Hum Genet (1995) 95:127–148

REVIEW ARTICLE

Ulrich Wolf

The genetic contribution to the phenotype

Received: 21 April 1994 / Revised: 16 September 1994

Abstract The phenotype is the result of ontogenetic development. This holds true also at the molecular level, because molecular biological processes take place within the organism. In ontogenesis, genetic and nongenetic factors interact in producing successive states, each of which is the prerequisite, and determines the conditions, for the next one to follow. In this interplay, genes are a necessary, but not sufficient, component. The structures already present, gradients, threshold values, positional relationships, and conditions of the internal milieu, are equally essential. Thus, even monofactorial traits can be considered to be of multifactorial causation, and the varying borderline conditions that arise during development add to the complexity. From this standpoint, it is not to be expected that a mutation has a consistent phenotypic outcome, and the genotype-phenotype relationship may be irregular. In the present review, genotypic heterogeneity versus phenotypic heterogeneity is discussed with the help of some selected examples of hereditary diseases. Conditions and mechanisms contributing to this heterogeneity are addressed. It is concluded that the genotype-phenotype relationship is neither unidimensional, programmatical nor hierarchical in a strict sense. Nevertheless, in particular cases, ontogenetic modification appears to be of minor significance, so that the phenotype of a mutation can be predicted with considerable accuracy. This is no surprise if, depending on the nature of the mutation and the physiological function of the gene affected, the genotype-phenotype relationship is direct. However, this relationship may also be consistent in more complex conditions. It is assumed that the total of the non-genetic influences (epigenetic, environmental) are usually so similar or are compensated by the organism to such an extent that the respective mutation acts as the major variable during ontogenetic development.

This article is dedicated to Professor Marco Fraccaro in friendship

U. Wolf
Institut für Humangenetik und Anthropologie
der Universität Freiburg, Breisacher Strasse 33,
D-79106 Freiburg i. Br., Germany

Introduction

Phenotypic variation forms the basis, and is the starting point, for genetic investigation. The range of variation comprises what is considered to be normal variability, and pathological conditions as well. The genetic approach assumes that phenotypic characters are either determined or influenced by the genetic constitution of an organism. Thus, either the structure of a single gene or the interaction between several genes is considered to be responsible for, or to contribute to, phenotypic variation. However, the analysis of the correlation between genotype and phenotype has to take into account various problems involved with the realization of the phenotype. There are a number of factors that can interfere with this process, by modifying particular steps or by changing the boundary conditions altogether. Among these, epigenetic modifications, the successively changing conditions during ontogenetic development, and influences of the external environment may be envisaged. The phenotype is, thus, the product of ontogenetic development rather than the mere consequence of the genetic constitution of the zygote. Ontogenesis takes place within a framework of conditions that form the scenario for the processes realizing the phenotype. The principles inherent in ontogenesis are not the subject of the present article, and will be addressed separately (U. Wolf, manuscript). Semantic problems have also to be considered, and the terms used should be defined in order to interpret properly the complex interactions. Indeed, genetic terminology is metaphoric to some extent; in particular, the language of informatics has been largely adopted, suggesting that biological systems function in an analogous way to a computer. Thus, the current terminology already carries concepts and interpretations in an unavoidable way.

Semantic considerations

If we study genotype-phenotype relationships, it should be defined first, on which level of the underlying processes we are dealing with a phenotype. The total DNA forming the genome is no doubt the genotype. As far as the DNA is transcribed, though differentially at many gene loci, and the RNA is translated after its processing, there exists a one-to-one specification between DNA and RNA, and between RNA and the amino acid sequence, while the structure of a functional protein may not be unequivocally determined by its amino acid sequence. Because of this specification, the phenotypic level could be defined as commencing with proteins. However, only part of the DNA is transcribed into RNA, and only part of the transcripts are processed to serve as the mRNA that is translated into amino acid sequences; other RNA fractions are functional end products, such as tRNA and rRNA, or they become degraded immediately. Thus, there are clear deviations from a one-to-one equivalence between DNA, RNA or protein, and these deviations vary between cells, organs, and individuals. RNA clearly differs from DNA, despite its complementary nature to DNA, in its qualitative and quantitative representation within a given cell, and consequently contributes to phenotypic variation. The phenotype can thus be defined as the total of markers or characters of an organism apart from the DNA itself.

The terms "hereditary" and "genetically determined" are generally used synonymously. A phenotypic character or marker is considered to be genetically determined if its distinction from other markers or characters has a mere genetic cause, i.e., if it can be traced to a definite structure or change of this structure at the DNA level. In contrast, however, heritability is a statistical description of phenotypic similarities between, e.g., parents and offspring. The occurrence of a particular character, e.g., a disease phenotype, in families and even its apparent transmission from one generation to the next need not necessarily be genetic or only genetic. It may be determined by various factors, including genetic influences, and is thus multifactorial. However, a character may also appear to be hereditary, not because of a particular mutation, but because developmental conditions and constraints are similar or because environmental factors operate in the same direction. In this case, the character is a phenocopy. Therefore, the mode of inheritance may not always be conclusive regarding whether a character is hereditary.

The term "epigenetics" is used within the context of ontogenetic development, and refers to the interactions between genes and their products, and the various other conditions composing the milieu required for developmental processes to take place. Epigenetic changes are the result of these interactions, and may contribute significantly to the phenotype.

A lasting debate has tried to single out the hereditary versus environmental portions in the realization of the phenotype. The concept of heritability refers to interindividual variability within families or populations, and attempts to define the genotypic variance as a fraction of the phenotypic (total) variance. This distinction is based on the assumption that endogenously programmed processes take place that are invariant, but that are modified by an interfering environment. The stability of a characteristic in heredity, however, depends on the stability and range of variation of the developmental process resulting in this characteristic, and this is not just a matter of the stability of the genotype, but also of that of all the other conditions involved. Thus, heredity and environment are neither alternative nor independent causes for the manifestation of a characteristic. The organism organizes its environment and the environment acts upon the organism's structural and functional organization by selective reciprocal interactions.

Ontogenetic development is considered to be a succession of stages in which each particular stage requires the realization of the preceeding one. The successive stages are the result of interactions between internal and external factors and conditions, and this process does not allow for the separation of a hereditary and an environmental portion. In addition, it is almost impossible to define whether a particular component belongs to the hereditary or environmental complex: a gene finds itself in the environment of other genes that may interact, there are position effects and gradients of inactivation, the prospective fate of a differentiating cell depends upon its spatial position within the embryo or tissue, etc. The organism does not function like a computer running a program and producing results that are independent from any variables outside that program. Development is a historical process, and therefore, each developmental step is unique, as are other historical events.

Exposition of the problem

When analyzing the genotype-phenotype relationship, nevertheless the problem will be to dissect, in the case of a monofactorial trait, the range of effect of a particular gene. In the case of a polygenic or multifactorial trait, attempts can be made to estimate the number and the nature of the genes involved and the possible contribution of each to the manifestation of the trait. In the light of the considerations mentioned above, however, the relationship between the genetic contribution and the phenotype, even in the case of Mendelian inheritance, is expected to be complex, initially at the protein level, and more so beyond.

An indication supporting this assumption can be derived from a comparison of the number of Mendelian traits known in man and the number of genes estimated to be contained in the human genome. The catalogue of McKusick et al. (1992) lists 5710 entries, whereas 60000–70000 genes are expected to be functional (Fields et al. 1994). Many genes, if changed or eliminated by mutation, will not result in a readily distinguishable phenotype. On the other hand, many genes will show a phenotypic mani-

festation, but escape phenotypic detection. The simplest explanation for this paradox is the assumption that mutations in different genes result in the same or a similar phenotype.

Thus, in the case of Mendelian characters, the following possibilities may be envisaged as contributing to a distortion of a one-to-one relationship between genotype and phenotype. (1) One and the same mutation may result in different phenotypes. (2) Mutations at different sites of the same gene may cause either the same or different phenotypes. (3) Mutations in different genes may result in the same phenotype. (4) A mutation may have no phenotype at all. In the case of polygenic characters, additive effects of the genes involved, interactions between genes, and indirect effects on gene expression should be considered.

Chromosome aberrations should also be taken into account because they produce recognizable, but overlapping, phenotypes. They may indeed provide some insights into the basis of multifactorial disease, just because of their relative nonspecificity and phenotypic overlap.

While the above mentioned possibilities will be examined by analyzing some examples at hand, it is foreseeable at the outset that the phenotype is not a mere consequence of the genotype or karyotype. The genotype itself and the chromosomes are not invariant entities. They are prone to mutations arising by endogenous mechanisms and exogenous agents. This is true both for germ cells and for somatic cells; in the latter case, mutations may contribute to the phenotype even if the constitutional genotype is not affected by the respective mutation.The expression of genes is modified, restricted, and canalized (Waddington 1942) by epigenetic interactions during development. Developmental constraints further narrow the spectrum of phenotypic manifestations. Moreover, in pathogenesis, secondary interactions, e.g., between metabolites, play a major part.

It can be envisaged that genetic and epigenetic interactions modify the phenotypic manifestations of genetic disorders in varying degrees depending on the number of genes involved and the types of mutations. Thus, in monofactorial traits, variation is expected to be smaller than in polygenic traits, and to be most pronounced in multifactorial and chromosomal disorders. Some modifying conditions will now be briefly addressed.

Mechanisms and conditions in development influencing the genotype-phenotype relationship

Somatic mutations

Mutations can have an effect on the phenotype; indeed, the main aim of the present article is to evaluate the relationship between genotypic and phenotypic change. If the mutations considered are constitutional, they may have consequences, depending on the kind of mutation, at any time during ontogenetic development and for any cell of the organism. However, somatic mutations may also show phenotypic manifestations, even if only in a circumscribed way. As in constitutional mutations, somatic mutations may be of various kinds, including gene mutations, duplications and deletions, amplification, expansion and reduction, and chromosome aberrations. These mutations result in a mosaic composition of "normal" (constitutional) and mutant cell lines.

Chromosomal mosaics, by definition, are of postzygotic origin. They are usually caused by mitotic non-disjunction and result in cell lines with mono- or polysomies of individual chromosomes. In rarer cases, mosaics of chromosome structure are also found. Compared with the respective constitutional aberrations, chromosomal mosaics are more mildly affected or even normal clinically (e.g., as in mosaic trisomy 21 or mosaic monosomy X). Trisomy of chromosome 8 is an example of a chromosome aberration that is only viable as a somatic mosaic (Riccardi 1977; de Grouchy and Turleau 1982).

Rearrangements of DNA sequences, as in the case of the immunoglobulin and T cell receptor genes, are to be considered as a regular process of cell differentiation and not as mutations. However, somatic mutations contribute considerably to immune cell and antibody diversity.

Trinucleotide repeat expansion is another change at the genomic level occurring not only during gametogenesis (see below), but also during postzygotic development resulting in a mosaic constitution. An expansion may contribute to the phenotype, depending on the number of repeats and the distribution of cells with different repeat sizes within the body. In myotonic dystrophy (DM), as in the other triplet repeat expansion syndromes, a progressively earlier age of onset and therefore increasing severity of the disease in successive generations is known. This anticipation is correlated with an increasing number of repeats of a CTG trinucleotide found within the DM gene. However, considerable variation in the relationship of repeat expansion and age of onset has been ascribed to the mitotic instability of the repeat, which results in somatic mosaics with different repeat lengths (Hunter et al. 1992). In Huntington disease, a CAG repeat is expanded, and repeat mosaicism occurs in a tissue-specific way, affected regions of the brain showing the largest repeat lengths (Telenius et al. 1994). Furthermore, somatic mosaics for varying sizes of a CGG repeat in the FMR-1 gene have been demonstrated in the fragile X syndrome (Wöhrle et al. 1993), but a correlation with the clinical phenotype has not yet been established. However, the degree of methylation of the repeats appears to influence the phenotype, and methylation mosaics may develop a milder form of the disease (P. Steinbach, personal communication).

Gene mutations occurring in somatic cells during development also contribute to phenotypic variation and may result in disease phenotypes of varying severity, depending on the relative abundance and the tissue distribution of mutant cells. Examples are presented in Cooper and Krawczak (1993, p. 296 ff), one of which shall be quoted here as an illustration. A lethal mutation in the COL1A1 gene resulting in osteogenesis imperfecta was present in mosaic form in a mildly affected male, whereas his son exhibited the full extent of the disease and was not

viable. It was concluded that the father also had germline mosaicism (Wallis et al. 1990). Similar findings have been reported for numerous other mutations.

Mutations in tumor susceptibility genes occurring during development or even later in the life of an organism can give rise to particular tumors. According to Knudson (1971), a heterozygote for a recessive tumor susceptibility gene is predisposed because the wild-type allele may become nonfunctional by somatic mutation or other mechanisms, resulting in the loss of heterozygosity and, in consequence, the tumor phenotype. Even a normal homozygote may develop the tumor if both alleles of a somatic cell lose their function successively. This hypothesis has been confirmed by abundant cases, including many different tumor susceptibility genes.

Mitochondrial diseases may also develop by somatic mutations and by processes of differential proliferation during lifetime (Wallace 1992). A mutation in the mitochondrial genome may be present as a minority population in the fertilized egg, or may arise later as a somatic mutation in a proliferating cell. The cell thus harbors a mixture of normal and mutant mitochondria (heteroplasmy) that segregate randomly during cell division. Subsequently, in certain cell types, mutant mitochondrial genomes may become a majority by chance, resulting in the onset of disease. In the case of deletions, defective mitochondrial DNA may also progressively increase because it is more rapidly replicated than full-size mitochondrial DNA (Wallace 1992). A wide spectrum of degenerative diseases may be caused by these mechanisms.

In connection with somatic mutations, environmental mutagens/carcinogens should be mentioned, some of which act specifically on certain cell types, tissues, or DNA sites. Some agents become mutagenic only after being processed by the metabolism, a mechanism that could be considered as epimutagenesis.

Thus, the genotype itself is not invariant during ontogenesis. Since somatic mutations can contribute to pathology, they may also influence normal phenotypic variation, apart from intrinsic mechanisms as in the case of the immune system.

Phenocopies

The occurrence of phenocopies demonstrates that phenotypic characters (disease phenotypes), which are known to be the consequence of mutations, can also be produced by factors other than genetic influences. There are cases in which the underlying mechanism is well understood. For example, adrenogenital syndromes can be caused by mutations resulting in 21-hydroxylase deficiency; however, a similar phenotype develops because of adrenal tumors in the mother during pregnancy (Calaf et al. 1994). Embryopathies caused by teratogens or other factors interfering with morphogenesis can mimic Mendelian traits, for example, in thalidomide embryopathy mimicking the Holt-Oram syndrome at least at a young age (Hurst et al. 1991), or cleft lip, deafness, and polydactyly. Congenital malformations associated with chromosome aberrations can also occur in individuals with a normal karyotype, only with a considerably lower frequency, and not necessarily in a combination indicative of a particular chromosome disorder (Shapiro 1983). Phenocopies thus clearly demonstrate, apart from the trivial fact that knowledge of the genotype cannot at once be derived from an individual phenotype, that similar discrete phenotypic characteristics can originate from categorically different causes.

Non-Mendelian inheritance

For a number of phenotypic characters known or assumed to have a monofactorial basis, deviations from Mendelian modes of inheritance are observed. This applies to genomic imprinting, X inactivation, and trinucleotide repeat expansion.

Genomic imprinting (or gametic imprinting; Barlow 1994) and X inactivation can be considered to be epigenetic mechanisms (Lyon 1993). These phenomena are basically reversible. They are transmitted during mitotic proliferation and may be temporal and/or tissue specific. X inactivation will be addressed below. Gametic imprinting results in the differential activity of alleles, depending on their parental origin (for a review, see Peterson and Sapienza 1993). Heterozygotes for a particular mutation (deletion) may therefore express the mutant phenotype only if the mutation was transmitted either by the father (as in the case of the Prader-Willi syndrome), or by the mother (as in the case of the Angelman syndrome). Thus, only one parental sex transmits the phenotype, a mode of inheritance that is incompatible with Mendelian genetics. Primary imprinting takes place in the gametes or in the early zygote, and is erased again (at the latest) in the germ cells. Postzygotic modifications are also involved, in particular DNA methylation, which appears to be secondary to gene expression/repression; epigenetic chromatin configurations, replication asynchrony, and transacting factors have also been discussed (Surani 1994).

Trinucleotide repeat expansion occurs during gametogenesis, or early postzygotic development. A particular disease phenotype becomes manifest, depending on the number of repeats expanded. The X-linked FMR-1 gene includes a tandemly arranged repetitive sequence of CGG trinucelotides. In patients affected by the fra-X syndrome, the CGG repeats are amplified to about 1000 or more. The syndrome can be transmitted not only by female heterozygote carriers, but also by unaffected (hemizygous) males who show expanded repeat numbers of 60–230 compared with normal controls who have an average repeat number of 29. Repeat expansion can occur during oogenesis of female carriers, but also at early postzygotic development, resulting in somatic mosaics as discussed above. Unaffected paternal carriers of X-linked diseases are not compatible with Mendelian genetics, and the phenomenon is explained by mechanisms occurring during development.

The triplet repeat expansion syndromes known so far are characterized by increased severity and/or an earlier

onset of the disease in successive generations, a phenomenon termed anticipation. The triplet repeats involved pass a labile stage of expansion, and the large number of repeats characteristic for the manifestation of the disease may be generated during gametic and early somatic development. The phenomenon of anticipation is another deviation from classical Mendelian genetics.

Nonspecific factors involved in signal transduction

Factors serving as signals for cell proliferation, cell differentiation, and various cellular responses are often nonspecific. They can bind to target structures (receptors or particular binding sequences) of different cells or cellular components, thereby stimulating different effects. The specificity required does not involve the signal factor itself, but rather the receptor, the machinery coupled with the receptor, or selective mechanisms at the target site. The signals may be gene products (proteins), e.g., proteohormones, growth factors, transcription factors, small peptides, other small molecules such as neurotransmitters and steroid hormones, or even ions. The signal transduction processes show that largely nonspecific factors are used as signals by the cell or within the cell; these signals are transduced in successively more specific ways, eventually resulting in a specific reaction or response. The processes cannot therefore be considered to be programmed by the genome, as they depend on the availability and the number of the factors required, feed back mechanisms and epigenetic interactions. Because of the mechanisms involved, signal transduction contributes considerably to phenotypic variation.

Spatio-temporal patterns

In the early postzygotic embryo, some cells are not yet committed to a specific pathway of differentiation; they are totipotent or pluripotent. However, at defined developmental stages, and depending on its position within the embryo, a particular cell becomes restricted in its potential, maintaining a committed state, named the positional value, even if transplanted into a different cellular environment. The positional value may be brought about by a concentration gradient of a morphogen that, through threshold effects and feedback amplification, stimulates differential gene expression. This process is usually not reversible; the cell "remembers" the effects to which it was exposed, during a sensitive period, in a particular spatial context. This process may recur several times, resulting in an increasingly refined pattern of positional values.

If a cell or blastemic tissue fails to become committed to a particular pathway of differentiation, it may follow a different morphogenetic pathway. For example, in the male embryo, the indifferent gonadal blastema, in the presence of the testis-determining factor (TDF), develops into a testis. The TDF gene has been shown to be expressed only during a short period, viz. at the time of primary testis differentiation. It has been hypothesized (Burgoyne 1989; McLaren 1991) that delayed TDF expression could be the reason for XY sex reversal. The supporting cell lineage, if not committed to the male pathway because of a "timing mismatch" of TDF expression, differentiates into follicular cells, and sexual development follows the female pathway. It can be easily imagined that, depending on the extent of the delay, various conditions of intersexuality could arise. The reasons for delayed gene expression may be genetic or epigenetic.

Chromosomal effects on the phenotype

X inactivation normally appears to be a random process taking place in early female embryogenesis of eutherian mammals. With the exception that some chromosome segments or individual genes escape inactivation, an entire X chromosome is inactivated. The mechanism of inactivation is not yet fully understood (McBurney 1993), and a single gene, the Xist gene, active on the inactive X chromosome and vice versa seems to be involved (Brown et al. 1991). Differential methylation plays a role in the expression of the Xist gene, indicating that X inactivation is developmentally controlled (Norris et al. 1994). The randomness of the process implicates that the number of cells committed to a particular developmental pathway, with either an active maternal or active paternal X chromosome, varies. If cells are not yet committed at the time of inactivation, further variation is introduced by growth dynamics. Since the imprint is stably transmitted to successive cell generations, the mosaic composition of a particular tissue, depending on the developmental processes, the size of the stem cell pool at the time of inactivation, and possible selection, may deviate considerably from the statistical mean, and an X-linked recessive phenotype may become manifest. Thus, stochastic events and epigenetic interactions can be constitutive for the phenotype.

Chromosomal imbalance arises from duplications and/or deficiencies of entire chromosomes or chromosome segments, the consequences of which have been extensively discussed by Epstein (1986). Primarily, gene dosage is affected, and therefore, the phenotypic outcome is assumed to be the result of gene dosage effects. These dosage effects may not be proportional, and also, because of regulatory impairment, genes on nonaffected chromosomes could be secondarily involved (Holtzman and Epstein 1992). Chromosomal disorders are characterized by congenital malformations and mental retardation, and each pathological trait when taken alone is nonspecific, and can be found in individuals with a normal karyotype. There is considerable overlap of malformation patterns between different chromosomal aberrations. For these and other reasons, the specificity of the relationship between chromosomal imbalance and the resulting phenotype has been debated, and the concept of disrupted homeostasis has been put forward (Shapiro 1983). This concept implies that chromosomal aberrations disrupt the genetic balance and, in consequence, the developmental physiol-

ogy; morphogenetic processes could thus become prone to impairment by intrinsic and extrinsic factors. Despite admitting that decreased developmental homeostasis is an intriguing concept, Epstein (1986) emphasizes that it must be ultimately based on the imbalance of particular genes. In view of the genetic, epigenetic, and environmental interactions taking place during the development of the phenotype, it is not surprising that phenotypic variability is larger, and hence overlap between chromosomal phenotypes is wider, in the gross genetic imbalance caused by chromosomal aberrations compared with single gene defects. The quotation by Epstein (1986) from Weiss (1973) can be repeated here, viz., that phenotypic features are merely "the last scene of a long play of interactions".

The biochemical network

Each individual process studied in development is embedded in a network of interactions between genes and their products, metabolites, nutrients, and other environmental factors. The view can be taken that genes do not have a superordinate role in this interplay (Nijhout 1990). While gene expression can be regulated (in the case of non-constitutively expressed genes), the function of gene products can also be regulated, e.g., by allosteric mechanisms, feedback inhibition, rate of turnover, etc. Metabolism and biosynthesis are processes that are as specific and well controlled as is gene expression. Some disease phenotypes become manifest only under environmental challenges to metabolism (pharmacogenetics). Therefore, because of the molecular and biochemical interactions producing the phenotype, it appears to be arbitrary to give the genetic part a privileged rank.

Selected examples of relationships between genotype and phenotype

The question to what extent a change at the DNA level is represented at the phenotypic level on a one-to-one scale shall now be more closely examined by inspection of some well-documented examples. A principal difficulty that cannot be overcome is the multiplicity of variables involved, most of which are beyond experimental control or even unknown. At the genetic level, the phenotypic outcome will be influenced by the respective genetic background. Although some examples are known that suggest the interference of modifier genes, interactions between various genes remain the major possibility in all cases, and it may be difficult or even impossible to differentiate between epistatic and epigenetic effects. This also applies to single copy genes with exactly defined mutations following Mendelian modes of inheritance (see, for example, Suthers and Davies 1992). Therefore, conclusions can only be approximate, if not speculative. A whole range of deviations from a one-to-one representation of a particular mutation in a disease phenotype is expected to be found, and the borderline cases will be of particular interest, i.e. the realization of such a direct relationship on one hand, and on the other hand the absence of any relationship. Whereas monofactorial traits will serve as the more appropriate models, some more complex conditions will also be discussed, because they may shed some light on the problems to be considered.

Monofactorial traits: polypheny at identical mutations

The term polypheny usually applies to mutations that affect one and the same gene, and that result in different phenotypes. It has only become possible to identify the nature of the respective mutations since the advent of DNA analysis, and surprisingly enough, it turned out that polypheny is not only observed in the case of mutations at different sites or of a different nature within the gene considered, but also in the case of identical mutations. Thus, phenotypic variation based on allelic heterogeneity (heteroallely) can now be discriminated from polypheny associated with identical mutations (homoallely), i.e., polypheny in its strict sense. This phenomenon is expected to provide the clearest examples for the interaction of other genes or non-genetic factors during the development of a particular phenotype. The various examples at hand may be classified according to the mechanisms involved, e.g., the influence of environmental factors, the interaction with modifier genes, the cases that remain to be clarified, etc. However, such a classification will be provisional at the present state of analysis, and may remain so to some extent, because gene expression and the modified or impaired function of gene products are not isolated processes, but depend on all other variables interacting to produce a particular phenotype.

In the following, some examples are provided to illustrate this phenomenon. When refering to clinical variability in the case of an identical mutation, it can hardly be avoided to mention also heteroallely, and therefore, some of the conditions discussed here also represent examples of allelic heterogeneity.

A classical example is the hemaglobin beta-S mutation resulting in the substitution of valine for glutamic acid at position 6 of the β-globin chain. In the homozygous state, this mutation usually causes sickle cell anemia, which shows wide variation in clinical severity in different patients. Epistatic effects are believed to be responsible for this variation, and indeed, different genetic backgrounds or coinheritance of other hemoglobinopathies have been found to be associated with differing clinical severity of the disease, as discussed in detail by Cooper and Krawczak (1993, p. 300 ff).

Defects in the metabolism of phenylalanine result in different clinical phenotypes that have been subdivided into classical phenylketonuria (PKU), moderate PKU, mild PKU, and non-PKU-hyperphenylalaninaemia (Güttler 1992). A closer differentiation can be made at the metabolic level. Activities of phenylalanine hydroxylase (PAH) vary between 0% and 50% of normal activity and are significantly correlated with blood phenylalanine lev-

els. DNA analysis of the PAH gene allows for a still closer differentiation, and more than 70 allelic mutations have been reported so far. Several different mutations result in similar phenylalanine levels, and thus, the spectrum of mutations can be closely correlated with metabolic phenotypes. In consequence, the metabolic phenotype can be predicted rather exactly by mutation analysis (Okano et al. 1991; Eisensmith and Woo 1992). However, genotype analysis does not allow, in all cases, a definitive prognosis of the clinical phenotype, which includes mental performance. In a family with three sibs affected by the same mutation and showing the same metabolic phenotype, two sibs who were never on a phenylalanine-restricted diet varied widely in their intellectual performance, one being normal, the other severely retarded. The third sib was on a diet and was also normal (Di Silvestre et al. 1991). These findings are based on haplotype analysis and not on DNA sequencing, and therefore, a remote possibility of sequence differences remains. However, apart from this and other examples (e.g., Primrose 1983), mutation analysis is insufficient for the long-term prognosis of the clinical phenotype (Langenbeck et al. 1988; Scriver 1991; Eisensmith and Woo 1992; Güttler 1992).

The severity of muscular dystrophy depends on the nature of the deletion in the dystrophin gene. According to the age of loss of ambulation, cases are classified as Duchenne muscular dystrophy (DMD) with early onset (up to 13 years) and Becker muscular dystrophy (BMD) with late onset (beyond 16 years); intermediate severity has also been defined (13–16 years), because the clinical spectrum is quasi-continuous. A reading frame hypothesis has been put forward based on DNA analysis (Monaco et al. 1988); it predicts that DMD results from frameshift deletions, and BMD from inframe deletions. This hypothesis is valid in 92% of all cases (Koenig et al. 1989). However, a number of cases with identical deletions show either DMD or the intermediate phenotype (Hodgson et al. 1989). Mental retardation and short stature are symptoms not rarely associated with these muscular dystrophies, but they have not been found to be correlated with specific deletions (Hodgson et al. 1989). Thus, other factors, genetic or epigenetic, are assumed to be responsible for these variations in the clinical phenotype. In addition, asymptomatic individuals are expected to occur having in-frame deletions in regions of the dystrophin gene where no or only rarely deletions have been found so far (Koenig et al. 1989). If confirmed, this would provide an example of mutations with no perceptible phenotypic consequences.

Amyloid polyneuropathy (McKusick et al. 1992, no. 176300.001) is characterized by extracellular deposition of amyloid with peripheral and autonomic neuropathy, and in some patients with vitreous opacities. In this syndrome, a dominant mutation has been identified in the transthyretin gene (TTR) localized on chromosome 18q12.1 (Sparkes et al. 1987). The mutation, a substitution of methionine for valine at position 30 (TTR-met^{30}), is one and the same in families originating from countries including Portugal, Japan and Sweden (Saraiva et al. 1983; Tawara et al. 1983; Nakazato et al. 1987; Holmgren et al. 1988b). Considerable clinical variation for this mutation has been reported, depending on the genetic background of the respective populations, but also within a population or even within families. Thus, the age of onset of the disease in Sweden is much later (mean 53 years) then in Portugal (mean 32 years) and Japan (mean 32 years) (Holmgren et al. 1988b). In the Swedish sample, cases developing vitreous opacities as the first symptom show a significantly later age of onset than those presenting with sensory and autonomous neuropathy as the first symptom (Sandgren et al. 1991). Age of onset also varies with the sex of the transmitting parent (Drugge et al. 1993). In two homozygous sibs with the TTR-met^{30} mutation, the brother was affected by typical polyneuropathy and vitreous amyloid, whereas symptoms of the disease could not be demonstrated in his sister (Holmgren et al. 1988a). These examples show that the TTR mutation, although present in all cases, does not determine the disease phenotype per se, and that interaction with other factors must be responsible for the clinical variation.

Retinitis pigmentosa (RP) comprises a group of hereditary retinal degenerations that are heterogeneous genetically and to a lesser extent clinically. In this disorder, a number of different genes have been shown to be affected by mutation (see below), and allelic heterogeneity has also been described (Lindsay et al. 1992; Berson 1993). Among the genes concerned is the peripherin/RDS gene (rds = retinal degeneration slow in the mouse model of RP); an autosomal dominant form of RP results if this gene is defective. In an affected family, a three base-pair deletion in the peripherin/RDS gene has been identified, which, in different family members, is associated with three distinct phenotypes. The proband presented with adult-onset RP, and of her three children, one daughter developed pattern dystrophy of the pigment epithelium, whereas the other daughter and the son exhibited fundus flavimaculatus (Weleber et al. 1993). No other mutations or polymorphisms could be detected in the peripherin/RDS and related genes (ROM1, rhodopsin) in this family, but the interaction of other (unknown) genes cannot be excluded.

Autosomal recessive proximal spinal muscular atrophy (SMA) is clinically classified according to the severity of the disease as type I (Werdnig-Hoffmann), type II (intermediate), and type III (Kugelberg-Welander), which is the mildest form (Dubowitz 1978). All three types have been mapped to the same chromosome region, viz., 5q12-q14. Variation in severity has also been reported to occur within families, and different SMA types have been described in the same sibship. Müller et al. (1992) analyzed four sibships each exhibiting the coexistence of SMA types II and III; allelic heterogeneity could be excluded. Thus, the clinical differences are each associated with an identical mutation in the SMA gene, and other genetic or environmental factors are probably involved.

Adrenoleukodystrophy (ALD) is another condition characterized by conspicuous clinical variation (Moser and Moser 1989); a putative gene has been assigned to the

distal part of chromosome Xq28 (Mosser et al. 1993). In two sibs with an identical deletion in this gene, one brother developed cerebral ALD at 8 years, whereas the other showed mild adrenal insufficiency only at 13 years. Secondary factors are assumed to influence this condition (Mosser et al. 1993).

Marfan syndrome, a dominantly inherited disorder of connective tissue, is caused by mutations in the fibrillin gene FBN1 on chromosome 15q21.1 (Human Gene Mapping 11 1991; Dietz et al. 1991). This condition shows a high penetrance, but great variability in the manifestation of skeletal, cardiovascular, and ocular abnormalities. Wide variability with respect to age of onset and severity of the disease was also observed in the affected members of a three-generation family with an identical mutation in FBN1 (Dietz et al. 1992). It is concluded that the disease phenotype is determined by additional factors.

Patients with Denys-Drash syndrome have severe abnormalities of the urogenital system resulting in nephropathy, and they may develop Wilms' tumor. In XY individuals, gonadal dysgenesis is the most common genital abnormality, and in consequence, they have a female phenotype. In the majority of cases, mutations in the Wilms' tumor suppressor gene (WT1), which codes for a zinc finger protein, have been identified. The most frequent mutation found so far (≈ 55%) converts amino acid residue ^{394}Arg to Trp, affecting a zinc finger domain that is critically involved in DNA recognition. Pelletier et al. (1991) report a number of cases with this mutation and a wide spectrum of genital anomalies; in particular, in one case, a normal ovary was found, whereas another individual had streak gonads. Similarly, Baird et al. (1992) state that "there is no obvious correlation between the type of mutation and phenotypic expression"in Denys-Drash syndrome. Coppes et al. (1992) present a patient with the ^{394}Arg to Trp mutation, which was transmitted by his unaffected father. If the mode of inheritance in Denys-Drash syndrome is autosomal dominant as suggested by the molecular data, this case exhibits incomplete penetrance. As pointed out by Hastie (1993), "this difference in penetrance is likely to be either a reflection of developmental plasticity in the genitourinary system or of genetic background effects".

XY gonadal dysgenesis (XYGD) is a sex-reversal syndrome with streak gonads and female genitalia. In some 15% of the cases, a mutation has been identified in the Y-linked SRY gene believed to code for the testis-determining factor (reviewed in Wolf et al. 1992). Although most mutations are de novo, three familial mutations have been described, including normal male carriers and patients (Hawkins et al. 1992; Jäger et al. 1992; Vilain et al. 1992). It is speculated that this incomplete penetrance could be due, among other reasons, to a threshold effect or interaction with different alleles of other sex-determining genes, or both. Thus, XYGD presents an example of identical mutations resulting in different phenotypes (normal and affected), and of different mutations (allelic and non-allelic) resulting in a uniform clinical phenotype.

There are numerous reports on the concomitant occurrence of XX males and true hermaphrodites within families (Berger et al. 1970; Fraccaro et al. 1979; Skordis et al. 1987; McElreavey et al. 1993a). Patients may have bilateral or unilateral testes or ovotestes and varying degrees of genital ambiguities. In the cases of McElreavey et al. (1993a) and Skordis et al. (1987), SRY has been shown to be absent, and the reasons for partial or complete sex-reversal are still unclear. However, there is evidence that several genes are involved in sex determination, and the quantitative variation of humoral factors may also play a part (reviewed in Wolf et al. 1992; McElreavey et al. 1993b).

Monofactorial traits: allelic heterogeneity

Allelic heterogeneity refers to mutations at different sites, or of a different nature in one and the same gene or its pertaining regulatory elements. Allelic mutations may or may not have a different phenotypic outcome. They may produce completely separate phenotypes, a spectrum of related phenotypes of varying severity, or the same phenotype, or they may show no recognizable phenotype at all. In view of the large number of allelic mutations identified in some conditions, the number of discernible phenotypes appears to be extremely low. In part, this will be a reflection of the parameters considered. Thus, from a clinical point of view, only a rough classification may be possible, whereas biochemical analysis may unravel finer differences that are of little clinical relevance, and at the molecular level, several different alterations will result in the same biochemical (metabolic) phenotype. Various mechanisms may be responsible for this convergence of primary differences to a small number of phenotypes at higher levels of complexity. Needless to say, the structural or functional loss of a gene product can be the consequence of different mutations converging to the same phenotype, and the same is true for a reduction of the biological activity of the gene product. Homeostatic mechanisms will compensate for some of the primary differences, and threshold effects will be the reason for "all or none" reactions, restricting the possible spectrum of phenotypes. Ontogenetic buffering systems may thus eliminate or diminish one or the other primary defect.

If allelic mutations lead to completely different phenotypes, functional differences of the altered gene product can be envisaged. Loss of function will have other consequences than gain of function, and in the latter case, various alternatives are conceivable. A few selected examples are specified in the following.

The consequences of allelic heterogeneity are best understood in the hemoglobinopathies. With the exception of sickle cell anemia, mutations in the globin genes or their regulatory elements are dominant because of the tetrameric structure of hemoglobin, which consists of two pairs of non-allelic subunits. Practically all types of mutations have been found, including those affecting the structure or synthesis of hemoglobin chains, and the functional consequences are well explained by the respective mutations. Thus, the hemoglobinopathies provide an example

of a straight forward relationship between genetic and phenotypic alterations; this is no surprise, since the disease phenotype is the immediate consequence of the impaired function of the gene product itself. Therefore, in the present context, hemoglobinopathies will not be discussed in more detail, and the reader is referred to the literature (e.g., Vogel and Motulsky 1986, p. 278 ff).

The primary physiological defect in cystic fibrosis (CF) is believed to be a reduced conductance of chloride ions in the epithelial tissues affected by the disease. More than 400 mutations in the gene for the chloride ion channel protein, the cystic fibrosis transmembrane conductance regulator (CFTR), have been reported to be responsible for its dysfunction. In consequence, fluid secretion and salt absorption are impaired, and patients can show, among other symptoms, pancreas insufficiency and chronic obstructive lung disease. A significant correlation has been observed between the different CFTR mutations and the level of pancreatic enzyme secretion, although the severity of pulmonary disease varies independently of the respective mutations. Thus, unrelated CF patients with the same genotype (e.g., homozygotes for ΔF508) show a striking variation in lung function. In contrast, significant concordance between affected siblings has been found within pedigrees (Dean and Santis 1994). This difference points to the influence of other factors, as yet unidentified. Other symptoms, such as meconium ileus, occurring in some 10% of CF patients with pancreatic insufficiency, are also not associated with specific genotypes. It is generally believed that the severity and progression of the CF phenotype is strongly modified by additional factors that may be genetic and nongenetic, rendering prognosis difficult (Kerem et al. 1990; Tsui 1992; Dean and Santis 1994).

In the CFTR gene, skipping of different exons has been found in clinically normal individuals; it occurs mostly in a mosaic form caused by alternative splicing. This may represent a polymorphism, or it may contribute to phenotypic variation as discussed by Cooper and Krawczak (1993, pp. 304, 305).

More than 90% of patients with osteogenesis imperfecta have mutations in one of the two structural genes for type I procollagen, viz., COL1A1 coding for proα1(I) and COL1A2 coding for proα2(I). McKusick et al. (1992) list 34 allelic mutations in the COL1A1 gene (McKusick et al. 1992, no. 120150), and 25 in the COL1A2 gene (McKusick et al. 1992, no. 120160). Type I procollagen is a trimeric molecule consisting of two proα1(I) and one proα2(I) chains. Therefore, mutations in COL1A1 may cause a more severe form of the disease. In general, mutations resulting in abnormal proα chains (qualitative changes) are more deleterious than those affecting the quantity of the gene product. It has also been noted that mutations in different regions of the gene produce different phenotypes, from early lethality to moderate or mild forms occurring later in life or even overlapping with normal variation. Mild to moderate forms are less predictable than severe forms, as discussed by Prockop et al. (1990). Clinical heterogeneity occurring within families has also been described; development of the phenotype is complex, as reviewed by Prockop et al. (1990) and Byers (1990).

Among peripheral neuropathies, Charcot-Marie-Tooth disease type 1A (CMT1A) is the most frequently occurring type. In nearly 70% of patients, a stable duplication of 1.5 Mb has been found in the CMT1A region of chromosome 17p11.2-p12. Within the CMT1A region, a gene coding for a peripheral myelin protein has been localized, viz., PMP22. In cases carrying the duplication, PMP22 is overexpressed, and hence a gene dosage effect is believed to be the cause of the disease. However, in a number of cases without the duplication, point mutations have been identified in the PMP22 gene. These point mutations are usually dominant. Nevertheless, a family with a recessive mutation in the PMP22 gene has also been described with a similar disease phenotype (Roa et al. 1993). Thus, different mechanisms, e.g., duplication and structural alteration, whether dominant or recessive, in the same gene result in a similar phenotype. Clinically, variation of the disease phenotype has been observed even among members of the same family, pointing to additional factors influencing the course of the disease (Patel 1993).

Androgen insensitivity syndromes (AIS) are caused by mutations in the androgen receptor (AR) gene located on Xq11-q12 (Brown et al. 1989) and coding for a ligand-activated transcription factor. Some 80 AR mutations have been described, resulting in a spectrum ranging from nearly normal female to nearly normal male phenotypes. A rough clinical classification differentiates between a complete form (CAIS, testicular feminization), a partial (or incomplete) form (PAIS, ambiguous genitalia), and a mild form (MAIS, male external genitalia). A genotype-phenotype relationship is not yet well established. Mutations affecting the DNA-binding domain and those affecting the androgen-binding domain may cause various forms of AIS. In CAIS and MAIS, phenotypic variation within families is low, whereas it is high in PAIS, which covers the whole range of male to female phenotypes; a threshold effect has been proposed for PAIS (Pinsky et al. 1994). A third domain, called the modulator or transactivational domain at the N-terminal, includes a CAG-repeat normally represented by 11–31 trinucleotides, but expanded to 40–62 or more trinucleotides in spinal and bulbar muscular atrophy (SBMA, Kennedy disease). SBMA is often associated with MAIS (LaSpada et al. 1991). As in other trinucleotide repeat expansion syndromes, the age of onset is inversely correlated with an increasing repeat number. It is proposed that the expansion alters the transcriptional regulatory competence of the modulator domain without altering the androgen-binding activity (Mhatre et al. 1993). In two male patients with breast cancer, constitutional point mutations have been identified in codons 607/608 of the DNA-binding domain associated with PAIS (Wooster et al. 1992; Lobaccaro et al. 1993). These mutations are believed to increase the risk of breast cancer, and it is speculated that either the protective effect of the androgen is lost, or a new function is gained by these mutations.

In the RET gene coding for a receptor tyrosine kinase, different allelic mutations have been reported to give rise

to four different syndromes (van Heyningen 1994). The RET gene functions as a protooncogene and has been mapped to chromosome 10q11.2 (Ishizaka et al.1989). The gene consists of 21 exons and comprises various domains, including an extracellular ligand-binding, a transmembrane, and an intracellular tyrosine kinase domain. Mutations in the cysteine-rich region of the extracellular domain near the transition to the transmembrane domain (exons 10, 11) can result in familial medullary thyroid carcinoma (FMTC) or multiple endocrine neoplasia type 2A (MEN2A) (Mulligan et al. 1993; Donis-Keller et al. 1993). In the more severe MEN2B, mutations have been identified in exon 16 of the tyrosine kinase domain (Hofstra et al. 1994). Finally, in Hirschsprung disease (HSRC), mutations at various locations of the gene have been identified, including deletions and loss-of-function point mutations (Romeo et al. 1994; Edery et al. 1994). All the mutations in these four syndromes are constitutive and dominant with variable penetrance. In the cancer syndromes, the mutations identified so far result in amino acid substitutions compatible with the generation of an altered gene product that, in a dominant way, may initiate an oncogenic mechanism. In contrast, in HSRC, a gene product of the mutant allele is absent, resulting in a gene dosage effect. Therefore, haplo-insufficiency of the RET gene is considered to be a major component in the causation of HSRC.

The three cancer syndromes are phenotypically related, but are manifested with different severity. FMTC is the mildest form and only the thyroid is affected; in MEN2A, medullary thyroid carcinoma and pheochromocytoma occur, and MEN2B patients develop ganglioneuromas and skeletal abnormalities, in addition to the symptoms of MEN2A. Identical mutations can also result in either FMTC or MEN2A (Donis-Keller et al. 1993). HSRC has rarely been observed associated with multiple endocrine neoplasia (Mahaffey et al. 1990), and the majority of cases do not develop these tumors. Thus, it appears that the different disease phenotypes associated with mutations in the RET gene are explained by mutations involving either a gain or a loss of function.

Monofactorial traits: locus heterogeneity

Locus heterogeneity is addressed if a similar disease phenotype is based on a defect in one of several genes at different map positions. It may be reflected by some clinical heterogeneity with an overlap of pathological characteristics. However, on mere clinical grounds, it is often difficult or even impossible to delineate different etiological entities, and in these cases, only genetic analysis allows for a nosologic classification. Thus, analogous to a class of allelic mutations, mutations in different genes converge to give similar or even identical disease phenotypes. The question arises as to why the genes involved do not compensate for each other. The situation is trivial if the respective genes are complementary, coding for different polypeptide chains forming a heteropolymeric protein. In other conditions, locus heterogeneity may be attributable to additive polygeny, because several genes are required for the formation of a particular feature, i.e., the respective gene products have differential functions. With respect to ontogeny, developmental pathways are canalized (Waddington 1942) and the competence of the cells or tissues concerned is limited, so that the range of possible responses is also narrowed. As a result, heterogeneous causes have a similar phenotypic effect.

Table 1 Autosomal genes associated with dominant RP-mutations

Gene symbol	Map location	
RP1	8p11–q21	
RP5	3q	
RP7 = RDS	6p21.2–cen	Peripherin
RHO = RP4	3q21–q24	Rhodopsin
adRP	7p	Inglehearn et al. 1993
adRP	7q	Jordan et al. 1993
adRP	17p	Greenberg et al. 1994
adRP	19q13.4	Al-Maghteth et al. 1994

Non-syndromic retinitis pigmentosa (RP) presents an example for all kinds of heterogeneity, i.e., polypheny at an identical mutation, heteroallely, and in particular, association with one of a multiplicity of different gene loci. Autosomal dominant, autosomal recessive, and X-linked modes of inheritance have been described. The number of independent gene loci is a matter of debate, and more genes are expected to be detected. At least eight autosomal genes with dominant RP-mutations have been identified (Chromosome Coordinating Meeting 1992; additional references are listed in Table 1).

For autosomal recessive RP, no particular gene has as yet been mapped, although it is the most frequent genetic type. However, mutations in the rhodopsin gene can also be recessive (Rosenfeld et al. 1992), and involvement of the RHO and RDS genes was excluded in a large pedigree showing autosomal recessive inheritance of RP (Bleeker-Wagemakers et al. 1992). Three different recessive genes on the X chromosome have been recorded (Chromosome Coordinating Meeting 1992), viz., RP2 (Xp11.4-p11.23), RP3 (Xp21.1), and RP6 (Xp21.3-p21.2). In addition, a number of candidate genes are under study, but mutations in these genes causing non-syndromic RP have not yet been reported. Among these genes is ROM1 (11q13), which codes for a transmembrane protein of the rod outer segment discs of the retina, as do the rhodopsin and peripherin RDS genes. A candidate for an autosomal recessive RP gene is PDEB (4p16.3), coding for the β-subunit of a cGMP phosphodiesterase; a mutation is known in the mouse homolog of this gene, and results in degeneration of the retinal rod photoreceptor cells in the homozygous state.

Clinically, RP can be classified into various types depending on the age of onset of visual impairment, including night blindness, narrowing of the visual field, and eventually complete visual loss. The mainly occurring

types are type 1 characterized by early loss of rod function, whereas cone function is lost much later in life, and type 2 with simultaneous regional (patchy) loss of both functions at various ages. Needless to say, clinical phenotypes can be differentiated in much more detail. However, no exact correlations between mutations in the different RP genes and the clinical variation of the retinopathies have been established. The same is true for allelic mutations in the RHO gene, in which up to 50 different mutations have been identified so far. Even one and the same mutation can result in inter- and intrafamilial variation (for a review, see Lindsay et al. 1992; Humphries et al. 1993; Berson 1993). These findings point to the influence of other factors than the respective mutation itself (in the case of clinical heterogeneity at the same mutation), or to constraints in the development of the retina resulting from a limited range of possible responses of the affected cells (in the case of a similar clinical phenotype at different mutations).

Locus heterogeneity is also extensive in congenital cataracts, and a clinical distinction between etiologically different forms is difficult. Autosomal dominant, autosomal recessive, and X-linked transmission has been reported. In autosomal dominant cataracts, a minimum of three gene loci has been assigned to different chromosomes, viz., CAE1 to 1q21–q25, CCL to 2q33–q35, and CTM to 16; however, observations in other pedigrees exclude these loci (Lund et al. 1992), and point to yet unidentified genes. Among others, the numerous crystallin genes (several α, β, and γ genes) are candidates for mutations resulting in cataracts. The morphologic similarities are ascribed to the limited "possible biological reactions of the lens expressing a cataract gene" (Lund et al. 1992).

The particularly complex interrelationship between phenotypical and etiological heterogeneity in microphthalmus and coloboma has been reviewed recently by Warburg (1991, 1992, 1993). The author distinguishes between three etiological classes, viz., genetic, acquired, and associations. These ocular anomalies may occur as isolated features or as part of a more complex symptomatology or a syndrome. The phenotype of microphthalmus and coloboma can be classified into a multiplicity of clinical variants (Warburg 1993), but this classification is etiologically irrelevant, and the features responsible, genetic or nongenetic, cannot be derived from the clinical picture. In the majority of cases, a genetic basis has been identified, and Warburg (1991) lists more than 100 genetic disorders that are monofactorial, including autosomal dominant and recessive, and X-linked syndromes that are associated with microphthalmus and coloboma. Considerable intrafamilial variation of symptoms has been reported (Warburg 1993). Microphthalmus and coloboma also occur in chromosome aberrations (Warburg and Friedrich 1987). Various teratogens, infections of the mother, and abnormal conditions during prenatal development can also cause these eye disorders. Finally, microphthalmus and coloboma can be found in associations believed to be causally nonspecific and pathogenetically not related (Warburg 1992).

Renal cystic disease has been found associated with a number of inherited disorders (Reeders 1992), the most common of which is polycystic kidney disease (PKD) affecting approximately 1 in 1000 individuals. PKD has also proved to be based on locus heterogeneity. An autosomal recessive form that has not yet been mapped can be differentiated clinically from a dominant form. Autosomal dominant PKD was ascribed to mutations in a gene designated PKD1 on chromosome 16p13.3 (Reeders et al. 1985), accounting for some 85% of cases. This gene has been cloned recently (European Polycystic Kidney Disease Consortium 1994). Because only a minority of nephrons is affected and the number of foci increases with age, it has been proposed that the constitutional mutation has no dominant effect, but a second event, "genetic or otherwise", at the somatic level is required to give rise to the disease (Reeders 1992). In some families, the gene responsible for the disease has been assigned to chromosome 4q13-q23 and named PKD2 (Peters et al. 1993; Kimberling et al. 1993). Furthermore, a family was studied showing no linkage of the disease to either chromosome 16 or 4, and the still unmapped gene responsible is to be named PKD3 (K. Zerres, personal communication). Clinically, these three autosomal dominant forms of PKD are similar with largely overlapping symptoms, PKD2 being milder and PKD3 more severe, whereas PKD1 is variable.

Charcot-Marie-Tooth disease (CMT) is another example showing extensive locus heterogeneity, including autosomal and X-linked dominant and recessive forms (for a review, see Vance 1991; Patel and Lupski 1994). In connection with allelic heterogeneity, one form, CMT1A, has been discussed above. Clinically, two main types are distinguished, viz., a slow nerve conduction type (CMT1) and a type with nearly normal conduction velocities (CMT2). In type 1, locus heterogeneity is well documented, whereas the clinical findings, though variable, are similar. In CMT1A, the gene PMP22 has been located on chromosome 17p12-p11.2, and a duplication within this gene or point mutations cause dominant CMT type 1; however, a recessive point mutation with a similar disease phenotype has also been described (Roa et al. 1993). A second locus, designated CMT1B, has been assigned to chromosome 1q22–q23 (Bird et al. 1982; Lebo et al. 1991). Affected individuals exhibit typical CMT1 and the inheritance is dominant. A gene for a myelin protein, P_O or MPZ (myelin protein zero), maps to the CMT1B locus, and in patients with CMT1B, mutations in MPZ have been identified suggesting that this is the disease gene (Kulkens et al. 1993; Hayasaka et al. 1993a). Mutations in the P_o gene can also be associated with the Dejerine-Sottas disease, a motor and sensory neuropathy similar to CMT, but with more severe symptoms (Hayasaka et al. 1993b). Thus, allelic heterogeneity also occurs at the P_o locus. CMT has also been found to be X-linked (CMTX) and has been mapped to Xq13.1 where the gene for connexin 32 (Cx32), a gap junction protein, is located. In families with CMTX, mutations have been identified in the Cx32 gene, suggesting that this gene has a crucial function in periph-

eral nerves (Bergoffen et al. 1993; Fairweather et al. 1994). Although CMTX mutations are dominant, X-linked recessive CMT mutations in other map positions may also exist (Ionasescu et al. 1991). Finally, three additional autosomal loci have been reported, viz., an autosomal recessive form on chromosome 8q (CMT4A; Ben Othmane et al. 1993a), a form of CMT2 on chromosome 1p35–p36 (CMT2A; Ben Othmane et al. 1993b), and a third form of autosomal dominant CMT1 that was excluded from linkage to chromosomes 17 and 1 and that was therefore named CMT1C (Chance et al. 1990). In total, three different proteins, viz., PMP22, MPZ, and Cx32, have thus been shown to be involved in CMT so far.

In familial Alzheimer disease (FAD), three different genetic loci have been identified, and a fourth is derived from the exclusion of linkage to these three loci. Clinically, only differences in the age of onset are observed. Mutations in the amyloid precursor protein gene APP on chromosome 21q21.2 account only for a small minority of cases (AD1; Goate et al. 1991; Murell et al. 1991). In over 70% of families, FAD is linked to 14q24.3 (AD3; Schellenberg et al. 1992). A third locus has been assigned to chromosome 19q (AD2; Pericak-Vance et al. 1991), and it has been debated whether the gene responsible could be that for apolipoprotein E (APOE) on 19q13.2 (Strittmatter et al. 1993; Corder et al. 1993). Exclusion of these three locations in some Volga-German families gives evidence for the existence of still another gene responsible for FAD (Martin 1993). Most late-onset cases are sporadic, and it is generally believed that there is only a minor genetic contribution, if any, to the manifestation of the disease. However, Bergem (1994) questions this assumption in her study of twins with a mean age of 79 years. Concordance rates in monozygotic twins compared with dizygotic twins are 83% versus 42%. Thus, late-onset cases may be sporadic because relatives die before the disease develops. However, in view of the high prevalence of Alzheimer's disease (almost 30% at the age of 90 years; Jorm et al. 1987), it is hard to believe that most of these cases are genetically determined.

Multifactorial traits

Multifactorial traits are characterized by an irregular mode of inheritance believed to be caused by the interaction of genetic (polygenic) and epigenetic factors, and the inner and outer environment. As we have seen, monofactorial traits are also the consequence of normal and abnormal gene expression in a particular developmental and environmental context. From this viewpoint, the interactions taking place in the case of multifactorial traits are more complex, but not of a different quality. In proceeding pathogenetic analysis, it may turn out that the vast majority of diseases involving genetic factors are multifactorial. The genetic component is addressed by terms such as increased risk, predisposition, or susceptibility for a certain disease. The genes involved can have a weaker or stronger disease association, and exposure to environmental factors (e.g., smoking, diet) acting on a particular genetic background can also predispose to disease. The phenotypic penetrance and expressivity also vary, depending on these and other variables. In consequence, it is not to be expected that the phenotypic manifestation of a multifactorial trait can be predicted from the genotype, even if, in a polygenic condition, the genotype is known. Because of their complexity of causation, multifactorial diseases are poorly defined, to date, in genetic terms; such diseases include cardiovascular conditions, diabetes, endogenous psychoses, and cancer (with some exceptions). In the present context, I shall select a few examples with a defined genetic contribution, preferentially those that can be considered borderline conditions between monofactorial and multifactorial causation.

Hirschsprung disease (HSCR) is a congenital intestinal agangliosis occurring as an isolated disorder affecting the distal segment of the colon, or associated with a large number of different congenital defects including chromosome aberrations (see Table 1 in Passarge 1993). Family data suggested a multifactorial causation, but dominant and recessive modes of inheritance with variable penetrance were also discussed (Passarge 1993). After a locus for HSCR was placed in the paracentric region of chromosome 10q by linkage information (Lyonnet et al. 1993; Angrist et al. 1993), the receptor tyrosine kinase gene RET on 10q11.2 was found to be affected by mutation in patients with HSCR (Romeo et al. 1994; Edery et al. 1994). These mutations cause loss of function, and therefore, the disease is believed to be the result of haplo-insufficiency in the heterozygotes. Thus, HSCR can be considered a monogenic dominant condition in these cases. However, some peculiarities remain to be explained. RET mutations were detected only in a minority of patients. Even in familial occurrence, carriers of the mutation were found to have no symptoms, and the severity of the disease phenotype varied within families. Because of incomplete penetrance, the risk to siblings of a proband with respect to being affected is low. The expression is extraordinarily variable, and the association with various malformation syndromes of heterogeneous origin is not yet understood. The sex modification with a sex ratio up to 4:1 is also exceptional. Even in the case of null mutations in the RET gene, other genes and nongenetic factors seem to interact. Furthermore, linkage studies have excluded the RET region in some families, and a second locus is assumed to exist in a pedigree with a recessive form of HSCR (Angrist et al. 1993). Indeed, a recessive gene for HSCR has now been assigned to chromosome 13q22 in a large inbred kindred (Puffenberger et al. 1994). In this pedigree, penetrance is reduced and the sex ratio is distorted. Because of deviations from single Mendelian recessive inheritance, modifier genes have been sought. Preliminary evidence for genetic modifiers on chromosome 21q22 and at the RET locus has been obtained. The involvement of chromosome 21 is of particular interest in view of the association between Down syndrome and HSCR. Thus, whereas the RET gene is to be considered a major HSCR locus, the disease appears to be heteroge-

neous, including allelic and locus heterogeneity, different modes of inheritance, interaction with modifier genes, and possible nongenetic causes. Therefore, the disease should remain to be classified as multifactorial. This example illustrates the fluid transitions from mono- to multifactorial disorders, and the terminology turns out to be somewhat arbitrary.

The manifestation of Hartnup disease depends on a mutation at the Hartnup locus coding for a polypeptide component of a transport system in the brush border membranes of epithelia. This system serves the renal and intestinal transport of a larger number of amino acids. Patients show pellagra-like symptoms and aminoaciduria. Although the mutation is autosomal recessive, only some 10% of mutant homozygotes are affected. The defect in the transport system predisposes to the disease, but clinical manifestation depends on various additional factors, including other genes and components of the environment, such as diet. Thus, the metabolic phenotype is monogenic, and the disease phenotype is multifactorial. Scriver (1988) coined the term "homeostatic phenotype" for such a condition. His interpretation is that the homeostasis of amino acids (interaction of polypeptides), which is controlled by genes other than the Hartnup gene, is lowered in affected families; in the presence of the Hartnup mutation, environmental influences may evoke the disease.

Physiology teaches us that the level of blood pressure is multifactorial and based on the interplay of an indeterminable number of endogenous and exogenous factors. Hypertension is believed to result from particular genetic backgrounds exposed to specific variables of the environment. In view of the unknown number of genes involved and the complex environmental interactions, it seems hopeless to trace individual genes contributing to hypertension. Nevertheless, detailed physiological knowledge has focused interest on proteins involved in the regulation of blood pressure, and genetic studies of some of these proteins have succeeded in identifying at least a "signpost in the labyrinth". Vascular tone is maintained by the renin-angiotensin system, and one component of the system is angiotensinogen serving as the substrate for renin. Blood pressure has been shown to be related to plasma angiotensinogen levels. The angiotensinogen gene (AGT) has been characterized and localized on human chromosome 1q42–q43. Allelic variants of AGT, in particular the allele T235, were found to be associated with hypertension (Jeunemaitre et al. 1992). These variants are believed to be involved in the pathogenesis of hypertension, at least as predisposing factors. Interestingly, the T235 allele has also been found associated with preeclampsia, a pregnancy-induced hypertensive condition (Ward et al.1993).

It may be debated whether or not familial hypercholesterolemia (FH) is multifactorial. Clearly, mutations in the low-density lipoprotein receptor gene (LDLR) result in autosomal dominant hypercholesterolemia type II (McKusick et al., no. 143890), and mutations in other genes produce a similar clinical phenotype (McKusick et al., no. 144400). Thus, FH could be considered an example of locus heterogeneity. In the case of LDL receptor mutations, heterozygotes develop coronary artery disease later in life, whereas the rare homozygotes are more severely affected and the disease occurs earlier. Extensive variation in clinical signs has been observed in both heterozygotes and homozygotes (Goldstein and Brown 1989), and can be attributed, at least in part, to allelic heterogeneity. However, marked clinical differences are also seen among homozygotes carrying one and the same mutation in both alleles, viz., a deletion resulting in complete loss of the receptor protein (Hobbs et al. 1987). One of the patients developed coronary artery disease at the age of 17 and survived until the age of 33, whereas another died at the age of three years. Circulating levels of LDL were extremely high in both cases. These differences in susceptibility or resistance are ascribed by Scriver (1988) to the other genes controlling lipid homeostasis, and to sex, blood pressure, and life habits. Thus, the clinical manifestation has a multifactorial causation.

Monozygotic twins

In monozygotic twins, occasionally only one twin may be affected by mutation, e.g., for trisomy 21 or DMD, and in these cases, the occurrence of phenotypic differences (discordance) is to be expected. However, discordance has also been observed when the twins are assumed to be identical genetically.

Leber's hereditary optic neuropathy (LHON) is linked to mutations in the mitochondrial genome (Wallace et al. 1988) and results in visual loss. Phenotypic variation including intrafamilial variability is high, even in the case of identical mitochondrial mutations, and modifier genes, epigenetic mechanisms and environmental influences are believed to, or have been shown to, contribute to this variation (Wallace 1993). Interestingly, a pair of monozygotic twins, identical in their nuclear and mitochondrial genomes including the most common mitochondrial mutation found in LHON at the 11778 position, were discordant at the clinical level for 6 1/2 years, the one twin being affected by optic neuropathy, the other not. Because of the apparent homoplasmy, this discordance is ascribed to differences in the occupational exposure to toxic substances (Johns 1993).

In classic Juvenile-onset diabetes (IDDM, McKusick et al. 1992, no. 222100), half of the monozygotic pairs were discordant when the index twin developed the disease before the age of 40 years; the discordance existed in half of these pairs for more than 10 years, so that the nonaffected twin was not expected to become diabetic later (Tattersall and Pyke 1972). Although the discordance is ascribed in large part to non-genetic etiology, evidence for genetic predisposition is provided by the occurrence, in three out of 31 pairs, of an affected first-degree relative.

Discordance in monozygotic twins for schizophrenia is also 50%. Among the discordant traits, an abnormal development of the hand ectoderm may be given here as an example (Bracha et al. 1991, quoted in Strohman 1993). This impairment occurs during the second trimester of

prenatal life when the distal upper limb is formed by migration of ectodermal cells, and at the same time, neuronal cells migrate from the periventricular germinal matrix to the cortex. These slight discordances of the hand ectoderm are considered to be the consequence of differential exposure of the twins to the uterine environment. Dysmorphological hand anomalies were found significantly more frequent in schizophrenic than in nonaffected twins.

A broad range of variation in concordance rates of monozygotic twins has been compiled and discussed by Vogel and Motulsky (1986) for various multifactorial diseases. Although the above examples simply show that multifactorial diseases are multifactorial, they also demonstrate the strong influence of nongenetic factors during the developmental realization of the phenotype.

Animal models

Genetic disorders in laboratory animals are widely used as models for homologous defects in the human species. For a comparison of the phenotypic effects of a mutation, it has to be established that homologous loci are mutated in both species. Because of the possibility of allelic heterogeneity, only those mutations resulting in homologous amino acid exchanges occurring in domains of homologous function, or in complete loss of function (null mutation) should be considered. If, under these criteria, phenotypic differences occur, they can be ascribed to interactions of factors other than the affected gene itself.

The majority of animal models are provided by the mouse, whether by spontaneous or induced mutation, or by the production of transgenic animals. Mouse models have been listed and critically discussed by Darling and Abbott (1992). Here, I will restrict myself to some examples of homologous null mutations showing significant phenotypic differences between the animal model and the human.

X-linked muscular dystrophy in the mouse has been shown to be caused by a mutation, mdx, in a gene homologous to the human dystrophin gene, and results in complete absence of the protein (Hoffman et al. 1987; Sicinski et al. 1989). In contrast to DMD, however, the mdx mouse develops no obvious muscle weakness, and because of muscle fiber regeneration, limb muscles do not degenerate progressively. Thus, although the mdx mouse is also affected by the mutation (Stedman et al. 1991), the difference in clinical severity compared with DMD is striking.

Lack of hypoxanthine guanine phosphoribosyltransferase (HPRT) causes the Lesch-Nyhan syndrome (McKusick et al. 1992, no. 308000). In contrast, in the mouse, HPRT deficiency has only minor effects (Finger et al. 1988). Differences in purine metabolism are beleived to be responsible for the almost complete absence of clinical features in the mouse mutant. Indeed, it has been shown that administration of an inhibitor of adenine phosphoribosyltransferase (APRT) to HPRT-deficient mice results in behavioral changes similar to those seen in the Lesch-Nyhan syndrome (Wu and Melton 1994). Therefore, it is assumed that in the mouse, the purine salvage pathway relies on APRT rather than HPRT.

An additional example is deficiency of carbonic anhydrase II. The human CA2 gene is homologous to the murine Car-2 locus. Homozygous patients for an autosomal recessive null allele exhibit osteoporosis with renal tubular acidosis (McKusick et al. 1992, no. 259730) and, in consequence, failure to thrive and have a short stature; they also develop cerebral calcification. In the mouse, homozygous mutants for a null allele also have renal tubular acidosis and are smaller than their unaffected siblings, but they do not develop osteoporosis or cerebral calcification (Lewis et al. 1988). Apparently, bone remodeling differs between the two species.

Using a targeting construct, Schuchhardt et al. (1994) produced a loss of function mutant in the ret gene, coding for a receptor tyrosine kinase, in the mouse. This gene is homologous to the human RET gene, which, if affected by loss of function mutations, causes or contributes to Hirschsprung disease. Although the mutations in the human are dominant, that in the mouse is recessive. The phenotypic consequences in affected individuals are partly similar but partly different between the two species. Essentially, the mouse is more severely affected than human patients. Homozygous mouse mutants die shortly after birth. The anomalies detected so far include the absence of enteric neurons throughout the digestive tract and the absence of or rudimentary kidney development. Whereas the enteric nervous system is affected in both species, the disorders in the mouse with a complete absence of the ret gene product are in contrast to Hirschsprung patients with aganglionic hindgut only. These differences are ascribed to the interaction of modifier genes and/or stochastic processes in development (van Heynigen 1994).

At the chromosomal level, the phenotypic differences of the XO condition between the human and the mouse are marked. The XO mouse exhibits no obvious somatic anomalies, in particular, no Turner phenotype, and it is fertile, although its reproductive life span is diminished (Lyon and Hawker 1973). Lethality of XO embryos in the mouse is about 35% (Searle 1989), whereas it is over 99% in the human (Hassold 1986). Several genes escaping X inactivation in the human undergo inactivation in the mouse, and so far only one gene, Smcx, has been shown conclusively to escape inactivation in the mouse (Agulnik et al. 1994). Gene dosage requirements may be different in the two species; however, unless so-called Turner genes are identified, this issue remains speculative.

The genotype-phenotype relationship

There are basically two contrasting positions, emphasized by Stent (1985). The one position assumes that the phenotype is the realization of a genetic program laid down in the genome, and that a single cell, the zygote, contains all the information necessary for ontogenesis. From this viewpoint, the genome is a unidimensional description of

the whole organism. The other position considers the phenotype as the result of interactions between a multiplicity of factors, of which genes represent only one category. Ontogenesis is seen as a historical rather than a programmatic process, being determined by genetic and epigenetic interrelationships that take place within and between cells, and on more complex levels of integration (Stent 1981; Oyama 1985; Nijhout 1990; Strohman 1993).

Considering the various examples analyzed above in order to trace the genetic contribution to the phenotype, the alternative put forward by Stent shall be discussed in the following subsections. (1) Is the genotype-phenotype relationship (GPR) unidimensional? (2) Is it programmatical? (3) Is it hierarchical?

Is the GPR unidimensional?

The term "unidimensional" is used here in the sense that a proportional projection exists between cause and effect, or in the present context, that a defined genetic structure is responsible for a defined phenotypic character. If this is so, the phenotype of a particular mutation can be predicted, and the phenotype can be reduced to the genotype. A possible way to study this question is to analyze the phenotypic effects of mutations, as attempted above.

In the case of chromosome aberrations, gene dosage deviates from the usually duplex representation, but specific effects of individual genes occurring, for example, in uniplex or triplex have hardly been established. It is to be assumed that chromosomal phenotypes are the consequence of genetic imbalance, but this imbalance need not necessarily be additive. Apart from gene products that are tolerated within a wide range of quantitative variation, e.g., enzymes, others, e.g., some controlling factors, act only at certain threshold concentrations, and still others, e.g., monomeric components of heteromeric proteins, if produced in unequal amounts, lead to a shift in the proportion of different polymers. In the latter two cases, genes on non-affected chromosomes may also be involved. The manifold possible consequences of chromosomal imbalance are comprehensively discussed by Epstein (1986). To account for the relative lack of specificity of phenotypes in chromosome aberrations, the concept of disrupted homeostasis has been put forward (Shapiro 1983), as discussed above. The concept implies that chromosomal imbalance results in a general developmental instability and labile physiological homeostasis; in addition to direct gene dosage effects, a predisposition for developmental disturbances is assumed that becomes manifest by epigenetic and environmental interactions. Although this concept is still vague, it appears plausible that the chromosomal phenotype is the result of multifactorial causation, including more specific effects that may account for some of the syndromologic differences, and more nonspecific effects that interfere with basic morphogenetic processes.

With respect to multifactorial traits, modifications of the phenotype manifesting in incomplete penetrance and variable expression are obvious. No doubt, the underlying mechanisms are heterogeneous. Whereas a particular combination of a number of recessive genes constituting the genetic background may be a prerequisite, the manifestation of a multifactorial trait will depend on genetic and environmental conditions. In epigenetics, chance may be contributive, in particular, if threshold effects are involved. In the case of quantitative variation, local concentrations of a factor, and in qualitative variations, e.g., higher concentrations required for a factor with reduced affinity, will be influenced by chance during development.

A basic difference exists phenomenologically between congenital malformations and late onset diseases. However, this difference need not necessarily have its origin in different mechanisms, but it may be attributable to different genetic constellations in combination with particular boundary conditions, e.g., temporary or chronic noxes.

The contribution of environmental conditions, in particular nutrition, life habits, exposure to potential mutagens, carcinogens, or teratogens, is well documented in some multifactorial traits. If these conditions vary, the manifestation of the trait may also vary. Thus, to some extent, the polygenic basis is only a predisposition for the occurrence of a particular defect that could also occur in individuals considered not to be disposed genetically.

If we admit the concept of disrupted homeostasis, it may be assumed that, in multifactorial diseases, particular morphogenetic processes or the homeostasis of particular metabolic pathways are also labile, depending on the nature of the genes involved. This would however occur in a more circumscribed and specific way than in chromosome aberrations.

When considering monofactorial traits, some clear examples of deviations from a proportional relationship between specific mutations and specific phenotypic anomalies can be found. Phenotypic variation in the case of one and the same mutation as defined in molecular terms indicates the interplay of additional factors. These may be the products of other genes (genetic background), metabolic interferences, and/or exogenous factors. If the gene affected is represented only once within the genome, if it fulfills a unique function, and if the gene product is totally lacking because of mutation, it is most probable that phenotypic variation is the result of epigenetic and/or exogenous action. Vice versa, if one and the same phenotype is produced by different mutations, these mutations may either affect or block the same pathway (which is trivial), or the genes concerned may have a nonspecific function, and developmental and/or metabolic constraints may canalize the phenotypic manifestation.

It is to be expected that, in dominant disorders, phenotypic variation is wider than in recessive disorders, because in the former, the wild-type gene product is also available. Although the proportional production rate may be the same in every cell, the concentrations of the products may show regional fluctuations depending on chance and resulting in the variable expressivity of a trait.

These findings and considerations touch on the monocausal disease concept. There are not only many interme-

diate steps between a mutation and its phenotypic manifestation, but this process is also not unidimensional in the sense that there would be a one-to-one projection between each step and the next one.

The manifestation of X-linked mutations in females is another example of epigenetic interactions. The size of the stem-cell pools at the developmental stages when random X-inactivation occurs, and differential clonal proliferation have been shown to be responsible for a disease phenotype, often of milder expression, in carrier females. Similarly, mitochondrial diseases can be the result of mutant mitochrondrial genomes present as a minority population in the zygote or also at later ontogenetic stages; the mutant genome may become a majority population by chance or by selection.

Twin studies may be considered crucial for defining the genetic contribution to the phenotype. Discordance of traits in monozygotic twins is well documented. Apart from genetic differences caused by mutational events affecting only one twin, phenotypic variation should be ascribed to epigenetic and environmental interactions. As discussed in detail by Vogel and Motulsky (1986, p. 209 ff), "chorionic transfusion syndrome" can occur in monozygotic twins, caused by anastomosis of blood vessels with the consequence of chronic malnutrition of the donor twin. This syndrome is believed to be the reason for birth-weight differences of more than 1000 g between the twins, and could also account for various malformations affecting one of the monozygotic twins only. Needless to say, discordance between monozygotic twins can be attributable to many other factors acting during pre- and postnatal life, as also discussed by Vogel and Motulsky (1986). Nevertheless, the fact that concordance for many complex parameters is high among monozygotic twins should be considered from the viewpoint that similarity of traits need not necessarily have a genetic or only genetic origin. As discussed above with respect to the heredity of a trait, developmental and environmental conditions, if operating in the same direction, will contribute to the similarity of a characteristic in related individuals, and these conditions will be even more alike in the case of monozygotic twins. Thus, twin studies, while referring to which characteristics are hereditary or not, cannot always differentiate between genetic and non-genetic contributions to the phenotype.

The comparative use of animal models is only of limited value for the problem under discussion, because genetic, developmental, and ecological conditions are different between species. However, these models show that mutations considered to be homologous can have quite a different outcome. Apart from the genetic context, in particular if no other genes are known to compensate for a mutation, epigenetic buffering systems must be at work when the phenotypic manifestation is milder in one species than in another.

A comparison between chromosome aberrations in different species depends on the size of conserved linkage groups. Here, the X chromosome forms an exception because it has been conserved in its entirety within the infraclass of eutherian mammals. The conspicuous phenotypic differences between human and murine XO females may be ascribed to differences in X-inactivation behavior; the genes that escape inactivation and that are candidates for the Turner phenotype are different in the mouse from the human. It will be interesting to determine whether these differences in inactivation behavior are the result of the position within the X chromosome of the genes concerned. The location of a gene, on the other hand, is the result of chance events during evolution if selection is not at work, and comparative gene mapping favors this view. Thus, the phenotypic differences between the XO conditions in man and mouse could be the result of random rearrangements of genes within the X chromosome during evolution.

In view of these considerations, a direct phenotypic manifestation of a mutation appears to be the exception rather than the rule. Clearly, the mutation, if it manifests itself, is the origin of a phenotypic change. However, the phenotypic outcome does not depend on the nature of the mutation only. The primary structural change, passed on into the network of developmental and metabolic pathways, can result in a spectrum of consequences ranging from the complete blocking of a pathway to the complete compensation of the defect, depending on the epigenetic interactions. A majority of phenotypic traits caused by mutation may also be inducible under extreme exposure to exogenous factors, showing that the epigenetic network is finally responsible for the outcome of the trait.

Since epigenetic processes have their own dynamics, it will not be possible to predict the phenotype exactly, even if the nature of a mutation is known. Here, we come to the borders of reductionism.

Is the GPR programmatical?

The idea of a DNA sequence as a program is closely connected with that of a unidimensional relationship between genotype and phenotype. A process follows a program if, in a succession of transformations, each step results from the previous one mechanically by the application of the same principle. In biological processes, this would mean that a complete structural correspondence exists between two patterns, the one being generated by the other. Such a relationship is found between nucleotide and amino acid sequences, the RNA processing being taken for granted. Indeed, the amino acid sequence can be deduced from the DNA sequence (cDNA) with considerable accuracy. However, the three-dimensional structure of a protein may take different configurations that are stable thermodynamically, and therefore, the one-to-one projection from the DNA level ends at the polypeptide level. Protein folding is a complex process involving various cofactors (Gething and Sambrook 1992).

In ontogenetic development, there are other quasi-programmatical structures, such as so-called positional code and the morphogenetic code. The positional code refers to the phenomenon of an ordered spatial arrangement or dis-

tribution of genes or gene products that transmit this order to cellular differentiation processes. Gradients in eggs and early embryos defining the various body axes of the developing organism are an example (Manseau and Schüpbach 1989). Moreover, the colinearity between homeotic genes and segmentation is striking. Here, the spatial order of genes, their temporal pattern of activity or repression, and embryonic pattern formation resulting in segmentation of various parts of the body correspond to each other (Gaunt 1991; Krumlauf 1994). The specific combination of murine Hox genes, each expressed in a prevertebral segment, has been termed the "Hox code" (Kessel and Gruss 1991). It has been suggested that colinearity reflects the course of evolution of the homeotic gene clusters (Sander 1994). If these clusters originated by duplication and subsequent diversification (Ohno 1970), the genes expressed earlier in ontogenesis could be older in evolutionary terms, whereas the duplicated derivatives became expressed later. Thus, evolutionary history would be the primary reason for this programmatic behavior.

A relationship between programmatical structures and morphogenetic processes can also be seen in cellular recognition attributable to differential cell surface structures. The selective combination in the qualitative and quantitative composition of cell adhesion molecules and their spatial distribution appears to be substantial for histo- and organogenesis. The cell surface has a programmatical structure that, if corresponding to that of other cells, results in interactions of various kinds, thereby initiating morphogenetic processes. Since the cell surface is topographically specifically structured, this phenomenon has been named the morphogenetic code.

Apart from these examples, signal transduction may be generally taken as evidence for the programmatic nature of development and differentiation. As addressed by these examples, programmatic processes in ontogenesis and those serving the maintenance of the functioning of an organism, are realized at various levels of complexity. However, it is not to be concluded that the organism achieves its organization and functioning because it follows a program deposited in the genome. The programmatic interactions taking place are themselves products of ontogenesis and the functioning of the organism, and they can be understood as epigenetic consequences of these processes. The program for polypeptides is not the genome as such, but rather the expressed genome, and differential gene expression is an achievement of ontogenesis and a consequence of intra-, inter-, and extracellular interactions. This also applies to the positional code as far as gene expression is concerned. Otherwise, the positional and morphogenetic codes are assembled during the course of ontogenesis, depending again on manifold interactions. All these programs have a specific spatio-temporal range of validity, and a universal program for the entire organism is not recognizable. It may even be doubted whether "program" is the right term for the structures generating isomorphic or quasi-isomorphic structures. The structure is considered a program only after it is realized. Thus, program in this context is an a posteriori description of a structure, and not an a priori instruction for generating a structure (Oyama 1985, p. 54). The program is to be equated with a process of self-organization that takes place only if all the interacting factors that are necessary and sufficient are present in spatio-temporal terms.

Is the GPR hierarchical?

If the realization of a phenotype is neither a unidimensional nor a programmed process, it may still be subjected to hierarchical organization. A hierarchy implies a gradual succession of increasing competence, and consequently control and subordination in one direction only. Therefore, it can be asked in which direction the processes under discussion take their course. Is it from the gene to the phene or vice versa, or is no direction maintained over a longer span?

Genes are essentially reactive (Oyama 1985). They react to various stimuli that need not necessarily be products of other genes. The way in which they react is not determined by the nature of the stimulus. The signal as such is nonspecific; it only interacts with a target or various targets (receptor, binding site), and the ligand-receptor interaction results directly or indirectly in either activation or repression of a gene or genes. If, however, cell surface receptors are transplanted onto other cells or if promotor regions are exchanged, then other genes become activated or repressed by the respective signal. Thus, genes are not instructed by a signal, but rather the nature of the cell or the gene determines the specificity of the response, in a reactive way. The specificity lies in the structural correspondence and in the reaction itself. The interactions are not hierarchical.

The relationships are similar at other levels of organization and complexity. The fate of a cell depends upon its position in the embryo, but only during a transient developmental period, and the cell may have another fate if it does not react during this period. Conditions change continuously depending on the interacting components, and each subsequent step is the consequence of the conditions reached by the foregoing step. One of the best studied examples of a morphogenetic process is embryonic pattern formation in *Drosophila*, which has proved to be a model for similar mechanisms acting in other species including mammals. Positional differentiation is provided by gradients of morphogenetic factors, and a particular structure is specified by a combination of concentration-dependent activation and repression events (Pankratz and Jäckle 1990). Among the segmentation genes, some regulate the expression of one another (Ingham 1988). These and other mechanisms, by consecutive steps, lead to a successive subdifferentiation of the embryo, and the interactions are not hierarchical. It would be arbitrary to say which component is placed over others: the gene, the gene product, the position in space, or the conditions of the physicochemical milieu. Control mechanisms interact by structural correspondence, and this interaction may result in other controlling factors that directly or after a succession

of steps return to the point of departure. Thus, controlling mechanisms are themselves controlled or control themselves, and the system is self-referential. It would be difficult to put such systems in a hierarchical order.

Conclusions

It appears that the realization of the phenotype is characterized by program-like, regulatory, stochastic, and historical processes that interact to form a network of relationships, rather than a linear succession of events. There is no fundamental design or program that is followed but rather the interactions taking place form the program. During the course of development, each state presupposes the foregoing state; each subsystem produces successively the prerequisites for its further development. The processes taking place are essentially reactive, depending on structural correspondences, and are therefore highly selective. Control phenomena and cascade mechanisms are not hierarchical, but self-referential. Thus, there is no hierarchy either from gene to phene or vice versa. The principles that nevertheless guarantee identity, autonomy, and integration of the organism are not subject to this review; however, they are in concordance with the view presented here (U. Wolf, manuscript).

For the reasons discussed, the genotype-phenotype relationship cannot be expected to follow strict laws, but rather to be irregular. Indeed, this relationship is highly prone to variation. The phenotype is not deducible from the genotype. The fact that variation is restricted to the extent that, within certain limits, individual phenotypes can be assigned to individual mutations and vice versa can be ascribed to the extensive similarity, if not identity, of the majority of conditions for the development and functioning of the organism, so that the respective mutation is the main variable within the system.

Acknowledgements This article was conceived during a fellowship spent at the Wissenschaftskolleg zu Berlin in 1989/90, and outlined during a sabbatical leave in early 1993 at the Collegio Cairoli, University of Pavia, at the invitation of Professor Marco Fraccaro. I am much indebted to Professor Winfrid Krone, Ulm, Dr. Susumu Ohno, Duarte, California, Professor Karl Sperling, Berlin, Professor Gunter S. Stent of the Wissenschaftskolleg zu Berlin, Professor Friedrich Vogel, Heidelberg, and Dr. Thomas F. Wienker, Freiburg/Berlin for stimulating discussions and/or suggestions. I am also grateful to Dr. Mette Warburg, Gentofte, and Dr. Peter Steinbach, Ulm, for valuable bibliographical hints.

References

Agulnik AI, Mitchell MJ, Mattei M-G, Borsani G, Avner PA, Lerner JL, Bishop CE (1994) A novel X gene with a widely transcribed Y-linked homologue escapes X-inactivation in mouse and human. Hum Mol Genet 3: 879–884

Al-Maghtheh M, Inglehearn CF, Kenn TJ, Evans K, Moore AT, Jay M, Bird AC, Bhattacharya SS (1994) Identification of a sixth locus for autosomal dominant retinitis pigmentosa on chromosome 19. Hum Mol Genet 3: 351–354

Angrist M, Kauffman E, Slaugenhaupt SA, Matise TC, Puffenberger EG, Washington SS, Lipson A, Cass DT, Reyna T, Weeks DE, Sieber W, Chakravarti A (1993) A gene for Hirschsprung disease (megacolon) in the pericentromeric region of human chromosome 10. Nature Genet 4: 351–356

Baird PN, Santos A, Groves N, Jadresic L, Cowell JK (1992) Constitutional mutations in the WT1 gene in patients with Denys-Drash syndrome. Hum Mol Genetics 1: 301–305

Barlow DP (1994) Imprinting: a gamete's point of view. Trends Genet 10: 194–199

Ben Othmane K, Hentati F, Lennon F, Ben Hamida C, Blel S, Roses AD, Pericak-Vance MA, Ben Hamida M, Vance JM (1993a) Linkage of a locus (CMT4A) for autosomal recessive Charcot-Marie-Tooth disease to chromosome 8q. Hum Mol Genetics 2: 1625–1628

Ben Othmane K, Middleton LT, Loprest LJ, Wilkinson KM, Lennon F, Rozear MP, Stajich JM, Gaskell PC, Roses AD, Pericak-Vance MA, Vance JM (1993b) Localization of a gene (CMT2A) for autosomal dominant Charcot-Marie-Tooth disease type 2 to chromosome 1p and evidence of genetic heterogeneity. Genomics 17: 370–375

Bergem ALM (1994) Heredity in dementia of the Alzheimer type. Clin Genet 46: 144–149

Berger R, Abonyi D, Nodot A, Vialatte J, Lejeune J (1970) Hermaphrodisme vrai et "garçon XX" dans une fratrie. Eur J Clin Biol Res 15: 330–333

Bergoffen J, Scherer SS, Wang S, Oronzi Scott M, Bone LJ, Paul DL, Chen K, Lensch MW, Chance PF, Fischbeck KH (1993) Connexin mutations in X-linked Charcot-Marie-Tooth disease. Science 262: 2039–2042

Berson EL (1993) Retinitis pigmentosa. Invest Ophthalmol Vis Sci 34: 1659–1676

Bird TD, Ott J, Giblett ER (1982) Evidence for linkage of Charcot-Marie-Tooth neuropathy to the Duffy locus on chromosome 1. Am J Hum Genet 34: 388–394

Bleeker-Wagemakers LM, Gal A, Kumar-Singh R, Born LI van den, Li Y, Schwinger E, Sandkuijl LA, Bergen AAB, Kenna P, Humphries P, Farrar GJ (1992) Evidence for nonallelic genetic heterogeneity in autosomal recessive retinitis pigmentosa. Genomics 14: 811–812

Brown CJ, Goss SJ, Lubahn DB, Joseph DR, Wilson EM, French FS, Willard HF (1989) Androgen receptor locus on the human X chromosome: regional localization to Xq11-12 and description of a DNA polymorphism. Am J Hum Genet 44: 264–269

Brown CJ, Lafreniere RG, Powers VE, Sebastio G, Ballabio A, Pettigrew AL, Ledbetter DH, Levy E, Craig IW, Willard HF (1991) Localization of the X inactivation center on the human X chromosome in Xq13. Nature 349: 82–84

Burgoyne PS (1989) Thumbs down for zinc finger? Nature 342: 860–862

Byers PH (1990) Brittle bones – fragile molecules: disorders of collagen gene structure and expression. Trends Genet 6: 293–300

Calaf J, Prats J, Esteban-Altirriba J (1994) Female pseudohermaphroditism caused by maternal hyperandrogenism. In: Martinez-Mora J (ed) Intersexual states. Doyoma, Barcelona, pp 187–197

Chance PF, Bird TD, O'Connell P, Lipe H, Lalouel J-M, Leppert M (1990) Genetic linkage and heterogeneity in type I Charcot-Marie-Tooth disease (hereditary motor and sensory neuropathy type I). Am J Hum Genet 47: 915–925

Chromosome Coordinating Meeting (1992) Cuticchia AJ, Pearson PL, Klinger HP (eds) Genome priority reports, vol 1. Basel, Karger

Cooper DN, Krawczak M (1993) Human gene mutation. Bios Scientific, Oxford

Coppes MJ, Liefers GJ, Higuchi M, Zinn AB, Balfe JW, Williams BRG (1992) Inherited WT1 mutation in Denys-Drash syndrome. Cancer Res 52: 6125–6128

Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261: 921–923

Darling SM, Abbott CM (1992) Mouse models of human single gene disorders. I. Nontransgenic mice. Bioessays 14: 359–366

Dean M, Santis G (1994) Heterogeneity in the severity of cystic fibrosis and the role of CFTR gene mutations. Hum Genet 93: 364–368

Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, Puffenberger EG, Hamosh A, Nanthakumar EJ, Curristin SM, Stetten G, Meyers DA, Francomano CA (1991) Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature 352: 337–339

Dietz HC, Pyeritz RE, Puffenberger EG, Kendzior Jr RJ, Corson GM, Maslen CL, Sakai LY, Francomano CA, Cutting GR (1992) Marfan phenotype variability in a family segregating a missense mutation in the epidermal growth factor-like motif of the fibrillin gene. J Clin Invest 89: 1674–1680

DiSilvestre D, Koch R, Groffen J (1991) Different clinical manifestations of hyperphenylalaninemia in three siblings with identical phenylalanine hydroxylase genes.Am J Hum Genet 48: 1014–1016

Donis-Keller H, Dou S, Chi D, Carlson KM, Toshima K, Lairmore TC, Howe JR, Moley JF, Goodfellow P, Wells Jr SA (1993) Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. Hum Mol Genet 2: 851–856

Drugge U, Andersson R, Chizari F, Danielsson M, Holmgren G, Sandgren O, Sousa A (1993) Familial amyloidotic polyneuropathy in Sweden: a pedigree analysis. J Med Genet 30: 388–392

Dubowitz V (1978) Muscle disorders in childhood. Saunders, London

Edery P, Lyonnet S, Mulligan LM, Pelet A, Dow E, Abel L, Holder S, Nihoul-Fékété C, Ponder BAJ, Munnich A (1994) Mutations of the RET proto-oncogene in Hirschsprung's disease. Nature 367: 378–380

Eisensmith RC, Woo SLC (1992) Molecular basis of phenylketonuria and related hyperphenylalaninemias: mutations and polymorphisms in the human phenylalanine hydroxylase gene. Hum Mutat 1: 13–23

Epstein CJ (1986) The consequences of chromosome imbalance. Cambridge University Press, Cambridge

European Polycystic Kidney Disease Consortium (1994) The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. Cell 77: 881–894

Fairweather N, Bell C, Cochrane S, Chelly J, Wang S, Mostacciuolo ML, Monaco AP, Haites NE (1994) Mutations in the connexin 32 gene in X-linked dominant Charcot-Marie-Tooth disease (CMTX1) Hum Mol Genet 3: 29–34

Fields C, Adams MD, White O, Venter JC (1994) How many genes in the human genome? Nature Genet 7: 345–346

Finger S, Heavens RP, Sirinathsinghji DJS, Kuehn MR, Dunnett SB (1988) Behavioural and neurochemical evaluation of a transgenic mouse model of Lesch-Nyhan syndrome. J Neurol Sci 86: 203–215

Fraccaro M, Tiepolo L, Zuffardi O, Chiumello G, Di Natale B, Gargantini L, Wolf U (1979) Familial XX true hermaphroditism and the H-Y antigen. Hum Genet 48: 45–52

Gaunt SJ (1991) Expression patterns of mouse hox genes: clues to an understanding of development and evolutionary strategies. Bioessays 13: 505–513

Gething M-J, Sambrook J (1992) Protein folding in the cell. Nature 355: 33–45

Goate A, Chartier-Harlin M-C, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L, Mant R, Newton P, Rooke K, Roques P, Talbot C, Pericak-Vance M, Roses A, Williamson R, Rossor M, Owen M, Hardy J (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 349: 704–706

Goldstein JL, Brown MS (1989) Familial hypercholesterolemia. In: Scriver C, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease, 6th edn. McGraw-Hill New York, pp 1215–1250

Greenberg J, Goliath R, Beighton P, Ramesar R (1994) A new locus for autosomal dominant retinitis pigmentosa on the short arm of chromosome 17. Hum Mol Genet 3: 915–918

Grouchy J de, Turleau C (1982) Atlas des maladies chromosomiques, 2nd edn. Expansion Scientifique Française, Paris

Güttler F (1992) Molecular basis for the phenotypic diversity of phenylketonuria. Pteridines 3: 5–10

Hassold TJ (1986) Chromosome abnormalities in human reproductive wastage. Trends Genet 2: 105–110

Hastie N (1993) Wilms' tumour gene and function. Curr Opin Genet Dev 3: 408–413

Hawkins JR, Taylor A, Goodfellow PN, Migeon CJ, Smith KD, Berkovitz GD (1992) Evidence for increased prevalence of SRY mutations in XY females with complete rather than partial gonadal dysgenesis. Am J Hum Genet 51: 979–984

Hayasaka K, Himoro M, Sato W, Takada G, Uyemura K, Shimizu N, Bird TD, Conneally PM, Chance PF (1993a) Charcot-Marie-Tooth neuropathy type 1B is associated with mutations of the myelin P_0 gene. Nature Genet 5: 31–34

Hayasaka K, Himoro M, Sawaishi Y, Nanao K, Takahashi T, Takada G, Nicholson GA, Ouvrier RA, Tachi N (1993b) De novo mutation of the meylin P_0 gene in Dejerine-Sottas disease (hereditary motor and sensory neuropathy type III). Nature Genet 5: 266–268

Heyningen V van (1994) One gene – four syndromes. Nature 367: 319–320

Hobbs HH, Brown MS, Russell DW, Davignon J, Goldstein JL (1987) Deletion in the gene for the low-density-lipoprotein receptor in a majority of French Canadians with familial hypercholesterolemia. N Engl J Med 317: 734–737

Hodgson S, Hart K, Abbs S, Heckmatt J, Rodillo E, Bobrow M, Dubowitz V (1989) Correlation of clinical and deletion data in Duchenne and Becker muscular dystrophy. J Med Genet 26: 682–693

Hoffman EP, Brown Jr RH, Kunkel LM (1987) Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell 51: 919–928

Hofstra RMW, Landsvater RM, Ceccherini I, Stulp RP, Stelwagen T, Luo Y, Pasini B, Höppener JWM, Ploos van Amstel HK, Romeo G, Lips CJM, Buys CHCM (1994) A mutation in the RET protooncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature 367: 375–376

Holmgren G, Haettner E, Nordenson I, Sandgren O, Steen L, Lundgren E (1988a) Homozygosity for the transthyretin-met^{30}-gene in two Swedish sibs with familial amyloidotic polyneuropathy. Clin Genet 34: 333–338

Holmgren G, Holmberg E, Lindström A, Lindström E, Nordenson I, Sandgren O, Steen L, Svensson B, Lundgren E, Gabain A van (1988b) Diagnosis of familial amyloidotic polyneuropathy in Sweden by RFLP analysis. Clin Genet 33: 176–180

Holtzman DM, Epstein CJ (1992) The molecular genetics of Down syndrome. In: Friedman T (ed) Molecular genetic medicine, vol 2. Academic Press, New York, pp 105–120

Human Gene Mapping 11 (1991) 11th International Workshop on Human Gene Mapping. Cytogenet Cell Genet 58

Humphries P, Farrar GJ, Kenna P (1993) Autosomal dominant retinitis pigmentosa: molecular, genetic and clinical aspects. Prog Retinal Res 12: 231–245

Hunter A, Tsilfidis C, Mettler G, Jacob P, Mahadevan M, Surh L, Korneluk R (1992) The correlation of age of onset with CTG trinucleotide repeat amplification in myotonic dystrophy. J Med Genet 29: 774–779

Hurst JA, Hall CM, Baraitser M (1991) The Holt-Oram syndrome. J Med Genet 28: 406–410

Ingham PW (1988) The molecular genetics of embryonic pattern formation in *Drosophila*. Nature 335: 25–34

Inglehearn CF, Carter SA, Keen TJ, Lindsey J, Stephenson AM, Bashir R, Al-Maghtheh M, Moore AT, Jay M, Bird AC, Bhattacharya SS (1993) A new locus for autosomal dominant retinitis pigmentosa on chromosome 7p. Nature Genet 4: 51–53

Ionasescu VV, Trofatter J, Haines JL, Summers AM, Ionasescu R, Searby C (1991) Heterogeneity in X-linked recessive Charcot-Marie-Tooth neuropathy. Am J Hum Genet 48: 1075–1083

Ishizaka Y, Itoh F, Tahira T, Ikeda I, Sugimura T, Tucker J, Fertitta A, Carrano AV, Nagao M (1989) Human RET proto-oncogene mapped to chromosome 10q11.2. Oncogene 4: 1519–1521

Jäger RJ, Harley VR, Pfeiffer RA, Goodfellow PN, Scherer G (1992) A familial mutation in the testis-determining gene SRY shared by both sexes. Hum Genet 90: 350–355

Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, Hunt SC, Hopkins PN, Williams RR, Lalouel J-M, Corvol P (1992) Molecular basis of human hypertension: role of angiotensinogen. Cell 71: 169–180

Johns DR, Smith KH, Miller NR, Sulewski ME, Bias WB (1993) Identical twins who are discordant for Leber's hereditary optic neuropathy. Arch Ophthalmol 111: 1491–1494

Jordan SA, Farrar GJ, Kenna P, Humphries MM, Sheils DM, Kumar-Singh R, Sharp EM, Soriano N, Ayuso C, Benitez J, Humphries P (1993) Localization of an autosomal dominant retinitis pigmentosa gene to chromosome 7q. Nature Genet 4: 54–58

Jorm AF, Korten AE, Henderson AS (1987) The prevalence of dementia: a quantitative integration of the literature. Acta Psychiatr Scand 76: 465–479

Kerem E, Corey M, Kerem B-S, Rommens J, Markiewicz D, Levison H, Tsui L-C, Durie P (1990) The relation between genotype and phenotype in cystic fibrosis – analysis of the most common mutation (ΔF_{508}). N Engl J Med 323: 1517–1522

Kessel M, Gruss P (1991) Homeotic transformations of murine vertebrae and concomitant alteration of Hox codes induced by retinoic acid. Cell 67: 89–104

Kimberling WJ, Kumar S, Gabow PA, Kenyon JB, Connolly CJ, Somlo S (1993) Autosomal dominant polycystic kidney disease: localization of the second gene to chromosome 4q13-q23. Genomics 18: 467–472

Knudson AG (1971) Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci USA 68: 820–823

Koenig M, Beggs AH, Moyer M, Scherpf S, Heindrich K, Bettecken T, Meng G, Müller CR, Lindlöf M, Kaariainen H, Chapelle A de la, Kiuru A, Savontaus M-L, Gilgenkrantz H, Récan D, Chelly J, Kaplan J-C, Covone AE, Archidiacono N, Romeo G, Liechti-Gallati S, Schneider V, Braga S, Moser H, Darras BT, Murphy P, Francke U, Chen JD, Morgan G, Denton M, Greenberg CR, Wrogemann K, Blonden LAJ, Paassen HMB van, Ommen GJB van, Kunkel LM (1989) The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. Am J Hum Genet 45: 498–506

Krumlauf R (1994) Hox genes in vertebrate development. Cell 78: 191–201

Kulkens T, Bolhuis PA, Wolterman RA, Kemp S, Nijenhuis S te, Valentijn LJ, Hensels GW, Jennekens FGI, Visser M de, Hoogendijk JE, Baas F (1993) Deletion of the serine 34 codon from the major peripheral myelin protein P_0 gene in Charcot-Marie-Tooth disease type 1B. Nature Genet 5: 35–39

Langenbeck, U, Lukas HD, Mench-Hoinowski A, Stenzig KP, Lane JD (1988) Correlative study of mental and biochemical phenotypes in never treated patients with classic phenylketonuria. Brain Dysfunction 1: 103–110

La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 352: 77–79

Lebo RV, Lynch ED, Wiegant J, Moore K, Trounstine M, Ploeg M van der (1991) Multicolor fluorescence in situ hybridization and pulsed field electrophoresis dissect CMT1B gene region. Hum Genet 88: 13–20

Lewis SE, Erickson RP, Barnett LB, Venta PJ, Tashian RE (1988) N-ethyl-N-nitrosourea-induced null mutation at the mouse Car-2 locus: an animal model for human carbonic anhydrase II deficiency syndrome. Proc Natl Acad Sci USA 85: 1962–1966

Lindsay S, Inglehearn CF, Curtis A, Bhattacharya S (1992) Molecular genetics of inherited retinal degenerations. Curr Opin Genet Dev 2: 459–466

Lobaccaro J-M, Lumbroso S, Belon C, Galtier-Dereure F, Bringer J, Lesimple T, Namer M, Cutuli BF, Pujo H, Sultan C (1993) Androgen receptor gene mutation in male breast cancer. Hum Mol Genet 2: 1799–1802

Lund AM, Eiberg H, Rosenberg T, Warburg M (1992) Autosomal dominant congenital cataract; linkage relations; clinical and genetic heterogeneity. Clin Genet 41: 65–69

Lyon MF (1993) Epigenetic inheritance in mammals. Trends Genet 9: 123–128

Lyon MF, Hawker SG (1973) Reproductive lifespan in irradiated and unirradiated chromosomally XO mice. Genet Res 21: 185–194

Lyonnet S, Bolino A, Pelet A, Abel L, Nihoul-Fékété C, Briard ML, Mok-Siu V, Kaariainen H, Martucciello G, Lerone M, Puliti A, Luo Y, Weissenbach J, Devoto M, Munnich A, Romeo G (1993) A gene for Hirschsprung disease maps to the proximal long arm of chromosome 10. Nature Genet 4: 346–350

Mahaffey SM, Martin LW, McAdams AJ, Ryckman FC, Torres M (1990) Multiple endocrine neoplasia type IIB with symptoms suggesting Hirschsprung's disease: a case report. J Pediatr Surg 25: 101–103

Manseau LJ, Schüpbach T (1989) The egg came first, of course! Trends Genet 5: 400–405

Martin JB (1993) Molecular genetics of neurological diseases. Science 262: 674–676

McBurney MW (1993) X chromosome inactivation: the feminine mystique continues. Bioessays 15: 825–826

McElreavey K, Vilain E, Abbas N, Herskowitz I, Fellous M (1993a) A regulatory cascade hypothesis for mammalian sex determination: SRY represses a negative regulator of male development. Proc Natl Acad Sci USA 90: 3368–3372

McElreavey K, Vilain E, Cotinot C, Payen E, Fellous M (1993b) Control of sex determination in animals. Eur J Biochem 218: 769–783

McKusick VA, Francomano CA, Antonarakis SE (1992) Mendelian inheritance in man. 10th edn. Johns Hopkins University Press, Baltimore London

McLaren A (1991) Development of the mammalian gonad: the fate of the supporting cell lineage. Bioessays 13: 151–156

Mhatre AN, Trifiro MA, Kaufman M, Kazemi-Esfarjani P, Figlewicz D, Rouleau G, Pinsky L (1993) Reduced transcriptional regulatory competence of the androgen receptor in X-linked spinal and bulbar muscular atrophy. Nature Genet 5: 184–188

Monaco AP, Berteleson CJ, Liechti-Gallati S, Moser H, Kunkel LM (1988) An explanation for the phenotypic differences between patients bearing partial deletions of the DMD locus. Genomics 2: 90–95

Moser HW, Moser AB (1989) Adrenoleukodystrophy (X-linked) In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease, 6th edn. McGraw-Hill, New York, pp 1511–1532

Mosser J, Douar A-M, Sarde C-O, Kioschis P, Feil R, Moser H, Poustka A-M, Mandel J-L, Aubourg P (1993) Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. Nature 361: 726–730

Müller B, Melki J, Burlet P, Clerget-Darpoux F (1992) Proximal spinal muscular atrophy (SMA) types II and III in the same sibship are not caused by different alleles at the SMA locus on 5q. Am J Hum Genet 50: 892–895

Mulligan LM, Kwok JBJ, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, Mole SE, Moore JK, Papi L, Ponder MA, Telenius H, Tunnacliffe A, Ponder BAJ (1993) Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature 363: 458–460

Murrell J, Farlow M, Ghetti B, Benson MD (1991) A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. Science 254: 97–99

Nakazato M, Steen L, Holmgren G, et al (1987) Structurally abnormal transthyretin causing familial amyloidotic polyneuropathy in Sweden. Clin Chim Acta 167: 341–342

Nijhout HF (1990) Metaphors and the role of genes in development. Bioessays 12: 441–446

Norris DP, Patel D, Kay GF, Penny GD, Brockdorff N, Sheardown SA, Rastan S (1994) Evidence that random and imprinted Xist expression is controlled by preemptive methylation. Cell 77: 41–51

Ohno S (1970) Evolution by gene duplication. Springer, Berlin Heidelberg New York

Okano Y, Eisensmith RC, Güttler F, Lichter-Konecki U, Konecki DS, Trefz FK, Dasovich M, Wang T, Henriksen K, Lou H, Woo SLC (1991) Molecular basis of phenotypic heterogeneity in phenylketonuria. N Engl J Med 324: 1232–1238

Oyama S (1985) The ontogeny of information. Cambridge University Press, Cambridge London New York New Rochelle Melbourne Sydney

Pankratz MJ, Jäckle H (1990) Making stripes in the *Drosophila* embryo. Trends Genet 6: 287–292

Passarge E (1993) Wither polygenic inheritance: mapping Hirschsprung disease. Nature Genet 4: 325–326

Patel PI (1993) Charcot-Marie-Tooth disease type 1A: mutational mechanisms and candidate gene. Curr Opin Genet Dev 3: 438–444

Patel PI, Lupski JR (1994) Charcot-Marie-Tooth disease: a new paradigm for the mechanism of inherited disease. Trends Genet 10: 128–133

Pelletier J, Bruening W, Kashtan CE, Mauer SM, Manivel JC, Striegel JE, Houghton DC, Junien C, Habib R, Fouser L, Fine RN, Silverman BL, Haber DA, Housman D (1991) Germline mutations in the Wilms' tumor suppressor gene are associated with abnormal urogenital development in Denys-Drash syndrome. Cell 67: 437–447

Pericak-Vance MA, Bebout JL, Gaskell Jr PC, Yamaoka LH, Hung W-Y, Alberts MJ, Walker AP, Bartlett RJ, Haynes CA, Welsh KA, Earl NL, Heyman A, Clark CM, Roses AD (1991) Linkage studies in familial Alzheimer disease: evidence for chromosome 19 linkage. Am J Hum Genet 48: 1034–1050

Peters DJM, Spruit L, Saris JJ, Ravine D, Sandkuij LA, Fossdal R, Boersma J, Eijk R van, Nørby, Constantinou-Deltas CD, Pierides A, Brissenden JE, Frants RR, Ommen G-JB van, Breuning MH (1993) Chromosome 4 localization of a second gene for autosomal dominant polycystic kidney disease. Nature Genet 5: 359–362

Peterson K, Sapienza C (1993) Imprinting the genome: imprinted genes, imprinting genes, and a hypothesis for their interaction. Annu Rev Genet 27: 7–31

Pinsky L, Trifiro M, Beitel LK, Kaufman M (1994) Molecular genetics of androgen insensitivity syndromes in humans. In: Wachtel SS (ed) Molecular genetics of sex determination. Academic Press, San Diego New York Boston London Sydney Tokyo Toronto, pp 341–365

Primrose DA (1983) Phenylketonuria with normal intelligence. J Ment Defic Res 27: 239–246

Prockop DJ, Baldwin CT, Constantinou CD (1990) Mutations in type I procollagen genes that cause osteogenesis imperfecta. Adv Hum Genet 19: 105–132

Puffenberger EG, Kauffman ER, Bolk S, Matise TC, Washington SS, Angrist M, Weissenbach J, Garver KL, Mascari M, Ladda R, Slaugenhaupt SA, Chakravarti A (1994) Identity-by-descent and association mapping of a recessive gene for Hirschsprung disease on human chromosome 13q22. Hum Mol Genet 3: 1217–1225

Reeders ST (1992) Multilocus polycystic disease. Nature Genet 1: 235–237

Reeders ST, Breuning MH, Davies KE, Nicholls RD, Jarman AP, Higgs DR, Pearson PL, Weatherall DJ (1985) A highly polymorphic DNA marker linked to adult polycystic kidney disease on chromosome 16. Nature 317: 542–544

Riccardi VM (1977) Trisomy 8: an international study of 70 patients. Birth Defects: Orig. Article Series XIII (3C): 171–184

Roa BB, Garcia CA, Pentao L, Killian JM, Trask BJ, Suter U, Snipes GJ, Ortiz-Lopez R, Shooter EM, Patel PI, Lupski JR (1993) Evidence for a recessive PMP22 point mutation in Charcot-Marie-Tooth disease type 1A. Nature Genet 5: 189–194

Romeo G, Ronchetto P, Luo Y, Barone V, Seri M, Ceccherini I, Pasini B, Bocciardi R, Lerone M, Kääriäinen H, Martucciello G (1994) Point mutations affecting the tyrosine kinase domain of the RET proto-oncogene in Hirschsprung's disease. Nature 367: 377–378

Rosenfeld PJ, Cowley GS, McGee TL, Sandberg MA, Berson EL, Dryja TP (1992) A null mutation in the rhodopsin gene causes rod photoreceptor dysfunction and autosomal recessive retinitis pigmentosa. Nature Genet 1: 209–213

Sander K (1994) Spuren der Evolution in den Mechanismen der Ontogenese – neue Facetten eines zeitlosen Themas. Leopoldina (R. 3) 39: 297–319

Sandgren O, Drugge U, Holmgren G, Sousa A (1991) Vitreous involvement in familial amyloidotic neuropathy: a genealogical and genetic study. Clin Genet 40: 452–460

Saraiva MJM, Costa PP, Birken S, Goodman DS (1983) Presence of an abnormal transthyretin (prealbumin) in Portuguese patients with familial amyloidotic polyneuropathy. Trans Assoc Am Physicians 96: 261–270

Schellenberg GD, Bird TD, Wijsman EM, Orr HT, Anderson L, Nemens E, White JA, Bonnycastle L, Weber JL, Alonso ME, Potter H, Heston LL, Martin GM (1992) Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. Science 258: 668–671

Schuchardt A, D' Agati V, Larsson-Blomberg L, Costantini F, Pachnis V (1994) Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor ret. Nature 367: 380–383

Scriver CR (1988) Cases are not incidence and vice versa. Genet Epidemiol 5: 481–487

Scriver CR (1991) Phenylketonuria – genotypes and phenotypes. N Engl J Med 324: 1280–1281

Searle AG (1989) Numerical variants and structural rearrangements. In: Lyon MF, Searle AG (eds) Genetic variants and strains of the laboratory mouse, 2nd edn. Oxford University Press, Oxford, pp 582–616

Shapiro BL (1983) Down syndrome – a disruption of homeostasis. Am J Med Genet 14: 241–269

Sicinski P, Geng Y, Ryder-Cook AS, Barnard EA, Darlison MG, Barnard PJ (1989) The molecular basis of muscular dystrophy in the *mdx* mouse: a point mutation. Science 244: 1578–1580

Skordis NA, Stetka DG, Mac Gillivray MH, Greenfield SP (1987) Familial 46,XX males coexisting with familial 46,XX true hermaphrodites in same pedigree. J Pediatr 110: 244–248

Sparkes RS, Sasaki H, Mohandas T, Yoshioka K, Klisak I, Sakaki Y, Heinzmann C, Simon MI (1987) Assignment of the prealbumin (PALB) gene (familial amyloidotic polyneuropathy) to human chromosome region 18q11.2–q12.1. Hum Genet 75: 151–154

Stedman HH, Sweeney HL, Shrager JB, Maguire HC, Panettieri RA, Petrof B, Narusawa M, Leferovich JM, Sladky JT, Kelly AM (1991) The mdx mouse diaphragm reproduces the degenerative changes of Duchenne muscular dystrophy. Nature 352: 536–539

Stent GS (1981) Strength and weakness of the genetic approach to the development of the nervous system. Annu Rev Neurosci 4: 163–194

Stent GS (1985) Thinking in one dimension: the impact of molecular biology on development. Cell 40: 1–2

Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD (1993) Apolipoprotein E: high-activity binding to β-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA 90: 1977–1981

Strohman RC (1993) Ancient genomes, wise bodies, unhealthy people: limits of a genetic paradigm in biology and medicine. Perspect Biol Med 37: 112–145

Surani MA (1994) Genomic imprinting: control of gene expression by epigenetic inheritance. Curr Opin Cell Biol 6: 390–395

Suthers GK, Davies KE (1992) Invited editorial: phenotypic heterogeneity and the single gene. Am J Hum Genet 50: 887–891

Tattersall RB, Pyke DA (1972) Diabetes in identical twins. Lancet II: 1120–1125

Tawara S, Nakazato K, Kangawa H, Matsuo T, Araki S (1983) Identification of amyloid prealbumin variant in familial amyloidotic polyneuropathy (Japanese type). Biochem Biophys Res Commun 116: 880–888

Telenius H, Kremer B, Goldberg YP, Theilmann J, Andrew SE, Zeisler J, Adam S, Greenberg C, Ives EJ, Clarke LA, Hayden MR (1994) Somatic and gonadal mosaicism of the Huntington disease gene CAG repeat in brain and sperm. Nature Genet 6: 409–414

Tsui L-C (1992) The spectrum of cystic fibrosis mutations. Trends Genet 8: 392–398

Vance JM (1991) Hereditary motor and sensory neuropathies. J Med Genet 28: 1–5

Vilain E, McElreavey K, Jaubert F, Raymond J-P, Richaud F, Fellous M (1992) Familial case with sequence variant in the testis-determining region associated with two sex phenotypes. Am J Hum Genet 50: 1008–1011

Vogel F, Motulsky AG (1986) Human genetics – problems and approaches, 2nd edn. Springer, Berlin Heidelberg New York

Waddington CH (1942) The canalization of development and the inheritance of acquired characters. Nature 150: 563–565

Wallace DC (1992) Mitochondrial genetics: a paradigm for aging and degenerative diseases? Science 256: 628–632

Wallace DC (1993) Mitochondrial diseases: genotype versus phenotype. Trends Genet 9: 128–133

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AMS, Elsas II LJ, Nikoskelainen EK (1988) Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 242: 1427–1430

Wallis GA, Starman BJ, Zinn AB, Byers PH (1990) Variable expression of osteogenesis imperfecta in a nuclear family is explained by somatic mosaicism for a lethal point mutation in the αI(I) gene (COLIAI) of type I collagen in a parent. Am J Hum Genet 46: 1034–1040

Warburg M (1991) An update on microphthalmos and coloboma. Ophthalmic Paediatr Genet 12: 57–63

Warburg M (1992) Update of sporadic microphthalmos and coloboma. Ophthalmic Paediatr Genet 13: 111–122

Warburg M (1993) Classification of microphthalmus and coloboma. J Med Genet 30: 664–669

Warburg M, Friedrich U (1987) Coloboma and microphthalmos in chromosomal aberrations. Ophthalmic Paediatr Genet 8: 105–118

Ward K, Hata A, Jeunemaitre X, Helin C, Nelson L, Namikawa C, Farrington PF, Ogasawara M, Suzumori K, Tomoda S, Berrebi S, Sasaki M, Corvol P, Lifton RP, Lalouel J-M (1993) A molecular variant of angiotensinogen associated with preeclampsia. Nature Genet 4: 59–61

Weiss PA (1973) Differentiation and its three facets: facts, terms and meaning. Differentiation 1: 3–10

Weleber RG, Carr RE, Murphey WH, Sheffield VC, Stone EM (1993) Phenotypic variation including retinitis pigmentosa, pattern dystrophy, and fundus flavimaculatus in a single family with a deletion of codon 153 or 154 of the peripherin/RDS gene. Arch Ophthalmol 111: 1531–1542

Wöhrle D, Hennig I, Vogel W, Steinbach P (1993) Mitotic stability of fragile X mutations in differentiated cells indicates early post-conceptional trinucleotide repeat expansion. Nature Genet 4: 140–142

Wolf U. Principles in ontogenesis. Manuscript 1993

Wolf U, Schempp W, Scherer G (1992) Molecular biology of the human Y chromosome. Rev Physiol Biochem Pharmacol 121: 147–213

Wooster R, Mangion J, Eeles R, Smith S, Dowsett M, Averill D, Barrett-Lee P, Easton DF, Ponder BAJ, Stratton MR (1992) A germline mutation in the androgen receptor gene in two brothers with breast cancer and Reifenstein syndrome. Nature Genet 2: 132–134

Wu C-L, Melton DW (1993) Production of a model for Lesch-Nyhan syndrome in hypoxanthine phosphoribosyl-transferase-deficient mice. Nature Genet 3: 235–240