RESEARCH ARTICLE

New Gene Variants Associated with the Risk of Chronic HBV Infection

Mengjie Fan[1] · Jing Wang[2,3] · Sa Wang[1] · Tengyan Li[3] · Hong Pan[3] · Hankui Liu[4,5] · Huifang Xu[4,5] ·
Daria V. Zhernakova[6] · Stephen J. O'Brien[6,7] · Zhenru Feng[8] · Le Chang[8] · Erhei Dai[9] · Jianhua Lu[9] ·
Hongli Xi[1] · Yanyan Yu[1] · Jianguo Zhang[4,5] · Binbin Wang[3] · Zheng Zeng[1]

Abstract

Some patients with chronic hepatitis B virus (HBV) infection failed to clear HBV, even persistently continue to produce antibodies to HBV. Here we performed a two stage genome wide association study in a cohort of Chinese patients designed to discover single nucleotide variants that associate with HBV infection and clearance of HBV. The first stage involved genome wide exome sequencing of 101 cases (HBsAg plus anti-HBs positive) compared with 102 control patients (anti-HBs positive, HBsAg negative). Over 80% of individual sequences displayed $20 \times$ sequence coverage. Adapters, uncertain bases > 10% or low-quality base calls (> 50%) were filtered and compared to the human reference genome hg19. In the second stage, 579 chronic HBV infected cases and 439 HBV clearance controls were sequenced with selected genes from the first stage. Although there were no significant associated gene variants in the first stage, two significant gene associations were discovered when the two stages were assessed in a combined analysis. One association showed rs506121-"T" allele [within the dedicator of cytokinesis 8 (*DOCK8*) gene] was higher in chronic HBV infection group than that in clearance group ($P = 0.002$, OR = 0.77, 95% CI [0.65, 0.91]). The second association involved rs2071676—A allele within the Carbonic anhydrase (*CA9*) gene that was significantly elevated in chronic HBV infection group compared to the clearance group ($P = 0.0003$, OR = 1.35, 95% CI [1.15, 1.58]). Upon replication these gene associations would suggest the influence of *DOCK8 and CA9* as potential risk genetic factors in the persistence of HBV infection.

Keywords Whole exome sequencing · HBV infection · DOCK8 · CA9 · Variation

Mengjie Fan, Jing Wang and Sa Wang have contributed equally to this work.

✉ Jianguo Zhang
zhangjg@genomics.cn

✉ Binbin Wang
wbbahu@163.com

✉ Zheng Zeng
zeng@bjmu.edu.cn

[1] Department of Infectious Diseases, Peking University First Hospital, Beijing 100034, China

[2] Department of Medical Genetics and Development Biology, School of Medical Basic, Capital Medical University, Beijing 100069, China

[3] Center for Genetics, National Research Institute for Family Planning, Beijing 100081, China

[4] BGI-Shenzhen, Shenzhen 518083, China

[5] BGI Genomics, BGI-Shenzhen, Shenzhen 518083, China

[6] Laboratory of Genomic Diversity, Center for Computer Technologies, ITMO University, St. Petersburg, Russia 197101

[7] Guy Harvey Oceanographic Center, Halmos College of Natural Sciences and Oceanography, Nova Southeastern University, Ft Lauderdale, FL 33004, USA

[8] Department of Laboratory Medicine, Peking University First Hospital, Beijing 100034, China

[9] The Fifth Hospital of Shijiazhuang, Shijiazhuang 050024, China

Abbreviations

ALT	Alanine transaminase
anti-HAV	Antibody to hepatitis A virus
anti-HBc	Antibody to hepatitis B core antigen
anti-HBe	Antibody to hepatitis B e antigen
anti-HBs	Antibody to hepatitis B surface antigen

anti-HCV   Antibody to hepatitis C virus
anti-HDV   Antibody to hepatitis D virus
anti-HEV   Antibody to hepatitis E virus
CA9   Carbonic anhydrase 9
CHB   Chronic hepatitis B
DNA   Deoxyribonucleic acid
DOCK8   Dedicator of cytokinesis 8
DP   Double positive
ELISA   Enzyme-linked immunosorbent assay
GWAS   Genome wide association study
HBeAg   Hepatitis B e antigen
HBsAg   Hepatitis B surface antigen
HBV   Hepatitis B virus
HBx   Hepatitis B x protein
HCC   Hepatocellular carcinoma
HDV   Hepatitis D virus
HEV   Hepatitis E virus
IgM   Immunoglobulin M
LLOD   Lower limit of detection
PCR   Polymerase chain reaction
SD   Standard deviation
SP   Single positive
TOF   Time of flight mass spectrometry
WES   Whole exome sequencing

Introduction

HBV infection is a serious worldwide public health issue. Despite the application of antiviral drugs such as nucleos(t)ide analogues, interferons (IFNs) and hepatitis B vaccine, there are still about ~ 257 million people worldwide who are chronic carriers of HBV (WHO 2017) and have a mortality risk from HBV-related liver diseases, such as liver cirrhosis, hepatic decompensation and hepatocellular carcinoma (Ding *et al.* 2016). In the natural course of HBV infection, less than 5% of cases in adulthood (WHO 2015) and 90% of neonates and 20%–60% of children under the age of 5 years fail to clear HBV and eventually develop into chronic HBV infection (Hoofnagle *et al.* 2007). The clinical manifestations of HBV infection vary among different infected subjects, ranging from acute self-limiting infection, inactive carrier state, fulminant hepatic failure to chronic hepatitis with potential diverse severe progression and complications (Wang *et al.* 2010). However, the factors that contribute to the persistence are unknown.

It was commonly believed that HBsAg and anti-HBs (protective antibody) seldom appear simultaneously in serum of the same patient. However, chronic HBV replication is able to occur despite the presence of anti-HBs in the serum of certain infected individuals. Coexistence of HBsAg and anti-HBs was first reported by Arnold *et al.* (1976), and later many other studies reported this phenomenon as well (Colson *et al.* 2007; Fu *et al.* 2017; Xiang *et al.* 2017). Chen *et al.* (2011) reported significant higher aa substitution diversity within HBV viral *S* gene, especially the MHR and the "a" determinant in patients who display coexistence of HBsAg and anti-HBs, most frequently in the s126, s129 and s130 sites. Chen *et al.* (2011) also found that the incidence of basal core promoter double mutations (A1762T/G1764A) in the coexistence group was higher than in controls. Hadiji-Abbes *et al.* (2015) reported that the C69R variant contributes to a conformational change that occur not only at mutation site but also in the immunodominant "a" region, causing a decrease on the binding affinity to anti-HBs. In addition to the viral factors, host factors may also influence the presence of this concomitant situation. We previous reported that variants within the *OAS3* gene was associated the presence of HBsAg and anti-HBs in patients with chronic HBV infection (Wang *et al.* 2018).

The pathogenesis and clinical manifestations of HBV infection are due to the interaction between virus replication and host immune responses to HBV-encoded antigens (Chisari *et al.* 2010). For viral factors, many studies had investigated virus genetic factors that may have influence on the progression of HBV infection. Researchers sequenced basal core promotor of HBV genome and found that patients with T1762/A1764 mutation were associated with progression of liver diseases, pathogenesis of HBV infection, and were more likely to develop HCC than controls (Kao *et al.* 2003). Several studies further confirmed that patients with T1762/A1764 mutations were associated with an increased risk of severe liver diseases including HCC (Kuang *et al.* 2004; Liu *et al.* 2006; Zheng *et al.* 2011). For host factors, Saxena *et al.* (2014) found that several genotypes in interleukin 6 (*IL-6*) ($-572/-597$) gene influence HBV disease progression. For example, the GA haplotype in -597G $>$ A of *IL-6* decreased the risk of hepatitis, cirrhosis, and subsequent HCC development among HBV carriers. Other cytokine genes such as interleukin-10 (*IL-10*), interleukin-28 (*IL-28*), transforming growth factor-β1 (*TGF-β1*), tumor necrosis factor-α (*TNF-α*) and migration-inhibitory factor *(MIF)* may also affect the susceptibility to HBV infection (Qi *et al.* 2009; Zhang *et al.* 2011a, Zhang *et al.* 2011b, Zhang *et al.* 2013; Zheng *et al.* 2012; Korachi *et al.* 2013; Zeng 2014). Several studies revealed that *HLA-DPA1*-A and *HLA-DPB1* alleles were associated with the viral clearance of HBV and HCC development (An *et al.* 2011; Hu *et al.* 2012). Earlier, Diepolder *et al.* (1998) found that patients with chronic hepatitis B (CHB) had lower *HLA-DR13* allele than in healthy controls, suggesting that the HLA class II allele *DR13* was associated with self-limiting of HBV infection.

Table 1 Inclusion and exclusion criteria.

Inclusion criteria

Cases (DP,chronic HBV infection)

 1. HBsAg, anti-HBc positive for at least 6 months and no history of hepatitis B vaccination

 2. anti-HAV, anti-HEV, HDAg negative and/or anti-HDV negative

 3. Anti-HCV negative, HCV RNA negative

 4. For DP cases in the first stage, anti-HBs positive for at least 6 months; for chronic HBV infection cases in the second stage, anti-HBs can be positive or negative

Controls (SP, HBV clearance)

 1. Anti-HBs and anti-HBc positive or anti-HBs positive and no history of hepatitis B vaccination; HBsAg negative

 2. HBV-DNA negative, anti-HAV, anti-HEV, HDAg negative and/or anti-HDV negative

 3. Anti-HCV negative, HCV RNA negative

Exclusion criteria*

 1. Evidence of past or current infection by HCV or HDV

 2. With other hepatitis virus infection

 3. Other systemic disease not related to HBV infection

 4. Age less than 18 for all cases and controls

 5. Not of Han ethnicity

DP Double positive; *SP* single positive.

*Excluded from enrollment if one or more of the exclusion criteria were met, applicable for all the two stage samples.

HBV infection development is a multifactorial process with implicated influences of both host and viral genetic determinants. Any factors that may influence the host's immunity towards hepatitis B virus are likely to affect the outcomes of HBV infection. However, previous studies mainly focused on the common host potential genetic factors, which may miss rare functional genetic variants. Our previous study (Wang *et al.* 2018) was focus on genetic factors that influence anti-HBs expression. To reveal additional associated variants, we conducted a two-stage GWAS-bioinformatic analyses aiming to find other potentially functional variants that influence HBV persistent infection.

Materials and Methods

Study Design and Population

We carried out a retrospective survey of HBV related diseases in Chinese Han population from May, 2002 to June, 2017 and conducted a study with two stages of bioinformatic analyses aiming to find the potential genes that may play a role in different status of HBV related diseases. In the first stage (Wang *et al.* 2018), 101 cases positive for both HBsAg and anti-HBs [double positive (DP)] and 102 control subjects who show positive for anti-HBs but negative for HBsAg [single positive (SP)] were included. All these subjects are age and gender matched and genotyped by whole exome sequencing (WES). For the second stage, we expanded our samples [579 cases who are chronic HBV infection and 439

controls who are HBV clearance (HBsAg negative but anti-HBs and anti-HBc positive)] and conducted a phenotypic analysis using time of flight mass spectrometry (TOF) to further validate the result of the first stage. All the samples included in this study were mainly obtained from Peking University First Hospital and the Fifth Hospital of Shijiazhuang. The inclusion and exclusion criteria applicable to all samples are listed in Table 1.

Case definitions of different status of HBV infection are in consistent with the criteria issued by the Association of Infectious Diseases of China in 2015 (Hou and Lai 2015). In briefly, the cases of these two stages are chronic HBV infection, the controls are HBV clearance. The study was approved by the Ethics Committee of Peking University First Hospital and the Fifth Hospital of Shijiazhuang. Before entering this research group, all the subjects had signed an informed consent.

Laboratory Examination

Virological and serological tests were processed at local sites. Serum HBsAg, anti-HBs, HBeAg, and anti-HCV were detected using the ARCHITECT I2000 test (Abbot, USA). HBsAg higher than 0.05 IU/mL and anti-HBs higher than 10 mIU/mL were defined as positive, respectively. HBV DNA was quantified using Roche Cobas Ampliprep/Cobas Taqman PCR with lower limit of detection (LLOD) of 20 IU/mL (Roche, USA) or commercial real-time polymerase chain reaction kit with LLOD of 100 IU/mL (Daan Company, China). Anti-HAV IgM,

HDV antigen, anti-HDV and anti-HEV were determined by commercially ELISA kits in China.

Library Preparation and Whole Exome Sequencing

Genomic DNA of host was extracted from peripheral blood clot using protocols of QIAamp DNA Blood Mini Kit (QIAGEN) or salting-out method conducted by Tianyi Huiyuan Company (http://www.dna1953.com.cn/index.html). After that, 2–3 micrograms of DNA per individual from the first stage of 101 cases and 102 controls were delivered to BGI (http://www.genomics.cn/index) and were then sequenced and analyzed using WES on Illumina Hiseq X-Ten. Detailed procedure can be seen in our published article (Wang *et al.* 2018). The quality of exon sequencing was strictly controlled to guarantee that $> 80\%$ of the targets were covered by at least $20 \times$. Reads containing adapters, uncertain bases (N) $> 10\%$ or low-quality bases (Phred < 5) $> 50\%$ were filtered out and the remaining clean sequence reads were then compared to the human reference genome hg19 (http://genome.ucsc.edu/, build 37.1) using BWA (http://bio-bwa.sourceforge.net/index.shtml, 0.7.15). In addition, the duplicate fragments were labeled using Picard (http://picard.sourceforge.net/).

SNP and Small Indel Detection

After base quality recalibration and local realignment around the potential Indel sites, the Genome Analysis Toolkit (GATK, http://www.broadinstitute.org/gatk/index.php, v3.6) was applied to call SNPs and Indels. Then VEP (http://grch37.ensembl.org/info/docs/tools/vep/index.html, release-77) and ExAC databases were used to conduct annotation, which provide information such as mutation allele frequency, gene variant consequences and altered function of protein.

GWAS Dataset Construction

After alignment, mutation detection and annotation of the clean data, we conducted further filtering to identify high-confidence variants in targeted sequence. Variants meeting all the following requirements were considered to be high-confident: (1) quality (QUAL) ≥ 100; (2) depth of coverage ≥ 6 and support variant reads ≥ 3; (3) pass the allele balance test (prop test $P > 0.0005$); (4) the interval of two variants > 5 bp. High-confidence variants of all samples were merged to one Variant Call Format (VCF) file using bcftools. The call rate threshold of final VCF file was set to 80% and variants that failed to achieve the threshold were excluded from this GWAS dataset. After that, a genetic association analysis was carried out with fisher's exact test between cases and controls in the first stage.

Genetic Factors Confirmation by Time of Flight Mass Spectrometry (TOF)

TOF (Griffin *et al.* 1999) is based on single base extension molecular reaction, different allele has different molecular weight and different flying time in the electric field, which makes it possible to classify. TOF is the most important technical platform for stage II study by GWAS, it can effectively avoid the false-positivity caused by traditional fluorescence signal technique, which can ensure more accurate and correct results. To further determine the potential gene variants found in the first stage of WES, we performed TOF among larger samples which composed of 579 cases with chronic HBV infection and 439 controls who are HBV clearance with anti-HBs and anti-HBc positive but HBsAg negative. Genomic DNA of host was extracted as mentioned above. We applied iPLEX GOLD (Sequenom MassARRAY) to design primers for PCR and the PCR products were then digested by SAP enzyme to remove free dNTPs from the system. After that, we had a single base extension reaction. Then we transferred the purified products to the 384-well Spectro-CHIP bio-array to conduct analysis using MALDI-TOF mass spectrometer. The raw data and genotype map were obtained by using TYPER4.0 software. After checking the completeness and correctness, the results were stored in appropriate storage media and submitted to the biological information room for analysis.

SNP Selection in Replication Study

In the first stage of WES analysis, we chose the first 150 potentially functional variations (missense mutation, frameshift mutation, stop-gained mutation and stop-lost mutation) ranked by P value. To further confirm the discovery-phase genetic factors, we searched the related genes of these 150 variations in databases (such as PubMed, EMbase, Cochrane) and chose those that may have a relationship with HBV related liver diseases. Combining consideration of primer design in the process of TOF, we selected 30 variants from the above 150 loci as candidate genes and analyzed whether there was genetic discrepancy between cases and controls.

Statistical Analysis

Regarding to the demographic and clinical characteristics of the subjects included in this study, Chi square test and *t*-test was applied using SPSS17.0 statistical software.

Single variant association analysis was conducted by PLINK 1.07 software using Fisher's exact test.

Results

Characteristics of Participants in This Study

Characteristics of participants in the first stage were delineated in our previously published article (Wang *et al.* 2018).

Characteristics of the 1018 subjects in the second stage who received a TOF analysis were described in Table 2. Individuals in the control group were significantly older than those in the case group ($P < 0.05$), whereas gender was well balanced between the two groups ($P > 0.05$). Compared to the control group, ALT, HBV DNA levels and HBeAg positive rate in the case group were significant higher ($P < 0.05$).

Genetic Analysis between Groups in the First Stage of WES

We performed a genome-wide association study of 58,336 polymorphism variants that have available authoritative transcript using Fisher's exact test in all samples of 101 cases and 102 controls and conducted a Bonferroni correction (significance level was set as $P < 0.05/58,336$) because of the existing multiple tests. However, no loci achieved this significance threshold. Figure 1 delineated the P value of all these single variants. The first 150 potentially functional variations ranked by P value are displayed in Supplementary Table S1. We also showed the characteristics of published CHB risk-associated SNPs (Chang *et al.* 2014) in Supplementary Table S2. However, those loci are not in the exome capture region, so the performance are unavailable in our first stage of WES.

Genetic Analysis between Groups in the Second Stage of TOF

In the first stage GWAS, we failed to detect any potentially functional association. In view of this, we expanded our sample sizes and performed this second stage (candidate gene analysis) using TOF in 30 leading associated variants to reveal an association with chronic HBV infection (Table 3). There were several sites that achieved significant difference, including rs11040923, rs2071676, rs2288868, rs4774113 and rs506121. SNPs rs2288868 and rs4774113 were excluded due to Hardy–Weinberg Equilibrium departure ($P < 0.05$) and $< 95\%$ call rate in the cases group respectively. SNP rs11040923 was also excluded due to discordant allele frequencies in stage 1 *vs* stage 2 populations.

Table 4 delineates allele discrepancies of rs506121, rs2071676, rs11040923 between cases and controls in the two stages. Patients who were HBsAg positive in the case group had elevated *DOCK8*—T allele (rs506121; $P < 0.05$) and *CA9*—A allele (rs2071676; $P < 0.05$) than that in clearance group in both stages. A meta-analysis to evaluate the performance of these three SNPs (Table 4), which suggested a similar result for SNPs rs506121 and rs2071676 ($P = 0.002$, OR = 0.77, 95% CI [0.65, 0.91]; $P = 0.0003$, OR = 1.35, 95% CI [1.15, 1.58], respectively).

Discussion

The outcomes of HBV infection can vary depending on differing viral and host genetic determinants factors. Previous studies of host genes mainly focused on common genes such as cytokines and HLA genes, however, rare gene may have an influence as well. Based on our previous study that mutation of *OAS3* gene may affect the presence of anti-HBs in patients with chronic HBV infection (Wang *et al.* 2018), we enlarged sample size and focus on

Table 2 Characteristics of subjects in the second stage of time of flight mass spectrometry.

Group	Cases (n = 579)	Controls (n = 439)	P value
Age, y			
Mean (x ± SD)	49.57 ± 14.74 (579)	61.93 ± 13.40 (439)	**0.000**
Range	18–84	18–96	
Male, % (n)	55% (317/579)	59% (261/439)	0.142
HBsAg positive, % (n)	100% (579)	0 (439)	**0.000**
anti-HBs positive, % (n)	6.8% (39/571)	100% (439)	**0.000**
HBeAg positive, % (n)	18% (103/565)	0 (0/439)	**0.000**
ALT, IU/mL, mean ± SD (n)	32.74 ± 60.06 (456)	21.88 ± 23.86 (333)	**0.000**
HBV-DNA, log IU/mL, mean ± SD (n)	1.63 ± 2.51 (277)	0.00 ± 0.00 (14)	**0.000**

P values less than 0.05 are indicated in bold

ALT Alanine transaminase; *anti-HBs* antibody to the hepatitis B surface antigen; *DP* double positive; *HBeAg* hepatitis B e antigen; *HBsAg* hepatitis B surface antigen; *SD* standard deviation
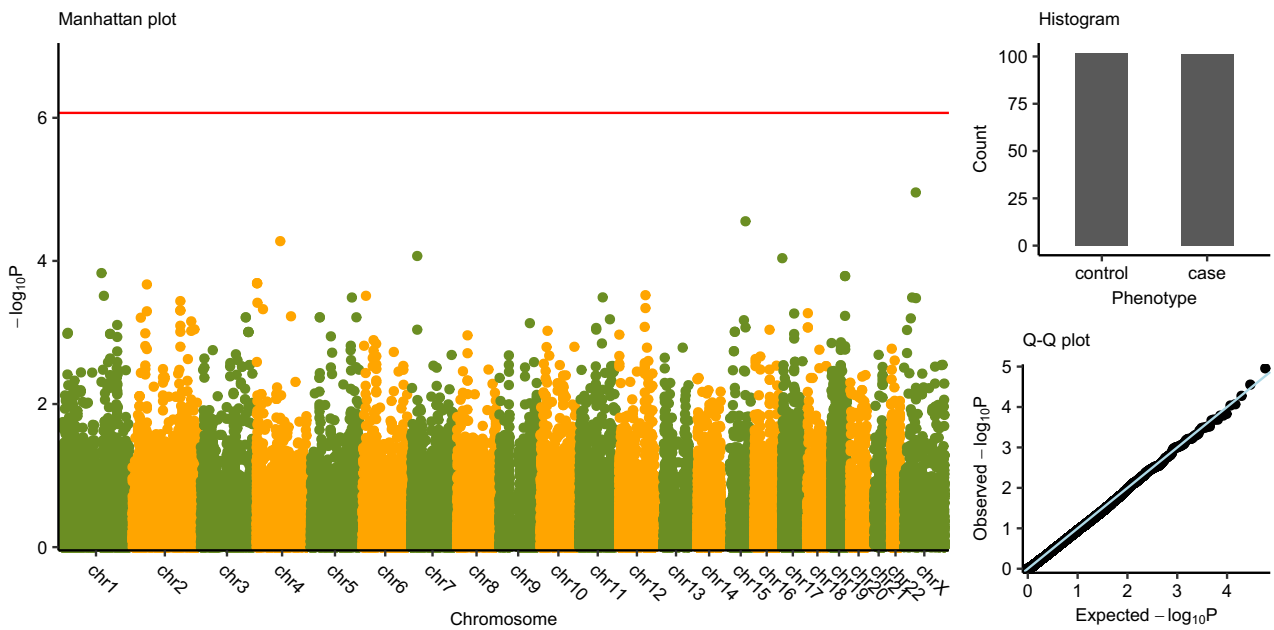
Fig. 1 **Manhattan plot** Genome-wide single variant association study based on the dataset of all single variants. X axis indicates the variant location at hg19, chromosomes separated by different colors; y axis indicates the $-\log_{10}(P \text{ value})$ from Fisher's exact test. The red line indicates the genome-wide significance threshold [y = $-\log_{10}(0.05/58{,}336)$]. Histogram indicates the number of case group and control group. Q–Q plot indicates the observed association *P* values and the expected *P* values followed the uniform distribution.

persistent HBV infection compared to HBV clearance to reveal new potential variants that may have an effect on the clearance or persistence of HBV infection.

In the first stage, we did not find any potentially functional gene variants that may have a relationship with HBV infection at the Bonferroni genome-wide significance level (Fig. 1). Reasons accounting for this may be as follows: first, exome sequencing only focuses on genetic variation in the exome region, and is thus very likely to neglect those lying in the noncoding region (Wang *et al.* 2018); second, statistical power of classical single-variant based association tests for low-frequency and rare variants is low when the sample sizes are small (Lee *et al.* 2014; Wang *et al.* 2018). In the following stage, we expanded our sample sizes and further conducted a TOF analysis to confirm the potential gene variants found in the discovery-phase. Two-stage design is the standard strategy of genome-wide association study for discovering and validating the candidate genetic makers. Stage 2 is a replication study which focuses on the statistical significance discovered in stage 1. Previous study demonstrated that joint analysis of replication-based study result in increased power to detect genetic association (Skol *et al.* 2006). And for evaluating the significance of association between genetic factors and affected-unaffected phenotypes, Chi square test, Fisher's exact test (Purcell *et al.* 2007) and mixed regression model (Zhou and Stephens 2012) are the most popular methods designed for population matched and un-matched study

respectively. In our study, we used Chi square test to perform the joint analysis on 30 variants in two populations. Combining the results of these two stages, we observed that cases in CHB group have significantly higher frequency of *DOCK8* -T allele and higher frequency of *CA9*—A allele than controls in clearance group (Table 4).

Autosomal recessive mutations in the *DOCK8* gene, located on chromosome 9p24.3, can result in *DOCK8* immunodeficiency (Betts *et al.* 2015), a syndrome predisposing carriers, to various viral or fungal infections (Zhang *et al.* 2009). *DOCK8* is a member of the *DOCK180* superfamily of atypical guanine-nucleotide exchange factors (GEF) (Cote and Vuori 2002) and can activate cell division cycle 42 (Cdc42) (Mizesko *et al.* 2013). Previously, Nishikimi *et al.* concluded that Cdc42 is a member of Rho GTPase, which functions as molecular switches by cycling between an inactive GDP-bound state and an active GTP-bound state (Nishikimi *et al.* 2013) and is important for reorganization of filamentous actin (F-actin) cytoskeleton in natural killer (NK) cells and dendritic cells (DCs) (Sinai *et al.* 2010; Harada *et al.* 2012). The loss of DOCK8 in mice may lead to DCs' less competitive to migrate from peripheral tissues to lymph nodes and act its role as an antigen-presenting cell, which being able to initiate an adaptive immune response (Krishnaswamy *et al.* 2015). Some studies demonstrated that there exhibits a similar phenomenon of a migration defect of macrophages and CD8 + T cells in the absence of DOCK8 (Kearney

Table 3 Alleles discrepancy of the selected 30 variants between cases and controls in the second stage of time of flight mass spectrometry.

Position	Case (n%)	Control (n%)	OR (95% CI)	P value
rs1048906	62.37	64.45	0.91 (0.76–1.10) (C/T)*	0.35
rs10821128	46.37	49.20	0.89 (0.75–1.06) (C/T)	0.21
rs11040923	67.00	62.4	1.22 (1.02–1.47) (A/G)	**0.035**
rs16932912	34.30	35.10	0.97 (0.80–1.16) (A/G)	0.74
rs17206365	70.80	71.60	0.96 (0.79–1.18) (A/T)	0.72
rs1870134	28.60	26.10	1.14 (0.93–1.39) (C/G)	0.21
rs2071676	52.60	46.70	1.27 (1.06–1.51) (A/G)	**0.009**
rs2073674	55.10	55.70	0.98 (0.82–1.16) (A/C)	0.79
rs2075688	100	100	– (C)	–
rs2272662	52.80	55.90	0.88 (0.74–1.05) (C/T)	0.19
rs2277603	77.90	81.30	0.81 (0.65–1.01) (A/G)	0.06
rs2288868	80.60	72.50	1.58 (1.28–1.94) (C/T)	**2.28E − 5**
rs2297879	45.20	49.20	0.85 (0.71–1.01) (C/T)	0.07
rs2302061	29.70	29.00	1.03 (0.85–1.25) (C/T)	0.77
rs3732487	46.00	49.00	0.89 (0.75–1.06) (G/T)	0.19
rs3733662	28.70	31.40	0.88 (0.73–1.06) (A/C)	0.19
rs3745535	34.60	36.10	0.94 (0.78–1.13) (A/C)	0.51
rs3779234	76.70	76.90	0.99 (0.80–1.22) (C/T)	0.92
rs3804769	16.30	17.10	0.94 (0.75–1.19) (C/T)	0.63
rs3815045	23.90	22.50	1.08 (0.88–1.33) (A/G)	0.49
rs3818123	46.30	48.20	0.93 (0.78–1.11) (C/T)	0.42
rs4629585	46.40	47.70	0.95 (0.79–1.13) (A/C)	0.56
rs4774113	17.60	21.40	0.79 (0.63–0.99) (G/T)	**0.04**
rs4938941	27.90	25.70	1.11 (0.91–1.36) (A/G)	0.29
rs506121	60.30	65.10	0.81 (0.68–0.97) (C/T)	**0.027**
rs553717	69.40	68.20	1.06 (0.88–1.28) (C/T)	0.56
rs723077	54.20	53.50	1.03 (0.86–1.23) (A/C)	0.75
rs760749	78.20	76.10	1.13 (0.92–1.39) (A/C)	0.26
rs8100856	44.90	45.10	0.99 (0.83–1.18) (C/T)	0.96
rs934945	71.80	75.10	0.85 (0.70–1.04) (C/T)	0.106

P values less than 0.05 are indicated in bold

OR Odds ratio; *CI* confidence interval.

*If the OR was calculated as C/T, then the frequency listed in the table is the frequency of C among all the subjects

Table 4 Allele discrepancy of rs506121, rs2071676, rs11040923 between cases and controls.

Polymorphisms	Stage	Case (n%)	Control (n%)	OR (95% CI)	P value
rs506121 (*DOCK8*)	1	55.90	68.10	0.59 (0.40–0.89) (C/T)*	0.014
	2	60.30	65.10	0.81 (0.68–0.97) (C/T)	0.027
	1 + 2 (meta)			0.77 (0.65–0.91) (C/T)	**0.002**
rs2071676 (*CA9*)	1	58.90	43.60	1.85 (1.25–2.75) (A/G)	0.002
	2	52.60	46.70	1.27 (1.06–1.51) (A/G)	0.009
	1 + 2 (meta)			1.35 (1.15–1.58) (A/G)	**0.0003**
rs11040923 (*DNHD1*)	1	63.40	77.00	0.52 (0.34–0.80) (A/G)	0.003
	2	67.00	62.40	1.22 (1.02–1.47) (A/G)	0.035
	1 + 2 (meta)			1.07 (0.91–1.27) (A/G)	0.40

P values less than 0.05 in meta-analysis are indicated in bold

DOCK8 Dedicator of cytokinesis 8; *CA9* carbonic anhydrase 9; *DNHD1* dynein heavy chain domain 1; *OR* odds ratio; *CI* confidence interval.

*If the OR was calculated as C/T, then the frequency listed in the table is the frequency of C among all the subjects

et al. 2017; Shiraishi *et al.* 2017), being unable to perform their function as clearing pathogen. It was previously described by some researchers that patients with DOCK8 deficiency exhibit an impaired T-cell, B-cell, NKT cell and RORγt + innate lymphoid cells (ILCs) function, which may affect the persistence of these cells (Boztug *et al.* 2012; Kearney *et al.* 2017). Jabara *et al.* concluded in their study that DOCK8 acts as an adaptor, which can associate toll like receptor 9 (TLR9) with Pyk2-Src-Syk-Stat3 signaling cascade via Myeloid differentiation primary-response protein 88 (MyD88) and that the lack of DOCK8 may result in TLR9 mis-localization, crippling the ability of TLR9 to activate Stat3 and disabling subsequent TLR9-driven B cell activation, proliferation and immunoglobulin production, which will impair the humoral response mediated by B cells (Jabara *et al.* 2012). Pei *et al.* (2014) reported that MyD88 plays a part in the signaling cascade of the innate immune response mediated by TLRs. Further, overexpression of MyD88 in liver cells or mouse model can inhibit HBV replication via downregulation of viral transcription or acceleration of the decay of viral pre-genomic RNA (Xiong *et al.* 2004; Li *et al.* 2010). Many studies had previously described that TLRs signaling pathways play an essential role in the control of HBV replication (Isogawa *et al.* 2005; Pei *et al.* 2014) by modulating the expression of microRNAs ///(Zhang *et al.* 2011a, Zhang *et al.* 2011b; Li and Shi 2013) or inducing antiviral cytokines (Isogawa *et al.* 2005). In a case–control study, researchers found TLR mutations in rs3804099 and rs4696480 were significantly associated with milder hepatitis activity among patients with chronic HBV infection, concluding that the activation of TLR pathways may further intensify the inflammation of hepatocytes (Lin *et al.* 2018). Ligands of TLR2, TLR3, TLR4, TLR7, and TLR9 in hepatoma cells have been reported to inhibit HBV replication (Xia *et al.* 2008). Taking the discoveries of previous studies and the results of our current study, we speculate that DOCK8 -T allele may increase persistence of HBV infection thereby impairing the immune system, TLRs function and the correlated signaling pathways.

Carbonic anhydrase (CA) is a family of zinc metalloenzymes and can efficiently catalyze the reversible processes of hydration-dehydration of CO_2 and HCO_3^-, which can help maintain the neutral pH in hypoxic condition. Hypoxia is commonly observed in tumor microenvironment and can lead to various genetic and adoptive responses that accommodates tumor growth (Yoo *et al.* 2010). Hua *et al.* (2017) suggested that CA9 variants pose a prognostic marker of hepatocellular carcinoma (HCC), and that CA9 allele regulate tumor growth and metastasis of HCC cells through controlling cell proliferation and mobility and the epithelial-mesenchymal transition (EMT) process in HCC cells. Hyuga *et al.* (2017) suggested that CA9 variants are associated with poor prognosis through regulation of the EMT process in HCC. Kang *et al.* (2015) reported in two cohorts that those with high CA9-positive had poorer prognosis after surgery for HCC than those with low CA9-positive, which suggested that CA9 expression may be a prognostic factor for survival. Holotnakova *et al.* (2010) demonstrated that HBx increased the CA9 promoter activity and that HBx involves a portion of HBV DNA integrated into hepatocyte chromosomes, which explains the role of HBx in HBV infection with HCC. Holotnakova *et al.* (2010) also hypothesized that CA9 could enhance the hepatocyte tolerance to ischemia, which can increase the viability of HBV infected hepatocytes and assist further development such as oncogenesis. Lastly, Wang *et al.* (2013) found variants of CA9 gene may change the host susceptibility to certain tumors leading to different outcomes.

In this study, we found the CA9—A allele to be elevated in chronic HBV infection (Table 4). CA9 is located on chromosome 9p12-13 and contains 11 exons, which can encode for the 459-amino-acid protein (Wang *et al.* 2013). Mutation at locus rs2071676 of CA9 gene results in the alteration of amino acid (NM_001216:c.G96A; p.Val33-Met), which may affect the function of this gene-encoded protein and mRNA conformation and thereafter have an impact on disease (Shen *et al.* 2018). We proposed that rs2071676 variants of CA9 gene might also be a risk factor for the chronicity of HBV infection, in a way mediated by possible alteration of protein function.

We conducted a two-stage analysis and demonstrated that SNP alleles of DOCK8 and CA9 gene may increase the risk of chronicity of HBV infection, which may serve as a marker for the management of HBV infection. Further functional studies are needed to measure the expression of these two gene-coded proteins in HBV-related diseases to help management of HBV infection.

Author Contributions ZZ, BBW and JGZ designed the study. MJF, SW, JW, TYL, HP, ZRF, LC, EHD, JHL, HLX and YYY collected samples and analyzed data. MJF, SW, JW, JGZ, BBW and ZZ analyzed and interpreted the data with the assistance of HKL, HFX, DVZ, and SJO. MJF and JW wrote the manuscript. ZZ revised and approved the final manuscript. All authors had full access to the final version of the report and agreed to the submission.

Compliance with Ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Animal and Human Rights Statement All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and with 1964 Helsinki declaration and later amendments or comparable ethical standards. The study was approved by the Ethics Committee of Peking University First Hospital and the Fifth Hospital of Shijiazhuang. Before entering this research group, all the subjects had signed an informed consent. This study does not contain any studies with animals performed by any of the authors.

References

An P, Winkler C, Guan L, O'Brien SJ, Zeng Z, Consortium HBVS (2011) A common HLA-DPA1 variant is a major determinant of hepatitis B virus clearance in Han Chinese. J Infect Dis 203:943–947

Arnold W, Hess G, Meyer zum Buschenfelde KH, Kosters W, Biswas R, Strauch M, Knolle J (1976) Simultaneous occurrence in the serum of hepatitis B surface antigen (HBsAg) and antibodies against HBsAg (Anti-HBs) of different subtypes (Serologic and fluorescence histologic studies). Verh Dtsch Ges Inn Med 82(1):405–407

Betts K, Abusleme L, Freeman AF, Sarmadi M, Fahle G, Pittaluga S, Cuellar-Rodriguez J, Hickstein D, Holland SM, Su H, Moutsopoulos NM (2015) A 17-year old patient with DOCK8 deficiency, severe oral HSV-1 and aggressive periodontitis - a case of virally induced periodontitis? J Clin Virol 63:46–50

Boztug H, Karitnig-Weiss C, Ausserer B, Renner ED, Albert MH, Sawalle-Belohradsky J, Belohradsky BH, Mann G, Horcher E, Rummele-Waibel A, Geyeregger R, Lakatos K, Peters C, Lawitschka A, Matthes-Martin S (2012) Clinical and immunological correction of DOCK8 deficiency by allogeneic hematopoietic stem cell transplantation following a reduced toxicity conditioning regimen. Pediatr Hematol Oncol 29:585–594

Chang SW, Fann CS, Su WH, Wang YC, Weng CC, Yu CJ, Hsu CL, Hsieh AR, Chien RN, Chu CM, Tai DI (2014) A genome-wide association study on chronic HBV infection and its clinical progression in male Han-Taiwanese. PLoS ONE 9:e99724

Chen Y, Qian F, Yuan Q, Li X, Wu W, Guo X, Li L (2011) Mutations in hepatitis B virus DNA from patients with coexisting HBsAg and anti-HBs. J Clin Virol 52:198–203

Chisari FV, Isogawa M, Wieland SF (2010) Pathogenesis of hepatitis B virus infection. Pathol Biol (Paris) 58:258–266

Colson P, Borentain P, Motte A, Henry M, Moal V, Botta-Fridlund D, Tamalet C, Gerolami R (2007) Clinical and virological significance of the co-existence of HBsAg and anti-HBs antibodies in hepatitis B chronic carriers. Virology 367:30–40

Cote JF, Vuori K (2002) Identification of an evolutionarily conserved superfamily of DOCK180-related proteins with guanine nucleotide exchange activity. J Cell Sci 115:4901–4913

Diepolder HM, Jung MC, Keller E, Schraut W, Gerlach JT, Gruner N, Zachoval R, Hoffmann RM, Schirren CA, Scholz S, Pape GR (1998) A vigorous virus-specific CD4 + T cell response may contribute to the association of HLA-DR13 with viral clearance in hepatitis B. Clin Exp Immunol 113:244–251

Ding F, Miao XL, Li YX, Dai JF, Yu HG (2016) Mutations in the S gene and in the overlapping reverse transcriptase region in chronic hepatitis B Chinese patients with coexistence of HBsAg and anti-HBs. Braz J Infect Dis 20:1–7

Fu X, Chen J, Chen H, Lin J, Xun Z, Li S, Liu C, Zeng Y, Chen T, Yang B, Ou Q (2017) Mutation in the S gene of hepatitis B virus and anti-HBs subtype-nonspecificity contributed to the co-

existence of HBsAg and anti-HBs in patients with chronic hepatitis B virus infection. J Med Virol 89:1419–1426

Griffin TJ, Hall JG, Prudent JR, Smith LM (1999) Direct genetic analysis by matrix-assisted laser desorption/ionization mass spectrometry. Proc Natl Acad Sci USA 96:6301–6306

Hadiji-Abbes N, Mihoubi W, Martin M, Karakasyan-Dia C, Frikha F, Gergely C, Jouenne T, Gargouri A, Mokdad-Gargouri R (2015) Characterization of C69R variant HBsAg: effect on binding to anti-HBs and the structure of virus-like particles. Arch Virol 160:2427–2433

Harada Y, Tanaka Y, Terasawa M, Pieczyk M, Habiro K, Katakai T, Hanawa-Suetsugu K, Kukimoto-Niino M, Nishizaki T, Shirouzu M, Duan X, Uruno T, Nishikimi A, Sanematsu F, Yokoyama S, Stein JV, Kinashi T, Fukui Y (2012) DOCK8 is a Cdc42 activator critical for interstitial dendritic cell migration during immune responses. Blood 119:4451–4461

Holotnakova T, Tylkova L, Takacova M, Kopacek J, Petrik J, Pastorekova S, Pastorek J (2010) Role of the HBx oncoprotein in carbonic anhydrase 9 induction. J Med Virol 82:32–40

Hoofnagle JH, Doo E, Liang TJ, Fleischer R, Lok AS (2007) Management of hepatitis B: summary of a clinical research workshop. Hepatology 45:1056–1075

Hou JL, Lai W (2015) The guideline of prevention and treatment for chronic hepatitis B: a 2015 update. Zhonghua Gan Zang Bing Za Zhi 23:888–905

Hu L, Zhai X, Liu J, Chu M, Pan S, Jiang J, Zhang Y, Wang H, Chen J, Shen H, Hu Z (2012) Genetic variants in human leukocyte antigen/DP-DQ influence both hepatitis B virus clearance and hepatocellular carcinoma development. Hepatology 55:1426–1431

Hua KT, Liu YF, Hsu CL, Cheng TY, Yang CY, Chang JS, Lee WJ, Hsiao M, Juan HF, Chien MH, Yang SF (2017) 3'UTR polymorphisms of carbonic anhydrase IX determine the miR-34a targeting efficiency and prognosis of hepatocellular carcinoma. Sci Rep 7:4466

Hyuga S, Wada H, Eguchi H, Otsuru T, Iwagami Y, Yamada D, Noda T, Asaoka T, Kawamoto K, Gotoh K, Takeda Y, Tanemura M, Umeshita K, Doki Y, Mori M (2017) Expression of carbonic anhydrase IX is associated with poor prognosis through regulation of the epithelialmesenchymal transition in hepatocellular carcinoma. Int J Oncol 51:1179–1190

Isogawa M, Robek MD, Furuichi Y, Chisari FV (2005) Toll-like receptor signaling inhibits hepatitis B virus replication *in vivo*. J Virol 79:7269–7272

Jabara HH, McDonald DR, Janssen E, Massaad MJ, Ramesh N, Borzutzky A, Rauter I, Benson H, Schneider L, Baxi S, Recher M, Notarangelo LD, Wakim R, Dbaibo G, Dasouki M, Al-Herz W, Barlan I, Baris S, Kutukculer N, Ochs HD, Plebani A, Kanariou M, Lefranc G, Reisli I, Fitzgerald KA, Golenbock D, Manis J, Keles S, Ceja R, Chatila TA, Geha RS (2012) DOCK8 functions as an adaptor that links TLR-MyD88 signaling to B cell activation. Nat Immunol 13:612–620

Kang HJ, Kim IH, Sung CO, Shim JH, Yu E (2015) Expression of carbonic anhydrase 9 is a novel prognostic marker in resectable hepatocellular carcinoma. Virchows Arch 466:403–413

Kao JH, Chen PJ, Lai MY, Chen DS (2003) Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. Gastroenterology 124:327–334

Kearney CJ, Randall KL, Oliaro J (2017) DOCK8 regulates signal transduction events to control immunity. Cell Mol Immunol 14:406–411

Korachi M, Ceran N, Adaleti R, Nigdelioglu A, Sokmen M (2013) An association study of functional polymorphic genes IRF-1, IFNGR-1, and IFN-gamma with disease progression, aspartate aminotransferase, alanine aminotransferase, and viral load in chronic hepatitis B and C. Int J Infect Dis 17:e44–49

Krishnaswamy JK, Singh A, Gowthaman U, Wu R, Gorrepati P, Sales Nascimento M, Gallman A, Liu D, Rhebergen AM, Calabro S, Xu L, Ranney P, Srivastava A, Ranson M, Gorham JD, McCaw Z, Kleeberger SR, Heinz LX, Muller AC, Bennett KL, Superti-Furga G, Henao-Mejia J, Sutterwala FS, Williams A, Flavell RA, Eisenbarth SC (2015) Coincidental loss of DOCK8 function in NLRP10-deficient and C3H/HeJ mice results in defective dendritic cell migration. Proc Natl Acad Sci USA 112:3056–3061

Kuang SY, Jackson PE, Wang JB, Lu PX, Munoz A, Qian GS, Kensler TW, Groopman JD (2004) Specific mutations of hepatitis B virus in plasma predict liver cancer development. Proc Natl Acad Sci USA 101:3575–3580

Lee S, Abecasis GR, Boehnke M, Lin X (2014) Rare-variant association analysis: study designs and statistical tests. Am J Hum Genet 95:5–23

Li Y, Shi X (2013) MicroRNAs in the regulation of TLR and RIG-I pathways. Cell Mol Immunol 10:65–71

Li J, Lin S, Chen Q, Peng L, Zhai J, Liu Y, Yuan Z (2010) Inhibition of hepatitis B virus replication by MyD88 involves accelerated degradation of pregenomic RNA and nuclear retention of pre-S/S RNAs. J Virol 84:6387–6399

Lin Y, Gao ZX, Shen X, Chen MJ, Li YT, Li SL, Lin HL, Zhao QF, Liu F, Niu JJ (2018) Correlation between polymorphisms in toll-like receptor genes and the activity of hepatitis B virus among treatment-naive patients: a case-control study in a Han Chinese population. BMC Infect Dis 18:28

Liu CJ, Chen BF, Chen PJ, Lai MY, Huang WL, Kao JH, Chen DS (2006) Role of hepatitis B viral load and basal core promoter mutation in hepatocellular carcinoma in hepatitis B carriers. J Infect Dis 193:1258–1265

Mizesko MC, Banerjee PP, Monaco-Shawver L, Mace EM, Bernal WE, Sawalle-Belohradsky J, Belohradsky BH, Heinz V, Freeman AF, Sullivan KE, Holland SM, Torgerson TR, Al-Herz W, Chou J, Hanson IC, Albert MH, Geha RS, Renner ED, Orange JS (2013) Defective actin accumulation impairs human natural killer cell function in patients with dedicator of cytokinesis 8 deficiency. J Allergy Clin Immunol 131:840–848

Nishikimi A, Kukimoto-Niino M, Yokoyama S, Fukui Y (2013) Immune regulatory functions of DOCK family proteins in health and disease. Exp Cell Res 319:2343–2349

Pei RJ, Chen XW, Lu MJ (2014) Control of hepatitis B virus replication by interferons and Toll-like receptor signaling pathways. World J Gastroenterol 20:11618–11629

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81:559–575

Qi P, Chen YM, Wang H, Fang M, Ji Q, Zhao YP, Sun XJ, Liu Y, Gao CF (2009) 509C > T polymorphism in the TGF-beta1 gene promoter, impact on the hepatocellular carcinoma risk in Chinese patients with chronic hepatitis B virus infection. Cancer Immunol Immunother 58:1433–1440

Saxena R, Chawla YK, Verma I, Kaur J (2014) IL-6(-572/-597) polymorphism and expression in HBV disease chronicity in an Indian population. Am J Hum Biol 26:549–555

Shen HP, Hsiao YH, Yang SF, Liu YF, Ko JL, Wang PH (2018) Single nucleotide polymorphisms and haplotypes of carbonic anhydrase 9 can predict invasive squamous cell carcinoma of uterine cervix. Int J Med Sci 15:587–594

Shiraishi A, Uruno T, Sanematsu F, Ushijima M, Sakata D, Hara T, Fukui Y (2017) DOCK8 protein regulates macrophage migration through Cdc42 protein activation and LRAP35a protein interaction. J Biol Chem 292:2191–2202

Sinai P, Nguyen C, Schatzle JD, Wulfing C (2010) Transience in polarization of cytolytic effectors is required for efficient killing and controlled by Cdc42. Proc Natl Acad Sci USA 107:11912–11917

Skol AD, Scott LJ, Abecasis GR, Boehnke M (2006) Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nat Genet 38:209–213

Wang B, Wang J, Zheng Y, Zhou S, Zheng J, Wang F, Ma X, Zeng Z, Consortium HBVS (2010) A study of TNF-alpha-238 and -308 polymorphisms with different outcomes of persistent hepatitis B virus infection in China. Pathology 42:674–680

Wang SS, Liu YF, Ou YC, Chen CS, Li JR, Yang SF (2013) Impacts of CA9 gene polymorphisms on urothelial cell carcinoma susceptibility and clinicopathologic characteristics in Taiwan. PLoS ONE 8:e82804

Wang S, Wang J, Fan MJ, Li TY, Pan H, Wang X, Liu HK, Lin QF, Zhang JG, Guan LP, Zhernakova DV, O'Brien SJ, Feng ZR, Chang L, Dai EH, Lu JH, Xi HL, Zeng Z, Yu YY, Wang BB (2018) Identified OAS3 gene variants associated with coexistence of HBsAg and anti-HBs in chronic HBV infection. J Viral Hepat 25:904–910

WHO (2015) Guidelines for the Prevention, Care and Treatment of Persons with Chronic Hepatits B Infection. https://www.who.int/hepatitis/publications/hepatitis-b-guidelines/en/

WHO (2017) GLOBAL HEPATITIS REPORT, 2017. https://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/

Xia C, Lu M, Zhang Z, Meng Z, Zhang Z, Shi C (2008) TLRs antiviral effect on hepatitis B virus in HepG2 cells. J Appl Microbiol 105:1720–1727

Xiang Y, Chen P, Xia JR, Zhang LP (2017) A large-scale analysis study on the clinical and viral characteristics of hepatitis B infection with concurrence of hepatitis B surface or E antigens and their corresponding antibodies. Genet Mol Res 16

Xiong W, Wang X, Liu X, Xiang L, Zheng L, Yuan Z (2004) Interferon-inducible MyD88 protein inhibits hepatitis B virus replication. Virology 319:306–314

Yoo H, Sohn S, Nam BH, Min HS, Jung E, Shin SH, Gwak HS, Lee SH (2010) The expressions of carbonic anhydrase 9 and vascular endothelial growth factor in astrocytic tumors predict a poor prognosis. Int J Mol Med 26:3–9

Zeng Z (2014) Human genes involved in hepatitis B virus infection. World J Gastroenterol 20:7696–7706

Zhang Q, Davis JC, Lamborn IT, Freeman AF, Jing H, Favreau AJ, Matthews HF, Davis J, Turner ML, Uzel G, Holland SM, Su HC (2009) Combined immunodeficiency associated with DOCK8 mutations. N Engl J Med 361:2046–2055

Zhang TC, Pan FM, Zhang LZ, Gao YF, Zhang ZH, Gao J, Ge R, Mei Y, Shen BB, Duan ZH, Li X (2011a) A meta-analysis of the relation of polymorphism at sites -1082 and -592 of the IL-10 gene promoter with susceptibility and clearance to persistent hepatitis B virus infection in the Chinese population. Infection 39:21–27

Zhang X, Zhang E, Ma Z, Pei R, Jiang M, Schlaak JF, Roggendorf M, Lu M (2011b) Modulation of hepatitis B virus replication and hepatocyte differentiation by MicroRNA-1. Hepatology 53:1476–1485

Zhang K, Pan X, Shu X, Cao H, Chen L, Zou Y, Deng H, Li G, Xu Q (2013) Relationship between MIF-173 G/C polymorphism and susceptibility to chronic hepatitis B and HBV-induced liver cirrhosis. Cell Immunol 282:113–116

Zheng JX, Zeng Z, Zheng YY, Yin SJ, Zhang DY, Yu YY, Wang F (2011) Role of hepatitis B virus base core and precore/core promoter mutations on hepatocellular carcinoma in untreated older genotype C Chinese patients. J Viral Hepat 18:e423–431

Zheng MH, Xiao DD, Lin XF, Wu SJ, Peng MM, Yu XY, Liu WY, Li LF, Shi KQ, Fan YC, Chen YP (2012) The tumour necrosis factor-alpha-238A allele increases the risk of chronic HBV infection in European populations. J Viral Hepat 19:e11–17

Zhou X, Stephens M (2012) Genome-wide efficient mixed-model analysis for association studies. Nat Genet 44:821–824