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REVIEW

Dominant versus recessive: Molecular mechanisms in metabolic disease

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Summary Inborn errors of metabolism used to be regarded as simple monogenic traits, but a closer look at how different alleles of a gene determine different phenotypes shows that the molecular mechanisms in the individual case are often complicated. Most metabolic disorders represent a spectrum of phenotypes from normal via attenuated to severe (and sometimes prenatally fatal), and disease manifestation is often influenced by other specific genetic or exogenous factors. The terms 'dominant' or 'recessive' relate to the functional consequences of differing alleles in the (compound) heterozygous individual; the terms are irrelevant for homozygous individuals and inappropriate for X-linked disorders. Mutations affecting the same amino acid residue may be associated with different inheritance patterns. True dominant inheritance in metabolism is rare; it may be found e.g. in tightly regulated biosynthetic pathways or when minor changes in metabolite concentrations have a functional effect. Some disorders such as erythropoietic protoporphyria show pseudodominant inheritance due to prevalent loss-of-function polymorphisms in the general population and are better acknowledged as recessive traits. The term 'variable expressivity' is not helpful with regard to autosomal recessive disorders when variable phenotypes are explained by different mutations in the respective gene. Clonal unmasking of a heterozygous mutation through somatic loss of the second allele, the main pathomechanism in inherited tumour predisposition syndromes, is rare in metabolic disorders, but focal congenital hyperinsulinism is a notable exception. Somatic mosaicism for an *OTC* gene mutation is given as an example of an apparently heterozygous mutation pattern in a boy with an X-linked disease.

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References to electronic databases: OMIM database of individual disorders: <http://www.ncbi.nlm.nih.gov/sites/entrez>
SNP database of genetic variants: <http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp>

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Abbreviations

ALAD	5-aminolevulinic acid dehydratase
ALAS1	5-aminolevulinic acid synthase 1
ASM	acid sphingomyelinase
DGGE	denaturing gradient gel electrophoresis
EIHI	exercise-induced hyperinsulinism
EPP	erythropoietic protoporphyria
FECH	ferrochelatase
GALT	galactose-1-phosphate uridyltransferase
GTA	α-(1→3)-*N*-acetylgalactosaminyltransferase
GTB	α-(1→3)-galactosyltransferase
MCAD	medium-chain acyl-CoA dehydrogenase
MCT1	monocarboxylate transporter 1
MHP	mild hyperphenylalaninaemia
OTC	ornithine transcarbamylase

PAH	phenylalanine hydroxylase
PBGD	porphobilinogen deaminase
PKU	phenylketonuria
UPD	uniparental disomy

Introduction: Archibald Garrod and the question of dominance and recessiveness

Archibald Garrod's concept of 'Inborn Errors of Metabolism' was developed at a time when the rediscovery of Gregor Mendel's experiments on the inheritance of physical characteristics in *Pisum sativum* (Mendel 1866) marked the beginning of modern human genetics. Several botanists including Hugo de Vries (1900), Carl Correns (1900) and Erich von Tschermak-Seysenegg (1900) had independently read Mendel's paper and confirmed his results in additional experiments. The importance of these observations was recognized by William Bateson (1900), who subsequently published an English translation of Mendel's work (Bateson 1902) and later coined several important terms including the term 'genetics' (Bateson 1907). In a footnote to a report on 'Experimental studies in the physiology of heredity', Bateson and Saunders (1902) pointed out that the familial occurrence of alkaptonuria, recently described by Garrod (1899, 1901), was compatible with a Mendelian recessive inheritance pattern. It was the first time that a sound scientific explanation had been suggested for the inheritance of a medical condition in humans.

The concept of recessive inheritance was immediately taken up by Archibald Garrod in his article on 'The incidence of alkaptonuria: A study in chemical individuality':

> Whether the Mendelian explanation be the true one or not, there seems to be little room for doubt that the peculiarities of the incidence of alkaptonuria and of conditions which appear in a similar way are best explained by supposing that, leaving aside exceptional cases in which the character, usually recessive, assumes dominance, a peculiarity of the gametes of *both* parents is necessary for its production. (Garrod 1902)

The inheritance of metabolic disorders as Mendelian traits was further discussed in Garrod's Croonian lectures a few years later (Garrod 1908). He pointed out that pseudodominant transmission of alkaptonuria from parent to offspring, observed in two families, is compatible with the disease being a 'recessive character' (as outlined by Bateson), while he regarded the more complicated inheritance of cystinuria as more compatible with this condition being a 'dominant characteristic'. Garrod even recognized the statistical problem of ascertainment bias in that a 3:1 distribution of affected and non-affected children was observed only in large families with a sufficient number of unaffected individuals (Garrod 1908).

While Garrod was the first person to describe a Mendelian inheritance pattern of a medical condition, the molecular basis of dominance and recessiveness was clearly beyond true understanding at a time when even the chromosomal basis of inheritance was only on the verge of being recognized (Sutton 1903). Mendel himself had taken a purely phenomenological (phenotype-based) approach in that he distinguished 'dominant', 'recessive' and 'hybrid' *characters* based on the observed appearance of the plant and the type of progeny generated (Mendel 1866). The second law of segregation in the offspring of hybrids was described by Mendel as A+2Aa+a, rather than AA+2Aa+aa. Bateson, who introduced the terms 'homozygous' and 'heterozygous', used the same principle with the letters D and R (the crossing of two hybrids DR resulting in 1D, 2DRs and 1R) (Bateson and Saunders 1902). He suggested the use of the morphology-based term 'allelomorphs' for 'unit-characters existing in antagonistic pairs' or even as a continuum of morphological characteristics. Indeed, Bateson only tentatively discusses the 'material' (molecular) basis of inheritance, comparing hybrids with chemical compounds:

> Remembering that we have no warrant for regarding any hereditary character as depending on a material substance for its transmission, we may (...) compare a compound character with a double salt, such as an alum, from which one or other of the metals of the base can be dissociated by suitable means, while the compound acid-radicle may be separated in its entirety, or again be decomposed into its several constituents. Though a crude metaphor, such an illustration may serve to explain the great simplification of the physiology of heredity to which the facts now point. (Bateson and Saunders 1902)

Bateson suggests the possibility of gametes being 'mosaic' in order to explain the localized appearance of 'dominant' features in an apparently pure 'recessive' plant, a phenomenon caused in fact by a mosaic somatic mutation. It was Sutton (1903) who questioned this concept and in his first systematic delineation of the 'chromosome theory' used AA and aa to represent the Mendelian characteristics A and a in their homozygous forms. Sutton concluded that not only in heterozygotes but also in homozygotes 'the paired

arrangement of the chromosomes indicates a dual basis for each character': 'It is probable that specific differences and individual variations are alike traceable to a common source, which is a difference in the constitution of homologous chromatin entities. Slight differences in homologues would mean corresponding, slight variations in the character concerned' (Sutton 1903).

Dominance and recessiveness thus are features of 'chromatin entities' (Sutton 1903) rather than morphological characters, or, using a different terminology, they are functional properties of *alleles*, i.e. DNA variants at a particular gene locus, rather than allelomorphs in the original Batesonian meaning. Although some human geneticists still emphasize that dominance and recessiveness are properties of a character (a phenotype) rather than an allele (Read and Donnai 2007), it may be argued that the genetic basis and inheritance of specific phenotypes is much better understood if dominance and recessiveness are regarded as properties of *genetic information* resulting in a certain function rather than the function itself.

It is essential to recognize, however, that dominance and recessiveness are not features of alleles per se but describe the functional relationship of two alleles in a heterozygous cell or organism with differing genetic information at a given locus. In other words, the terms describe how two different alleles compete for determination of the clinical picture. This general principle was already stated by Mendel (1866), who clearly defined dominant and recessive in relation to the effects observed in hybrids:

> Experiments which in previous years were made with ornamental plants have already affording evidence that the hybrids, as a rule, are not exactly intermediate between the parental species. With some of the more striking characters, those, for instance, which relate to the form and size of the leaves, the pubescence of the several parts, etc., the intermediate, indeed, is nearly always to be seen; in other cases, however, one of the two parental characters is so preponderant that it is difficult, or quite impossible, to detect the other in the hybrid. (...) Henceforth in this paper those characters which are transmitted entire, or almost unchanged in the hybridization, and therefore in themselves constitute the characters of the hybrid, are termed the dominant, and those which become latent in the process recessive. The expression "recessive" has been chosen because the characters thereby designated withdraw or entirely disappear in the hybrids, but nevertheless reappear unchanged in their progeny, as will be demonstrated later on. (Bateson 1902)

Dominant and recessive as medical terms are thus best regarded as categories that help to understand or predict a phenotype in a (compound) heterozygous individual and to understand or predict the inheritance of a phenotype within a family. It is also necessary to distinguish dominance and recessiveness in the individual or family from the effects on the cellular level as there may be fundamental differences. How this all relates to the genetic basis of inborn errors of metabolism is the main focus of this article.

Function dominates over lack of function

Bateson (1909) recognized that an understanding of the inheritance pattern of a condition may 'contribute to a proper understanding of the pathology':

> If, for example, a disease descends through the affected persons, as a dominant, we may feel every confidence that the condition is caused by the operation of a factor or element added to the usual ingredients of the body. In such cases there is something *present*, probably a definite chemical substance, which has the power of producing the affection. (...) On the contrary, when the disease is recessive we recognize that its appearance is due to the absence of some ingredient which is present in the normal body. So, for example, albinism is almost certainly due to the *absence* of at least one of the factors, probably a ferment, which is needed to cause the excretion of pigment; and, as Garrod has shown, alkaptonuria must be regarded as due to the absence of a certain ferment which has the power of decomposing the substance alkapton. (Bateson 1909)

The question still needs to be put why heterozygotes for an enzyme deficiency usually have the same phenotype as homozygotes for the wild type. Most enzymes are present in amounts considerably in excess of what is required to maintain metabolic equilibrium, and protein synthesis and enzyme activity may be further stimulated by rising substrate concentrations. In addition, Kacser and Burns (1981) showed that in multi-enzyme metabolic pathways even a large change in the activity of a single enzyme results in only a negligible change in flux (Fig. 1). Enzyme activity in heterozygotes may be approximately 50% of normal, but this has little effect on the actual metabolic flux *in vivo*. In consequence there is usually little difference in the actual substrate concentration between homozygous wild type and heterozygous mutation carriers in metabolic disorders such as phenylketonuria (Knox

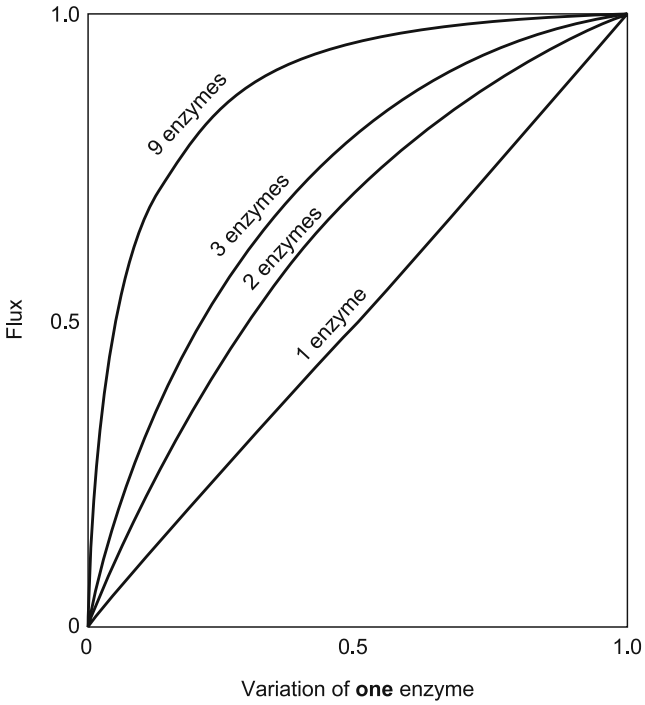

Fig. 1 Effect of reduced enzyme activity on flux in a multi-enzyme pathway. In a multi-step pathway, the overall effect of reduced activity of a single enzyme is influenced by the total number of enzymes. In a 9-enzyme pathway, 50% reduction in the activity of one enzyme leads to less than 10% reduction in flux. (From Kacser and Burns 1981, reprinted with permission of the Genetics Society of America)

and Messinger 1958), and only an (almost) complete loss of enzyme function usually causes a rise in substrate concentrations (Kacser et al 1973) (Fig. 2). For practical purposes, most inborn errors of metabolism are indeed inherited as recessive traits and may be regarded as such in genetic counselling. Nevertheless, the improved understanding of the molecular basis of various metabolic disorders in the last 20 years has shown that Mendelian traits are not quite as simple as sometimes assumed (Scriver and Waters 1999), and that the molecular mechanisms in the individual case are often complicated.

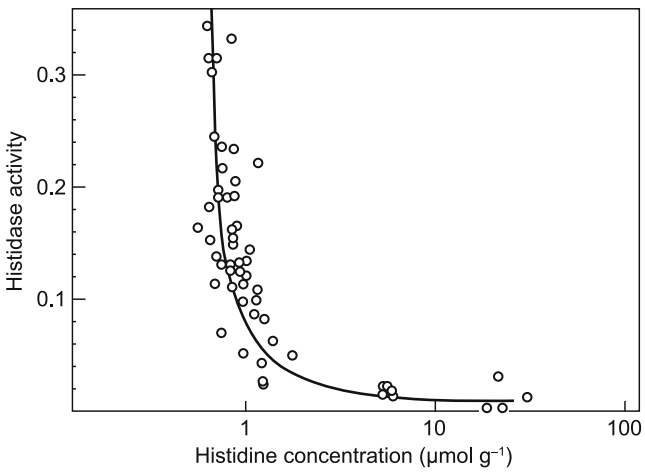

Fig. 2 Effect of reduced enzyme activity on substrate concentration. In a histidinaemia mouse model, only a severe reduction of hepatic histidase activity close to zero causes a rise in histidine concentrations in the liver. (From Kacser et al 1973, reprinted by permission from Macmillan Publishers Ltd)

Mutations with higher residual function have a dominant effect in compound heterozygous patients: phenylketonuria and MCAD deficiency

It is now well accepted that with regard to metabolic disorders a dichotomy of 'disease' versus 'normal' is an oversimplification. Most metabolic disorders are caused by a large number of different mutations that may have different functional effects. While some mutations such as nonsense mutations or many splicing mutations are *null mutations* that cause a complete loss of protein (enzyme) function, others may leave residual enzyme activity. The enzymatic and (if untreated) the metabolic phenotype in any patient is determined primarily by the genotype, i.e. the combined effect of the mutations on both copies of the respective gene. This was studied in detail for phenylalanine hydroxylase (PAH) deficiency which was known to occur in a spectrum of severities ranging from classical severe phenylketonuria (PKU) via moderate and mild PKU to mild hyperphenylalaninaemia not requiring treatment (MHP) (Güttler 1980). It was shown that homozygosity or compound heterozygosity for null mutations always causes classical PKU (Eisensmith and Woo 1992). On the other hand, some mutations with associated high residual PAH activity on one gene copy always cause MHP regardless of the nature of the mutation on the other gene copy (Guldberg et al 1994; Zschocke et al 1994). Such mutations thus *dominate* over more severe mutations in compound heterozygous individuals (just as function generally dominates over lack of function, and wild-type alleles dominate over mutant alleles). Subsequently a system was developed that allows classification of mutations according to the phenotype in 'functional hemizygotes', i.e. patients with a known null mutation on one gene copy (Guldberg et al 1998) (Fig. 3). This system does not take into account the possibility of a functionally relevant interaction of two stable missense mutations in a homomultimer, and disregards the effects that other factors (such as the concentration of the cofactor tetrahydrobiopterin in PAH deficiency) may have on the enzyme stability or activity. Nevertheless, this approach to the prediction of genotype–phenotype

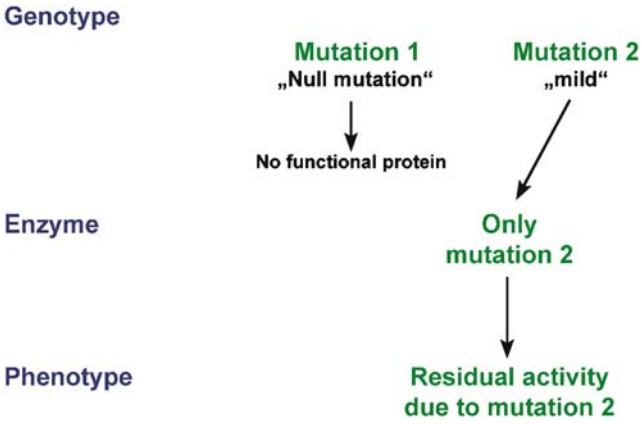

Fig. 3 Functional hemizygosity. Compound heterozygosity for a null mutation leading and a stable missense mutation with residual activity leads to 'functional hemizygosity': the milder mutation only will be assembled in the enzyme (frequently a homomultimer) and will determine the phenotype

correlations has been shown to work quite well, particularly for very severe and very mild mutations, and is applicable not only to PAH deficiency but to the great majority of metabolic disorders.

The fact that metabolic disorders represent a spectrum of enzyme deficiencies from severe via attenuated to harmless can cause difficulties in determining the need for clinical intervention when a diagnosis is made prior to the appearance of clinical symptoms. This is particularly true for the expanded neonatal screening programmes based on tandem-MS analysis that in effect represent a form of *predictive* testing for a large number of metabolic disorders. For example, presymptomatic diagnosis of severe forms of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is of clear, potentially life-saving advantage as it facilitates early intervention in catabolic risk situations. On the other hand, neonatal screening also identifies a considerable number of children with 'mild MCAD deficiency' associated, for example, with a prevalent mutation Y67H (c.199T>C) on one of the two gene copies (Andresen et al 2001; Zschocke et al 2001). Both genetically and enzymatically this attenuated variant is clear 'MCAD deficiency', but the diagnosis may cause more harm than good as no published individual with this kind of genotype has had clinical problems of the disease. Different countries have different ways of dealing with this dilemma, and the neonatal screening programme in the USA, for example, is more proactive than the more cautions approach in Great Britain. In any case it is essential to recognize that there is no 'either disease or normal' but a spectrum of phenotypes reflected in differences in likelihood of metabolic decompensation and the need for treatment. Identification of a specific mutation associated with high residual activity (such as Y67H) may at some stage be regarded as a useful dominant marker of mild MCAD deficiency that does not require treatment, just like MHP in PAH deficiency. However, this is difficult to prove in prospective studies as patients with diagnosed MCAD deficiency will always be 'treated', for example with extra vigilance in fasting periods. For the time being it is prudent to be on the safe side and recommend avoidance of prolonged fasting regardless of the genotype.

Co-dominance in a congenital disorder of glycosylation: the ABO blood group

The ABO blood group system best illustrates the principle that dominant and recessive describe the functional effects of two different alleles in a (compound) heterozygous individual, and not properties of an allele per se. The different blood groups effectively represent a congenital disorder of glycosylation involving terminal fucose-α1→2-galactose residues of complex carbohydrate structures expressed at the surface of erythrocytes or other cells. Blood group A represents oligosaccharides in which *N*-acetylgalactosamine is attached to the galactose residue, while in blood group B galactose is linked to this residue. Two different glycosyltransferases are responsible for these reactions: α-(1→3)-*N*-acetylgalactosaminyltransferase (GTA, EC 2.4.1.40) generates the A agglutinogen, while α-(1→3)-galactosyltransferase (GTB, EC 2.4.1.37) generates the B agglutinogen (Fig. 4). Interestingly, both enzymes are encoded by the same gene, the ABO gene on chromosome 9q34.2 (Yamamoto et al 1990b). The functional alleles coding for GTA and GTB, respectively, show sequence differences that affect four critical amino acid residues of the enzyme: Gly/Arg176, Gly/Ser235, Leu/Met266 and Ala/Gly268 (Yamamoto et al 1990a). GTB has a significantly smaller active site cleft that cannot accommodate the more bulky substrate UDP-GalNAc, while the active site in GTA is too large for UDP-Gal as a substrate (Alfaro et al 2008; Patenaude et al 2002). Finally, there is a common frameshift deletion c.258delG, which causes a complete loss of the functional protein; this allele (here denoted GT'O') is associated with the blood group O. Individuals who have two different alleles coding for GTA and GTB, respectively, will generate both A and B agglutinogen; they have blood group AB. GTA and GTB alleles are thus *co-dominant* towards each other (Fig. 4b). In contrast, individuals with two different alleles coding for GTA (or GTB) and the null allele, respectively, will generate A (or B) agglutinogen only.

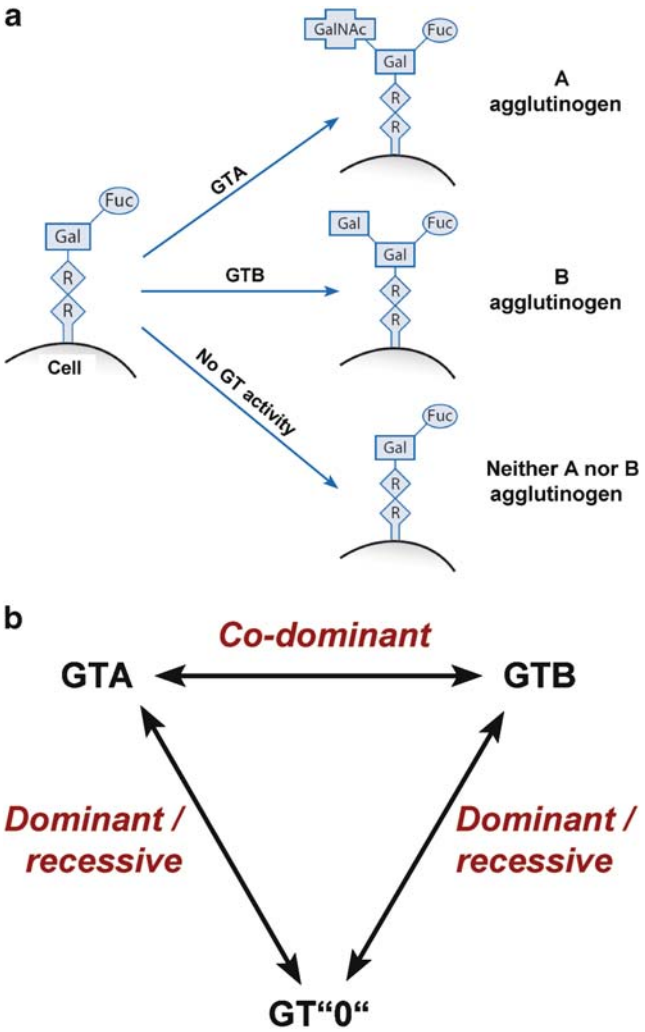

Fig. 4 Dominance and co-dominance in the ABO blood group system. (**a**) The glycosylation enzymes α-(1→3)-*N*-acetylgalactosaminyltransferase (GTA, EC 2.4.1.40) and α-(1→3)-galactosyltransferase (GTB, EC 2.4.1.37) are encoded by different alleles of the ABO gene and give rise to the blood group agglutinogens A and B. Another allele in this gene contains a null mutation and in the homozygous state leads to blood group O. (**b**) Alleles coding for GTA and GTB are co-dominant towards each other but are dominant over the recessive allele GT'O'

GTA and GTB alleles are *dominant* over the null allele; just as carrier status for a null allele in recessive disorders has little or no functional effect.

Pseudodominant inheritance caused by prevalent loss-of-function alleles in the general population: erythropoietic protoporphyria

Garrod already observed that a recessive disorder may occur in successive generations if an affected individual happens to have children with a heterozygous carrier for the same disease (Garrod 1908). This *pseudodominant* inheritance is well known to all clinicians who care for a large number of families with metabolic disorders. For example, the obvious likelihood that a European individual with PKU will have a partner who is carrier for PKU is 1:30–1:50, the carrier frequency in the European population (2–3%). It is important to note that the clinical severity of the disease in the affected child can differ from the severity in the parent: If the affected parent has an attenuated form of the disease because of compound heterozygosity for a mild and a severe mutation, and the parent's partner is carrier for a severe mutation, the child may have a severe or attenuated form of the disease depending on which mutation was inherited from the affected parent. This mechanism should not be confused with the variable expressivity in dominant disorders, where the clinical picture can be different in individuals with the same genotype. Similarly, variable penetrance in dominant disorders should not be confused with variable presence or absence of a pseudodominant disorder in different mutation carriers of the same family. True dominant disorders show variable penetrance and expressivity because of chance events or genetic or non-genetic factors unrelated to the specific disease gene. In contrast, clinical variability of the phenotype in pseudodominant disorders is fully explained by the presence of different mutations or variants within the disease gene.

Sequencing the coding region of a gene may not be sufficient for solving pseudodominant inheritance in a family as there may be intronic variants that affect expression or splicing and in consequence protein function. This is the case in erythropoietic protoporphyria (EPP), the most common form of the primarily erythropoietic porphyrias. It is caused by a deficiency of ferrochelatase (FECH), which inserts ferrous iron into protoporphyrin IX to generate haem (Fig. 5). The disease usually presents with marked photosensitivity of the skin although (potentially fatal) liver disease may occur; no drug or other environmental factor is known to precipitate attacks. Familial occurrence of EPP in different generations led to the conclusion that it was a dominant disorder with reduced penetrance (Reed et al 1970), even though inconsistencies were noted at an early stage (Gasser-Wolf 1965). Autosomal recessive inheritance was demonstrated for cases of severe EPP with potentially fatal liver failure (Sarkany et al 1994). The inheritance patterns were finally clarified by Gouya and co-workers (2002) who identified a common intronic polymorphism IVS3–48T>C (SNP rs2272783) that is associated with increased frequency of aberrant splicing and reduced ferrochelatase activity. This variant has an allele frequency of

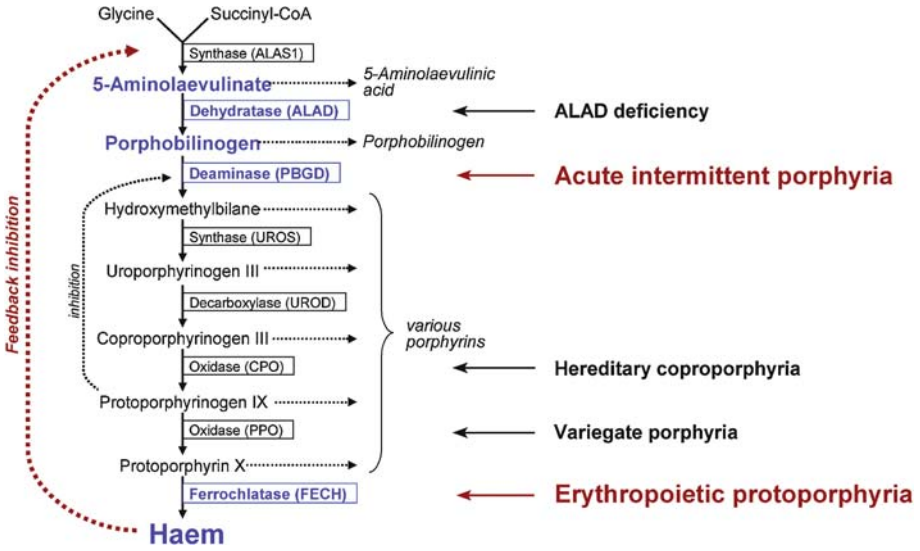

Fig. 5 The hepatic haem biosynthetic pathway. Acute porphyrias are characterized by accumulation of neurotoxic 5-aminolevulinic acid and porphobilinogen. They may be caused either by primary porphobilinogen deaminase (PBGD) deficiency (acute intermittent porphyria, the most common acute porphyria) or 5-aminolevulinic acid dehydratase (ALAD) deficiency, or by secondary inhibition of PBGD in hereditary coproporphyria or variegate porphyria. Increased production of true porphyrins that cause photosensitivity is found in enzyme deficiencies beyond PBGD. The most common erythropoietic porphyria is erythropoietic protoporphyria, the genetic defect of the last step of haem biosynthesis. Haem deficiency causes upregulation of 5-aminolevulinic acid synthase 1 (ALAS1), the rate-limiting first step in hepatic haem biosynthesis

4% or higher in European populations and particularly in Japan, but appears to be rare in individuals of sub-Saharan African origin (HapMap CEPH sample at www.ncbi.nlm.nih.gov/SNP/, build 129; Gouya et al 2006; Nakano et al 2006). The molecular data confirmed a three-allele system previously postulated by Went and Klasen (1984), comprising (a) the wild-type allele, (b) various rare disease-causing mutations that cause severe loss of enzyme function, and (c) the splicing variant IVS3−48C, which is prevalent in the general population. The molecular data thus show that EPP is *always inherited as an autosomal recessive trait*, caused by loss-of-function variants on both copies of the *FECH* gene. Compound heterozygosity for a severe mutation and the prevalent IVS3−48C variant causes the much more frequent attenuated, photosensitivity type of the disease, while the combination of two severe mutations causes a more severe phenotype with potentially fatal liver disease. Unfortunately, the incorrect term 'dominant' for the attenuated form of EPP is still used in recent publications (Egger et al 2006; Gouya et al 2006; Holme et al 2007; Nakano et al 2006). If anything, it is pseudodominant inheritance that causes recurrence of the disease in several generations of a family.

It is important to note that the three-allele system outlined for EPP refers to the presence of three different allele types in the same gene in the general population and should not be confused with triallelic inheritance of disorders caused by a combination of three mutations in two different genes, as in Bardet–Biedl syndrome. Triallelic inheritance has not yet been reported for metabolic disorders, although clinical manifestation of some disorders may be influenced by genetic variants in other pathways (Vockley 2008). This, however, is not a focus of the present article.

Attenuated forms caused by compound heterozygosity for a known disease-causing mutation and a loss-of-function variant that is prevalent in the general population have been recognized in many metabolic disorders. Possibly the earliest example is the Duarte (D2) allele of galactose-1-phosphate uridyltransferase (GALT), which is found with a frequency of 5–10% in European populations (Tyfield et al 1999). The Duarte allele is associated with a 50% reduction in GALT activity (Beutler et al 1965) caused by a 4 bp deletion in the promoter region of the *GALT* gene (Kozak et al 1999; Trbušek et al 2001). Compound heterozygosity for the Duarte allele and a severe *GALT* mutation is recognized in neonatal screening for galactosaemia but does not require treatment. Intermittent or 'mild' trimethylaminuria, or fish odour syndrome, caused by a deficiency of flavin-containing monooxygenase type III (FMO3) was found to be due to compound

heterozygosity for a severe mutation and a prevalent 'variant allele' [E158K+E308G] of the *FMO3* gene that has an allele frequency of up to 20% in some populations (Zschocke et al 1999). Clinical symptoms (malodour) in this attenuated form of FMO3 deficiency depend on external factors such as intake of choline-rich foods. Interestingly, homozygosity for the variant allele is not usually associated with symptoms but may explain malodour in children on treatment with carnitine, which is converted into trimethylamine (unpublished data). Again there is a continuous spectrum from severe enzyme deficiency via mild deficiency to normal, as found in principle for all metabolic disorders.

Semidominant inheritance: familial hypercholesterolaemia

All conditions discussed in the preceding section are inherited as autosomal recessive traits but the discrimination from dominant traits becomes blurred when minor clinical effects may be found in heterozygous individuals who carry a mutation and a wild-type allele. For some recessive disorders such effects may be observed only in special circumstances: heterozygote advantage (overdominant selection) at some stage in the past is assumed for cystic fibrosis carriers (Pier et al 1998; Wiuf 2001) and has been suggested for PKU (Krawczak and Zschocke 2003). The term *overdominance* is used to describe the situation when the phenotype of the heterozygote is 'fitter' than either homozygote. The effect may be restricted to very particular circumstances and is normally of little clinical relevance. Overdominance is difficult to prove but may be postulated from the distribution pattern of disease mutations in different populations (Krawczak and Zschocke 2003). Another, more fundamental question is that of the definition of the wild-type allele: is it the most frequent allele or the allele with the highest function? This is not easy to answer, as exemplified by the ABO blood group system, where the O allele characterized by complete loss of gene function is the prevalent allele in many populations, or by galactosaemia where the rare Los Angeles (D1) allele is associated with increased enzyme activity due to a gain-of-function mutation.

In most recessive and dominant disorders alike, different genotypes cause a spectrum of phenotypes from normal via mild to severe, and the only difference is that, in dominant disorders, clinical symptoms are a regular feature in the heterozygote. A much more severe phenotype is usually observed in individuals who are homozygous for a loss-of-function mutation, and the term *semidominant* (or incomplete dominant) inheritance is used to describe this phenomenon. A prominent example is familial hypercholesterolaemia (FH) caused by mutations in the low-density-lipoprotein receptor (*LDLR*) gene. Heterozygous LDLR deficiency is characterized by moderately elevated cholesterol concentrations in blood and an increased risk for premature atherosclerosis and cardiovascular disease; individuals with homozygous *LDLR* gene mutations, in contrast, show extremely high blood cholesterol concentrations and may suffer a myocardial infarction at early school age. Again, disease severity and age of manifestation are influenced by the individual *LDLR* mutation. We previously reported monozygous twins with a very early clinical diagnosis of homozygous LDLR deficiency (Zschocke and Schaefer 2003) (Fig. 6). Blood LDL cholesterol concentrations in the twins were 27.8 and 30.5 mmol/L (1080 and 1180 mg/dl) and thus even higher than in other reported patients. The excessively high LDL cholesterol is partly explained by the nature of the mutation W577R (c.1729T>C) identified in this family. This mutation affects a highly conserved YWTD repeat that constitutes part of the LDLR β-propeller. Confocal microscopy and western blot experiments revealed that the mutation produces a class 2a receptor that is completely retained in the ER, indicating complete loss of protein function (Soufi et al, submitted). Consequently the homozygous twins are unable to produce any LDLR protein that reaches the cell surface, in contrast to other homozygous patients with other missense mutations that allow expression of stable LDLR with residual function on the cell. It is not surprising that the most severe coronary atherosclerosis is observed in homozygous patients with the lowest residual LDL receptor activity and the highest plasma LDL cholesterol levels (Bertolini et al 1999). A similar correlation between type of mutation and LDL cholesterol concentrations was also reported for heterozygous individuals (Bertolini et al 2000), although there are other genetic and non-genetic factors that influence cholesterol concentrations and the development of clinical symptoms (Alonso et al 2008; Jansen et al 2002, 2005). In conclusion, LDLR deficiency is a dominant disorder because even minor elevation of blood cholesterol has a potential phenotypic effect. In a metabolic system with a direct correlation between protein function and clinical phenotype, reduced function is immediately noticeable and thus dominates over normal function. Disease severity represents a continuous spectrum with a much more severe phenotype in homozygous individuals than heterozygous mutation carriers, reflecting a semidominant inheritance pattern.

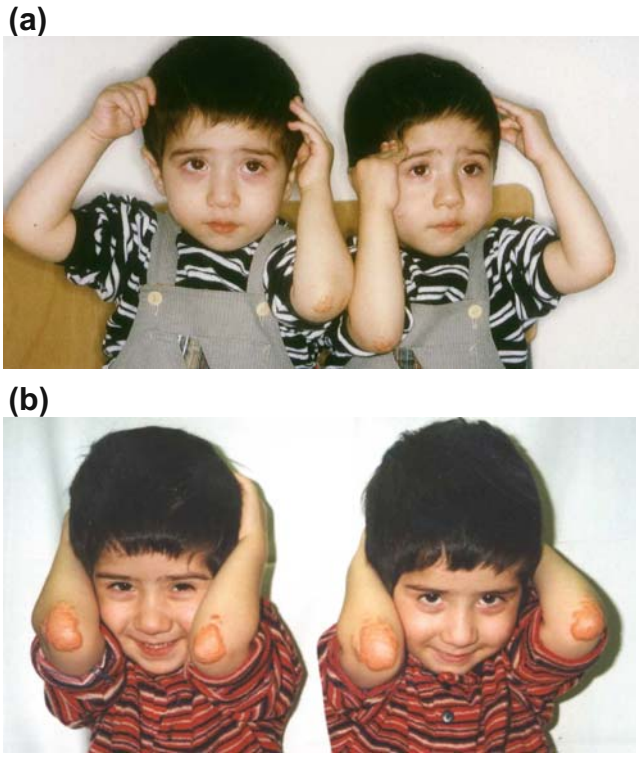

Fig. 6 Homozygous LDL receptor deficiency in monozygotic twins. Xanthomas dramatically increased in size from age 2 years (Fig. 6a) to 3 years (Fig. 6b). At the time of the second photograph, regular lipid apharesis was initiated which led to reversal of lipid deposition and a continuous decrease in the size of the xanthomas (not shown). The twins are currently well at the age of 11 years

True dominance with similar clinical phenotypes in heterozygous and homozygous mutation carriers, as observed in Huntington disease (Narain et al 1999), has not been reported in metabolic disorders.

Dominant inheritance in a highly regulated biosynthetic pathway: acute porphyrias

Most enzymes involved in the *breakdown* of molecules have a high capacity regulated by substrate concentrations, and a 50% activity reduction due to heterozygosity for a loss-of-function mutation does not usually lead to relevant metabolic effects. Therefore, as discussed above, deficiencies of such enzymes are generally inherited as autosomal recessive traits. *Biosynthetic* pathways, in contrast, may need to be tightly controlled in order to secure sufficient amounts of product but avoid potentially harmful overproduction. Such pathways are thus intrinsically less stable, and partial genetic deficiencies in heterozygous individuals are more likely to lead to clinical symptoms. A well-known example of such a disorder, inherited as a dominant trait, is acute intermittent porphyria (AIP).

Regulation of haem biosynthesis in erythrocyte progenitor cells and the liver, respectively, shows important differences with a direct impact on disease manifestation in the porphyrias (Ajioka et al 2006). In the liver, haem is mainly required for enzymes, e.g. of the cytochrome P450 system, and haem biosynthetic enzymes are turned over rapidly to adapt to changing metabolic needs. In contrast, erythrocyte progenitor cells show a high steady-state level of haem biosynthesis that depends primarily on the availability of iron. Several enzymes in the haem biosynthetic pathway (Fig. 5) have two different promoters that allow differential expression. There are two isozymes of the rate-limiting first enzyme in the pathway, 5-aminolevulinic acid synthase (ALAS), coded by two different genes. The hepatic isozyme ALAS1 is highly regulated and has an extremely short half-life of 1–3 h in the liver of different animals (Anderson et al 1981). Synthesis and mitochondrial translocation of ALAS1 is inhibited by subcellular 'free haem' not incorporated into haemoproteins, while haem depletion caused, for example, by increased cytochrome P450 synthesis in response to certain drugs leads to a multifold increase in ALAS1 activity.

AIP is caused by a 50% reduction in the activity of porphobilinogen deaminase (PBGD), the third step in haem biosynthesis, due to heterozygous loss-of-function mutations in the *PBGD* gene. The disease typically presents with intermittent attacks of abdominal pain and other neurovisceral and circulatory disturbances, although some patients have isolated neurological or psychiatric abnormalities. AIP is diagnosed through increased urinary and faecal excretion of the porphyrin precursors 5-aminolevulinic acid and porphobilinogen, particularly at the time of acute symptoms (Anderson et al 2005). Remarkably, only a minority of mutation carriers (possibly fewer than 10%) develop clinical symptoms even though there is no difference in residual PBGD activity in symptomatic and asymptomatic heterozygotes (Badminton and Elder 2005; Puy et al 1997; Strand et al 1972). At an early stage it was recognized that disease manifestation in the acute porphyrias is triggered by a variety of precipitating factors such as drugs (in particular inducers of cytochrome P450 enzymes) hormones (progesterone), nutritional factors (low cellular glucose levels) as well as smoking, alcohol and stress (Anderson et al 2005; Stokvis 1889). Symptoms in acute intermittent porphyria arise when an increased demand for hepatic haem cannot be met by the limited capacity of the biosynthetic pathway, determined in this case by half-normal PBGD activity. In this situation, depletion of cellular haem causes disinhibition of

the rate-limiting enzyme ALAS1 and results in the production of excessive amounts of porphyrin precursors. Neurological symptoms are thought to be due to precursor toxicity rather than haem deficiency, although the exact pathomechanism is still unknown (Solis et al 2004). In conclusion, AIP is a dominant disorder because (1) a half-normal PBGD activity is not sufficient to meet demands when flux through the biosynthetic pathway is upregulated, and (2) accumulating substrates are highly toxic.

There are very few reports of children with more severe PBGD deficiency caused by homozygosity or compound heterozygosity for mutations in the *PBGD* gene. Clinical symptoms include severe mental retardation and central and peripheral neurological disturbances (Beukeveld et al 1990; Solis et al 2004). Mutations identified in such patients leave significant residual activity and are rarely associated with clinical symptoms in heterozygous carriers. It is possible that homozygosity for null mutations in the *PBGD* gene is not compatible with life.

Two other acute porphyrias, hereditary coproporphyria and variegate porphyria caused by deficiencies of coproporphyrinogen oxidase (CPO) and protoporphyrinogen oxidase (PPO), respectively, are also inherited as autosomal dominant traits. Attacks are associated with accumulation of 5-aminolevulinic acid and porphobilinogen, probably due to inhibition of PBGD (Meissner et al 1993); accumulation of other porphyrins causes photosensitivity, which may be the only symptom in these disorders. The fourth acute porphyria, 5-aminolevulinic acid dehydratase (ALAD) deficiency, is inherited as an autosomal recessive trait: normal activity of ALAD greatly exceeds that of the other enzymes in the pathway, and a much more severe deficiency (<5% normal) is required for the development of neurovisceral symptoms (Bird et al 1979; Gross et al 1998).

Dominant inheritance associated with reduced metabolite transport or production: neurometabolic disorders

In some conditions, full metabolic capacity is required for the provision of adequate amounts of particular metabolites, and reduced product concentrations in heterozygous individuals result in clinical symptoms. Heterozygous mutations in the *GLUT1* gene coding for the glucose transporter across the blood–brain barrier cause reduced availability of glucose in CSF and brain (hypoglycorrhachia), and severe epileptic encephalopathy. It can be successfully treated with ketogenic diet, providing an alternative energy substrate (Klepper and Leiendecker 2007). Complete loss of GLUT1 function caused by homozygous null mutations is thought to be embryonically lethal. Tetrahydrobiopterin (BH_4) is required as a cofactor for several hydroxylases including phenylalanine hydroxylase and tyrosine hydroxylase. Severe deficiency of one of the enzymes of BH_4 biosynthesis causes 'atypical PKU', a combination of elevated phenylalanine concentrations and neurotransmitter disturbances; the respective conditions are inherited as autosomal recessive traits (Thony and Blau 2006). In contrast, heterozygous mutations in the *GCH1* gene coding for GTP cyclohydrolase I, the first enzyme in BH_4 biosynthesis, cause dopa-responsive dystonia (Segawa syndrome), a condition well treatable with oral levodopa, and inherited as a dominant trait (Ichinose et al 1994; Segawa et al 2003; Thony and Blau 2006). Like all disorders with a 'semidominant' inheritance pattern, GTP cyclohydrolase I deficiency is associated with a continuous spectrum of clinical phenotypes from attenuated to severe, with manifestation in the heterozygote sometimes explained by a dominant negative effect (Hwu et al 2000) and intermediate presentations caused by specific genotypes (Horvath et al 2008; Hwu et al 1999; Nardocci et al 2003).

Dominant negative effect in a multimeric protein complex: congenital hyperinsulinism

Heterozygous missense mutations affecting structural proteins frequently cause dominant disorders because a misfolded but stable protein produced by the mutant allele interferes with the function of the normal protein produced by the normal allele. The phenotypic consequences are often more severe than in complete loss of allele function caused by a heterozygous deletion or null mutation that generates no or unstable protein. This *dominant negative* effect is well known, fore example from osteogenesis imperfecta in which the severe, prenatally lethal type II is usually caused by *COL1A1/2* missense mutations affecting glycine residues important for helix formation, resulting in stable misfolded procollagen fibres. The much milder type I associated with increased likelihood of fractures but normal stature in adults, on the other hand, is usually caused by mutations that completely remove gene function and result in reduced amount of structurally normal collagen 1 (Byers 2001).

A dominant negative effect has also been demonstrated in some metabolic disorders that involve

multimeric enzymes. Co-expression of the common galactosaemia-mutation Q188R and GALT wild-type was found to be associated with a reduction of enzyme activity to 15%, much lower than the (expected) half-normal activity observed when a different mutation (R333W) was co-expressed with wild-type. Neither heterodimer varied significantly from the wild-type with regard to apparent K_m for two substrates, although Q188R/WT but not R333W/WT heterodimers demonstrated significantly increased thermal sensitivity (Elsevier and Fridovich-Keil 1996). Q188R not only affects enzymatic function in the active site in which it is inserted but also appears to interfere with the active site of the other subunit via perturbation of the interface geometry (Marabotti and Facchiano 2005). A dominant negative effect was also reported for mutation R385S in 3-methylcrotonyl-CoA carboxylase (MCC) deficiency (Baumgartner et al 2004). Negative dominant effects in enzyme disorders are limited to specific missense mutations and are more difficult to recognize than in disorders of structural proteins. It is important to realize that although dominant negative missense mutations in heterozygotes have a more severe impact on the phenotype than null mutations, homozygosity for a dominant negative mutation could be associated with residual function and thus may have a less severe impact on the phenotype than homozygosity for a null mutation (provided that homozygous mutations are compatible with life).

A remarkable example of a dominant negative effect in a 'metabolic' disorder is found in congenital hyperinsulinism. This disease is characterized by non-physiological release of insulin from the pancreatic β-cell when blood glucose concentrations are low. It is frequently caused by malfunction of the inwardly rectifying ATP-sensitive potassium (K_{ATP}) channel in the β-cell, closure of which causes cell depolarization and insulin exocytosis. The K_{ATP} channel is a hetero-multimer consisting of four Kir6.2 subunits encoded by the *KCNJ11* gene, surrounded by four SUR1 subunits encoded by the *ABCC8* gene (Nichols 2006). Both genes are located on chromosome 11p15. The deficiency of the K_{ATP} channel is usually inherited as an autosomal recessive trait caused by loss-of-function mutations in either of these genes (more frequently *ABCC8*; Gloyn et al 2006); by contrast, heterozygous *gain-of-function* mutations in *KCNJ11* cause neonatal diabetes mellitus, a dominant trait due to permanent repression of insulin release (Gloyn et al 2004). Very few cases of dominantly inherited hyperinsulinism caused by heterozygous mutations in either *ABCC8* or *KCNJ11* have been reported (Huopio et al 2000; Lin et al 2006; Thornton et al 2003). Mutations affecting the same amino acid residue, or single amino acid deletions affecting adjacent residues, may be associated with different inheritance patterns: for example, the *ABCC8* mutation R1353H results in SUR1 protein that is normally expressed on the cell surface and acts as a dominant mutation, causing partially disrupted receptor function, in contrast to the mutation R1353P which is not expressed at the plasma membrane and acts as a non-functional recessive mutation (Magge et al 2004). Pinney and co-workers (2008) reviewed and analysed the effects of all known dominantly inherited K_{ATP} mutations, including 11 *ABCC8* and 3 *KCNJ11* mutations. All of them showed normal (or at most mildly impaired) channel assembly and trafficking to the cell surface. Overexpression of dominant *ABCC8* mutations revealed significantly diminished or absent response to MgADP or diazoxide, which normally trigger opening of the K_{ATP} channel, whilst dominant *KCNJ11* mutations resulted in low or absent conductivity of the K_{ATP} channel. Under simulated conditions of heterozygosity these effects were still noticeable, but the wild-type subunits were sufficient to confer

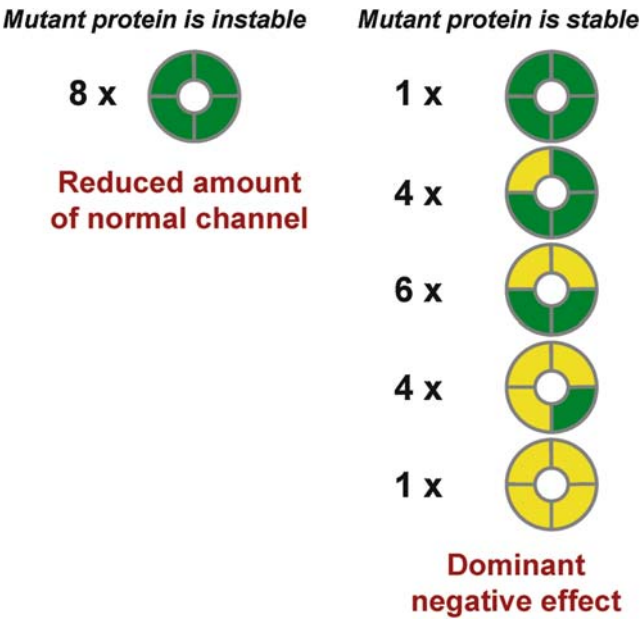

Fig. 7 Schematic diagram of a dominant negative effect in a homotetrameric membrane channel. Heterozygosity for a null mutation that produces an instable protein would be expected to lead to a reduced number of structurally normal channels. In contrast, heterozygosity for a dominant negative mutation leading to random integration of stable but misfolded proteins would affect a much greater proportion of channels; in theory, only 1 out of 16 channel homotetramers would consist of four normal subunits and would function normally

partial channel function or response to channel agonists. Considering that each K_{ATP} channel consists of four SUR1 and Kir6.2 subunits and that mutant and wild-type forms are randomly assembled in heterozygous individuals, the chance of a completely normal K_{ATP} channel (with four normal subunits) is 1/16 when 50% of the produced subunits are stable mutants with a dominant negative effect (Fig. 7). Presence of a heterozygous dominant mutation therefore renders the great majority of K_{ATP} channels in a β-cell non-functioning or resistant to channel agonists. In contrast, heterozygous null mutations that cause, for example, an unstable protein are expected to lead to at most a 50% reduction of normally regulated K_{ATP} channels in the β-cell, which is not associated with a clinical phenotype.

Dominant activating mutations that disturb highly regulated cellular functions: congenital hyperinsulinism

In most metabolic pathways, mutations associated with an increased activity of an enzyme do not cause a clinical phenotype. In some instances, however, cellular functions depend on exact regulation of product concentrations, and overactivation of a relevant metabolic step causes clinical symptoms. For example, opening and closure of the K_{ATP} channel involved in insulin release is regulated by the ATP concentration in the pancreatic β-cell. Heterozygous activating mutations of either glucokinase or glutamate dehydrogenase lead to high intracellular ATP despite normal or low blood glucose and in consequence to hyperinsulinism (Glaser et al 1998; Stanley et al 1998). Both conditions, inherited as autosomal dominant traits, are well treatable with the potassium channel activator diazoxide. A related pathomechanism was recently reported in exercise-induced hyperinsulinism (EIHI) characterized by inappropriate insulin secretion during anaerobic exercise or on pyruvate load. EIHI is caused by activating mutations in the promoter of the *SLC16A1* gene coding for the monocarboxylate transporter 1 (MCT1) and again is inherited as an autosomal dominant trait. The MCT1 is not normally expressed in the pancreatic β-cell. Mutations found in EIHI patients abolish transcriptional silencing of the gene and lead to inappropriate expression of the transporter in the pancreatic β-cell, permitting pyruvate uptake and metabolism particularly under anaerobic conditions. The consequences are increased intracellular ATP concentrations and hyperinsulinism (Otonkoski et al 2007).

Transcriptional silencing of an imprinted gene: Niemann–Pick disease

Another mechanism by which transcriptional silencing may affect manifestation of a metabolic disease was reported for Niemann–Pick disease types A and B caused by the deficiency of acid sphingomyelinase (ASM). The *SMPD1* gene encoding this enzyme is paternally imprinted, and ASM activity therefore appears to be largely determined by the function of the maternally inherited gene copy. Niemann–Pick disease is normally inherited as an autosomal recessive trait but heterozygous individuals that inherit a mutation from the mother may sometimes develop symptoms (Simonaro et al 2006). It is possible that imprinting is restricted to certain organs only and that additional factors may be necessary for carrier manifestation, but this awaits further study.

Dominant gain of function caused by clonal loss of the second (maternal) allele: focal congenital hyperinsulinism

The mechanisms of dominance and recessiveness discussed so far are similar in the sense that there is a *spectrum* of phenotypes explained by the specific underlying genotype combined with other genetic and non-genetic factors, and associated with variable disease manifestation in heterozygous, compound heterozygous and homozygous individuals. However, a fundamentally different mechanism of dominance is found in many of the most common dominant disorders including familial cancer predisposition syndromes and neurocutaneous syndromes (hamartoses or phakomatoses). The underlying pathomechanism in these disorders is clonal cell proliferation due to the loss of a growth-inhibiting factor such as a tumour suppressor gene. The retinoblastoma protein pRb encoded by the *RB1* gene, for example, has a central role in many cellular processes including cell cycle regulation, DNA damage response and repair, DNA replication, cell differentiation, and apoptosis. Deficiency of pRb contributes to tumorigenesis through increased cell proliferation rates and decreased differentiation potential (Classon and Harlow 2002). It is important to recognize that, on a cellular level, pRb deficiency is an autosomal recessive trait, with loss of both gene copies required for pathogenesis. Two mutational events (two 'hits') affecting the two copies of the *RB1* gene are required in the development of retinoblastoma. As the probability of two independent somatic mutation events affecting both copies of a gene in the same cell (clone) is small, sporadic

retinoblastomas are rare tumours. In families with an inherited heterozygous mutation in the *RB1* gene, however, only one additional somatic mutation on the other allele is required for cellular loss of pRb function. Considering the huge number of retinoblast cells involved in eye development, the probability that this 'second hit' will take place in one of them is rather high. The 'two-hit hypothesis' (Knudson 1971, 2001) explains the high frequency of cancers in a wide range of tumour disposition syndromes, although it is now recognized that additional genomic changes are required, for example for the development of retinoblastomas (Corson and Gallie 2007). These conditions are inherited as dominant traits because the probability of a somatic second (and third, fourth, etc.) mutational event in one of the huge numbers of cells (clones) is high. Tumorigenesis, however, is a *stochastic* event at least partly determined by chance. Variability in disease manifestation cannot be explained by the genotype (in combination with specific exogenous factors) alone, leading to truly variable penetrance and expressivity (and these terms are best restricted to such disorders).

Dominance caused by clonal cell proliferation due to loss of a growth-inhibiting factor is rather unlikely in metabolic disorders, but owing to a unique combination of factors is the cause of focal congenital hyperinsulinism. As mentioned above, the genes for the two subunits of the K_{ATP} channel required for regulation of insulin release are located on the distal short arm of chromosome 11. In close proximity is a group of imprinted genes including the maternally imprinted *IGF2* gene, expressed on the paternal allele only, and the paternally imprinted *H19* gene, expressed on the maternal allele only. *IGF2* codes for insulin-like growth factor 2, a member of the insulin family of polypeptide growth factors which are autocrine regulators of cell proliferation and mediate cell growth (Rodriguez et al 2007). *H19* encodes a 2.3 kb non-coding RNA that can function as a primary microRNA precursor, counteracts IGF2 and has a growth-repressing effect (Cai and Cullen 2007; Gabory et al 2006). Imbalances in the H19/IGF2 region have been implicated in a range of conditions with reduced or enhanced growth patterns, including Silver–Russell syndrome which in approximately 1/3 of cases is associated with loss of methylation and thus overexpression of *H19* (Abu-Amero et al 2008) and Beckwith–Wiedemann syndrome, which is associated with growth-enhancing epigenetic changes in the region such as *H19*-hypermethylation and overexpression of *IGF2* (Cooper et al 2005). Clonal loss of the maternal allele of this region in pancreatic β-cells causes adenomatous cell growth, which usually goes unnoticed. If there happens to be a *ABCC8* or *KCNJ11* mutation on the paternal allele, however, the new cell clone will be hemizygous for this mutation, unable to synthesize a normal K_{ATP} channel, and will oversecrete insulin (Fig. 8). The consequence is focal congenital hyperinsulinism that is treatable by surgical removal of

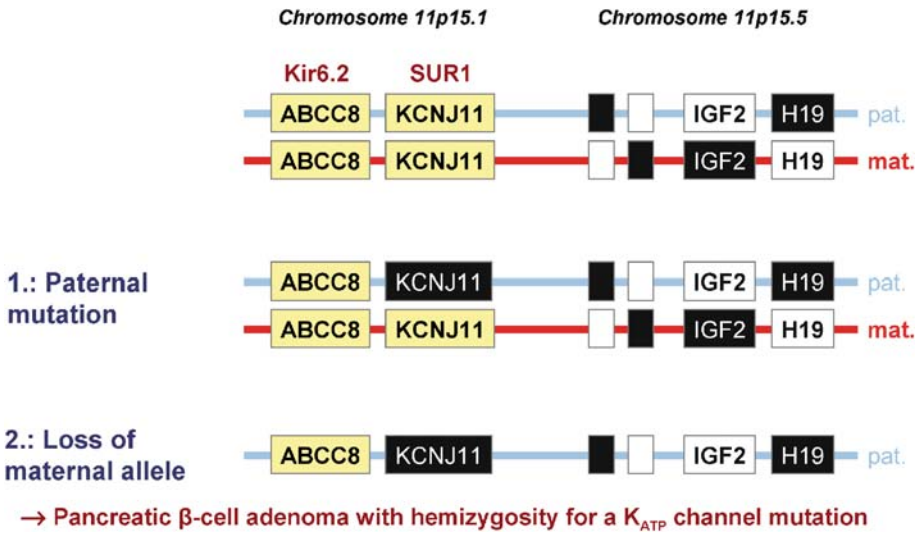

Fig. 8 Pathogenesis of focal congenital hyperinsulinism. The *ABCC8* and *KCNJ11* genes coding for the two subunits of the pancreatic K_{ATP} channel are located on chromosome 11p15.1, approximately 15 MB proximal to a group of growth-regulating imprinted genes on chromosome 11p15.5 that includes the genes *H19* and *IGF2*. Genes that are expressed on the paternal allele (imprinted on the maternal allele) stimulate growth, while expressed genes on the maternal allele inhibit growth. Focal congenital hyperinsulinism may develop in a fetus who inherited a K_{ATP} channel mutation from his father and in whom a 'second hit' caused loss of the distal short arm of the maternally inherited chromosome 11 in a pancreatic β-cell. The result would be a cell clone with increased adenomatous cell growth (loss of the maternal allele) and uncontrolled insulin secretion (hemizygous K_{ATP} mutation)

the pancreatic adenomatous lesion (de Lonlay et al 1997; Verkarre et al 1998). Although the underlying mutation is 'recessive', the condition itself should be regarded as an autosomal dominant trait with an unusual inheritance pattern as only paternal origin of the mutation can lead to the disease. Penetrance is low as familial occurrence appears to be rare and has not been mentioned in large case series (de Lonlay-Debeney et al 1999). Expressivity is variable and there may be multifocal disease due to independent second hits on the maternal allele (Giurgea et al 2006). Genetic counselling should take into consideration the rare possibility that both parents could be carriers for *ABCC8* or *KCNJ11* mutations, particularly when there is consanguinity, and siblings in the same family could have focal ('dominant') and diffuse ('recessive') hyperinsulinism by different disease mechanisms (Valayannopoulos et al 2007).

The constellation of "recessive" mutations in the same "metabolic" gene causing a dominant tumour predisposition syndrome and a severe recessive systemic disease is also found in fumarase deficiency. Mutations on both copies of the fumarate hydratase (*FH*) gene cause a severe encephalopathy with death in early childhood (Gellera et al 1990; Coughlin et al 1998). Available data indicate that in these cases, at least one of the two alleles is associated with residual function; homozygosity for null mutations with complete loss of fumarase function may be embryonic lethal (Alam et al 2003). In contrast, heterozygosity for *FH* gene mutations is the cause of a tumour disposition syndrome characterised by e.g. multiple cutaneous and uterine leiomyomas (MCUL) or hereditary leiomyomatosis and renal cell cancer (HLRCC) (Tomlinson et al 2002). Tumours develop when an inherited severe (null) mutation on one copy of the *FH* gene is unmasked by clonal loss of the wild type allele as a second hit (Alam et al 2003); the exact pathogenesis is still poorly understood (Gottlieb and Tomlinson 2005). A similar, more well-known example is *BRCA2*: homozygosity for specific mutations that retain partial BRCA2 function causes Fanconi anaemia type D1, while heterozygosity for the same mutations confers an increased risk for the development of breast and other cancers (Alter et al 2007; Howlett et al 2002). Homozygous null mutations in the *BRCA2* gene are thought to be embryonically lethal.

An unusual form of diffuse hyperinsulinism was observed in a patient with somatic mosaicism for paternal uniparental disomy (UPD) of the chromosome segment 11p15.1 (replacement of this region on the maternal chromosome with a paternal copy, which in consequence is duplicated while the maternal copy is deleted) (Hussain et al 2008). Mosaic UPD was found in cells from all tissues including lymphocytes and fibroblasts. The pancreas contained some β-cells with homozygosity for the paternal mutation, resulting in hyperinsulinism, and others with normal heterozygosity for the familial mutation. There was no adenomatous hyperinsulinism as the chromosomal region 11p15.5 was not involved and normally imprinted, and the paternal origin of the UPD was of no functional relevance. Hyperinsulinism in this family would thus be described as atypical autosomal recessive ('*reduction to homozygosity*') as has been reported for a large number of other conditions (Engel 2006), albeit not usually in mosaic form.

X-linked metabolic disorders—beyond dominance and recessiveness

Male individuals have only one X chromosome, and mutations in X-chromosomal genes are thus *hemizygous* and not hetero- or homozygous. Similarly, in females random X-inactivation (lyonization) (Lyon 1962) in each cell results in usage of only one copy of most X-chromosomal genes and thus *functional hemizygosity*. There is one major difference between males and females with regard to X-inactivated genes: in males all cells use the same gene copy, while females are functional mosaics with regard to usage of either of the two available X-chromosomal gene copies. In consequence, the terms dominant and recessive are inappropriate with regard to the cellular effect of typical X-chromosomal genes as only one allele is expressed, and there is no 'functional relationship of differing alleles' in the same cell. Even with regard to the clinical picture in heterozygous females, these terms are probably more misleading than helpful and should be avoided (Dobyns et al 2004).

When the concept of X-chromosomal inheritance was developed at the beginning of the 20th century it was heavily influenced by observations in animal systems such as *Drosophila* (Morgan 1910; Morgan et al 1915; Wilson 1911); only much later was it realized that there is no X-inactivation in *Drosophila* and the mechanism of sex determination and the expression of X-chromosomal genes is different (Parkhurst and Meneely 1994). Traditionally, disorders affecting secreted proteins (such as coagulation factors, e.g. haemophilia) or other functions in which loss of function in some cells may be compensated by normal function in other cells have been called X-chromosomal recessive; heterozygous females are supposed to be asymptomatic. Disorders where clonal loss of function causes recognizable changes in the clinical

phenotype have been called X-chromosomal dominant; heterozygous females are symptomatic whereas hemizygosity in males may be incompatible with life (e.g. Rett syndrome). The reality is much more complicated, as most X-linked disorders may give rise to at least some clinical symptoms in at least a minority of heterozygous females. Different reasons for the great variability of disease manifestation in X-linked conditions have been listed by Dobyns and co-workers (2004).

In some inborn errors of metabolism such as pyruvate dehydrogenase deficiency (Chun et al 1995; Dahl 1995) or Fabry disease (Deegan et al 2006; Wilcox et al 2008), the majority of heterozygous females are affected. In others such as ornithine transcarbamylase (OTC) deficiency (Batshaw et al 1986; Maestri et al 1998) or adrenoleukodystrophy (Deon et al 2008; Maier et al 2002) only a minority show relevant clinical symptoms. There are also metabolic disorders like Lesch–Nyhan syndrome or Hunter syndrome in which heterozygous carriers are almost always asymptomatic. The explanations of the clinical manifestation in females are highly variable and require individual assessment of the underlying mutations as well as the effects of metabolic disturbances (substrate accumulation, product deficiency, etc.) caused by specific enzyme deficiencies at cellular, organ and systemic levels (Dahl 1995). An important pathogenic factor in most X-linked disorders is skewed X-inactivation leading to expression of the non-functional mutant allele in a disproportionate number of cells. This is frequently a stochastic event, and it is therefore impossible to predict the clinical severity of, for example, OTC deficiency when carrier status is found in prenatal diagnosis. Manifestation of severe disease in a heterozygous female in exceptional cases may be due to complete inactivation of the normal allele caused by an unrelated genetic factor such as a structural aberration involving the X-chromosome (Rinat et al 2006). In conclusion, the terms 'X-linked dominant' or 'X-linked recessive' should be avoided and replaced by the term 'X-linked inheritance'.

It has been suggested that the milder clinical phenotype in heterozygous females compared with hemizygous males could be described with the term 'incomplete' dominance or recessiveness (Siemens 1925), but the standard use of 'incomplete dominant' or semidominant refers to the effect of half-normal

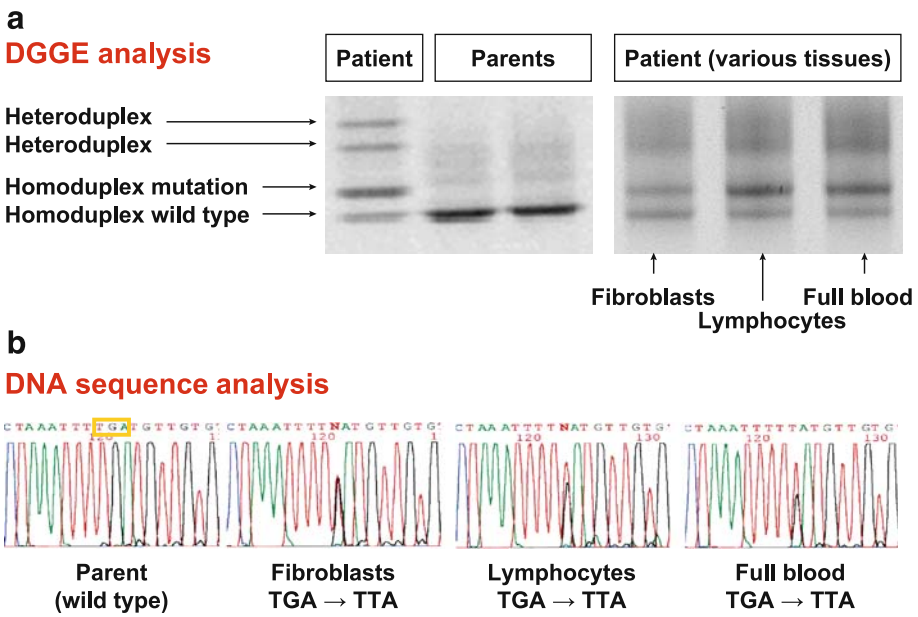

Fig. 9 Mosaicism for mutation X355L (c.1064G>T) in exon 10 of the *OTC* gene. (**a**) Denaturing gradient gel electrophoresis (DGGE) in DNA extracted from peripheral blood in a male infant with suspected OTC deficiency revealed an apparently 'heterozygous' mutation in exon 10 of the *OTC* gene that was not detected in the parents. Closer examination of the gel picture showed that mutant and wild-type homoduplexes had different band intensities (normally they should be exactly the same), indicating a higher proportion of mutant gene copies. Band intensities were equal in DNA extracted from fibroblasts. (**b**) Sequencing of exon 10 of the *OTC* gene confirmed mosaicism for a hemizygous mutation that was absent in the parents. The mutant peak (T) in relation to the wild-type peak (G) was slightly higher in lymphocytes/full blood than in fibroblasts, confirming the results of the DGGE analysis. The mutation changes the stop codon of the *OTC* gene into a triplet coding for leucine and is expected to lead to the insertion of 14 additional amino acids at the C-terminus of the protein. Klinefelter syndrome was excluded by the variable proportions of mutant and wild-type alleles in different cell types (and by chromosome analysis)

function in an individual heterozygous for a mutation in an autosomal gene, rather than the various mechanisms that explain clinical symptoms of X-linked disorders in heterozygous females.

Atypical, attenuated manifestation of an X-linked disease in a male patient may occasionally be due to somatic mosaicism for a pathogenic mutation caused by a mutation event in the (post-zygotic) early phases of embryonic development (Fig. 9). In this situation some of the body's cells are hemizygous mutant, others are hemizygous normal. The functional consequences are similar to those in heterozygous females where one or the other allele is inactivated, but the genetic basis is fundamentally different and the term 'heterozygous' is inappropriate. The correct diagnosis of somatic mosaicism is important for genetic counselling as the *de novo* mutation event in the affected boy excludes carrier status in the mother. There is also a reduced risk (less than 100%) for carrier status in the daughters of the patient as only a proportion of germ cells are expected to carry the mutation. Affected grandsons, however, would be expected to show a (much) more severe disease than the mosaic grandfather.

Conclusion

The molecular basis of monogenic disorders and the inheritance patterns in families are much better understood if 'dominant or recessive' and 'autosomal or X-chromosomal' are regarded as independent categories. Dominance and recessiveness relate to the functional consequences of (compound) heterozygosity for differing alleles at a particular locus in the individual. The term dominant should be reserved for disorders where individuals with a heterozygous mutation and one normal 'wild-type' allele show clinical symptoms. Even these rather straightforward definitions are not simple, for example with regard to the definition of the wild-type allele. Mutations affecting the same amino acid residue, or single amino acid deletions affecting adjacent residues, may be associated with different inheritance patterns. True dominant disorders should be distinguished from other conditions such as erythropoietic protoporphyria where a phenotype is caused by compound heterozygosity for severe loss-of-function mutations and a mild variant (which may be a prevalent polymorphism). The inheritance pattern in these cases is better acknowledged as autosomal recessive or pseudodominant. Indeed, most metabolic disorders represent a spectrum of phenotypes from normal via attenuated to severe (and sometimes prenatally fatal, such as homozygosity for null mutations in Smith–Lemli–Opitz syndrome), and disease manifestation is often influenced by other genetic or exogenous factors. The terms 'attenuated' or 'variant' disease may be preferable to 'mild disease' when symptoms even in less-severe forms may be debilitating and should not be belittled as 'mild'. The term 'variable expressivity' is not helpful with regard to autosomal recessive disorders in which variable phenotypes are explained by variable types of mutations in the respective gene. Understanding the molecular basis of monogenic disorders in the diploid individual helps to understand the disorders themselves and should assist in the management and treatment of patients.

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