Host Genomics in Infectious Diseases

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Understanding mechanisms by which genetic variants predispose to complications of infectious diseases can lead to important benefits including the development of biomarkers to prioritize vaccination or prophylactic therapy. Family studies, candidate genes in animal models, and the absence of well-defined risks where the complications are rare all can point to genetic predisposition. The most common approach to assessing genetic risk is to conduct an association study, which is a case control study using either a candidate gene approach or a genome wide approach. Although candidate gene variants may focus on potentially causal variants, because other variants across the genome are not tested these studies frequently cannot be replicated. Genome wide association studies need a sizable sample and usually do not identify causal variants but variants which may be in linkage disequilibrium to the actual causal variant. There are many pitfalls that can lead to bias in such studies, including misclassification of cases and controls, use of improper phenotypes, and genotyping errors. These studies have been limited to common genes and rare variants may not be detected. As the use of next generation sequencing becomes more common, it can be anticipated that more variants will be confirmed. The purpose of this review article is to address the issue of genomics in infectious diseases with an emphasis on the host. Although there are a plentitude of studies that focus on the molecular characteristics of pathogens, there are far fewer studies that focus on the molecular characteristics of pathogens, there are far fewer

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Introduction

The purpose of this review article is to address the issue of genomics in infectious diseases with an emphasis on the host. Although there are a plentitude of studies that focus on the molecular characteristics of pathogens, there are far fewer
studies that address the role of human genetics in the predisposition to infection or more commonly its complications. This paper will review both the approaches used to study host genetics in humans and the pitfalls associated with some of these methods. The focus will be on human disease and therefore discussion of the use of animal models will be limited to those where there are genes that have been replicated in humans. The paper will focus on common genetic variants that account for complex traits such as infectious diseases using examples from flaviviruses.

Why study genetic determinants for infectious diseases?

The first question to address is what are the potential benefits of studying genetic variants in humans. While it is true that the virulence of pathogens such as bacteria and viruses often has a molecular basis and this to a limited extent has been addressed as being capable to increasing complication rates in humans, less is known about the human host as derived from human genetic studies. For example, while there have been numerous studies to suggest that the H5N1 influenza virus increases complication rates in humans, much less is known about genetic variants that predispose humans to complications of influenza [1-3]. There are good arguments to support determining genetic variants associated with infectious diseases. The first is that doing so will lead to a better understanding about the mechanism of disease. As discussed below, this is not an easy goal to attain even if significant genetic variant is confirmed to be associated with disease. This is because the marker itself may not be causal but may be linked to another maker that has yet to be determined. However, if a causal variant is eventually discovered, this could lead to functional work to better understand the precise mechanism.

From a practical standpoint, knowledge about genetic variants associated with the risk of infectious complications could lead to the development of biomarkers to predict which individuals will be at high risk for complications of disease. This itself however is not a trivial task, developing biomarkers can take years as both analytic and clinical sensitivity and specificity need to determined and validated. However, it is an area that is lacking in the field of infectious diseases. Having accurate biomarkers could help target individuals for preferential vaccination or prophylactic therapy.

What are clues for genetic susceptibility to infection?

For certain illnesses, family histories give clues to genetic susceptibility. For infectious diseases, exposure to infection in families is common so it can be more difficult to separate exposure from actual susceptibility to infection. This is the situation with tuberculosis where it is difficult to separate risk for tuberculous infection from exposure compared to genetic role. However, using disease due to tuberculous and comparing this to infection would help define genetic predisposition. A study of mortality among adoptees demonstrated a nearly six fold increase in risk of an infectious disease cause of death in those where one of their biological parent died of an infectious disease before the age of 50 years [4]. Twin studies have been an important source of knowledge for genetic predisposition. Although there are not many examples of such studies, influenza is a notable exception. That is, using genealogy data from Utah, it was possible to estimate a greater complication rate in twins due to influenza [5].

One of the most important clues for possible genetic susceptibility to infections, is the paucity of well-defined risk factors. Serious clinical illness due to West Nile virus emerged rather dramatically in North America beginning with a large outbreak in New York City in 1999. Since that time West Nile virus (WNV) emerged as an important human pathogen in North America where it eventually became reported in a majority of states and provinces in the U.S. and Canada [6]. Although the incidence of reported cases has changed from year to year, the severe complications that can occur in infected cases remain a concern. For example, for West Nile virus infection only 1 in 150 individuals who are infected develop severe illness such as meningitis or encephalitis [7]. This is suggestive for a genetic susceptibility in humans. Although the incidence of severe neurological syndromes increases with age and with immunosuppression, there is an absence of other well-defined risk factors, again suggesting that here is an underlying genetic predisposition to complications of disease. For dengue, the situation is similar. Dengue virus (DENV) is found in tropical and sub-tropical regions around the world, predominantly in urban and semi-urban areas [8]. The public health burden is huge; it is estimated that 250 million people or two fifths of the world’s population are at risk from this virus. The World Health Organization currently estimates that there may be 50 million cases of DENV infection worldwide annually [9]. Although the vast majority of dengue virus infections result in no symptoms or a mild febrile illness, approximately 500,000
dengue cases progress to life-threatening disease causing 20,000 to 25,000 deaths annually. The clinical presentation may include fever and an influenza-like syndrome characterized by headache, retro-ocular, and joint pain, rash, and lymphadenopathy; known as classic dengue or "break-bone fever" [10, 11]. Following the febrile phase, the disease may progress to dengue hemorrhagic fever (DHF) characterized by thrombocytopenia and pleural and abdominal effusions and dengue shock syndrome (DSS) (DHF with evidence of systemic hypoperfusion).

Less than 2% of individuals infected with dengue develop dengue hemorrhagic fever (DHF). This suggests that host genetic factors may play an important role. Indeed, there is an absence of DHF in the Haitian population despite hyper-endemic transmission of dengue virus serotypes. Similarly, multiple dengue virus serotypes circulate in West Africa, but there have been no reports of DHF. Furthermore, blacks were less likely to be hospitalized during Cuban dengue virus epidemics. Although pre-existing immunity may be a confounding factor, these reports suggest that genetic predisposition is an important factor as well.

Evidence from animal models can suggest human genetic loci that predispose to infectious disease. Unlike dengue, where there is no animal model, there have been experiments in mice to try to establish a locus for susceptibility for West Nile virus. Innate resistance to flavivirus-induced morbidity and mortality was first demonstrated in mice in the 1920s and showed monogenic autosomal dominant inheritance. These mice are susceptible to infections with other viruses but are resistant to all flaviviruses. Furthermore, within the mouse genus Mus, susceptibility to West Nile virus experimental infection is completely correlated with the occurrence of a point mutation resulting in the truncation of the 2′-5′-oligoadenylate synthetase (2′-5′-OAS) L1 isoform. This would suggest that this enzyme is relevant in WN pathogenesis through an effect restricting viral replication in target tissues [12]. Indeed, the cluster of genes encoding 2′-5′-oligoadenylate synthetases (2′-5′-OAS) has for many years been seen as a prominent candidate locus since they encode a multimer member family of IFN-inducible proteins known to play an important role in the established endogenous antiviral pathway.

**What are the most common approaches to study genetic variants for infectious diseases?**

Although family transmission studies can be conducted as well as analysis of rare immune deficiency syndromes [13-15], the most common way to genetic variants that predispose to infectious diseases is by association studies. The approach is to compare the frequency of alleles in cases to controls. That is, a case can be defined as a patient who developed a complication to infection while the control would be someone who might evidence of infection (e.g. serological evidence) but did not develop complications. Such an approach would ensure that both cases and controls were exposed in a similar manner to the pathogen and the difference in outcome could be inferred to be due to genetic variant in the host assuming the pathogen in cases and controls were similar.

There are two broad types of association studies, candidate gene studies or whole genome association studies (GWAS). Candidate gene studies have an underlying specific hypothesis that a particular variant or variants are causal, that is that typically, a variant (i.e a single nucleotide polymorphism or SNP) at such a locus leads to an amino acid change that ultimately predisposes the patient to disease. Variants in vitamin D receptor provide a good example. The vitamin D receptor mediates the immunoregulatory effects of 1,25-dihydroxyvitamin D$_3$ (1,25 D$_3$), which activates monocytes, stimulating cellular immune responses and suppressing immunoglobulin production and lymphocyte proliferation. The C allele of a SNP at position 352 of the VDR gene has been associated with tuberculoid leprosy, clearance of hepatitis B infection, and resistance to pulmonary tuberculosis [16, 17]. In a study conducted in Vietnam, 327 children admitted to hospital in Vietnam with dengue shock syndrome were compared to 251 ethnically matched healthy controls [18]. Frequency of the variant VDR.I352 was 2% in cases compared to 3% in controls suggesting a possible protective effect (OR: 0.48, 95% CI: 0.21 to 1.09, P = 0.056). It is notable that this study did not have an adequate sample size as is the case with many genetic studies. Such studies are best considered as hypothesis generating and provide preliminary data to support larger studies.

In contrast to candidate gene studies, a GWAS study is based on a genome wide screen for variants that typically will locate a variant which itself may not be causal but may be linked (i.e. in linkage disequilibrium) to the causal variant. These studies are based on the common disease-common variant model which is based on comparisons of common alleles (> 5%). Although candidate gene studies used to be more common, as the cost of chips declined, the number of GWAS studies with large sample sizes has increased. One recent example is a GWAS study of 2,008 children with DSS and 2,018 controls from Vietnam, a susceptibility locus at MICB (major histo-
compatibility complex (MHC) class I polypeptide-related sequence B) was identified [19]. It was within the broad MHC region on chromosome 6 but outside the class I and class II HLA loci (rs3132468, $P_{	ext{meta}} = 4.41 \times 10^{-11}$, per-allele odds ratio (OR) = 1.34 (95% confidence interval: 1.23-1.46) [20]. Another locus identified was within PLCE1 (phospholipase C, epsilon 1) (rs3765524, $P_{	ext{meta}} = 3.08 \times 10^{-10}$, per-allele OR = 0.80 (95% confidence interval: 0.75-0.86). This study is typical of many GWAS studies because the genetic variants that were found to be associated with disease were completely unexpected and certainly not a hypothesis that the investigators were testing. It is also typical in that these variants are likely not causal themselves but perhaps linked to causal variants.

What are possible pitfalls of association studies?

One of the most important considerations is to choose appropriate cases and controls. This is a similar principal to other types of case control studies. As mentioned above, exposure to an infectious agent can confound the relationship between variants and disease. Therefore, selecting individuals with microbiologic evidence of exposure to infection is often worthwhile. While it can be argued that there may not be a difference between exposed individuals who do not become ill and non-exposed individuals, there is always a possibility for genetic factors to predispose to infection (as opposed to complications) and this would argue for the choice of exposed individuals as controls in order to focus genetic variants associated with complications. For example, a recent GWAS study for West Nile virus compared cases with neuroinvasive disease to controls who were symptomatic (i.e. had West Nile fever) with serologic evidence of infection but who did not have complications [20]. It is also extremely important to carefully define the phenotype for cases and for the controls. There is empiric evidence that misclassification of the phenotype can have a profound effect on the results [21]. Defining the phenotype well is also important because it can have an impact on replication studies. That is, using a different phenotype in a replication study may lead to lack of replication of a particular variant because the original definition was not used and it may have biological implications [22]. While assessing the effect of a variant across various ethnic groups is a laudable goal this may lead to bias if the structure of the population is not taken into account. This is because of alleles at a particular locus can change from population to population and if it not balanced cases and controls lead to spurious associations due to allelic differences i.e the relationship between the disease and the candidate gene is confounded by the ancestry of the population [23]. So in this case a particular disease may be more common in a population with a high frequency of a particular allele but the disease is not due to the allele but due to another factor. Approaches to guard against such population stratification is to either study one particular ancestry or to using principal components analysis to adjust for the population structure.

Having excellent quality control procedures for genotyping is imperative. Quality checks based on the sex and ethnicity of the phenotype compared to what has been genotyped. It is also important to have a high call rate (correctly attributing a SNP that has been genotyped to the appropriate genotype). Sometimes unbeknownst to the investigator, two or more individuals in the cohort being tested may be related (known as cryptic relatedness). Since the methods for analysis are based on unrelated or independent individuals only one of the related individuals can be kept in the analysis. Genotyping error can also occur when the distribution of cases and controls on plates is not at random. For example, when all cases are genotyped separately from controls differential failure of SNPs in cases compared to controls may create an imbalance that can lead to biased results.

As mentioned above, sample size is often a limitation in infectious diseases genetic association studies. Unlike conditions that are highly prevalent in many populations, such as diabetes or high cholesterol, it can very challenging and expensive to create large cohorts of individuals with complications of infectious diseases. Because of this, it can be difficult to obtain adequate sample sizes. Obtaining an adequate sample size is key because in a GWAS the $P$ value for significance is $5 \times 10^{-8}$, in order to account for the high number of SNPs being compared which typically is over 500,000 to 2 million SNPs. Generally speaking, investigators should aim to have at least 1,000 cases and 1,000 controls in the first phase of a GWAS.

Examples from the flavivirus literature

Some challenges mentioned above are evident in examples of association studies using flaviviruses. For West Nile virus, one candidate gene studied 33 West Nile virus infected individuals who had developed either fever, meningitis, or encephalitis [24]. They were compared to 60 healthy controls from an available database and therefore were unlikely to
have been exposed to the virus. The study identified one synonymous SNP in OASL exon 2 as being at significantly higher frequency in the cases compared to the controls ($P < 0.0044$). However, no adjustment was made for multiple testing and there was no attempt at replication. An important principle in genetic studies is that results, either of candidate gene studies or association studies, should be replicated in an independent population. The $P$ value needs to be adjusted for multiple testing because of the large number of comparisons being made.

More recently, another candidate gene study examining OAS gene cluster alone again suggested a predisposition to WNV infection [25]. This study compared OAS variants in a cohort of symptomatic individuals (fever, meningitis, encephalitis) and asymptomatic but infected individuals identified through a blood donor bank to a non-infected cohort. There were no genetic variants associated with severity of disease, in keeping with a larger association study recently reported [20]. However, the authors reported that a SNP (rs10774671) was significantly more frequent in West Nile virus infected than non-infected individuals ($P = 0.0002$). Limitations include the fact that there was no replication in a separate cohort, although this SNP did have an effect on viral replication in an ex-vivo model of primary human lymphoid tissue [25].

One study compared symptomatic to asymptomatic individuals with WNV infection and found that SNPs in the interferon pathway (IRF3 and MX1) were associated with symptoms [26]. In contrast to other studies that examined complications, they also found that OAS1 was associated with an increased risk for encephalitis and paralyis. An association between symptomatic WNV disease and homozygosity for the CCR5Δ32 mutation in the chemokine receptor gene CCR5 was initially reported [27, 28]. However, this association was not replicated but was suggestive of a link to clinical manifestations of infection with CCR5Δ32 mutation [29]. Moreover, in a recent large association study (560 neuroinvasive cases and 950 controls and a replication cohort of 264 cases and 296 controls) no such evidence for an effect was noted [20]. One possible reason for the discrepancy is that the latter study compared cases to controls all of whom were symptomatic when infected with WNV. In contrast, the published reports compared cases to controls with no symptoms.

**Future directions for GWAS in the field of infectious diseases**

To date, although there have been many important association studies that have led to biological insight in the field of infectious diseases, the vast majority of results, similar to those in other fields, have been relatively low effect sizes (odds ratio < 2). Such results do not provide an optimal basis for screening since the risk of disease complications may already be quite low (as with flaviviruses < 1%) and a low odds ratio will not lead to a substantial increase in absolute risk. However, with the advent of next-generation sequencing, we may be able to anticipate the discovery of more rare alleles that may have a greater effect size and thus help bridge the gap between biological discovery and progress in clinical medicine [30].

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