BEYOND MENDEL: AN EVOLVING VIEW OF HUMAN GENETIC DISEASE TRANSMISSION

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Methodological and conceptual advances in human genetics have led to the identification of an impressive number of human disease genes. This wealth of information has also revealed that the traditional distinction between Mendelian and complex disorders might sometimes be blurred. Genetic and mutational data on an increasing number of disorders have illustrated how phenotypic effects can result from the combined action of alleles in many genes. In this review, we discuss how an improved understanding of the genetic basis of multilocus inheritance is catalysing the transition from a segmented view of human genetic disease to a conceptual continuum between Mendelian and complex traits.

HUMAN GENETICS AND DISEASE

PHENYLKETONURIA
An inborn error of metabolism that is caused by lack of the enzyme PAH that converts phenylalanine to tyrosine. If left untreated, it causes abnormally high phenylalanine levels and severe, progressive mental retardation, but can be prevented by neonatal screening and a low phenylalanine diet from an early age.

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The rapid identification of genes that are associated with human disease has revolutionized the field of medical genetics, providing more accurate diagnostic, prognostic and potential therapeutic tools. In addition, an improved understanding of the molecular aetiology of genetic disorders is also altering our perception of disease transmission. The classical model that is used for the discovery of many single-gene disorders is founded on the assumption that the spread of a trait in families is synonymous with the transmission of a single molecular defect. Although some traits are still recognized to be inherited in a monogenic fashion — with individual alleles segregating into families according to Mendel's laws — the number of disorders for which the phenotype can be satisfactorily explained by mutations at a single locus is now diminishing.

It has been suggested that compartmentalizing genetic disorders into monogenic and multifactorial might be an oversimplification[1]. Several studies even indicate that our view of diseases as monogenic might actually be a conceptual artefact[2,3]. Here, we discuss how oligogenic traits were recognized, and what tools can be applied to detect them and dissect their genetic basis. We also review the current molecular models for complex inheritance and illustrate how genetic and mutational data are moving the proposed 'paradigm shift'[4] away from simple models of disease transmission. Despite our improved understanding of multilocus inheritance, the study of true polygenic disorders remains challenging. Nevertheless, the expansion of Mendelian concepts and the construction of theoretical models of increased complexity is an important initial step towards understanding the genetic and molecular basis of multifactorial traits.

From the beginning

PKU and hyperphenylalaninaemia. PHENYLKETONURIA (PKU) was one of the first genetic disorders for which the biochemical defect was identified long before the advent of polymorphic markers and the ability to carry out genetic analysis in families. A defect in the hepatic enzyme phenylalanine hydroxylase (PAH) was recognized during the 1950s (REF. 5), and by 1960, the detection of hyperphenylalaninaemia was offered in neonatal screening tests. This provided not only an early diagnosis, but also the opportunity to treat the affected individuals[6] (also reviewed in REFS 7,8). Unfortunately ~ 1% of patients did not respond well to the traditional therapy, leading to the realization that, although defects at the *PAH* locus are present in most PKU cases, both

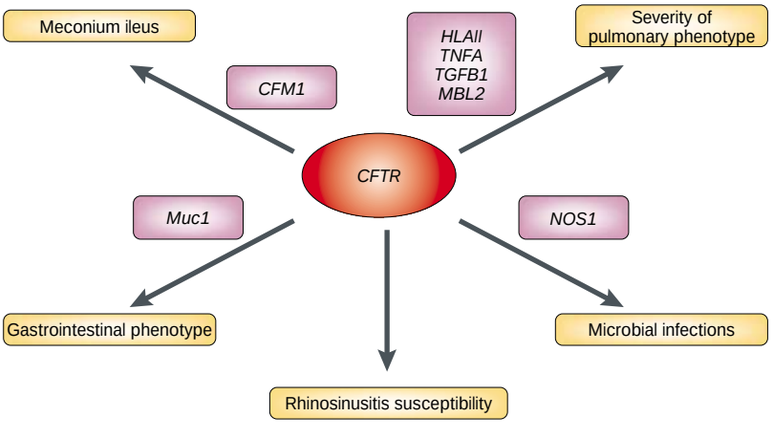

Figure 1 | **Complexity in monogenic diseases.** Mutations in *CFTR* almost always cause the CF phenotype. Owing to modification effects by other genetic factors, the presence and nature of mutations at the *CFTR* locus cannot predict what the phenotypic manifestation of the disease will be. Therefore, although CF is considered a Mendelian recessive disease, the phenotype in each patient depends on a discrete number of alleles at different loci. Meconium ileus describes the obstruction at birth of the small and/or large intestine (ileus) with the first faecal excretion (meconium). CF, cystic fibrosis; *CFTR*, cystic fibrosis transmembrane conductance regulator; *CFM1,* cystic fibrosis modifier 1; HLA-II, MHC class II antigen; *MBL2*, mannose-binding lectin (protein C) 2; *NOS1*, nitric oxide synthase 1; *TGFB1*, transforming growth factor-β1; *TNFA*, tumour necrosis factor-α encoding gene.

allelic heterogeneity and mutations at other loci might also account for the trait in some cases[8]. In 1983, the mapping and cloning of the *PAH* gene[9] not only confirmed this biochemical observation, but also showed substantial locus and allelic heterogeneity. In the next decade, the expectation of genetic heterogeneity was substantiated by the discovery of hyperphenylalaninaemia mutations in loci that affect TETRAHYDROBIOPTERIN synthesis or recycling[10]. Consistent with the then emerging view that the genetic complexity in PKU was significantly higher than originally expected for a monogenic disease, several studies reported extensive phenotypic variability, even in the presence of identical genotypes[1,11]. Although the inheritance of mutant alleles follows a Mendelian segregation pattern, the identification of mutations in any of the genes that cause hyperphenylalaninaemia cannot predict the phenotype of the patient, indicating that genetic factors and the environment might be important modulating agents.

From biochemical models to pure genetics

Cystic fibrosis. By contrast to PKU — in which the defect was first defined (at least in part) by biochemical means — cystic fibrosis (CF) represents an early example of the use of pure genetic models to identify the mutated gene. On the basis of the observed autosomal-recessive inheritance in families, the gene *CFTR* (cystic fibrosis transmembrane conductance regulator) was first mapped in humans to chromosome 7q31.2 (REF. 12). The *CFTR* gene was cloned[13], fuelling speculation that mutation analyses might be sufficient to predict the clinical outcome of patients. The analyses of *CFTR* mutations in large and ethnically diverse cohorts indicated that the initial hypothesis was an oversimplification of the true genetic nature of this phenotype, particularly with respect to the substantial phenotypic variability observed in some CF patients. For instance, although *CFTR* mutations show a degree of correlation with the severity of pancreatic disease, the severity of the pulmonary phenotype — which is the main cause of mortality — is difficult to predict (for recent reviews, see REFS 2,14–18).

Such realization of the limitations of a pure monogenic model prompted an evaluation of more complex inheritance schemes. This led to the mapping of a modifier locus for the intestinal component of CF in both human and mouse[19,20]. Further phenotypic analysis led to the association of low-expressing mannose-binding lectin (*MBL*; also known as *MBL2*) alleles, *HLA* (human leukocyte antigen) class II polymorphisms, variants in tumour necrosis factor-α (*TNFA*) and transforming growth factor-β1 (*TGFB1*) with pulmonary aspects of the disease[21–24], the correlation of intronic nitric-oxide synthase 1 (*NOS1*) polymorphisms with variability in the frequency and severity of microbial infections[25], and the contribution of mucin 1 (*Muc1*) to the gastrointestinal aspects of the CF phenotype in mice[26] (FIG. 1).

Recently, further layers of complexity have been discovered for both *CFTR* and its associated phenotype. First, heterozygous CF mutations have been associated with susceptibility to rhinosinusitis, an established multifactorial trait[27]. Second, and perhaps most surprising, a recent study has reported that some patients with a milder CF phenotype do not have any mutations in *CFTR*. This indicates that the hypothesis that *CFTR* gene dysfunction is a requisite for the development of CF might not always be true[28].

The emergence of oligogenic disorders

The difficulty in establishing a phenotype–genotype correlation, as exemplified by PKU and CF, indicates that, although Mendelian models are useful for identifying the primary genetic cause of familial disorders, they might be incomplete models of the true physiological and cellular nature of the defect[1,3,4]. Recently, numerous disorders that were characterized initially as being monogenic are proving to be either caused or modulated by the action of a small number of loci (TABLE 1). These disorders are described as 'oligogenic' disorders — a continuously evolving concept that encompasses a broad spectrum of phenotypes that are neither monogenic nor complex. By contrast to polygenic traits — which are thought to result from poorly understood interactions between many genes and the environment — these oligogenic disorders remain primarily genetic in aetiology, but require the synergistic action of mutant alleles at a small number of loci. Many examples indicate that a conceptual continuum exists between classical Mendelian and complex traits[29,30]. The position of any given disorder along this continuum depends on three main variables: whether a major locus contributes markedly to the phenotype, the number of loci involved and the extent of environmental participation. CF belongs near the beginning of the conceptual continuum, not because of the small number of phenotypic modifiers (there are at least six),

TETRAHYDROBIOPTERIN
Phenylalanine hydroxylase (PAH) is an oxygenase that couples an electron from a tetrahydrobiopterin cofactor (BH_4) and an oxygen atom to hydroxylate phenylalanine to form tyrosine. Consequently, any defects in BH_4 biosynthesis impair PAH function.

but owing to the prevalence of a major locus, *CFTR*, which accounts for most of this phenotype. Schizophrenia, on the other hand, resides in the middle of the continuum because the substantial genetic predisposition (40–50% phenotypic concordance in twin studies) is probably conferred by a relatively small number of genes, with eight mapped potential loci and a handful of proposed candidate genes (for a recent review, see REF. 31). The far end of the spectrum might contain traits that influence behaviour or cognition, the genetic basis of which is too poorly understood (and often controversial) to quantify.

Table 1 | Examples of human oligogenic disorders

Syndrome or trait	OMIM number	Primary locus	Secondary locus	Effect	References
Non-syndromic recessive deafness (DFNB1)	220290	*GJB2*	*GJB6*	Digenic	102
Non-syndromic recessive deafness (DFNB26)	605429	*DFNB26*	*DFNM1*	Suppressor	103
Usher syndrome type I	276903	*USH3*	*MyoVIIA*	Synergistic	104
Non-syndromic dominant deafness	601842	*DFNA12*	*DFNA2*	Digenic/additive	105
Waardenburg syndrome type II and ocular albinism	103470	*MITF*	*TYR*	Digenic	106
Retinitis pigmentosa	180721	*RDS*	*ROM1*	Digenic	44
Limb-girdle muscular dystrophy 2A	114240	*CAPN3*	Postulated	Penetrance/expressivity	107
Dysfibrinogenaemia causing recurrent thrombosis	134820	*FGA*	*FGG*	Digenic	108
Bardet–Biedl syndrome	209900	*BBS6/BBS2* *BBS2/BBS4*	*BBS2/BBS6* *BBS4/BBS2*	Digenic "triallelic" Digenic "triallelic"	50 46
Hirschsprung disease	142623	*RET* *RET*	*GDNF* 3p21;19q12	Digenic Penetrance/risk	85 59
Junctional epidermolysis bullosa	150310	*LAMB3*	*COL17A1*	Digenic	61
Cystinuria type III	600918	*SLC7A9*	*SLC3A1*	Digenic?	109
Becker muscular dystrophy	159991	*DMD*	*MYF6*	Severity modifier	110
Breast cancer	175100	*BRCA*	*APC*	Modifier/risk	111
Spinal muscular atrophy	603011	*SMN1*	*H4F5*	Candidate severity modifier	112
Cystic fibrosis	603855	*CFTR*	*CFM1*	Suppressor	19
Breast and ovarian cancer	113705	*BRCA1*	*HRAS1*	Penetrance/risk	113
Familial amyotrophic lateral sclerosis	147450	*SOD1*	*CNTF*	Severity modifier	33
Familial hypercholesterolaemia	143890	*LDLR*	13q	Suppressor	114
Familial Mediterranean fever	249100	*MEFV*	SAA1	Pleiotropy	115
Maternally inherited deafness	561000	12S ribosomal	*D8S277*	Penetrance	116
Melanoma	600160	*CDKN2A*	*MC1R*	Penetrance/risk	117
Van der Woude syndrome	604547	*VWS*	17p11.2	Penetrance	118
Type I von Willebrand disease	601628	*VWF* *VWF*	ABO blood group *Galgt2*	Penetrance Modifier	119 120
Nephrotic syndrome	256300	*NPHS1*	*NPHS2*	Digenic	66
Autosomal-dominant glaucoma	137750	*MYOC*	*CYP1B1*	Severity modifier	74
Congenital disorder of glycosylation type Ia	212065	*PMM2*	*ALG6*	Severity modifier	94
Alzheimer's disease	104300	*APP*	*TGFB1*	Severity modifier	121
Familial adenomatous polyposis	175100	*Apc*	*Mom1, Cox2, cPLA2*	Protection	38,122,123
Alagille syndrome	118450	*Jag1*	*Notch2*	Digenic (mouse model)	90

ALG6, dolichyl-P-Glc:$Man_9GlcNAc_2$-PP-dolichylglucosyltransferase; *APC*/*Apc*, adenomatosis polyposis coli; *APP*, amyloid-β(A4) precursor protein; *BBS2/4/6*, Bardet–Biedl syndrome 2/4/6; *BRCA1*, breast cancer 1; *CAPN3*, calpain 3, (p94); *CDKN2A*, cyclin-dependent kinase inhibitor 2A; *CFM1*, cystic fibrosis modifier 1; *CNTF*, ciliary neurotrophic factor; *CFTR*, cystic-fibrosis transmembrane-conductance regulator; *COL17A1*, collagen, type XVII, α1; *Cox2*, cyclooxygenase 2; *cPLA2*, phospholipase A2; *CYP1B1*, cytochrome P450, subfamily I (dioxin-inducible), polypeptide 1; *D8S277*, Diamond–Blackfan anaemia 2; *DFNA2*, potassium voltage-gated channel, KQT-like subfamily, member 4; *DFNA12*, tectorin-α; *DFNB26*, deafness, autosomal recessive 26; *DFNM1*, deafness modifier 1; *DMD*, dystrophin; *FGA*, fibrinogen, A α-polypeptide; *FGG*, fibrinogen, γ-polypeptide; *Galgt2*, UDP-*N*-acetyl-α-D-galactosamine:(*N*-acetylneuraminyl)-galactosyl-*N*-acetylglucosaminylpolypeptide-β1, 4-*N*-acetylgalactosaminyltransferase; GDNF, glial-cell-derived neurotrophic factor; *GJB*, gap-junction protein β; *H4F5*, small EDRK-rich factor 1A (telomeric); *HRAS1*, v-Ha-ras Harvey rat sarcoma viral oncogene homologue; *Jag1*, jagged 1; *LAMB3*, laminin-β3; *LDLR*, low-density-lipoprotein receptor; *MC1R*, melanocortin 1 receptor; *MEFV*, Mediterranean fever; *MITF*, microphthalmia-associated transcription factor; *Mom1*, phospholipase A2, group IIA; *MYF6*, myogenic factor 6; *MyoVIIA*, deafness modifier 1; *MYOC*, myocilin, trabecular meshwork inducible glucocorticoid response; *Notch2*, Notch gene homologue 2; *NPHS,* nephrosis 1/2; *PMM2*, phosphomannomutase 2; *RDS*, retinal degeneration, slow; *RET*, ret proto-oncogene; *ROM1*, retinal-outer-segment membrane protein 1; *SAA1*, serum amyloid A1; SLC7A9/*3A1*, solute carnér family 7, member 9/solute carrier family 3, member 1; *SMN1*, survival of motor neuron 1; *SOD1*, superoxide dismutase; *TGFB1*, transforming growth factor, β1; *TYR*, tyrosinase; *USH3*, Usher syndrome 3; VWF, von Willebrand factor; VWS, Van der Woude syndrome.

Establishing oligogenicity

It is not surprising that our understanding of genetic disorders decreases precipitously the further we deviate from Mendelian models. However, the application of statistical and molecular tools that were developed using monogenic systems has shown some success in the modelling and/or cloning of genes that are involved in oligogenic traits.

In the oligogenic disorders that have been characterized, several complementary approaches have been applied to show oligogenicity and to clone the loci responsible for the disease. Methodologically, we broadly recognize four lines of investigation: phenotype–genotype correlations, phenotypic differences in an animal model of the disease that are dependent on the genetic background, the identification of *bona fide* mutations that do not conform to monogenic inheritance and the establishment of linkage to more than one locus or the failure to detect linkage using Mendelian models.

Phenotype–genotype correlations. After the discovery of a disease gene, a natural first step is to investigate the mutational spectrum in large patient cohorts. This leads to attempts to correlate specific mutations (or classes of mutation, such as nonsense versus missense) with various phenotypic aspects, such as severity and age of onset. The frequent inability to draw conclusions from such studies prompts the expansion of the monogenic model of disease transmission to account for other factors. Difficulties in predicting the phenotype of PKU and CF patients from mutational data are early examples of observations that now abound in the literature. For instance, in familial amyotrophic lateral sclerosis (FALS) — a neurological disorder that is transmitted primarily as a dominant trait[32] — a family was reported in which the mother, son and daughter carried the same V148G mutation in the gene encoding copper/zinc superoxide dismutase 1 (SOD1). The mother developed FALS at the age of 54 and died at 55, the daughter was asymptomatic at 35, but the son developed severe FALS at the age of 25 and died within 11 months. This prompted Giess and colleagues to screen for candidate modifiers that might have an impact on the age of onset. They reported that the affected son also carried a homozygous null mutation in the ciliary neurotrophic factor (*CNTF*) gene, thereby implicating *CNTF* as a modifier of FALS[33] (FIG. 2a). Naturally, because these observations were based on the findings from a single family, it remains unclear how commonly *CNTF* modulates the FALS phenotype. Interestingly, the same *CNTF* mutation had been reported previously in Japanese patients with various neurological disorders. However, as no correlation with the disease could be drawn on the basis of the Mendelian model of transmission, the mutation was concluded not to be causally related to the disease[34]. In retrospect, we propose that the neurological disease in these Japanese patients might be attributable to mutations in *CNTF* and other loci.

Phenotypic differences in animal models. Recapitulating human phenotypes in experimental animal models allows the human mutation to be isolated and examined in a fixed genetic background. This has been a powerful tool for both establishing oligogenicity and mapping the loci that are involved in this phenomenon. FAMILIAL ADENOMATOUS POLYPOSIS (FAP) is caused by mutations in the adenomatous polyposis coli (*APC*) gene and is a disorder that is thought to lie early in the continuum of phenotypic causality, representing a classic example of such modelling. The phenotype of the dominant ENU (ethyl nitrosourea mouse mutant *Min* (multiple intestinal neoplasia), caused by a point mutation in the *Apc* gene[35], was shown to be modulated by a second locus, *Mom1* (modifier of *Min*), with respect to the number of tumours that developed[36,37]. Genetic analysis of the modification effect on the *Min* locus led to the mapping of *Mom1* to mouse chromosome 4 (REF. 38; see REF. 17 for a more extensive description).

Another example of this approach is the recent cloning of a modifier locus for the phenotype caused by the mouse *tubby* (*tub*) mutation. This spontaneous, autosomal-recessive mutation is characterized by obesity, insulin resistance and sensory defects, including retinal degeneration and hearing loss. The *tub* phenotype was reported in 1990 (REF. 39) and positional cloning identified it as being caused by mutations in the *Tub* gene, which encodes a novel protein of unknown function[40,41]. However, genetically controlled modifications of the *tub* phenotype were observed, in which the modifier conferred protection against hearing loss. QUANTITATIVE TRAIT LOCUS (QTL) analysis then mapped the modifier of tubby hearing 1 (*moth1*) to mouse chromosome 2 and showed that wild-type *Moth1* alleles, present in the AKR/J, CAST/EiJ and 129P2/OlaHsd strains, provided this protection to *tub* mice in a dominant fashion[42]. On the basis of this information, the *Moth1* gene was cloned and shown to correspond to the microtubule-associated protein 1a (*Map1a*) gene[43].

Disparities between mutations and Mendelian models. By contrast to directed modelling studies that revealed oligogenicity, many of the oligogenic traits that were initially thought to be purely monogenic — such as cases in which mutations were found to contradict the classical Mendelian model — have been detected by fortuitous means. RETINITIS PIGMENTOSA (RP), a genetically and clinically heterogeneous disease that can be inherited as an autosomal-dominant, autosomal-recessive or X-linked trait, represents the first clear example of how an expansion of the Mendelian model has resolved conflicting mutational and genetic data. Using a digenic model of disease transmission, Kajiwara and colleagues showed that heterozygous mutations in both the retinal outer segment membrane protein 1 (*ROM1*) gene and the peripherin/retinal degeneration slow (*RDS*) gene were required in some seemingly dominant RP pedigrees to cause disease[44] (FIG. 2b). In three families, the RP phenotype was shown to segregate with a L185P missense mutation in *RDS* and a 1-bp deletion at codon 114 in *ROM1*, with individuals carrying either of the two mutations being asymptomatic[45].

FAMILIAL ADENOMATOUS POLYPOSIS
(FAP). The development of numerous adomatous polyps in the colon that might progress to carcinomas.

QUANTITATIVE TRAIT LOCUS
(QTL). A genetic locus or chromosomal region that contributes to variability in complex quantitative traits (such as height or body weight), as identified by statistical analysis. Quantitative traits are typically affected by several genes and by the environment.

RETINITIS PIGMENTOSA
(RP). A group of both clinically and genetically heterogeneous hereditary retinal degeneration disorders that are caused by the death of both rod and cone photoreceptors, leading to a complete loss of vision.

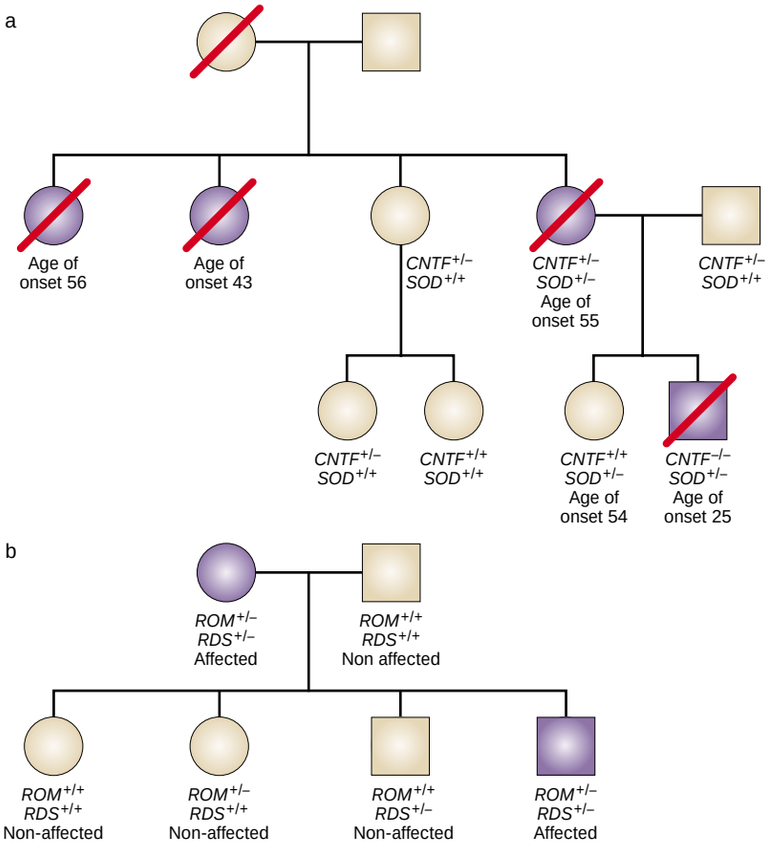

Figure 2 | **Idealized pedigrees showing examples of complex phenotypic modulation.** **a** | A *CNTF*-null allele modulates the age of onset of the dominantly transmitted disease amyotrophic lateral sclerosis (ALS). Mutations in both *SOD1* and *CNTF* lead to early-onset ALS (age 25) and death within 11 months (the third-generation male in the diagram). The third-generation female with a *SOD1* mutation but no *CNTF* mutation did not present with the disorder until the age of 54. **b** | A pedigree of digenic inheritance showing how retinitis pigmentosa occurs only in individuals who have inherited one mutation in each of *ROM1* and *RDS*. Heterozygotes for either mutant allele are asymptomatic. *CNTF*, ciliary neurotrophic factor; *RDS*, retinal degeneration slow; *ROM1*, retinal outer segment membrane protein 1; *SOD1*, superoxide dismutase 1. Circles indicate females; squares indicate males; diagonal red line across symbol indicates lethality.

BARDET–BIEDL SYNDROME (BBS). A rare and genetically heterogeneous disorder that is characterized primarily by obesity, retinal dystrophy, polydactyly, hypogenitalism, learning difficulties and renal malformations.

HAPLOTYPE ANALYSIS
The study of the pattern of descent of a combination of alleles at different sites on a single chromosome (known as a haplotype). It is used for the identification of recombination events between markers and traits during linkage studies, thereby establishing the boundaries of the location of a phenotype-associated locus.

Depending on the level of influence that each of the two loci exerts on the phenotype, digenic inheritance can be considered as either synergistic or modifying. Typically, those traits that segregate in families in a dominant fashion probably manifest a synergistic effect, whereas recessive disorders might present a modifying effect. Naturally, variation between these two models are likely to manifest in an allele-and-context-dependent manner.

A recent example of a recessive trait, the non-Mendelian inheritance of which was established by mutational data, is BARDET–BIEDL SYNDROME (BBS), a genetically heterogeneous disease that is thought to be caused by recessive mutations in one of at least six genes. Mutational and HAPLOTYPE ANALYSIS of *BBS6*, the first BBS gene to be cloned[46,47], indicated that some mutations did not conform to the expected recessive transmission. In one CONSANGUINEOUS pedigree in particular, a heterozygous A242S mutation was transmitted from the father to both of his children, only one of whom was affected, although haplotype analysis indicated that both siblings had inherited the same maternal chromosome (FIG. 3a). Therefore, even if the mother carried an undetected mutation, she would have to have transmitted it to both siblings, which would lead to an unaffected individual carrying two heterozygous *BBS6* mutations. This would indicate that, in some families, more than two mutant alleles might be required to manifest BBS. Indeed, the affected sibling was homozygous across the critical interval of another BBS gene, *BBS2*, indicating that this pedigree might segregate two *BBS2* mutations and one *BBS6* mutation[48]. The positional cloning of *BBS2* (REF. 49) confirmed this hypothesis. Consistent with the haplotype data, a homozygous Y24X *BBS2* mutation was detected in the affected but not the unaffected sibling[50], indicating the existence of another variation of digenic inheritance, 'triallelic' inheritance, which initially seemed to be transmitted as a recessive trait[50]. Consistent with this oligogenic mode of disease transmission, three mutations at two loci were found in three more BBS pedigrees, including one family in which the patient inherited two nonsense *BBS2* mutations and a nonsense *BBS6* mutation, and his unaffected brother had two *BBS2* nonsense mutations but was wild type for *BBS6* (REF. 50; FIG. 3b).

Linkage studies. Statistical tools for modelling the genetic interactions between loci and environmental factors are proliferating rapidly. Although mathematical analyses of oligogenicity are beyond the scope of this discussion (see REFS 51,52 for in-depth analyses), it is important to recognize that the modified use of traditional linkage approaches remains a useful tool for the study of oligogenic diseases, especially if a major locus that contributes greatly to the phenotype is known.

This is exemplified by recent developments in Hirschsprung disease (HSCR), a congenital disorder that is characterized by the variable absence of ENTERIC GANGLIA. On the basis of the extent of aganglionosis, two main phenotypic groups can be distinguished: short-segment HSCR (S-HSCR) and the more severe long-segment HSCR (L-HSCR)[53]. Autosomal-dominant inheritance with incomplete PENETRANCE has been proposed for L-HSCR, whereas complex inheritance that involves an autosomal-recessive trait has been observed in S-HSCR. Oligogenicity has been established in both HSCR variants by virtue of several factors: a recurrence risk that varies from 3 to 25%, depending on the length of aganglionosis and the sex of the patient; HERITABILITY values close to 100%, which indicates an exclusively genetic basis; significant clinical variability and reduced penetrance; and non-random association of hypomorphic changes in the endothelin receptor type B (*EDNRB*) with rearranged during transfection (*RET*) polymorphisms and HSCR[54] (for a comprehensive discussion; see REF. 55). So far, a combination of linkage, positional-cloning studies and functional candidate gene analyses has identified eight HSCR genes (for a review, see REF. 56), of which the proto-oncogene *RET*[57,58] is thought to be the main predisposing locus, particularly in families with a high incidence of L-HSCR[59].

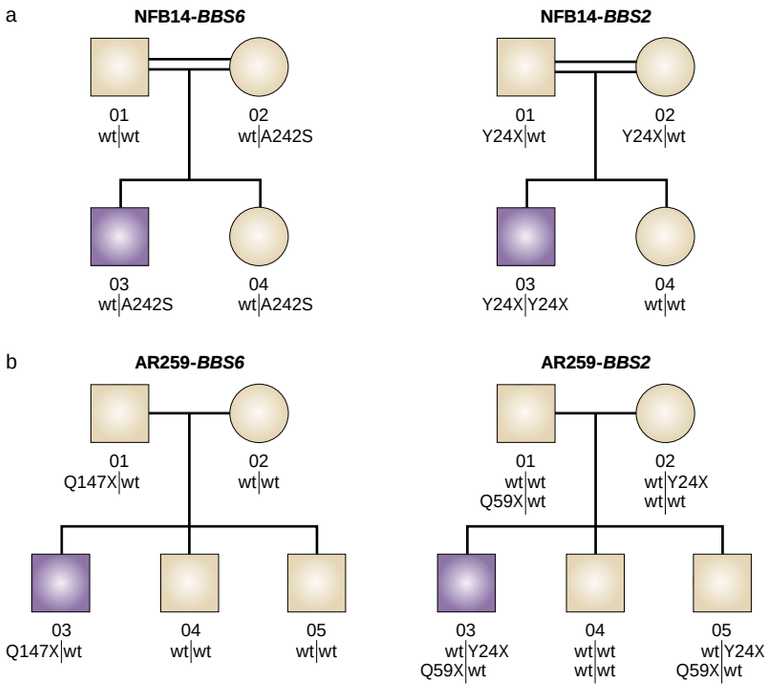

Figure 3 | **Triallelic inheritance. a** | In the consanguineous pedigree NFB14 both the affected (03) and the unaffected (04) individuals carry the same mutation (A242S) in the Bardet–Biedl syndrome gene, *BBS6*. Only the affected sibling is homozygous for a nonsense mutation (Y24X; X indicates a stop codon) in *BBS2*. The double line that links the couple in the first generation indicate consanguineity. **b** | Three mutations at two loci are necessary for pathogenesis in this pedigree, as the affected sibling (03) has three nonsense mutations (Q147X in *BBS6,* and Y24X and Q59X in *BBS2*) and the unaffected sibling (05) has two nonsense *BBS2* mutations, but is wild-type for *BBS6*.

CONSANGUINEOUS
Descended from a recent common ancestor.

ENTERIC GANGLIA
Parasympathetic mass of nerve tissue (ganglia) in the colon.

PENETRANCE
The proportion of affected individuals among the carriers of a particular genotype. If all individuals who have a disease genotype show the disease phenotype, then the disease is said to be "completely penetrant".

HERITABILITY
The proportion of the variation in a given characteristic or state that can be attributed to genetic factors.

PARAMETRIC LINKAGE
Parametric analyses are statistical tests for linkage that use assumptions such as mode of transmission, allele frequencies and penetrance.

The non-Mendelian transmission of HSCR has hindered the identification of predisposing modifier loci by conventional linkage approaches. When both PARAMETRIC and NON-PARAMETRIC LINKAGE (NPL) studies were carried out on a group of 12 L-HSCR families, weak LOD SCORES and NPL values were observed on 9q31. However, on the basis of the hypothesis that only milder *RET* mutations could be associated with another locus, families were categorized according to the *RET* mutational data. Significant linkage on 9q31 was detected when families with potentially weak *RET* mutations were analysed independently[60], indicating that mild *RET* alleles, in conjunction with alleles at an unknown gene on chromosome 9, might be required for pathogenesis.

The mode of inheritance in S-HSCR has proved to be more complex than L-HSCR, and has required further adjustments to the linkage strategies. Recently, the application of model-free linkage, without assumptions about the numbers and inheritance mode of segregating factors, showed that a three-locus segregation was both necessary and sufficient to manifest S-HSCR, with *RET* being the main locus, and that the transmission of susceptibility alleles was additive[59].

The impact of oligogenicity on genetic research

The inheritance patterns observed in disorders such as Hirschsprung disease illustrate the power of both expanded models of disease inheritance that account for reduced penetrance and phenotypic variability and the ability of these models to genetically map loci involved in oligogenic diseases — a first step towards identifying genes that underly them. More importantly, the establishment of non-Mendelian models has caused a change of perception in human genetics which, in turn, has accelerated the discovery of oligogenic traits.

Although genetic studies have clearly been useful, most of the success in this field has relied on the availability of many genes that result in the same or similar phenotypes in an apparently monogenic way. Therefore, it is not surprising that the genetic basis of non-Mendelian inheritance has been demonstrated more frequently in genetically heterogeneous disorders. The key to the discovery of digenic inheritance in RP and BBS was not only the identification of mutations that conflicted with the familial transmission of the trait, but also the availability of several genes, each of which had independently been shown to cause the same phenotype. Given that human patient cohorts are not always sufficiently informative to conduct genetic studies, these 'candidate-gene' approaches (once complex inheritance has been established) show great promise. This is exemplified by junctional epidermolysis bullosa (JEB), a group of recessive disorders of the skin that range in severity from lethal (JEB Herlitz) to milder forms, such as generalized atrophic benign epidermolysis bullosa (GABEB). In one family, the proband presented with a severe phenotype that had aspects of both JEB Herlitz and GABEB, which led the investigators to query the genes known to cause these disorders, including collagen XVII (*COL17A1*) and the β3-subunit of laminin 3 (*LAMB3*). This resulted in the detection of two *COL17A1* nonsense mutations (L855X and R1226X) and a heterozygous *LAMB3* mutation (R635X) in the patient[61].

Recently, digenic inheritance has been proposed for NEPHROTIC SYNDROME. Nephrin (*NPHS1*) and podocin (*NPHS2*) have been shown to cause two types of nephrotic syndrome, the Finnish congenital nephrotic syndrome (CNF) and autosomal-recessive familial focal segmental glomerulosclerosis (SRN1)[62–64]. Functional studies have indicated that these two proteins interact physically[65], and the recognition that nephrotic syndrome has substantial clinical and genetic variability prompted Koziell and colleagues to screen both *NPHS1* and *NPHS2* in patients with this disorder[66]. Consistent with the expectation of oligogenicity, four patients were identified who carried mutations in both genes in a model similar to triallelic inheritance[66].

The nature and effect of oligogenic mutations

Although mapping and cloning loci is a major challenge, defining oligogenic mutations is often substantially more problematic. A key hurdle that can prove difficult to overcome is establishing the specific alterations in the DNA sequence as deleterious. Fortunately, the effect of 'major' mutations, such as deletions, insertions, splice-site changes and nonsense alterations, is relatively easy to both predict and test.

By contrast, predisposing or protecting alleles, the effect of which on the phenotype is often subtle, is considerably more difficult. Some mutations might be mild coding sequence alterations, might lie in the regulatory regions of transcripts such as 5′ and 3′ terminal regions and introns or might lie in control elements that are several kilobases away from the transcript. In the absence of alternative means of assaying the mutagenic effect of an allele, it can be challenging to conclude using genetic and population data that a particular gene is causal to the phenotypic modulation.

The digenic inheritance in BBS exemplifies this problem. Shortly after the discovery of triallelic inheritance between *BBS2* and *BBS6*, the third gene for this disorder, *BBS4*, was identified[67]. Subsequent mutational and genetic analyses indicate that mutations in *BBS4* also interact with mutations at other loci, including the possibility that two *BBS4* mutant alleles and two *BBS2* mutant alleles are necessary for pathogenesis in some pedigrees. One patient had two *BBS2* T560I mutations and two *BBS4* A364E mutations, whereas his asymptomatic mother and brother were T560I/T560I, A364E/wt. In another three pedigrees, only a single *BBS4* mutant allele was found (L327P, N165H and S457I), and each pedigree was excluded by haplotype analysis from having a second mutation in *BBS4*[68]. In the absence of functional information, determining which, if any, of these alterations are pathogenic is problematic. It is not possible to distinguish between rare benign variants and subtle mutations, or, in the absence of complete knowledge of all key regulatory elements in *BBS4*, can it be claimed that all potential pathogenic variations have been detected.

GLAUCOMA is another example that shows the difficulty in linking DNA variations with pathogenic causality. Mutations in several genes have been associated with different types of glaucoma, including myocilin mutations (*MYOC*) in juvenile open-angle glaucoma[69] (JOAG) and *CYP1B1* (cytochrome P450, subfamily I, polypeptide I) in primary congenital glaucoma[70] (PCG). Oligogenicity in glaucoma has been suspected because of substantial inter- and intrafamilial phenotypic variability[71], and because of the identification of non-expressing individuals who carry two mutant alleles without developing the disease[72,73]. Recently, a pedigree was reported in which glaucoma segregated in an autosomal-dominant fashion. In this family, patients with a heterozygous G399V mutation in *MYOC* and a heterozygous R368H mutation in *CYP1B1* manifested the disease at a mean age of 27 years, whereas individuals with only the heterozygous *MYOC* mutation developed glaucoma at a mean age of 51 years (REF.74). Moreover, a V432L variant in *CYP1B1*, which is thought to be a polymorphism, was also found in the same family. The leucine allele has been proposed to affect the ability of the protein to hydroxylate 17β-oestradiol, a target of *CYP1B1* some studies have reported a decrease in catalytic efficiency[75–77], whereas others have reported the converse[78]. Collectively, the *MYOC* and *CYP1B1* genetic and mutational data indicate that *CYP1B1* is probably a modifier locus. However, in the absence of functional data for some variants and none for others it is difficult to discriminate whether some of these alleles are pathogenic are in LINKAGE DISEQUILIBRIUM with the true mutation or are the product of random GENETIC DRIFT in the population. Furthermore, evaluation of the effect of particular alleles in isolation might provide limited information, as the true biochemical defect could be unmasked only by modelling the synergistic action of several mutations acting both in *cis* and in *trans*.

Examining oligogenic alleles in model organisms, such as the mouse, can overcome some of these difficulties, particularly because the effect of an allele can be tested in a genetically homogeneous background. Such experimental animal models are particularly useful when some information on the gene function is available or when a tissue/organ phenotype has been established. For instance, the protection afforded by *moth1* in the *Tub* hearing phenotype seems to be attributable to a shorter Ala-Pro repeat region in *Map1a*, whereby the protective strains 129P2/OlaHsd and CAST/Ei contain a Map1a protein with two and five repeat subunits fewer than the B6 strain, respectively[43]. Genetic and sequencing data alone cannot distinguish between a mutation at this site versus linkage disequilibrium between a polymorphism and the actual modifier mutation, which might or might not map to the same gene. However, partial rescue of the *Tub* hearing phenotype with a *Map1a* transgene containing the 129P2/OlaHsd allele, provides strong evidence that *Map1a* is *moth1*. Co-immunoprecipitation studies also indicated that the observed differences between the various *Map1a* alleles might result in different binding affinities for specific proteins[43], although further work is required to substantiate this intriguing hypothesis.

Molecular mechanisms of oligogenicity

By contrast to genetic modelling of oligogenicity, of which there are now numerous examples (TABLE 1), the paucity of functional data for many of the genes that cause human disease means that the molecular basis of such phenomena is poorly understood.

One of the best-studied illustrations for the molecular basis of complex inheritance is the digenic interaction of *ROM1* and *RDS* that causes RP. These two proteins form homodimers, which in turn interact to form tetrameric complexes[79] that are important for the structural integrity of photoreceptors in the retina[80,81]. The digenic *RDS* mutation prevents the formation of functional RDS–RDS homocomplexes[82,83], with the null *ROM1* mutation further decreasing the amount of functional complexes available, and is postulated to lead to photoreceptor degeneration[82,83].

Other examples in which synergistic mutations at discrete loci underlie a direct interaction between their encoded proteins illustrates the same principle. The genetic interaction of *NPHS1* and *NPHS2* in nephrotic syndrome could be attributed to the direct interaction of the carboxyl terminus of *NPHS2* with *NPHS1* (REFS 65,84). However, the effect of the mutations on the stability and function of this complex

NON-PARAMETRIC LINKAGE
Non-parametric approaches are statistical procedures that are not based on models, or assumptions pertaining to the distribution of the quantitative trait.

LOD SCORE
(Base 10 'logarithm of the odds' or 'log-odds'). A method of hypothesis testing. The logarithm of the ratio between likelihoods under the null and alternative hypotheses.

NEPHROTIC SYNDROME
Malfunction of the renal glomerular filtration barrier (a structure in the glomerulus that is responsible for protein filtration) that lead to the loss of plasma proteins.

GLAUCOMA
The abnormally elevated pressure in the liquid that fills the anterior part of the eye (the aqueous humour).

LINKAGE DISEQUILIBRIUM
The condition in which the frequency of a particular haplotype for two loci is significantly greater than that expected from the product of the observed allelic frequencies at each locus.

GENETIC DRIFT
Random fluctuations in the allele and, less commonly, the phenotype frequencies, as genes are transmitted from one generation to the next.

requires clarification. Finally, receptor–ligand relationships might also underlie the physiological basis for some types of complex inheritance. Oligogenicity in some Hirschsprung families is attributed to mutations in both *RET* and the glial-cell-derived neurotrophic factor (*GDNF*) gene[85]; with *RET* forming part of a multisubunit receptor of *GDNF*[86,87]. Likewise, phenotypic modulation in ALAGILLE SYNDROME, a disease caused by HAPLOINSUFFICIENCY of the jagged (*JAG1*) gene product[88,89], has been attributed in part to mutations in *NOTCH2*, which encodes a receptor for JAG1 (REF. 90).

These examples illustrate the general principle of non-allelic non-complementation, whereby mutations in two different genes can behave as alleles of the same locus by causing or exacerbating the same phenotype. This phenomenon has been established in various organisms and in numerous physiological processes, including cuticle development, transcriptional regulation, neuron outgrowth and cytoskeletal motility[91]. The two main models that explain non-allelic non-complementation are the dosage model and the poison model[92,93]. According to the dosage model, the simultaneous decrease in dosage at two loci is required to manifest the phenotype (FIG. 4a,b). In the

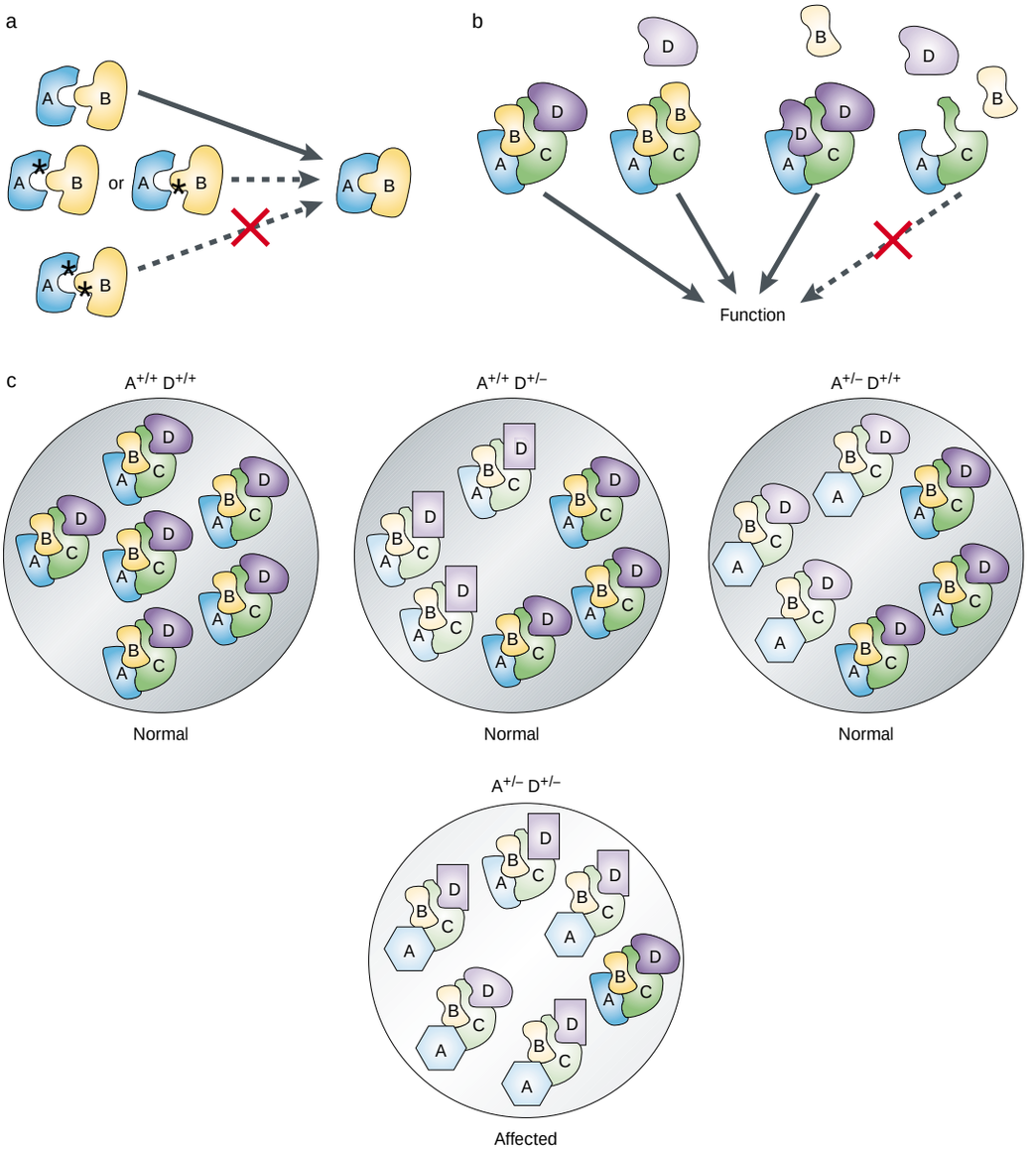

Figure 4 | **Models of non-allelic complementation. a, b** | The direct-interaction dosage model. **a** | Mutations at one locus (mutated proteins are indicated by asterisks) are not sufficient to disrupt the formation of the complex between proteins A and B, although the strength of the interaction might be reduced (dashed line). A further mutation in protein B causes disruption of the complex (red cross), resulting in a detectable phenotype. **b** | A similar model involving proteins B and D, which are members of the same multi-subunit complex but do not interact directly. **c** | The poison model. Mutations in protein A disrupt the complex, although enough functional units remain to maintain function. A further mutation (or mutations) in protein D disrupts more units, resulting in the disruption of a physiological process and the generation of a cellular phenotype.

ALAGILLE SYNDROME
A dominantly inherited disorder that is characterized primarily by a scarcity of bile ducts in the liver. Other features include heart, eye, kidney and skeletal abnormalities, as well as defects in the central nervous system.

HAPLOINSUFFICIENCY
A gene dosage effect that occurs when a diploid requires both functional copies of a gene for a wild-type phenotype. An organism that is heterozygous for a haploinsufficient locus does not have a wild-type phenotype.

poison model, a mutation impairs the protein complex, while retaining function, probably because enough functional complexes remain available to the cell. A second mutation at another protein of the same complex further disrupts the already reduced number of normal complexes and leads to an observable effect (FIG. 4c). Ligand–receptor relationships (such as JAG1–NOTCH2) might conform to the dosage model, whereas the poison model could describe phenomena such as digenic RP.

Molecular models of oligogenicity need not be confined to proteins that interact directly. In *Caenorhabditis elegans*, non-allelic complementation has been observed between UNC-13 and synaptobrevin, two proteins that bind to the same complex but that are not directly associated with each other[91]. In other models, two proteins might act at different stages of the same pathway; in such cases, a mutation in each protein contributes quantitatively to the progressive dysfunction of the pathway until a crucial threshold is reached and a disease phenotype is observed (FIG. 5). This is exemplified by some congenital disorders of glycosylation, whereby mutations in *ALG6* and *PMM2*, each encoding an enzyme involved in a different part of the post-translational-modification process, might be required to manifest the more severe form of the disease[94]. Finally, mutations at different positions of the same protein network, or in independent but synergistic networks, might also result in oligogenic phenotypes. There is no doubt that, as our understanding of the nature and composition of protein networks and signalling pathways increases (for example, see REFS 95,96), new examples will surface, and our molecular models to describe oligogenicity will improve in both accuracy and sophistication.

The road ahead

Oligogenic disorders represent both a unique challenge and an opportunity. The realization that many of the genetic disorders that were described previously as monogenic are in fact the product of defects at a small number of loci creates conceptual and practical problems. First, it requires the reorganization of our approach to studying human genetic disorders, starting by dissociating the mode of inheritance in families (that is, Mendelian segregation of traits) from the mode of inheritance of specific disease alleles. As most mutations probably exert a quantitative effect on the phenotype, labelling mutant alleles as dominant or recessive is often an oversimplification.

Similarly, diagnoses and patient management can be impeded by oligogenic phenomena[97]. In most cases, although the likelihood of disease onset that is attributable to a mutant allele can be predicted, the phenotypic outcome cannot. For example, the onset of FALS at age 25 or age 54 are two different scenarios, each having a profound impact on the life choices of the patient (FIG. 2a). In other cases, the absence of CF mutations might not be sufficient to exclude CF or, on the contrary, the presence of two mutations in one gene might not always predict the onset of disease, as is the case for some triallelic BBS pedigrees (FIG. 3b). Consequently, the diagnosis and management of genetic diseases will continue to be a case-by-case process and to rely heavily on epidemiological data for each disease and its associated alleles.

Recognition of these limitations is the first step towards a solution. A better understanding of the molecular mechanisms of oligogenicity will probably enhance our ability to make accurate genotype-based phenotypic predictions and to then estimate more clearly the effect of the environment. Furthermore, the identification of loci, the concurrent dysfunction of which leads to a specific phenotype, represents an important tool for research in protein function and cellular pathways.

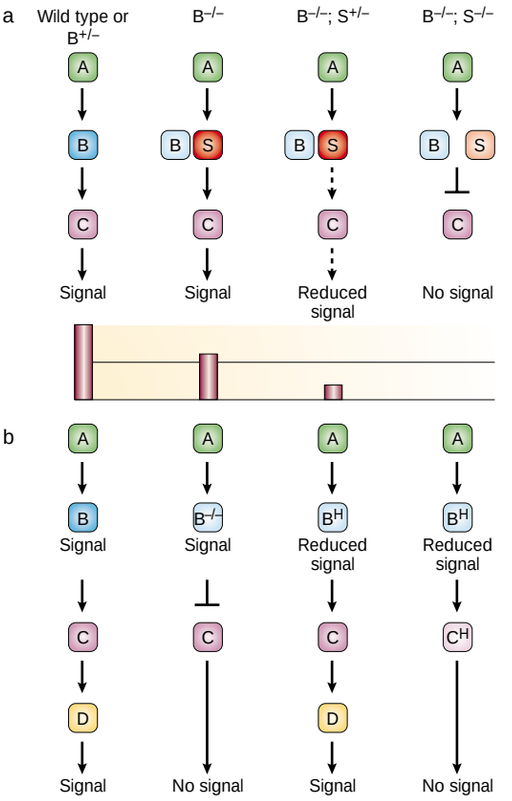

Figure 5 | **Idealized pathway-complementation schemes.** **a** | Signal transduction between protein A and protein C (first column) is disrupted by the homozygous loss of function of protein B (second column). The substitution of the function of protein B by S rescues the pathway. Heterozygous mutations in S decrease the efficiency of the pathway and might cause an observable phenotype (third column). By contrast, loss of S causes a complete loss of signal (last column). The histogram illustrates the relative strength of the signal that is associated with each genotype. **b** | Dosage-dependent effects on members of the same pathway. Null alleles of any of proteins A–D cause a loss of signal (a mutation in B is shown in the second column). However, hypomorphic (H) mutations in B might maintain the signal, albeit at a reduced strength (third column); a second hypomorphic mutation has the same effect as a null mutation of any member of the pathway (last column). This phenomenon has been termed 'synergistic heterozygosity' in the context of metabolic disease[102].

Perhaps the greatest opportunity stems from the promise that an expansion of monogenic concepts can assist in the modelling of multifactorial disorders, which are of great socio-economic relevance. The identification of rare, monogenic or oligogenic phenotypes that are present in complex disease has already led to the identification of susceptibility loci for complex traits, such as the presence of some CF alleles in rhinosinusitis[27] and the association of *ABCA4* (ATP-binding cassette, subfamily A, member 4) alleles with age-related macular degeneration[98–101]. Although analysing the complex interactions between numerous genes and the environment remains a distant goal, modelling the interaction of a discrete number of loci and understanding the phenotypic consequences of such interactions is a small, yet significant step in the right direction.

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