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Molecular Epidemiology of Hepatitis B Viral Serotypes and Genotypes in Taiwan

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Key Words

Hepatitis B virus · Serotype · Genotype · Surface gene · Molecular epidemiology

Abstract

Subtypes of hepatitis B virus (HBV) have specific geographic distributions and can serve as epidemiological markers. The relationship of HBV serotypes and genotypes in Taiwan and their correlation with the domiciles of origin in 122 patients with chronic HBV infection were investigated. The serotype of HBV was determined by comparing the surface gene encoding amino acids 22–148 of the major surface protein with published sequences. Genotyping of HBV was performed by polymerase chain reaction-restriction fragment length polymorphism. Serotype *adw* accounted for 70% (85/122) of all HBVs, with the remaining belonging to serotype *adr*. All *adr* HBVs were genotype C, regardless of the patient's domicile. Of the 85 *adw* HBVs, 69 (81%) were genotype B, 10 (12%) were genotype C, 5 (6%) were genotype F and only 1 (1%) was genotype A. In the 31 patients originating from mainland China, the prevalence of *adr*/genotype C was higher than in the 91 Taiwanese patients (15/31 vs. 22/91; $p < 0.05$). The distribution of the HBV serotypes and genotypes was not significantly different between 17 patients born in Taiwan (6 *adw*/genotype B, 2 *adw*/genotype C, 1 *adw*/genotype F and 8 *adr*/genotype C) and 14 patients born in mainland China (5 *adw*/genotype B, 2 *adw*/genotype C and 7 *adr*/genotype C). Our results indicate that in Taiwan, most HBVs of serotype *adw* are genotype B, and all HBVs of serotype *adr* are genotype C. Patients with origins in mainland China have a higher proportion of serotype *adr*/genotype C infection.

Introduction

Hepatitis B virus (HBV) infection is a major health problem worldwide, affecting approximately 350 million persons [9]. The serological heterogeneity of the HBV has been established, and HBV isolates are classified into four major serotypes according to the antigenic determinants of hepatitis B surface antigen [3, 5, 12–14, 15, 16, 24, 26]. These serotypes are *adw, ayw, adr* and *ayr,* which are defined by two mutually exclusive determinant pairs, *d/y* and *w/r,* and a common determinant *a.* Alternatively, a genetic classification based on the comparison of complete HBV genomes has defined seven genotypes (A–G) [16, 21, 25]. Several studies have proposed the geographic

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distribution of each genotype [10, 17, 29]. Genotype A is predominant in northern Europe; genotypes B and C are confined to populations with origins in eastern Asia and the Far East; genotype D is found worldwide, but prevails in the Mediterranean area, the Near and Middle East and south Asia; genotype E is indigenous to western sub-Saharan areas; genotype F is likely to be present in populations with origins on the American continent, and the newly identified genotype G is found in France and the USA.

The correlation of the four serotypes with the seven genotypes has been studied previously [15, 18, 25]. Genomes encoding *adw* are found in genotypes A–C, F and G, while the genomes encoding both *adr* and *ayr* occur in genotype C, along with *adw*. However, these results are still incomplete because the isolates analyzed were small in number and were limited to certain geographic areas. In addition, the HBV isolates of the same serotype/genotype are generally believed to have evolutionary relationships, and can be used for tracing the route of HBV transmission and geographic migration of HBV carriers [1, 4, 12, 14]. Taking advantage of the high prevalence of HBV infection in Taiwan and the different origins of inhabitants in Taiwan, we studied the interrelation between HBV serotypes and genotypes in Taiwan and their correlation with the origins of these patients.

Patients and Methods

Patients

Serum samples from 122 patients (88 men and 34 women; mean age 48.1 years, range 21–78 years) at different stages of chronic HBV infection (33 asymptomatic hepatitis B surface antigen carriers, 31 patients with chronic hepatitis, 22 with liver cirrhosis and 36 with hepatocellular carcinoma) followed at the National Taiwan University Hospital were used for virological assays.

For epidemiological study of HBV infection, the geographic distribution of these patients was determined as previously described [2]. Briefly, the geographical origin of the patient was determined according to the father's place of origin (domicile) rather than the place of birth. Those who were born in Taiwan and whose ancestors came from southern mainland China more than 100 years ago were defined as having their domicile of origin in Taiwan. Those whose origins were in mainland China, i.e. they or their parents moved from mainland China to Taiwan after the Second World War, were further classified into those originating from northern mainland China and those from southern mainland China, with the Yangtze River as the dividing line. Because HBV is mainly transmitted from the mother to the newborn perinatally, the patient's place of birth was also taken into account for the mainlanders and was determined by the year of birth. If the year of birth was before the year 1945, the mainlanders were presumed to be born in mainland China and living in Taiwan. In contrast, the place of birth was presumably Taiwan if the mainlanders were born in or after 1945. Accordingly, 31 (25%) patients originated from mainland China. Twenty-seven of the 31 mainlanders originated from southern mainland China, of whom 15 (56%) were presumably born and living in Taiwan. Only 4 mainlanders originated from northern mainland China in the present study, and 2 of them were presumed to be born and living in Taiwan.

Determination of the HBV Serotype and Genotype

Because monospecific antibodies were not available in Taiwan, the HBV serotypes were alternatively determined by phylogenetic analysis. Previous studies showed that most HBV strains in Taiwan were *adw* and *adr* [26]. We thus determined the serotypes by comparing part of the surface gene amplified from our patients with the published sequences of serotypes *adw* and *adr* [28]. Briefly, serum viral DNA was extracted using commercially available kits (QIAamp DNA Blood Mini Kit, Qiagen, Chatsworth, Calif., USA) according to the manufacturer's instructions. The part of the surface gene encoding amino acids 22–148 of the major surface protein was amplified by single-round or nested polymerase chain reaction (PCR). Then the amplified PCR products were directly sequenced using fluorescence-labeled primers with an automatic sequencer (Model 377A, Applied Biosystems, Foster City, Calif., USA). Sequencing conditions were specified in the protocol for the Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems). After comparing the deduced amino acid sequence with published consensus sequences, the corresponding HBV strain was classified into serotype *adw* or *adr*.

Genotyping of HBV was performed by PCR-restriction fragment length polymorphism of the surface gene of HBV as previously described [7, 10]. Briefly, the extracted DNA was amplified for the fragment of the HBV genome between nucleotide positions 256 and 796. The PCR products were subsequently treated with restriction enzymes. After incubation, the samples were run on a 3% agarose gel and stained by ethidium bromide. Six genotypes (A–F) of HBV were identified by the restriction patterns of DNA fragments.

To avoid false positivity in PCR, strict precautions were followed [8].

Statistical Analyses

Data were analyzed by χ^2 analysis with Yates' correction, or χ^2 analysis of contingency table where appropriate. A p value of <0.05 was considered statistically significant.

Results

Distribution of Serotypes and Genotypes

Our results confirmed that all of the HBV strains in this study belonged to serotype *adw* or *adr*, because the amino acid at position 122 of the major surface protein was lysine in all sequences. Serotype *adw* predominated in this study and accounted for 70% (85/122) of all HBVs; the remaining 37 HBVs were *adr* (table 1). The results of HBV genotyping were consistent with our previous data showing that genotypes B and C prevail in Taiwan [21]. The genotype distribution was as follows: A 1 (1%); B 69 (57%); C 47 (39%), and F 5 (4%) (table 1).

Correlation between Serotypes/Genotypes and Patients' Place of Origin/Birth

Overall, serotype *adw* predominated. Of the 85 *adw* HBVs, 69 (81%) were genotype B, 10 (12%) were genotype C, 5 (6%) were genotype F and 1 was genotype A. However, the ratio of *adw* to *adr* in the Taiwanese was much higher than that in the mainlanders (69/22 vs. 16/15; $p < 0.05$). The ratio of genotype B to C in the Taiwanese was also higher than that in the mainlanders (58/28 vs. 11/19; $p < 0.05$). All 37 *adr* HBVs were genotype C, irrespective of the origins of the patients. Therefore, the increased prevalence of genotype C in the mainlanders was mainly attributed to the codominance of *adr*/genotype C. Subgroup analysis showed that among serotype *adw*, the ratios of genotype B to C were not significantly different between the mainlanders and the Taiwanese (11/4 vs. 58/6; $p > 0.05$). However, the distribution and interrelation of HBV serotypes and genotypes of the northern mainlanders (1 *adw*/genotype B, 1 *adw*/genotype C and 2 *adr*/genotype C) were similar to those of the southern mainlanders (10 *adw*/genotype B, 3 *adw*/genotype C, 1 *adw*/genotype F and 13 *adr*/genotype C).

In the 27 patients who had origins in southern mainland China, 14 HBVs were *adw* and 13 HBVs were *adr*. The interrelation and distribution of HBV serotypes and genotypes were similar between 12 southern mainlanders presumably born in mainland China (5 *adw*/genotype B, 1 *adw*/genotype C and 6 *adr*/genotype C) and 15 southern mainlanders presumably born in Taiwan (5 *adw*/genotype B, 2 *adw*/genotype C, 1 *adw*/genotype F and 7 *adr*/genotype C) (table 2).

Table 1. Distribution of HBV serotypes and genotypes in 122 Taiwanese patients

Genotype	Serotype	
	adw (n = 85; 70%)	*adr* (n = 37; 30%)
B	69 (81)	
C	10 (12)	37 (100)
Other	6 (7)	0 (0)

Figures in parentheses represent percentages.

Discussion

Both serotype and genotype can serve as epidemiological markers of HBV infection. Most people living in Taiwan have moved from different parts of the Chinese mainland at different time points. Thus, it is interesting to clarify if the combination of serotype and genotype can serve as a more useful epidemiological marker of HBV infection in Taiwan. In this study, we established the interrelation of HBV serotypes and genotypes and their possible correlation with origins of different populations

Table 2. Correlation of HBV serotype and genotype with the geographic origin of patients

Origin of patient	Total cases	Serotype				
		adw			*adr*	
		genotype B	genotype C	others	genotype C	others
Northern mainland China[1]	4	1	1	0	2	0
Born in mainland China[2]	2	0	1	0	1	0
Born in Taiwan[2]	2	1	0	0	1	0
Southern mainland China[1]	27	10	3	1	13	0
Born in mainland China[2]	12	5	1	0	6	0
Born in Taiwan[2]	15	5	2	1	7	0
Taiwan	91	58	6	5	22	0
Total	122	69	10	6	37	0

Origin of patient was determined according to the father's place of origin (domicile) [2].

[1] The Yangtze River was the line dividing northern and southern mainland China.

[2] Mainlanders born before the year 1945 were presumed to be born in mainland China. Those born in or after 1945 were presumably born in Taiwan.

in Taiwan. To make the epidemiological map more complete, further genotyping studies in aboriginal HBV carriers in Taiwan are ongoing in our laboratories.

Our data clearly demonstrated a close interrelatedness between serotypes and genotypes of HBV. Serotype *adw* accounted for 80% of HBVs in the Taiwanese patients. Serotype *adw* can be genotype A, B, C, F or G in different geographic areas [7, 15–19, 21–23, 25]. In the present study, we consistently documented that 80% of the Taiwanese *adw* HBVs were genotype B, 10% of them were genotype C and the remaining were genotypes A and F. In contrast, all serotype *adr* HBVs were genotype C, as previously described [11, 18].

We have previously shown that *adr* was confined mostly to carriers originating from mainland China north of the Yangtze River, and *adw* was likely to be present in populations from Taiwan and from regions south of the Yangtze River [26, 27]. In agreement with previous results, the serotype *adw* predominated in the present study, which enrolled subjects mostly with origins in Taiwan and southern mainland China. However, the distribution of HBV serotypes was not well correlated with the origin of the patients. In patients originating from southern mainland China, there was a higher proportion of serotype *adr*. The inadequate number of mainlanders enrolled in the present study was a limitation to clearly defining the relationship between serotype and place of origin of our patients. Furthermore, the assumption that the location where HBV infection was acquired equaled the place of origin for the mainlanders in this study could introduce information bias. Since vertical transmission is the most important mode of spreading HBV, the domicile of origin of the patients in this study may not represent the true source of the acquired HBV, which instead could be more accurately depicted from the place of origin of their mothers. A future large-scaled epidemiological study, including detailed information on patients' mothers, may clarify this important issue.

The rate of HBV nucleotide substitution per site per year remains almost constant as long as the gene function remains unchanged and in the absence of immune selection [6, 20]. Thus, HBV isolates of the same serotype/genotype are generally believed to have evolutionary relationships and can be used to trace the route of HBV transmission and geographic migration of HBV carriers [1, 4]. In the present study, we tried to evaluate whether the combination of serotype and genotype could be a more useful epidemiological marker of HBV infection. Serotype *adr*/genotype C existed in all populations, and thus we did not expect that this strain could serve as a marker to trace the source of HBV infection. Serotype *adw*, in contrast, can be genotype B or C in patients of different origins. Unfortunately, *adw*/genotype B and *adw*/genotype C again did not pinpoint the specific place of origin of the patients in the present study. Taken together, these results show that combining serotypes and genotypes might not provide useful information for tracing HBV infection in Taiwan.

In conclusion, HBV serotype *adw* predominated in the Taiwanese patients, with 80% of them being genotype B. All HBVs of serotype *adr* were exclusively genotype C. Patients with origins in mainland China had a higher proportion of serotype *adr*/genotype C infection. Further studies are needed to clarify the relation between serotype/genotype and the place of origin of patients or clinical significance. In the present study, combining HBV serotype and genotype did not seem to provide additional information for tracing the route of HBV transmission.

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