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ORIGINAL ARTICLE

Basic Study

# Hepatitis B virus infection and genotype in asymptomatic people from 10 ethnic groups in Yunnan, China

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## Abstract

**AIM:** To evaluate the infection and genotype distribution of hepatitis B virus (HBV) in ethnic groups in Yunnan, China.

**METHODS:** Two thousand five hundred and eighty-four asymptomatic local people from 10 ethnic groups were investigated in Yunnan, China. Infection and genotype distribution were evaluated by serological and genetic methods. Genotyping was verified by sequencing. Ethnic genotype distribution was compared by proportion test.

**RESULTS:** Four types of infection model based on HBV serum markers were identified, and the average HBV infection rate was 5.7% in those asymptomatic local people. The genotype prevalence was 59.6% for B, 21.1% for C and 19.3% BC; subgenotypes Ba, Cs and Ce were identified in this study. Hepatitis B surface antigen-positive rate and the proportion of genotype B were significantly lower in ethnic groups with a northern origin compared to those with a southern origin (50% *vs* 73.9%, $P = 0.037$; 4.2% *vs* 10.5%, $P = 0.000$).

**CONCLUSION:** Genotype B is dominant and genotype BC has high occurrence in asymptomatic local ethnic groups in Yunnan. HBV infection status and genotype distribution may associate with ethnic origin.

**Key words:** Hepatitis B virus; Infection and genotype; Ethnic distribution; BC genotype; Yunnan; China



**Core tip:** Hepatitis B virus (HBV) infection and genotype distribution were evaluated in asymptomatic local people from 10 ethnic groups in Yunnan, China. The genotype prevalence rate was 59.6% for B, 21.1% for C and 19.3% BC; hepatitis B surface antigen-positive rate and the proportion of genotype B were significantly lower in ethnic groups with a northern origin compared to those with a southern origin. Our results suggested that HBV infection status and genotype distribution may associate with ethnic origin. It may also give some hint on understanding virus evolution.

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## INTRODUCTION

Hepatitis B is a global public health threat. This chronic disease leads to the development of liver cirrhosis (LC) and hepatocellular carcinoma (HCC)[1]. According to official World Health Organization website, one-third of the world's population, or approximately 2 billion people, have been infected with hepatitis B virus (HBV), and there are approximately 350 million people with chronic HBV worldwide. The situation is even worse in China: the prevalence of chronic HBV infection is up to 10%-20% in the population, which account for three-quarters of cases in the world[2], and half a million people are killed by HBV each year[3]. Although acute HBV infection has declined due to implementation of vaccination, HBV-related complications are still increasing[4].

Based on its genomic structure and sequence differences, HBV has been divided into nine genotypes, A-I, as well as corresponding subgenotypes[5-9]. The various HBV genotypes are associated with differences in pathogenicity[10], disease progression[11], and responses to antiviral drugs[12]. Studies have found that the distribution of HBV genotypes/subgenotypes has remarkable geographical characteristics[13], which may relate to the anthropologic history of the region[14].

Moreover, DNA recombination is a significant and relatively frequent event in the evolution of HBV[15]. There have been increasing reports regarding HBV hybrids or mixed genotypes[16], wherein part of the viral genome of a certain HBV genotype is replaced by the corresponding part of another HBV genotype. The existence of AD genotype is reported in Italy and South Africa, BC is identified in Southeast Asia, and CD is limited to China[16]. Reports in China show that mixed genotype occurs more in those regions with multi-ethnic society, suggesting that this genotype distribution may be related to both geography and ethnicity[17].

Yunnan is located in southwest China. It is characterized by the highest geographic diversity as well as the highest biodiversity and the most diverse mixture of ethnic groups in China[18]. The distinct geographical location and cultural background of Yunnan province result in the diverse epidemiological characteristics of blood-borne HBV infection in this region. However, ethnic distribution of infection and genotypes remains unclear[4].

In this study, we assessed the HBV genotype distribution and infection status in asymptomatic people from 10 ethnic groups in Yunnan, China; 6 of them (Bai, Lisu, Achang, Mosuo, Naxi and Dulong) were judged to originate from Shanxi, Qinghai and Gansu regions in northern China 4000-5000 years ago[18]. Because C dominates the HBV distribution in northern China while B is dominant in southern China[2], we hypothesized that genotype B dominates in Yunnan. Moreover, if historical origin impacted genotype distribution, those people with a northern ethnic background are likely to have a lower genotype B prevalence.

## MATERIALS AND METHODS

### *Materials*

Intravenous blood samples were collected from 2584 asymptomatic people (1153 males and 1431 females, villagers without any sign of HBV infection) from 10 ethnic groups in west Yunnan. Their ethnic background was identified with household registration. Only those with consistent ethnic records in 3 generations are included. Their sera were separated and stored at -20 ℃ for later analysis of the infection status and genotypes/subgenotypes.

We used the following toolkits for our analysis: ELISA kits (Shanghai Kehua Bio-engineering company); Taq DNA polymerase (Bio Bisic Inc); the restriction endonucleases *Stu* Ⅰ, *Hpa* Ⅰ, *Bst*E Ⅱ, and *Bcn* Ⅰ (MBI Fermentas); 10 × polymerase chain reaction (PCR) Buffer ($Mg^{2+}$) (Bio Bisic Inc.); $MgCl_2$ (Bio Bisic Inc.); medical virological Tris base (Bio Bisic Inc.); DNA Marker D (Bio Bisic Inc.); agarose gel (United States AMRESCO); boric acid, EDTA, dNTPs, and primers (Shanghai Sangon Inc.); PCR amplification instrumentation (Perkin Elmer 9600, United States);

**Table 1 The primer sequences for identifying the hepatitis B virus genotypes/subgenotypes**

| Gene identification | PCR | Primer | Primer sequence | Site |
|---|---|---|---|---|
| Genotype (A-F) | 1st PCR | P1 (s) | 5′-TCA CCA TAT TCT TGG GAA CAA GA-3′ | nt2823–2845 |
| | | S1-2 (as) | 5′-CGA ACC ACT GAA CAA ATG GC -3′ | nt685–704 |
| | 2nd PCR | B2 (s) | 5′-GGC TCM AGT TCM GGA ACA GT-3′ | nt67–86 |
| | mixA | BA1R (as) | 5′-CTC GCG GAG ATT GAC GAG ATG T-3′ | nt113–134 |
| | | BB1R (as) | 5′-CAG GTT GGT GAG TGA CTG GAG A-3′ | nt324–345 |
| | | BC1R (as) | 5′-GGT CCT AGG AAT CCT GAT GTT G-3′ | nt 165–186 |
| | mixB | BD1 (s) | 5′-GCC AAC AAG GTA GGA GCT-3′ | nt 2979–2996 |
| | | BE1 (s) | 5′-CAC CAG AAA TCC AGA TTG GGA CCA-3′ | nt 2955–2978 |
| | | BF1 (s) | 5′-GYT ACG GTC CAG GGT TAC CA-3′ | nt 3032–3051 |
| | | B2R (s) | 5′-GGA GGC GGA TYT GCT GGC AA-3′ | nt 3078–3097 |
| Subgenotype B (B1 and B2) | 1st PCR | PC1 (s) | 5′-CAT GCA ACT TTT TCA CCT CTG CCT-3′ | nt1813-1836 |
| | 2nd PCR | PC2 (s) | 5′-ATT AGA CCT ATT GAT TGG AAA GT-3′ | nt1861-1881 |
| | | COR-HBV (as) | 5′-GAG TGC GAA TCC ACA CTC CA-3′ | nt2285-2266 |
| C (C1 and C2) | 1st PCR | HBV964F (s) | 5′-ATT AGA CCT ATT GAT TGG AAA GT-3′ | nt964-986 |
| | 2nd PCR | HBV970F2 (s) | 5′-CCT ATT GAT TGG AAA GTA TGT CA-3′ | nt970-992 |
| | | HBV1272R (as) | 5′-AGT ATG GAT CGG CAG AGG AG-3′ | nt1272-1253 |

(s): Sense; (as): Anti-sense; M: A or C; Y: C or T; HBV: Hepatitis B virus; PCR: Polymerase chain reaction.

an electrophoresis groove (DYCR-31D, Beijing Liuyi Instrument Factory); and the Syngene Automatic Image Analysis System (GGMID2, Britain's Gene Inc).

## Methods

**Detection of serum markers for hepatitis B:** Serum markers for hepatitis B were tested with an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions. The kit contained HBsAg, HBsAb, HBeAg, HBeAb and HBcAb.

**HBV DNA extraction:** HBV DNA was extracted using the methods described previously[19] and stored at -80 ℃.

**Primer design and synthesis:** Primer sequences were listed in Table 1, and primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai).

**Identification of the HBV genotypes and subgenotypes:** Genotypes were identified according to the methods described in an earlier study[20]. Specifically, the following procedure was followed with a 40 μL reaction system for the first round of PCR amplification. Cycle parameters were as follows: an initial 10 min denaturation at 94 ℃ followed by 40 cycles of amplification at 94 ℃ for 20 s, 55 ℃ for 20 s, and 72 ℃ for 60 s, and strand synthesis at 72 ℃ for 7 min. The second-round PCR was divided into two groups, A and B. Two milliliters of the first-round PCR products were used in the second round of PCR under the same reaction system but with the primers from group A (B2, BA1R, BB1R and BC1R2) and group B (B2R, BD1R, BE1R and BF1R). Both groups A and B were treated with the same cycle parameters, which were denaturation at 95 ℃ for 10 min, 20 cycles of amplification at 94 ℃ for 20 s, 58 ℃ for 20 s, and 72 ℃ for 30 s, followed by an additional 20 cycles of 94 ℃ for 20 s, 60 ℃ for 20 s, and 72 ℃ for 30 s, and an extension at 72 ℃ for 7 min. Finally, the PCR products were electrophoresed on a 2.5% agarose gel, stained with ethidium bromide and checked under UV light.

The HBV genotypes were determined based on the size of amplified fragments; products of 68 bp, 281 bp, 122 bp, 119 bp, 167 bp and 97 bp were considered as genotypes A, B, C, D, E and F, respectively. A mixed genotype was considered when its products consisted of any of the two aforementioned fragments.

**Subgenotypes:** The method for identifying subgenotypes was adapted from that described in the literature[20]. The first round of PCR amplification used a 20 μL reaction system containing 2.0 μL of 10 × buffer, 0.4 μL of 0.2 mmol/L dNTPs, 0.4 μL of each 0.2 μmol/L primer (genotype B: PC1 and COR and genotype C: HBV964F and HBV1272R), 2.0 μL of 0.5 U/μL Taq DNA polymerase, 3.0 μL of serum for template, and 11.8 μL dd$H_2O$. The following cycle parameters were used: denaturation at 94 ℃ for 1 min, 30 cycles of amplification at 94 ℃ for 40 s, 55 ℃ for 30 s, and 72 ℃ for 50 s, and a final extension at 72 ℃ for 7 min. The second-round PCR required 2 μL of the first-cycle PCR products as a template with the same reaction system and cycle parameters but different primers (genotype B: PC2 and COR and genotype C: HBV970F and HBV 1272R). The products (5 μL) of the second round were digested with the corresponding restriction endonucleases (Ba/Bj: *Stu* Ⅰ/*Hpa* Ⅰ; Cs/Ce: *Bst*E Ⅱ/*Bcn* Ⅰ), and the subgenotypes were identified according to the fragments of the digested products under a UV light following electrophoresis. A sequencing approach was adopted to determine the subgenotypes of the non-digested products.

**Sequencing:** To verify the accuracy of the genotype-specific PCR method, products from 5 samples of the first-round PCR, including 3 cases of genotype B and 2 cases of genotype C, were selected to determine

**Table 2 Hepatitis B surface antigen-positive rates in the 10 ethnic groups in Yunnan Province**

| Ethnic group | Number | Age | Origin[1] | Gender | | HBsAg | |
|---|---|---|---|---|---|---|---|
| | | | | Male *n* (%) | Female *n* (%) | Positive | Rate (%) |
| Han | 165 | 16.01 ± 14.47 | - | 87 (52.7) | 78 (47.3) | 7 | 4.2 |
| Dai | 459 | 38.41 ± 18.57 | S | 159 (34.6) | 300 (65.4) | 43 | 9.4[2] |
| Bulang | 100 | 36.24 ± 18.11 | S | 54 (54.0) | 46 (46.0) | 7 | 7.0 |
| Pumi | 61 | 34.90 ± 18.44 | S | 33 (54.1) | 28 (45.9) | 15 | 24.6[2] |
| Achang | 347 | 30.19 ± 18.46 | N | 154 (44.4) | 193 (55.6) | 16 | 4.6 |
| Mosuo | 234 | 31.61 ± 18.14 | N | 101 (43.0) | 133 (57.0) | 15 | 6.4[2] |
| Naxi | 196 | 8.78 ± 18.32 | N | 100 (51.0) | 96 (49.0) | 2 | 1.0 |
| Dulong | 305 | 31.51 ± 18.66 | N | 143 (46.9) | 162 (53.1) | 10 | 3.3 |
| Bai | 401 | 32.78 ± 17.12 | N | 178 (44.4) | 223 (55.6) | 22 | 5.5 |
| Lisu | 316 | 18.24 ± 18.02 | N | 144 (45.6) | 172 (54.4) | 10 | 3.2 |
| Total | 2584 | 28.35 ± 18.66 | | 1153 (44.6) | 1431 (55.4) | 147 | 5.7 |

[1]Historical ethnic origin; [2]Indicates those with higher than average values. N: From north China; S: From south China; -: Han has no specific origin; HBsAg: Hepatitis B surface antigen.

**Table 3 The distribution of the genotypes and subgenotypes in different ethnic groups**

| Ethic group | Total | Source[1] | Genotype | | | | Subgenotype | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | B | C | B + C | Sum | Ba | Cs | Ce |
| Han | 165 | - | 1 | 0 | 1 | 2 | 1 | 0 | 0 |
| Dai | 459 | S | 13 | 4 | 1 | 18 | 13 | 3 | 0 |
| Bulang | 100 | S | 0 | 0 | 1 | 1 | 0 | 1 | 0 |
| Pumi | 61 | S | 4 | 0 | 0 | 4 | 3 | 0 | |
| Achang | 347 | N | 2 | 0 | 4 | 6 | 3 | 1 | 0 |
| Mosuo | 234 | N | 3 | 3 | 1 | 7 | 0 | 0 | 0 |
| Naxi | 196 | N | 1 | 0 | 0 | 1 | 1 | 0 | 0 |
| Dulong | 305 | N | 1 | 1 | 0 | 2 | 1 | 1 | 0 |
| Bai | 401 | N | 6 | 3 | 2 | 11 | 4 | 3 | 0 |
| Lisu | 316 | N | 3 | 1 | 1 | 5 | 0 | 1 | 1 |

[1]Historical ethnic origin. N: From north China; S: From south China; -: Han has no specific origin.

the sequence of the HBV S region. The PCR-amplified products were purified, cloned and directly sequenced by TAKARA Biotechnology (Dalian Co., Ltd). DNASTAR was used to analyze the homology between the sequencing genotype results and the standard GenBank strain sequences, and a phylogenetic tree was built.

**Statistical analysis:** $\chi^2$ test and proportion test were performed with R Statistics (The statistical methods of this study were reviewed by Ren GP of Dali University).

### *Ethics statement*

Written informed consent was obtained from all adult participants and from their parents or guardians for minors/children in the study [All procedures in this study were approved by the Medical Ethics Committee of Dali University (No. 2007005)].

## RESULTS

### *Infection status*

The overall positive rate of HBsAg in this study was 5.7% in average. Infection rates in Dai, Pumi, Bulang and Mosuo were higher than average, especially for Pumi (24.6%). Detailed infection status for the different ethnic groups is listed in Table 2.

The HBsAg positive rate was 10.5% in ethnic groups originating from south China and 4.2% for those with a northern origin, which had a significant difference ($P$ = 0.000).

### *Genotype/subgenotype distribution*

The genotype-specific PCR method was employed to detect the HBV genotypes. Fifty-seven samples were positive, including 34 cases of genotype B (59.6%), 12 cases of genotype C (21.1%), and 11 (19.3%) cases of BC. All ethnic groups had genotype B except Bulang. Achang had the highest BC occurrence. No C or BC was found in Pumi and Naxi. Detailed distribution of the genotypes and subgenotypes in different ethnic groups is listed in Table 3.

Genotype B accounted for 50% of those with a northern origin; C, 25%; and BC, 25%. For those ethnic groups with a southern origin, the genotype distribution was 73.9% for B, 17.4% for C, and 8.7% for BC. There was a statistically significance difference in the proportion of B genotype between groups of different origins ($P$ = 0.037). No difference was noted in the genotype prevalence for genotypes C ($P$ = 0.250) and BC ($P$ = 0.061).

Regarding the subgenotypes, a total of 37 samples

**Table 4 The distribution of the hepatitis B virus genotypes/subgenotypes in different serum marker models**

| Serum marker mode | Genotype | | | | Subgenotype | | | |
|---|---|---|---|---|---|---|---|---|
| | B | C | B + C | Subtotal | Ba | Cs | Ce | Subtotal |
| HBsAg+HBeAg+HBcAb+ | 22 | 6 | 6 | 34 | 15 | 7 | 1 | 23 |
| HBsAg+HBeAb+HBcAb+ | 7 | 4 | 2 | 13 | 6 | 3 | 0 | 9 |
| HBsAg+HBcAb+ | 3 | 0 | 3 | 6 | 2 | 1 | 0 | 3 |
| HBeAg+ | 2 | 2 | 0 | 4 | 1 | 1 | 0 | 2 |
| Total | 34 | 12 | 11 | 57 | 24 | 12 | 1 | 37 |

HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; HBcAb: Hepatitis B core antibody; HBeAb: Hepatitis B e antibody.

were genotyped; 24 of them were subgenotype Ba, while 12 were subgenotype Cs and 1 was subgenotype Ce.

### *Serum markers*

There were 4 types of HBV serum infection marker model for all samples, namely, HBsAg+HBeAg+HBcAb+, HBsAg+HBeAb+HBcAb+, HBsAg+HBcAb+ and HBeAg+ (Table 4). Comparison of the genotypes and subgenotypes for the 4 models did not show statistically significant difference ($P$ = 0.303; $P$ = 1.000).

### *Genotype validation*

The sequences of 2 standard strains of genotype C (EU306694 and EU439011) and 2 standard strains of genotype B (EU439019 and EU43902) from Yunnan province were downloaded from GenBank. Three cases of genotype B (No. KMS6330, KMS6337 and KMS6339) and 2 cases of genotype C (No. KMS6329 and KMS6333) in this study were compared with the standard strains. The homology of KMS6330, KMS6337 and KMS6339 with the standard B strain was 98%-99%, and their homology with the standard C strain was 87%-92%. The homology of KMS6329 and KMS6333 with the standard C strain was 98%-99% and 87%-94% with the standard B strain. Therefore, the genotype classification should be valid.

## DISCUSSION

The overall positive rate of HBsAg in this study was 5.7% in average, which is lower than the average level in China (7.18%)[21]. However, it reached 24.6% in Pumi people. Moreover, all 4 sites with a positive rate higher than the average level were concentrated in a remote village of west Yunnan. This pattern is quite consistent with the distribution pattern of ethnic groups in Yunnan, which is characterized by a mixed distribution on a large scale and an isolated distribution on a small scale.

The HBV genotype distribution pattern in China is dominated by genotype C in the north and B in the south; main genotypes were C, B and BC, and their rates were 50.99%, 35.58%, and 6.07%, respectively[22]. The genotypes B and C distribution was consistent with this pattern, and genotype B was dominant with a prevalence of 59.6% in this study. However, Wang *et al*[4] and Kang *et al*[23] found that genotype C dominated in their samples. Wang *et al*[4] reported that genotype B (33.3%), genotype C (62.5%), genotype I (2.78%) and C/D (1.39%) in 80 samples of patients in middle and east Yunnan; Kang *et al*[23] reported 76.9% C, 15.4% B, 5.1% D and 2.5% I in 2216 samples from people who had a physical examination in Kunming city. Since Kunming is the capital of Yunnan province; thus, those studies focusing on patient instead asymptomatic people in big cities with large migration population may cause a biased conclusion. Genotype C proportion is much higher that reported by Zhu and Dong[23]. We think our study may represent the origin distribution of genotypes in Yunnan better, because all samples were collected from asymptomatic people in west Yunnan, where is underdeveloped than other part of Yunnan. Besides the overall distribution of genotype distribution in this study, comparison between ethnic groups with different origins also showed a pattern consistent with overall pattern in China. The prevalence of genotype B in ethnic groups with a northern origin was much lower than that with a southern origin (50% *vs* 73.9%). Thus, ethnic/genomic background may have driven the HBV genotype distribution of a certain human population. Although there was no difference in the distribution of genotype B, the BC distribution nearly showed a statistically significant difference ($P$ = 0.061), which may be due to the small sample size and the fact that the study subjects were from an asymptomatic population.

A much higher prevalence of BC (19.3%) was found in this study, especially for those with a northern origin. This result is consistent with the 26.1% BC frequency reported in a previous study[17] in southwest China (Sichuan Province) that included minority groups (Tibetan people). Higher infection in ethnic groups with a southern background, and the lower infection rate in ethnic groups with a northern origin in a region (in which B dominates) may reflect the physical-anthropological factors contributing to the HBV infection distribution. This also suggested that recombination is an important way for HBV genotypes to adapt to people with different genomic backgrounds. The BC genotype may be a strategy that allows genotype C to survive; when the familiar hosts adapt to a different habitat, it can be a transition expression or another adaption type. One may expect

a higher prevalence of the mixed genotype in regions with ethnic group migration, which agrees well with the finding that the CD hybrid is the dominant genotype in a population with frequent migration[24]. A recent paper reviewed 16 studies on HBV genotype distribution in 20 minority groups in China, and it shows that recombination was found in 13/20 ethnic groups[25].

The HBV divergence in humans and apes was estimated to occur in the last 6000 to 7000 years[26]. However, most estimations of the time to the most recent ancestor of human HBV fell in the range of 2000-4000 years[15]. This is consistent with ethnic migration time estimation (4000-5000 years) based on a linguistics study in Yunnan[18]. Wang *et al*[4] suggested that distribution of HBV in Yunnan is also impacted by other provinces of China with an estimation around 1900s, when Yunnan started its development. Thus, HBV genotype distribution can be shaped by both historical genomic backgrounds of people and economic development.

Additionally, there were 37 cases of HBV sub-genotype positivity in the identified genotype samples, of which there were 24 cases of Ba subgenotype, 12 cases of Cs subgenotype and 1 case of Ce subgenotype. This is consistent with other studies in China[2]. However, we did not find the Bj subgenotype in this study, which is likely due to a small sample size.

In conclusion, our study not only found that the genotype distribution in asymptomatic people is related to their ethnic origin but also found different HBV infection rates in different ethnic groups. A recent study showed that the ethnogeographical project can link ethnic origins and HBV genotype distribution in patients[27]. Ethnic epidemiological studies with systematic spatial sampling that account for the migration history and socioeconomic factors (such as an ethnic group's travel), may improve our understanding of the evolution of HBV. Such studies will improve disease prevention and clinical treatment, because HBV transmission is affected by increasing interactions among different ethnic groups. More systematic sampled epidemiological research is needed in Yunnan, which is a hub between China and southeast Asia and a reserve representing historical issues.

## COMMENTS

### Background

Hepatitis B is a global public health threat. It is even worse in China. Hepatitis B virus (HBV) has been divided into nine genotypes, and various HBV genotypes are associated with differences in pathogenicity, disease progression, and responses to antiviral drugs. Studies have found that the distribution of HBV genotypes/subgenotypes has remarkable geographical characteristics, and it may relate to the anthropologic history of the region. Yunnan is located in southwest China. It is characterized by the highest geographic diversity as well as the highest biodiversity and the most diverse mixture of ethnic groups in China. However, ethnic distribution of infection and genotypes remains unclear.

### Research frontiers

Ethnogeographical project can link ethnic origins and HBV genotype distribution in patients. Thus, ethnic epidemiological studies should be a hotspot to improve our understanding of the evolution of virus in the future.

### Innovations and breakthroughs

Former studies focusing on patients in big cities with large migration population may cause a biased conclusion. This study focuses on asymptomatic people in an underdeveloped region, and included ethnic origin as a key factor to understand HBV genotype distribution. Results showed that HBV genotype distribution may relate to ethnic origin thousands years ago.

### Applications

Results from this study may give some hint on evolution of HBV, and it also has potential contribution to the prevention and management of HBV infection.

### Terminology

Hepatitis B is an infectious disease caused by the HBV which affects the liver. It can cause both acute and chronic infections.

### Peer-review

This manuscript describes a prospective epidemiological study on the infection and genotype of hepatitis B virus in Yunnan, China. The authors have clearly outlined their hypothesis for the study. The study design and methods were described in a very detailed manner, especially regarding sample testing techniques.

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