

Structural organization and sequence diversity of the complete nucleotide sequence encoding the *Plasmodium malariae* merozoite surface protein-1

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Supplemental Table S1 Sequencing primers for *PmMSP1*

Primer Name	Sequence (5' → 3')	Direction	Positions after GenBank no. FJ824669
PmMSP1-F1	CTATATTATCAAGTTTAATTCAAA	Forward	(-25) – (-2)
PmMSP1-F2	TTGCTCGACGAAATAAAGGAAATTG	Forward	844 - 868
PmMSP1-F3	CGGAACAGGAGAACAATCCGAAC	Forward	1863 - 1885
PmMSP1-F4	GAAACTCAGGAAATTGGAATCGTT	Forward	2515 - 2538
PmMSP1-F5	ACAAGCAGTACTAATCATATTAAG	Forward	3436 - 3460
PmMSP1-F6	TCAATTTAAACATGCAACTTC	Forward	4350 - 4370
PmMSP1-R1	ATCCTTCTTTTCTGTCTTTTTTTC	Reverse	1 - 24
PmMSP1-R2	TCAACATCTAATAGTTTATACG	Reverse	4394 - 4415
PmMSP1-R3	ATTTTCAACTTCTTTCTTTTCTGC	Reverse	3565 - 3588
PmMSP1-R4	TTGATATGACACACATAAGCAGT	Reverse	2593 - 2615
PmMSP1-R5	CTTGATTTTTTCTTCCTCCAGTTTTTTC	Reverse	1968 - 1995
PmMSP1-R6	ATCTGTAAATAGACCATCCAT	Reverse	1006 - 1026

Supplemental Table S2 Haplotype and nucleotide diversity in the *MSP1* genes of *P. malariae*, *P. ovale curtisi*, *P. ovale wallikeri*, *P. knowlesi*, *P. vivax* and *P. falciparum* from diverse endemic areas of Thailand

Gene	n	H	$h \pm \text{S.D.}$	$\pi \pm \text{S.E.}$	$\pi_S \pm \text{S.E.}$	$\pi_N \pm \text{S.E.}$	References
<i>PmMSP1</i>	35	20	0.941 ± 0.021	0.01135 ± 0.00136	0.01014 ± 0.00151	0.01166 ± 0.00116	This study
<i>PocMSP1</i>	5	3	0.700 ± 0.218	0.00129 ± 0.00040	0.00124 ± 0.00081	0.00129 ± 0.00038	1
<i>PowMSP1</i>	5	4	0.900 ± 0.161	0.00024 ± 0.00015	0.00074 ± 0.00056	0.00010 ± 0.00010	1
<i>PkMSP1</i>	12	10	0.970 ± 0.044	0.04532 ± 0.00251	0.05475 ± 0.00442	0.04251 ± 0.00207	2
<i>PvMSP1</i>	207	108	0.930 ± 0.015	0.04842 ± 0.00172	0.05699 ± 0.00423	0.04594 ± 0.00245	3, 4
<i>PfMSP1</i>	80	24	0.890 ± 0.025	0.04569 ± 0.00699	0.05139 ± 0.00303	0.04192 ± 0.00161	5-7

n, number of taxon; H, number of haplotypes; h , haplotype diversity; π , nucleotide diversity, π_S , nucleotide diversity at synonymous site; and π_N , nucleotide diversity at nonsynonymous site.

Z-tests of the hypothesis that π , π_S or π_N in *PmMSP1* equals the corresponding value in *PocMSP1*, *PocMSP1*, *PkMSP1*, *PvMSP1* or *PfMSP1*: all p values < 0.00001 .

Z-tests of the hypothesis that π , π_S or π_N in *PkMSP1* equals the corresponding value in *PvMSP1* or *PfMSP1*: all p values > 0.05 .

References:

1. Putaporntip, C., Hughes, A.L. & Jongwutiwes, S. Low level of sequence diversity at merozoite surface protein-1 locus of *Plasmodium ovale curtisi* and *P. ovale wallikeri* from Thai isolates. *PLoS One* **8**, e58962 (2013).
2. Putaporntip, C., Thongaree, S. & Jongwutiwes, S. Differential sequence diversity at merozoite surface protein-1 locus of *Plasmodium knowlesi* from humans and macaques in Thailand. *Infect. Genet. Evol.* **18**, 213-9 (2013).
3. Putaporntip, C. *et al.* Mosaic organization and heterogeneity in frequency of allelic recombination of the *Plasmodium vivax* merozoite surface protein-1 locus. *Proc. Natl. Acad. Sci. USA.* **99**, 16348-16353 (2002).
4. Jongwutiwes, S., Putaporntip, C. & Hughes, A.L. Bottleneck effects on vaccine-candidate antigen diversity of malaria parasites in Thailand. *Vaccine* **28**, 3112-7 (2010).
5. Tanabe, K. *et al.* Allelic dimorphism-associated restriction of recombination in *Plasmodium falciparum msp1*. *Gene* **397**, 153-160 (2007).
6. Tanabe, K. *et al.* Within-population genetic diversity of *Plasmodium falciparum* vaccine candidate antigens reveals geographic distance from a Central sub-Saharan African origin. *Vaccine* **31**, 1334-1339 (2013).
7. Tanabe, K. *et al.* *Plasmodium falciparum*: Genetic diversity and complexity of infections in a isolated village in Western Thailand. *Parasitol. Int.* **64**, 260-266 (2015).

Supplemental Figure S1. Amino acid substitutions in the putative 42 KDa- and 19 KDa-fragments of PmMSP1 relative to the two parental strains of PfMSP1 (MAD20 and K1).

PfMSP1-MAD20	NKKVNEALKS	YENFLPEAK-	VTTVVTTPQP	DVTPSPLSVR	VSGSSGSTKE	ETQIPTSGSL	LTELQQVVQL	QNYDEEDDSL	VVLPIFGESE	DNDEYLDQVV	1304													
PfMSP1-K1	NTDVNNALES	YKKFLPEGTD	VATVSE---	-----	-SGSDTLEQS	QPKKPASTHV	GAESNTITTS	QNVDEVDV	IIVPIFGESE	EDYDDLQVV	1251													
PmMSP1	NEEVNKALNV	YEELLPK---	--QISTEEQP	D-----	NAL	ADGTENATEG	AEVRAATAES	LVQGE----	DEYPPEEVDEV	IVFPVIGKKE	KE-NPLDQIT	1377												
variants	---K.													
← 42 KDa fragment																								
PfMSP1-MAD20	TGEAISVT-M	DNILSGFENE	YDVIYLKPLA	GVYRSLKKQI	EKNIITFNLN	LNDILNSRLK	KRKYFLDVLE	SDLMQFKHIS	SNEYIIEDSF	KLLNSEQKNT	1403													
PfMSP1-K1	TGEAVTPSVI	DNILSKIENE	YEVLYLKPLA	GVYRSLKKQL	ENNVMTFNVN	VKDILNSRFN	KRENFKNVLE	SDLIPYKDLT	SSNYVVKDPY	KFLNKEKRDK	1351													
PmMSP1	KGQAETKQ-D	DNILKPIITNE	YEVLYIKPLA	GVYRVLKQI	GDQIDAFNSN	LTNALDTRKK	KRTYFLDVLN	SDLIQFKHAT	SDSYIIKDPY	KLLDVDKKAK	1476													
variants													
PfMSP1-MAD20	LLKSYKYIKE	SVENDIKFAQ	EGISYIEKVL	AKYKDDLESI	KKVIKEEKEK	FPSSPPTTPP	SPAKTDEQKK	ESKFLPFLT	IETLYNNLVN	KIDDYLINLK	1503													
K PfMSP1-K1	FLSSYNYIKD	SIDTDINFAN	DVLGYKILS	EKYKSDLDSI	KKYI-----	-----	----NDKQGE	NEKYLPLLN	IETLYKTVD	KIDLFVIHLE	1431													
PmMSP1	LIGSYKYIVS	AIEKDITSAE	NGVEYDKMT	KLYKTQLEAV	KSAAEAQKE	-----	----GDKKTE	NEKYIPFLT	MOTLYENLLN	KINGNIINLK	1562													
variants	K.....	-----	----.....													
← 19 KDa fragment																								
PfMSP1-MAD20	AKINDCNVEK	DEAHVKITKL	SDLKAIDDKI	DLFKNTNDFE	AIKKLINDDT	KKDMLGKLLS	TGLV-QNFPN	TIISKLIEGK	FQDMLNIS-Q	HQC	VKKQ	CPE	1601											
PfMSP1-K1	AKVLNYTYEK	SNVEVKIKEL	NYLKTIQDKL	ADFKKNNFV	GIADLSTDYN	HNNLLTKFLS	TGMVFENLAK	TVLSNLLDGN	LQGMLNIS-Q	HQC	VKKQ	CPQ	1530											
PmMSP1	TLITNCNLEK	DAVNITISKL	TEYSKFDEKI	EMFKNSKN--	-----	EKDIA	SSGILDILKQ	KGLVNKNEST	KIISELLGVD	SNALLNISAK	HAC	TETKYPE	1655											
variants	D.....	-----											
PfMSP1-MAD20	NSG	CFRHLDE	REE	CK	CLLNY	KQEGDK	CVEN	PNPT	CNENNG	G	CADAT	C	TE	EDSGSSRKKI	T	C	CTKPDSY	PLFDGIF	C	SS	SNFLGISFLL	ILMLILYSFI	1701	
PfMSP1-K1	NSG	CFRHLDE	REE	CK	CLLNY	KQEGDK	CVEN	PNPT	CNENNG	G	CADAK	C	TE	EDSGSNGKKI	T	C	CTKPDSY	PLFDGIF	C	SS	SNFLGISFLL	ILMLILYSFI	1630	
PmMSP1	NAG	CYRYEDG	KEVWR	CLLNY	KLVDGG	CVED	EEPS	CQVNNG	G	CAPEAN	C	TK	GDD	----	NKI	V	CAC	NAPYSE	PIFEGVF	C	GS	SSFLGLSLLL	AALLIMFNLL	1751
variants	E..D.	V..K.

Note: GenBank accession numbers are X05624, X03371 and FJ824669 for PfMSP1 (MAD20 and K1 strains) and PmMSP1 (Cameroon strain). Variants include 35 Thai PmMSP1 sequences (GenBank accession nos. OM525734- OM525768). Dots represent identical residues with PmMSP1 (FJ82669). Dashes are deletions. Cysteine residues forming epidermal growth factor (EGF)-like domains 1 and 2 are highlighted in red and blue residues, respectively. Positively and negatively selected amino acids are highlighted in black and green, respectively. Amino acid positions are shown on the right.


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ITEEEGKINA LKGDVDSKLE KFKKIEGEFK PLEKIFYDER LDNSITTENF EKFLSKRTEY LITEKNLLESS SYELSKALVK KLKKQLMYLE DYSLRKEVFD EEVNHFNCLD 550
*****
DRB1*12:02
DRB1*15:02
DQA1*01:01/DQB1*03:01
DQA1*01:01/DQB1*03:03
DQA1*01:02/DQB1*03:01
DQA1*01:02/DQB1*03:03
DQA1*03:02/DQB1*03:01
DQA1*03:02/DQB1*03:03
DQA1*06:01/DQB1*03:01
DQA1*06:01/DQB1*03:03
DQA1*06:01/DQB1*05:01
DQA1*06:01/DQB1*05:02
*****

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LQLNADIHKL ESEIKRKENL LTVVDTLKFS DVVELOVQKV LLGKKIGQLK NVEAFLQAK LKETFHPIQA YGTGEQSEPY YLIALKREID KLNISIPKIE EMLKNEKKLE 660
*****
DRB1*12:02
DRB1*15:02
DQA1*01:01/DQB1*03:01
DQA1*01:01/DQB1*03:03
DQA1*01:02/DQB1*03:01
DQA1*01:02/DQB1*03:03
DQA1*03:02/DQB1*03:01
DQA1*03:02/DQB1*03:03
DQA1*06:01/DQB1*03:01
DQA1*06:01/DQB1*03:03
DQA1*06:01/DQB1*05:01
DQA1*06:01/DQB1*05:02
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→← Block VI

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EEKIKAAAQN VSGHVSAGE TSNHSGSSGG GGSTQIVTIT PSTITITAITTS SQIVSVGETG SAQAQAQPOP QPQPQPQTQT QTQTQPQAAG ATGTPQSGGQ SGQSGQSGAE 770
*****
DRB1*12:02
DRB1*15:02
DQA1*01:01/DQB1*03:01
DQA1*01:01/DQB1*03:03
DQA1*01:02/DQB1*03:01
DQA1*01:02/DQB1*03:03
DQA1*03:02/DQB1*03:01
DQA1*03:02/DQB1*03:03
DQA1*06:01/DQB1*03:01
DQA1*06:01/DQB1*03:03
DQA1*06:01/DQB1*05:01
DQA1*06:01/DQB1*05:02
*****

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→← Block VII

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GITETITGITG QAGITGITEPQ AAAAGPQAEI TATPGQAGAA GAAGPQAEIT GTPGQAGAAG PQTETEVEET QEIGIVVPTL SKLQYLEKLY DFLKTAYVCH INILVNNSTM 880
*****
DRB1*12:02
DRB1*15:02
DQA1*01:01/DQB1*03:01
DQA1*01:01/DQB1*03:03
DQA1*01:02/DQB1*03:01
DQA1*01:02/DQB1*03:03
DQA1*03:02/DQB1*03:01
DQA1*03:02/DQB1*03:03
DQA1*06:01/DQB1*03:01
DQA1*06:01/DQB1*03:03
DQA1*06:01/DQB1*05:01
DQA1*06:01/DQB1*05:02
*****

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NETLLQOYKL KIEEDKKLLE KCDQLDLLFN VQNNLQVMYS MYDSVSNVLQ NOYKELNOKE MIYNIYKLVK KNDKLNKFLN LTANSAAASS ALPPPPSVPP AVPPASQQPQ 990
***** *
DRB1*12:02
DRB1*15:02 *
DQA1*01:01/DQB1*03:01 *****
DQA1*01:01/DQB1*03:03 *****
DQA1*01:02/DQB1*03:01 *****
DQA1*01:02/DQB1*03:03 *****
DQA1*03:02/DQB1*03:01 *****
DQA1*03:02/DQB1*03:03 *****
DQA1*06:01/DQB1*03:01 *****
DQA1*06:01/DQB1*03:03 *****
DQA1*06:01/DQB1*05:01 *****
DQA1*06:01/DQB1*05:02 *****

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Block VIII

→← Block IX

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FQAALPAQPPQ AAVPAQSQAT VPAQSQAAMP AITQSSSVSA PTGTINGASPA TPVAPAGSEN AIQLKANDNE DDANELDFDI DDIYIKYLEQ VSKYDENFKN FIESKKDIIN 1100
DRB1*12:02
DRB1*15:02 *****
DQA1*01:01/DQB1*03:01 *****
DQA1*01:01/DQB1*03:03 *****
DQA1*01:02/DQB1*03:01 *****
DQA1*01:02/DQB1*03:03 *****
DQA1*03:02/DQB1*03:01 *****
DQA1*03:02/DQB1*03:03 *****
DQA1*06:01/DQB1*03:01 *****
DQA1*06:01/DQB1*03:03 *****
DQA1*06:01/DQB1*05:01 *****
DQA1*06:01/DQB1*05:02 *****

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KMSESEWKEL GEEINTLKQD IQSSFDNFCK YKCLKLERLLK KKNKITSSTN HIKEYSILKA QLLRKNILN NPRHLVAAFV VFFNKIEAE KKEVENALKN TDIMLKYYKA 1210
*****
DRB1*12:02
DRB1*15:02 *****
DQA1*01:01/DQB1*03:01 *****
DQA1*01:01/DQB1*03:03 *****
DQA1*01:02/DQB1*03:01 *****
DQA1*01:02/DQB1*03:03 *****
DQA1*03:02/DQB1*03:01 *****
DQA1*03:02/DQB1*03:03 *****
DQA1*06:01/DQB1*03:01 *****
DQA1*06:01/DQB1*03:03 *****
DQA1*06:01/DQB1*05:01 *****
DQA1*06:01/DQB1*05:02 *****

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RIKYYISEAF PLKTITEQSL QKEINYLHLE KFKVYSRLEG RIKKMLNLEK ENITYLSGGL HHVLTTELKEI INDKTYTGYT HIKNNEEVNK ALNVYEELLP KQISTEEQPD
*****
DRB1*12:02 *****
DRB1*15:02 *****
DQA1*01:01/DQB1*03:01 *****
DQA1*01:01/DQB1*03:03 *****
DQA1*01:02/DQB1*03:01 *****
DQA1*01:02/DQB1*03:03 *****
DQA1*03:02/DQB1*03:01 *****
DQA1*03:02/DQB1*03:03 *****
DQA1*06:01/DQB1*03:01 *****
DQA1*06:01/DQB1*03:03 *****
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DQA1*06:01/DQB1*05:02 *****

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DRB1*12:02
 DRB1*15:02
 DQAI*01:01/DQBI*03:01 *****
 DQAI*01:01/DQBI*03:03 *****
 DQAI*01:02/DQBI*03:01 *****
 DQAI*01:02/DQBI*03:03 *****
 DQAI*03:02/DQBI*03:01 *****
 DQAI*03:02/DQBI*03:03 *****
 DQAI*06:01/DQBI*03:01 *****
 DQAI*06:01/DQBI*03:03 *****
 DQAI*06:01/DQBI*05:01 *****
 DQAI*06:01/DQBI*05:02 *****

NALADGTENA TEGAEVRAAT AESLVQGEDE YPEEVDEVIV FPIVGGKEKE NPLDQITKGQ AETKQDDNLL KPITNEYEVL YIKPLAGVYR VLRKQIGDQI DAFNSNLINA 1430

DRB1*12:02
 DRB1*15:02
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 DQAI*01:01/DQBI*03:03 *****
 DQAI*01:02/DQBI*03:01 *****
 DQAI*01:02/DQBI*03:03 *****
 DQAI*03:02/DQBI*03:01 *****
 DQAI*03:02/DQBI*03:03 *****
 DQAI*06:01/DQBI*03:01 *****
 DQAI*06:01/DQBI*03:03 *****
 DQAI*06:01/DQBI*05:01 *****
 DQAI*06:01/DQBI*05:02 *****

LDTRKKKRTY FLDVLSNDLI QFKHATSDSY IIKDPYKLLD VDKKAKLIGS YKYIVSAIEK DITSAENGVE YYDKMTKLYK TQLEAVKSAI AEAQKEGDKK TENEKYIPFL 1540

DRB1*12:02
 DRB1*15:02
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 DQAI*01:01/DQBI*03:03 *****
 DQAI*01:02/DQBI*03:01 *****
 DQAI*01:02/DQBI*03:03 *****
 DQAI*03:02/DQBI*03:01 *****
 DQAI*03:02/DQBI*03:03 *****
 DQAI*06:01/DQBI*03:01 *****
 DQAI*06:01/DQBI*03:03 *****
 DQAI*06:01/DQBI*05:01 *****
 DQAI*06:01/DQBI*05:02 *****

TNMQTLYENL LNKINGNIIN LKTLITNCNL EKDAVNITIS KLTEYSKFDE KIEMFKNSKN EKDIASSGIL DILKQKGLVN KNESTKIISE LLGVDSNALL NISAKHACTE 1650

DRB1*12:02
 DRB1*15:02
 DQAI*01:01/DQBI*03:01 *****
 DQAI*01:01/DQBI*03:03 *****
 DQAI*01:02/DQBI*03:01 *****
 DQAI*01:02/DQBI*03:03 *****
 DQAI*03:02/DQBI*03:01 *****
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 DQAI*06:01/DQBI*03:03 *****
 DQAI*06:01/DQBI*05:01 *****
 DQAI*06:01/DQBI*05:02 *****

TKYPENAGCY RYEDGKEVWR CLLNYKLVGD GCVDEEPPSC QVNNGGCAPE ANCTKGDDNK IVCACNAPYS EPIFEGVFCG SSSFLGLSLL LAALLIMFNL L 1751

Supplemental Figure S3. Schematic representation of malarial MSP1s, depicting homologous regions (broken lines) across *Plasmodium* species. Open and filled boxes are conserved sequences and variable non-repeat regions within each locus. Boxes with checkerboard, horizontal brick, solid diamond and horizontal line are repeat regions. The schemes are drawn in approximate proportion to the gene length.



