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Sequence Note

Molecular Characterization of the HIV Type 1 *vpr* Gene in Infected Chinese Former Blood/Plasma Donors at Different Stages of Diseases

CHENGLI SHEN,[1,2] PHALGUNI GUPTA,[1] HAO WU,[2] XINYUE CHEN,[2] XIAOJIE HUANG,[2] YUSEN ZHOU,[3] and YUE CHEN[1]

ABSTRACT

The HIV-1 *vpr* regions were PCR amplified and sequenced from eight long-term nonprogressors' (LTNP) and seven AIDS patients' DNA samples of a cohort of HIV-1-infected Chinese plasma/blood donors (PBDs). Sequence analysis revealed that the patients' HIV-1 *vpr* sequences belong to HIV-1 subtype B and there were no differences in the divergence of *vpr* sequences between these two groups of patients. Similarly, in the deduced amino acid sequences, no significant differences have been detected in *vpr* functional domains from patients at different stages of the disease. Moreover, the predicted binding motifs of HLA A2 and A11 were highly conserved among patients' *vpr* amino acid sequences. These results show that *vpr* may not play an important role in HIV-1 pathogenesis in different stages of Chinese patients and may have important implications in developing *vpr*-related treatments suitable for HIV-1-infected PBDs.

In central China, because of unsanitary blood/plasma collecting activities in the early and mid-1990s, many poor farmers were infected with HIV-1.[1,2] Sequence analysis of the HIV-1 indicates that the infected plasma/blood donors (PBDs) harbor HIV-1 subtype B′, which is closely related to Thailand B virus.[3,4] We previously analyzed the HIV-1 *env* region and *nef* gene from chromosomal DNA isolated from peripheral blood mononuclear cells (PBMCs) of these patients in Henan province of central China. No significant differences have been detected in *nef* nucleotide sequences or functional motifs with different disease stages.[5] However, the C2–C5 sequences in the *env* region from AIDS patients clustered differently from those long-term nonprogressors (LTNPs) in the phylogenetic tree and showed a significantly higher divergence and lower diversity compared to those from the LTNP subjects.[4] In addition, *env* sequences from AIDS patients carry more syncytia-inducing (SI) viral genotypes than those present in LTNP subjects. This raises the possibility that HIV-1 from AIDS patients may be genetically and biologically different compared to that present in LTNP subjects. To further characterize HIV-1 harbored in the PBDs, we have sequenced and analyzed the HIV-1 viral protein R (*vpr*) gene.

Vpr is a 96-amino acid auxiliary protein that is well conserved in HIV-1, HIV-2, and SIV.[6] Despite its small size, vpr plays a multifunctional role in viral pathogenesis and is associated with disease progression. It is an important component of the reverse transcription complex (RTC) in the initiation step of the reverse transcription process.[7,8] By influencing the accuracy of the reverse transcription, vpr modulates the *in vivo* mutation rate of HIV-1.[9,10] Furthermore, by promoting direct or indirect interactions with the cellular machinery, it regulates the nucleocytoplasmic shuttling and enhances the transport of the viral DNA into the nucleus of nondividing cells.[11–13] In addition, vpr protein, a regulator of various cell genes,[14] can also

[1]Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania 15261.
[2]Department of Infectious Diseases, Capital University of Medical Science Youan Hospital, Beijing, China.
[3]Department of Pathogenic Molecular Biology, Beijing Institute of Microbiology and Epidemiology, Beijing, China.

Table 1. Clinical and Epidemiological Data of Infected Chinese Blood Donors Enrolled in the Study

Patient number	*Date of blood collection*	*CD4 counts at the time of blood collection*	*Clinical symptoms at the time of blood collection*
CN H1	1/15/2004	742	LTNP
CN H2	3/17/2004	655	LTNP
CN H6	12/31/2003	580	LTNP
CN H7	10/17/2003	565	LTNP
CN H10	1/24/2004	585	LTNP
CN H41	10/27/2003	686	LTNP
CN H53	9/14/2003	814	LTNP
CN H77	11/20/2004	737	LTNP
CN L1	3/17/2004	195	AIDS
CN L3	12/31/2003	158	AIDS
CN L4	9/21/2003	151	AIDS
CN L5	11/24/2003	116	AIDS
CN L7	10/11/2003	130	AIDS
CN L8	7/22/2003	122	AIDS
CN L10	2/15/2004	160	AIDS

induce an arrest in the G2 phase of the cell cycle of infected proliferating human and simian T cells[15,16] and apoptosis of CD4 T cells.[17] Although researchers have characterized the *vpr* gene in HIV-1 circulating in Africa and Western countries, very little is known about the genetic characterization of the *vpr* gene of HIV-1 in infected Chinese PBDs. In this report, we provide information on the genetic characterization, the sequence variability of the functional domains, and the HLA binding motifs of HIV-1 *vpr* from Chinese PBDs at different stages of diseases.

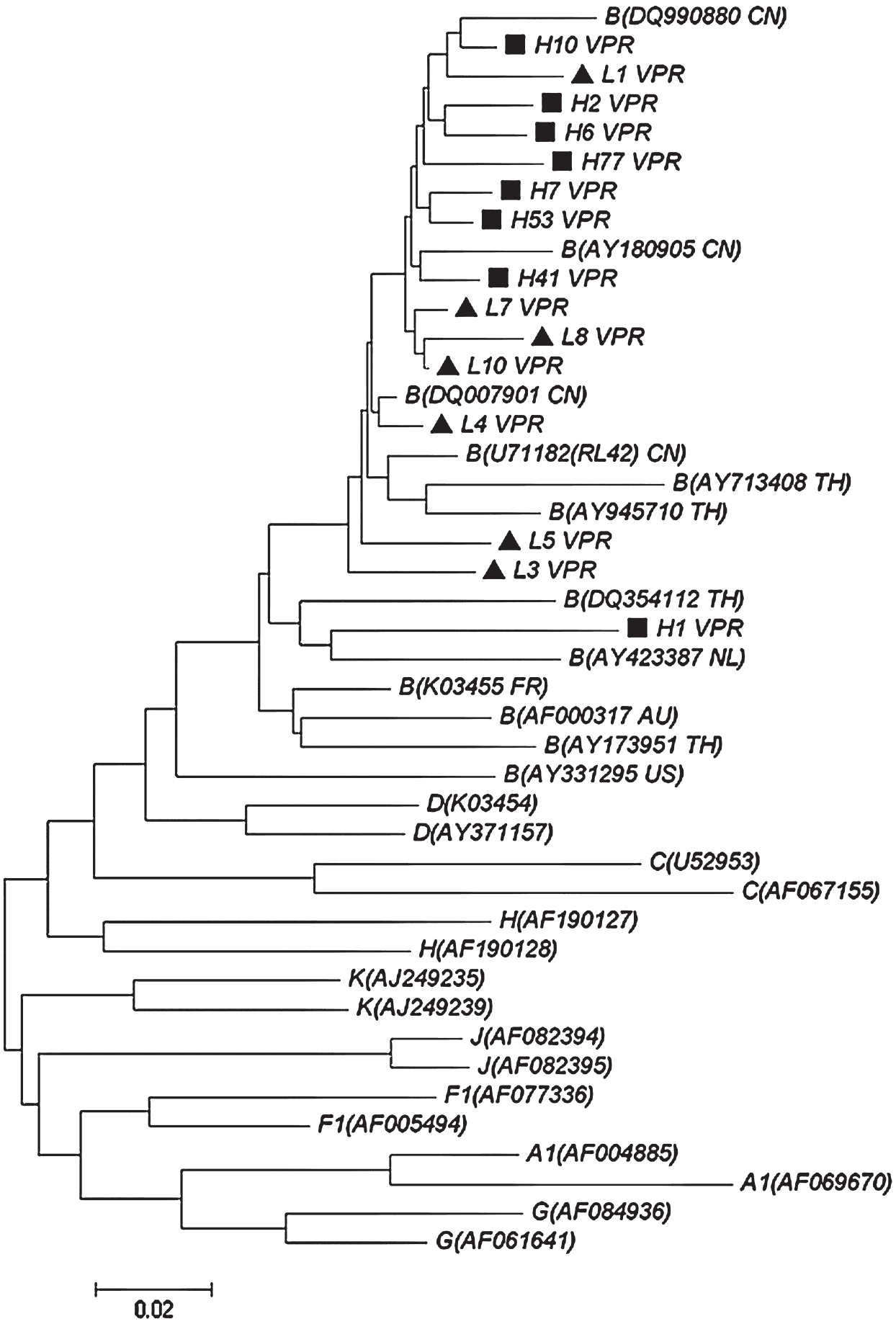
Fifteen HIV-1-infected PBDs were included in the present study. All of these patients were diagnosed as HIV-1 positive more than 10 years prior to sampling and never received any antiretroviral therapy. Blood samples were collected between November 2003 and April 2004. The patients' CD4 cell counts and clinical symptoms were monitored at the time of sample collection. Eight LTNP patients (H1, H2, H6, H7, H10, H41, H53, and H77) with CD4 cell counts ranging from 814 to 565 and seven AIDS patients (L1, L3, L4, L5, L7, L8, and L10) with CD4 cell counts ranging from 195 to 116 were included in this study (Table 1). High-molecular-weight chromosomal DNA was isolated from the PBMCs using a DNA purification kit (Qiagen). DNA was then subjected to polymerase chain reaction (PCR) amplification using primer pairs of outer forward (VPR_FO: gaatggaggagaaagagatatagcacacaag) and outer reverse (VPR_RO: gctgaatactatggtccacacaactattgc) followed by a nested PCR amplification using primers of vpr interior forward (VPR_F: ggacgtgtagttagccctagttgtgattatc) and interior reverse (VPR_R: tcctgccataggagatgcctaagc) to amplify the full-length *vpr* gene. Purified PCR products were sequenced using the forward interior primer. PCR negative controls were utilized with each PCR run to detect any possible contamination.

Full-length *vpr* open reading frame (ORF) sequences from all patients were aligned with reference *vpr* sequences from different HIV-1 subtypes available from the Los Alamos HIV-1 databases by the Clustal W multiple sequence alignment program from the Mega 4.0 software package. The phylogenetic tree was constructed by the neighbor-joining method as implemented in Mega 4.0 and evaluated by the bootstrap resampling method using 1000 replicates. All *vpr* sequences were tightly clustered with Chinese B followed by Thailand B and intermingled together irrespective of disease stage (Fig. 1). This result is in agreement with previous reports that former blood donors were harboring HIV-1 subtype B′, which is genetically related to Thailand B.[18,19]

Analysis of viral divergence between each patient HIV-1 and the predecessor Thailand B AY713408 using the Kimura two-parameter model in Mega 4.0 software revealed no difference between the asymptomatic patients and AIDS patients (data not shown). This is consistent with a previous report[20] that shows a similar degree of overall genetic divergence of *vpr* identified in the different groups of patients with no significant correlation between the degree of genetic divergence and the rate of disease development. In addition, immune selection pressure in different disease stages may have a different impact on the synonymous/nonsynonymous (dS/dN) mutation ratio and changes in glycosylation sites of *vpr*. However, analysis of the dS/dN ratio and potential glycosylation sites did not reveal any significant difference in the dS/dN ratio and glycosylation sites of *vpr* between patients at different stages of the disease (data not shown).

Although there were some reports of an association between *vpr* gene insertions, deletions, length polymorphisms, and premature stop codon with disease progression,[21–23] other re-

FIG. 1. Phylogenetic analysis of 15 HIV-1 complete *vpr* genes from Chinese infected blood donors (▲, low CD4 patients; ■, high CD4 patients). The tree was constructed by the neighbor-joining method as implemented in Mega 4.0. The validity of the branching orders was estimated with 1000 replicates. Reference strains were obtained from the Los Alamos HIV database: subtype B′ vpr sequences from China are AY180905, U71182 (RL42), DQ990880, and DQ007901; subtype B vpr sequences from Thailand are AY713408, AY945710, DQ354112, and AY173951.


B(DQ990880 CN)
H10 VPR
L1 VPR
H2 VPR
H6 VPR
H77 VPR
H7 VPR
H53 VPR
B(AY180905 CN)
H41 VPR
L7 VPR
L8 VPR
L10 VPR
B(DQ007901 CN)
L4 VPR
B(U71182(RL42) CN)
B(AY713408 TH)
B(AY945710 TH)
L5 VPR
L3 VPR
B(DQ354112 TH)
H1 VPR
B(AY423387 NL)
B(K03455 FR)
B(AF000317 AU)
B(AY173951 TH)
B(AY331295 US)
D(K03454)
D(AY371157)
C(U52953)
C(AF067155)
H(AF190127)
H(AF190128)
K(AJ249235)
K(AJ249239)
J(AF082394)
J(AF082395)
F1(AF077336)
F1(AF005494)
A1(AF004885)
A1(AF069670)
G(AF084936)
G(AF061641)
0.02

searchers[20] found that HIV-1 sequences in LTNPs contained the full-length *vpr* with an intact ORF. Similar to the latter report, all *vpr* sequences in our study had an intact ORF, which encoded 96 amino acids regardless of disease stages.

The deduced vpr amino acid sequences were further analyzed for the functional domains reported previously.[24,25] The structure of vpr is characterized by three well-defined α-helices: amino acids 17–33, 38–50, and 56–77 surrounded by flexible N- and C-terminal domains. Vpr N- and C-terminal domains have been reported to maintain the viral protein functional interaction between vpr and a host peptidylprolyl *cis/trans* isomerase (PPIase) that might regulate the *cis/trans* interconversion of the imidic bond within the proline residues (position 5, 10, 14, and 35) of vpr *in vivo*,[26] and induce cell cycle arrest in the G2 phase within the serine residues (positions 79, 94, and 96),[9,27,28] respectively. The first α-helix (amino acids 17–33) region is essential for nuclear localization, virion packaging,[25] and the nuclear entry of vpr.[29] Proline at 35, which lies between the first and second helices, is important for the interaction between peptidylpropyl isomerase cyclophilin A and vpr, and a mutation at position 35 changes the structure of vpr and results in a decreased encapsidation.[30] The second α-helix (38–50) region is associated with the oligomerization of vpr and the translocation of the preintegration complex (PIC) to the perinuclear region,[29,31] and mutation of W38, L39, L42, and I46 resulted in a drastic reduction in the virion incorporation of vpr[31] and the mutation of residues V57, I61, L64, L68, H71, and G75 affected vpr nuclear localization. The mutant of vpr W54, which lies between the second and third α-helices, affects the interaction of vpr with the nuclear form of uracil DNA glycosylase (UNG2).[9] The third helix (56–77) is related to dimerization via a leucine-zipper mechanism. The 71-H(S/F)RIG-75 motif in the third helix acts as a signal for DNA interactions.[32] In our study, all the proline and serine residues in the N-terminal or C-terminal of vpr, the structure and function-related important acidic residues at amino acid positions 17, 21, 24, 25, and 29, four

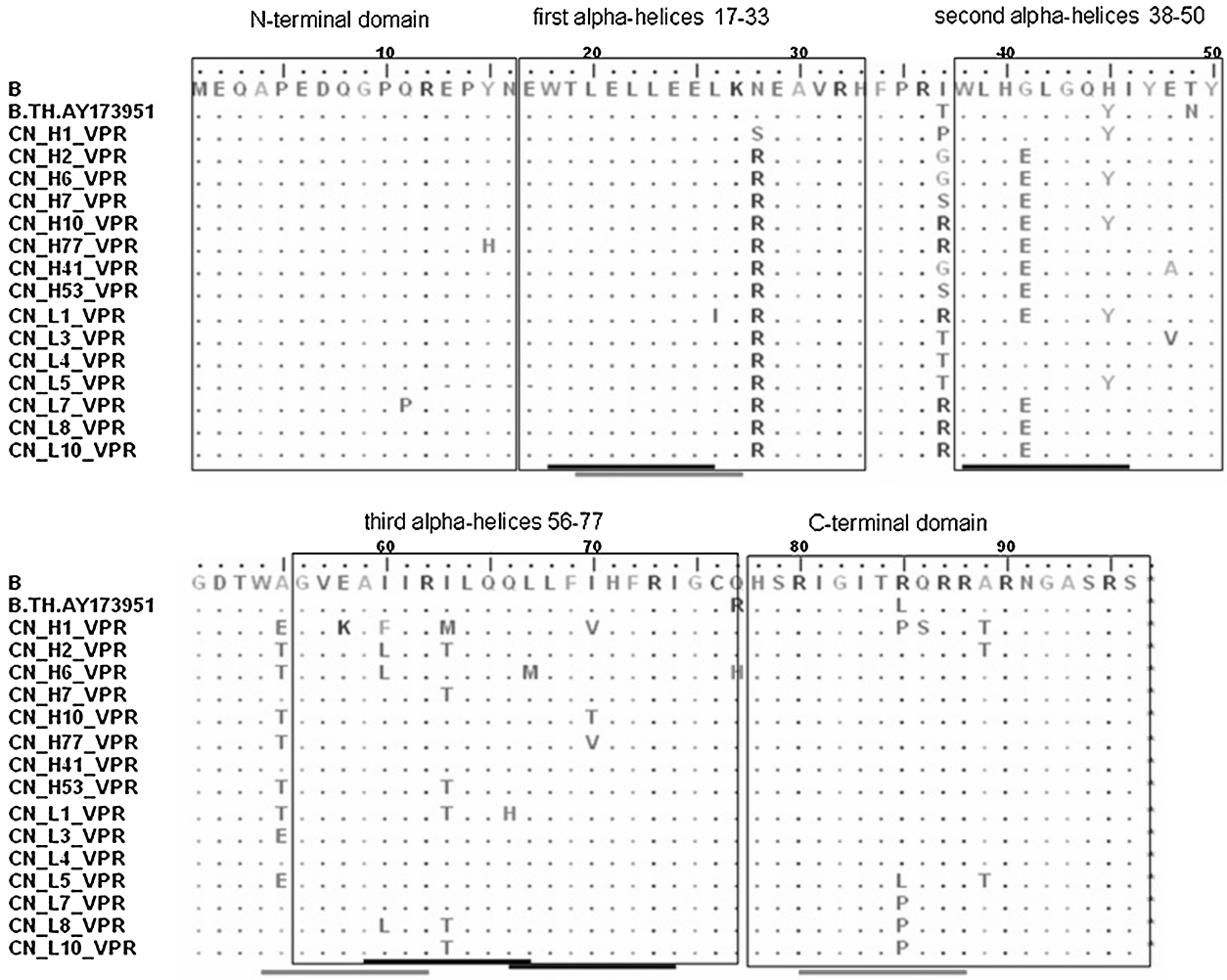


FIG. 2. Alignments of the deduced amino acid sequences of *vpr* genes from infected Chinese blood donors (H1-L10) with the reference *vpr* genes of Consensus B and Thailand B (AY173951) subtypes (obtained from the Los Alamos HIV database) using alignment Explorer/Clustal in Mega 4.0 package. A dot represents identical residues. Significant protein domains are labeled and the best defined CTL epitopes for HIL A2 (black) and A11 (grey) are underlined. Boxed areas are various functional domains within the vpr protein.

hydrophobic polar leucines at amino acid positions 20, 22, 23, and 26, and alanine at amino position 30 in the first α-helices[24] are all highly conserved in LTNP and AIDS patients of PBDs (Fig. 2). P35 lying between the first and second helices, W54 lying between the second and third helices, all functional residues in the second and third helices, and the H(S/F)RIG motif in the third helix are very well conserved in all 15 patients, regardless of disease stages (Fig. 2).

Recently, some investigators showed that the mutations of three amino acid residues at position 73, 77, and 80, which interact with the first cytoplasmic loop of the adenine nucleotide translocator (ANT), are associated with disease progression. Moreover, the R77Q mutation occurs more frequently in long-term nonprogressor patients,[9,33,34] while Fischer *et al.* stated that the R77Q mutation was associated with the viral subtype more than with disease progression.[35] In our report, R73 and R80 are highly conservative and the R77Q mutation appeared in all PBD patients regardless of disease stages. The R77Q mutation is more common in HIV-1 B-infected patients and is not associated with stages of the disease. However, more *vpr* sequence data are needed to confirm these results.

Since vpr is involved in the early steps of the HIV-1 infection cycle, anti-vpr cytotoxic T lymphocyte (CTL) activity may play an important role in controlling HIV replication in primary infection. Selection of significant vpr epitopes depends on HIV-1 subtype and human HLA types. HLA-A2 and HLA-A11 are the dominant MHC Class I subtypes in PBDs.[36–38] The binding sites of HLA-A2 and HLA-A11 in all deduced vpr amino acid sequences were predicted by the NIH prediction program based on the estimated disassociation time at http://bimas.dcrt.nih.gov/molbio/hla_bind/. All the predicted binding motifs were highly conserved with slight alterations observed in LTNP and AIDS patients (Fig. 2). This information has important implications in developing an epitope-based vaccine in vpr suitable for Chinese blood donors.

In summary, we characterized the *vpr* genes from 15 infected Chinese blood donors at different stages of the disease and all of them belong to subtype B′. No significant differences have been detected in vpr nucleotide sequences or functional motifs in the deduced amino acid sequences with different stages of disease. These results indicate that vpr may not be the major contributor for disease progression in Chinese PBDs. In view of the fact that the *vpr* gene has been widely linked to disease progression and pathogenesis in subtype B-infected patients, it is surprising that we did not detect any *vpr* genetic segregation or significant changes in functional motifs in Chinese patients at different stages of disease. It is important to investigate more patients to further confirm this conclusion.

GENBANK ACCESSION NUMBERS

The GenBank accession numbers of the nuclotide sequences reported in this paper are EU267012–U267026.

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Address reprint requests to:
Yue Chen
University of Pittsburgh Graduate School of Public Health
Department of Infectious Diseases and Microbiology
Pittsburgh, Pennsylvania 15261

E-mail: cheny@pitt.edu