Host susceptibility to persistent hepatitis B virus infection

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Abstract

Genetic epidemiology researches such as twin studies, family-clustering of hepatitis B virus (HBV) infection studies and ethnic difference studies have provided the evidence that host genetic factors play an important role in determining the outcome of HBV infection. The opening questions include which human genes are important in infection and how to find them. Though a number of studies have sought genetic associations between HBV infection/persistence and gene polymorphisms, the candidate gene-based approach is clearly inadequate to fully explain the genetic basis of the disease. With the advent of new genetic markers and automated genotyping, genetic mapping can be conducted extremely rapid. This approach has been successful in some infectious diseases. Linkage analysis can find host genes susceptible to HBV and is of great clinical importance.

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Key words: Hepatitis B virus; Susceptibility; Association study; Linkage study

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INTRODUCTION

Hepatitis B virus (HBV) infection is a serious global health problem, with 2 billion people infected worldwide, and 350 million suffering from chronic HBV infection. HBV infection results in 500,000 to 1.2 million deaths per year caused by chronic hepatitis, cirrhosis, and hepatocellular carcinoma and is the 10th leading cause of death worldwide. The mechanisms of persistent HBV infection are not fully understood, but they seem to involve several aspects and the genetic component, in particular, is still controversial. Early studies by Blumberg et al. have also suggested a recessive mode of inheritance for HBV viral persistence, but this is perhaps an oversimplification giving the more recent advances in knowledge of the effect of maternal viral infection and the transmissibility of the virus.

Generally, exposure to HBV can cause a broad spectrum of infection. Ninety to ninety-five percent of adults infected with HBV can eliminate the virus and only 5%-10% of them become chronic HBV carriers. 20%-30% of chronic HBV carriers develop chronic hepatitis B (CHB) and 5% of them develop liver cirrhosis and hepatocellular carcinoma in a long term of disease course. Some rare cases result in a fulminant infection in which the liver is rapidly overwhelmed and ultimately fails. What factors determine why one develops a life-threatening infection, whereas another carries HBV as a harmless commensal or limits the infection to a clinical trivial episode? There is evidence that host genetic factors play an important role in determining the outcome of HBV infection.

EPIDEMIOLOGY EVIDENCE FOR HUMAN GENETIC SUSCEPTIBILITY TO PERSISTENT HBV INFECTION: TWINS, FAMILY-CLUSTERING OF HBV INFECTION AND ETHNIC DIFFERENCES

Twin studies

Studies of susceptibility to diseases in identical and non-identical twins are extremely useful in evaluating the importance of inherited or environmental factors in disease susceptibility. If the concordance rates for infection and clearance of HBV are significantly higher in monozygotic (MZ) than in dizygotic (DZ) twins, the process of HBV infection and persistence is more genetically decisive. Lin et al. studied 289 pairs of MZ twins, 102 pairs of DZ twins and 375 pairs of age-sex-matched singleton controls and found that there is a significant difference in the concordance of HBV infection between MZ and DZ twins and controls, suggesting that the genetic influence occurs in response to HBV infection. Xu et al. also found that not only the concordance rate of infection, but the concordance of clinical phenotype and serological
patterns between MZ and control groups is significantly different, indicating that genetic factors influence not only susceptibility to infection but also clinical outcome.

Genetic factors not only influence host response to HBV infection, but also affect the response to HB vaccine. Hohler et al.\(^{[1]}\) prospectively studied and vaccinated 202 twin pairs with a combined recombinant HBsAg vaccine and found that the heritability of anti-HBs immune response is 0.61, which means that 60% of the phenotypic variance of responsiveness to HB vaccine can be explained by genetic effect and 40% by environmental effect.

**Family-clustering of HBV infection**

Most of us do not inherit single-gene diseases. We all, however, inherit slightly different variants of each of our pairs of 30,000 genes. These differences may determine whether we are more or less likely to develop particular health problems or diseases than other people. Genes are shared within families. Because we inherit genes from our parents, a parent who has inherited a particular gene mutation generally means that each child has a fifty-fifty chance of having the same mutation. In fact, many cases of diseases not showing the clear inherited patterns of single-gene diseases, show family clustering patterns that are due, at least in part, to genetics. Substantial genetic epidemiology studies indicate that HBV spreads in families. The familial occurrence of HBV infection has been established in some ethnic groups. Ohbayashi et al.\(^{[2]}\) have reported 3 Japanese families in which 36 of the 54 members are HBsAg positive. Of these, some are healthy carriers while others have liver cirrhosis and hepatocellular carcinoma. Similar observations have been reported in American\(^{[3]}\), European\(^{[4]}\) and Asian\(^{[5,6]}\) continents.

This observed familial clustering may stem from inherited defects in specific genes, from shared environmental exposures among family members or from interaction between specific genetic and environmental factors. If a trait has a genetic basis, the relatives of affected individuals will be affected more frequently than the relatives of unaffected people, and the prevalence of disease decreases from monozygotic (MZ) twin to the first-, second- and third-degree relatives. If the disorder has an environmental basis only, the possibility of infection in each family member is equal\(^{[7]}\). Tong et al.\(^{[8]}\) reported that HBV markers are detected more frequently in blood relatives than in non-blood relatives of the index cases in family. Wang et al.\(^{[9]}\) also showed that HBsAg carrier rate decreases in the order of the first, second and third degree relatives, indicating that it is the defect gene shared by family members that produces the epidemiological characteristics of family-clustering HBV infection.

**Ethnic differences**

Another method used to investigate the role of host genetics in infectious diseases is to look for differences in clinical disease and immune response between different ethnic groups having equal exposure to the same pathogen. Carrilho et al.\(^{[10]}\) determined the frequency of HBV markers of genetically related (consanguineous) and non-genetically related (non-consanguineous) Brazilian families of Asian origin and Western origin and found that the occurrence of HBsAg is significantly higher \( (P < 0.0001) \) in family members of Asian origin (81.8%) than in those of Western origin (36.5%), which is in line with the high HBsAg prevalence in Asian countries and the relatively low HBsAg prevalence in Western countries\(^{[21]}\). Though the Asians live in Brazilia, a country with a low HBsAg prevalence, and the environment has changed, disease-related genes remain shared within the ethnic group, indicating that Asians possess the HBV susceptible gene(s). This is why they are more susceptible to HBV.

Tong et al.\(^{[20]}\) tested family members of Asian and non-Asian patients for HBV markers, and found that Asian family members have a significant increase in HBsAg (34% higher) and antibodies to HBsAg or to hepatitis B core antigen (50% higher) compared with the non-Asian family members. Moreover, birthplace, either in Asia or in United States, does not influence the frequency of antigenemia. In China, the prevalence of HBsAg is 19.1% in Mongoloid populations\(^{[22]}\), and 10% in Chinese Han populations in the same area. These studies have provided important insights into the fact that different ethnic in the same region have different HBV epidemiological characteristics and the same races in the different region share the same prevalence of HBV markers, indicating that genetic factors may play a role in maintaining the frequency of HBV infection and persistence. Moreover, molecular epidemiology study has identified several genetically determined differences between races.

Taken together these epidemiological data provide strong evidence for a genetic predisposition to HBV infection and raise the questions of which human genes are important in infection and how to find them.

**TWO METHODS USED TO IDENTIFY HBV SUSCEPTIBLE GENES**

Analysis of the human genome has focused primarily on variations that occur between people in their DNA sequence.\(^{[23]}\) Because these differences contribute to the differences in our susceptibility to developing specific diseases, naturally occurring genetic variations in the human genome are frequently found (about every 3 to 500 bp) most often in the form of a change from one base to another, namely a single nucleotide polymorphism (SNP)\(^{[24]}\). Other common forms of variation include microsatellite where a short sequence, usually a dinucleotide repeat is bound, so that one person might have 10 and 12 copies of the repeating motif and others have 9 and 11 copies. If the repeating sequence is longer, the motif is known as a minisatellite.\(^{[25]}\) They are widely used to determine similarities and differences of human and hunter disease-related genes. Because this kind of genetic variations often varies between individuals (i.e., it is highly polymorphic), microsatellites are particularly informative in the genetic sense\(^{[24]}\). Analysis of genetic susceptibility to HBV infection aims to link these DNA variations (the genotype) with a particular HBV infection (the phenotype). HBV infection and clearance are complex traits\(^{[27]}\), meaning that the genetic contribution to them is not inherited in a
simple Mendelian manner and several polymorphic genes exert effects on the outcome. Many possible approaches to mapping the genes underlying complex traits fall broadly into two categories: candidate gene-based association studies and genome-wide linkage studies.

**Association studies**

Association studies compare the frequency of alleles or genotypes of a particular variant between disease cases and controls to link the genotype with the particular phenotype. Such studies are widely used to investigate inflammatory and infectious diseases. Repeat sequences, such as those of microsatellites, lend themselves less well to association studies because they are intrinsically unstable and may undergo considerable mutations over successive generations and disease-modifying polymorphisms may have arisen many hundreds of generations previously. SNPs, on the other hand, are stable, common and increasingly amenable to high throughput automated genotyping. A number of studies have sought genetic associations between HBV infection/persistence and gene polymorphisms (Tables 1 and 2).

| Gene/loci       | Population | Sample size | P value | Reference |
|-----------------|------------|-------------|---------|-----------|
| HLA A 0301      | Caucasian  | 563         | 0.0005  | [30]      |
| HLA-DRB1 1302   | Caucasian  | 563         | 0.03    | [30]      |
| HLA-DRB1 1302   | Gambian    | 638         | 0.012   | [31]      |
| HLA-DRB1 1101/1104 | Chinese   | 190         | 0.0145  | [32]      |
| HLA-DQA1 0301   | Chinese    | 190         | 0.0167  | [32]      |
| HLA-DR13        | Korean     | 1272        | < 0.001 | [33]      |
| TNF-alpha-238 GG genotype | Chinese | 895         | 0.041   | [34]      |
| TNF-alpha-308 A | Korean     | 1400        | < 0.001 | [35]      |
| TNF-alpha-857 TT genotype | Chinese | 355         | 0.02    | [36]      |
| CTLA-4-172 C    |            | 0.06        |         | [37]      |
| CTLA-4-49 G     |            | 0.02        |         | [37]      |
| CCR5 59029 G allele | Chinese | 377         | 0.001   | [38]      |

**Table 2 Gene polymorphisms associated with susceptibility to chronic hepatitis B**

| Gene/loci       | Population | Sample size | P value | Reference |
|-----------------|------------|-------------|---------|-----------|
| HLA B 08        | Caucasian  | 563         | 0.03    | [30]      |
| HLA B 44-Cw 1601| Caucasian  | 563         | 0.02    | [30]      |
| HLA B 44-Cw 0501| Caucasian  | 563         | 0.006   | [30]      |
| HLA-DRB1 0301   | Chinese    | 190         | 0.0074  | [32]      |
| HLA-DRB1 1301/2 |            |             |         |           |
| HLA-D6         | Korean     | 1272        | < 0.001 | [33]      |
| HLA-DQA1 0501   | Chinese    | 190         | 0.0157  | [32]      |
| HLA-DQA1 0501   | African American | 91 | 0.05   | [40]      |
| HLA-DQB1 0301   | African American | 91 | 0.01   | [40]      |
| HLA-DQB1 0301   | Chinese    | 190         | 0.0075  | [32]      |
| TNF-alpha-863 A | Korean     | 1400        |         | [35]      |
| TNF-alpha-238 GG genotype | Chinese | 355         | 0.02    | [36]      |
| TNF-alpha-857 CC genotype | Chinese | 895        | < 0.001 | [34]      |
| IFN-gamma A/A   | Chinese    | 77          |         | [42]      |
| CTLA-4+6230 A   |            | 0.04        |         | [37]      |
| CCR5 59029 A allelic genotype | Chinese | 377         | 0.002   | [38]      |
| ESR1 29 T/T genotype | Chinese | 2318       | < 0.001 | [43]      |

The huge variation in clinical response to identical infecting pathogens is due to the combined effects of genetic variation both in the infecting pathogen and in the infected host. Its ability to mount an effective immune response to infection is a powerful evolutionary selection pressure, contributing to human genetic diversity. The advantage of a flexible immune response, allowing an efficient response to diverse pathogens without damage to the host, is reflected in marked genetic variability of immune-related genes among (both in DNA sequence and in protein structure) in the entire human genome.

The prototype region for genetic association studies is the human leukocyte antigen (HLA) loci involved in antigen processing and presentation. HLA associated with infections such as AIDS, tuberculosis, leprosy, malaria, and persistence of hepatitis-C virus has been well-described. This is most obvious within the HLA region, where functional variation has arisen as a strategy to combat pathogen antigenic diversity. Indeed in HBV infection, maximal HLA variation appears to have a direct protective effect, individuals with the most different alleles at class II have the lowest HBV disease progression and the lowest mortality (a “heterozygous advantage”). Conversely, lack of HLA diversity (a “homozygous disadvantage”) may increase the susceptibility to HBV infection among isolated communities. The extensive linkage disequilibrium across some HLA regions makes it difficult to localize specific disease-associated polymorphisms, although the HLA allelic association has allowed identification of critically pathogenic epitopes in some diseases, which might act as potential vaccine candidates.

Disease associations involving loci outside the HLA region are also valuable in identifying the functional molecular basis underlying infectious disease resistance. For example, HIV uses various chemokine receptors as cofactors for CD4 binding to gain entry into human leukocytes. A functional polymorphism of the chemokine receptor CCR5, which is essential for HIV entry into macrophages, results in a truncated nonfunctional protein that confers highly significant protection against HIV susceptibility in the homozygous state and slows disease progression in heterozygotes. Chang et al. have developed the association between CCR5 and HBV infection, though the biological process and significance in HBV infection need to be further studied.

**Shortcoming of association studies in susceptible gene hunting**

The number of studies seeking to identify genes that influence susceptibility to persistent HBV infection has greatly increased since we entered the “post-genomic” era. These studies are fuelled by the unlimited availability of...
SNPs, the relative ease of performing genotyping assays based on PCR technology, and the desire to identify major disease susceptibility gene(s). Literature is now littered with unreproducible genetic association studies that confuse the readers and have an understandable impact on the willingness of editors to accept further manuscripts for publication[55]. Nature Genetics published an editorial in 1999 that set out a list of criteria for genetic association studies[56]: plausible biological context, rigorous phenotypic selection (case selection), independent replication, rigorous genotyping, low P values, appropriate statistical analysis, and transmission disequilibrium test. Up to now, few candidate genes can fully meet the criteria.

Candidate gene-based association studies rely on having predicted the identity of the correct gene or genes, usually on the basis of biological hypotheses or the location of the candidate within a previously determined region of linkage[57]. Even if these hypotheses are broad (for example, involving the testing of all genes in the insulin-signaling pathway), they will, at best, identify only a fraction of genetic risk factors for diseases in which the pathophysiology is relatively well understood. When the fundamental physiological defects of a disease are unknown, the candidate-gene approach is clearly inadequate to fully explain the genetic basis of the disease[58]. In 2004, Hepatology editor appealed for less hypothesis-driven association studies that result in a negative or weak correlation[59].

### Linkage studies

Linkage is the tendency for genes and other genetic markers to be inherited together because of their location near one another on the same chromosome. Linkage studies classically seek to identify microsatellite markers that are inherited more commonly than expected by siblings who have the disease of interest (“affected sibling pairs”) [60]. Genetic linkage analysis is a powerful tool to detect the chromosomal location of disease genes[58]. A linkage study is to use a large number of families to look for regions of linkage to a disease, which suggest the presence of loci containing genes that may predispose to this disease. Linkage studies have the advantage of making no supposition about which genes might be involved in a disease, in that they merely identify stretches of chromosome around the microsatellite markers and can be used to examine the entire genome (a “whole genome screen”) [58].

With the advent of new genetic markers and automated genotyping, genetic mapping can be conducted extremely rapid[28,59]. This approach has been successful in some infectious diseases (Table 3), but no report on such similar scans for HBV viral persistence is available. Recently a research team of Xi’an Jiaotong University has collected 327 HBV-infected subjects of 32 family pedigrees from a remote village (data not published), which makes it possible to find chromosome regions containing determinant(s) of persistent HBV infection. Their results will be reported soon.

### CLINICAL IMPLICATION OF GENETIC STUDIES OF HBV INFECTION

Studies of the genetic determinants for HBV susceptibility can reveal fundamental data concerning the human immune system. The ultimate goal of such studies is the identification of critical immunologic mechanisms in the disease process to develop specific therapeutic interventions. As the precise immune deficiency is identified, it may be possible to “bypass” the identified immune deficiency with a specific therapy.

A specific genetic defect has been identified in rarer single gene defects, which may offer preconception genetic counseling to affected families. In complex diseases it might ultimately be possible to identify patients whose risk factors make them candidates for targeted therapies. Once the genotypic markers for a poor outcome of HBV infections are found, they in combination with rapid genotyping technology may allow more intensive therapies for those patients who are at the greatest risk of poor outcome and death[70,71]. The potential to target drug treatment, both in terms of identifying patients most likely to benefit clinically and in terms of predicting those who are susceptible to either favorable or adverse pharmacologic outcome, is of great importance. It is conceivable that in the future our understanding of host genetics will largely influence our therapeutic response to HBV-infected patients and determine our choice of both preventive and curative interventions.

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