氏名	Le Thi Thanh Thuy（レイ ティ タン トゥイ）
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学位論文名	Distribution of Genotype/Subtype and Mutational Spectra of the Surface Gene of Hepatitis B Virus Circulating in Hanoi, Vietnam （ハノイ（ベトナム）における B 型肝炎ウィルス表面遺伝子の遺伝子型/血清学的亜型の分布と変異の解析）
論文審査委員	（主査） 教　授　藤堂　　剛 （副査） 教　授　森　　康子　　教　授　杉本　　央

論　文　内　容　の　要　旨

[Objective]

Hepatitis B virus (HBV) is divided into nine serological types (subtype) and seven genotypes (A to G). The genotypes and subtypes have been suggested as useful clinical and epidemiological markers, and in addition genotype with subtype correlate strongly with ethnicity. On the other hand, analysis of mutations in surface (S) gene has provided important information for the improvement of diagnosis, potential vaccines and therapeutic antibody in HBV endemic area. Vietnam is one of the high HBV endemic countries with 14% to 26% hepatitis B surface antigen (HBsAg) positive carriers. However, sufficient information on the molecular epidemiology of HBV is not available except in Ho Chi Minh City (the southern Vietnam) and for refugees entering United States. We therefore undertook a study on sequencing of the 219 nucleotide of S gene of HBV for patients with chronic hepatitis in Hanoi, in order to determine the distribution of genotypes and subtypes and to analyze spectra of mutations. The site of 219 nt includes “a” determinant region that binds HBV antibody.

[Materials and Methods]

Blood samples were collected from hepatitis patients in Vietnam, December 2001 and subjected to examine HBsAg. Among HBsAg positive patients, 40 patients with HBV DNA positive in the sera were selected ; 34 patients with chronic hepatitis, 4 with acute hepatitis and 2 with hepatocellular carcinoma. All the patients had not been vaccinated. They received traditional medication except hepatocellular carcinoma cases treated with anticancer medicine. DNA was extracted from the sera and PCR was performed for 219 nucleotides. The PCR products were used for direct sequencing. Genotypes were determined by phylogenetic analysis using MEGA program version 2.1 and by genotype-specific probe assay (GSPA) using SMITEST (HBV Genotype Detection Kit, Genome Science Laboratories Co. Ltd., Tokyo, Japan). Subtypes were classified by the deduced aa residues (Magnius and Norder, 1995).

[Results]

Among 30 samples determined by phylogenetic analysis, 23 and 7 were genotypes B and C, respectively. Of the remaining 10 samples, 7 were of genotype B and one was mixture of genotype B and C by GSPA method. Consequently, 75% (30/40) were genotype B, 17.5% (7/40) were C and 2.5% (1/40) was B and C mixture. Two samples were not analyzed because of insufficient amount of DNA. Among 40 samples, 25 were classified as *ayw1* (63%), 7 as *adr* (18%), and 1 as *adw2* subtype. Three samples were classified as mixture of subtypes *ayw1* and *ayr*, *ayw1* and *adw2*, and *adw2* and *adw3*. As for the rest 4 samples having mixture of two nucleotides at amino acid residues for subtype determinations, it was not possible to determine a defined subtype.

The nt sequences of the samples with genotype B and C were compared to those of their reference ones. Surprisingly, 24 out of 40 samples (60%) have at least one mixture of two different nucleotides at certain nucleotide positions. Total numbers of base substitutions were 55. Missense mutations were five-fold more prevalent than silnet mutations in the genotype B with *adw2* and with mixture of *adw2* and *adw3*, and C with *adr*, while the ratio of missense to silent mutation as 13：20 in genotype B with *ayw1*. Amino acid sequences of the samples with genotype B and of the samples with genotype C were compared with their reference aa sequences. In the 28 genotype B samples, except two samples, 19 aa substitutions were found. Three M133L (mutations) and one P127T (variation) were in the first and three T141I (variations) in the second loop of the “a” determinant. Other 4 mutations and 8 variations were outside of “a” determinant. In the 7 genotype C with *adr* samples, there were 5 aa changes ; two I126T (variations), one I126M (new mutation) and one T131N (variation) in the first loop and one A166G (mutation) at the outside of the “a” determinant.

[Conclusion]

1. On the basis of 219 nt sequence in the S gene, genotype B with *ayw1* is the predominant genotype/subtype (63%) in Hanoi, Vietnam followed by genotype C with *adr* (18%).
2. Quasi-species nature of HBV was strongly reflected as overlapping two different nucleotides at certain nt positions in 24 (60%) samples.
3. Fifty five base substitutions have been detected. One new mutation and 3 others were found in the first loop of “a” determinant region, however no mutation was found in the second loop where most of the vaccine associated mutations were arisen. Five new variants were found in this study.

論文審査の結果の要旨

B型肝炎ウイルス抗体陽性者がベトナムでは高率（14～26%）であるにもかかわらず、分子疫学の報告は皆無である。そこで、ハノイにおける40名（慢性肝炎：34、急性肝炎：４、肝がん：２）のB型肝炎患者の血清よりHBV-DNAを抽出後、抗体結合領域を含む部分をPCR-direct sequence法により塩基配列を決定した。遺伝子型と血清学的亜型（Subtype）は治療薬の感受性、変異はワクチン有効性、に関連している。結果はハノイにおける遺伝子型-Subtypeは *B-ayw1* ; 63%、*C-adr* ; 18%がの主であった。*B-ayw1* ベトナム特異的である。突然変異に関しては、新変異が１個、３個の同変異が “a” determinant region、first loop で、６個が “a” region 以外の領域で検出された。Second loop領域では検出されなかった。First loop 領域変異は自然変異が多く、Second loop 領域変異はワクチン関連変異と報告されている。したがって、ハノイでみつかった変異の起原は自然突然変異である可能性があり、現在使用のワクチンの有効性が示唆された。

以上の結果は学位の授与に値すると考えられる。