

MODIFIER GENES IN MICE AND HUMANS

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An emerging theme of studies with spontaneous, engineered and induced mutant mice is that phenotypes often depend on genetic background, implying that genetic modifiers have a role in guiding the functional consequences of genetic variation. Understanding the molecular and cellular basis by which modifier genes exert their influence will provide insights into developmental and physiological pathways that are critical to fundamental biological processes, as well as into novel targets for therapeutic interventions in human diseases.

INBRED STRAINS

Mouse strains generated through systematic inbreeding, which results in virtual genetic homozygosity.

It is becoming increasingly evident that traits that are inherited in a simple Mendelian fashion can have phenotypes that differ in subtle or profound ways¹⁻⁶. Many Mendelian traits that result in birth defects, adult diseases and other genetic disorders vary in diverse biological features such as age-of-onset, severity and other associated phenotypic properties. In fact, the consistent expression of traits seems to be the exception in humans and experimental species such as laboratory mice. Among the several causes of variable phenotypes for Mendelian traits are alternative alleles, environmental factors and modifier genes. Examples of allelic and environmental variability are numerous and well characterized^{4,7-11}. Evidence for modifier effects comes from a range of phenotypes in human families or among different INBRED STRAIN backgrounds that cannot be explained by alleles of the disease gene or by environmental factors, and when the presence of at least one independent gene accounts for the modified phenotype. Although examples of genetic modifiers are limited in humans, they are frequent in laboratory mice and are expected to be a common cause of phenotypic variability in humans.

Phenotype modification occurs when the expression of one gene alters the expression of another gene^{1,2}. Modification could involve any aspect of a trait, from the primary action of the target gene (transcription), through to intermediate phenotypes at the molecular or cellular levels, to end phenotypes at the organ, system or organismal levels. Targets of modifier effects could be

spontaneous mutations, engineered mutations produced by transgenesis or gene targeting, or chemical- or radiation-induced mutations. In laboratory animals, modifier effects are usually attributed to genetic background and can be inherited as Mendelian or polygenic traits. In most cases, the genetic basis for modification is unknown; in some cases, modifier genes have been mapped; in several cases, candidate genes for modifier effects are being evaluated; and in a few cases modifier genes have been identified.

These modifiers provide insight into genes that underlie diverse developmental and physiological processes. Direct interactions between proteins can be discovered by using the yeast two-hybrid system¹², mass spectroscopy¹³ and other related methods. But genetic studies remain one of the most powerful ways to find both indirect and direct interactions. In this review, I discuss the various kinds of modifier gene and their phenotypic effects in mice and humans, and evaluate how modifier studies in model organisms can inform research into modifiers that determine the severity of human diseases.

Modifier effects

Genetic modifiers can affect penetrance, dominance modification, expressivity and pleiotropy (FIG. 1). Depending on the nature of the phenotypic effect, modifiers might cause more extreme (enhanced) phenotypes, less extreme (reduced) phenotypes, novel (synthetic) phenotypes or wild-type (normal) phenotypes, as dis-

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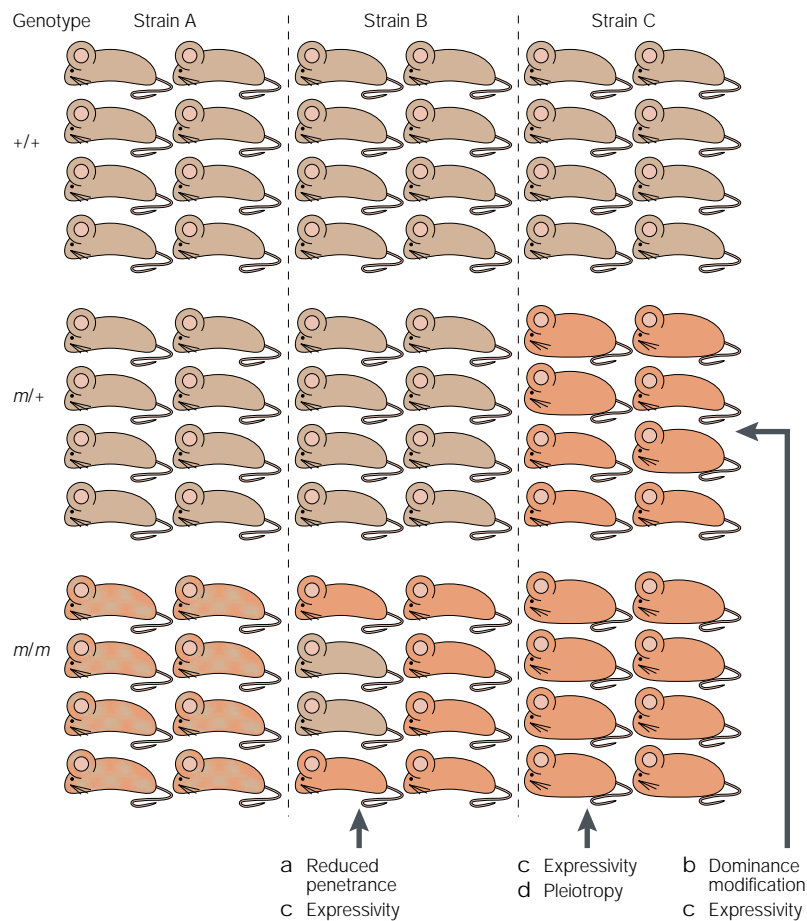


Figure 1 | Types of modification. The *m* mutation arose on the Strain A background in which it has recessive effects on variegation of the coat colour. The mutation was then transferred to the Strain B and C backgrounds by repeated backcrosses and by either genotypic or phenotypic selection. The resulting congenic strains have novel phenotypes that involve penetrance, dominance modification, expressivity and pleiotropy. **a** | Penetrance measures the frequency of affected mice among carriers. In mutant homozygotes, penetrance is 100% on the Strain A and Strain C backgrounds, but is reduced to 75% on the Strain B background, regardless of the nature of the variegated coat. **b** | Dominance modification describes the change in dominance. On the Strain A and Strain B backgrounds, none of the *m*/*+* heterozygotes is phenotypically affected, whereas on the Strain C background, all heterozygotes are affected, indicating a change from a recessive trait to a semidominant trait. **c** | Expressivity describes the extent to which a trait is affected in mice with a variegated coat colour. Genetic modifiers from the Strain B and C backgrounds change the pattern of variegation from coarse, as on the Strain A background, to fine. **d** | Pleiotropy describes the variety of traits in affected mice with the mutant gene. On the Strain A and Strain B backgrounds, mutants show only the variegated coat, whereas on the Strain C background, mutants show a change in body shape, as well as a variegated coat.

CONGENIC STRAINS
 Mouse strains in which a chromosome segment from one inbred strain background has been transferred to another inbred strain background by repeated backcrossing and selection, either for the phenotype of interest or for genotypes of marker loci that flank the mutant gene of interest.

cussed below and in FIG. 1. Epistasis, which occurs when an allele of one gene masks the phenotype of another gene^{14,15}, is also a form of genetic modification. To distinguish genetic modifier effects from other forms of phenotypic modification, we assume that individuals of the same genotype are evaluated on different genetic backgrounds but under similar environmental conditions.

Penetrance is a measure of the frequency of affected individuals among the carriers of a particular genotype. It is therefore a characteristic of a population of individuals and can often be reduced among individuals with the same genotype for a particular Mendelian trait. In mice, the presence of modifier genes that affect pene-

trance becomes evident when the frequency of affected mice varies among CONGENIC STRAINS in which the same mutant allele has been transferred to different genetic backgrounds, or among segregating crosses that involve different combinations of strains (FIG. 1a). In humans, individuals with a 'disease' genotype who are nevertheless unaffected are evidence of reduced penetrance. If linkage analysis of these unaffected individuals reveals evidence for an independent gene, the basis for reduced penetrance is a genetic modifier.

Dominance depends on many factors, including alleles, genotypes, genetic background and environmental conditions. Dominance of a particular genotype often varies with genetic background, even under similar environmental conditions. A trait that is inherited in a dominant manner on one background can be inherited as a semidominant or recessive trait on another background (FIG. 1b). The ability of genetic background to modulate dominance, under the same environmental conditions, suggests that independent genetic factors determine whether mutant heterozygotes are affected.

Expressivity describes the extent to which particular structures or processes are affected by a particular genotype. The trait might be enhanced or reduced; it might even be suppressed so that mutants seem to be almost normal. The action of genetic modifiers is evident when the extent to which a trait is affected varies among genetic backgrounds (FIG. 1c). Expressivity is relevant only in individuals in which the trait is penetrant.

Pleiotropy refers to the diverse phenotypic effects of a single-gene mutation. Genetic modifiers of pleiotropy result in different combinations of traits on different genetic backgrounds (FIG. 1d). These modifiers can also lead to novel phenotypes that are found only on particular genetic backgrounds. Such a mutation might affect structures and functions that seem to be developmentally and physiologically unrelated, the interrelationships of which might only become evident once the gene defect is identified.

Environmental factors that affect the expression of a trait can be mistaken as an effect of genetic modifiers. These factors can cause a trait to be strongly expressed in one environment but not to be expressed in another environment. Distinguishing genetic and environmental modifiers, although complicated, can be resolved if genetically identical individuals are evaluated on different genetic backgrounds in similar environments. It is therefore essential that modifier genes are studied under similar environmental conditions, or at least that non-genetic effects are taken into account.

FIGURE 2 shows the ways in which background genes modify pleiotropy, penetrance, dominance modification and expressivity to modulate phenotypes. The key components of this model are: first, the distribution of trait values for individuals of the three genotypes at the modified gene; and second, the threshold that determines whether the trait is expressed in individuals of a particular genotype. Genetic variation in the modifier gene affects the position of the phenotypic threshold relative to the trait distributions. By contrast, genetic variation in the modified target gene affects the position

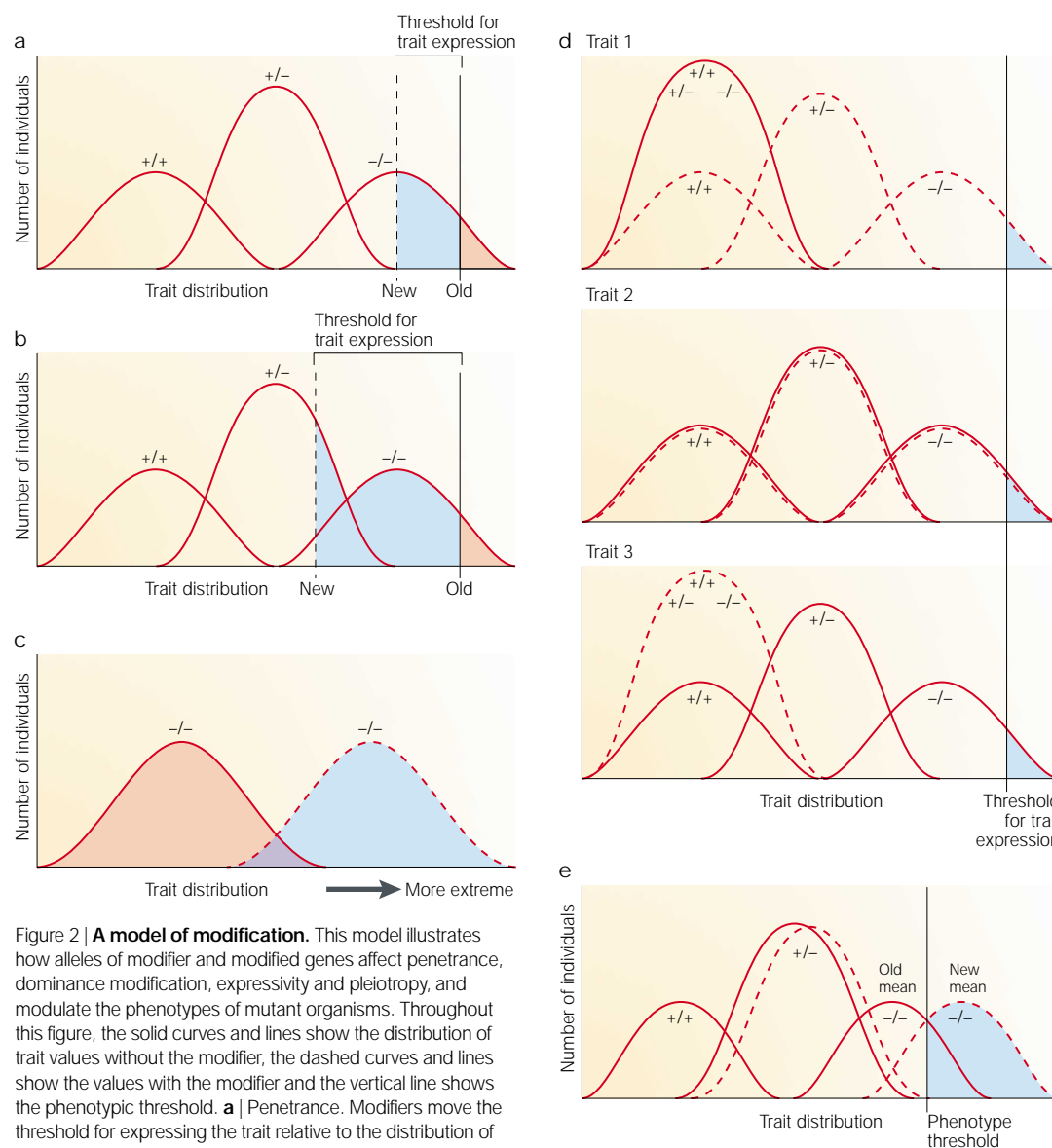


Figure 2 | A model of modification. This model illustrates how alleles of modifier and modified genes affect penetrance, dominance modification, expressivity and pleiotropy, and modulate the phenotypes of mutant organisms. Throughout this figure, the solid curves and lines show the distribution of trait values without the modifier, the dashed curves and lines show the values with the modifier and the vertical line shows the phenotypic threshold. **a** | Penetrance. Modifiers move the threshold for expressing the trait relative to the distribution of trait values. By moving the threshold, a greater (or smaller) proportion of the mutant homozygotes are affected, thereby increasing (or reducing) penetrance. **b** | Dominance modification. Modifiers move the threshold for expressing the trait relative to the distribution of trait values. By moving the threshold into (or out of) the range of the heterozygotes, some mutant heterozygotes are (or are no longer) affected, thereby converting a trait with recessive effects into one with semi-dominant effects (or the converse depending on the direction in which the threshold moves). **c** | Expressivity. The location of the distribution of trait values for mutant homozygotes or mutant heterozygotes, or both, but not for the wild-type homozygotes is shifted relative to the phenotypic threshold. **d** | Pleiotropy. Modifiers determine the particular traits that are affected in mutant organisms. Any of three traits can be found in individuals with a mutant gene, depending on the action of a modifier gene. When the modifier is absent, only traits 2 and 3 are observed, whereas when the modifier is present, only traits 1 and 2 are observed. Only expression of trait 2 is independent of the modifier. **e** | Phenotypic changes resulting from different alleles of the disease gene. In this case, modifier genes are not involved. The alternative alleles result in a slight shift in the distribution of trait values for the heterozygotes and a more dramatic shift for homozygotes.

of the trait distribution of the mutant homozygotes, heterozygotes or both relative to the phenotype threshold (FIG. 2e), which is assumed to be fixed in cases for which only genetic variation in the modified gene is considered. Additive effects can be distinguished from modifier effects by whether the distribution of trait values is affected for all genotypes or only for selected genotypes, because only additive effects shift the distribution of trait values for all genotypes. Although it does

not explain the mechanisms of modification, this model outlines the various ways in which modifiers modulate fundamental genetic properties to influence the expression of phenotypic traits.

Modifier genes in mice

Many examples of modifier effects can be found in the classical literature of mouse genetics^{1,2}. Recently, mutants generated by gene targeting have revealed

Table 1 | Examples of modifier genes and their phenotypic effects

Target modified gene	Modifier gene	Modifier effect	Nature of the modified phenotype	References
Examples in mice (allele)				
disorganization (<i>Ds</i>)	Genetic background (C57BL/6J or C3H)	Penetrance	Whether or not an affected mouse shows <i>Ds</i> -like birth defects	16
undulated (<i>Pax1^{un}</i>)	Genetic background (C57BL/6J × CBA)	Dominance modification	Presence or absence of foramina transversaria	19, 20
short-ear (<i>Bmp5^{se}</i>)	Genetic background (C57BL/6J or CBA)	Dominance modification	Presence or absence of foramina transversaria	19, 20
brachyury (<i>T</i>)	Genetic background (selection experiment based on mixed background)	Expressivity	Tail length	23
<i>Cftr</i> knockout	<i>Cfm1</i>	Suppressor	Suppresses meconium ileus	65
<i>Apc^{min}</i>	Phospholipase A ₂ , group IIA (<i>Mom1</i>)	Suppressor	Reduces polyp number	70
<i>Apc^{d716}</i>	<i>Cox2</i> (knockout)	Suppressor	Reduces polyp number	78
<i>Apc^{d716}</i>	<i>cPLA₂</i>	Suppressor	Reduces polyp number	79
<i>Pax3^{Sp}</i>	Fidgetin — fidget (<i>Fign^f</i>)	Suppressor	Suppresses spina bifida	26
ashen (<i>Rab27a</i>)	<i>dsu</i>	Suppressor	Coat colour suppressor	29
leaden (<i>ln</i>)	<i>dsu</i>	Suppressor	Coat colour suppressor	29
ruby-eye (<i>ru</i>)	<i>dsu</i>	Suppressor	Coat colour suppressor	29
ruby-eye 2 (<i>ru2</i>)	<i>dsu</i>	Suppressor	Coat colour suppressor	29
Patch (<i>Ph</i>)	<i>Pax1^{un}</i>	Novel phenotype	Spina bifida occulta in <i>Ph/+ un/un</i> mice	32
<i>Pax3^{Sp}</i>	Curly tail (<i>ct</i>)	Novel phenotype	More extreme spina bifida in <i>Sp/+ ct/ct</i> mice	44
Examples in humans (gene/disease)				
Peripherin 1 (<i>PRP1</i>)	<i>ROM1</i>	Dominance modification	Retinitis pigmentosa in <i>PRP1/+</i> heterozygotes	47
Familial hypercholesterolaemia (FH)	Unnamed modifier on 13q	Suppressor	Reduces cholesterol level in FH	50
Familial Mediterranean fever (FMF)	Serum amyloid A (SAA)	Pleiotropy	Whether FMF cases show renal amyloidosis	51
Cystic fibrosis transmembrane conductance regulator (<i>CFTR</i>)	Cystic fibrosis (<i>CFM1</i>)	Suppressor	Suppresses meconium ileus	66
Non-syndromic deafness (<i>DFNB26</i>)	At least one gene on chromosome 7	Penetrance	Suppresses deafness	45
Mitochondrial 12S ribosomal gene	At least one gene near D8S277	Penetrance	Suppresses deafness	46

(*Apc*, adenomatous polyposis coli; *Cfm1*, cystic fibrosis modifier 1; *Cox*, cyclooxygenase; *cPLA₂*, cytosolic phospholipase A₂; *dsu*, dilute suppressor; *Pax*, Paired box transcription factor; ROM, rod outer segment protein.)

SYNDACTYL
Fused digits.

POLYDACTYL
Supernumerary digits.

FORAMINA TRANSVERSARIA IMPERFECTA
An opening for nerves and blood vessels in the transverse process of the cervical vertebrae. The presence and size of this aperture (called a foramen) is variable.

many important examples of modifier genes. These and other examples show that modifier effects are common and involve most aspects of mouse biology (TABLE 1).

Penetrance. The phenotype of mice with the **disorganization** (*Ds*) mutation is an example of a trait in which modifiers affect penetrance but not other aspects of the phenotype. Penetrance varies considerably among inbred strains, from almost zero on the C57BL/6J inbred background to 10% on the A background to as high as 89% on the C3H background¹⁶. Disorganization also has perhaps the most striking variation in expressivity of any gene in the mouse^{16,17}. Each affected mouse has unique birth defects, and no two affected mice have identical phenotypes. Any body part can be affected in

diverse manners from agenesis to malformation and extranumerary structures. Features such as extra, missing, cystic or fused kidneys, SYNDACTYL or POLYDACTYL, or missing or ectopic limbs are common. Variable expression, which is an inherent feature of the mutation, is seemingly unaffected by genetic background and apparently results from random somatic events¹⁸.

Dominance modification. A classic example of dominance modification involves a variable feature of the cervical vertebrae. These vertebrae have FORAMINA TRANSVERSARIA IMPERFECTA (FTI), with variability in the presence and size of the foramen, depending on the Mendelian mutant that is present and on the genetic background. In both **undulated** and **short-ear** mice, which are caused

by mutations in *Pax1* and *Bmp5*, respectively, the presence of FTI in heterozygotes depends on genetic background. For example, FTI are common in short-ear heterozygotes on the C57BL/6J background, but are absent in short-ear heterozygotes on the CBA background^{19,20}. A recent example of dominance modification involves the modifier of deaf waddler (*mdfw*) gene, which modifies hearing loss in deaf waddler heterozygotes²¹.

Expressivity. Mice that are heterozygous for the *T* mutation in the *brachyury* gene have a short tail and homozygotes die during embryonic development²². Although *T*+ heterozygotes usually have a short tail, the extent of tail-shortening varies considerably among genetic backgrounds. On a mixed background, the tail length in some *T*+ heterozygotes is very similar to that of their wild-type siblings, whereas other *T*+ heterozygotes have an exceptionally short tail. Selection experiments starting with a genetically mixed founder population show that it is possible to select a line in which most *T*+ mice have a very short tail and another line in which most *T*+ mice have a nearly normal tail²³. These modifiers did not affect the length of the tail in the wild-type controls, showing that the experiment did not simply select for longer tails in both wild-type and *T*+ mice. So, genes in different strains can have profound consequences on the expressivity of the short-tail phenotype.

An example of a modifier gene that normalizes a phenotype can be seen in the interaction between the *splotch* and *fidget* mutations. *Splotch* results from a mutation in the paired box gene *Pax3*, and homozygous *Pax3*^{Sp} embryos die mid-gestation with neural tube defects. *Fidget* results from a mutation in the *fidgetin* (*Fign*) gene²⁴. *Fidget* mutant homozygotes show head-tossing, circling, hypersensitivity to sound, corneal lesions, absence of the bony labyrinth and semicircular canals of the inner ear, and other neurological and morphological abnormalities²⁵. In *splotch*–*fidget* double-mutant homozygotes, both the incidence and severity of neural tube defects are greatly reduced²⁶. The contrasting effects of the *splotch* and *fidget* mutations on cell-cycle dynamics might restore normal development to the neural tube²⁶. The *splotch* mutation is thought to increase the length of the cell cycle²⁷, whereas the *fidget* mutation is thought to decrease it²⁶. The combination of the two mutations in double homozygous mutant mice could restore normal cell-cycle dynamics. Now that both the *splotch* and *fidget* genes have been cloned, it should be possible to revisit this unique modifier effect and determine the molecular and cellular basis for the interaction.

Another example of a modifier gene that normalizes phenotypes involves the *dilute suppressor mutation*. *Dilute* is one of the classic coat-colour mutations in the mouse. It is a recessive mutation that causes a lighter coat colour in mice with a pigmented coat and results from mutations in the myosin 5a (*Myo5a*) gene. In mice that are homozygous for the *dilute* suppressor (*dsu*) gene, the coat colour of *dilute* mice resembles that of non-*dilute* mice²⁸. *dsu* also suppresses the coat-colour mutants *ashen* (*Rab27a*) and *leaden* (*ln*), as well as the two ruby-eye colour mutants, *ruby-eye* (*ru*) and *ruby-*

eye 2 (*ru2*), but it does not affect the coat colour of several other mutants²⁹. In *dilute*, *ashen* and *leaden* mutant mice, melanocyte numbers are reduced and their dendritic processes are smaller, resulting in the retention of pigment granules in the body of the melanocytes and the characteristic *dilute* coat colour³⁰. *dsu* increases melanocyte numbers and restores normal morphology and normal coat colour in *dilute*, *ashen* and *leaden* mice. The mechanism by which *dsu* restores normal eye colour in the ruby eye-colour mutants is uncertain and the identity of the *dsu* gene is unknown.

Pleiotropy. Mice that are homozygous for the *tubby* (*tub*) mutation are obese, deaf and blind³¹. Although *tubby* mice are obese on all genetic backgrounds, hearing loss depends on unlinked modifier genes³¹. *Tubby* mice obtained from intercrosses between a C57BL/6J congenic strain that was homozygous for *tub* and either of two unrelated strains were tested for hearing loss by measuring their auditory brainstem responses. A gene, called modifier of *tubby* hearing 1 (*moth1*), was shown to control 50% of the genetic association of hearing loss with obesity in *tubby* mice³¹. Thus an unlinked gene controls hearing loss as a pleiotropic consequence of the *tubby* mutation.

An example of a novel phenotype that is not found in single mutant mice is the anatomical localization and histological nature of spina bifida in mice mutant for both *patch* and *undulated*³². *Patch* (*Ph*) is a deletion that encompasses the platelet-derived growth factor receptor- α (*Pdgfra*) gene³³ and *undulated* (*un*) is a mutation in the *Pax1* transcription factor³⁴. *Patch* (*Ph*+) heterozygotes lack pigmentation in the lumbosacral region; homozygotes develop subcutaneous blisters that flank the midline of the developing neural tube and do not survive embryonic development because of SPINA BIFIDA OCCULTA along the entire vertebral column^{35,36}. *Undulated* (*un/un*) homozygotes develop vertebral defects that result in KYPHOSIS and tail kinks^{37,38}. In addition to the pigmentation anomaly and the *undulated* tail, which are expected in *patch* and *undulated* mice with either of these mutations, all *Ph*+ *un/un* double-mutant mice, and only the double mutants in these crosses, have spina bifida occulta that is restricted to the lower thoracic and lumbar regions. Histological analysis indicates that the subcutaneous blisters that flank the midline in *patch* homozygotes are moved to the midline, where they seem to interfere with vertebral fusion in the lumbar region of these double-mutant mice³².

A closely related example involves neural tube defects in *splotch*–*curly-tail* double mutant mice. Homozygous *Pax3*^{Sp} mice die mid-gestation from neural tube defects that include RACHISCHISIS in either the hindbrain or lumbosacral region^{39–41}. *Curly-tail* (*ct*) is a recessive mutation that causes spina bifida (and occasionally ANENCEPHALY), which is strikingly similar in its developmental characteristics to many cases of spina bifida in humans^{42,43}. In *Pax3*^{Sp} *ct/ct* double-mutant mice, embryos develop with neural tube defects that are more severe but different in their developmental origins than those found in either single mutant alone⁴⁴. The phenotype of these double

SPINA BIFIDA OCCULTA

Failure of vertebral fusion but when skin covers the open arches and without the protrusion of a hernia.

KYPHOSIS

The abnormal curvature of the thoracic spine.

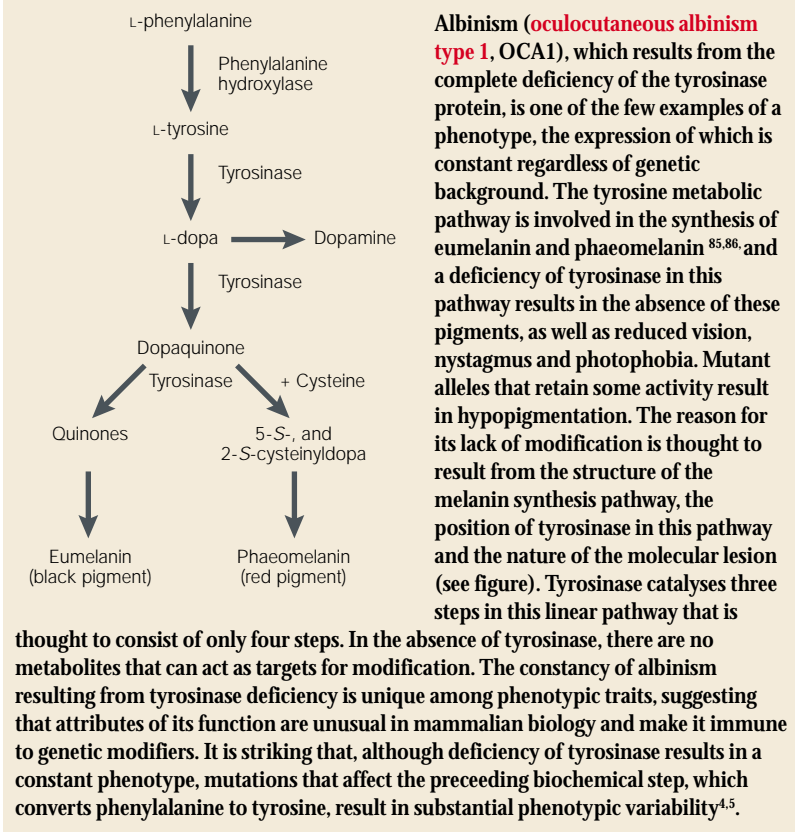
RACHISCHISIS

A form of spina bifida in which the spinal cord is split.

ANENCEPHALY

Absence of the greater part of the brain, often with skull deformity.

Box 1 | Albinism — an unmodified trait?



on chromosome 7 protects these individuals from hearing loss and accounts for reduced penetrance. Evidence for a nuclear modifier of a mitochondrial disease has also been recently reported⁴⁶. A chromosome 8 gene, near D8S277, has been reported to modify the penetrance of the A1555G mutation in the **mitochondrial 12S ribosomal gene**, a mutation that causes maternally inherited deafness⁴⁶. Additional linkage studies are required to verify this provocative observation.

Dominance modification. Peripherin-1 (*PRP1*) is a gene that exemplifies dominance modification. Some individuals that are heterozygous for *PRP1* develop **retinitis pigmentosa**, even though only homozygous individuals are expected to be affected⁴⁷. In these cases, alleles at the unlinked rod outer segment protein 1 gene (*ROM1*) act together with *PRP1* variants to cause retinitis pigmentosa in double heterozygotes. So, an allele that normally has a recessive effect on retinitis pigmentosa acts as a dominant when a particular allele of the unlinked *ROM1* gene is present.

Expressivity. A possible example of a modifier gene that reduces expressivity involves a gene that lowers cholesterol levels in individuals who are predisposed to **familial hypercholesterolaemia**. Familial hypercholesterolaemia is an autosomal dominant trait that affects one person in 500 and causes significantly elevated cholesterol levels⁴⁸. Familial hypercholesterolaemia heterozygotes are predisposed to premature cardiovascular disease and familial hypercholesterolaemia homozygotes often die of cardiovascular disease during the first two decades of life. Some individuals in familial hypercholesterolaemia families have low density lipoprotein (LDL) levels that are ~25% lower than expected, raising the possibility of a modifier gene with cholesterol-lowering effects⁴⁹. A recent study mapped this modifier to chromosome 13q in several populations, verifying the existence of a modifier gene and demonstrating its general effects in these populations⁵⁰. However, this study also provided evidence that the gene on 13q reduces the LDL level in individuals with normal LDL levels, as well as those with familial hypercholesterolaemia, raising the possibility that the 13q gene has an additive rather than a modifier effect. In general, modifier genes that suppress disease could represent an important way to exploit endogenous mechanisms to suppress or perhaps even to prevent disease.

Pleiotropy. RENAL AMYLOIDOSIS is a variable complication that is associated with **familial Mediterranean fever**⁵¹ (FMF). FMF is an autosomal recessive trait that is common in individuals in or derived from Mediterranean populations and is characterized by the recurrence of fever and SEROSAL INFLAMMATION. It results from a mutation in the **pyrin/marenostrin** gene⁵². Amyloidosis is an important complicating factor that can lead to renal failure⁵³. However, the variable association between amyloidosis and FMF raises the possibility of genetic modifiers. A recent study showed that a polymorphism in **serum amyloid A** accounts in part for the variable association of amyloidosis with FMF⁵¹.

mutant mice raises the possibility that an additive phenotypic effect does not necessarily reveal genes that interact directly⁴⁴. However, interactions, whether direct or indirect, cannot be excluded given our modest understanding of many molecular, cellular and developmental processes.

Modifier genes in humans
Modifier effects are remarkably common in humans and model organisms. In fact, it is probably exceptional for phenotypes to present identically in all cases (for an apparent exception, see BOX 1). In this part of the review, I evaluate the evidence for modifier genes in humans and restrict the discussion to those cases for which there is evidence that independent genes control penetrance, dominance, expressivity or pleiotropy of Mendelian traits (TABLE 1). In the absence of this direct evidence, the cause of phenotypic variability is uncertain.

Penetrance. Although reduced penetrance is a common feature of many Mendelian traits, there are few available examples of variability being caused by independent genes. However, one example has recently been published — a dominant modifier of the non-syndromic deafness gene near *DFNB26* (REF. 45). Most individuals who are homozygous for the *DFNB26* gene on chromosome 4q31 have **non-syndromic hearing loss**. However, several individuals in one of the families in which *DFNB26* was mapped are homozygous for the *DFNB26* haplotype but nevertheless have normal hearing. A dominant modifier

RENAL AMYLOIDOSIS
A metabolic disorder associated with the deposition of amyloid (a protein-polysaccharide complex) in the kidney.

SEROSAL INFLAMMATION
Inflammation of the membranes that line the chest and peritoneal cavities and that enclose the lungs, the heart, the main blood vessels and the gut.

PANCREATIC INSUFFICIENCY
The inadequate functioning of the pancreas that results from the blockage of the pancreatic duct, which prevents the secretion of pancreatic fluids.

MECONIUM ILEUS
Obstruction of the intestine by mucus.

BILIARY CIRRHOSIS
Cirrhosis of the liver that results from the obstruction and inflammation of the bile ducts and from the chronic retention of bile.

Case studies of genetic modifiers

Modifier genes discovered in experimental species, such as laboratory mice and rats, are often relevant to the study of human diseases. Genetic linkages discovered in one species can guide the search for linkage in humans, as exemplified by the first example below. The second example shows how the identity of modifier genes in experimental species can reveal developmental or physiological pathways that modulate phenotypes in humans.

Compensation for CFTR deficiency. Cystic fibrosis is one of the most common human autosomal recessive diseases⁵⁴. Mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*), which encodes a membrane-bound chloride ion channel, cause impaired fluid and salt secretion in various tissues. These mutations can result in mucus accumulation in the airways, PANCREATIC INSUFFICIENCY, MECONIUM ILEUS, BILIARY CIRRHOSIS, impaired salt resorption in sweat glands, chronic bronchopulmonary infections and infertility. The presentation of these clinical features of cystic fibrosis can vary as can age-of-onset, disease severity and other disease features. This variability results partly from the numerous mutations that have been identified in the *CFTR* gene^{54–56} and partly from unlinked genetic modifiers⁵⁷. In particular, few patients with cystic fibrosis suffer from meconium ileus. The lack of association between meconium ileus and specific *CFTR* mutations raises the possibility that independent genetic factors control the occurrence of meconium ileus in patients with cystic fibrosis^{58–60} — an example of pleiotropy modification.

To create a mouse model of cystic fibrosis, gene-targeting technology was used to make mice that are deficient in the mouse *Cftr* protein^{61–63}. Homozygous mutant mice die soon after birth because of mucus accumulation in the intestine^{61,62,64}. Although failure to thrive occurs on most genetic backgrounds, *Cftr*-deficient mice live for many months on some backgrounds, implying that modifier genes modulate disease severity⁶⁵. Linkage studies have revealed a single locus with semidominant effects that account for most, but not all, of the variation in viability. *Cftr*-deficient mice that do not have the modifier gene die soon after birth, mice heterozygous for the modifier gene usually survive until weaning, and mice homozygous for the modifier survive for several months longer than mice of the same *Cftr* genotype that lack the modifier. This modifier reduces the amount of mucus in the intestine and restores relatively normal membrane electrophysiology, perhaps because of a calcium-activated chloride conductance channel that reduces mucus accumulation in the intestine⁶⁵. This cystic fibrosis modifier gene is located near the centromere of mouse chromosome 7; the corresponding location in the human genome is 19q13.2–q13.4. Associations were tested between meconium ileus, a pulmonary phenotype and markers on this chromosome segment in patients with cystic fibrosis. Strong evidence for linkage was found for meconium ileus but not for the pulmonary phenotype, suggesting that the occurrence of meconium ileus with cystic fibrosis depends on mutations in this

chromosome 19 gene⁶⁶. This is a striking example of a modifier gene, the discovery of which in the mouse led to the successful predictions of the location of a corresponding gene in humans.

Colon cancer susceptibility in *Mom1* mice. Susceptibility to ADENOMATOUS POLYPS characterizes the cancer predisposition syndrome called **familial adenomatous polyposis** (FAP). It is an autosomal dominant trait with variable penetrance and expressivity, which can be explained partly by the hundreds of germline and somatic mutations in the adenomatous polyposis coli (*APC*) gene that underlie the disease and partly by independent genetic factors⁶⁷. Mutations in the *APC* gene contribute to the transformation of normal epithelial cells to benign adenomas, a critical step in the progression to colon cancer. A survey of chemically mutagenized mice revealed an induced mutant, originally called *Min* for 'multiple intestinal neoplasia', with an increased incidence of polyps⁶⁸. Mapping studies identified the mouse homologue of *APC* as a candidate gene and sequencing studies revealed a nonsense mutation in the mouse *Apc* gene⁶⁹, thereby establishing the *Apc^{Min}* mouse as a model for studies of FAP and the aetiology of spontaneous polyps.

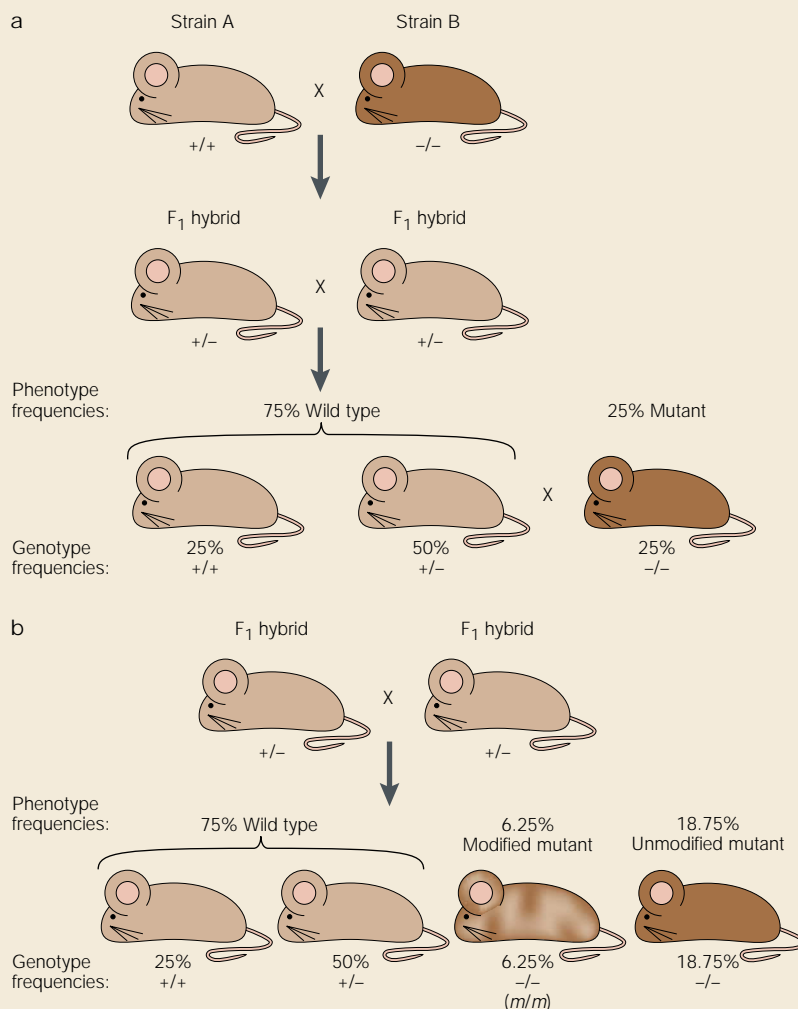
The *Apc^{Min}* mouse model has made important contributions to the development of preventative treatments for polyps because of a modifier gene that modulates polyp number and that was discovered as a result of linkage studies to map the *Apc^{Min}* mutant. During the course of these mapping studies, William Dove and colleagues discovered that the number of polyps is markedly reduced when *Apc^{Min}* mice on a C57BL/6J background are crossed to mice of the AKR strain⁷⁰. *Apc^{Min}* mice on a C57BL/6J background have numerous polyps, AKR/J mice with the *Apc^{Min}* mutation have few polyps, and F₁ hybrids between the two strains (with the *Apc^{Min}* mutation) have an intermediate number of polyps, indicating that a modifier gene with semidominant effects modulates polyp number. This modifier gene, originally called *Mom1* for 'modifier of Min', was subsequently mapped to the distal portion of chromosome 4. It explains 50% of the genetic variance in polyp number, indicating that other modifier genes with weaker effects on polyp number probably exist.

The evaluation of candidate genes for *Mom1* revealed a polymorphism in the secretory phospholipase A₂ (*Pla2g2a*) gene. *Pla2g2a* belongs to a large family of phospholipases that hydrolyse phosphoglycerides to yield fatty acids and lysophospholipids. This gene family contributes not only to the metabolism of phospholipids but also to inflammatory responses. Strains with the *Mom1* variant that reduces polyp number have a normal *Pla2g2a* gene, whereas strains with the variant that fails to suppress polyp number have a deletion in the *Pla2g2a* gene and lack enzymatic activity⁷¹. Complementation of high polyp number was evaluated in transgenic mice to test whether the normal *Pla2g2a* gene accounts for the *Mom1* phenotype⁷². As expected, a significant reduction in polyp number was observed. However, the reduction was not restored to wild-type levels, indicating either that transgene

Box 2 | Finding modifier genes

Two general strategies can be used to discover modifier genes in laboratory mice. Typically, an inbred strain that carries a Mendelian mutation is crossed to other inbred strains and progeny are examined for variation in the mutant phenotype (see figure). Panel a shows segregation of a recessive trait without modifiers, panel b the segregation of the same recessive trait, but in crosses with a strain that has a single recessive modifier gene (*m*). Another approach is to transfer a mutant gene from one inbred strain background to another inbred background, through repeated backcrossing and selection to make a 'congenic strain'. In these cases, the phenotype associated with the mutant gene is evaluated on a different but defined genetic background. Both kinds of study have provided classic examples of modifier genes.

Establishing that an inbred strain has a modifier gene is challenging, partly because of lack of previous evidence that a particular strain has a modifier until a single gene defect is introduced and partly because of the large sample sizes that are required. Generally, there is no way to know whether a particular strain has a modifier without making the linkage crosses or congenic strains. As a result, several crosses involving many progeny and many phenotype tests are needed, depending on the penetrance of the trait and the number of modifier genes that are involved^{60,65}. Alternatively, six to ten generations of backcrosses are needed to assess the mutant phenotype on different genetic backgrounds. Information about which strains have, or do not have, the modifier gene, is invaluable when evaluating candidate genes — strains with the modifier should have alleles that are distinct from those in strains that do not have the modifier.



expression might have failed to recapitulate fully wild-type functions or that other closely linked genes affect polyp number and that the *Mom1* phenotype results from the cumulative action of several closely linked genes. Although genes in the corresponding portion of the human genome seem to have a modifier that accounts for variation in FAP presentation⁷³, polymorphisms in the homologue of the *Pla2g2a* gene do not appear to account for significant variation in susceptibility to colon cancer⁷⁴.

The finding that *Pla2g2a* modulates polyp number in mice has nevertheless been informative given the pathway in which it acts. *Pla2g2a* is part of the prostaglandin synthesis pathway. Aspirin, sulindac and other non-

steroidal anti-inflammatory agents markedly reduce susceptibility to polyps, perhaps by interfering with cyclooxygenase-2 (*Cox2*) activity in the prostaglandin pathway⁷⁵⁻⁷⁷. The involvement of *Cox2* and the prostaglandin pathway is supported by the observation that *Apc^{min}* mice deficient for either *Cox2* or cytosolic phospholipase A₂ (*cPLA₂*) have reduced polyp numbers^{78,79}. Although *Mom1* did not identify a gene that modulates susceptibility to colon cancer to a significant extent in humans, it did successfully identify a pathway that is pharmacologically relevant to the management of colon cancer. So genetic variants in mice do not also need to identify genetic variants in humans for them to be useful in understanding and treating human diseases.

ADENOMATOUS POLYPS
Benign growths that arise from the lining of the colon or rectum, which can protrude into the intestinal lumen.

Conclusion

A recent paper questions the impact of genetics and genomics on health care because penetrance confounds genetic analysis of simple and especially of complex genetic traits⁸⁰. Although this viewpoint is unnecessarily pessimistic^{81–84}, the burden of proof should rightly be on the genetics community to demonstrate the contribution that genetics, and genetic modifiers in particular, can make to the diagnosis, treatment and prevention of common human diseases. The evidence for modifier genes in humans and mice, and examples of mouse modifier genes that have guided the search for modifier genes in humans and led to therapeutically relevant biochemical pathways, suggests that factors that complicate inheritance of Mendelian traits can be effectively studied to great advantage.

The availability of the sequence of the mouse genome will resolve a key challenge in establishing the identity of modifier genes (current strategies for identifying modifier genes are summarized in BOX 2). These modifiers, which are interesting as phenotypic phenomena, illustrate the importance of dissecting the complex networks of gene interactions that underlie variation in organismal biology. In particular, the identities of the modifier and modified genes provide insights into the molecular, biochemical and cellular mechanisms for modification. With the reported completion of the sequencing of a draft version of the mouse genome (see link to **Celera**), large numbers of genetic markers will become available with which to determine the precise chromosomal location of genetic modifiers. The sequenced mouse genome, together with the sequence of the human genome, should allow the location of the corresponding gene in the human genome to be accurately predicted. The sequence of the mouse and human genomes will revolutionize the analysis of modifier genes and variable phenotypes.

Modifier genes provide insights into the mechanisms by which organisms modulate biological processes to accommodate the adverse effects of genetic mutations. Carrier individuals that are free of disease or that show a substantially 'normal' phenotype are examples of developmental and physiological accommodation of disease risk despite inherited susceptibility to disease^{50,51,66}. These modifier genes often have at least two alleles, one of which exacerbates disease, and one that suppresses disease. These alleles can be thought of as being 'disease promoting' and 'disease suppressing', respectively. These disease-suppressing modifier genes move the phenotypic threshold for expression of traits so that fewer carrier individuals are affected. New disease therapeutics could be based on mimicking and perhaps enhancing the effects of naturally occurring genetic modifiers. Understanding the basis for variable disease presentation in general, and for the suppression of disease in particular, could improve the prediction, treatment and perhaps even the prevention of human diseases.

Links

DATABASE LINKS **disorganization** | **undulated** | **short-ear** | **Pax1** | **Bmp5** | **mdfw** | **T mutation** | **brachyury** | **splotch** | **Pax3** | **Fidget** | **Fig** | **dilute suppressor mutation** | **Myo5a** | **dsu** | **ashen** | **Rab27a** | **leaden** | **In** | **ruby-eye** | **ru** | **ruby-eye 2** | **ru2** | **tubby** | **Patch** | **Pdgfr α** | **undulated** | **Curly-tail** | **DFNB26** | **mitochondrial 12S ribosomal gene** | **PRP1** | **retinitis pigmentosa** | **ROM1** | **familial hypercholesterolaemia** | **familial Mediterranean fever** | **pyrin/marenostrin** | **serum amyloid A** | **cystic fibrosis** | **CFTR** | **Cfr** | **familial adenomatous polyposis** | **APC** | **Min** | **Apc** | **Pla2g2a** | **Cox2 activity in the prostaglandin pathway** | **Cox2** | **cytosolic phospholipase A₂**

FURTHER INFORMATION **Mouse genome sequencing at Celera**

ENCYCLOPEDIA OF LIFE SCIENCES **Genotype–phenotype relationships**

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Acknowledgements

I thank A. Matin for insightful discussions about modifier genes, N. Robin for sharing examples of modifier genes, and M. Drumm and M. Taketo for comments on portions of this manuscript. This work was supported by grants from the NIH, by a grant from the Keck Foundation to the Department of Genetics, and by a grant from the Howard Hughes Medical Institute to the Case Western Reserve University School of Medicine.