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Molecular Genetic Characterization of the Merozoite Surface Protein 1 Gene of *Plasmodium vivax* from Reemerging Korean Isolates[▽]

So-Hee Kim,[1] Seung-Young Hwang,[2] Jeong Hwan Shin,[3,4] Chi-Sook Moon,[5] Dong-Wook Kim,[5] and Weon-Gyu Kho[1,2,6]*

Department of Malariology,[1] Paik Institute for Clinical Research,[3] and Department of Parasitology,[2] Department of Laboratory Medicine,[4] Department of Internal Medicine,[5] and Mitochondrial Research Group (Frontier Inje Research for Science and Technology),[6] Inje University College of Medicine, 633-165 Gaegum-dong, Jin-gu, Busan 614-735, South Korea

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***Plasmodium vivax* merozoite surface protein 1 (PvMSP-1) has been considered a major candidate for the development of an antimalaria vaccine, but the molecule exhibits antigenic diversity among isolates. The extent of genetic polymorphism in the region between interspecies conserved blocks 4 and 5 (ICB4 and ICB5) of the PvMSP-1 gene was analyzed for 30 Korean isolates. Two genotypes, SK-A and SK-B, were identified on the basis of amino acid substitution. Almost all the amino acid sequences of the Korean isolates were nearly identical to those of the Solomon Island isolate Solo-83 (97.8 to 99.9% similarity) and Philippine isolates Ph-79, Ph-52-2, and Ph-49 (97.3 to 99.8% similarity). Also, we report two sequences in the isolates that were characterized on the basis of restriction fragment length polymorphism (RFLP). The RFLP profiles following digestion with the DraI restriction enzyme produced two distinguishable patterns. This study might be the first report of the region between ICB4 and ICB5 of the MSP-1 gene of *P. vivax* in South Korea.**

Plasmodium vivax malaria represents a major public health problem for many tropical and subtropical countries, which has been exacerbated by the expansion of drug-resistant strains (2, 10, 29, 30). The enormous toll of mortality caused by *Plasmodium falciparum* has tended to overshadow the public health importance of *P. vivax*. For this reason and on account of technical difficulties, relatively little investigation has been done toward the development of a vaccine against *P. vivax* (27). One of the major problems in vaccine development is the antigenic diversity of the vaccine candidates. The critical emerging problem is that the host response to one allele is not very effective against parasites expressing different allelic forms (8, 25). Therefore, genetic variation studies for the antigens of vaccine candidates are very important for *P. falciparum* and *P. vivax*. The polymorphism of potential malaria vaccine targets is rather greater for *P. vivax* than for *P. falciparum*. Also, the growing resistance of *P. vivax* strains to chloroquine is spurring the development of a vaccine against *P. vivax* malaria.

The study of polymorphism is important not only for establishing the antigenic repertoire of isolates from regions where malaria is endemic but also for elucidating the mechanisms by which antigenic diversity is generated. The WHO declared in 1979 that malaria had been eradicated in Korea, but in 1993 (4), a case of malaria in a soldier working in the Demilitarized Zone (the border area between North and South Korea) of the Republic of Korea was reported. After 1993, the number of malaria cases expanded exponentially each year, with 3,932 patients diagnosed in 1998 (15). Current epidemiological results suggest that the malaria that has reemerged did not originate from overseas. All indigenous cases of malaria are due to *P. vivax*, with the occasional imported case of *P. falciparum*. However, the genetic characteristics of the reemergent Korean strain are not known at present.

In previous studies, *P. vivax* circumsporozoite protein (13), *P. vivax* Duffy binding protein (12), and *P. vivax* apical membrane antigen 1 (6) showed genotypes with at least two new phenotypes among Korean isolates. However, the extent of genetic diversity of Korean *P. vivax* isolates is not accurately known at present, due to the fact that very few polymorphic markers are available for studying *P. vivax*.

P. vivax merozoite surface protein 1 (PvMSP-1) is a well-characterized antigen whose diversity is maintained by host immune selection pressure (20). There is extensive allelic diversity of MSP-1 among isolates (5, 11, 19, 21), and this polymorphism may hamper the development of an effective vaccine against malaria. The polymorphism of PvMSP-1 has been considered to result from interallelic recombination in nature (20). Although the polymorphism of *P. falciparum* MSP-1 is well characterized, little is known about *P. vivax* MSP-1. To contribute useful information regarding genetic diversity and to facilitate rational vaccine design, the polymorphism of PvMSP-1 in Korean isolates was investigated in this study. In addition, we also describe the molecular phylogenetic characteristics of Korean *P. vivax* isolates.

MATERIALS AND METHODS

Isolation of parasite genomic DNA. Blood samples were collected from 30 patients who contracted *P. vivax* malaria in Yonchon-gun, Kyonggi-do, in 1998. All patients were diagnosed by microscopic examination in the malaria clinic of Busan Paik Hospital, Inje University. Thin films were prepared for each patient and were stained using the Diff-Quick stain kit (International Reagents, Japan). An expert microscopist examined the slides using an oil immersion lens at a magnification of ×1,000. The parasites in the smear were counted against 200 leukocytes (7). For the parasite density estimation, it was assumed that there were 8,000 leukocytes in 1 μl of blood (3). This study protocol was approved by

* Corresponding author. Mailing address: Department of Malariology, Paik Institute for Clinical Research, and Department of Parasitology, Inje University College of Medicine, 633-165 Gaegum-dong, Jin-gu, Busan 614-735, South Korea. Phone: 82-51-890-6731. Fax: 82-51-894-6709. E-mail: wgkho@inje.ac.kr.

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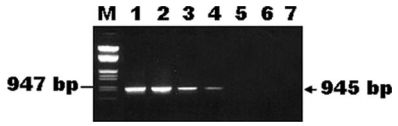

FIG. 1. Sensitivity of PCR for the PvMSP-1 gene. The PCR-generated fragment from the ICB4-to-ICB5 region of the PvMSP-1 gene was electrophoresed on a 1% agarose gel and stained with ethidium bromide. Infected whole blood was serially diluted with normal whole blood. The initial level of *P. vivax* parasitemia was 0.02%. Lanes show results for the following numbers of parasites: 1,100 (lane 1), 110 (lane 2), 11 (lane 3), 1.1 (lane 4), 0.11 (lane 5), and 0.011 (lane 6). Lane 7, negative control (human genomic DNA); lane M, λ DNA/EcoRI+HindIII (MBI Fermentas, Amherst, NY), used as a DNA size marker.

the Institutional Review Board of Inje University College of Medicine, Busan, Korea.

Parasite DNA was extracted from 0.1 ml of EDTA-treated blood samples by using a QIAamp DNA blood kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The extracted DNA was dissolved in 0.2 ml TE (10 mM Tris-HCl [pH 7.4] and 1 mM EDTA [pH 8.0]) buffer and was stored at −70°C until use.

PCR conditions and cloning into T-vectors. A DNA fragment encompassing interspecies conserved blocks 4 to 5 (ICB4 to ICB5) of PvMSP-1 was amplified by PCR in a 20-μl reaction mixture containing extracted *P. vivax* DNA, 200 μM each deoxynucleoside triphosphate, 0.5 pM each primer, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.0 mM $MgCl_2$, and 2.5 U of *Taq* polymerase (Takara, Kyoto, Japan). The PCR primers were PvMSPF1 (5′-GCCAAGACGGTGAACTTCG ACCTG-3′) (nucleotides 1081 to 1104; positions based on the Belem sequence [GenBank accession no. M60807]) and PvMSPR1 (5′-CTTGTCAATTTCCCT TTTGAGGAC-3′) (positions 2025 to 2002). The reaction involved 35 cycles consisting of 35 s of denaturation at 94°C, 35 s of annealing at 64°C, and 35 s of extension at 72°C, with a 5-min final extension at 72°C. The amplicons were analyzed by electrophoresis on a 1% agarose gel (Seakem LE agarose; FMC, Rockland, ME) in 0.5× TAE running buffer (20 mM Tris-acetate, 0.5 mM EDTA) containing 0.05% ethidium bromide.

Each amplified DNA fragment was purified using the Qiaex II gel extraction kit (Qiagen), ligated into the pGEM-T Easy vector (Promega, Madison, WI), and transformed into *Escherichia coli*. Recombinant plasmid DNA was extracted with a QIAprep spin miniprep kit (Qiagen) and was used in DNA sequencing.

Sequencing and gene analysis. The nucleotide sequence was determined by the dideoxynucleotide chain termination method using an ABI PRISM Dye Terminator cycle sequencing core kit (Perkin-Elmer) and an automated DNA sequencer (Applied Biosystems model 377A; Perkin-Elmer). Primers for sequencing were T7, SP6, PvMSP1F, and PvMSP1R. Nucleotide substitutions were verified by direct sequencing of newly amplified PCR products from the same isolates, which apparently do not contain clonal mixtures.

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Belem   1111: CTGTTTACGGACGCAGAGGAGTTGGAGTACTACTTGAGGGAGAAGGCAAAGATGGCCGGCACGCTAATCATCCCAGAAAGCACCAAATCAGCAGGCACCCCTGGAAAGACAGTTCCAACC 1230
SK-A    1111: -----C--CA----------A----------------------------------G----------G----------A-GTG--T-GA-T---A-C--G---------- 1230
SK-B    1111: -----C--CA----------A----------------------------------G----------G----------A-GTG--T-GA-T---A-C--G---------- 1230
Sal-1   1111: --------T----------------------T---------------------------------------------------------------------------- 1230

Belem   1231: CTGAAAGAGACCTACCCACACGGAATAAGCTACGCTTTAGCAGAAAACAGTATTTATGAACTGATAGAAAAAATTGGATCTGATGAAACATTTGGTGATTTGCAAAATCCAGATGATGGA 1350
SK-A    1231: ------C----------T----------------------------C-----C---------------------------------------------------------- 1350
SK-B    1231: ------C----------T----------------------------C-----C---------------------------------------------------------- 1350
Sal-1   1231: ---------------------------------------------------------------------------------------------------------------- 1350

Belem   1351: AAGCAACCGAAGAAGGGAATCCTCATTAATGAAACAAAGAGGAAAGAATTGCTGGAAAAAATTATGAATAAAATTAAGATAGAAGAAGACAAATTGCCCAACCTAAAAAAAGAATTGGAG 1470
SK-A    1351: ----------------------------------------------------------------------------------------------------AC--- 1470
SK-B    1351: ----------------------------------------------------------------------------------------------------AC--- 1470
Sal-1   1351: ----------------------------------------------------------------------------------------------------AC--- 1470

Belem   1471: GAAAAATATAAGGTGTACGAGGCAAAGGTTAATGAGTTCAAACCAGCATTTAATCACTTTTATGAGGCAAGACTGGACAACACCCTTGTTGAAAACAAATTTGATGAATTTAAACCAAA 1590
SK-A    1471: ----------------------------------------------------------------------------------------------------<------ 1590
SK-B    1471: ----------------------------------------------------------------------------------------------T-----G-AA---<------ 1590
Sal-1   1471: ----------------------------------------------------------------------------------------------T-----G-AA--- 1590

Belem   1591: AGGGAGGCATATATGGAGGAGAAGAAAAAACTAGAGAGCTGCTCCTACGAACAGAACACCAATCTGATTAACAAGTTGAAAAAACAACTGACCTACTTGGAGGACTACGTGTTAAGAAAA 1710
SK-A    1591: >>>>>>>>>>>>>>>---------------------------------------------------------------------------------------------- 1710
SK-B    1591: --A>>>>>>>>>>>>>---------A----------------------------------C----------A--A----------T--------- 1710
Sal-1   1591: --A----------------------------A---------------------G----------C----------A--A----------T--------- 1710

Belem   1711: GACATCGCCGACGATGAAATTAAACACTTCAGTTTCATGGAGTGGAAATTAAAGAGCGAAATTTATGATCTAGCCCAGGAAATCCGAAAAAACGAAAACAAGCTCACCGTTGAAAACAAA 1830
SK-A    1711: ---------------------------------------------------------------------------------------------------------- 1830
SK-B    1711: ---------------------------------------------------------------------------------------------------------- 1830
Sal-1   1711: ----------------------------------------------------------------------------------------------A----------- 1830

Belem   1831: TTCGACTTCTCCGGGGTTGTGGAAGGACAAGTACAAAAGGTATTGATAATCAAAAAAATTGAGGCTCTAAAGAATGTCCAGAATCTTCTTAAGAATGCCAAGGTGAAGGACGACCTGTAC 1950
SK-A    1831: ----------------------TT---------------------------------------------------------------------------------- 1950
SK-B    1831: ----------------------TT---------------------------------------------------------------------------------- 1950
Sal-1   1831: ----------------------TT---------------------------------------------------------------------------------- 1950

Belem   1951: GTTCCAAAGGTGTATAATACAGGCGAGAAACCTGAGCCCTACTACTTG 1998
SK-A    1951: A---------------C---------------------------- 1998
SK-B    1951: A---------------C---------------------------- 1998
Sal-1   1951: A---------------C---A------------------------ 1998
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FIG. 2. Comparisons of the nucleotide sequences of the ICB4-to-ICB5 region of PvMSP-1 from Korean isolates (SK-A and SK-B) and strains Belem and Sal-1. Dashes indicate the same nucleotide as that for strain Belem. Nucleotide sequences corresponding to the genotype-specific primers MSPR1 (for SK-A) and MSPR2 (for SK-B) are underlined and indicated by arrows, respectively. An arrowhead indicates the DraI restriction enzyme site for RFLP.

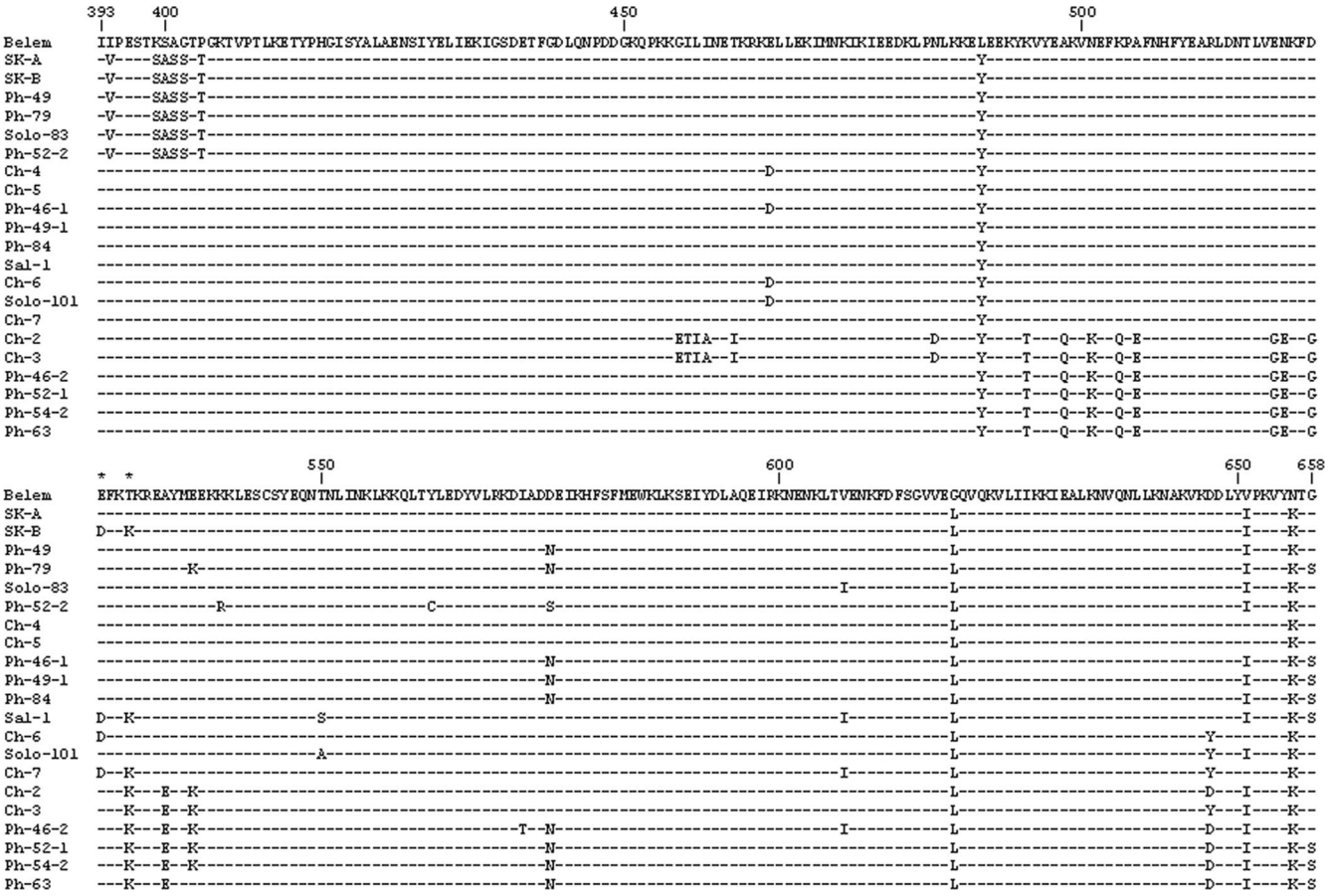

FIG. 3. Alignment of deduced amino acid sequences of the ICB4-to-ICB5 region of PvMSP-1 in Korean isolates (SK-A and SK-B), strains Belem and Sal-1, and isolates from the Philippines (Ph), China (Ch), and the Solomon Islands (Solo). Asterisks indicates differences in sequence between the SK-A and SK-B genotypes. Amino acid positions are given above the sequences.

Preliminary pairwise sequence alignment and comparison were performed using GeneJockey II (Biosoft Co.) and the BLAST program of the NCBI databases (NIH, Bethesda, MD). A multiple-sequence alignment was constructed with Clustal-X (28). WDNASIS (version 2.5; Hitachi, Japan) was used for translating DNA sequences into amino acid sequences and for predicting protein secondary structure. A phylogenetic tree was constructed by the neighbor-joining method (26) using the Clustal-X program, and the evolutionary distances were calculated by Kimura's two-parameter method (14). One thousand replicates of bootstrapping were used for neighbor-joining analysis to obtain relative support for internal nodes. The sequence data of the Korean isolates were analyzed and compared with the published sequence of *P. vivax* (20).

Genotype-specific PCR and PCR-RFLP. In order to determine the genotypes of the Korean isolates by using convenient techniques, we developed type-specific PCR and restriction fragment length polymorphism (RFLP) assays. For type-specific PCR, the primers were MSPF1, MSPR2 (5′-CATATATGCCTCC CTTTTGGTT-3′) (nucleotides 495 to 474; positions based on the Korean isolate SK-A sequence [GenBank accession no. AF451297]), and MSPR3 (5′-TCCAT ATATGCCTCTCTTTTTTTC-3′) (positions 497 to 474, based on the Korean isolate SK-B sequence [GenBank accession no. AF451298]). The reaction involved 35 cycles consisting of 35 s of denaturation at 94°C, 35 s of annealing at 64°C, and 35 s of extension at 72°C, with a 5-min final extension at 72°C. The amplicons were analyzed by electrophoresis on a 1% agarose gel.

RFLP analysis was performed on PCR amplification products with DraI restriction enzymes (Promega). Suitable restriction enzymes were identified with the aid of a computer program (GeneJockeyII). For each isolate, 10 U of each enzyme and 10 μl of amplification products were used in a total volume of 20 μl of buffer supplied with restriction enzymes. Digestion was performed at 37°C for 3 h. Five microliters of restricted sample was then mixed with 2 μl loading buffer and transferred to a 1% agarose gel together with a 100-bp DNA ladder (Promega) for fragment size determination. The conditions for analysis were those described above.

RESULTS

P. vivax malaria patients were all in their twenties (ages, 21.51 ± 0.74 years for those with SK-A isolates and 21.32 ± 0.90 years for those with SK-B isolates) and male. The typing of Korean *P. vivax* isolates indicated that the parasitemias with the SK-A and SK-B genotypes were not significantly different (data not shown). All of the patients were detected in Yonchon-gun, but the exact infection area and time were unknown due to the prolonged incubation period of the Korean isolates (W. G. Kho, presented at the Symposium on Recent Reemerging Diseases, Korean Academy of Medicine, 1997).

By blood smear analysis, the levels of parasitemia ranged from 0.004% to 0.28%, with a mean parasitemia of 0.073%; 208 to 13,888 parasites were amplified per reaction. Although at varying concentrations, the PvMSP-1 gene was successfully amplified in all cases after PCR. PCR amplification produced DNA fragments of 945 bp, corresponding to positions 1081 to 2025 of the strain Belem sequence, which was expected from the sequences. Size polymorphisms and multiple bands were not observed (data not shown). No amplification was observed

TABLE 1. Pairwise comparison of percentages of nucleotide sequence difference in the 888-bp ICB4-to-ICB5 region of PvMSP-1 between Korean isolates with genotype SK-A or SK-B and other isolates

Strain(s)	% Difference between the nucleotide sequence of the ICB4-to-ICB5 region and that of Korean isolates with genotype:	
	SK-A	SK-B
Belem	3.0	4.2
Sal-1	3.9	2.8
Philippine isolates		
Ph-46-1	2.8	3.9
Ph-46-2	4.8	5.3
Ph-49	1.2	2.4
Ph-49-1	2.5	3.6
Ph-52-1	4.7	5.2
Ph-52-2	1.6	2.7
Ph-54-2	4.7	5.2
Ph-63	4.7	5.2
Ph-79	1.5	2.6
Ph-84	2.6	3.7
Solomon Islands isolates		
Solo-83	1.1	2.2
Solo-101	3.4	3.4
Chinese isolates		
Ch-2	5.6	6.1
Ch-3	5.6	6.1
Ch-4	2.8	3.9
Ch-5	2.1	3.3
Ch-6	3.3	3.5
Ch-7	3.9	2.8
Korean isolates of genotype:		
SK-A		1.1
SK-B	1.1	

with human genomic DNA alone (data not shown). We analyzed the sensitivity of the PCR with whole blood that was serially diluted with uninfected human whole blood. The results show that as few as 1 to 10 parasites per μl of whole blood were detectable (Fig. 1). This corresponds to a parasitemia of 2.0×10^{-5} to 2.0×10^{-4}%.

An 888-bp region of each PCR product (excluding primer regions) was analyzed for polymorphism. The DNA sequences of the 30 isolates were determined (Fig. 2), and amino acid sequences were deduced (Fig. 3). Two genotypes, SK-A (GenBank accession no. AF451297) and SK-B (GenBank accession no. AF451298), were identified on the basis of the grouping of variation in the nucleotides and their corresponding amino acids. Of 30 isolates, 15 isolates had the SK-A genotype and 15 had the SK-B genotype. The Korean isolates had >94.4% nucleotide sequence similarity, which distinguished them from strains from other regions. These strains showed the greatest similarity to the Solomon Islands isolate (97.8 to 99.9%) and Philippine isolates Ph-79, Ph-52-2, and Ph-49 (97.3 to 99.8%). Pairwise comparison between the MSP-1 sequences of *P. vivax* showed differences ranging from 1.1 to 6.1% (Table 1).

Comparison of the region between ICB4 and ICB5 of PvMSP-1 with that in strain Belem demonstrated 10 differences in SK-A and 12 differences in SK-B among 266 amino acids. Ten of these differences, at positions 394 (Ile/Val), 399 (Lys/Ser), 400 (Ser/Ala), 401 (Ala/Ser), 402 (Gly/Ser), 404 (Pro/Thr), 489 (Leu/Tyr), 619 (Leu/Tyr), 651 (Val/Ile), and 656 (Asn/Lys), were shared by SK-A and SK-B. Differences at positions 526 (Glu/Asp) and 529 (Thr/Lys) were found only in SK-B (Fig. 3).

A neighbor-joining tree based on alignment of PvMSP-1 nucleotide sequences is presented in Fig. 4. Two Korean isolates formed a cluster with a Solomon Islands isolate (Solo-83) and three Philippine isolates (Ph-79, Ph-52-2, and Ph-49) in a well-supported clade; however, the two genotypes of the Korean isolates were distinct from previously described genotypes of *P. vivax*.

The strategy for primer design was such that the sense primer was common to the SK-A and SK-B genotypes, while the antisense primer was genotype specific, yielding amplification of an isolate with a specific genotype (Fig. 2). PCR amplification produced DNA fragments of 495 bp and 497 bp for the SK-A and SK-B genotypes, respectively; these were the sizes expected from their sequences. A PCR product was obtained specifically only when DNA from the corresponding genotype was present in the reaction mixture (Fig. 5). The sensitivity of the genotype-specific PCR shows that as few as 1 to 10 parasites per μl of whole blood were detectable (Fig. 5).

Fragments amplified from the region between ICB4 and ICB5 of PvMSP-1 were analyzed with DraI. The RFLP profiles following digestion with the DraI restriction enzyme produced two distinguishable patterns. As shown in Fig. 6, isolates of genotype SK-A yielded fragments of 501 and 444 bp, while isolates of genotype SK-B yielded a fragment of 945 bp with no cutting.

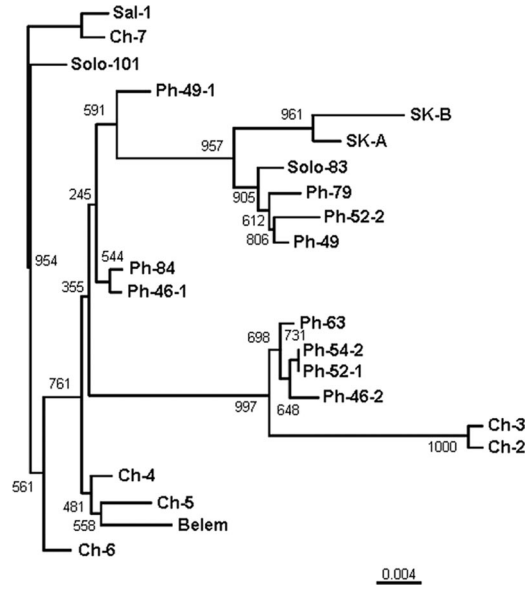

FIG. 4. Neighbor-joining phylogenetic tree constructed with the nucleotide sequences of the ICB4-to-ICB5 region of the PvMSP-1 gene from two Korean isolates, strain Sal-1, strain Belem, 11 isolates from the Philippines (Ph), 2 isolates from the Solomon Islands (Solo), and 6 isolates from China (Ch). The scale bar represents the estimated number of nucleotide substitutions per nucleotide site. Bootstrap values based on 1,000 bootstrap replicates are given at each node.

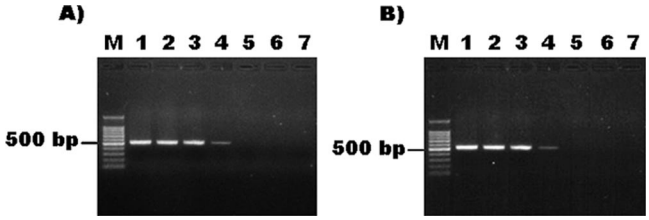

FIG. 5. Sensitivity of genotype-specific PCR for the ICB4-to-ICB5 region of the PvMSP-1 gene. (A) SK-A genotype; (B) SK-B genotype. Infected whole blood was serially diluted with normal whole blood. The initial level of *P. vivax* parasitemia was 0.02%. Lanes show results for the following numbers of parasites: 1,100 (lanes 1), 110 (lanes 2), 11 (lanes 3), 1.1 (lanes 4), 0.11 (lanes 5), and 0.011 (lanes 6). Lanes 7, negative control; lanes M, 100-bp DNA ladder (Promega), used as a DNA size marker.

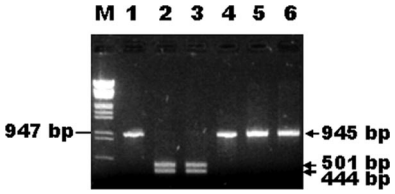

FIG. 6. PCR-RFLP results for the PvMSP-1 gene from Korean isolates. RFLP analysis was performed using the DraI restriction enzyme (Promega). Lane M, DNA size marker (λ DNA/EcoRI+HindIII; MBI Fermentas); lane 1, undigested SK-A genotype of PvMSP-1; lanes 2 and 3, digested SK-A genotype; lane 4, undigested SK-B genotype of PvMSP-1; lanes 5 and 6, digested SK-B genotype.

DISCUSSION

PvMSP-1 is available for antigenic and genetic variation studies of *P. vivax* populations. Analysis of specific gene regions of PvMSP-1 derived from parasite isolates from several areas suggests interallelic recombination, thus supporting that the MSP-1 gene in *P. vivax* is polymorphic in nature, as in *P. falciparum* (1). Cheng et al., using the ICB4-to-ICB5 region derived from isolates from China and the Philippines, have shown variation clusters, which do not match any of the allele types described (5).

The polymorphism pattern of Korean isolates was very similar to that of a Solomon Islands isolate (Solo-83), which has been reported by Cheng et al. (5). This finding raises the question of the existence of a third PvMSP-1 allelic family. The high extent of polymorphism may account for the new polymorphism in areas of endemicity, but Korean isolates of parasites have shown limited polymorphism in previous studies. It has been suggested that the antigenic polymorphism of PvMSP-1 is the result of either meiotic recombination or gene conversion (9). Malaria parasites are diploid only for a brief period following the fertilization of macrogametes with microgametes in the mosquito midgut. Since the next nuclear division is meiosis, restoring the haploid state, recombination can take place only at this stage in the mosquito, and only if a mosquito has simultaneously ingested gametes from mixed strains. Few data examining the recent intensity of malaria transmission in Korea are available. It is believed that the opportunity for malaria transmission by mosquitoes might be strictly limited, since transmission takes place during a limited season, from May to October, and the transmission ability of the vector mosquito may be extremely low (22). Also, the frequency of human-vector (*Anopheles sinensis*) contact is very low, because *A. sinensis* is highly zoophilic (23), there is widespread application of personal protection against mosquito bites (17), and *A. sinensis* has very low to moderate longevity in Korea (16). It is difficult to produce a secondary case from a primary patient, because the human blood index of *A. sinensis* was 0.007 in an area of endemicity in 1999 (24). Taking into account the epidemic characteristics of malaria, genetic changes may occur more slowly in Korea than in areas of high endemicity. Therefore, our data support the hypothesis that a third PvMSP-1 allelic type may exist. Also, the data indicate that recombination is an important mechanism in the generation of PvMSP-1 allelic diversity, but we failed to detect a third allelic type resulting from recombination of the SK-A and SK-B genotypes among Korean isolates.

Attempts are currently being made to measure transmission intensity using genetic information. Genetic variation in areas of hyperendemicity has been found to be greater than that in areas of hypoendemicity (1, 18). Previous studies of the genetic variation within the polymorphic region of PvMSP-1 showed several patterns among the isolates. A high frequency of genetic variation in high-transmission-intensity areas was also observed (5, 21). Concerning the extent of the genetic polymorphism of the ICB4-to-ICB5 region of PvMSP-1 according to geographical region, 2 genotypes were found in 30 patients in Korea, while 10 genotypes were found in 18 patients in the Philippines (5). The difference both in the number of genotypes and in the positions changed in the amino acid sequence between Korea and other countries may be due to the difference in the intensity of malaria transmission. It is believed that genetic variation of the *Plasmodium* sp. gene is frequent in areas of high transmission, because the variations may occur mainly due to continuous immune pressure or selection. Malaria is endemic in the Philippines, an area that has shown high transmission intensity for a long time.

In phylogenetic analyses, the Korean isolates seem to share some molecular similarity with previously characterized Solomon Islands isolates and several Philippine isolates. However, the Korean isolates apparently represent a distinct genotypic group. Interestingly, the two genotypes of PvMSP-1 found in Korea were related to the two genotypes of *P. vivax* circumsporozoite protein, *P. vivax* Duffy binding protein, and *P. vivax* apical membrane antigen 1 (6, 12, 13). This finding strongly suggests that the two strains of *P. vivax* coexist in the area of endemicity in Korea. Consideration of the epidemiology of malaria in Korea suggests that the two genotypes have existed in Korea for a relatively long time. However, the possibility that a third genotype exists in Korea cannot be completely ruled out.

These results suggest that the PvMSP-1 of Korean isolates has little polymorphism and that two genotypes of *P. vivax* coexist in the areas of endemicity in Korea. In the Republic of Korea, RFLP analysis and genotype-specific PCR, rather than DNA sequencing, would be useful for classifying the genotypes of *P. vivax*.

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