Genetic Modifiers and Oligogenic Inheritance

Maria Kousi and Nicholas Katsanis

Center for Human Disease Modeling, Duke University, Durham, North Carolina 27710

Correspondence: katsanis@cellbio.duke.edu

Despite remarkable progress in the identification of mutations that drive genetic disorders, progress in understanding the effect of genetic background on the penetrance and expressivity of causal alleles has been modest, in part because of the methodological challenges in identifying genetic modifiers. Nonetheless, the progressive discovery of modifier alleles has improved both our interpretative ability and our analytical tools to dissect such phenomena. In this review, we analyze the genetic properties and behaviors of modifiers as derived from studies in patient populations and model organisms and we highlight conceptual and technological tools used to overcome some of the challenges inherent in modifier mapping and cloning. Finally, we discuss how the identification of these modifiers has facilitated the elucidation of biological pathways and holds the potential to improve the clinical predictive value of primary causal mutations and to develop novel drug targets.

Since the mapping of the first locus for autosomal dominant retinitis pigmentosa (RP) on the long arm of chromosome 3 (McWilliam et al. 1989) and the subsequent identification of rhodopsin (RHO) as the causal transcript (Dryja et al. 1990), the genetic dissection of disorders in which retinal degeneration comprises a core phenotypic component has accelerated rapidly, leading to the report of more than 250 loci and genes cataloged in RetNet (as of May 2014; see https://sph.uth.edu/retnet/disease.htm). These advances have had a clear contribution to both our understanding of photoreceptor biology and in providing genetic diagnoses to families. At the same time, however, post hoc knowledge of disease-causing genes and mutations had a modest impact on the predictive ability of prognosis with regard to key aspects of disease expressivity, such as age-of-onset, rate of progression, severity, and manifestation of other comorbidities. Numerous reports exist in the literature in which patients with the same primary mutation show stark differences in phenotypic expressivity, with the more striking examples coming from nonidentical twin studies in which patients that grow under a shared environment and carry the same primary mutation display distinct phenotypes, most likely because of genetic determinants that act as modifiers (Walia et al. 2008). The example above is not an isolated oddity. Accumulating clinical and genetic evidence now suggests that, even for classically monogenic disorders, the view that the expressivity of the phenotype is exclusively the property of dominant or recessive mutations at a single locus...
might be an oversimplification of the biological phenomena (Dipple and McCabe 2000; Badano and Katsanis 2002; Schäffer 2013).

The concept of modifier genes and alleles is not new (Haldane 1941). Nonetheless, the field of retinal degeneration disorders has been in a unique position to lead the efforts to understand genetic architecture, causality, and variable expressivity. This is in part because of (1) large and well-phenotyped multiethnic cohorts; (2) a substantial (and growing) mutational target of almost 300 genes, each known to be necessary for retinal biogenesis, homeostasis, and/or function; and (3) the availability of in vitro and in vivo tools to model genetic interaction phenomena. Here we will discuss the concepts of modifier genes and alleles, including their evolving terminology; we will highlight examples that illustrate the still episodic, yet significant, advances in dissecting the genetic basis of modification of penetrance and expressivity; and we will discuss possible avenues toward improving our interpretative ability of such events.

PROPERTIES OF GENETIC MODIFIERS

A critical step in understanding genetic modification phenomena consists of providing a clear definition for the term itself. Phenomena described by terms such as epistasis, oligogenic inheritance, or genetic interaction/modification, are all effective synonyms of the same process, namely, the effect of one gene/allele on the phenotypic outcome of a second gene/locus. A distinction that can aid better defining these genetic phenomena consists in establishing whether the primary locus is both necessary and sufficient to cause disease. If yes, then the presence of allele(s) at a second locus/gene exerts purely a modifying role on the severity of the phenotype as defined by the rate of disease progression; the severity of the phenotype with regard to its pleiotropy; or in the manifestation of endophenotypes that necessitate genetic interactions; if not, then the requirement of an allele in a second gene to manifest pathology defines a case of digenic or oligogenic inheritance (Fig. 1). In general, in the context of dissecting modifier phenomena, four major parameters play a prominent role:

1. Allelic heterogeneity. Phenotypic variability can stem from mutations in different genes that contribute to disease endophenotypes. However, simpler models of genetic heterogeneity and their influence on phenotypic expressivity should first be considered. Locus and allelic heterogeneity are highlighted in Leber congenital amaurosis (LCA), a disorder for which at least 11 causative genes have been reported under a recessive paradigm (reviewed in Davis and Katsanis 2012). In addition to genetic heterogeneity, LCA is also an example of allelic heterogeneity. For example, nonsense and frameshift, as well as missense mutations in CEP290 drive disorders ranging between nonsyndromic LCA to Joubert syndrome (JBTs) to the more severe Meckel Gruber syndrome (MKS) phenotype. These observations have reasonably suggested that modifier genetic factors account for this marked variance in clinical presentation (den Hollander et al. 2006, 2008; Sayer et al. 2006; Valente et al. 2006; Baala et al. 2007; Frank et al. 2007; Perrault et al. 2007). However, a discrete intronic mutation (c.2991+1655A>G) in CEP290 that creates a strong splice-donor site and inserts a cryptic exon in the CEP290 mRNA resulting in an aberrantly spliced transcript, appears to be sufficient to cause LCA (den Hollander et al. 2006, 2008; Sayer et al. 2006; Valente et al. 2006; Baala et al. 2007; Frank et al. 2007; Perrault et al. 2007). These data argue that, at least in some instances, this mutational event alone is sufficient to explain the disease without necessarily evoking the action of additional alleles. In this example, recurrence of the specific mutation has facilitated that argument. However, typically bereft of such data, dissecting phenotype–genotype correlations within a primary causal locus from modifier effects can be challenging, especially because most such correlations are made on the basis of a few mutations.

on penetrance and/or expressivity can vary from a “monogenic” model of modification, in which one modifier can account for most of the observed variability, to a more complex model in which more than one genetic determinant interacts with the primary disease locus. Although identification of major or “panmodifiers” is feasible, the dissection of more than one gene modulating the phenotype caused by the primary locus is challenging, due primarily to lack of genetic power. An example illustrating the mapping of a “pan-modifier” was described in a Bardet-Biedl syndrome (BBS) cohort of 226 patients not preselected for mutations at any specific locus. Within this group, an allele in CCDC28B was associated with a more severe BBS phenotype, a discovery based on association analy-
sis and transmission disequilibrium testing (TDT) and dissected further by biochemical studies (Badano et al. 2006). Had the modifying effect of CCDC28B been specific to a particular BBS locus, unmasking of such a genetic interaction would have been difficult. In contrast to “panmodifiers,” the interplay of multiple modifiers, each of which has a variable strength of effect on the final disease expression is probably common, but the most challenging to unravel. A good example is cystic fibrosis, a life-shortening recessive disorder in which 90% of the affected patients die because of obstructive pulmonary disease (Cutting 2010). Despite the majority of patients carrying identical genotypes (homozygosity for CFTR F508del), striking differences in lung function have been described (Kerem et al. 1990). Candidate modifier studies reported that functional variants in MBL2 and alleles in the promoter and first exon of TGFb1 were associated with lower lung function measures (Garred et al. 1999; Drumm et al. 2005; Darrah et al. 2010). More recently, genome-wide association studies revealed additional modifiers of lung function; IL-8, IFRD1 and ENDR4 associated with neutrophil function (Vanscoy et al. 2007; Hillian et al. 2008). Taken together, the aforementioned genetic modifiers are believed to alter lung disease severity by affecting the ability of the host to tolerate infection, which can be further modified by nongenetic determinants such as air pollutants, secondhand smoking, or even access to medical care and treatment (Rubin 1990; Goss et al. 2004). Under such a model, each modifier explains only a small portion of the variability, similar to the genetic risk factors involved in complex traits.

3. **Modifiers in the same locus/region with the disease driver.** Genetic modifiers in cis with the disease driver gene might be considered to be variants tagging and being transmitted in linkage disequilibrium with the primary mutation. An example of a modifier in cis comes from several studies showing that variants within FTO were reported to confer susceptibility to developing obesity (Dina et al. 2007; Frayling et al. 2007; Scuteri et al. 2007; Church et al. 2010). Although FTO was shown to regulate body mass, the variants identified appeared to have no effect on the expression and stability of the protein (Klotzing et al. 2008; Grunnet et al. 2009). Instead, the variants within the FTO region are regulating the expression of a transcription factor located in cis, IRX3 that is now thought to potentially act as the primary susceptibility gene for developing obesity (Smemo et al. 2014). An example of trans modification within the same locus was described in a pedigree diagnosed with erythropoietic protoporphyria (EPP), in which the patients carry a splice donor site deletion resulting in skipping of exon 10 (Delx10) of ferrochelatase (FECH). When this change is accompanied by an intragenic variant (IVS3-48C), the phenotype is aggravated with the levels of ferrochelatase in those patients being significantly lower than in asymptomatic carriers (Gouya et al. 1996, 2006). The mechanism underlying this phenomenon consists in IVS3-48C modulating the use of a constitutive aberrant acceptor splice site on FECH resulting in an aberrantly spliced mRNA that is subject to nonsense-mediated decay and therefore leading to FECH deficiency (Gouya et al. 2002).

4. **Frequency of the modifying variants in the general population.** Assuming that a modifier allele alone is not sufficient to induce pathology, it might be relatively common in the general population, thus complicating the dissection of phenotypically relevant versus benign variants. In the study that identified an allele that confers susceptibility to Hirschsprung disease (HSCR), the causal variant had a minor allele frequency (MAF) of 0.45 in Asia, 0.25 in Europe and was virtually absent (0.01) in the African population (Emison et al. 2005). Unmasking such common variants may require the use of multiple methods; in this case the combinatorial use of careful phenotyping combined with human genetic, comparative genomic, functional, and population genetic analyses.
(Emison et al. 2005). At a second tier, there are alleles of intermediate frequency in the general population. An example of this is the p.A229T allele in \textit{RPGRIP1L} which aggravates the retinal phenotype in ciliopathy patients, present in 3% of Northern Europeans (Khanna et al. 2009). Finally, rare variants that are enriched in patients compared with controls can have a modifying effect on disease expressivity and penetrance. In age-related macular degeneration (AMD), in which most primary driver variants are common, rare alleles (MAF\textless{}1\%) within the complement pathway can alter the disease phenotype. The first example described the rare p.R1210C mutation in \textit{CFH} as a penetrant allele that confers high risk to AMD and was followed by the discovery of an additional five risk and 15 protective variants through a study that evaluated components of the complement pathway (Raychaudhuri et al. 2011; Seddon et al. 2013). Although it is easier to establish the relevance of a rare variant to disease causality or predisposition, the same is not true for common variants that are overrepresented in the control population. In the context of genetic modification the true debate is not how common or rare a variant is, but rather how those variants work together and what is their cumulative effect (Gibson 2012).

**CHALLENGES IN IDENTIFYING MODIFIER GENES/ALLELES**

In contrast to the remarkable progress in identification of disease causing genes, the dissection of oligogenic and epistatic phenomena is lagging, in part because of the reduced power of traditional genetic analyses to uncover such phenomena. Beyond methodological limitations, there are additional challenges to consider:

1. **Availability of accurate clinical evaluation and longitudinal studies.** The identification of genetic modifying phenomena relies heavily on the detailed characterization of the aspect(s) of the phenotype under modification. It is thus imperative to have detailed clinical longitudinal information. Historically, cohorts have been characterized qualitatively for the presence/absence of a trait (i.e., RP or macular degeneration), with since-point proxies used as an imperfect means of expressing disease progression; these have included age of onset of a specific phenotype (Badano et al. 2006), thickness of the outer nuclear layer (ONL) (Danciger et al. 2008), or number of photoreceptors (Haider et al. 2008). The literature however, is populated by incomplete phenotypic information on the cases presented, often focusing on specific aspects of disease. In BBS, a multisystemic disorder characterized by rod–cone dystrophy, central obesity, polydactyly, and retinal degeneration, a cardinal symptom of the disorder, renal dysfunction had been overlooked (Brattgard 1949; Landau et al. 1949). A reason for this could have been that, although renal structural abnormalities might be present, only a small fraction of the patients develop symptoms consistent with kidney functional impairment that would prompt clinical evaluation (Magro and Peres 1970; Labrune et al. 1974; Gershoni-Baruch et al. 1992; Beales et al. 1999). In the case of \textit{RPGRIP1L}, thorough description of the retinal phenotype across a cohort of patients with syndromic disorders falling under the umbrella of the ciliopathies, led to the dissection of the p.A229T allele as a variant associated with increased loss of photoreceptors, whereas absence of this change was proposed to have a potential protective effect from RP (Khanna et al. 2009).

2. **Nongenetic factors.** The effect of nongenetic factors poses a major confounder. In humans, one way to determine whether the phenotypic variability observed is caused by genetic modifiers or environmental factors is to compare inter- and intrafamilial variability. A second approach is that of comparing the phenotype in monozygotic (MZ) and dizygotic (DZ) twins that share the same environmental influence. One of the best characterized examples of nongeneti-
ic determinants leading to disease manifestation comes from studies linking pregnancies complicated by fetal neural tube defects (NTDs) with folate insufficiency, and the evidence that periconceptional folate supplementation can reduce the risk of NTDs and other congenital abnormalities (Laurence et al. 1980; Tolarova 1982; Czeizel and Dudás 1992; Wald et al. 2001).

STRATEGIES FOR CLONING GENETIC MODIFIERS

The examples of disease modifier identification existing in the literature have used a plethora of techniques and approaches (Fig. 2), typically combining several lines of investigation:

1. **Linkage analyses.** If nuclear families with individuals carrying the same primary locus mutation and displaying discordant phenotypes are available, linkage analyses to identify alleles identical by descent (IBD) in individuals sharing a specific trait or endophenotype can be performed. The same can be applied in sib pairs concordant or discordant for a given phenotype. Knowing the primary disease causing mutation, linkage analysis for the detection of modifier loci can be performed by evaluating the sample set for qualitative phenotypes (mutation carriers with the phenotype, mutation carriers without the phenotype, and mutation carriers with more severe disease form), or for quantitative phenotypes (i.e., age of onset). One of the reasons that could account for the inability to map modifiers is that, especially in human studies, large pedigrees are often necessary to map the regions harboring the genes of interest. This is exemplified in Fuchs corneal dystrophy (FCD), in which genome-wide linkage in a large multigenerational pedigree carrying the TCF8 p.Q840P allele that drives disease, revealed a second locus on 9p that, when combined with TCF8 p.Q840P results in a more severe form of disease (Riazuddin et al. 2010). Even when large pedigrees are available, variable intrafamilial phenotypic expression, in association with the difficulty to identify families with uniform phenotypes, can complicate mapping of modifiers. To address this, screening pedigrees from genetic isolates can prove beneficial. Such a study was performed on the Menonite genetic isolate to identify genes underlying HSCR (Carrasquillo et al. 2002). Using a genome-wide association study coupled with multipoint-linkage disequilibrium analysis, which searches for association arising from common mutation ancestry, linkage over known HSCR loci was detected. However, this signal was insufficient to explain disease manifestation (Carrasquillo et al. 2002). Instead, it was an epistatic interaction between the receptor tyrosine kinase (RET) and the endothelin receptor type B (EDNRB) that was necessary for disease manifestation (Carrasquillo et al. 2002).

2. **Candidate locus association studies.** The advantage of this approach consists mainly in that data from singletons can be compared with other unrelated patients with similar phenotypes. A limitation of this approach is that once association is established, it is difficult to discern whether it is truly owing to the polymorphism/marker identified, to a variant that is in linkage disequilibrium (LD) with the marker used, or to an unknown confounding factor that might not even be genetic (Génin et al. 2008). An alternative is family-based association and TDT, in which the preferential transmission of alleles from parent to affected offspring is evaluated (Spelman et al. 1993). In HSCR, linkage of modifiers has been shown consistently to the RET locus, harboring the main HSCR driver, RET, although in most of these studies no coding mutations could be identified (Bolk et al. 2000; Borrego et al. 2000; Gabriel et al. 2002; Garcia-Barceló et al. 2003; Sancandi et al. 2003). In these studies, it was hypothesized that the genetic determinant conferring increased susceptibility to HSCR when combined with mutations in other loci, would lie within the RET locus (Emison et al. 2005). As such, a combination of human ge-
**Figure 2.** Examples of strategies for cloning modifiers. 

**A.** Linkage analysis, with the affected family members manifesting specific endophenotypes or differential disease severity. By evaluating the sample set for the endophenotype or trait of interest, the candidate modifier loci can be mapped. The snapshot of chromosome 9 in panel A is from the UCSC browser (http://genome.ucsc.edu/).

**B.** Association studies compare patient cohorts with (gray figures) or without (white figures) a modifier of disease severity/expressivity, to obtain a signal for the locus/position that harbors the modifying allele(s).

**C.** The candidate gene approach evaluates whether genes whose products operate within the same pathway can exert a modifying effect on each other. The example illustrated comes from the observation that mutations in PRPH2 and ABCA4 can aggravate disease severity when combined with primary disease mutations in ROM1.

**D.** The system-based hypothesis evaluates the likelihood of specific genes to physically interact or manifest similar expression profile changes in control versus affected individuals to build genetic networks that drive disease pathogenesis. Such network interactions among genes that cause the same or similar disorders is exemplified by BBS, in which the responsible genes are shown to operate and interact together.

**E.** In transcriptome analyses, heatmaps compare changes in expression levels of genes, between healthy and affected individuals.

**F.** The use of mouse congenic strains to map modifiers in a more simplified genetic background. This figure was prepared using Servier Medical Art (http://www.servier.com/Powerpoint-image-bank).
netic, comparative genomic, functional and population genetic analyses were undertaken to show that the variant sought was an intronic change within a conserved enhancer sequence in intron 1 of RET (Emison et al. 2005). A similar approach was used to show the overtransmission of the CCDC28B modifier in BBS (Badano et al. 2006).

3. Candidate gene approach. One strategy to identify genetic modifiers is by applying a hypothesis-based candidate gene approach through the screening of genes that operate within the same biological pathways/processes as the primary disease driver gene(s). Such hypotheses can be formulated either through current experimental or in silico knowledge of gene networks or from evidence from model organisms. Using this approach, Poloschek and colleagues screened 15 family members for mutations in genes known to cause retinal degeneration. They identified heterozygous mutations in PRPH2, ABCA4, and ROM1; family members carrying the p.R172W mutation in PRPH2 were severely affected, patients carrying heterozygous alterations in only one of those genes displayed a milder phenotype and patients with heterozygous mutations in all three genes evaluated, show an even more severe form of disease (Poloschek et al. 2010). Another example based on the candidate gene approach involved the identification of the genetic interaction between NPHP1 and AHI1, with NPHP1 accounting for the nephronophthisis phenotype and the AHI1 p.R830W allele being the major determinant driving the retinal phenotype in the NPHP cohort (Louie et al. 2010).

4. System-based candidates. This approach requires a priori knowledge of the specific genes driving the pathogenesis of the trait of interest. In the human disease network hypothesis, genes associated with similar disorders show higher likelihood of physical interactions and higher expression profiling similarity between their products (Goh et al. 2007). Such an interaction network was constructed for 54 proteins involved in 23 distinct types of human inherited ataxias (Lim et al. 2006). Within this network, different ataxia-causing proteins showed physical interactions and revealed common cellular pathways potentially relevant to disease (Lim et al. 2006). To test the robustness of this approach, the ataxia network was evaluated for its ability to unravel both modifiers and disease drivers. Two major modifiers of spinocerebellar ataxia type 1 (SCA1), ataxin-2 (ATXN2) and RNA binding protein with multiple splicing (RBPMS) were present on the “ataxia-genome” map as nodes that shared strong physical interactions with ataxin-1 (ATXN1) (Lim et al. 2006). BBS represents another example of this approach, with more than 20 causative genes participating in the function of the cilium and thus showing that they are related to each other at a higher level of cellular and organismal organization (Davis and Katsanis 2012). As such, other proteins localizing and operating within the cilium emerge as candidates to either drive disease pathology or modulate phenotypic expressivity. Starting with the bona fide genes that can cause BBS under a recessive paradigm in some families, and thus representing de facto contributors to the molecular etiology of BBS, an excess of pathogenic heterozygous BBS alleles were found in families with recessive mutations in other BBS loci (Katsanis et al. 2001; Badano et al. 2003; Beales et al. 2003; Stoetzel et al. 2006, 2007; Leitch et al. 2008). These phenomena were not restricted to genes mutated in BBS under a recessive paradigm; the BBS modifier CCDC28B was screened based on its interaction and colocalization with multiple BBS proteins, in addition to the fact that it was belonging to the ciliary proteome and hence operating in the same system as the other BBS proteins (Li et al. 2004; Badano et al. 2006; Gherman et al. 2006).

5. Transcriptome analyses. Methodologies that evaluate the expression of proteins such as microarrays and RNA-seq present powerful tools for studying the transcriptional landscape of a given cell or tissue.
tome analyses compare the expression levels of proteins between different phenotypic groups, such as control versus disease organism. The comparison can be applied to the whole transcriptome of the tissue of interest, or it can be combined with other methodologies such as linkage analyses, to identify only the genes that have altered expression within the locus of interest. Whole transcriptome comparison was performed between four siblings (two of which were asymptomatic) and two unrelated patients affected by spinal muscular atrophy (SMA), all of which carried identical SMN1/SMN2 genotypes (Oprea et al. 2008). One transcript showing statistically significant differential expression between the individuals with discordant phenotypes was plastin 3 (PLS3), a protein that elevates the F actin levels and promotes axonogenesis, comprising the first protective modifier in SMA reported (Oprea et al. 2008). The protective effect of PLS3 was illustrated further in a conditional mouse model overexpressing PLS3 into an SMA background (SMA_{PLS3}), with the motor neurons of the SMA_{PLS3} mice showing restored actin dynamics, increased size of the somata, increased quantal content of the neuromuscular junctions, axon stabilization, increase in the size of innervated muscle fibers, and overall prolonged survival (Ackermann et al. 2013; Lyon et al. 2014).

6. Considering whole-exome data in toto. A more unbiased approach to screen for genetic modifiers is through interrogation of the whole exome. Advances in massively parallel sequencing over the past years have made such analyses feasible. The caveat in using such large-scale screens lies in the small sample size available to screen for modifiers, the failure to correct for multiple hypotheses testing when several variants and genetic markers are examined, as well as the effects of population stratification (Gusella and MacDonald 2009). Nonetheless, the advantage of using these technologies consists mainly in the fact that they provide an unbiased approach. This is illustrated by recent studies in Gordon Holmes syndrome (GHS), a rare form of cerebellar ataxia associated with hypogonadotropic hypogonadism. Analysis of whole-exome sequencing (WES) identified rare alleles in a large consanguineous pedigree and when the genetic findings were coupled to functional studies concluded that, in that family GHS was hypomorphic caused by combinatorial mutations in two independent genes (RNF216 and OTUD4), both of which participate in the ubiquitin proteasome pathway (Margolin et al. 2013). There are several conclusions that can be drawn by this study: (1) if significant linkage was obtained over the locus of one or both of the genes identified, it would have most probably led to the mapping of the one of the genes of this interaction, leaving the second locus unexplored; (2) if the epistatic interaction in GHS had not been underlaid by homozygous hits in both genes, but rather was mediated by a homozygous hit in the primary locus and a heterozygous hit in the second or epistatic locus, it would have likely been missed by “typical” WES allele filtering; (3) without the use of the in vivo functional model uncovering the functional synergy of the two genes, RNF216 would have been proposed as the gene underlying GHS because of identification of mutations in this gene in unrelated patients, leading to the false conclusion that OTUD4 is not relevant to disease.

7. Modifiers in inbred and mutation-induced mouse strains. The most successful approach to map genetic modifiers are mouse models that show phenotypic divergence caused by their different genetic background. A study illustrating the power of this approach to identify genetic modifiers consisted of crossing C57BL/6j-tub/tub mice that display retinal and cochlear degeneration (Heckenlively et al. 1995; Ohlemiller et al. 1997) with AKR/J+/- animals. In the F2 generation, the C57BL/6j:AKR/J-tub/tub mice were protected from retinal degeneration, having an almost normal ONL thickness and number of photoreceptors when compared with the C57BL/6j-tub/tub littermates (Ikeda
GENETIC MODIFIERS OF RETINAL DEGENERATION IN HUMANS

The report of a nuclear family with a 3 bp deletion in peripherin (RDS) presenting with three distinct clinical phenotypes (RP, pattern macular dystrophy, and fundus flavimaculatus; Table 1), provided the first example of complex inheritance in retinal degeneration (Welleber et al. 1993). The identification of additional families with RDS mutations and significant inter- and intrafamilial variability ensued (Apfelstedt-Sylla et al. 1995). Kajiwara and colleagues then chose three clinically variable families carrying the heterozygous RDS p.L185P allele; the investigators undertook a candidate gene approach and screened for mutations in the gene encoding the retinal outer segment membrane protein 1 (ROM1), whose homodimer protein products complex with those encoded by RDS (Goldberg and Molday 1996). This study revealed that only family members that were double heterozygotes for the RDS p.L185P alleles and the ROM1 p.114fsX developed RP, whereas individuals that were heterozygous for either one of these mutations did not (Kajiwara et al. 1994; Rivolta et al. 2002). The mechanism through which such a genetic interaction can affect the clinical phenotype has also been elucidated, revealing that RDS mutant subunits fail to homodimerize and that the formation of functional complexes is further decreased by the frameshifting mutation in ROM1, thus leading to the ultimate degeneration of photoreceptors in the retina (Goldberg and Molday 1996; Loewen et al. 2001). The effect of the modifier allele was established in a zebrafish model in which it scored as pathogenic, and in a yeast two-hybrid screen in which the mutant RPGRIP1L was exerting a significantly lower affinity to form complexes with the retinitis pigmentosa GTPase regulator protein (RPGR), with which it inter-
acts (Khanna et al. 2009). It is thought that the observed photoreceptor degeneration in patients with the modifying RPGRIP1L p.A229T allele are because of the attenuation of the RPGRIP1L–RPGR interaction (Khanna et al. 2009). The observation that RPGRIP1L contributes modifying alleles was shown further in X-linked RP caused by mutations in RPGR (Fahim et al. 2011). Affected males from 56 families with RPGR mutations were genotyped for common alleles in each of RPGRIP1L, RPGRIP1L, CEP290, and IQCB1 based on the fact that they all interact with RPGR as determined by coimmunoprecipitation experiments (Boylan and Wright 2000; Otto et al. 2005; Chang et al. 2006; Khanna et al. 2009). Two alleles, the IQCB1 p.I393N and the common RPGRIP1L p.K744Q, showed significant association with disease severity, with the minor allele in IQCB1 and the common allele in RPGRIP1L being associated with more severe disease (Fahim et al. 2011).

In Usher syndrome, a genetically heterogeneous recessive disease characterized by hearing loss and RP, a genetic modifier of the retinal phenotype was identified by blasting homologous proteins to harmonin causing Usher syndrome subtype 1C (USH1C) and whirlin underlying USH2D that cross-link other Usher proteins to form a multimolecular complex in the photoreceptors and hearing cells (Kremer et al. 2006; Ebermann et al. 2007). PDZD7 was identified and screened for mutations in patients with an Usher syndrome diagnosis (Ebermann et al. 2010; Table 1). Although no changes in PDZD7 were identified in Usher patients with any molecular diagnosis, in familial cases with mutations in known Usher genes, PDZD7 seemed to be contributing to a digenic mode of inheritance and to also be contributing modifying alleles (Ebermann et al. 2010). For example, in a family with two USH2A (p.C1447QfsX29) affected sisters, the one that also carried a heterozygous frameshift mutation in PDZD7 (p.R56PfsX24) displayed an earlier age of onset and more severe progression of RP, whereas in a distinct case of a family with two affected offspring, only the individual with a heterozygous USH2C (GPR98 p.A5713LfsX3) mutation and a second heterozygous hit in PDZD7 is affected, suggesting that GPR98 and PDZD7 seem to be participating in a digenic mode of inheritance (Ebermann et al. 2010). Similar to the study identifying RPGRIP1L as a genetic modifier, the direction of effect of the PDZD7 alleles as pathogenic and the digenic inheritance of GPR98 and PDZD7 were confirmed in a zebrafish in vivo model for RP (Ebermann et al. 2010).

BBS has contributed substantially to understanding the contribution of genetic modifiers to disease (Katsanis et al. 2001; Badano et al. 2006; Table 1). The disorder is genetically and clinically heterogeneous, with well-documented examples of inter- and intrafamilial phenotypic variability (Carmi et al. 1995; Riise et al. 1997). Although initially reported to follow an autosomal recessive mode of inheritance, BBS was the first disorder to follow a “triallelic” model of disease transmission with three alleles at two independent loci being required for disease manifestation (Katsanis et al. 2001). More specifically, in a nuclear family with one affected and two unaffected siblings, compound heterozygosity in BBS2 for the nonsense alleles p.Y24X and p.Q59X was not sufficient to cause the phenotype, but required the presence of a third allele at a second BBS locus, BBS6 p.Q147X, which acts essentially as a modifier of penetrance (Katsanis et al. 2001). A second example of genetic modification coming from the study of BBS samples is the identification of CCDC28B p.C430T as a modifier affecting RP; the patients that carried p.C430T displayed an earlier onset of RP and accelerated disease progression (Badano et al. 2006). In addition to contributing modifying alleles, CCDC28B has also been postulated to represent an example of complex inheritance because it seems to be required for disease manifestation in a family with BBS1 p.M390R homozygous siblings and also asymptomatic father. It is therefore hypothesized that the third allele (p.C430T) located in CCDC28B might be required for disease manifestation or be acting as a modifier of penetrance similar to the BBS6 and BBS2 epistatic interaction (Beales et al. 2003; Badano et al. 2006).
GENETIC MODIFIERS OF RETINAL DEGENERATION IN MICE

The cloning of modifiers in human genetic disorders is confounded by genetic and allelic heterogeneity. Some of these challenges can be overcome in model organisms. Laboratory mice in particular, have been successfully used to map modifier genes and alleles in a wide range of genetic disorders (Table 2). Although in most successful studies the modification of the primary phenotype is because of the effects of a single locus, independent from the primary locus (Danciger et al. 2000; Ikeda et al. 2002a), mapping of modifiers can be complicated when the modification results from the combined effects of several genes at discrete loci (Table 2) (Ikeda et al. 2002a).

The first report of a genetic modifier of retinal degeneration in mice was the identification of protective alleles in the rd5 or tubby mouse for both the hearing and retinal degeneration phenotypes (Danciger et al. 2000; Ikeda et al. 2002a,b). The tubby mouse has been proposed as animal model for Usher syndrome 1, BBS and Alström syndrome because of the phenotypic convergence of the clinical phenotypes of retinal and cochlear degeneration, and maturity onset insulin-resistant obesity (Coleman and Heckenlively et al. 1995). Originally, the profound hearing loss in B6-\(\text{tub/tub}\) mice was fully rescued in F2 \(\text{tub/tub}\) homozygotes in protective strains (Ikeda et al. 1999, 2002a). Linkage analysis and subsequent sequencing identified 10 amino acid substitutions and an Ala-Pro repeat length difference in the microtubule-associated protein 1A (\(\text{Mtap1a}\)) residing within the modifier of \(\text{tubby}\) hearing 1 (\(\text{moth1}\)) locus on chromosome 2, between the B6-\(\text{tub/tub}\) and the \(\text{tub/tub}\) homozygotes in protective strains (Ikeda et al. 1999, 2002a). \(\text{Mtap1a}\) was suggested to play a role in vesicular trafficking of proteins to the neuronal synaptic junctions and further work demonstrating differential binding affinities of \(\text{Mtap1a}\) with the postsynaptic marker PSD95 support a role of the \(\text{Mtap1a}\) modifier gene in synaptic maintenance and architecture (Brenman et al. 1998; Halpain and Dehmelt 2006). The second modifier hunt in the tubby strain consisted in the identification of the protective allele for the retinal degeneration phenotype of this model (Ikeda et al. 2002a). The degree of retinal degeneration was quantitatively assessed by measuring the thickness of the ONL and the number of photoreceptors in a defined area of the ONL between C57BL/\(6\)-\(\text{tub/tub}\) and \(\text{tub/tub}\) homozygotes in the protective AKR/J strain (Ikeda et al. 2002a). Linkage analysis comparing the two strains identified a significant signal on chromosome 11, a locus termed modifier of \(\text{tubby}\) retinal degeneration 1 (\(\text{moth1}\)) whereby a recessive mode of inheritance was assumed for the mapping of the protective allele and two suggestive signals on chromosomes 2 and 8, where dominant protective alleles were likely to segregate (Ikeda et al. 2002a). Interestingly, the locus on chromosome 2 identified in the study for the retinal degeneration modifier, maps to the same locus as \(\text{moth1}\) harboring the \(\text{Mtap1a}\) hearing modifier (Ikeda et al. 2002a,b). Subsequent screening of \(\text{Mtap1a}\) revealed nonsynonymous sequence alterations between the C57BL/\(6\)-\(\text{tub/tub}\) and AKR/J-\(\text{tub/tub}\) mice, suggesting that the same gene contributes protective alleles that account for the amelioration of both the hearing and retinal degeneration phenotypes of the \(\text{tubby}\) mouse (Maddox et al. 2012).

The use of ONL thickness as a quantitative trait that reflects retinal damage to perform linkage analysis led to the successful identification of a protective modifier in the \(\text{Rpe65}\) mouse model for light-induced photoreceptor damage, corresponding to human LCA (Akhmedov et al. 2000). The two strains compared were C57BL/\(6\)-\(\text{c(2J)}\) (\(\text{c2J}\)) albino mice that showed less damage to their photoreceptors after exposure to prolonged light than BALB/c mice and other albino strains, suggesting that the \(\text{c2J}\) strain was contributing protective allele(s) (Danciger et al. 2000). The strongest linkage signal was on chromosome 3; subsequent analyses let to the mapping of \(\text{Rpe65}\), in which p.Leu450 was present on the BALB/c strain and was associated with light-induced photoreceptor damage, although the \(\text{c2J}\) strain carried the...
Table 1. Genetic modifiers in retinal degeneration from studies in humans

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Primary disease driver</th>
<th>Second-site modifier</th>
<th>Clinical aspect affected by modifier</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Earlier onset of RP; accelerated disease progression</td>
<td>Badano et al. 2006</td>
</tr>
<tr>
<td>Ciliopathies</td>
<td></td>
<td>GUCA1A (p.P50 L)</td>
<td>Disease severity</td>
<td>Downes et al. 2001</td>
</tr>
<tr>
<td>LCA</td>
<td>CEP290</td>
<td>AHII (p.N811K and p.H758P)</td>
<td>Neurological phenotype; disease severity</td>
<td>Coppitieters et al. 2010</td>
</tr>
<tr>
<td></td>
<td>AIPL1 (p.Q163X)</td>
<td>CRX (p.T273L)</td>
<td>Earlier disease onset; disease severity</td>
<td>Zernant et al. 2005</td>
</tr>
<tr>
<td></td>
<td>AIPL1 (p.R302 L)</td>
<td>CRB1 (p.R1331H)</td>
<td>Accelerated disease progression</td>
<td>Zernant et al. 2005</td>
</tr>
<tr>
<td></td>
<td>RPE65 (p.E102X)</td>
<td>GUCY2D (p.I573V)</td>
<td>Disease severity</td>
<td>Zernant et al. 2005</td>
</tr>
<tr>
<td>LCA</td>
<td>GUCY2D (p.S448X)</td>
<td>CRX (p.A158T)</td>
<td>Development of macular coloboma in both eyes</td>
<td>Zernant et al. 2005</td>
</tr>
<tr>
<td>Macular dystrophy</td>
<td>PRPH2</td>
<td>ROM1 (p.R172 W or p.R229H)</td>
<td>Disease severity; loss of rod function</td>
<td>Poloschek et al. 2010</td>
</tr>
<tr>
<td>Nephronophisis</td>
<td>NPHP1</td>
<td>AHII (p.R830 W)</td>
<td>Retinal degeneration</td>
<td>Louie et al. 2010</td>
</tr>
<tr>
<td>Oguchi’s disease/autosomal recessive RP</td>
<td>SAG (p.383fsX)</td>
<td>Unknown</td>
<td>Development of macular degeneration and golden-yellow fundus reflex</td>
<td>Nakazawa et al. 1998</td>
</tr>
<tr>
<td>Retinoschisis (X-linked)</td>
<td>XLRS1 (p.125fsX)</td>
<td>Unknown</td>
<td>Disease severity and progression</td>
<td>Tantri et al. 2003</td>
</tr>
<tr>
<td>RP (X-linked)</td>
<td>RPGR</td>
<td>RPGRIP1L (p.R744Q)</td>
<td>Disease severity</td>
<td>Fahim et al. 2011</td>
</tr>
<tr>
<td>RP (X-linked)</td>
<td>RPGR</td>
<td>IQCB1 (p.1393N)</td>
<td>Disease severity</td>
<td>Fahim et al. 2011</td>
</tr>
<tr>
<td>RP (autosomal dominant)</td>
<td>PRPF31</td>
<td>CNOT3</td>
<td>Disease penetrance through transcriptional repression</td>
<td>Venturini et al. 2012</td>
</tr>
</tbody>
</table>

Continued
p.Met450 that appeared to be driving the protection against light-induced damage (Danciger et al. 2000). The protective effect of Rpe65 p.Met450 was also confirmed in other forms of retinal degeneration (Samardzija et al. 2006) and was supported further by introducing the allele in a c-Fos\textsuperscript{2/2} mouse (Wenzel et al. 2003). c-Fos is essential for light-induced photoreceptor apoptosis because of a sustained increase in the DNA-binding activity of AP-1 (Hafezi et al. 1997; Wenzel et al. 2000) and as a result c-Fos\textsuperscript{−/−} mice appear resistant to light-induced damage (Wenzel et al. 2000). When the endogenous Rpe65 gene (carrying p.Met450) in c-Fos\textsuperscript{−/−} mice is substituted by Rpe65 p.Leu450, Rpe65 p.Leu450 resulted in an increase in the levels of rhodopsin and therefore led to light-induced photoreceptor loss despite the protective role of c-Fos deficiency (Wenzel et al. 2003).

A third successful application of linkage analysis for the mapping of a quantitative trait locus (QTL) was performed in Rs1 mouse model for X-linked retinoschisis (XLRS) (George et al. 1995; Johnson et al. 2010). XLRS is a form of juvenile-onset macular degeneration, whereby there is splitting of the retina (schisis) and loss of synaptic transmission (George et al. 1995; Johnson et al. 2010). When C57BL/6J Rs1\textsuperscript{tmge} mice were backcrossed into the AKR/J background, a recessive modifier seemed to be protecting Rs1\textsuperscript{tmge} mice from retinoschisis.
<table>
<thead>
<tr>
<th>Disorder</th>
<th>Primary disease driver/mouse model</th>
<th>Second-site modifier</th>
<th>Phenotypic aspect affected by modifier</th>
<th>Strains involved</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrous retrolental tissue</td>
<td>p53</td>
<td>Not identified</td>
<td>Disease penetrance</td>
<td>C57BL/6J, 129/Sv</td>
<td>Ikeda et al. 1999</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>BMP4</td>
<td>Not identified</td>
<td>Disease severity</td>
<td>C57BL/6J, BlA, CAST/Ei, C3H, AKR/J, BALB/C, 129/SvEvTac</td>
<td>Hong et al. 1999</td>
</tr>
<tr>
<td>Iris atrophy/Glaucoma</td>
<td>Ipd</td>
<td>Isa (Ty r p1)</td>
<td>Disease manifestation</td>
<td>C57BL/6J, DBA/2J</td>
<td>Chang et al. 1999</td>
</tr>
<tr>
<td>Iris pigment dispersion</td>
<td>Ipd</td>
<td>Not identified</td>
<td>Disease penetrance</td>
<td>AKXD-28/Ty</td>
<td>Anderson et al. 2001</td>
</tr>
<tr>
<td>Iris stromal atrophy</td>
<td>Isa</td>
<td>Not identified</td>
<td>Disease severity; increased cell death</td>
<td>AKXD-28/Ty</td>
<td>Anderson et al. 2001</td>
</tr>
<tr>
<td>LCA</td>
<td>Rd16</td>
<td>Bbs6</td>
<td>Variability in photoreceptor degeneration</td>
<td>C57BL/6J, 129/Sv</td>
<td>Rachel et al. 2012</td>
</tr>
<tr>
<td>Ocular retardation</td>
<td>Chx10</td>
<td>Not identified</td>
<td>Disease penetrance</td>
<td>CASA/Rk</td>
<td>Bone-Larson et al. 2000</td>
</tr>
<tr>
<td>Oguchi disease (stationary night blindness)</td>
<td>Arrestin⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻<del>-</del>-~ Arrestin⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻<del>-</del>-~</td>
<td>C57BL/6J, 129/Sv</td>
<td>Chen et al. 1999</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinoschisis</td>
<td>Rs1</td>
<td>Tyr (Mor1)</td>
<td>Disease penetrance</td>
<td>C57BL/6J, AKR/J</td>
<td>Johnson et al. 2010</td>
</tr>
<tr>
<td>RP (recessive)</td>
<td>Pde6a</td>
<td>Not identified</td>
<td>Disease severity; photoreceptor loss rate</td>
<td>G3 A.B6-Tyr + / J</td>
<td>Sakamoto et al. 2009</td>
</tr>
<tr>
<td>RP1</td>
<td>Rp1h</td>
<td>Not identified</td>
<td>Disease severity</td>
<td>(N6) B6.129S, DBA.129S(B6), A.129S(B6)</td>
<td>Liu et al. 2009</td>
</tr>
<tr>
<td>RP</td>
<td>Tub or Rd5</td>
<td>Mtp1a (motr1)</td>
<td>RP penetrance; disease progression</td>
<td>B6.Cg-rd5/rd5, AKR/J</td>
<td>Ikeda et al. 2002b; Maddox et al. 2012</td>
</tr>
<tr>
<td>Retinal degeneration</td>
<td>Rd3</td>
<td>Not identified</td>
<td>Onset and progression of photoreceptor degeneration</td>
<td>RBE/Dn, Meta- In(1)Rk, Rb/(11.13)4Bnr, In-30</td>
<td>Heckenlively et al. 1993</td>
</tr>
</tbody>
</table>

Continued
(George et al. 1995; Johnson et al. 2010). Linkage analysis identified the modifier for Rs1 (Mor1) locus on chromosome 7 (Johnson et al. 2010). Within the critical interval mapped in AKR/J Rs1*tmgc1 mice, a recessive mutation in tyrosinase (Tyrc-2J) was identified as the Mor1 gene responsible for protection from the development of the schisis phenotype (George et al. 1995; Johnson et al. 2010).

**CONCLUSIONS AND FUTURE PERSPECTIVES**

Knowledge of the primary disease-causing mutation is rarely coupled by accurate prognosis. In many instances, the clinical phenotype of a patient with an identified driver mutation can either be ameliorated on the presence of protective alleles or exacerbated when disease-promoting alleles are present in the genome. Despite the numerous reports supporting the presence and importance of genetic modifiers, this field has not progressed as rapidly compared with the identification of primary disease drivers. One of the reasons for this difference, is our inability to recognize interactions among alleles and variants (Eichler et al. 2010). This is true not only for disorders considered to be monogenic but also for complex traits, in which “missing heritability,” the fraction of the genetic contribution not explained/explainable by single-locus analysis for rare or common alleles, has been attributed, in part, to the presence of locus interactions and modifiers (Marchini et al. 2005; Zuk et al. 2012). It is possible to consider epistasis and genetic modification to uncover additional genetic contributions; in a study of ankylosing spondylitis, a common form of inflammatory arthritis, nonancestral ERAP1 alleles have been shown to interact with disease predisposition alleles in HLA-B27 to confer protection from disease manifestation (Evans et al. 2011). The protective role of ERAP1 was validated further in a second multifactorial disorder, psoriasis, uncovering the interaction of this gene with HLA-Cw6, one of the major psoriasis susceptibility genes (Genetic Analysis of Psoriasis Consortium & the Wellcome Trust Case Control Consortium 2, 2010). Despite the advances in the sequencing technologies, the number of successful genetic modifier identification studies remains small to date owing to many reasons such as the limited disease population sizes, genetic heterogeneity, the low frequency at which any particular combination of alleles is present in the population, and incomplete clinical descriptions (Flint and Mackay 2009). Animal models have provided a powerful tool for the identification of genetic modifiers. Congenic mouse strains have facilitated the mapping of protective and disease-aggravating alleles in a number of cases, some of which have been illustrated here. Zebrafish in vivo assays have aided in the functional annotation and

![Table 2. Continued](image-url)
direction of effect of candidate alleles arising by such screens.

The most efficient method to unravel phenomena of genetic modification and oligogenic or complex inheritance, is to interrogate the identified candidate alleles starting from a discrete number of likely interacting loci. Higher throughput technologies provide a unique opportunity toward this goal; a complete genetic profile of cohorts or even specific cell types such as photoreceptors in retinal disorders can be generated through whole-exome or whole-genome sequencing providing the opportunity to mind the same data set multiple times to dissect specific and discrete endophenotypes associated with a genetic condition and its progression, and the development of induced pluripotent stem (IPS) cell technology offers the promise to provide differentiated products from individual human subjects. Although each of these methodologies appear promising, the majority of successful genetic modifier identification reports have relied on the combinatorial use of such tools. Shedding light into the field of genetic modifiers will lead to a more insightful comprehension of the affected pathways and cellular processes. Understanding the basis of disease is the only way toward designing new and more efficient therapeutic paradigms, improve prognosis and even prevent disease manifestation by attempting to mimic the effect of modifiers that affect disease penetrance.

REFERENCES


Cold Spring Harbor Perspectives in Medicine

www.perspectivesinmedicine.org

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

M. Kousi and N. Katsanis

Cite this article as Cold Spring Harb Perspect Med 2015;5:a017145


Liu Q, Savilev A, Pierce EA. 2009. The severity of retinal degeneration in Rp1h gene-targeted mice is dependent
Genetic Modifiers and Oligogenic Inheritance


