Project (Internet address http://hugo.gbd.org), maintains a European head office in London (e-mail address: hugo@hugo-europe.org.uk).

Within the framework of the BIOMED research programme of the European Union there are countless research associations of scientists of the member states. Worthy of mention here is the Concerted Action of Genetic Services in Europe (CAGSE), which, under the leadership of Professor Rodney Harris of the University of Manchester, UK, has recently undertaken a review of the range of medical genetics services on offer in Europe (including a large number of countries outside the EU). A summary report will appear in one of the next issues of the "European Journal of Human Genetics".

Of immediate practical use is the European Directory of DNA Laboratories (EDDNAL), Internet address http://www.eddnal.com, which has a list of 280 service providers of genetic tests in numerous European countries and is updated at regular intervals (currently 385 different congenital diseases).

9 Prospects for the future – the biochip

The technical complexity and therefore the costs of carrying out genetic tests will fall considerably in the future and thereby they will become accessible to a broader spectrum of the population. Science and industry are working intensively on the automation of complex analytical steps. It will no longer be necessary to wait several weeks or even months for the results of genetic tests, which is still often the case today, but they will be available in a matter of days or possibly even hours. Whether one day such test kits can be obtained at the chemist's to be used in a do-it-yourself manner and thereby anonymously is still pure speculation and depends on advances in gene technology and public interest.

However, already underway is the development of the so-called biochip (gene chip, genetic chip), which can revolutionize gene technology just as the computer chip once revolutionized the electronics industry.

1. **Principle**: Similar to electronic chips, all possible variants of the DNA strand under investigation can be placed on the surface of a silicon chip (see Fig. 14). The DNA under investigation is replicated by PCR (polymerase chain reaction) and at the same time labelled by fluorescent substances which are incorporated in the DNA. When the DNA under investigation and the chip DNA coincide, a positive reaction is produced under ultraviolet radiation. Samples can be taken from finger prints, hair or drops of blood. In this way it is now possible to automatically analyse 20,000 to 30,000 genetic properties on 1 cm^2.

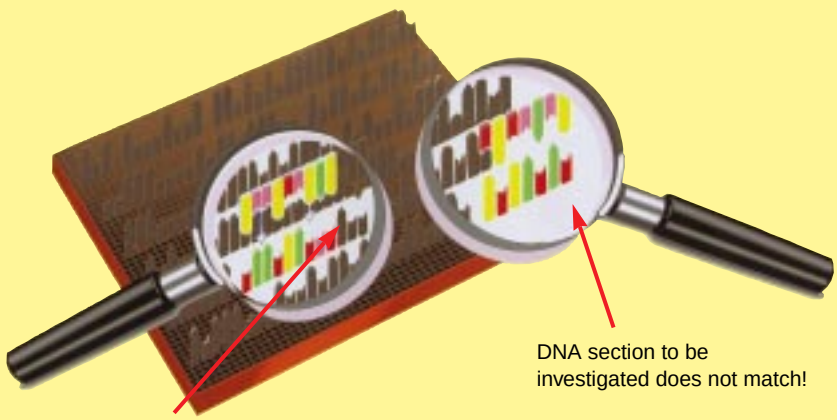


Figure 14

2. **Development:** The market leader in this development is a company in California which is collaborating intensively with several large international pharmaceutical corporations. In April 1996 it developed a so-called gene chip scanner. This system can be used to scan 65,000 different samples within 5 minutes. A leading scientist in this company has stated that within 1 to 2 years this number of samples will be increased to 400,000.

3. **Application:** The first biochip was used to search for mutations of the HIV virus and so to reveal potential resistance to treatment with protease and reverse transcriptase inhibitors. Several dozen prototypes are being tested at the moment for feasibility. As things stand at present, this new development looks likely to be used in the following areas:

- biomedical basic research
- screening for genetic predisposition to certain types of cancer (breast, ovaries, colon, melanoma, thyroid)
- monitoring the course of cancer (prognostic tests)
- monogenic diseases
- measurement of gene expression
- detection of infectious diseases avoiding extensive cell culture studies (tuberculosis, hepatitis C, chlamydia)
- monitoring antibiotics treatment for multiple resistance

4. **Impact:** With the DNA chip, routine diagnosis is reduced to a few minutes. Hundreds of experiments can be carried out at the same time. Apart from the speed, the advantage of this technique is a considerable reduction in costs and an increase in information. As a result, many genetic tests which were previously found to be too insensitive can in the future be developed in such a way that they are completely viable for screening.

Future applications will have a strong impact on the whole of medicine and also on insurance medicine, because to an extent not yet conceivable, early diagnostic tests (predisposition tests, screening tests), and also therapeutic analyses (“Which is the most effective drug?”) may become available. The time required for testing drugs prior to registration could be considerably reduced. Using the biochip, highly complex interactions, dependencies and information flows between individual genes can be investigated (**functional genetics**).

10 Conclusions

At present, the diseases that can be identified by genetic tests are almost exclusively monogenic diseases. There are practically no tests currently available for polygenic or multifactorial anomalies. In the light of our knowledge today, these would only have minor predictive value in individual cases because of the probability of environmental factors contributing to disease.

Powerful predictive genetic tests exist for accurately assessing the risks associated with the following monogenic diseases with a significant incidence in the population (prevalence 1–2%):

- Alzheimer's (several familial forms)
- hereditary breast cancer
- hereditary non-polyposis colon cancer (HNPCC – Lynch syndrome)
- thrombophilia (factor V Leiden deficiency)
- monogenic lipid metabolism disorders (LDL receptors, apolipoprotein B, apolipoprotein E, lipoprotein lipase)

The genetic tests for these anomalies are in part very complex and unsuitable for medically indicated screening in the general population. As described more closely in Section 3.1 opportunities for genetic testing in the population – mostly associated with family planning – are still only rarely taken. Thus the antiselection potential for the insurance sector is currently low.

Nevertheless, in view of the growing impact of genetics in medicine ("genetization"), it must be assumed that, in the future, genetic parameters will replace many conventional diagnostic investigative procedures as standard practice because of their objectivity.

11 Glossary of genetics

This glossary contains a small selection of the most important concepts occurring in genetics. It is meant as a quick guide.

Allele: different alternative forms of the same gene. Every human always has two alleles for the same gene (double set of chromosomes).

Autosomes: chromosomes present in the same number in men and women. They should be distinguished from the sex chromosomes X and Y. The concept autosomal (autosomal dominant or autosomal recessive inheritance) refers to genes and chromosome segments which lie on the autosomes.

Base pairing: In a DNA double strand there are always two bases opposite each other: adenine to thymine and cytosine to guanine.

Bases: constituents of the nucleotides. The genetic information is stored in the order of the four bases, adenine, thymine, guanine and cytosine.

Carrier: carrier of a genetic mutation, in recessive inheritance without increased risk of a genetic disease.

Cell nucleus: the “control centre” of a cell surrounded by a membrane. It contains the chromosomes.

Centromere: chromosome region at the centre of the chromosomes. At this point each of the already duplicated chromosomes is pulled into one of the two developing daughter cells.

Chromatin: material from which the chromosomes are made: DNA, proteins (histones and non-histone proteins).

Chromosome: the structures of the cell nucleus containing the hereditary material.

Clone: a collection of genetically identical cells or organisms.

Codon: sequence of three nucleotides (triplet) of DNA or RNA, which is responsible for translation into a specific amino acid.

DNA (deoxyribonucleic acid): a double-stranded giant molecule which stores the genetic information in its nucleotide sequence.

DNA sequence: linear sequence of nucleotide units.

Dominant: an allele which will “prevail” in the heterozygote state.

Exon: sequence section of a gene which is repeated in the mature m-RNA and contains the information for the amino acid sequence of the protein.

Expression: transformation of the information coded in a gene into a biological activity.

Gene: basis for inheritance. A gene consists of an individual nucleotide sequence which determines the amino acid make-up of proteins.

Gene bank: collection of cloned DNA fragments which represents the complete genome or parts of it.

Gene technology: procedures for changing the genetic information.

Gene therapy: treatment of illnesses by altering the genetic information in human cells (e.g. infiltrating an extra gene – gene transfer). A distinction is made here between so-called somatic gene therapy (intervention only in body cells) and germ line cell therapy, which can lead to hereditable changes.

Genetic fingerprinting: utilization of DNA polymorphisms for producing a genotype specific to the individual. There is a characteristic pattern for every person.

Genome: the entire genetic information of a cell or a living organism (in humans: about 3 thousand million base pairs per single chromosome set).

Germ line: cell sequence from the fertilized egg cell to the germ cells (egg or sperm cells) of the new life form.

Gonosomes (sex chromosomes): In men the gonosomes consist of an X and a Y chromosome; in women the gonosomes consist of two X chromosomes. The Y chromosome therefore determines the sex.

Hemizygosity: presence of a single allele since the gene location only occurs once (on the X chromosome of the male sex).

Heterogeneity: The same illness results in different patients from mutations at different gene locations (**locus heterogeneity**) or from different mutations at the same gene site (**allelic heterogeneity**).

Heterozygosity: presence of two different alleles at the same gene site.

Homozygosity: The two alleles at the same gene location are identical.

Hybridization: combining two single strands to a complementary DNA double strand.

Intron: (“intervening sequences”) non-coded section of a gene (or m-RNA).

Karyotype: the chromosome set of an individual (in humans 23 chromosome pairs) (see also Fig. 1).

Meiosis: special division of the cell nuclei in the germ cell precursor cells which leads to reduction of the chromosome set from the diploid state (double set) to the haploid state (single set).

Messenger RNA: messenger molecule which takes the genetic information for a protein from the cell nucleus to the place for protein biosynthesis.

Mitosis: process in the cell nucleus which leads to the production of daughter cells which have the same composition as the mother cells.

Molecular genetics: the science which investigates the inheritance processes at the level of molecules (DNA).

Mutation: alteration of the genetic material.

Nucleotide: basic units of DNA, comprising a sugar (ribose, deoxyribose), a purine or pyrimidine base and a phosphate group.

PCR (polymerase chain reaction): procedure for replicating DNA sections in the test tube, in other words a type of “molecular copying machine”.

Penetrance: the probability or frequency with which the action of a gene is manifested (e.g. number of affected persons out of all those carrying the pathological genes).

Phenotype: the way an individual appears (e.g. blood group, hair colour, blood pressure), which results from the genotype and environmental influences.

Polygenic traits: traits which derive from several genes.

Polymorphism: gene modifications occurring in the population (mutations) which in general do not produce any disease.

Protein: As a rule proteins consist of polypeptide subunits = amino acid chains. The information for the sequence of amino acids is coded by the genetic information of the DNA.

Purine bases: adenine (A), guanine (G).

Pyrimidine bases: cytosine (C), thymine (T).

Recessive: genetic expression of alleles at a gene location which is only manifested in the homozygote state.

Replication: identical duplication of DNA.

Ribonucleic acid (RNA): nucleic acid which differs from DNA in that instead of the base thymine, the base uracil is systematically substituted.

Sequencing of DNA: determination of the sequence of nucleotides within DNA.

Telomere: the end of a chromosome.

Transcription: the first stage of protein biosynthesis by formation of a messenger RNA (m-RNA) to transfer the DNA information from the nucleus to the cytoplasm.

Translation: second stage of protein biosynthesis. Here the genetic information transferred by the m-RNA is read off and converted to a corresponding sequence of amino acids.

12 Index

Sources of figures

Title page	Boehringer Ingelheim, pharmaceutical company, Ingelheim
Fig. 1	Centre for Human Genetics, Medical University of Hannover, Prof. Schmidtke
Fig. 2	E. Passarge, Taschenatlas der Genetik, Thieme Verlag
Fig. 3	E. Passarge, Taschenatlas der Genetik, Thieme Verlag
Fig. 5	E. Passarge, Taschenatlas der Genetik, Thieme Verlag
Fig. 6	E. Passarge, Taschenatlas der Genetik, Thieme Verlag
Fig. 8	E. Passarge, Taschenatlas der Genetik, Thieme Verlag
Fig. 10	Centre for Human Genetics, Medical University of Hannover, Prof. Schmidtke
Fig. 12	Centre for Human Genetics, Medical University of Hannover, Prof. Schmidtke