

## Host genetics and the outcome of hepatitis B viral infection

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### Abstract

Hepatitis B Virus (HBV) infection can result in numerous different clinical outcomes. A complex combination of environment, and viral and host genetic factors play a critical role in determining both susceptibility to HBV persistence and the course of infection. Evidence is presented that suggests that host genetic factors play an important role in determining the outcome of HBV infection. This data from various groups demonstrates that multiple genes play a role in determining hepatitis B viral clearance or persistence. However, to identify all the relevant variants that affect the outcome of infection, alternative strategies such as genome-wide association studies with large sample sizes will be required to define the majority of the relevant polygenes.

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### 1. Introduction

Hepatitis B is a globally important disease. Approximately one million deaths in the world each year are attributable to the end stage sequelae of persistent HBV infection. HBV infection results in persistence of the hepatitis B surface antigen (HBsAg) in around 10% of cases; the remaining 90% of infections are considered to be acute infections, although some rare cases do result in a fulminant infection in which the liver is rapidly overwhelmed and ultimately fails. The mode of transmission of the infection varies from a mainly vertical transmission in the perinatal period in South East Asia, to horizontal transmission via an unknown route in sub-Saharan Africa. It is considered that in Africa by the age of seven, more than half of the population will have been infected [1], whereas around 15% of children fail to clear the virus.

Persistence of the hepatitis B virus can, in some cases, lead to end stage complications of infection such as liver cirrhosis, liver failure or primary liver cancer (hepatocellular carcinoma or HCC). Around 17% of Chinese HBV

persistent cases present with clinical manifestations of disease such as liver damage from active replicating HBV infection and elevated liver enzymes [2]. Factors known to influence the outcome of infection are not as yet fully understood, but may be classified into three categories, virological factors, immunological factors and host genetic factors. Virological factors such as viral load, viral genotype and mutations in the viral genome itself are known to be important. For example, the hepatitis B virus mutates rapidly and uses this variability as an escape mechanism from the host's immune response.

Immunological factors, such as the adaptability of the host's immune response to the rapidly evolving T cell epitopes, are clearly important in the role of the humoral and cell-mediated immune responses in controlling the virus [3,4]. By far the most research thus far has been done on these two types of factors, but an area of increasing interest is that of host genetic factors.

Evidence for a role for host genetics was originally provided by a twin study of HBV disease concordance in Chinese twins [5] in which the concordance of HBsAg carriage was significantly higher in monozygotic twins when compared to dizygotic twins and pairs of singleton births. Additionally a report from Turkey described the presentation of monozygous twin brothers with HBV

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associated HCC who were diagnosed simultaneously [6]. Early studies by Blumberg et al. also suggested a recessive mode of inheritance for HBV viral persistence, but this is perhaps an oversimplification given the more recent advancements in the knowledge of the effect of maternal viral infection and the transmissibility of the virus [7]. Further evidence of a genetic component to determining the outcome of HBV infection is provided by the discovery of a difference in the ratio of male: female persistent infections [8]. Males are approximately 1.5 times more likely to develop a chronic infection than females. One factor that may explain this is the apparently slower rate of HBsAg disappearance from the plasma in males [9].

## 2. Host genetics and HBV outcome

Several different, yet complementary approaches to the identification of genetic variation important in the course of infectious disease progression have been taken. A recent method has been to use large numbers of families to look for regions of linkage to a disease, which suggest the presence of locus containing genes that may predispose to infection. This approach has been successful in other infectious diseases such as malaria, TB and leprosy, but as yet any such similar scans for HBV viral persistence remain unreported [10–15]. By far the most common approach used for hepatitis B viral persistence has been to look for association in candidate genes using case-control studies. In general, large sample sets are needed to detect even moderate genetic effects in order to eliminate the possibility of false positive associations. Often, this has not been the case, leading to examples of inconsistent findings in genetic association studies, a problem not specific to the study of infection [16]. However, publication bias may have resulted in many negative findings going unreported. A major problem of this candidate gene approach is in the problem of selection of appropriate candidates but the record of successful guessing in infectious diseases in general is reasonably good, perhaps because genes related to infection resistance have higher levels of variation than most.

The vast majority of published studies of HBV persistence relate to the role of the major histocompatibility complex, or MHC, in determining the outcome of infection. The most convincing evidence refers to associations between HBV carriage and MHC class II molecules. Antigen presenting cells present fragments of pathogens such as viruses to CD4+ T helper cells in the cleft of the MHC class II molecules, enabling the CD4+ cells to support the effector arm of the immune response by secreting cytokines and proliferating. In a study of Gambians it was found that the allele DRB1\*1302 was associated with clearance of the virus [17] and this finding was subsequently replicated in a European population [18] and a study of Caucasians from the US [19]. A study in Qatar found that DR2 was associated

clearance and DR7 with persistence of the hepatitis B virus [20]. Whilst this report might at first glance seem inconsistent, the frequencies of alleles in these populations vary tremendously perhaps explaining the apparent inconsistency.

Class I MHC molecules also form enticing candidate genes for the analysis of HBV persistence. Hepatocytes infected with HBV present viral antigens via class I molecules to CD8+ cytotoxic T cells (CTL). In acute or self-limiting infections, HBV-specific CTLs can be easily detected in peripheral blood [21,22]. However, in established persistent infections CTL are not detectable in the liver despite the presence of CD8+ lymphocytes there, suggesting that polymorphism of class I loci have the power to influence the ability of the host to clear HBV. Still, studies demonstrating convincing class I association with HBV persistence are rare. In a study of US Caucasians, Thio et al. found two alleles from the MHC were associated with HBV outcome [19]. Allele A\*0301 was associated with viral clearance whilst B\*08 was associated persistent infection.

Non-MHC genes have also proved interesting and successful candidates for association studies of hepatitis B viral persistence. The vitamin D receptor (VDR) is expressed on the surface of white blood cells and activation of this receptor is thought to influence the immune response. A number of single nucleotide polymorphisms (SNPs) in the gene that encodes this receptor are thought to influence transcription efficiency of the gene. An allele at one such SNP in the VDR gene which is thought to increase the efficiency of transcription was reported to be associated with the clearance of HBV in Gambians [23].

Studies of the promoter of the tumour necrosis factor alpha gene (TNF $\alpha$ ) have revealed the presence of a number of polymorphisms that influence the amount of gene transcribed. In a study of Europeans it was found that a SNP at position –238 (with respect to the transcription initiation site) was associated with HBV infection outcome, although the allele associated with clearance in this study was correlated with higher TNF $\alpha$  secretion [24].

Cytotoxic T-lymphocyte antigen 4 (CTLA4) is an inhibitory receptor expressed by T lymphocytes that acts as a negative regulator of T cell responses [25]. Thio et al. investigated the role of this gene with respect to its influence on the vigor of the T cell response to hepatitis B infection. They used a novel approach in which common polymorphisms at a locus are genotyped in order to recreate the haplotype distribution in the population [26]. The haplotype distribution across this gene does differ between those with persistent HBV and those who have cleared, although not significantly. However, when analysing the haplotypes individually two of the haplotypes were associated with clearance of HBV and the most common haplotype was associated with persistent HBV infection, suggesting that variation in this gene does indeed influence the ability to clear HBV.

### 3. Concluding remarks

There are other aspects of HBV infection, beyond the scope of this review, that are also interesting from a geneticists' point of view. Studies of the genetic determinants of HBV vaccine response or development of hepatocellular carcinoma are underway and results from these may provide interesting insights into not only the particular aspect under consideration, but of the mechanisms of HBV infection in general. Indeed studies have already demonstrated overlap between the factors that predispose to persistent HBV infection and non-response to HBV vaccination [19].

An important factor to consider in the analysis of HBV persistence is that results, particularly those involving populations recruited in the developing world, may be confounded by the presence of other infectious diseases. One striking example is demonstrated by Thursz et al., who reported a significant association between HBV persistence and severe malaria infection in The Gambia [27].

Strategies for studying the outcome of HBV infection could, with the advent of new technology and the release of both the draft human genome sequence [28] and the data from the HapMap project [29], undergo vast changes. Whole genome wide screens of SNPs for associations are now firmly within the realms of possibility allowing the discovery of many new genes that influence outcome of HBV infection. Such data should provide far greater insights into HBV disease pathogenesis than has been possible with the few dozen candidate genes that have been evaluated to date. It is also feasible, given the advancements in technology and the drop in genotyping costs, to now study many more SNPs than were traditionally analysed which will considerably increase the number of genes that can be investigated. Additionally, the use of microarray technology could enable the identification of novel candidate genes on the basis of differential expression e.g. in HBV persistent cases versus those who have cleared the infection [30]. Finally, clues as to what genes affect response to infection in humans are being found in the analysis of outcome of infection in model organisms such as mice or drosophila [31–33]. A final clue to the identification of genes important in HBV disease outcome could be suggested by the analysis of which host genes the virus chooses to insert itself into. A recent report detected the insertion of the HBV genome in a patient with acute infection had integrated into a TNF induced protein, which given the central role of TNF $\alpha$  in HBV infection provides an intriguing suggestion that this may be worth further investigation [34].

Evidence is amassing that the outcome of HBV infection is, at least in part, under the control of many polygenes. However, it is clear that host genetics alone cannot account for the large amount of individual and ethnic differences in response to HBV infection. For example, it has been shown that the age of acquisition of infection is a major factor as

this differs greatly between sub-Saharan Africa and economically developed countries [35]. It is therefore likely that answers to the riddle of the dichotomous outcome of HBV will be provided by a complex analysis of many host genetic factors, along with those provided by the virus and the environment. Answers to this puzzle will aid in the future development of therapeutic and preventative strategies for HBV infection and disease progression.

### References

- [1] Ryder RW, Whittle HC, Wojcickowsky T, Moffat WM, Baker BA, Sarr E, et al. Screening for hepatitis B virus markers is not justified in West African transfusion centres. *Lancet* 1984;2(8400):449–52.
- [2] Wang FS. Current status and prospects of studies on human genetic alleles associated with hepatitis B virus infection. *World J Gastroenterol* 2003;9(4):641–4.
- [3] Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. *Annu Rev Immunol* 1995;13:29–60.
- [4] Guidotti LG, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol* 2001;19:65–91.
- [5] Lin TM, Chen CJ, Wu MM, Yang CS, Chen JS, Lin CC, et al. Hepatitis B virus markers in Chinese twins. *Anticancer Res* 1989;9(3):737–42.
- [6] Demir G, Belentepe S, Ozguroglu M, Celik AF, Sayhan N, Tekin S, et al. Simultaneous presentation of hepatocellular carcinoma in identical twin brothers. *Med Oncol* 2002;19(2):113–6.
- [7] Hann HW, Kim CY, London WT, Whitford P, Blumberg BS. Hepatitis B virus and primary hepatocellular carcinoma: family studies in Korea. *Int J Cancer* 1982;30(1):47–51.
- [8] London WT, Drew JS. Sex differences in response to hepatitis B infection among patients receiving chronic dialysis treatment. *Proc Natl Acad Sci U S A* 1977;74(6):2561–3.
- [9] Craxi A, Montano L, Goodall A, Thomas HC. Genetic and sex-linked factors influencing HBs antigen clearance: I. Nonimmune clearance in inbred strains of mice. *J Med Virol* 1982;9(2):117–23.
- [10] Bellamy R, Beyers N, McAdam KP, Ruwende C, Gie R, Samaai P, et al. Genetic susceptibility to tuberculosis in Africans: a genome-wide scan. *Proc Natl Acad Sci U S A* 2000;97(14):8005–9.
- [11] Jepson A, Sisay-Joof F, Banya W, Hassan-King M, Frodsham A, Bennett S, et al. Genetic linkage of mild malaria to the major histocompatibility complex in Gambian children: study of affected sibling pairs. *BMJ* 1997;315(7100):96–7.
- [12] Marquet S, Abel L, Hillaire D, Dessein A. Full results of the genome-wide scan which localises a locus controlling the intensity of infection by *Schistosoma mansoni* on chromosome 5q31–q33. *Eur J Hum Genet* 1999;7(1):88–97.
- [13] Mira MT, Alcais A, Van Thuc N, Thai VH, Huong NT, Ba NN, et al. Chromosome 6q25 is linked to susceptibility to leprosy in a Vietnamese population. *Nat Genet* 2003;33(3):412–5.
- [14] Siddiqui MR, Meisner S, Tosh K, Balakrishnan K, Ghei S, Fisher SE, et al. A major susceptibility locus for leprosy in India maps to chromosome 10p13. *Nat Genet* 2001;27(4):439–41.
- [15] Tosh K, Meisner S, Siddiqui MR, Balakrishnan K, Ghei S, Golding M, et al. A region of chromosome 20 is linked to leprosy susceptibility in a South Indian population. *J Infect Dis* 2002;186(8):1190–3.
- [16] Colhoun HM, McKeigue PM, Davey Smith G. Problems of reporting genetic associations with complex outcomes. *Lancet* 2003;361(9360):865–72.
- [17] Thursz MR, Kwiatkowski D, Allsopp CE, Greenwood BM, Thomas HC, Hill AV. Association between an MHC class II allele and clearance of hepatitis B virus in the Gambia. *N Engl J Med* 1995;332(16):1065–9 [see comments].

- [18] Hohler T, Gerken G, Notghi A, Lubjuhn R, Taheri H, Protzer U, et al. HLA-DRB1\*1301 and \*1302 protect against chronic hepatitis B. *J Hepatol* 1997;26(3):503–7.
- [19] Thio CL, Thomas DL, Karacki P, Gao X, Marti D, Kaslow RA, et al. Comprehensive analysis of class I and class II HLA antigens and chronic hepatitis B virus infection. *J Virol* 2003;77(22):12083–7.
- [20] Almarri A, Batchelor JR. HLA and hepatitis B infection. *Lancet* 1994;344(8931):1194–5 [see comments].
- [21] Bertoletti A, Ferrari C, Fiaccadori F, Penna A, Margolskee R, Schlicht HJ, et al. HLA class I-restricted human cytotoxic T cells recognize endogenously synthesized hepatitis B virus nucleocapsid antigen. *Proc Natl Acad Sci U S A* 1991;88(23):10445–9.
- [22] Waters JA, O'Rourke S, Schlicht HJ, Thomas HC. Cytotoxic T cell responses in patients with chronic hepatitis B virus infection undergoing HBe antigen/antibody seroconversion. *Clin Exp Immunol* 1995;102(2):314–9.
- [23] Bellamy R, Ruwende C, Corrah T, McAdam KP, Thursz M, Whittle HC, et al. Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the vitamin D receptor gene. *J Infect Dis* 1999;179(3):721–4.
- [24] Hohler T, Kruger A, Gerken G, Schneider PM, Meyer zum Buschenfelde KH, Rittner C. A tumor necrosis factor-alpha (TNF-alpha) promoter polymorphism is associated with chronic hepatitis B infection. *Clin Exp Immunol* 1998;111(3):579–82.
- [25] Shevach EM. CD4+ CD25+ suppressor T cells: more questions than answers. *Nat Rev Immunol* 2002;2(6):389–400.
- [26] Thio CL, Mosbruger TL, Kaslow RA, Karp CL, Strathdee SA, Vlahov D, et al. Cytotoxic T-lymphocyte antigen 4 gene and recovery from hepatitis B virus infection. *J Virol* 2004;78:11258–62.
- [27] Thursz MR, Kwiatkowski D, Torok ME, Allsopp CE, Greenwood BM, Whittle HC, et al. Association of hepatitis B surface antigen carriage with severe malaria in Gambian children. *Nat Med* 1995;1(4):374–5.
- [28] Bentley DR. The Human Genome Project—an overview. *Med Res Rev* 2000;20(3):189–96.
- [29] The International HapMap Project. *Nature* 2003;426(6968):789–96.
- [30] Bryant PA, Venter D, Robins-Browne R, Curtis N. Chips with everything: DNA microarrays in infectious diseases. *Lancet Infect Dis* 2004;4(2):100–11.
- [31] Lazzaro BP, Scurman BK, Clark AG. Genetic basis of natural variation in *D. melanogaster* antibacterial immunity. *Science* 2004;303(5665):1873–6.
- [32] Reiling N, Holscher C, Fehrenbach A, Kroger S, Kirschning CJ, Goyert S, et al. Cutting edge: toll-like receptor (TLR)2- and TLR4-mediated pathogen recognition in resistance to airborne infection with *Mycobacterium tuberculosis*. *J Immunol* 2002;169(7):3480–4.
- [33] Wasserman SA. Nature's fortress against infection. *Nat Immunol* 2004;5(5):474–5.
- [34] Murakami Y, Minami M, Daimon Y, Okanou T. Hepatitis B virus DNA in liver, serum, and peripheral blood mononuclear cells after the clearance of serum hepatitis B virus surface antigen. *J Med Virol* 2004;72(2):203–14.
- [35] Thio CL, Carrington M, Marti D, O'Brien SJ, Vlahov D, Nelson KE, et al. Class II HLA alleles and hepatitis B virus persistence in African Americans. *J Infect Dis* 1999;179(4):1004–6.