

## G1957C variation in Core Promoter and Precore/Core regions of Hepatitis B virus isolated from men who have sex with men (MSM) in Surakarta, Central Java, Indonesia

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**Abstract.** Prasetyo AA, Sariyatun R, Prasetyo AD, Yudistiro I, Sidhajati RA. 2014. G1957C variation in Core Promoter and Precore/Core regions of Hepatitis B virus isolated from men who have sex with men (MSM) in Surakarta, Central Java, Indonesia. *Nusantara Bioscience* 6: 107-110. Little is known about HBV isolates in Indonesian men who have sex with men (MSM) community. Thus, this study was aimed to analyze the genetic profile of HBV isolated from MSM in Indonesia, particularly in Surakarta, Central Java. A total of 205 blood samples were examined by polymerase chain reaction (PCR) targeting the partial Core Promoter (CP) and Precore/Core (preC/C) regions. Three samples were found positive by PCR. The amplicons were sequenced and subjected to molecular analysis. The genetic profile of all HBV isolated in Indonesia (n= 122) were also included for molecular analysis. All HBV isolated from the MSM were genotype B3. G1957C (C195S) was found in all HBV isolated from the MSM community and subject with sex with man history. Several disease-related mutations were observed among HBV sequences from Indonesia, including T1753C, A1762T, G1764A, A1846T, and G1896A which existed in 7.2% (9/125), 17.6% (22/125), 19.2% (24/125), 16.8% (21/125), and 24.8% (31/125) sequences, respectively; however, none were present in HBV isolated from the MSM. In conclusion, various mutations, including those related to disease, were found in Indonesian HBV isolates. G1957C variation was unique in the HBV isolated from the MSM.

**Key words:** Central Java, core, HBV, Indonesia, MSM.

### INTRODUCTION

Hepatitis B virus (HBV) has remained the cause of global health problem. HBV infects two billion people and 500,000-700,000 patients die annually due to liver disease related to HBV infection (Gasim et al. 2013). HBV is a hepatotropic DNA virus with four partially overlapping open reading frames (ORFs) which encode the virus surface (PreS1, PreS1, S), core (Precore, Core), Pol, and X proteins (Gerlich 2013). During replication, due to the lack of proofreading activity, mutation occurs with the rate of  $(3.2-7.9) \times 10^{-5}$  nucleotide substitutions/replicative cycle or approximately 100 times higher than other DNA viruses (Rodriguez-Frias et al. 2013). In Indonesia, a number of studies have reported the high variability of HBV strains in the country, as shown by the discoveries of numerous novel subgenotype, including C6 and D6 (Utsumi et al. 2009), B7, B8, B9, C7 (renamed as C8), C9, C10 (Nurainy et al. 2008; Mulyanto et al. 2009, 2010; Thedja et al. 2011), and C11-C16 (Mulyanto et al. 2011, 2012).

Viral genetics holds important roles in determining the outcome of HBV infection, thereby continuous studies of the viral genetics were crucial (Shi et al. 2012). Previously, several studies in Indonesia reported the molecular analysis of HBV isolates in the country, nevertheless the isolates were mostly from the Eastern region of Indonesia, while

isolates from Western part of Indonesia, as Central Java Province, were limited. Moreover, most of the isolates were fetched from general populations, clinical patients, or blood donors. Fewer data are available for men who have sex with men (MSM) community, which have been thought as a high-risk population for sexual transmitted diseases as HBV (Ruan et al. 2009). Therefore, this study was aimed to analyze HBV strains from MSM in Surakarta, Central Java, Indonesia. All HBV isolates in Indonesia were also analyzed for comparison and to provide insight about the national profile of HBV isolates in Indonesia. The concern was directed to the virus Core Promoter (CP) and Precore/Core (preC/C), the mutable regions of the viral genome which are known to associate with HBeAg seroconversion and HBV disease remission rates (Liu et al. 2009; Zoutendijk et al. 2013).

### MATERIALS AND METHODS

#### Sample collection

Since 2009, A-IGIC (Infection, Genomic, Immunology and Cancer) Research Group of Sebelas Maret University, Indonesia has performed a molecular epidemiology study of human blood borne virus, including that of the HBV, by collecting the epidemiological and clinical data and blood

samples from the high risk communities in Surakarta, Central Java, Indonesia. Approval was obtained from the institutional ethical committee review boards of the Faculty of Medicine of Sebelas Maret University and Dr. Moewardi General Hospital, Surakarta, Indonesia. Written informed consent was obtained from all individuals participating in the study. All procedures were conducted according to the principles of the Declaration of Helsinki. From MSM community, until 2011 as many as 205 blood samples have been collected by respondent driven sampling method from 205 young adult MSM. All blood samples were fractionated, aliquoted, and kept frozen until analyzed. In point of the HBV, all blood samples were screened by using molecular assays.

### HBV molecular detection and sequencing

Viral nucleic acid was extracted from 200 µl of plasma by using PureLink Viral RNA/DNA Kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Molecular detection was performed using GoTaq Green Master Mix (Promega, Madison, WI). A portion of CP and preC/C regions were amplified using the primer pair KL-6 5'-GGA AAG AAG TCA GAA GGG A-3' (nucleotide 1974-1956; the nucleotide numbering was referring to isolate under accession number M54923) and KL-28 5'-GAG ACC ACC GTG AAC GCC-3' (nucleotide 1611-1628), as previously described (Flodgren et al. 2000). PCR products were subjected to electrophoresis in 2% agarose gels, stained with ethidium bromide, and visualized under ultraviolet illumination. The PCR products were purified from agarose gels, and the nucleotide sequences were determined using the respected primers for the CP and preC/C regions.

### Genotype/subgenotype characterization

The sequencing results were submitted to BLAST to check their similarities to related strains in GenBank/EMBL/DDBJ. Subsequently, our sequences along with all HBV isolates in Indonesia and sequences with highest BLAST score were aligned against reference strains using Clustal W (Larkin et al. 2007). The phylogenetic tree was constructed using MEGA5 software based on the neighbor-joining method with 1000 bootstrap replicates (Hall 2013). The frequency of nucleotide substitution of each base was estimated by the Kimura two-parameter method. Recombination event was examined using the NCBI viral genotyping tool (<http://www.ncbi.nih.gov/projects/genotyping/formpage.cgi>).

### Nucleotide sequence accession numbers

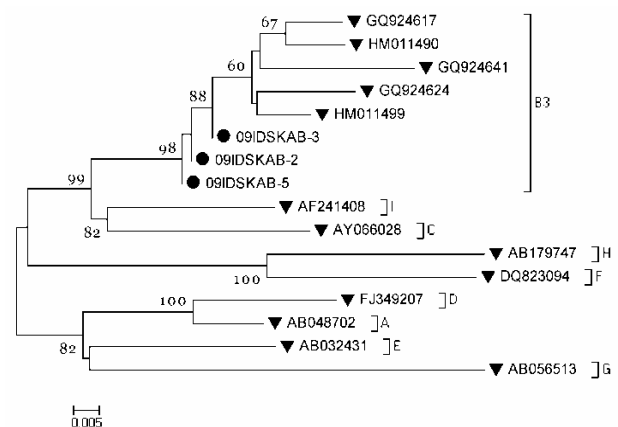
The sequences generated in this study were deposited in GenBank/EMBL/DDBJ under accession numbers JQ965947-JQ965949. In comparison to the national level, all HBV sequences from Indonesia were involved in the analysis, which consisted of accession numbers AB033554-AB033555, AB493827-AB493848, AB540582-AB540585, AB554014-AB554025, AB560661-AB560662, AB644280-AB644287, AB713527-AB713532, AP011084-AP011108, D00331, EF473971-EF473977, EU921418-EU921419, GQ358136-GQ358159, JQ429078-JQ429082,

JQ965950, and M54923 (n=122).

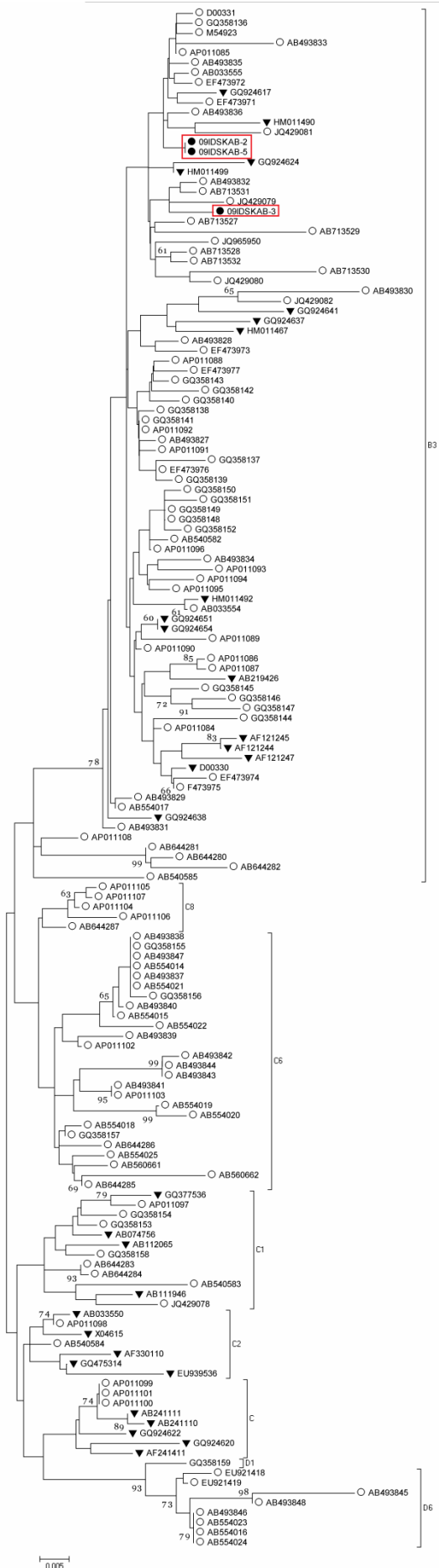
## RESULTS AND DISCUSSION

In 2009, it was estimated that there were 115,968 MSM in Central Java Province, and 4,740 of them were living in Surakarta city (Ministry of Health Republic of Indonesia, 2009). In this study, we successfully collected 205 blood samples from MSM community in Surakarta. None of the subjects showed HBV-related disease. Among them, three (09IDSKAB-2, 09IDSKAB-3, and 09IDSKAB-5) were positive by PCR detecting HBV CP-preC/C regions, thereby generating three CP-preC/C sequences. In BLAST search, the CP-preC/C sequences of 09IDSKAB-2, 09IDSKAB-3, and 09IDSKAB-5 possessed the identity of 99.72%, 99.45%, and 99.72% to AB493835, AP011085, and AB493835, respectively. AB493835 was B3 strain from Papua (Utsumi et al. 2009), while AP011085 was B strain from Jakarta (Mulyanto et al. 2009). All isolates from the MSM obtained in this study were B3 strains (Figure 1, 2), without any evidence of intergenotype recombination. This result supported a previous report that HBV isolates circulating in the Western part of Indonesia, as Surakarta, were predominantly genotype B3 strains (Thedja et al. 2011).

Analysis of all HBV sequences from Indonesia with the present sequences revealed the presence of some disease-related mutations, which consisted of T1753C, A1762T, G1764A, A1846T, and G1896A. T1753C was identified in 7.2% (9/125) sequences. T1753C caused I127T substitution and was reported to correlate with the enhancement of transactivation and anti-proliferation activity of HBx protein (Tanaka et al. 2006) and the emergence of hepatocellular carcinoma (HCC) (Chotiyaputta and Lok 2009; Thongbai et al. 2013). A1762T and G1764A appeared in 17.6% (22/125) and 19.2% (24/125) sequences, respectively. The appearance of A1762T/G1764A double



**Figure 1.** The phylogenetic tree of HBV isolates from MSM in Central Java, Indonesia (●; 09IDSKAB-2, 09IDSKAB-3, 09IDSKAB-5) and reference sequences of each HBV genotype (▼).



**Figure 2.** The phylogenetic tree of HBV isolates from MSM in Central Java, Indonesia (●; 09IDSKAB-2, 09IDSKAB-3, 09IDSKAB-5), all HBV isolates in Indonesia based on CP-preC/C sequences (○; AB033554-AB033555, AB493827-AB493848, AB540582-AB540585, AB554014-AB554025, AB560661-AB560662, AB644280-AB644287, AB713527-AB713532, AP011084-AP011108, D00331, EF473971-EF473977, EU921418-EU921419, GQ358136-GQ358159, JQ429078-JQ429082, JQ965950, M54923), and reference sequences of each HBV genotype/subgenotype (▼).

	1726	1727	1730	1752	1753	1754	1877	1957
M54923	T	A	C	T	G	A	G	T
09IDSKAB-2	A	.	.	.	.	.	.	C
09IDSKAB-3	.	.	.	.	.	.	T	C
09IDSKAB-5	A	.	.	.	.	.	.	C
AB493845	A	A	.	C	.	.	.	.
AB493846	A	A	.	C	.	.	.	.
AB493847	A	A	.	C	.	.	.	.
AB554016	A	A	.	C	.	.	.	.
AB554023	A	A	.	C	.	.	.	.
AB554024	A	A	.	C	.	.	.	.
AP011099	A	A	.	C	.	.	.	.
AP011100	A	A	.	C	.	.	.	.
AP011101	A	A	.	C	.	.	.	.
EU921418	A	A	.	C	.	.	.	.
EU921419	A	A	.	C	.	.	.	.
AB033554	.	.	.	.	.	.	.	.
AB033555	A	.	.	.	.	.	.	.
AB493827	.	.	.	.	.	.	T	.
AB493828	A	.	.	.	.	.	.	.
AB493829	A	.	.	.	.	.	T	.
AB493830	.	.	.	.	.	.	A	G
AB493831	A	C	.	.	.	.	.	.
AB493832	.	.	.	.	.	.	T	.
AB493833	.	.	.	.	.	.	C	.

**Figure 3.** The part of alignment result of nucleotides in CP and preC/C regions showing the G1957C variation of HBV isolates from MSM in Central Java, Indonesia (09IDSKAB-2, 09IDSKAB-3, 09IDSKAB-5), and another HBV isolates in Indonesia. Sequence numbering was in accord with M54923 (genotype B isolate). Dot represents identical nucleotide to those in M54923.

mutation has been reported to increase viral replication and the risk of HCC and liver cirrhosis (LC) (Liao et al. 2012). Both A1762T and G1764A were also suggested to be a predictive marker for progression of liver damage (Heriyanto et al. 2012; Kitab et al. 2012). A1846T was observed in 16.8% (21/125) sequences. T1846T caused H158L substitution and was known to associate with cirrhosis (Yin et al. 2011). Meanwhile, G1896A existed in 24.8% (31/125) sequences. This variation was known to increase the risk for severe disease (Liao et al. 2012). Nevertheless, a recent study implied that G1896A substitution alone seemed not to have direct pathogenic role (Malik et al. 2012). While numerous disease-related mutations were observed in HBV sequences from Indonesia, none of them appeared among the HBV sequences from the MSM community.

Some variations were prevalent among HBV sequences from the MSM community. The most frequent variant was G1957C, which would induce C195S substitution. Uniquely, this variation existed in all HBV isolated from

the MSM, but none of other sequences from Indonesia except JQ965950 which was also isolated from subject from correctional facility in Central Java with sex with man history, previously reported in GenBank by A-IGIC research group (Figure 3). The impact of this variation is still unknown. Other prevalent variation identified was C1726A, which was found in 09IDSKAB-2, 09IDSKAB-5, and another 60.8% (76/125) sequences from Indonesia. This variation, together with T1717(C/G), would correspond T118N substitution. This substitution has been associated with severity of liver disease (Utama et al. 2009).

### CONCLUSION

In conclusion, various variants were found in HBV sequences from Indonesia, including them previously reported to associate with the outcome of HBV infection. We also found that G1957C variation was unique in the HBV isolated from the MSM. Overall, the data reported in this study will enrich the molecular data of HBV isolates in Indonesia, particularly from MSM, which have remained limited. Further studies with larger samples and areas are required in order to serve more detailed information about the genetic profile of HBV isolates in Indonesia.

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