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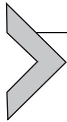

CHAPTER FOUR

Quantitative Genetics in the Study of Virus-Induced Disease

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Abstract

While the role of viral variants has long been known to play a key role in causing variation in disease severity, it is also clear that host genetic variation plays a critical role in determining virus-induced disease responses. However, a variety of factors, including confounding environmental variables, rare genetic variants requiring extremely large cohorts, the temporal dynamics of infections, and ethical limitation on human studies, have made the identification and dissection of variant host genes and pathways difficult within human populations. This difficulty has led to the development of a variety of experimental approaches used to identify host genetic contributions to disease responses. In this chapter, we describe the history of genetic associations within the human population, the development of experimentally tractable systems, and the insights these specific approaches provide. We conclude with a discussion of recent advances that allow for the investigation of the role of complex genetic networks that underlie host responses to infection, with the goal of drawing connections to human infections. In particular, we highlight the need for robust animal models with which to directly control and assess the role of host genetics on viral infection outcomes.

Advances in Virus Research, Volume 88
ISSN 0065-3527
http://dx.doi.org/10.1016/B978-0-12-800098-4.00004-0

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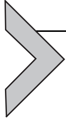
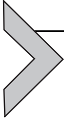


1. BACKGROUND

Genetic variation within populations has long been known to be critical in determining population-wide phenotypic variation. Indeed, since before Darwin (Vorzimmer, 1963), there has been an awareness that the range of phenotypes within a population can be influenced by heritable factors, which we now know as genetics. This is particularly true for the infectious disease field where there is abundant evidence that host genetic variation contributes to differences in disease susceptibility and outcome between individuals (e.g., Albright, Orlando, Pavia, Jackson, & Cannon Albright, 2008; Rau, Baur, & Geier, 2012; Vannberg, Chapman, & Hill, 2011). Furthermore, the use of genetic approaches to identify variant host genes that affect susceptibility to specific viruses has resulted in significant advances in our understanding of how viruses cause disease while also opening up new therapeutic avenues. For example, the determination that persons with a deletion in the HIV coreceptor CCR5 were highly resistant to HIV infection (Huang et al., 1996) informed the development of vaccines and therapies against HIV (van Lunzen, 2007). Despite the undeniable importance of host genetic variation in influencing host susceptibility to viral infection, to date, only a handful of polymorphic genes have been definitively identified and mechanistically characterized for their impact on virus-induced disease in humans (Everitt et al., 2012; Huang et al., 1996; Lindesmith et al., 2003; Zhang et al., 2013), and as a field, we are just beginning to understand how complex interactions between multiple genes and environmental factors influence host susceptibility to viral infection.

Therefore, the objective of this chapter is to illustrate some of the key genetic approaches and tools to facilitate the identification and classification of these genetic variants. These approaches include a variety of genetic mapping approaches, the development of genetically modified experimental systems, the characterization of naturally occurring genetic variants within experimental systems, and the development of powerful new computational approaches. We will also discuss how these approaches have influenced our understanding of virus–host interactions and end by discussing some recent advances within the field that promise to significantly enhance our ability to identify and study variant host genes that influence susceptibility to virus-induced disease.



2. APPROACHES TO GENETIC MAPPING

Genetic mapping is the process by which the physical location of a genetic factor is identified on chromosomes. A number of such approaches exist and were first popularized during the early twentieth century following T.H. Morgan's development of the initial genetic linkage map in *Drosophila* (Rubin & Lewis, 2000). Briefly, genetic mapping involves utilizing recombination and traceable genetic markers to identify regions in the genome that contain genetic elements of interest (Fig. 4.1). Historically, these approaches utilized phenotypic mutations, such as eye color in fruit flies, as the traceable markers allowed determination of the physical location of the genetic element responsible for a second phenotype. However, modern genetic mapping strategies more commonly use known genetic variants within the genome, such as single-nucleotide polymorphisms (SNPs), as markers for defining the location of a polymorphic gene of interest. The basic types of mapping approaches that have been used are linkage analysis and association studies, which will be discussed later. However, for readers who are interested in more in depth discussion of these approaches, we also refer you to several excellent reviews (Hill, 1996; McCarthy & Hirschhorn, 2008; Suarez & Cox, 1985).

As initially conceived, linkage analysis was most appropriate for the identification of highly penetrant, Mendelian genes (e.g., binary traits) segregating within some form of family pedigree or pedigrees. Individuals would be typed at each marker, with many initial linkage studies (Morgan & Lynch, 1912) using phenotypic markers, though as the field of genetics advanced, various molecular markers were used (Wilson et al., 2005). Within each family, linkage disequilibrium (LD) or the nonrandom association between the trait and various markers is assessed (Fig. 4.1). As physical distance between the markers and target gene increases, recombination will breakdown LD, leading to random association between traits and markers. Therefore, the highest degree of LD within the population helps identify the rough physical location of the gene of interest.

Quantitative trait locus (QTL) mapping was developed as an extension of linkage analysis, with the recognition that many traits were quantitative traits (Paterson, 1995). That is, instead of a single, highly penetrant or Mendelian gene being largely responsible for the traits of interest, a trait is influenced by multiple genetic elements, as well as by nongenetic environmental and

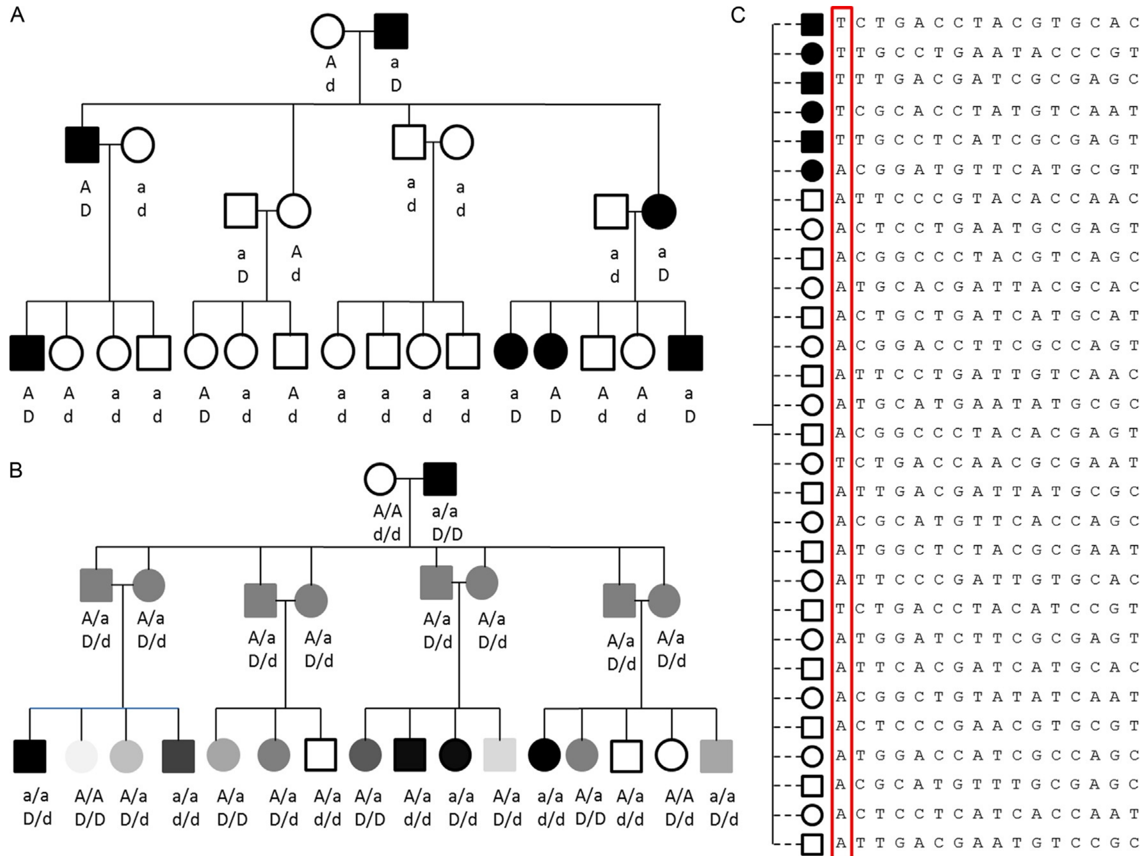


Figure 4.1 See figure caption on next page bottom.

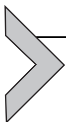
demographic factors. QTL studies utilize a similar framework to linkage analysis but instead examine the relationship between markers and statistically significant differences in a phenotype within a population. These relationships indicate that a gene or genetic element linked to the marker influences the phenotype of interest (Fig. 4.1). While moderate sample sizes are needed for linkage analysis of Mendelian traits, much larger samples are needed for QTL analysis, and these sample sizes depend on the underlying architecture of the trait of interest. Therefore, QTL analyses have largely been limited to more controllable experimental systems (e.g., mice (Georges, 1997) and rice (Yano & Sasaki, 1997)), where large numbers of crosses between individuals can be generated and screened for phenotypic variation.

In contrast to linkage analysis, association studies are useful for identifying common, low-pentrance alleles with broad distribution within the population. Association studies were made possible with the availability of dense SNP maps made available by the human genome sequencing project. While linkage relies on limited recombination breaking down LD between relatively distant markers and the gene impacting the trait of interest within family groups, association studies rely on the breakdown in LD over evolutionary timescales within nonrelated cohorts of individuals. As a result of this history of recombination, only SNPs that are either directly causative or very closely linked to the causative variant influencing the phenotype are detected in these approaches (Fig. 4.1). Indeed, the large number

Figure 4.1—Cont'd Different genetic mapping approaches. (A) Family pedigree containing a trait used for linkage mapping. The trait of interest (black individuals) is mapped by comparing trait to markers (A/a and D/d markers below individuals) within this family. Note that the trait is tightly linked to the D markers. (B) Experimental pedigree to identify QTL contributing to a continuous disease trait. Founder lines show a clear difference in phenotypes (black square and white circle at top of pedigree). F1 offspring all have intermediate phenotypes (gray individuals in middle of pedigree). F2 animals show a range of phenotypes (grayscale intensity). QTL analysis seeks to explain some portion of phenotype (grayscale) differences based on markers. In this case, individuals with A markers tend to be lighter in shade than those with *a* markers. (C) GWAS studies examine large pools of individuals without family structure (e.g., that are only evolutionarily distantly related). Within such a population, only SNPs that are very close to causative polymorphisms (or are in fact the causative polymorphisms) will avoid recombination breakdown of unequal associations. Therefore, by examining large numbers of SNPs in this population, those tightly associated with the phenotype will be identified. In this example, the first SNP (highlighted in red box) is associated with disease (black individuals). 5/6 individuals with the disease have a T at the SNP. In contrast, only 1/23 individuals without the disease have a T at the SNP.

of SNPs used for these studies ensures that the relationship between a causative polymorphism and the tested SNPs is very tight (McCarthy & Hirschhorn, 2008).

The two major approaches to association studies relevant to virology research are candidate gene analysis and genome-wide association studies (GWAS). Both approaches utilize dense SNP maps to find individual SNP sites that are significantly associated with phenotypic variation in the trait of interest, with associated SNPs being described by the increased risk of a disease outcome they confer. GWAS, as their name implies, are designed to screen the whole genome for genetic variants that contribute to variation in a phenotype within a population. For these studies, once data on variation in a phenotypic trait have been collected from a population, genome-wide SNP maps from these same individuals are used to conduct association analysis. These genome scans statistically test whether specific regions of the genome are significantly associated with variation in a phenotype. If the test population is sufficiently large and importantly the SNP map is sufficiently dense, for some phenotypes, it is possible to identify the specific polymorphisms (e.g., SNPs, insertions, or deletions) that are responsible for driving the phenotypic variation. In contrast, candidate gene studies are designed to test whether variation in a specific gene or genetic element is associated with variation in a specific phenotype. For this type of candidate analysis, target SNPs, which are likely to impact expression and or function of the gene, are tested for a statistically significant association with a phenotype of interest.



3. GENETIC MAPPING OF VIRAL DISEASE RELATED GENES WITHIN THE HUMAN POPULATION

With the rediscovery of Mendel's studies and the birth of the field of genetics, major efforts were put into the developments of genetic linkage maps to facilitate the identification of genomic locations of genes contributing to a variety of phenotypes. By the early 1970s, marker sets were largely available for the human population, allowing for linkage studies directly related to human diseases. Though not specifically directed toward understanding the susceptibility to virus-induced disease, these types of linkage studies did result in the identification of loci associated with variation in virus-induced type I interferon (IFN) responses (Tan, Creagan, & Ruddle, 1974), as well as the identification of variants in a wide range of immunoregulatory genes (Levine, Stember, & Fotino, 1972). These include components of the human leukocyte antigen (HLA) locus, including genes

encoding major histocompatibility complex molecules and components of the host complement cascade (Rittner, 1976), which were later shown to contribute to variation in antiviral immune responses and susceptibility to virus-induced disease. An additional set of studies were conducted on the role of host genetic variation in driving longer-term diseases that were facilitated by viruses, such as the role that host genetics plays on Epstein–Barr virus (EBV) infection and cancer development (Simons et al., 1975).

One of the more notable results to come out of linkage studies was the eventual identification of a set of rare X chromosome mutations in an SH2 domain gene causing Duncan's disease, which confers extreme susceptibility to EBV infection with 100% mortality due to associated cancer and uncontrolled lymphoproliferation. Through a series of studies (Sanger et al., 1990; Schuster, Dohrmann, & Kreth, 1991; Skare et al., 1989) utilizing linkage analysis, the causal locus for this disease was narrowed down to a region of the X chromosome, and a large (cytogenetically visible) deletion was identified in an affected individual. Further examination of this individual's relatives allowed for the identification of the gene *SH2D1A* as the potential causal gene for this disease. Examination of *SH2D1A* in other, unrelated individuals who had Duncan's disease showed that several had early stop codons or deletions, strongly pointing to mutations in this gene as causing Duncan's disease (Coffey et al., 1998). It also helped advance the understanding of T-cell activation (Tangye, Nichols, Hare, & van de Weerdt, 2003) and NF-kappa B signaling (Sylla et al., 2000). Such early studies illustrated the power and potential of genetic mapping approaches; however, they proved the exception rather than the rule in early linkage studies.

One of the problems with these initial linkage and QTL studies was that large populations of related individuals at equivalent infection stages were difficult to collect, thereby making it difficult to quantify those infection-associated phenotypes necessary for efficient mapping studies. Furthermore, while some traits had genes of large effect controlling phenotypic outcomes, many immunophenotypes are likely governed by multiple genetic factors. Even with the unfortunate increase in cohorts of chronically infected individuals with stable phenotypes (e.g., human immunodeficiency virus (HIV) viral load), linkage studies with sufficient power to identify host genes associated with variation in susceptibility to viral infections, though possible, were still rare. Though these studies often found variants associated with the HLA complex to be critical for variation in viral susceptibility (Carbonara et al., 1983; Cramp et al., 1998; Just et al., 1995; Kaslow et al., 1990; Kruskall, Alper, Awdeh, Yunis, & Marcus-Bagley, 1992;

Shaw & Biddison, 1979; Uno, Kawano, Matsuoka, & Tsuda, 1988), the identification of variants at other loci was limited (Hohler et al., 1998, reviewed overall in Hill, 1998). Furthermore, for acute viral infections, which lack the stable phenotypes associated with chronic infections, such as viral load, these types of approaches have very poor success for identifying variant host genes associated with susceptibility to viral infections.

In the mid-2000s, a powerful new approach was able to be used in human populations, GWAS. As mentioned earlier, this novel approach was developed to identify multiple genetic variants across the whole genome that either directly contribute or are very tightly linked to phenotypic variation. With the availability of the human genome sequence, and then dense SNP maps, researchers were able to identify genetic variants that were either causative or very closely linked to causative variants for a large and growing number of biomedical traits (Yang, Kon, & DeLisi, 2013). Despite this novel powerful approach, the drawback was the large number of samples needed for these analyses, limiting utility for some viral diseases. As with earlier linkage and QTL studies, the availability of large cohorts of chronically infected HIV and hepatitis C virus (HCV) patients with relatively stable viral loads provided useful data sets for identifying polymorphic host genes associated with variation in viral loads or other chronic disease conditions. GWAS studies continued to identify associations with HLA in response to a number of viral disease phenotypes, including HIV susceptibility (Limou et al., 2009), control (International HIV Controllers Study et al., 2010), and replication in macrophages (Bol et al., 2011), progression to HCV-induced liver cirrhosis (Urabe et al., 2013), the development of EBV-specific IgG responses (Rubicz et al., 2013), and hepatitis B virus (HBV) clearance (Nishida et al., 2012) and infection risk (Mbarek et al., 2011). GWAS studies were also able to identify associations with SNPs outside of HLA for a number of viral disease responses including associations between several genes and HCV-induced liver fibrosis (Patin et al., 2012), IFN-gamma responses to smallpox vaccine (Kennedy et al., 2012), HBV disease progression (Liu et al., 2011), and HIV susceptibility (Le Clerc et al., 2009). Several other GWAS have been conducted for more general immunologic phenotypes of interest to researchers of viral disease. These studies have found associations both inside and outside of HLA for a number of phenotypes, such as circulating C3 and C4 (Yang et al., 2012), circulating monocyte levels (Crosslin et al., 2013), and IgG production (Liao et al., 2012).

Perhaps, the most impressive and illustrative example of the power of GWAS approaches was in the identification and validation of *IL28B* as a

key regulator of HCV. Three independent GWAS of the response of HCV-infected individuals to treatment with pegylated IFN and ribavirin, a treatment that has variable efficacy across the population found that (Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009) SNPs near *IL28B* were associated with positive response to anti-HCV treatment, with further analysis of peripheral blood mononuclear cells suggesting that the variant affected expression of *IL28B*. A follow-up study (Thomas et al., 2009) further confirmed the role of *IL28B* not only in response to antivirals but also in spontaneous natural clearance of HCV. Indeed, genotyping of a highly diagnostic SNP in *IL28B* is now a standard aspect prior to prescription of HCV treatment, highlighting one of the initial promises of personalized/genetic medicine as it relates to viral disease.

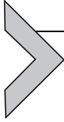
Though GWAS approaches represent a powerful tool for identifying common genetic variants in large populations, they also have limitations that can affect their application to certain types of viral disease processes. First, GWAS analysis requires access to large cohorts of individuals where quantifiable measures of the virus-induced disease process are available. Therefore, with some exceptions where there is a tight enough relationship between polymorphisms and disease response variation, such as a study identifying genetic associations with dengue shock syndrome (Khor et al., 2011), viral outbreak situations with relatively small sample sizes of affected individuals probably may not be sufficiently powered to utilize GWAS. Furthermore, when studying naturally acquired infections where the readouts are transitory (e.g., acute viral loads) or where environmental factors, including viral challenge dose, preexisting immunity, or nutritional status may confound the analysis, it may be difficult to achieve sufficient power to achieve statistically significant associations (e.g., Zhou et al., 2012). GWAS approaches are dependent upon the genetic variation affecting the phenotype of interest being due to common variants that are widely distributed within the population. If variation in response to a virus is being driven by rare genetic variants that have arisen *de novo* in individuals or small family groups and not generally distributed through the population, it is unlikely that GWAS approaches would achieve sufficient statistical power to identify these types of variants, and reversion to classical linkage approaches or novel exome-sequencing approaches (more below) is needed. Lastly, a more general problem with GWAS is that many of the found associations have been with intergenic or intronic regions (McCarthy & Hirschhorn, 2008). What remains an open question is how many of these associated SNPs are linked to causative nearby structural polymorphisms versus those involved in

noncoding RNA regulatory networks, such as miR-22's role in HCV pathogenesis (Janssen et al., 2013).

In light of these limitations of GWAS in regard to viral pathogenesis, researchers of viral disease have largely utilized more targeted candidate gene or pathway association studies to investigate how variation in specific genes or pathways affects viral disease. Unlike the unbiased GWAS, candidate gene-based association studies are focused on genes or pathways that are suspected to play a role in the pathogenesis of a specific viral pathogen based on prior evidence from other studies, including clinical observations or manipulative studies in experimental systems. Investigators then choose a limited set of genes and select variants in these genes that are known to or likely to affect either expression or function of the gene for interrogation with viral disease phenotypes. Importantly, because of the smaller cohorts of individuals needed, it is possible to identify associations with acute viral disease or those that had limited outbreaks. For example, due to binding interactions between DC-SIGN and L-SIGN and severe acute respiratory syndrome (SARS) coronavirus, it was hypothesized that SARS would interact with *ICAM3* and its signaling network. A candidate gene study was able to show that a nonsynonymous variant within *ICAM3* was associated with increased lymphocyte circulation during SARS infection (Chan et al., 2007). Many similar studies have been performed with both acute (Ermers et al., 2011; He et al., 2006; Morales-Garcia et al., 2012; Soundravally & Hoti, 2008) and chronic infections (Clark et al., 2012; Lo et al., 2013; Luo et al., 2012; Naggie et al., 2012; Talledo et al., 2012), as well as adaptive responses to viral vaccines (White et al., 2012).

While such targeted association studies allow for more precise investigations of the role that specific genes play in viral infections, they do have significant limitations. Specifically, by not interrogating the entire genome of these individuals, false associations (those generated by genomic structure) might be identified. True associations can also be masked by genetic structure elsewhere in the genome. Furthermore, since candidate gene studies are based on preexisting knowledge, it is likely that novel or previously uncharacterized pathways will be ignored by this approach. Nonetheless, these targeted association studies represent a powerful approach to begin identifying genes relating to more acute and/or limited viral infections and can be used to narrow down and more intensely interrogate the impact that variation in specific genes or pathways has on virus-induced disease processes. For example, a candidate gene study (Ovsyannikova et al., 2011) investigating SNPs in *CD46* and *SLAM* impacting measles vaccine

responses was able to better understand the role of SNPs in these genes enhancing innate responses and cytokine production as a mechanism for vaccine response. Furthermore, as more high-throughput siRNA screens (Karlas et al., 2010; Rose et al., 2011; Zhang, Katz, Gwinn, Dowling, & Khoury, 2009) are used, such studies can provide increased sets of rational candidates to probe within human systems.



4. EXPERIMENTAL APPROACHES TO STUDYING HOST GENETIC INFLUENCES ON VIRAL DISEASES

In response to several of the difficulties encountered in identifying host genes and genetic variants in the human population, a variety of experimental models have been used to identify variant genes that affect susceptibility to virus-induced disease. These experimental systems provide several advantages, including the ability to conduct more invasive and mechanistic studies than are possible in humans, greater control of potentially confounding environmental factors, and the availability of reproducible populations that permit analysis of phenotypic variation over time or across different experimental conditions (e.g., viral doses or different viral variants). These systems include resources developed to interrogate the function of specific genes using either reverse genetics (e.g., knockout mice) or forward genetics (e.g., *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis), and though these approaches have played key roles in advancing our understanding of the role the specific host genes play in the pathogenesis of a number of viruses, they have also been reviewed extensively elsewhere (e.g., Davies, Turner, & Klein, 2001; Georgel, Du, Hoebe & Beutler, 2008; Mordstein, Michiels, & Staeheli, 2010; Tecle, White, & Hartshorn, 2005). Therefore, we will only briefly discuss these approaches and will instead focus our discussion on systems tailored to investigate natural genetic variants segregating either between inbred lines of classic lab model organisms or within genetically variant populations (e.g., inbred line screens, F2 and backcrosses, consomic and congenic lines, and genetic reference populations (GRPs); Fig. 4.2).

4.1. Reverse and forward genetic systems

Reverse genetic approaches, such as targeted gene knockouts in mice, have provided major insights into the role that specific host genes play in either protecting from or promoting virus-induced disease. Furthermore, these approaches have clarified that most responses to viral infection are highly

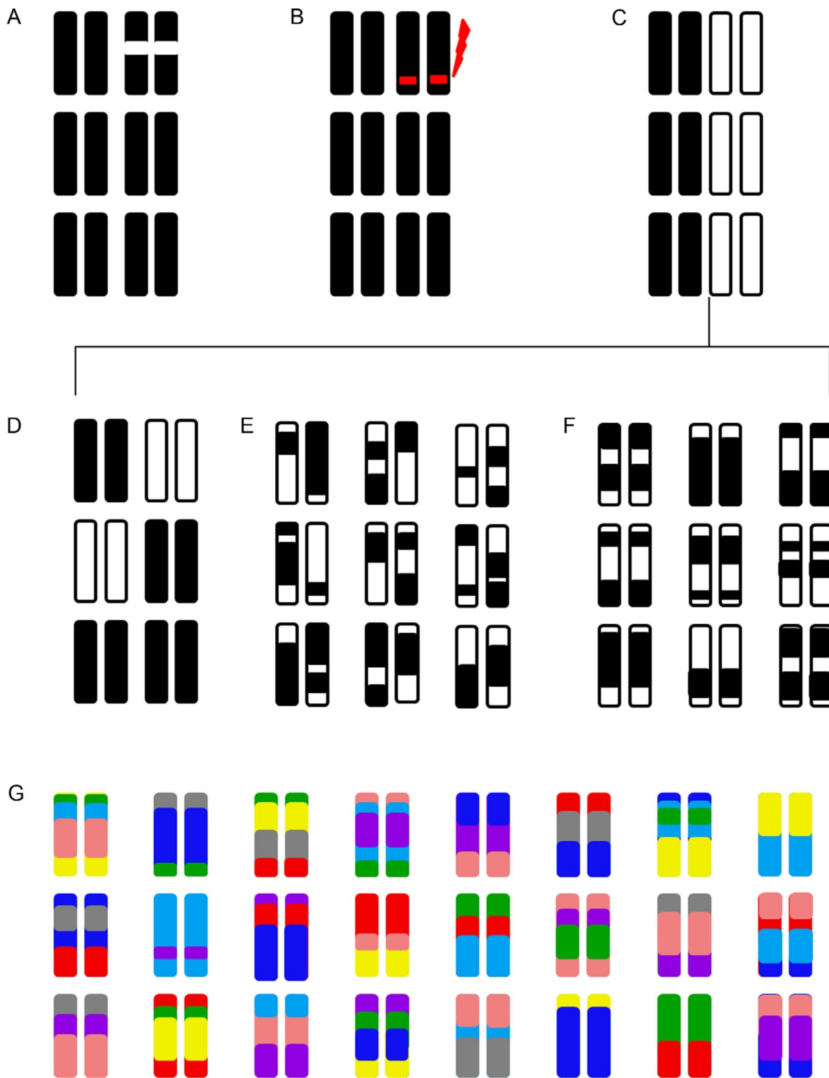


Figure 4.2 Experimental models for dissection of host genetic impact of viral disease. A number of different experimental approaches have been utilized to better understand the role of host genetics on viral disease. (A) Genetic knockout systems. Complete disruption of a gene product, where the contrast is between the wild-type inbred strain and the mutant strain. Both lines are isogenic except at the disrupted gene. (B) ENU mutagenesis. Random mutagenesis can produce variants that affect gene function. Again, by isolating and fixing the mutation, a direct comparison can be made to the original wild-type inbred strain, as the two lines will be isogenic except at the mutation of interest. (C) Comparisons between two inbred strains can reveal distinct disease

(*Continued*)

dependent on multiple host genes, which reflects the fact that the immune system is a highly complex network regulated by several interacting pathways. For example, a variety of studies have shown that genes relating to the Toll-like receptor (TLR) pathways (Majde, Kapas, Bohnet, De, & Krueger, 2010; Nasirudeen et al., 2011), IFN signaling (Durbin, Hackenmiller, Simon, & Levy, 1996; Hwang et al., 1995; Keller et al., 2006), and complement activity (Kotwal, Miller, & Justus, 1998; Mehlhop et al., 2007; Morrison, Simmons, & Heise, 2008) are all critical for either promoting or muting the host response to various viral infections and that for a given viral infection, disruption of different genes within the same pathway often results in similar immunophenotypes. Furthermore, these studies have also been important for illustrating the fact that the same immune pathway can play very different roles in the pathogenesis of different viruses, demonstrating the complexity of pathogen–host interactions. For example, while complement is critical for protection from encephalitic alphaviruses, such as Sindbis virus (Hirsch, Griffin, & Winkelstein, 1980) and Venezuelan equine encephalitis virus (Brooke, Schafer, Matsushima, White, & Johnston, 2012), it enhances disease during Ross River virus infection (Gunn et al., 2012; Morrison et al., 2008).

Figure 4.2—Cont'd responses (as in Boon et al., 2011; Srivastava et al., 2009; Zumbrun et al., 2012). Such results show that one or a number of unidentified genetic factors cause these differences. A variety of approaches can then identify these factors. (D) Consomic panels create novel inbred lines that contain an entire genome of one founder strain, except that each consomic line contains a single chromosome from the other founder strain. Disease measurements across such panels are useful for identifying and honing in (through the use of congenic animals) on genes of large effect or Mendelian genes affecting disease outcomes (Burgio et al., 2007). (E) By crossing animals from two founder lines together, and then breeding the F1 animals together, large pools of F2 animals can be quickly generated for QTL mapping studies. These F2 crosses result in individuals that are all genetically related, but each with a distinct (and incompletely inbred) genome. (F) In order to compare across QTL studies, and to integrate experimental replication and control into genetic mapping approaches, several recombinant inbred panels have been created (e.g., the BxD, AxB, and BxA panels in mouse and several panels in rice and *arabidopsis*). Each line is completely inbred, and its genome is a mosaic of the two founder lines. (G) The collaborative cross is a new recombinant inbred panel derived from eight highly genetically diverse founder strains. Thus, each CC line's genome is a mosaic of eight founder lines. At any given locus, up to eight alleles can be segregating in this population enabling both QTL mapping and more robust modeling of human genome-wide variation.

In addition to these reverse genetic approaches, studies utilizing forward genetic approaches, typically ENU mutagenesis, have further illustrated the complex role of multiple interacting genes and pathways (Cook, Vinuesa, & Goodnow, 2006; Hoebe, 2009). While knockout studies are directed toward dissecting the function of known genes, forward genetic approaches can be used both to investigate the function of specific genes by selecting specifically for hits within the gene of interest and also to provide an opportunity to perform an unbiased screen of the whole genome for genes that contribute to specific phenotypes, such as resistance to murine cytomegalovirus infection (Cook et al., 2006). While many of the initial findings from ENU mutagenesis studies have identified largely Mendelian, or genes of large effect, this type of analysis has identified key components of important immune pathways, such as the TLR pathways (Brandl et al., 2010; Hoebe & Beutler, 2008). ENU has also highlighted the knowledge that multiple allelic (Beutler et al., 2006; Crozat et al., 2006; Siggs et al., 2010) variants within a single gene can disrupt multiple different aspects of a gene's activity, such as work disassociating immunomodulating activities of *Ikbkg* from a role in development (Siggs et al., 2010).

4.2. Using natural genetic variation to identify host genes affecting viral pathogenesis

While forward and reverse genetic approaches have provided important insights into the role that host genes play in the pathogenesis of a number of viral infections, the use of naturally occurring genetic variation within experimental populations has also led to the identification of novel host genes and pathways that affect the host response to viral infection. A wide variety of studies have shown that there is significant variation in response to viral infection between different genetic backgrounds of mice (Staeheli, Grob, Meier, Sutcliffe, & Haller, 1988), plants (Meyer et al., 2009) and other animal systems (Li, Boroevich, Koop, & Davidson, 2011; Rothschild, 2004). Studies in a variety of these systems have utilized classical linkage approaches, while others have leveraged the increased precision and population sizes of experimental populations to utilize QTL analysis to identify host genes associated with specific virus susceptibility phenotypes. These include the use of a variety of mapping approaches and genetic techniques, including F2 and backcrosses, congenic and consomic mouse lines, and a variety of recombinant inbred panels (Fig. 4.2). Because of the additional experimental precision of many of these systems, host factors contributing to both endemic viral pathogens and experimental

models of human pathogens have been identified within these analyses. However, despite the strength of genetic mapping approaches in experimental systems, relatively few such studies have been conducted in systems relevant to human health.

In contrast, QTL studies have been widely used in agricultural systems (Gebhardt & Valkonen, 2001; Hill, 2012; Meyer et al., 2009; Reiner et al., 2002; Rothschild, 2004) with a focus on the development of breeding strategies (Ordon et al., 2004; Spelman & Bovenhuis, 1998) designed to increase disease resistance against damaging pathogens. QTL studies have also been conducted in other nonmodel systems to identify mosquito polymorphisms contributing to susceptibility to both La Crosse (Gomez-Machorro, Bennett, del Lourdes Munoz, & Black, 2004) and dengue viruses (Bosio, Fulton, Salasek, Beaty, & Black, 2000). Such approaches can lead to the development of novel strategies of control of vector-borne pathogens.

While QTL and consomic/congenic mapping approaches in mouse models of human disease have been somewhat limited, these approaches have provided important insights into the role of host genetic variation and its effects on the pathogenesis of a number of acute viral infections, such as Sindbis virus (Thach, Kleeberger, Tucker, & Griffin, 2001), influenza (Boivin et al., 2012; Boon et al., 2009; Nedelko et al., 2012), respiratory syncytial virus (Stark et al., 2010), Sendai virus (Simon et al., 2009), and West Nile virus (Mashimo et al., 2002). In addition, a number of studies in more chronic infections such as Theiler's virus (Bieber et al., 2010; Bureau et al., 1993), mouse cytomegalovirus (MCMV) (Scalzo, Fitzgerald, Simmons, La Vista, & Shellam, 1990; Scalzo et al., 1995; Stadnisky, Manichaikul, Lundgren, & Brown, 2009), gammaherpesvirus (Hardy et al., 2001), and viral-induced tumor development (Velupillai et al., 2012) have been conducted.

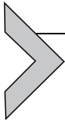
There are several examples of virus resistance genes where a single large effect size gene is largely responsible for the viral phenotype, yet despite the presence of well-characterized, large effect size loci, it is often difficult to use experimental mapping studies to narrow down loci to causative variants. However, there are important exceptions to this issue in the identification of *Oas1b* in West Nile virus infection (Mashimo et al., 2002; Perelygin et al., 2002; Simon-Chazottes et al., 2011) and of *Ly49H* in MCMV infection (Scalzo et al., 1995, 2003). This process is difficult and time-consuming, as illustrated by the identification of *Oas1b* as the causative gene underlying flavivirus resistance in the mouse (Perelygin et al., 2002). The flavivirus resistance locus, *flv*, had been known of as early

as the 1920s, and a congenic inbred strain carrying the resistance allele was developed, with the position of the resistance allele narrowed to a region on chromosome 5, including very tight linkage to a microsatellite marker. A large (300 kb) BAC contig was assembled based off of a probe with this marker. Exon trapping and cDNA selection from this contig were then utilized to identify a small number of genes within the region. Analysis of the susceptible C3H/He mouse strain, and its congenic *flv*-resistant strain, was able to identify polymorphisms in two of these genes, with sequence analysis of various susceptible and resistant mouse lines identifying a C820T transversion encoding a premature stop codon of *Oas1b* in susceptible lines. Follow-up studies in primary cells (Lucas et al., 2003) and in a transgenic mouse model (Simon-Chazottes et al., 2011) confirmed the role of variant *Oas1b* alleles in protection against flavivirus. Despite these difficulties, such positional cloning advances are critical for advancing understanding of viral pathogenesis, since these set the stage for investigating how variant alleles in genes affect specific aspects of virus-induced disease processes.

While a single gene, such as *Oas1b*, can explain much of the observed variation in some viral phenotypes, it is also clear that for most, if not all, virus-induced disease processes, multiple host genome regions contribute to variation in phenotypes such as disease resistance, mortality, or recovery (Bieber et al., 2010; Boon et al., 2009; Nedelko et al., 2012; Simon et al., 2009). Several studies have found multiple genome regions contributing to these processes. For example, a study of highly pathogenic avian influenza (H5N1) in the BxD recombinant inbred panel was conducted (Boon et al., 2009), as clear differences in survival time, disease, and LD_{50} were identified between the BxD progenitor strains C57BL/6J and DBA/2J. Animals from a panel of 66 BxD lines were then challenged with a dose that was lethal for DBA/2J animals, yet sublethal for C57BL/6J animals. This allowed for the identification of 5 QTL, which acted largely additively to each other to influence survival and mortality rate following infection. They also were able to determine that lines with severe responses had higher production of several proinflammatory cytokines in the lungs early postinfection, including TNF-α, CCL2, and type I IFN. By utilizing transcriptional responses from the lungs of C57BL/6J and DBA/2J animals, and narrowing in on transcripts that were differentially induced between these strains following infection, high-priority candidates were identified underneath the various QTL, including hemolytic complement, *Hc*, or *C5*. Analysis of congenic

mouse lines differing only at *Hc* confirmed the role of complement in H5N1 infection.

Two types of results from mouse QTL studies highlight further aspects of the genetic complexity of host responses to viral infections and also illustrate some of the potential difficulties in studying viral infections in human populations and the need for experimental systems. First, several studies across viruses (Boivin et al., 2012; Butterfield et al., 2003) have shown that QTL impacting viral responses can be sex-specific. Second, a study of influenza (Nedelko et al., 2012)-induced disease found that QTL contributing to the host response were limited to specific timepoints in the infection process, where the QTL contributing to clinical disease were seen at days 5 or 6 postinfection, but no evidence for this QTL carried over to later timepoints. Without precise experimental control, such host loci would presumably be missed and highlight the critical need for experimental precision.



5. THE COLLABORATIVE CROSS AS AN INTEGRATIVE GENETIC MAPPING AND SYSTEMS BIOLOGY PLATFORM

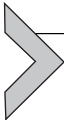
QTL studies in a variety of systems illustrated the utility of genetic mapping approaches and the value of exploring systems containing natural genetic variation (Boon et al., 2009). Similarly, ENU studies (Siggs et al., 2010) illustrated the importance of examining multiple, functionally different alleles at a gene (i.e., allelic series), which also replicates some of the abundant within-gene allelic diversity found within the human population (Boisson-Dupuis et al., 2012). In the early 2000s, a group of researchers began creating a novel mouse GRP to expand the types of genetic mapping studies that could be done, increase the allelic diversity and genetic variation within this panel, and provide more directed integration between human population-level diversity and GWAS results with *in vivo* animal models and systems genetic tools being developed. The collaborative cross (CC) recombinant inbred panel (Collaborative Cross Consortium, 2012) was derived from eight founder strains (the classical lab strains A/J, C57BL/6J, 129s1/SvImJ, NOD/ShILtJ, and NZO/HiLtJ as well as three wild-derived inbred strains CAST/EiJ, PWK/PhJ, and WSB/EiJ). Animals from these strains were bred together in a funnel design, with resultant offspring being bred together to generate a novel inbred line, whose genome is a

mosaic of contributions of the eight founder strains. This process was repeated many times, with the positions of the eight founders in the funnel shuffled to avoid sex chromosome and mitochondrial biases. As with other GRPs (e.g., BxD and AxB), this process created a set of inbred lines that are related to each other but share no recombination events (e.g., each line is related yet independent).

The CC presents several advantages over previous GRPs in that the size of the panel, the breeding design, and the precise selection of the eight founder lines have meant that there is a level of genetic variation within the CC that is roughly equivalent to common variants present within the human population (~45 million SNPs), albeit with a minor allele frequency of ~12.5% (Collaborative Cross Consortium, 2012; Valdar, Flint, & Mott, 2006). In contrast to other GRPs, which have typically been derived from two founder strains, the eight founder strains of the CC mean that (a) this genetic variation is located throughout the genome (i.e., there are no genetic blind spots in this panel) and (b) there can be up to eight functionally distinct alleles at genes within the CC. When taking these data, as well as the size of the CC panel into account, it is clear that the CC can be used to recapitulate many aspects of the human population, allow for the identification of QTL with subtle effects on disease phenotypes (Valdar et al., 2006), and also maintain experimental control and manipulation.

Although the CC and their related resources such as the diversity outbred population (Svenson et al., 2012) are relatively novel, a number of studies of biomedically important traits, including fungal infections and hematologic parameters, have confirmed the utility of the CC for genetic mapping and modeling of outbred populations, such as humans (Aylor et al., 2011; Durrant et al., 2011; Kelada et al., 2012; Mathes et al., 2011). Relevant for studies of viral disease, two studies (Bottomly et al., 2012; Ferris et al., 2013) utilized incompletely inbred animals from a number of CC lines (known as the PreCC population) to study the host response to influenza infection within the CC. In examining a variety of virological, pathological, and transcriptional responses across the entire PreCC population (Ferris et al., 2013), a number of QTL were identified influencing different aspects of the host response to infection. Illustrating the importance of allelic variants, a major QTL was found over *Mx1*. *Mx1* is a well-known anti-influenza gene (Haller, Staeheli, & Kochs, 2007). However, as there are five distinct *Mx1* alleles segregating within the CC, a novel allele that differentiated its antiviral effects from its ability to promote clinical disease resistance was identified in the PreCC study (Ferris et al., 2013). Despite the major role

of *Mx1* in controlling influenza-associated disease, a number of other QTL were identified that contributed to a variety of pathological phenotypes including pulmonary edema and excessive neutrophil infiltration, both hallmarks of severe human influenza infections. A study of extreme responders (those with exceptionally severe disease or high resistance to influenza infection) within this PreCC population (Bottomly et al., 2012) was able to identify and validate a large number of gene expression or eQTL differentiating these classes of animals, and further analysis of these transcripts was able to point to a potential role for the IFN-induced gene *Ifi2712a* as being a potential regulator of a variety of transcriptional responses relating to extreme influenza responses. Although not utilizing CC animals, but instead animals from the CC founder strains, two studies (Peng et al., 2010, 2011) were able to investigate transcriptional responses to both influenza and SARS infection and were able to show both mouse strain-specific and virus infection type-specific transcriptional difference. Furthermore, evidence from both these studies suggests that noncoding RNA species were critical in regulating these host responses. Such results resonate with the preponderance of GWAS studies finding associated SNPs in intergenic regions (McCarthy & Hirschhorn, 2008) and suggest that the CC might provide a powerful tool dissecting these regions.



6. LOOKING FORWARD

Several recent studies (Seok et al., 2013) have critiqued the use of the mouse as a tool for the study of human biomedical traits. Experimental models of viral pathogenesis (e.g., mouse models) that contain genetic diversity represent a critical resource for our understanding of host genetic contributions to viral pathogenesis for several reasons. First, such models can allow for the identification of pathologies that are difficult to logistically and ethically collect in human populations yet play critical roles in pathology (e.g., pulmonary edema during influenza infection (Ferris et al., 2013) and resident tissue titers (Boon et al., 2011)). Second, by being able to assess disease phenotypes at multiple timepoints in genetically identical individuals allows for the linking of genetic control of early disease events to later pathological outcomes (Nedelko et al., 2012). Finally, such experimental systems can inform as to the roles of critical host pathways and polymorphisms when there are extremely small human samples, limiting conclusions that can be drawn by more typical linkage or association approaches (Boon et al., 2009; Zumbrun et al., 2012).

This is not to say that research should remain solely focused on experimental models. Indeed, technological and computational advances have allowed for a variety of highly insightful new approaches into the study of human genetic responses to infection. Whole genome sequencing and whole exome sequencing have also opened up new possibilities for using this approach to identify variant genes that are associated with variation in susceptibility to viral infection. Though still in the early stages of application, these approaches combined with genome-wide linkage analysis have already been used to identify large effect genetic deficiencies associated with susceptibility to severe bacterial infections (Bogunovic et al., 2012). The utility of whole exome sequencing for the identification of causal mutations in ENU mutagenesis projects has also been pointed out (Andrews et al., 2012). Similarly, the availability of whole genome sequence data from the founder animals of both the CC and the BxD and AxB panels (Keane et al., 2011) has allowed for honing in on candidate genes (Ferris et al., 2013; Kelada et al., 2012) and SNPs (Durrant et al., 2011) underneath QTL. These technological and computational advances should shorten the time frame in all genetic mapping studies between initial discovery and positional cloning of causative variants.

Advances in computing and high-throughput molecular profiling have also led to the advent of systems biology approaches. These approaches have been used within human cohorts to identify a number of critical pathways and genes controlling responses to a variety of pathogens and/or vaccinations. Innate responses were found to be critical in the response to dengue fever, but not dengue hemorrhagic fever (Ubol et al., 2008). Similarly, clear profiles of responses were seen to distinguish influenza responses in a population of infected humans (Zaas et al., 2009). Studies of vaccine responses to yellow fever (Querec et al., 2009) as well as influenza vaccines (Nakaya et al., 2011) highlighted several critical regulators of protection, antibody production, and antiviral neutralizing activity following vaccination in human cohorts. Such approaches can guide the development of candidate association studies. Such systems approaches have also been used in various experimental models to identify critical roles for airway epithelial cells in the development of the cytokine storm during H5N1 influenza infection (Li, Bankhead, et al., 2011; McDermott et al., 2011) and also a critical role for NK cells in controlling West Nile virus dissemination (Suthar et al., 2013). Although not explicitly done yet, there has been interest (Law, Korth, Benecke, & Katze, 2013) in utilizing systems approaches in complex yet genetically defined populations such as the CC.

The approaches described within this chapter and earlier represent exciting new tools with which to better understand the role of host genetic variants on viral disease. These approaches can also allow for the integration of human, mouse, and other experimental systems together. However, as our knowledge from various sources has increased, so too has our awareness of specific challenges that need to be addressed.

While numerous GWAS studies have been conducted for a variety of studies, many of the current GWAS studies (McCarthy & Hirschhorn, 2008) have only identified main effect loci (those of small effect yet highly penetrant and working across a range of genetic backgrounds). It is thought that much of the "missing" genetic variation is due to epistatic or gene-by-gene interactions (i.e., specific allelic combinations at two or more loci interact to produce unexpected phenotypic outcomes). Indeed, such gene-by-gene (Ferris et al., 2013), gene-by-environment, and gene-by-demographic interactions (Boivin et al., 2012) have been identified in those limited QTL studies conducted in models of human disease. Furthermore, many studies (e.g., Verschoor, Brockman, Knipe, & Carroll, 2001) have demonstrated that cross talk and interconnectedness between arms of the immune response are widespread. These results suggest that approaches to identify and analyze complex gene interactions are critical for the advancement of the field.

Previous experimental studies within single inbred mouse lines have shown that variant viruses can interact very differently (Cilloniz et al., 2010). However, genetic mapping studies of influenza virus in mice (Boivin et al., 2012; Ferris et al., 2013), including two within the identical BxD panel (Boon et al., 2009; Nedelko et al., 2012), suggest that viral variants interact with different host variants, adding to the complex virus–host interactions in promoting disease. As mapping studies within the human population require large populations of individuals, there is the risk that viral variants will segregate within these human cohorts, clouding the roles of given host polymorphisms.

Though there are certainly challenges associated with defining complex host genetic interactions that determine susceptibility to viral pathogens in both humans and in model systems, recent advances in genetic analysis methods and resources for studying complex genetic interactions suggest that the field will see significant advances in the identification of polymorphic host genes and genetic elements that drive variation in the response to viral pathogens. Furthermore, by carefully integrating human studies with advanced animal models, it should be possible to perform mechanistic

predictive genetic studies to determine how interactions between polymorphic host genes and viral pathogens influence disease outcome. Ultimately, this type of research may lead to more effective vaccines and therapies that showed enhanced efficacy in outbred populations.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Alan Whitmore for helpful discussion. M. T. F. and M. T. H. were supported by U19 AI100625 from NIH.

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