The Use of Mouse Models to Study Genetic Modifiers of Neurological Disease

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Abstract

Neurological disorders are a significant public health concern affecting as many as one billion people worldwide, and this number is expected to grow in the coming years. Many neurological disorders exhibit some degree of heritability, and susceptibility genes have been identified for several of the heritable disorders. A common feature of heritable neurological disorders is phenotype heterogeneitya observed in families carrying identical mutations at disease genes or loci. This is an indication that genetic modifiers may be influencing the disease phenotype. Genetic modifiers are variation(s) in loci or genes that, when inherited along with a primary disease-causing mutation, alter some aspect of the disease phenotype. Genetic modifiers are prevalent among a wide variety of neurological diseases. Genetic modifiers increase the phenotypic complexity of neurological diseases, making diagnoses and treatment more difficult. Identifying genetic modifiers can enhance our understanding of neurological diseases and reveal new therapeutic targets. Mouse models of neurological disease are an excellent resource for the identification and characterization of genetic modifiers, as they can help circumvent many of the problems that are encountered when studying genetic modifiers in humans. This review highlights some of the ways in which mouse models can be used in conjunction with human studies to enhance our understanding of neurological diseases.

Keywords

Neurological Disease
Mouse Models
Modifier genes
Genetics
Complex Traits

Introduction

Neurological disorders are a significant public health concern. As many as one billion people worldwide are affected by neurological disorders, and this number is expected to increase in the coming years[1]. Many neurological disorders are heritable to some degree, and in recent years susceptibility genes have been identified for several neurological disorders[a]. In most cases, variation in the primary susceptibility gene has not been sufficient to explain the full range of phenotypes observed in the affected population. A common feature of heritable neurological disorders is phenotype heterogeneity observed among and between families carrying identical mutations at disease genes or loci. This indicates that additional factors contribute to the phenotype heterogeneity exhibited by many neurological disorders. Among the factors that can contribute to phenotype heterogeneity are environmental influence, stochastic events, and genetic modifiers, which are the focus of this review[2]. Understanding how genetic modifiers influence neurological disease phenotypes can enhance our knowledge of disease processes by uncovering disease-related pathways. Components of these pathways represent potential targets for novel therapies, which could improve the lives of patients with neurological disorders. This review highlights some of the ways in which genetic modifiers influence neurological disorder phenotypes and includes a discussion on the use of mouse models for the identification and characterization of genetic modifiers.

Genetic modifiers are genes or loci that alter the phenotypic expression of other, non-allelic[b] genes or loci (target genes). In the context of neurological disorders, variation in genetic modifiers generally does not have a noticeable phenotypic effect unless it is inherited in the presence of a pathogenic variant of a non-allelic susceptibility gene or locus (target genes) (see Fig. 1). For example, alleles that modify Huntington's disease (HD) do not produce their own discrete phenotypes in the absence of the pathogenic CAG repeats in the Huntingtin gene. However, when they are inherited in the presence of CAG repeats, they can affect the age of onset of HD[3]. Genetic modifiers are prevalent among a wide variety of inherited neurological disorders.

a. The proportion of phenotype heterogeneity that can be explained by genetic variation.

b. Located at different genetic loci.

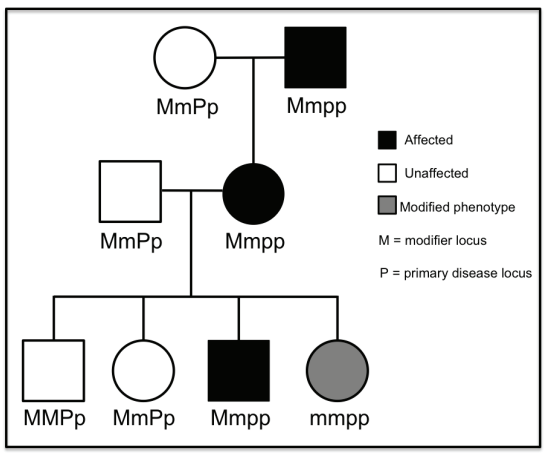

Figure 1. Phenotype heterogeneity within a family. In this representative family pedigree both the primary disease mutation and the modifier exhibit recessive modes of inheritance. Alleles at the modifier locus (designated M or m) segregate independently from alleles at the primary disease locus (designated P or p). In this particular case the phenotype is not modified unless two recessive alleles are inherited at both loci.

Disorders for which genetic modifiers have been implicated include: epilepsy, amyotrophic lateral sclerosis (ALS), Alzheimer disease, HD, tuberous sclerosis, and Hirschsprung disease, among others[4-9]. When present, genetic modifiers increase the complexity of the disease phenotype. This can complicate both the diagnoses and treatment of patients. Therefore, it is important to understand the ways in which genetic modifiers influence disease phenotypes.

Genetic modifiers can interact with target genes at any level of biological function to alter disease phenotypes in a wide variety of ways. Scnm1, one of the earliest modifiers of a neurological phenotype to be identified, acts at the level of transcription. Scnm1 modifies the neurologic movement disorder phenotype of $Scn8a^{medJ/medJ}$ mice, which results from a splice site mutation in Scn8a that leads to improper splicing and a reduction in functional Scn8a sodium channels[10]. Scnm1 is an RNA splicing factor that normally facilitates the proper splicing of Scn8a transcript. Buchner et al. identified a mutation in Scnm1 that exacerbates the $Scn8a^{medJ/medJ}$ phenotype by further reducing the amount of correctly spliced Scn8a transcript[11, 12]. Kcnv2, a genetic modifier of a seizure phenotype in the $Scn2a^{Q54}$ mouse model of epilepsy, works at the system level. $Scn2a^{Q54}$ mice have an epilepsy phenotype due to a gain-of-function mutation in the Scn2a sodium channel that results in excess sodium current [13]. Kcnv2 is a potassium channel subunit that reduces Kv2.1-mediated delayed rectifier potassium current. The exacerbation of the epilepsy phenotype is likely a result of a decrease in this delayed-rectifier potassium current [14]. This is an example in which the modifier gene (Kcnv2) does not directly interact with the target gene (Scn2a); instead it perturbs the system in which the target gene operates. There seems to be no limitations on the manner in which genetic modifiers can interact with their targets, or on the phenotypic effects that can result from these interactions. Specific examples of these interactions are too numerous to mention, but other phenotypic properties that can be altered include penetrance[c], disease progression, age of onset, and severity of the disease[15].

Genetic modifiers can offer insight into disease processes to help us better understand neurological diseases. The identification of the gene encoding microtubule-associated protein 1a (Mtap1a) as a modifier of hearing loss in tubby mice is a good example. Tubby mice have hearing loss as a result of a mutation in the tub gene. Before the identification of Mtap1a, the function of the tub gene was unknown. Ikeda et al. identified sequence polymorphisms in Mtap1a that were required for the hearing loss phenotype of tubby mice. These sequence polymorphisms reduced the binding efficiency of Mtap1a to Psd95, a gene encoding a synaptic scaffolding protein that helps coordinate synaptic function. These observations provided some of the earliest evidence of tub gene involvement in synaptic function[16]. Another example is the aforementioned discovery of Scnm1 as a modifier of the $Scn8a^{medJ/medJ}$ phenotype, which demonstrated that genes involved in mRNA splicing can modulate the phenotypic effects of splice-site mutations. This is of particular importance as splice site mutations are believed to compose approximately 10% of human disease mutations [17]. Identifying genetic modifiers can help us discover novel, disease-related pathways. These pathways not only help us to better understand pathogenic processes, but they may also contain therapeutic targets that could help us to better treat patients with neurological diseases. Therefore, studying genetic modifiers can be an important inroad to the successful treatment of neurological disorders. However, studying genetic modifiers in humans is challenging for a variety of reasons. Using mouse models of neurological disease can help researchers to circumvent some of these challenges. This review highlights some of the ways in which mouse models can facilitate the study of genetic modifiers of neurological disease.

Identifying Genetic Modification

The first step in the study of genetic modifiers is to establish that genetic modification is occurring. In hu-

c. The fraction of individuals with a particular genotype that express the associated phenotype.

mans, genetic modification is manifested as phenotype heterogeneity among or between families or populations that carry the same genotype at a primary disease gene or locus (see Fig.1). It can be difficult to distinguish between genetic modifiers and environmental sources of phenotype heterogeneity in humans. To do so, one must show that a portion of the phenotype heterogeneity is heritable. In HD, for example, there is considerable variability in the age of onset among patients with equivalent CAG repeat expansions in the HTT gene. It has been estimated that factors other than the length of the expansion account for approximately 30-50% of the total variability in age of onset[3, 18-20]. Using a large, well-characterized cohort of Venezuelan HD patients, Wexler et al. were able to show that a portion of this variability was heritable[3]. Oftentimes, large, well-characterized human cohorts are not available. In such cases, mouse models of the disease of interest can be employed. Genetic modification in mice is manifested as strain-dependent phenotype variability. Because mice can be reared and evaluated in similar environments, this strain-dependent variation is sufficient to establish that genetic modification is occurring[2]. Mouse models of neurological diseases frequently exhibit strain-dependent phenotypes. For example, the Hdh^{Q111} knock-in mouse model of HD exhibits several HD-related phenotypes that vary depending on genetic background, including: intergenerational repeat instability, somatic repeat instability, nuclear accumulation of full-length mutant huntingtin, and intranuclear N-terminal huntingtin inclusions[21]. Using mouse models to establish genetic modification can save researchers valuable time and money.

Genetic Mapping

Once it has been established that genetic modification of a disease phenotype is occurring within a population, genetic mapping is used to identify the genomic locations of the modifying genes/loci. Genetic mapping requires DNA samples from large, well-characterized populations of affected individuals, which are frequently unavailable in human populations. As an alternative approach, genetic mapping can be done in mouse models. This approach allows for the use of strategic breeding to take advantage of strain-dependent phenotypes to identify modifier loci. Once modifier loci/genes have been identified in mice, then researchers can screen a smaller number of patients for variants in the homologous loci/genes, thereby circumventing the need for large populations of human patients. This combination of genetic mapping and candidate gene screening was used to identify Kcnv2 as a modifier of epilepsy in mice and for the subsequent identification of two novel KCNV2 variants in pediatric epilepsy patients[14]. This approach has been used to successfully identify a number of other modifier loci/genes in mice and humans as well.

Forward Genetic Screen

For any potential modifier loci, the genetic mapping approach requires that there be genetic variation between individuals at that locus. Without this variation, there will be no discernible differences in phenotype with which to map the locus[22]. A forward genetic screen employs the use of a mutagen to induce polymorphisms throughout the genome, including potential modifier loci. This approach is commonly used in lower model organisms for pathway analysis, but it can also be used in mice to identify genetic modifiers. Using this approach in mice increases the number of potential modifiers that can be identified. Instead of relying on natural genetic variation between inbred mouse strains, N-ethyl-N-nitrosourea (ENU) is used to induce mutations throughout the genome. Mice carrying ENU-induced mutations can be crossed with any mouse model of interest to produce progeny that carry the primary disease-causing mutation along with ENU-induced mutations in potential modifier loci. These progeny are screened for phenotype modification, and standard genetic mapping is employed to identify modifier loci. This approach was first used by Matera et al. to identify a modifier of hypopigmentation in a Sox10 haploinsufficient[d] mouse model of Waardenburg syndrome[23].

Candidate Gene Approach

Genetic mapping and forward genetic screens are both unbiased approaches to identifying genetic modifiers. These approaches maximize the number of modifiers that can be identified. However, they can be time-consuming, even in mice. A less time-consuming alternative is the candidate gene approach. This approach involves screening candidate genes for genetic variation that is inherited along with the altered phenotype. Reducing the number of genes interrogated can increase statistical power, resulting in a reduced number of mice or patients required for the study. This can save both time and money, and is the approach most often used in human studies. Several modifiers of tuberous sclerosis complex phenotypes have been identified in humans by screening genes that interact with the tuberin-hamartin complex formed by TSC1 and TSC2, the target genes in which the primary tuberous sclerosis mutations occur[9]. Additionally, a number of different studies have identi-

d. A condition in which one allele is not sufficient for normal function.

fied putative modifiers in pathways believed to be involved in HD, including: glutamatergic transmission, protein degradation, gene transcription, stress response/apoptosis, lipoprotein metabolism, axonal trafficking, and energy metabolism[24-31]. Similarly, several modifiers of ALS have been identified in ALS-related pathways[32]. This approach can also be effective in mouse models. Cantrell et al. made use of this approach to identify Ednrb, a modifier of aganglionosis[e], in the $Sox10^{Dom}$ mouse model of Hirschsprung disease[8]. Instead of searching the whole genome for possible modifiers, they restricted their search to genes involved in the endothelin signaling pathway based on the knowledge that mutations in this pathway had been previously shown to cause Hirschsprung disease in humans[33]. For this approach to be effective in mice or humans, such previous knowledge is required to inform the search. This means that the search is generally restricted to genes found in pathways already known to be involved in the disease. Thus, modifiers identified using this method may not be as informative as modifiers identified using one of the unbiased approaches.

Validating Genetic Modifiers

Although genetic mapping and candidate gene screening establishes an association between a modifier gene and phenotype variation, this does not imply a causal relationship. In order to validate a putative modifier, the genetic variation at a modifier locus/gene must be shown to be sufficient to alter the phenotype of interest. This is commonly demonstrated by expressing the putative modifier as a transgene in the relevant mouse model. This approach was used to validate Kcnv2 as a quantitative modifier of the $Scn2a^{Q54}$ seizure phenotype. Several Kcnv2 transgenic mouse lines expressing different levels of the Kcnv2 transgene transcript were developed and bred to $Scn2a^{Q54}$ transgenic mice to produce double transgenic mice expressing $Scn2a^{Q54}$ and various levels of the Kcnv2 transcript. A comparison of the seizure phenotypes of each mouse line demonstrated that increased Kcnv2 expression is sufficient to exacerbate the $Scn2a^{Q54}$ epilepsy phenotype[4]. Mouse models are also useful for validating genetic modifiers that were identified in humans. A study by Giess et al. used this approach to validate CNTF as a modifier of ALS, which was first identified by screening candidate modifier genes in a family with ALS resulting from a SOD-1 mutation. To determine whether a CNTF deficiency could modify ALS onset, they crossbred hSOD-1G93A mice with $CNTF^{-/-}$ mice and compared disease onset to that of hSOD-1G93A mice expressing wild-type CNTF. They found that the CNTF-deficient mice had an earlier disease onset, validating CNTF as a modifier of the SOD-1 ALS phenotype[5]. This study demonstrates the benefit of combining both mouse and human approaches to study genetic modifiers.

e. The absence of parasympathetic ganglion cells in the myenteric plexus of the digestive system.

Considerations for Using Mouse Models

When using mouse models to study modifiers of human diseases, there are several considerations that one must take into account. First, not all modifiers identified in mouse models will be relevant to human diseases. This is because genetic and cellular pathways are not always conserved between mice and humans. Therefore, it is necessary to use caution when drawing conclusions from mice about human diseases. Second, there may be modifiers present in humans that cannot be identified in mice. This could occur because the homologous gene is not present in mice; because the pathways are not conserved; or because the imbred mouse strains are not polymorphic at the relevant locus. Third, mouse models of human disease may not recapitulate every aspect of the human phenotype. Even when the underlying mutation is the same, mouse model phenotypes can differ from human phenotypes. When observing mouse phenotypes, it is important to pick one that is conserved in humans. Even with these limitations in mind, mouse models remain an indispensable tool for studying genetic modifiers of human disease.

The Future of the Search for Genetic Modifiers

Large-scale, high-throughput sequencing techniques are developing at a rapid pace and becoming cheaper by the day. These techniques can greatly improve the speed and efficiency with which genetic modifiers can be identified. There are a number of ion channel variants that have been associated with epilepsy, and it is known that these variants can interact to influence epilepsy phenotypes[34]. Traditionally, these interactions have been tested one at a time. Recently, Klassen et al. performed exome sequencing on 237 ion channel genes and created ion channel variant profiles of individuals with sporadic idiopathic epilepsy and unaffected individuals, revealing a surprising degree of genetic complexity. Such an approach would not have been feasible just a few years ago. In the future, powerful techniques like these may help us to unravel some of the complexity underlying neurological diseases. Yet, with these techniques come new challenges. Though the discovery of genetic modifiers will come much faster than it has in the past, each putative modifier must be validated. As the complexity of the data increases, so must the means with which to evaluate it.

And with all of this new data come hypotheses that must be tested. Though high-throughput sequencing methods stand to decrease our reliance on mouse models for the discovery of modifiers, we will need mouse models more than ever to validate newly discovered modifiers and to test the hypotheses that we derive from them.

Conclusion

Genetic modifiers are a major contributor to the phenotypic heterogeneity observed in a wide variety of neurological diseases. Identifying modifier genes and elucidating the mechanisms by which they influence their targets is an important step in understanding neurological diseases. Mouse models are an indispensable resource in which to identify and characterize genetic modifiers. Knowledge gleaned through the skillful use of mouse models can be used to inform human studies, saving time and resources. When used thoughtfully and in combination with human studies, mouse models can help elucidate disease-related pathways, giving insight into pathogenic mechanisms. Knowledge of genetic modifiers and the pathways in which they operate can yield new therapeutic targets for the treatment of neurological disorders.

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