Human Molecular Genetics, 2004, Vol. 13, Review Issue 2 **R225–R233**
doi:10.1093/hmg/ddh254

Mechanisms of non-Mendelian inheritance in genetic disease

Veronica van Heyningen* and Patricia L. Yeyati

MRC Human Genetics Unit, Western General Hospital, Edinburgh EH4 2XU, UK

Received July 23, 2004; Revised and Accepted July 26, 2004

Single gene disorders with Mendelian inheritance patterns have contributed greatly to the identification of genes and pathways implicated in genetic disease. In these cases, molecular analysis predicts disease status relatively directly. However, there are many abnormalities which show familial recurrence and have a clear genetic component, but do not show regular Mendelian segregation patterns. Defining the causative gene for non-Mendelian diseases is more difficult, and even when the underlying gene is known, there is uncertainty for prenatal prediction. However, detailed examination of the different mechanisms that underlie non-Mendelian segregation provides insight into the types of interaction that regulate more complex disease genetics.

Mendelian inheritance patterns are well-established, and readily recognizable as 'textbook' examples, for many single gene diseases (1), and a few digenic cases (2–4). However, in most clinical genetics settings many cases are seen where the disease diagnosed is well known to have a strong genetic component, and show some familial recurrence, but no clear Mendelian inheritance. Such cases clearly pose additional problems in counselling and the estimation of recurrence risk. Here, we review some of the different molecular mechanisms that lead to such irregular inheritance patterns, focussing mostly on diseases where at least one implicated gene and some underlying mutations have been identified. It is useful to attempt to categorize the different ways in which the observed inheritance patterns are generated (Table 1) and then to consider in more detail some examples in each category.

Some detailed molecular mechanisms underlying non-Mendelian inheritance patterns will be unfolded below, but first some general concepts need to be clarified. Incomplete penetrance (5), where not all mutation carriers present with the expected phenotype, is commonly observed in human family studies. This forms a continuum with variable expressivity, which can be so extreme that subtle manifestations of carrier status are sometimes only identified with hindsight (6). Even some of the most classical Mendelian traits, like cystic fibrosis (CF), show complex variation (7,8). A significant proportion of variability can be ascribed to allelic differences (9,10), some of which will be cryptic regulatory variation, influencing gene expression levels (11,12). The existence of widespread variation is not surprising, as gene products fulfil their function through finely tuned interactions with other cellular components, often showing some degree of threshold requirement (7). Each component is subject to regulation, and variation, at every stage: transcription, splicing, translation (13), protein folding, oligomerization, translocation and compartmentalization within the cell or export from it (14). Subsequently, there is controlled turnover, through well-policed pathways of destruction (15,16). When focussing on gene products, we mostly think of proteins, but should increasingly remember that many cellular processes are regulated by RNA molecules, or at the RNA level (17). The complexity of the proteome is strongly affected by controlled alternative splicing, a mechanism often altered or damaged through sequence variation or mutation. Protein folding, association and sub-cellular localization, also require some helper systems, such as molecular chaperones, which often double as stress response proteins (18), and these are very likely implicated in extreme phenotypic variability, and hence segregation pattern variability (19,20).

SPORADIC OCCURRENCE OF GENETICALLY LETHAL SINGLE GENE ANOMALIES

An apparently sporadic pattern of disease incidence is observed if virtually all cases arise as a result of new mutation. No parent to child inheritance of the phenotype is seen, generally because the affected individuals are consistently unable to reproduce through infertility or for physical or social reasons. Occasionally sibling recurrence is seen in such cases, if

*To whom correspondence should be addressed. Tel: +44 1314678405; Fax: +44 1314678456; Email: v.vanheyningen@hgu.mrc.ac.uk

the parent in whom the new mutation arose has gonadal mosaicism, which is sometimes accompanied by somatic mosaicism. Such recurrent new mutations are seen in Apert syndrome, with craniosynostosis and severe syndactyly caused by gain-of-function paternal mutations (21) at specific sites in the *FGFR2* gene (22). A significant proportion of severe bilateral anophthalmias is caused by *de novo* loss-of-function mutations in the early neural and eye development gene *SOX2* (23). Predicted loss of function mutations were recently identified in another developmental anomaly with no vertical transmission, Cornelia de Lange syndrome, in the human orthologue, *NIPBL*, of the Drosophila Nipped-B protein, with homology to sister chromatid cohesion protein SCC2 and a possible role in promoter-enhancer interactions (24,25). Most early onset severe cases of congenital central hypoventilation syndrome (CCHS) are associated with polyalanine expansion mutations in the paired-like homeobox gene *PHOX2B* (26). Frameshift changes in *PHOX2B* were also found in CCHS, including a single nucleotide deletion inherited from an asymptomatic mother (27). Some CCHS patients had additional manifestations of autonomic nervous system disease, such as Hirschsprung disease (HSCR) (26,27), and in two cases coincident *RET* and *GDNF* missense changes were seen (26), which may act as modifiers of the CCHS phenotype.

OLIGOGENIC DISEASE WITH INCOMPLETE PENETRANCE, PHENOTYPIC VARIABILITY AND LOCUS HETEROGENEITY

Oligogenic inheritance patterns are increasingly recognized, as the techniques of population studies are adapted, or even developed using these simpler, more clearly familial disease models. HSCR is phenotypically variable aganglionosis (failure of sympathetic innervation) of the colon. Long-segment L-HSCR, sometimes syndromic, is caused by coding sequence mutations at one of several loci including *RET*, *GDNF*, *SOX10*, *EDN3* and *EDNRB* (28,29). Short segment S-HSCR (80% of cases) reveals complex segregation patterns involving contributions from at least three loci, one of which is unequivocally the *RET* gene on chromosome 10q11; multiplicative interaction with other loci at 3p21 and 19q12, encompassing currently unidentified genes, has been proposed (29). Distinct *RET* mutations in the extracellular domain are preferentially associated with S-HSCR, and it is clear that mutations outside the coding region are missed (29).

Holoprosencephaly is characterized by incomplete separation of the forebrain into distinct halves, and is usually associated with craniofacial features (30). Many cases are apparently sporadic, but some show familial recurrence. Severity of phenotypes is extremely variable, even within families, from prenatal lethality with cyclopia, to hypertelorism, or the presence of a single central incisor; 36% of obligate carriers have no clinical phenotype (31). A number of causative genes have been identified, with *SHH*, *ZIC2*, *SIX3* and *TGIF* most frequently involved, but only ~20% of HPE cases have revealed mutations in any of the eight identified genes (31–33). *SHH* mutations are most frequently associated with familial disease, and some cases have mutations in two of the known genes (31,33), suggesting gene interaction. It should be noted that generally only coding region mutations are sought, but for *SHH* at least, complex *cis*-regulatory regions are known (34,35), and these may harbour disease-causing mutations with unknown phenotypic spectrum or penetrance. As *SHH* protein is cholesteroylated, related mutations, dietary cholesterol and drugs which interfere with its metabolism may influence the incidence of HPE (36–38).

LOW-PENETRANCE DISEASE PREDISPOSITION WITH IDENTIFIED GENETIC MODIFIER EFFECT

Hereditary haemochromatosis (HH) is tissue damage resulting from excessive iron storage, mostly involving homozygosity for mutations at different loci (39). Rare juvenile forms exist with severe mutations in *HJV*, a protein of unknown function (40,41), and recently homozygous mutations in the antibacterial inflammatory peptide hepcidin (*HAMP*) (42,43) were identified. The more common, late onset, forms of HH are most frequently found in individuals homozygous or compound heterozygous for two common variants (C282Y and H63D) of the MHC-linked protein HFE. However, the penetrance of these mutations is low, particularly in premenopausal women. It was recently found that double heterozygosity for *HFE* and *HAMP* mutations leads to much higher frequency and sometimes earlier and more severe symptoms (39,44). Interactions with other loci are also expected, making HH another unpredictable oligogenic disease. Considerable controversy surrounds proposals for population screening for these common potentially detrimental alleles.

PHENOTYPE MODIFICATION BY THE WILD-TYPE ALLELE VARIANT PRESENT *IN TRANS* WITH THE MUTANT ALLELE

Variation in expression of the 'normal' allele opposite to a mutant one can influence the penetrance of a mutant phenotype. Examples of this include incomplete penetrance at RP11, one of the dominant retinitis pigmentosa loci, where mutations in the ubiquitously expressed splicing factor *PRPF31* were shown to cause disease, but only in individuals carrying a high expressing 'wild-type' allele (45). The mechanism underlying variable expression at the normal allele is not clear, but regulatory polymorphisms are likely (12). A similar situation has also been identified in erythropoietic protoporphyria (EPP) where mutations in the ferrochelatase gene (*FECH*) fail to lead to a deficiency phenotype, when the allele *in trans* carries a single nucleotide variant in intron 3, that strengthens a cryptic splice site, the more frequent utilization of which leads to reduced levels of normally spliced mRNA (46). Although it has not been discussed in this mechanistic category, the observation of some cases of congenital absence of the vas deferens (CAVD) associated with *CFTR* mutations also fit this model. Most individuals with this phenotype have no overt CF phenotype, though occasionally they have one of the signs of CF (bronchiectasis, or nasal polyps), and some have a partial conductive chloride transport defect (9,47,48). A few cases have two *CFTR* mutations, one of which is generally a known mild allele, but others

are heterozygous for one severe (or sometimes mild) allele. In some cases the mild allele is a variant wild-type allele with a stretch of only five thymidines (T5) instead of seven (T7) or nine (T9). The T5 allele produces a proportion of transcripts with an in-frame deletion of exon 9. It is the most frequent second allele in CAVD, often found *in trans* to ΔF508, the most common severe allele, but which generally does not manifest a heterozygote phenotype. CAVD is, of course, doubly abnormal in its inheritance pattern, as vertical transmission is unlikely with an infertility phenotype, at least until the recent advent of assisted reproductive technologies, such as intracytoplasmic sperm injection.

REDUCED PENETRANCE ALLELES AT TUMOUR SUPPRESSOR LOCI

Germ line mutations at tumour suppressor loci predispose to familial cancer, although in accordance with the two-hit hypothesis there is a requirement to lose the normal allele of the gene in tumour tissue (49). Searching the literature on the best known of the tumour suppressor loci suggests, however, that in most cases the second hit is not rate limiting, so that once a predisposing mutation is present the inheritance pattern for the relevant malignancy is essentially dominant Mendelian. There are, however, specific alleles at some well known tumour suppressor loci that give rise to highly incomplete penetrance, sometimes accompanied by unusual phenotypes. In Brazil, where the incidence of paediatric adrenocortical carcinoma (ACC) is 10–15-fold higher than elsewhere, a *TP53* allele, with a recurrently arising R337H missense change, has been associated with non-Mendelian familial aggregations of ACC, but no other cancer types (50). A pH-dependent molecular mechanism has been proposed for the tissue-specific function of this relatively mild mutation (51). Other distinct, specifically ACC-associated, incompletely penetrant *TP53* mutations have also been identified (52). These families do not manifest the multiple cancer types generally associated with the broader *TP53*-associated Li-Fraumeni syndrome, where most common mutations lie in the DNA-binding domain (53). Splice donor site mutations in *RB1*, leading to an in-frame deletion of exon 13, have been described in two families with low-penetrance presentation of mainly unilateral retinoblastoma, which in one family was associated with later onset lipomas (54). A number of other reduced penetrance *RB1* mutations have been reported (55,56).

Hereditary non-polyposis colon cancers (HNPCC) associated with DNA repair enzyme mutations in *MLH1* and *MLH2* are quite highly penetrant in males (80%), but less so in females (40%) (57), where oestrogen is thought to play a protective role. Some specific low-penetrance alleles of *MLH1* have been reported, including the D132H missense variant which is found at fairly high frequency in Jewish populations (58). This allele is associated with late onset tumours (average age 70 years), and it is thought to act through a dominant negative mechanism, with no loss of heterozygosity and no microsatellite instability (58). *MSH6* mutations with reduced (~58%) penetrance have been identified in a cohort of HNPCC-spectrum cases which were not initially selected for familial occurrence (59).

KNOWN OR UNDEFINED ENVIRONMENTAL TRIGGER REQUIRED FOR EMERGENCE OF THE PHENOTYPE

Infection-dependent inflammatory triggers have long been suggested to be required in a number of diseases where a significant genetic component has been demonstrated, for example through twin studies, and familial recurrence is clearly observed, but non-Mendelian. In Type 1 juvenile-onset insulin dependent diabetes mellitus (IDDM), heterozygosity for HLA class 2 alleles *DR3/DR4* (old nomenclature) was established as the strongest risk factor many years ago. Although the molecular and biological mechanisms are not understood, aberrant antigen presentation in viral infection has been suggested. The genotype at a VNTR site closely linked to the insulin gene, has additional modifier effect on the probability of developing anti-islet antibodies by the age of 4 years (60,61). Recent work on inflammatory bowel disease, which again shows familial clustering but no clear inheritance pattern, has successfully identified a gene initially named *NOD2*, now *CARD15*, which carries fairly common variants (predominantly two missense changes and a frame shift) that are strongly associated with the development of Crohn's disease and ulcerative colitis. The relative risk of developing these phenotypes is only 2-fold for heterozygotes but 30–40-fold for compound heterozygotes or homozygotes (62). The cytoplasmic CARD15 protein is an intracellular sensor of bacterial peptidoglycan and plays a role in the response to bacterial antigens (63). Recently, using linkage studies with large numbers of affected sib pairs, two further IBD-associated loci were identified, one encoding adjacent cation transporters, *SLC22A4* and *SLC22A5*, which interact with CARD15 (64), the other an intracellular scaffold protein, *DLG5*, implicated in maintaining cell shape and polarity (65). Immune responsiveness to bacterial antigens is also implicated in several spondyloarthropathies, like ankylosing spondylitis, which are strongly associated with the presence of the *HLAB27* genotype at the major histocompatibility locus of affected individuals (66). Acute intermittent porphyria is a low-penetrance disease, with variants in the haeme biosynthetic enzyme hydroxymethylbilane synthase (*HMBS*), with several different predisposing alleles, some arising repeatedly. The 10–20% of individuals with reduced enzyme activity develop intermittent attacks of sometimes fatal neurovisceral dysfunction, precipitated by drugs, alcohol, starvation and stress (67).

Several ion-channel genes are implicated in the aetiology of Long QT (LQT) syndrome, involving cardiac pacemaking anomalies, associated with episodes of bradycardia, tachycardia, fainting attacks (syncope) and sudden death (68). Many mutation carriers are asymptomatic, sometimes with a phenotype detectable only by electrocardiogram (69–71). Identifying carriers by molecular analysis can save lives through appropriate drug treatment or provision of a cardiac pacemaker. Psychological and physical stress are among the triggers for the most severe symptoms, and different implicated genes may be associated with predominantly different episodic triggers (72). Hypertrophic cardiomyopathy mutations, also frequently associated with sudden death, are similarly quite often present in asymptomatic, but at risk

carriers (73,74). Other channelopathies with incomplete penetrance include some epilepsies (75,76).

SYNDROMES WITH SOME INCOMPLETELY PENETRANT COMPONENTS

Some mutations lead to complex phenotypes with multiple components some of which are fully penetrant, for example deafness associated with *GATA3* loss-of-function mutations, whereas other aspects such as overt parathyroid disease is observed only in some mutation carriers, although measurable hypocalcaemia is often present, and cryptic renal abnormalities may also be found (77). As many different deafness genes are known, it is only when the rare phenotypic component is observed in a family the correct causative gene will be considered. Another recently reported example describes the identification of an X-linked synapsin (*SYN1*) mutation in a family with epilepsy where some of the affected males have normal intelligence, whereas others have various combinations of epilepsy, learning difficulty, macrocephaly and aggressive behaviour (78). The authors originally debated whether a single gene disorder could account for the phenotypic spectrum in this family.

In some extreme cases, the phenotypic disease signs can be so mild and variable that diagnosis of affected status is sometimes only made following careful examination of defined mutation carriers, if at all. Such situations have been reported for holoprosencephaly (33), and tuberous sclerosis, where *TSC1* mutations may lead to very mild phenotypes in some cases, whereas *TSC2* is generally more severe (6). A specific *SHH* mutation, associated in several families solely with a single central incisor, has been identified (79). Incomplete penetrance is seen in families with capillary malformation, where *RASA1* mutations have been identified, and each family has at least one individual with ateriovenous malformation, ateriovenous fistula or Parkes Weber syndrome, although some obligate carriers show only mild, commonly observed cutaneous anomalies (80).

IMPRINTING DISEASES

Imprinting anomalies, such as Beckwith–Wiedemann syndrome (BWS) and Prader Willi/Angelman syndrome (PW/AS) generally arise sporadically. However, where familial recurrence is seen, the inheritance pattern is not Mendelian. Any heritable mutations will reveal effects only when inherited from the appropriate parent. Duplications including the *IGF2* gene, for example, only lead to overgrowth and other BWS-related anomalies, when inherited paternally. Loss of imprinting is generally sporadic, although there are suggestions from animal models that the parental imprints may be incompletely erased in the germ line (81) and epigenetic changes can be heritable (82), but patterns of inheritance will not be fully Mendelian. Biallelic *IGF2* and *H19* expression is frequently seen in some human tissues, without phenotypic abnormality, and it has been recently proposed that imprinting status may be modulated by environmental and nutritional factors (83). The relative frequency of the different types of BWS 'mutations' is described by Weksberg *et al.* (84). A novel genetic mechanism for BWS is also presented showing the occurrence of excess discordant female monozygotic twins, in whom the body tissues, but not the shared haemopoietic system, of the affected twin shows altered methylation of the maternal *KvDMR1*, in the upstream CpG island region of *KCNQ1OT1*, and biallelic expression of the gene itself. For PW/AS, both heritable deletions and epigenetic alterations were also revealed in an analysis of 136 cases (85). Diseases associated with other imprinted regions, such as transient neonatal diabetes on chromosome 6q24, also reveal different genetic origins: paternal uniparental disomy, paternally inherited duplications and methylation defects in a CpG island imprinting region (86), none of which show Mendelian inheritance as imprinting and epigenetic modifications are involved.

PARADOXICAL INHERITANCE PATTERNS

Interesting rare cases of unusual segregation patterns are seen in some specific diseases: for example in one large consanguineous kindred in eastern Quebec, cases of glaucoma involving the K423E allele of *TIGR* (trabecular meshwork-inducible glucocorticoid response) gene, are only found in heterozygotes (87). Presumably the homozygotes are able to form functional dimers, leaving them unaffected. A slightly similar situation was recently reported when the underlying defect in craniofrontonasal syndrome was reported (88,89). This X-linked phenotype was shown to be caused by mutations in ephrin B1 (*EFNB1*), a transmembrane ligand for the ephrin receptor tyrosine kinases. Heterozygous females are more severely affected than hemizygous mutant males. It is suggested that this ligand–receptor system plays a role in establishing tissue-boundaries, and the abnormalities are more severe in females with random X-inactivation where mutant and wild-type ligand-bearing tissues abut each other. The mouse *Efnb1* knock-out model recapitulates the greater female severity (89).

SEGREGATION DISTORTION

Repeated finding of an excess of affected offspring, significantly over the expected 50%, from an autosomal dominant disease phenotype is the rare, but interesting finding in the case of chromosome 10q24-linked split-hand/split-foot malformation (*SHFM3*) (90). There is an excess of affected sons from affected fathers, but the number of affected daughters from these fathers is also increased. An implicated gene has been tentatively identified through repeated disruption/partial duplication of the dactylin gene in seven independent families (91). The molecular mechanism of the mutation is not clear, but together with similar physical gene disruptions identified in mouse models (92), the suggestion of long-range control disruption should be considered.

ANTICIPATION DISTORTS SEGREGATION PATTERN

Classically, anticipation through sudden trinucleotide repeat expansion can increase the severity, and therefore the apparent penetrance, of a disease in a family. The archetypal examples

Table 1. Mechanisms of non-Mendelian inheritance patterns with examples

Inheritance pattern	Mechanism	Examples
1. Apparently sporadic occurrence, no vertical transmission	Recurrent *de novo* mutation, unable to reproduce	Apert syndrome *FGFR2* (21,22), anophthalmia *SOX2* (23), Cornelia de Lange *NIPBL* (24,25), congenital central hypoventilation *PHOX2B* (26,27)
2. Non-Mendelian patterns of inheritance incomplete penetrance	a. Identified gene–gene interactions required: incompletely penetrant digenic/oligogenic disease	Hirschsprung disease (28,29), holoprosencephaly (30–38), haemochromatosis (39–44)
	b. Interaction with identified alleles/variants at same locus	Retinitis pigmentosa (*PRPF31* mutn) (45), erythropoietic porphyria *FECH* (46), congenital absence of vas deferens *CFTR* (9,47,48)
	c. Reduced penetrance alleles at tumour suppressor loci, requiring second hit	Selected mutations in: *TP53* (50–53), *RB1* (54–56); mismatch repair genes: *MLH1* (57,58), *MSH2* (57), *MSH6* (59)
	d. Environmental trigger required: infections, chemical/drug-induced triggers, stress	Type 1 diabetes (60,61), inflammatory bowel disease (62–65); spondyloarthropathies (66), acute intermittent porphyria *HMBS* (67); LQT syndrome (68–72); hypertrophic cardiomyopathies (73,74), epilepsies (75,76)
3. Complex multi-component phenotypes	Some fully penetrant, others incompletely-confusing segregation patterns	Deafness and hypoparathyroidism *GATA3* (77), epilepsy, aggressive behaviour *SYN1* (78)
4. Variable expressivity	Variable expressivity so extreme that affected status may be missed	*TSC1* (6), HPE (33,79), *RASA1* (80)
5. Imprinting diseases	a. Rare inherited variants: only give rise to phenotype when inherited from the appropriate parent; alteration in epigenetic organization	Beckwith Wiedemann *BWS* (81–84), Prader Willi and Angelman *PWS/AS* (85), transient neonatal diabetes *TND* (86)
	b. Frequent occurrence of discordant monozygotic twins	*BWS* (84)
6. Paradoxical inheritance	Heterozygotes most severely affected	Myocilin glaucoma *TIGR* (87), craniofrontonasal syndrome *EFNB1* (88,89)
7. Excess affected cases	Segregation distortion	Split-hand split-foot malformation *SHFM3* (90–92)
8. Anticipation through generations	Triplet repeat expansion; telomere shortening	Myotonic dystrophy *DM* (93), X-linked mental retardation *FMRP* (94), spinocerebellar ataxia *SCA8* (95), dyskeratosis congenita *TERC* (96)
9. Non-Mendelian segregation of telomeric microsatellites	Inheritance patterns of cryptic telomeric alleles	Idiopathic mental retardation (97)
10. Mitochondrial disease	Cytoplasmic maternal inheritance, heteroplasmy leads to unpredictable severity and tissue involvement	Multiple anomalies caused by mitochondrial mutations leading to: muscle, heart, kidney, retinal disease and diabetes (98–100)

of intergenerational instability that give rise to unusual patterns of inheritance, are fragile X syndrome, affecting the *FMRP* gene and myotonic dystrophy (DM). The underlying pathological mechanisms are still debated but are probably distinct, although both may ultimately function through RNA-level control (93,94). Recently, an untranslated CTG expansion was shown to be involved in spinocerebellar ataxia 8 (*SCA8*), which is a neurodegenerative disease generally with very low penetrance, although a more completely penetrant family was also described (95), and the molecular mechanism has not been clarified. Anticipation is also reported in dyskeratosis congenita where the underlying mutation is in the RNA component of telomerase (*TERC*), and the increasing disease severity through the generations is imposed through the co-inheritance of the *TERC* mutation with the progressively shortened telomere length (96).

OTHER MECHANISMS

Non-Mendelian segregation of polymorphic microsatellite markers for the telomeric regions of multiple chromosomes, can be used to flag up possible cryptic telomeric rearrangements, associated with idiopathic mental retardation (97). The major original non-Mendelian segregation patterns were produced by mitochondrially inherited disease mechanisms. These, extensively reviewed diseases affecting many high energy-consuming tissues, such as retina, heart, kidney and muscle, show maternal inheritance, as classically only oocytes contain mitochondria (98–100).

CONCLUDING REMARKS

We have touched upon several distinct mechanisms implicated in non-Mendelian inheritance patterns in human disease. Each case covered has at least one identified gene aetiologically implicated, allowing discussion of at least some aspects of the underlying molecular mechanism. Many more examples are known with no underlying gene or pathway defined. The cases discussed are summarized in Table 1. Some general themes to pull together these apparently diverse mechanisms are presented in Figure 1. Oligogenic diseases with no regular recognizable segregation patterns are emerging increasingly, where phenotypes owing to specific mutations at one or a few loci are modified through a number of cellular and environmental mechanisms. Distinct processes participating in general cellular metabolism play key roles: cell cycle and proliferation control (101), transcriptional machinery (102) and splicing control (103) are emerging as major modifying

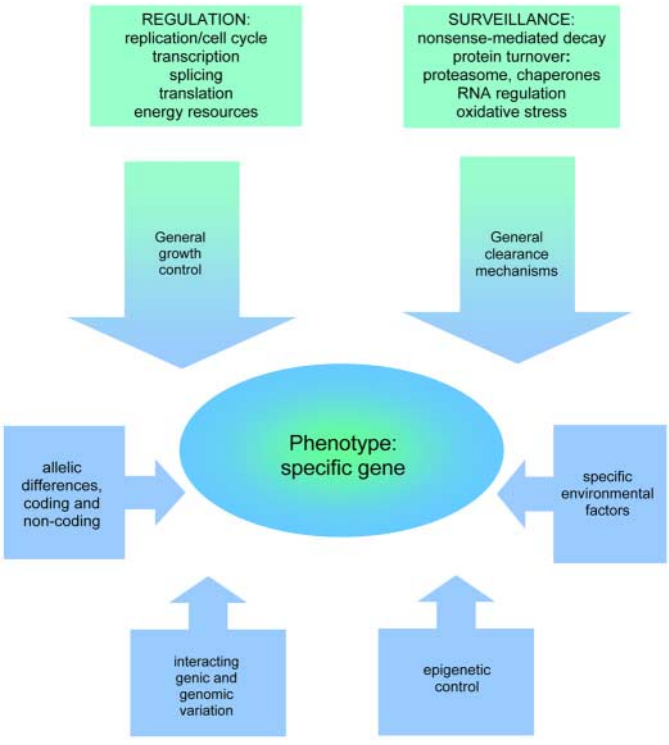

Figure 1. Factors which can influence the phenotypic outcome of a particular mutation. Genetic, epigenetic and environmental components can play a role in modifying the outcome of specific mutants at a defined locus. Variation in normal regulated cellular processes, and in the surveillance systems, contribute to the end phenotype.

mechanisms, translational regulation (104) and the machinery of energy management through oxidative control are also important players (105). In addition, surveillance mechanisms have been imposed throughout evolution, so that mutant proteins and aberrant RNAs are dealt with through defined pathways: prematurely truncated proteins are subject to nonsense-mediated decay if produced from a multi-exonic gene (106); protein turnover is regulated by the components of the proteasome pathway and ubiquitinylation (107); aberrantly folded proteins are carefully chaperoned by the stress-response system (108); a major new area is our growing insight into the many different levels of RNA-mediated regulation of gene expression (17); and pathways controlling the damaging effects of oxidative stress and the generation of free radicals are emerging as key mechanisms in aging and decay (109). Naturally, all these systems are plastic and subject to genetic and environmental variation. It is becoming clear that there are many allelic variants throughout the genome, and these are not just coding variants, but differences in transcriptional control through promoter and enhancer differences are increasingly emerging (12,110). Some *cis*-regulatory elements act at great genomic distance and can be pinpointed through evolutionary sequence conservation across mammals or broader vertebrate classes (111,112). This approach may ultimately be useful for understanding mechanisms of regulatory variation. Gene expression is clearly subject to complex epigenetic control (113), and we are just beginning to understand the rules involved in the modulation of chromatin structure (114). Finally, environmental variation plays a major role in modulating all aspects of gene expression (115), but individual responses to environmental factors are under partial genetic control. Each mutation seen in any individual is unique in its physiological, genomic and environmental and spatiotemporal context, so that ultimately pure Mendelian inheritance does not exist. There is a continuum between 'single gene' disorders and oligo- and multigenic regulation, with the constant superimposition of environmental factors, whose effect may be modulated through stress response pathways (116,117). The study of molecular mechanisms in non-Mendelian genetic disease, with some insight into the pathways affected, provides a useful bridge from single gene anomalies to complex disease.

ACKNOWLEDGEMENTS

The authors thank Dian Donnai and Andrew Wilkie for helpful advice and discussion on some of the examples used.

REFERENCES

1. Nussbaum, R.L., McInnes, R.R. and Willard, H.F. (2001) *Genetics in Medicine*. W.B. Saunders Company, Philadelphia.
2. Kajiwara, K., Berson, E.L. and Dryja, T.P. (1994) Digenic retinitis pigmentosa due to mutations at the unlinked peripherin/RDS and ROM1 loci. *Science*, **264**, 1604–1608.
3. Pallares-Ruiz, N., Blanchet, P., Mondain, M., Claustres, M. and Roux, A.F. (2002) A large deletion including most of GJB6 in recessive non syndromic deafness: a digenic effect? *Eur. J. Hum. Genet.*, **10**, 72–76.
4. Savage, D.B., Agostini, M., Barroso, I., Gurnell, M., Luan, J., Meirhaeghe, A., Harding, A.H., Ihrke, G., Rajanayagam, O., Soos, M.A. *et al.* (2002) Digenic inheritance of severe insulin resistance in a human pedigree. *Nat. Genet.*, **31**, 379–384.
5. Nadeau, J.H. (2001) Modifier genes in mice and humans. *Nat. Rev. Genet.*, **2**, 165–174.
6. Langkau, N., Martin, N., Brandt, R., Zugge, K., Quast, S., Wiegele, G., Jauch, A., Rehm, M., Kuhl, A., Mack-Vetter, M. *et al.* (2002) *TSC1* and *TSC2* mutations in tuberous sclerosis, the associated phenotypes and a model to explain observed *TSC1*/*TSC2* frequency ratios. *Eur. J. Pediatr.*, **161**, 393–402.
7. Dipple, K.M. and McCabe, E.R. (2000) Phenotypes of patients with 'simple' Mendelian disorders are complex traits: thresholds, modifiers, and systems dynamics. *Am. J. Hum. Genet.*, **66**, 1729–1735.
8. Badano, J.L. and Katsanis, N. (2002) Beyond mendel: an evolving view of human genetic disease transmission. *Nat. Rev. Genet.*, **3**, 779–789.
9. Jezequel, P., Dubourg, C., Le Lannou, D., Odent, S., Le Gall, J.Y., Blayau, M., Le Treut, A. and David, V. (2000) Molecular screening of the *CFTR* gene in men with anomalies of the vas deferens: identification of three novel mutations. *Mol. Hum. Reprod.*, **6**, 1063–1067.
10. Caspi, M., Coquelle, F.M., Koifman, C., Levy, T., Arai, H., Aoki, J., De Mey, J.R. and Reiner, O. (2003) *LIS1* missense mutations: variable phenotypes result from unpredictable alterations in biochemical and cellular properties. *J. Biol. Chem.*, **278**, 38740–38748.
11. Cowles, C.R., Hirschhorn, J.N., Altshuler, D. and Lander, E.S. (2002) Detection of regulatory variation in mouse genes. *Nat. Genet.*, **32**, 432–437.
12. Pastinen, T., Sladek, R., Gurd, S., Sammak, A., Ge, B., Lepage, P., Lavergne, K., Villeneuve, A., Gaudin, T., Brandstrom, H. *et al.* (2004) A survey of genetic and epigenetic variation affecting human gene expression. *Physiol. Genomics*, **16**, 184–193.
13. Proudfoot, N.J., Furger, A. and Dye, M.J. (2002) Integrating mRNA processing with transcription. *Cell*, **108**, 501–512.
14. Zimber, A., Nguyen, Q.D. and Gespach, C. (2004) Nuclear bodies and compartments: functional roles and cellular signalling in health and disease. *Cell Signal.*, **16**, 1085–1104.

15. Weissman, A.M. (2001) Themes and variations on ubiquitylation. *Nat. Rev. Mol. Cell Biol.*, **2**, 169–178.
16. Lehner, B., Sanderson, C.M. (2004) A protein interaction framework for human mRNA degradation. *Genome Res.*, **14**, 1315–1323.
17. Mattick, J.S. (2004) RNA regulation: a new genetics? *Nat. Rev. Genet.*, **5**, 316–323.
18. Young, J.C., Moarefi, I. and Hartl, F.U. (2001) Hsp90: a specialized but essential protein-folding tool. *J. Cell Biol.*, **154**, 267–273.
19. Rutherford, S.L. and Lindquist, S. (1998) Hsp90 as a capacitor for morphological evolution. *Nature*, **396**, 336–342.
20. Csermely, P. (2001) Chaperone overload is a possible contributor to 'civilization diseases'. *Trends Genet.*, **17**, 701–704.
21. Goriely, A., McVean, G.A., Rojmyr, M., Ingemarsson, B. and Wilkie, A.O. (2003) Evidence for selective advantage of pathogenic *FGFR2* mutations in the male germ line. *Science*, **301**, 643–646.
22. Wilkie, A.O., Slaney, S.F., Oldridge, M., Poole, M.D., Ashworth, G.J., Hockley, A.D., Hayward, R.D., David, D.J., Pulleyn, L.J., Rutland, P. *et al.* (1995) Apert syndrome results from localized mutations of *FGFR2* and is allelic with Crouzon syndrome. *Nat. Genet.*, **9**, 165–172.
23. Fantes, J., Ragge, N.K., Lynch, S.A., McGill, N.I., Collin, J.R., Howard-Peebles, P.N., Hayward, C., Vivian, A.J., Williamson, K., van Heyningen, V. and FitzPatrick, D.R. (2003) Mutations in *SOX2* cause anophthalmia. *Nat. Genet.*, **33**, 461–463.
24. Krantz, I.D., McCallum, J., DeScipio, C., Kaur, M., Gillis, L.A., Yaeger, D., Jukofsky, L., Wasserman, N., Bottani, A., Morris, C.A. *et al.* (2004) Cornelia de Lange syndrome is caused by mutations in NIPBL, the human homolog of *Drosophila melanogaster* Nipped-B. *Nat. Genet.*, **36**, 631–635.
25. Tonkin, E.T., Wang, T.J., Lisgo, S., Bamshad, M.J. and Strachan, T. (2004) *NIPBL*, encoding a homolog of fungal Scc2-type sister chromatid cohesion proteins and fly Nipped-B, is mutated in Cornelia de Lange syndrome. *Nat. Genet.*, **36**, 636–641.
26. Amiel, J., Laudier, B., Attie-Bitach, T., Trang, H., de Pontual, L., Gener, B., Trochet, D., Etchevers, H., Ray, P., Simonneau, M. *et al.* (2003) Polyalanine expansion and frameshift mutations of the paired-like homeobox gene *PHOX2B* in congenital central hypoventilation syndrome. *Nat. Genet.*, **33**, 459–461.
27. Matera, I., Bachetti, T., Puppo, F., Di Duca, M., Morandi, F., Casiraghi, G.M., Cilio, M.R., Hennekam, R., Hofstra, R., Schober, J.G. *et al.* (2004) *PHOX2B* mutations and polyalanine expansions correlate with the severity of the respiratory phenotype and associated symptoms in both congenital and late onset Central Hypoventilation syndrome. *J. Med. Genet.*, **41**, 373–380.
28. Chakravarti, A. and Lyonnet, S. (2001) *The Metabolic and Molecular Bases of Inherited Disease*. McGraw-Hill, New York.
29. Gabriel, S.B., Salomon, R., Pelet, A., Angrist, M., Amiel, J., Fornage, M., Attie-Bitach, T., Olson, J.M., Hofstra, R., Buys, C. *et al.* (2002) Segregation at three loci explains familial and population risk in Hirschsprung disease. *Nat. Genet.*, **31**, 89–93.
30. Muenke, M. and Beachy, P.A. (2001) *The Metabolic and Molecular Bases of Inherited Disease*. McGraw-Hill, New York.
31. Ming, J.E. and Muenke, M. (2002) Multiple hits during early embryonic development: digenic diseases and holoprosencephaly. *Am. J. Hum. Genet.*, **71**, 1017–1032.
32. Dubourg, C., Lazaro, L., Pasquier, L., Bendavid, C., Blayau, M., Le Duff, F., Durou, M.R., Odent, S. and David, V. (2004) Molecular screening of *SHH*, *ZIC2*, *SIX3*, and *TGIF* genes in patients with features of holoprosencephaly spectrum: Mutation review and genotype–phenotype correlations. *Hum. Mutat.*, **24**, 43–51.
33. Nanni, L., Ming, J.E., Bocian, M., Steinhaus, K., Bianchi, D.W., Die-Smulders, C., Giannotti, A., Imaizumi, K., Jones, K.L., Campo, M.D. *et al.* (1999) The mutational spectrum of the sonic hedgehog gene in holoprosencephaly: SHH mutations cause a significant proportion of autosomal dominant holoprosencephaly. *Hum. Mol. Genet.*, **8**, 2479–2488.
34. Belloni, E., Muenke, M., Roessler, E., Traverso, G., Siegel-Bartelt, J., Frumkin, A., Mitchell, H.F., Donis-Keller, H., Helms, C., Hing, A.V. *et al.* (1996) Identification of *Sonic hedgehog* as a candidate gene responsible for holoprosencephaly. *Nat. Genet.*, **14**, 353–356.
35. Boffelli, D., Nobrega, M.A. and Rubin, E.M. (2004) Comparative genomics at the vertebrate extremes. *Nat. Rev. Genet.*, **5**, 456–465.
36. Edison, R.J. and Muenke, M. (2004) Central nervous system and limb anomalies in case reports of first-trimester statin exposure. *N. Engl. J. Med.*, **350**, 1579–1582.
37. Shim, Y.H., Bae, S.H., Kim, J.H., Kim, K.R., Kim, C.J. and Paik, Y.K. (2004) A novel mutation of the human 7-dehydrocholesterol reductase gene reduces enzyme activity in patients with holoprosencephaly. *Biochem. Biophys. Res. Commun.*, **315**, 219–223.
38. Roux, C., Wolf, C., Mulliez, N., Gaoua, W., Cormier, V., Chevy, F. and Citadelle, D. (2000) Role of cholesterol in embryonic development. *Am. J. Clin. Nutr.*, **71**, 1270S–1279S.
39. Merryweather-Clarke, A.T., Cadet, E., Bomford, A., Capron, D., Viprakasit, V., Miller, A., McHugh, P.J., Chapman, R.W., Pointon, J.J., Wimhurst, V.L. *et al.* (2003) Digenic inheritance of mutations in *HAMP* and *HFE* results in different types of haemochromatosis. *Hum. Mol. Genet.*, **12**, 2241–2247.
40. Lanzara, C., Roetto, A., Daraio, F., Rivard, S., Ficarella, R., Simard, H., Cox, T.M., Cazzola, M., Piperno, A., Gimenez-Roqueplo, A.P. *et al.* (2004) Spectrum of hemojuvelin gene mutations in 1q-linked juvenile hemochromatosis. *Blood*, **103**, 4317–4321.
41. Lee, P.L., Beutler, E., Rao, S.V. and Barton, J.C. (2004) Genetic abnormalities and juvenile hemochromatosis: mutations of the *HJV* gene encoding hemojuvelin. *Blood*, **103**, 4669–4671.
42. Delatycki, M.B., Allen, K.J., Gow, P., MacFarlane, J., Radomski, C., Thompson, J., Hayden, M.R., Goldberg, Y.P. and Samuels, M.E. (2004) A homozygous *HAMP* mutation in a multiply consanguineous family with pseudo-dominant juvenile hemochromatosis. *Clin. Genet.*, **65**, 378–383.
43. Matthes, T., Aguilar-Martinez, P., Pizzi-Bosman, L., Darbellay, R., Rubbia-Brandt, L., Giostra, E., Michel, M., Ganz, T. and Beris, P. (2004) Severe hemochromatosis in a Portuguese family associated with a new mutation in the 5′UTR of the *HAMP* gene. *Blood*, in press.
44. Bridle, K.R., Frazer, D.M., Wilkins, S.J., Dixon, J.L., Purdie, D.M., Crawford, D.H., Subramaniam, V.N., Powell, L.W., Anderson, G.J. and Ramm, G.A. (2003) Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homoeostasis. *Lancet*, **361**, 669–673.
45. Vithana, E.N., Abu-Safieh, L., Pelosini, L., Winchester, E., Hornan, D., Bird, A.C., Hunt, D.M., Bustin, S.A. and Bhattacharya, S.S. (2003) Expression of *PRPF31* mRNA in patients with autosomal dominant retinitis pigmentosa: a molecular clue for incomplete penetrance? *Invest. Ophthalmol. Vis. Sci.*, **44**, 4204–4209.
46. Gouya, L., Puy, H., Robreau, A.M., Bourgeois, M., Lamoril, J., Da, S., V, Grandchamp, B. and Deybach, J.C. (2002) The penetrance of dominant erythropoietic protoporphyria is modulated by expression of wildtype *FECH*. *Nat. Genet.*, **30**, 27–28.
47. Dohle, G.R., Veeze, H.J., Overbeek, S.E., van den Ouweland, A.M., Halley, D.J., Weber, R.F. and Niermeijer, M.F. (1999) The complex relationships between cystic fibrosis and congenital bilateral absence of the vas deferens: clinical, electrophysiological and genetic data. *Hum. Reprod.*, **14**, 371–374.
48. Dohle, G.R., Halley, D.J., Van Hemel, J.O., van den Ouwel, A.M., Pieters, M.H., Weber, R.F. and Govaerts, L.C. (2002) Genetic risk factors in infertile men with severe oligozoospermia and azoospermia. *Hum. Reprod.*, **17**, 13–16.
49. Knudson, A.G., Jr (1989) The ninth gordon hamilton–fairley memorial lecture. Hereditary cancers: clues to mechanisms of carcinogenesis. *Br. J. Cancer*, **59**, 661–666.
50. Ribeiro, R.C., Sandrini, F., Figueiredo, B., Zambetti, G.P., Michalkiewicz, E., Lafferty, A.R., DeLacerda, L., Rabin, M., Cadwell, C., Sampaio, G. *et al.* (2001) An inherited p53 mutation that contributes in a tissue-specific manner to pediatric adrenal cortical carcinoma. *Proc. Natl Acad. Sci. USA*, **98**, 9330–9335.
51. DiGiammarino, E.L., Lee, A.S., Cadwell, C., Zhang, W., Bothner, B., Ribeiro, R.C., Zambetti, G. and Kriwacki, R.W. (2002) A novel mechanism of tumorigenesis involving pH-dependent destabilization of a mutant p53 tetramer. *Nat. Struct. Biol.*, **9**, 12–16.
52. Varley, J.M., McGown, G., Thorncroft, M., James, L.A., Margison, G.P., Forster, G., Evans, D.G., Harris, M., Kelsey, A.M. and Birch, J.M. (1999) Are there low-penetrance TP53 alleles? evidence from childhood adrenocortical tumors. *Am. J. Hum. Genet.*, **65**, 995–1006.
53. Varley, J.M. (2003) Germline TP53 mutations and Li-Fraumeni syndrome. *Hum. Mutat.*, **21**, 313–320.

54. Genuardi, M., Klutz, M., Devriendt, K., Caruso, D., Stirpe, M. and Lohmann, D.R. (2001) Multiple lipomas linked to an *RB1* gene mutation in a large pedigree with low penetrance retinoblastoma. *Eur. J. Hum. Genet.*, **9**, 690–694.
55. Klutz, M., Horsthemke, B. and Lohmann, D.R. (1999) *RB1* gene mutations in peripheral blood DNA of patients with isolated unilateral retinoblastoma. *Am. J. Hum. Genet.*, **64**, 667–668.
56. Lohmann, D.R., Gerick, M., Brandt, B., Oelschlager, U., Lorenz, B., Passarge, E. and Horsthemke, B. (1997) Constitutional *RB1*-gene mutations in patients with isolated unilateral retinoblastoma. *Am. J. Hum. Genet.*, **61**, 282–294.
57. Mitchell, R.J., Farrington, S.M., Dunlop, M.G. and Campbell, H. (2002) Mismatch repair genes *hMLH1* and *hMSH2* and colorectal cancer: a HuGE review. *Am. J. Epidemiol.*, **156**, 885–902.
58. Lipkin, S.M., Rozek, L.S., Rennert, G., Yang, W., Chen, P.C., Hacia, J., Hunt, N., Shin, B., Fodor, S., Kokoris, M. *et al.* (2004) The *MLH1 D132H* variant is associated with susceptibility to sporadic colorectal cancer. *Nat. Genet.*, **36**, 694–699.
59. Buttin, B.M., Powell, M.A., Mutch, D.G., Babb, S.A., Huettner, P.C., Edmonston, T.B., Herzog, T.J., Rader, J.S., Gibb, R.K., Whelan, A.J. and Goodfellow, P.J. (2004) Penetrance and expressivity of *MSH6* germline mutations in seven kindreds not ascertained by family history. *Am. J. Hum. Genet.*, **74**, 1262–1269.
60. Todd, J.A. and Farrall, M. (1996) Panning for gold: genome-wide scanning for linkage in type 1 diabetes. *Hum. Mol. Genet.*, **5**, 1443–1448.
61. Walter, M., Albert, E., Conrad, M., Keller, E., Hummel, M., Ferber, K., Barratt, B.J., Todd, J.A., Ziegler, A.G. and Bonifacio, E. (2003) IDDM2/insulin VNTR modifies risk conferred by IDDM1/HLA for development of Type 1 diabetes and associated autoimmunity. *Diabetologia*, **46**, 712–720.
62. Russell, R.K., Nimmo, E.R. and Satsangi, J. (2004) Molecular genetics of Crohn's disease. *Curr. Opin. Genet. Dev.*, **14**, 264–270.
63. Girardin, S.E., Hugot, J.P. and Sansonetti, P.J. (2003) Lessons from Nod2 studies: towards a link between Crohn's disease and bacterial sensing. *Trends Immunol.*, **24**, 652–658.
64. Peltekova, V.D., Wintle, R.F., Rubin, L.A., Amos, C.I., Huang, Q., Gu, X., Newman, B., Van Oene, M., Cescon, D., Greenberg, G. *et al.* (2004) Functional variants of OCTN cation transporter genes are associated with Crohn disease. *Nat. Genet.*, **36**, 471–475.
65. Stoll, M., Corneliussen, B., Costello, C.M., Waetzig, G.H., Mellgard, B., Koch, W.A., Rosenstiel, P., Albrecht, M., Croucher, P.J., Seegert, D. *et al.* (2004) Genetic variation in DLG5 is associated with inflammatory bowel disease. *Nat. Genet.*, **36**, 476–480.
66. Reveille, J.D. (2004) The genetic basis of spondyloarthritis. *Curr. Rheumatol. Rep.*, **6**, 117–125.
67. von Brasch, L., Zang, C., Haverkamp, T., Schlechte, H., Heckers, H. and Petrides, P.E. (2004) Molecular analysis of acute intermittent porphyria: mutation screening in 20 patients in Germany reveals 11 novel mutations. *Blood Cells Mol. Dis.*, **32**, 309–314.
68. Keating, M.T. and Sanguinetti, M.C. (2001) Molecular and cellular mechanisms of cardiac arrhythmias. *Cell*, **104**, 569–580.
69. Berthet, M., Denjoy, I., Donger, C., Demay, L., Hammoude, H., Klug, D., Schulze-Bahr, E., Richard, P., Funke, H., Schwartz, K. *et al.* (1999) C-terminal HERG mutations: the role of hypokalemia and a KCNQ1-associated mutation in cardiac event occurrence. *Circulation*, **99**, 1464–1470.
70. Huang, L., Bitner-Glindzicz, M., Tranebjaerg, L. and Tinker, A. (2001) A spectrum of functional effects for disease causing mutations in the Jervell and Lange–Nielsen syndrome. *Cardiovasc. Res.*, **51**, 670–680.
71. Benson, D.W., Wang, D.W., Dyment, M., Knilans, T.K., Fish, F.A., Strieper, M.J., Rhodes, T.H. and George, A.L. Jr (2003) Congenital sick sinus syndrome caused by recessive mutations in the cardiac sodium channel gene (*SCN5A*). *J. Clin. Invest.*, **112**, 1019–1028.
72. Schwartz, P.J., Priori, S.G., Spazzolini, C., Moss, A.J., Vincent, G.M., Napolitano, C., Denjoy, I., Guicheney, P., Breithardt, G., Keating, M.T. *et al.* (2001) Genotype–phenotype correlation in the long-QT syndrome: gene-specific triggers for life–threatening arrhythmias. *Circulation*, **103**, 89–95.
73. Watkins, H. (1998) Genotype: phenotype correlations in hypertrophic cardiomyopathy. *Eur. Heart J.*, **19**, 10–12.
74. Seidman, J.G., Seidman, C. (2001) The genetic basis for cardiomyopathy: from mutation identification to mechanistic paradigms. *Cell*, **104**, 557–567.
75. Gourfinkel-An, I., Baulac, S., Nabbout, R., Ruberg, M., Baulac, M., Brice, A. and LeGuern, E. (2004) Monogenic idiopathic epilepsies. *Lancet Neurol.*, **3**, 209–218.
76. Steinlein, O.K. (2004) Genetic mechanisms that underlie epilepsy. *Nat. Rev. Neurosci.*, **5**, 400–408.
77. Nesbit, M.A., Bowl, M.R., Harding, B., Ali, A., Ayala, A., Crowe, C., Dobbie, A., Hampson, G., Holdaway, I., Levine, M.A. *et al.* (2004) Characterization of *GATA3* mutations in the hypoparathyroidism, deafness, and renal dysplasia (HDR) syndrome. *J. Biol. Chem.*, **279**, 22624–22634.
78. Garcia, C.C., Blair, H.J., Seager, M., Coulthard, A., Tennant, S., Buddles, M., Curtis, A. and Goodship, J.A. (2004) Identification of a mutation in synapsin I, a synaptic vesicle protein, in a family with epilepsy. *J. Med. Genet.*, **41**, 183–186.
79. Nanni, L., Ming, J.E., Du, Y., Hall, R.K., Aldred, M., Bankier, A. and Muenke, M. (2001) SHH mutation is associated with solitary median maxillary central incisor: a study of 13 patients and review of the literature. *Am. J. Med. Genet.*, **102**, 1–10.
80. Eerola, I., Boon, L.M., Mulliken, J.B., Burrows, P.E., Dompmartin, A., Watanabe, S., Vanwijck, R. and Vikkula, M. (2003) Capillary malformation–arteriovenous malformation, a new clinical and genetic disorder caused by *RASA1* mutations. *Am. J. Hum. Genet.*, **73**, 1240–1249.
81. Kearns, M., Preis, J., McDonald, M., Morris, C. and Whitelaw, E. (2000) Complex patterns of inheritance of an imprinted murine transgene suggest incomplete germline erasure. *Nucl. Acids Res.*, **28**, 3301–3309.
82. Rakyan, V.K., Blewitt, M.E., Druker, R., Preis, J.I. and Whitelaw, E. (2002) Metastable epialleles in mammals. *Trends Genet.*, **18**, 348–351.
83. Waterland, R.A. and Jirtle, R.L. (2003) Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol. Cell. Biol.*, **23**, 5293–5300.
84. Weksberg, R., Shuman, C., Caluseriu, O., Smith, A.C., Fei, Y.L., Nishikawa, J., Stockley, T.L., Best, L., Chitayat, D., Olney, A. *et al.* (2002) Discordant *KCNQ1OT1* imprinting in sets of monozygotic twins discordant for Beckwith–Wiedemann syndrome. *Hum. Mol. Genet.*, **11**, 1317–1325.
85. Buiting, K., Gross, S., Lich, C., Gillessen-Kaesbach, G., el Maarri, O. and Horsthemke, B. (2003) Epimutations in Prader–Willi and Angelman syndromes: a molecular study of 136 patients with an imprinting defect. *Am. J. Hum. Genet.*, **72**, 571–577.
86. Temple, I.K. and Shield, J.P. (2002) Transient neonatal diabetes, a disorder of imprinting. *J. Med. Genet.*, **39**, 872–875.
87. Morissette, J., Clepet, C., Moisan, S., Dubois, S., Winstall, E., Vermeeren, D., Nguyen, T.D., Polansky, J.R., Cote, G., Anctil, J.L. *et al.* (1998) Homozygotes carrying an autosomal dominant *TIGR* mutation do not manifest glaucoma [letter]. *Nat. Genet.*, **19**, 319–321.
88. Wieland, I., Jakubiczka, S., Muschke, P., Cohen, M., Thiele, H., Gerlach, K.L., Adams, R.H. and Wieacker, P. (2004) Mutations of the *ephrin-B1* gene cause craniofrontonasal syndrome. *Am. J. Hum. Genet.*, **74**, 1209–1215.
89. Twigg, S.R., Kan, R., Babbs, C., Bochukova, E.G., Robertson, S.P., Wall, S.A., Morriss-Kay, G.M. and Wilkie, A.O. (2004) Mutations of ephrin-B1 (*EFNB1*), a marker of tissue boundary formation, cause craniofrontonasal syndrome. *Proc. Natl Acad. Sci. USA*, **101**, 8652–8657.
90. Ozen, R.S., Baysal, B.E., Devlin, B., Farr, J.E., Gorry, M., Ehrlich, G.D. and Richard, C.W. (1999) Fine mapping of the split-hand/split-foot locus (*SHFM3*) at 10q24: evidence for anticipation and segregation distortion. *Am. J. Hum. Genet.*, **64**, 1646–1654.
91. de Mollerat, X.J., Gurrieri, F., Morgan, C.T., Sangiorgi, E., Everman, D.B., Gaspari, P., Amiel, J., Bamshad, M.J., Lyle, R., Blouin, J.L. *et al.* (2003) A genomic rearrangement resulting in a tandem duplication is associated with split hand–split foot malformation 3 (*SHFM3*) at 10q24. *Hum. Mol. Genet.*, **12**, 1959–1971.
92. Sidow, A., Bulotsky, M.S., Kerrebrock, A.W., Birren, B.W., Altshuler, D., Jaenisch, R., Johnson, K.R. and Lander, E.S. (1999) A novel member of the F-box/*WD40* gene family, encoding dactylin, is disrupted in the mouse dactylaplasia mutant. *Nat. Genet.*, **23**, 104–107.
93. Ranum, L.P. and Day, J.W. (2004) Myotonic dystrophy: RNA pathogenesis comes into focus. *Am. J. Hum. Genet.*, **74**, 793–804.

94. Jin, P., Zarnescu, D.C., Ceman, S., Nakamoto, M., Mowrey, J., Jongens, T.A., Nelson, D.L., Moses, K. and Warren, S.T. (2004) Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. *Nat. Neurosci.*, **7**, 113–117.
95. Ikeda, Y., Dalton, J.C., Moseley, M.L., Gardner, K.L., Bird, T.D., Ashizawa, T., Seltzer, W.K., Pandolfo, M., Milunsky, A., Potter, N.T. *et al.* (2004) Spinocerebellar ataxia type 8: molecular genetic comparisons and haplotype analysis of 37 families with ataxia. *Am. J. Hum. Genet.*, **75**, 3–16.
96. Vulliamy, T., Marrone, A., Szydlo, R., Walne, A., Mason, P.J. and Dokal, I. (2004) Disease anticipation is associated with progressive telomere shortening in families with dyskeratosis congenita due to mutations in TERC. *Nat. Genet.*, **36**, 447–449.
97. Colleaux, L., Rio, M., Heuertz, S., Moindrault, S., Turleau, C., Ozilou, C., Gosset, P., Raoult, O., Lyonnet, S., Cormier-Daire, V. *et al.* (2001) A novel automated strategy for screening cryptic telomeric rearrangements in children with idiopathic mental retardation. *Eur. J. Hum. Genet.*, **9**, 319–327.
98. Schwartz, M. and Vissing, J. (2003) New patterns of inheritance in mitochondrial disease. *Biochem. Biophys. Res. Commun.*, **310**, 247–251.
99. Cummins, J.M. (2000) Fertilization and elimination of the paternal mitochondrial genome. *Hum. Reprod.*, **15**, 92–101.
100. Chinnery, P.F. and Turnbull, D.M. (2000) Mitochondrial DNA mutations in the pathogenesis of human disease. *Mol. Med. Today*, **6**, 425–432.
101. Murray, A.W. (2004) Recycling the cell cycle: cyclins revisited. *Cell*, **116**, 221–234.
102. Taatjes, D.J., Marr, M.T. and Tjian, R. (2004) Regulatory diversity among metazoan co-activator complexes. *Nat. Rev. Mol. Cell Biol.*, **5**, 403–410.
103. Cartegni, L., Chew, S.L. and Krainer, A.R. (2002) Listening to silence and understanding nonsense: exonic mutations that affect splicing. *Nat. Rev. Genet.*, **3**, 285–298.
104. Calkhoven, C.F., Muller, C. and Leutz, A. (2002) Translational control of gene expression and disease. *Trends Mol. Med.*, **8**, 577–583.
105. Ramakrishna, R., Edwards, J.S., McCulloch, A. and Palsson, B.O. (2001) Flux-balance analysis of mitochondrial energy metabolism: consequences of systemic stoichiometric constraints. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **280**, R695–R704.
106. Maquat, L.E. (2004) Nonsense-mediated mRNA decay: splicing, translation and mRNP dynamics. *Nat. Rev. Mol. Cell Biol.*, **5**, 89–99.
107. Goldberg, A.L. (2003) Protein degradation and protection against misfolded or damaged proteins. *Nature*, **426**, 895–899.
108. Whitesell, L., Bagatell, R. and Falsey, R. (2003) The stress response: implications for the clinical development of hsp90 inhibitors. *Curr. Cancer Drug Targets*, **3**, 349–358.
109. Lane, N. (2003) A unifying view of ageing and disease: the double-agent theory. *J. Theor. Biol.*, **225**, 531–540.
110. Yan, H., Yuan, W., Velculescu, V.E., Vogelstein, B. and Kinzler, K.W. (2002) Allelic variation in human gene expression. *Science*, **297**, 1143.
111. Kleinjan, D.A., Seawright, A., Schedl, A., Quinlan, R.A., Danes, S. and van Heyningen, V. (2001) Aniridia-associated translocations, DNase hypersensitivity, sequence comparison and transgenic analysis redefine the functional domain of *PAX6*. *Hum. Mol. Genet.*, **10**, 2049–2059.
112. Lettice, L.A., Heaney, S.J., Purdie, L.A., Li, L., de Beer, P., Oostra, B.A., Goode, D., Elgar, G., Hill, R.E. and de Graaff, E. (2003) A long-range Shh enhancer regulates expression in the developing limb and fin and is associated with preaxial polydactyly. *Hum. Mol. Genet.*, **12**, 1725–1735.
113. Wolffe, A.P., Matzke, M.A. (1999) Epigenetics: regulation through repression. *Science*, **286**, 481–486.
114. Dellino, G.I., Schwartz, Y.B., Farkas, G., McCabe, D., Elgin, S.C. and Pirrotta, V. (2004) Polycomb silencing blocks transcription initiation. *Mol. Cell*, **13**, 887–893.
115. Mone, S.M., Gillman, M.W., Miller, T.L., Herman, E.H. and Lipshultz, S.E. (2004) Effects of environmental exposures on the cardiovascular system: prenatal period through adolescence. *Pediatrics*, **113**, 1058–1069.
116. Rutherford, S.L. and Lindquist, S. (1998) Hsp90 as a capacitor for morphological evolution. *Nature*, **396**, 336–342.
117. Prohaszka, Z. and Fust, G. (2004) Immunological aspects of heat-shock proteins—the optimum stress of life. *Mol. Immunol.*, **41**, 29–44.