Infection, Genetics and Evolution 54 (2017) 39–46

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Genetic diversity in the C-terminus of merozoite surface protein 1 among *Plasmodium knowlesi* isolates from Selangor and Sabah Borneo, Malaysia

Nan Jiun Yap [a,b], Xiang Ting Goh [a], Anson V. Koehler [b], Timothy William [c], Tsin Wen Yeo [d,f], Indra Vythilingam [a], Robin B. Gasser [b,e], Yvonne A.L. Lim [a,*]

[a] Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
[b] Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Victoria, Australia
[c] Jesselton Medical Centre, Kota Kinabalu, 88300, Sabah, Malaysia
[d] Communicable Diseases Centre, Institute of Infectious Disease and Epidemiology, Tan Tock Seng Hospital, Moulmein Road, 308433 Singapore, Singapore
[e] State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, China
[f] Lee Kong Chian School of Medicine, Nanyang Technological University, 11 Mandalay Road, Singapore, 308232, Singapore

ARTICLE INFO

Article history:
Received 25 March 2017
Received in revised form 12 June 2017
Accepted 16 June 2017
Available online 17 June 2017

Keywords:
Malaria
Malaysia
Plasmodium knowlesi
Merozoite surface protein-1
Genetic variation

ABSTRACT

Plasmodium knowlesi, a malaria parasite of macaques, has emerged as an important parasite of humans. Despite the significance of *P. knowlesi* malaria in parts of Southeast Asia, very little is known about the genetic variation in this parasite. Our aim here was to explore sequence variation in a molecule called the 42 kDa merozoite surface protein-1 (MSP-1), which is found on the surface of blood stages of *Plasmodium* spp. and plays a key role in erythrocyte invasion. Several studies of *P. falciparum* have reported that the C-terminus (a 42 kDa fragment) of merozoite surface protein-1 (MSP-1_{42}; consisting of MSP-1_{19} and MSP-1_{33}) is a potential candidate for a malaria vaccine. However, to date, no study has yet investigated the sequence diversity of the gene encoding *P. knowlesi* MSP-1_{42} (comprising *Pk-msp-1*$_{19}$ and *Pk-msp-1*$_{33}$) among isolates in Malaysia. The present study explored this aspect. Twelve *P. knowlesi* isolates were collected from patients from hospitals in Selangor and Sabah Borneo, Malaysia, between 2012 and 2014. The *Pk-msp-1*$_{42}$ gene was amplified by PCR and directly sequenced. Haplotype diversity (Hd) and nucleotide diversity (π) were studied among the isolates. There was relatively high genetic variation among *P. knowlesi* isolates; overall Hd and π were 1 ± 0.034 and 0.01132 ± 0.00124, respectively. A total of nine different haplotypes related to amino acid alterations at 13 positions, and the *Pk*-MSP-1_{19} sequence was found to be more conserved than *Pk-msp-1*$_{33}$. We have found evidence for negative selection in *Pk-msp-*$_{42}$ as well as the 33 kDa and 19 kDa fragments by comparing the rate of non-synonymous versus synonymous substitutions. Future investigations should study large numbers of samples from disparate geographical locations to critically assess whether this molecule might be a potential vaccine target for *P. knowlesi*.

1. Introduction

Malaria, caused by protists of the genus *Plasmodium*, is transmitted by mosquitoes and remains one of the most important parasitic diseases in the world, with >3 billion people at risk of infection and about 200 million human cases of malaria occurring each year (WHO, 2015). The annual malaria-associated mortality is approaching a staggering 43, 8000 people, with mortality primarily in children of less than five years of age (WHO, 2015). The recent recognition of *Plasmodium knowlesi*, a zoonotic malaria parasite, as one of the causative agents of human malaria has made the abatement of global incidence of this disease more challenging (Singh et al., 2004; Cox-Singh et al., 2008).

Plasmodium knowlesi is transmitted by mosquitoes to humans from two major monkey reservoir hosts, the long-tailed (*Macaca fascicularis*) and pig-tailed (*M. nemestrina*) (Knowles and Das Gupt, 1932; Garnham, 1966). To date, human-to-human transmission has not been reported. The parasite is unique in that it has a short life cycle of 24 h, enabling a rapid progression of disease (Daneshvar et al., 2009; Cox-Singh et al., 2010). Importantly, it can be very virulent in human patients, associates with high parasitaemia, and can cause severe complications and death (Cox-Singh et al., 2008, 2010). Although globally the prevalence and incidence of human infection with *P. knowlesi* are much less than those caused by *P. falciparum* and *P. vivax* (80–95%) (Stępień, 2014), recent reports of severe and fatal consequences of knowlesi malaria in humans (Cox-Singh et al., 2010; Lau et al., 2011) highlight the public importance

* Corresponding author.
E-mail addresses: robinbg@unimelb.edu.au (R.B. Gasser), limailian@um.edu.my (Y.A.L. Lim).

http://dx.doi.org/10.1016/j.meegid.2017.06.019
1567-1348/

Table 1
Sources of blood samples containing *Plasmodium knowlesi* from Malaysia.

Amino acid haplotype	Isolate	Accession number	Year of collection	Place of collection
1	PeninKKB2	KX881363	2012	Hospital Kuala Kubu Bahru, Selangor
2	PeninKKB4	KX881364	2012	Hospital Kuala Kubu Bahru, Selangor
	SBBorneo3	KX894505	2014	Hospital Queen Elizabeth II, Kota Kinabalu, Sabah
3	PeninKKB1	KX881365	2012	Hospital Kuala Kubu Bahru, Selangor
4	PeninKKB5	KX881366	2012	Hospital Kuala Kubu Bahru, Selangor
	SBBorneo4	KX894506	2014	Hospital Queen Elizabeth II, Kota Kinabalu, Sabah
5	PeninSgB1	KX881367	2013	Hospital Sungai Buloh, Selangor
6	SBBorneo1	KX881368	2014	Hospital Queen Elizabeth II, Kota Kinabalu, Sabah
7	SBBorneo2	KX881369	2014	Hospital Queen Elizabeth II, Kota Kinabalu, Sabah
	SBBorneo5	KX894507	2014	Hospital Queen Elizabeth II, Kota Kinabalu, Sabah
8	PeninSely1	KX881370	2013	Hospital Selayang, Selangor
9	PeninKKB3	KX881371	2012	Hospital Kuala Kubu Bahru, Selangor

of this simian parasite, particularly in Malaysia. In 2016, *P. knowlesi* comprised 69% of the malaria cases reported in Malaysia (Ministry of Health, Malaysia, unpublished reports).

A study conducted by William et al. (2014) described the changing epidemiology of malaria in Sabah Borneo, and suggested a more than ten-fold increase in incidence of *P. knowlesi* infection in humans (from 59 in 2004 to 703 in 2011, 815 in 2012 and 996 in 2013). Besides Malaysian Borneo (Singh et al., 2004; Cox-Singh et al., 2008; Daneshvar et al., 2009; Lau et al., 2011; William et al., 2011) and Peninsular Malaysia (Cox-Singh et al., 2008; Kantele et al., 2008; Vythilingam et al., 2008; Lee et al., 2010), *P. knowlesi* infection in humans has also been reported in Southeast Asian regions such as Singapore (Ng et al., 2008; Ong et al., 2009; Jeslyn et al., 2011), Thailand (Putaporntip et al., 2009; Sermwittayawong et al., 2012), Myanmar (Zhu et al., 2006; Jiang et al., 2010), the Philippines (Luchavez et al., 2008), Indonesia (Figtree et al., 2010; Sulistyaningsih et al., 2010), Vietnam (Eede et al., 2009) and Cambodia (Khim et al., 2011).

Despite the significance of *P. knowlesi* malaria in these geographical regions, there is limited information about the genetic variation within the parasite (Putaporntip et al., 2013). Recent multilocus microsatellite genotyping of *P. knowlesi* from diverse regions of Malaysia indicated the presence of three major subpopulations of *P. knowlesi*, including two divergent clusters of human cases in Malaysian Borneo (associated with long-tailed macaques and pig-tailed macaques) and a third cluster in humans in Peninsular Malaysia, with most of the infections from wild long-tailed macaques sampled in Kelantan (Divis et al., 2017). Our focus here was to explore sequence variation in an immunogenic molecule called the merozoite surface protein-1 (MSP-1), which is found on the surface of blood stages of *Plasmodium* spp. and plays a key role in erythrocyte invasion (Holder and Freeman, 1984; Holder et al., 1992). Consequently, MSP-1 has been recognised as a vaccine candidate (Holder, 2009).

However, *Plasmodium* MSP-1 is known to exhibit sequence diversity among isolates, which might be the result of selective pressure by host immune responses (Tanabe et al., 1987; Putaporntip et al., 2002; Miahipour et al., 2012). In *Plasmodium* species, *msp*-1 gene encodes a 190 kDa precursor protein which undergoes a two-step proteolytic cleavage during merozoite maturation. It is cleaved into four major fragments of 83-, 42-, 38-, 30-kDa (MSP-1_{83}, MSP-1_{42}, MSP-1_{38} and MSP-1_{30}), which remain on the merozoite surface as a glycosylphosphatidylinositol-anchored complex. Before erythrocyte invasion, the MSP-1_{42} fragment undergoes a second cleavage, resulting in the 33- and 19-kDa (MSP-1_{33}

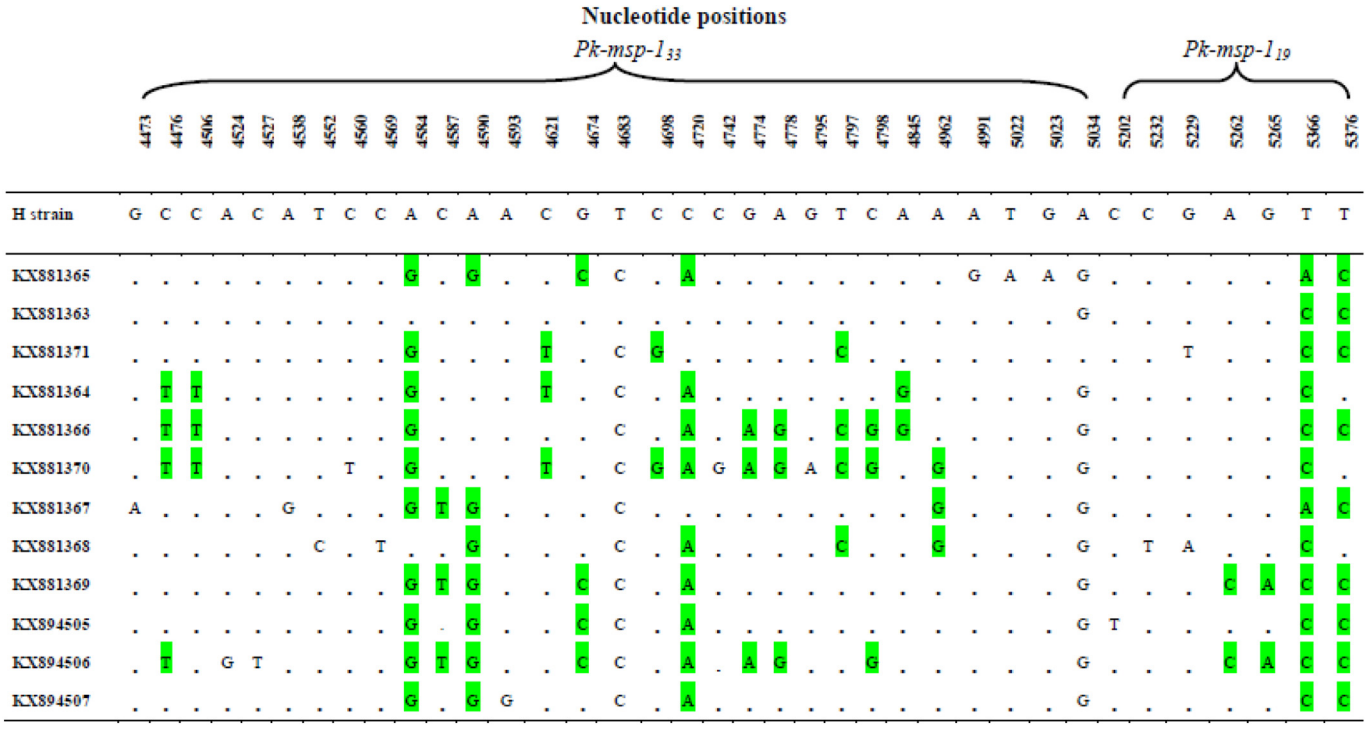

Fig. 1. Nucleotide variants of *Pk-msp-1*$_{42}$ (940 bp) representing *Plasmodium knowlesi* isolates from Malaysia. In total, 37 nucleotide alterations were detected in 12 isolates. Identical nucleotides are marked as dots, while polymorphic with parsimony informative sites are shaded in light green.