

## **Genetic diversity of circumsporozoite protein in *Plasmodium knowlesi* isolates from Malaysian Borneo and Peninsular Malaysia**

### **ABSTRACT**

Understanding the genetic diversity of candidate genes for malaria vaccines such as circumsporozoite protein (csp) may enhance the development of vaccines for treating *Plasmodium knowlesi*. Hence, the aim of this study is to investigate the genetic diversity of non-repeat regions of csp in *P. knowlesi* from Malaysian Borneo and Peninsular Malaysia. A total of 46 csp genes were subjected to polymerase chain reaction amplification. The genes were obtained from *P. knowlesi* isolates collected from different divisions of Sabah, Malaysian Borneo, and Peninsular Malaysia. The targeted gene fragments were cloned into a commercial vector and sequenced, and a phylogenetic tree was constructed while incorporating 168 csp sequences retrieved from the GenBank database. The genetic diversity and natural evolution of the csp sequences were analysed using MEGA6 and DnaSP ver. 5.10.01. A genealogical network of the csp haplotypes was generated using NETWORK ver. 4.6.1.3. The phylogenetic analysis revealed indistinguishable clusters of *P. knowlesi* isolates across different geographic regions, including Malaysian Borneo and Peninsular Malaysia. Nucleotide analysis showed that the csp nonrepeat regions of zoonotic *P. knowlesi* isolates obtained in this study underwent purifying selection with population expansion, which was supported by extensive haplotype sharing observed between humans and macaques. Novel variations were observed in the C-terminal non-repeat region of csp. The csp non-repeat regions are relatively conserved and there is no distinct cluster of *P. knowlesi* isolates from Malaysian Borneo and Peninsular Malaysia. Distinctive variation data obtained in the C-terminal non-repeat region of csp could be beneficial for the design and development of vaccines to treat *P. knowlesi*.