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Identified OAS3 gene variants associated with coexistence of HBsAg and anti-HBs in chronic HBV infection

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Abstract

The underlying mechanism of coexistence of hepatitis B surface antigen (HBsAg) and hepatitis B surface antigen antibody (anti-HBs) is still controversial. To identify the host genetic factors related to this unusual clinical phenomenon, a two-staged study was conducted in the Chinese Han population. In the first stage, we performed a case-control (1:1) age, gender matched study of 101 cases with concurrent HBsAg and anti-HBs and 102 controls with negative HBsAg and positive anti-HBs using whole exome sequencing. In the second validation stage, we directly sequence the 16 exons on the OAS3 gene in two dependent cohorts of 48 cases and 200 controls. Although in the first stage, a genome-wide association study of 58,563 polymorphism variants in 101 cases and 102 controls found no significant loci (P-value ≤0.05/58563), and neither locus achieved a conservative genome-wide significance threshold (P-value ≤5e-08), gene-based burden analysis

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

showed that OAS3 gene rare variants were associated with the coexistence of HBsAg and anti-HBs. (P-value =4.127e-06 ≤0.05/6994). 16 rare variants were screened out from 21 cases and 3 controls. In the second validation stage, one case with a new different rare variant was identified. Fisher's exact test of all 149 cases and 302 controls showed that the rare coding-sequence mutations were more frequent in cases versus controls [P-value=7.299e-09, OR=17.27, 95% CI (5.01-58.72)]. Protein-coding rare variations on the OAS3 gene are associated with the coexistence of HBsAg and anti-HBs in patients with chronic HBV infection in Chinese Han population.

Keywords

OAS3; coexistence of HBsAg and anti-HBs; whole exome sequencing; rare variants

Introduction

In the natural course of HBV infection, 90-95% of healthy adults would develop immunity and clear the infection, typically characterized by the disappearance of HBsAg with or without the occurrence of antibodies against HBsAg (anti-HBs) in the serological profile; whereas 90% of neonates and 20-60% of children under the age of 5 years would fail to achieve viral clearance and develop into chronic HBV infection, classically featured by the detection of serum circulating HBsAg for more than 6 months or even lifelong. (1) Anti-HBs is host-produced protective antibody homologous to the HBsAg and is able to neutralize HBsAg, leading to clearance of infectious HBV particles from peripheral blood. Generally, serum HBsAg is a marker of HBV infection, and anti-HBs is an indicator of immunity against HBV infection, both of which should not be detected concomitantly in sera of the same individual with present HBV infection in routine clinical practice. However, several recent studies with a large sample size performed in different geographic areas showed that the prevalence of this special phenomenon among HBsAg positive subjects is approximate 2-9% (2-10), illustrating that the coexistence of HBsAg and anti-HBs may be a universal serological pattern in chronic HBV infection in spite of the low incidence.

The mechanisms underlying the concurrent presence of HBsAg and anti-HBs have not been well delineated and understood. Originally, the coexistence of HBsAg and anti-HBs was simply regarded as superinfection with a different subtype of HBV. (11, 12) After the discovery of vaccine-induced HBsAg escape mutants in 1990,(13) a growing number of studies reported a significantly higher amino acid variability in major hydrophilic region (MHR) of the S gene, especially in the “a” determinant, from carriers with coexistence of HBsAg and anti-HBs than those with HBsAg but without concomitant anti-HBs and concluded that the coexistence of HBsAg and anti-HBs is associated with the accumulation of HBsAg escape mutant due to immune pressure. The “a” determinant is the main target of protective anti-HBs and a single or multiple mutations in or around the “a” determinant may result in alteration of the antigenicity of HBsAg and lead to reduced or even abolished binding of neutralizing antibodies, thus eliciting the detection of both HBsAg and anti-HBs in the serum at the same time. (2-8, 10) Contradictorily, along comes the study by Zhang et al, which showed that the frequencies of amino acid substitutions and/or variations were comparable in patients with and without anti-HBs in the HBsAg sequences, indicating that

the pattern of concurrent HBsAg and anti-HBs was not associated with the emergence of HBsAg mutants. (9)

The current studies related to coexistence of HBsAg and anti-HBs mainly focus on the viral factors and some possible explanations for this special serological pattern were proposed. However, some reported cases have mutations in the HBsAg sequence but without anti-HBs in the serum and some cases without HBsAg mutation have concurrent HBsAg and anti-HBs. This indicates HBV mutants may not be the only reason of the occurrence of anti-HBs in patients with chronic HBV infection and there's maybe some other unknown factors involved. The clearance of HBV, marked by the occurrence of homologous anti-HBs, is the result of a dynamic balance between viral replication and host immune response and studies have shown that other than viral and environmental factors, host genetic factors may play a key role in the clearance of HBV. (14) Therefore, we postulated that other than virus mutation, the host genetic factors might also have an influence on the occurrence of anti-HBs in chronic HBsAg carriers, leading to the coexisting of HBsAg and anti-HBs. So, we conducted a two-stage association study with an aim to identify genetic variations related to this uncommon phenomenon, expecting to further elucidate the clearance of HBV and the occurrence of anti-HBs.

The study was approved by the Ethics Committee of Peking University First Hospital and the Fifth Hospital of Shijiazhuang, and was conducted in accordance with the ethics principles of the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained from all the patients at the participating institution.

Materials and Methods

See Supplementary Materials and Methods.

Results

Characteristics of participants

The characteristics of the 203 subjects in the first stage are summarized in Table 2. All participators are ethnic Han. Gender (Male: 63.34% vs. 50.98%, P-value >0.05) and age (42.36±12.23 vs. 43.89±3.77, P-value >0.05) between two groups were well balanced. The mean alanine transaminase (ALT) level in case group was significant higher than in control group (92.66±186.80 vs 26.20±24.02, P-value ≤0.05) as well as HBV DNA positive rate (50.00% vs 0, P-value ≤0.05) and mean HBV DNA levels (2.62±2.92 vs 0.00±0.00 $\log_{10}$ IU/ml, P-value ≤0.05) as a consequence of the inclusion of not only inactive carriers but also patients with chronic hepatitis B (CHB). 26 patients in the case group were known used to take antiviral agents before the samples were collected.

Genome-wide single variant association study

We performed a genome-wide association study of 58,563 polymorphism variants with minor allele frequency > 0.05 after QC filtered in 101 cases and 102 controls. Genetic association analysis was carried out with Fisher's exact test. No loci achieved a conservative significance threshold after Bonferroni correction for multiple testing (P-value ≤

0.05/58563) neither any locus achieved a conservative genome-wide significance threshold (P-value ≤5e-08). The all variants with P-value are displayed in Supplementary Figure 1.

Gene-based burden study

We evaluated whether cases were more likely to carry low-frequency functional variants in a gene compared with controls by performing a gene-based burden analysis of genome-wide low-frequency variants from 101 cases and 102 controls. Variants were annotated using the variant effect predictor tool. The dataset consists of loss-of-function (LOF) variants, missense variants, synonymous variants and splice region variants. The mutation allele frequency (MAF) cutoffs was restrictive 0–5% in control population, 0-5% in ExAC-EAS database and 0-5% in KG-EA database. Only the genes carried more than 5 variants were included into the panel for gene-based burden analysis. After filtering, the panel consists of 6,994 genes which carried more than 5 low-frequency functional variants. Gene-based burden analysis was carried out with weighted-sum statistic method developed by Madsen and Browning. (15) The all genes with P-value are displayed in figure 1. We found that OAS3 were achieved a conservative significance threshold after Bonferroni correction of 6,994 genes testing (P-value =4.127e-06 ≤0.05/6994). The variation distribution is shown in Supplementary Figure 2. 16 rare variants (ExAC-EAS<0.01) with potentially functional variation on OAS3 gene were screened out (Table 3) and are distributed among 24 patients including 21 DP cases and 3 SP controls.

The follow-up validation results

In the second stage, we sequenced and analyzed the coding region of all 16 exons on OAS3 gene in two independent cohorts of 48 DP cases and 200 SP controls respectively. The characteristics of the two cohorts are shown in Table 4. After filtering the high-frequency variants, one DP case was found with a new rare stop-codon variation (NM_006187.3: c. 3161G>A; p.1054Trp>X) on the OAS3 gene close to the stop-codon variation (ENST00000228928.7: c.3167G>A; p.1056Trp>Ter) discovered in whole exome sequencing study. (Figure 2)

In total, 17 rare variants on the OAS3 gene were identified and distributed among 22 DP cases and 3 controls from the four cohorts. A Fisher exact test was then carried out among four cohorts combined together, i.e., 149 DP cases and 302 SP controls. The result showed that DP case group has a significant higher rate of rare variants than the SP controls. [P-value=7.299e-09, OR=17.27, 95% CI (5.01-58.72)]

Discussion

The coexistence of HBsAg and anti-HBs is a special pattern of HBV serological profile and many previous studies have reported and studied this uncommon phenomenon. The underlying mechanism remains controversial although a variety of explanations revolving around viral factors for this phenomenon were developed. (16, 17) Among these, two main hypotheses can be summarized predicated on existing point of views. One hypothesis states that it is associated with mutations in the PreS/S gene, especially the “a” determinant and even the polymerase region (18-20). Mutations within the “a” determinant may occur under

selective pressure induced by host immunity, antiviral therapy, HBV active or passive immunization. (21-24) Another hepothesis declares that the coexistence of HBsAg and anti-HBs is associated with the presence of heterologous subtype-specific anti-HBs but not with mutations in the S gene region. (9, 12)

However, because of the difference of the study population including numbers, races, HBV genotypes, inclusion criteria and the sensitivity and specificity of commercial assaying kits, results from different studies are not entirely comparable and consistent. The current studies related to the coexistence of HBsAg and anti-HBs mainly focused on the viral mutation but overlooked the influence of the host genetics. It's well established that the host genetic factors play a key role in determining the outcomes of HBV infection. With an aim to identify the host genetic genes involved in the coexistence of HBsAg and anti-HBs in patients with chronic HBV infection, we designed this two-stage study and identified OAS3 gene variants are associated with coexistence of HBsAg and anti-HBs in Chinese Han population.

Although in the first stage of our study, genetic association analysis failed to find loci that would achieve a conservative genome-wide significance threshold after Bonferroni correction ($P \leq 0.05/58563$), the results from gene-based burden analysis of rare variants showed OAS3 were achieved a conservative significance threshold after Bonferroni correction of 6,994 genes testing ($P \leq 0.05/6994$). This may be because: first, GWAS usually does not have the power to detect all variants, only the ones with the biggest effects. Traditional GWAS mainly focused on the identification of common genetic variants by collecting them and then performing a series of single-marker tests where each variant is tested individually to discover associations. But only a small portion of disease heritability is explained by common variants, and studies consider that analyses of low-frequency ($1\% \leq MAF < 5\%$) and rare ($MAF < 1\%$) could explain additional disease risk or trait variability. Since each rare variant is present in only a small number of individuals, single-marker tests have low power to identify these variants involved in complex disease. (25) Second, although next-generation deep sequencing provides an unparalleled opportunity to investigate the roles of low-frequency and rare variants in complex diseases, the statistical power of classical single-variant based association tests for low-frequency and rare variants is low unless sample sizes or effect sizes are very large (26), yet the size of samples in this study is small as a result of limited funds and eligible samples. Third, noncoding regions can play an important role in complex diseases and traits and it has been shown that most GWAS loci lie in noncoding regions. (26) The limitation that exome sequencing only captures genetic variation in the exome may also explain the failure to find significant loci using case-control association analysis. Accounting for these, a group wise association tests that group rare variants in genes should be considered as methods to boost the power of studies on rare variants. Therefore, burden analysis is employed. It evaluates association for multiple variants in a biologically relevant region, such as a gene, instead of testing the effects of single variants, as is commonly done in GWAS and eventually identify OAS3 gene has a significant difference between the two groups. (15)

Oligoadenylate synthetases (OAS) are pattern-recognition receptors for viral dsRNA, a common pathogen-associated molecular pattern for many types of RNA and DNA viruses.

Viral infections produce dsRNA which stimulates OAS to produce 2′, 5′-oligoadenylates (2-5A) from ATP. The 2-5A-dependent ribonuclease L (RNase L) was activated upon binding to 2-5A and was then able to degrade viral and cellular RNAs, inhibits protein synthesis, and restricts the replication and spread of diverse viruses. The OAS/ RNase L pathway is one of main effector pathways of the interferon (IFN)-mediated antiviral response and plays an important role in the innate immune response. In humans, there are four OAS genes, all stimulated by IFN, but only three of these (OAS1, OAS2, and OAS3) encode catalytically active proteins. (27) Genetic variants in the OAS genes are known to affect OAS activity and are associated with severity and/or outcomes of many kinds of viral infections, such as hepatitis C virus (HCV) infection, (28, 29), tick-borne encephalitis (TBE), (30) dengue virus, (31) enterovirus-71, (32) West Nile virus (33) and HBV (34-36). In particular to hepatitis B disease, multiple viral and host factors are reported to affect host immune and antiviral responses and, thus, are associated with variable disease outcomes. For the host factors, other than the widely reported and studied MHC I and MHC II class polymorphisms, as well as tumor necrosis factor-alpha (TNF-a), IFN, and interleukin (IL), (37) the present study showed that variations in the OAS3 gene might affect the outcomes of chronic HBV infection as well. The expression of antiviral enzyme OAS may be affected by polymorphisms in OAS3 gene and thus the IFN-induced OAS/ RNase L pathway could not be fully activated, leading to insufficient antiviral effects. This maybe a possible reason for the patients with coexistence of HBsAg and anti-HBs but failed to clear the HBsAg. Therefore, in the next step, we expect to examine the quantity and activity of OAS enzymes in the serum to test this hypothesis.

There are several limitations in this study. First, the sample size is small. Second, Antiviral therapy and HBV mutants are confounding factors in this study. But most of the cases in DP group were HBV-DNA negative at the entry of the study and sera of some of the HBV-DNA positive cases weren't available, thus making the virus sequencing impossible. Also, 26 cases were undergoing antiviral therapy at the time of the study. Actually, virus sequencing of S gene was carried out in 4 DP cases with rare variants, of which two cases possessed no variation in the S gene while the other two did (Data not shown). One of the case with both virus variation in the S gene and genomic variant in the OAS3 gene once take antiviral therapy of interferon and entecavir before the coexistence of HBsAg and anti-HBs appears in the serological profile. Third, noncoding regions can play an important role in complex diseases and traits, but exome sequencing captures genetic variation only in the exome region. The occurrence of anti-HBs in patients with chronic HBV infection could be the cumulative effect of variations on OAS3 gene combined with allele on introns or different genes. Therefore, deep whole genome sequencing should be considered in future study.

In this study, we demonstrate that OAS3 gene variants are associated with coexistence of HBsAg and anti-HBs in Chinese Han population through a burden study of rare variants and a subsequent validation study. Further functional study of OAS3 gene is needed to fully evaluate its contribution to CHB infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ALT	alanine transaminase
Anti-HBc	hepatitis B core antibody
anti-HBe	hepatitis B e antibody
anti-HBs	hepatitis B surface antibody
anti-HCV	antibodies against HCV
anti-HDV	antibodies against HDV
CHB	chronic hepatitis B
DP	double positive
GATK	genome analysis toolkit
GWAS	genome-wide association study
HCC	hepatocellular carcinoma
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HDV	hepatitis D virus
HDVAg	HDV antigen
HEV	hepatitis E virus
LLOD	lower limit of detection
LOF	loss-of-function
MAF	mutation allele frequency
2-5A	2′,5′-oligoadenylates
OAS	Oligoadenylate synthetases
MHR	major hydrophilic region
RNase L	ribonuclease L

PCR	polymerase chain reaction
SD	standard deviation
ULN	upper limit of normal
SP	single positive
WES	whole exome sequencing

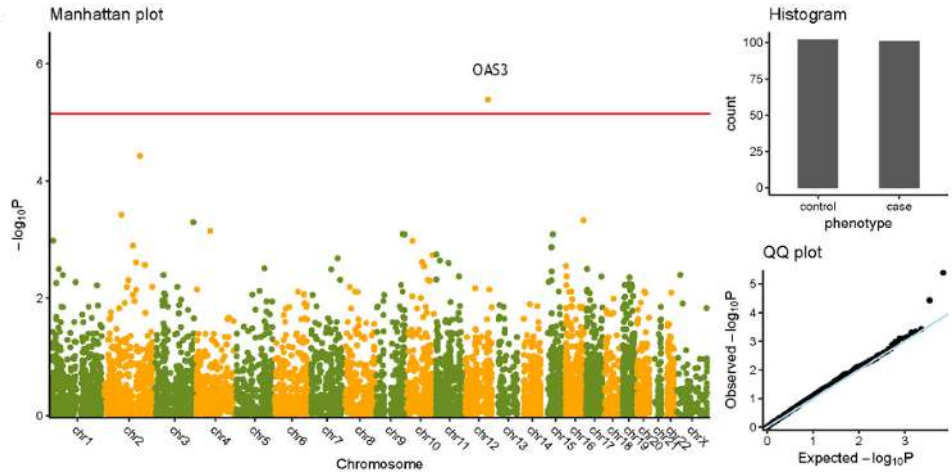

Fig. 1. Manhattan plot

We perform gene-base burden analysis base on the dataset of low-frequency functional variants. X axis indicates the variant location at hg19, chromosomes separated by different colors; y axis indicates the -log10 (P-value) from burden analysis. The red line indicates the genome-wide significance threshold (y=-log10(0.05/6994)). Candidate gene as shown with names (OAS3) on top. 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.

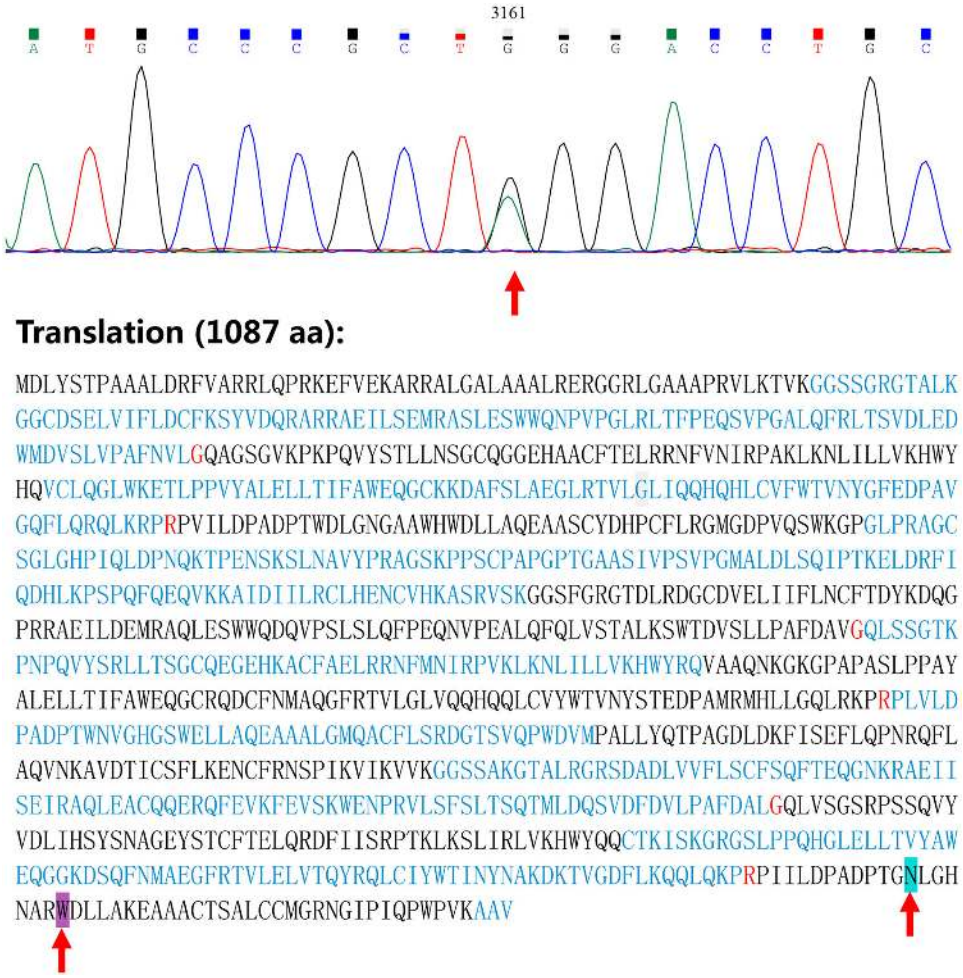

Fig. 2. Peak map

Peak map of variation (NM_006187.3: c.3161G>A) and its amino acid change (p. 1054W>X) as shown in purple. This stop-codon variation is close to the one (ENST00000228928.7: c.3167G>A; p.1056Trp>Ter) discovered using exome sequencing in the first stage as shown in blue.

Table 1

Inclusion criteria and exclusion criteria

Inclusion criteria		
Cases (DP)	**1**	HBsAg, HBsAb and anti-HBc positive for at least 6 months and no vaccination history;
	2	anti-HAV, anti-HEV, HDAg negative and/or anti-HDV negative;
	3	Anti-HCV negative;
Control (SP)	**1**	Anti-HBs and anti-HBc positive or anti-HBs positive and no vaccination history;
	2	HBV-DNA negative, anti-HAV, anti-HEV, HDAg negative and/or anti-HDV negative;
	3	Anti-HCV negative.

Exclusion criteria *	
1	Evidence of past or current infection by HCV or HDV;
2	Age less than 18 for all cases and controls;
3	Other systemic disease not related to HBV infection;
4	With other hepatitis virus infection;
5	Not of Han ethnicity.

* Excluded from enrollment if one or more of the exclusion criteria were met; Applicable for all the samples; DP: Double positive; SP: Single positive.

Table 2
Characteristics of 101 DP cases and 102 SP controls in whole exome sequencing study

Group	DP cases (n=101)	SP controls (n=102)	P-value
Male, %(n)	63.34 (64/101)	50.98 (52/102)	0.075
Age, y, mean±SD (n)	42.36±12.23 (101)	43.89±3.77 (102)	0.230
HBeAg positive, %(n)	56.44 (57/101)	0 (0/114)	0.000
HBsAg, IU/ml, mean±SD (n)	3329.78±5719.41 (100)	Negative (102) *	
HBsAb, IU/ml, mean±SD (n)	110.03±188.95 (100)	Positive (102) *	
ALT, IU/ml, mean±SD (n)	92.66±186.80 (92)	26.20±24.02 (90)	0.001
Normal ALT, % (n)	60.87 (56/92)	91.84 (90/98)	0.000
HBV-DNA, $\log_{10}$ IU/ml, mean±SD (n)	2.62±2.92 (92)	0.00±0.00 (102)	0.000
HBV DNA positive, %(n)	50.00 (46/92)	0 (0/102)	0.000
Antiviral therapy, n	26	0	
Interferon	9	0	
Telbivudine	2	0	
Adefovir	2	0	
Entecavir	10	0	
Entecavir+Adefovir	2	0	
Tenofovir	1	0	

*Note: only shows negative or positive in database. ALT, alanine transaminase; HBV, hepatitis B virus; SD, standard deviation; DP, double positive; SP, single positive. ALT upper limit of normal (ULN): 40IU/ml.

Table 3

16 rare genetic variants found in the OAS3 gene in whole exome sequencing study

Chr	Position	cDNA	Protein	Consequence	ExAC-EAS	101 DP cases	102 SP controls
chr12	113376421	c.86C>T	p.Ala29Val	Missense variant	NA	1 het	0
chr12	113379466	c.269A>G	p.Gln90Arg	Missense variant	0.000765	1 het	0
chr12	113379591	c.394C>T	p.Arg132Cys	Missense variant	NA	1 het	0
chr12	113379599	c.402A>G	NA	Synonymous variant	0.00586	5 het	2 het
chr12	113379688	c.491G>A[a]	p.Gly164Glu[b]	Missense variant	NA	1 het	0
chr12	113384545	c.637-3C>T	NA	Splice region variant	0.000588	2 het	0
chr12	113384668	c.757C>T	p.Arg253Ter	Stop gained	0.000128	1 het	0
chr12	113385843	c.969delC	p.Tyr324MetfsTer7	Frameshift variant	NA	1 het	1 het
chr12	113400602	c.1979G>A	p.Gly660Glu	Missense variant	0.000382	1 het	0
chr12	113400619	c.1996C>T	p.Gln666Ter	Stop gained	NA	0	1 het
chr12	113400686	c.2063T>C	p.Leu688Pro	Missense variant	NA	1 het	0
chr12	113402109	c.2299C>T	p.Arg767Cys	Missense variant	0.00616	1 het	0
chr12	113402173	c.2363G>A	p.Cys788Tyr	Missense variant	NA	1 het	0
chr12	113403705	c.2560C>T	p.Arg854Trp	Missense variant	0.000259	1 het	0
chr12	113403782	c.2637G>A	NA	Synonymous variant	0.00383	3 het	0
chr12	113407469	c.3167G>A	p.Trp1056Ter	Stop gained	0.0018	1 het	0

Nucleotide numbering (“c.”) reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence ENST00000228928.7 and ENST00000551007.1. The initiation codon is codon 1. Protein numbering (“p.”) refers to sequence ENSP00000228928.7 and ENSP00000449299.1 (b). “Chr” refers to chromosome and “het” refers to heterozygous changes. ExAC-EAS refers to the East Asian population of ExAC (Exome Aggregation Consortium) dataset. DP, double positive; SP, single positive.

Table 4
Characteristics of the 48 DP cases and 200 SP controls in the second stage

Group	DP cases (n = 48)	SP controls (n=200)	P-value
Male, %(n)	56.25 (27/48)	67.00 (134/200)	0.161
Age, y, mean±SD (n)	42.58±17.35 (48)	61.62±12.66 (200)	0.000
ALT, IU/ml, mean±SD (n)	82.62±104.44 (35)	23.19±31.56 (145)	0.002
Normal ALT, % (n)	57.14 (20/35)	90.34 (131/145)	0.000
HBeAg positive, % (n)	45.24 (19/42)	0 (0/200)	0.000#
HBsAg, IU/ml, mean±SD (n)	7287.00±19134.67 (48)	0.01±0.01 (200)	0.000
HBsAb, IU/ml, mean±SD (n)	87.36±174.05 (48)	244.70±325.94 (200)	0.000
HBV-DNA, $\log_{10}$ IU/ml, mean±SD (n)	4.39±3.22 (24)	0.00±0.00 (5)	
HBV-DNA positive, %(n)	70.83 (17/24)	0 (0/5)	

#Fisher's exact test, two-sided value. DP, double positive; SP, single positive.

ALT ULN: 40IU/ml

HBV-DNA LLOD: <100IU/ml