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## Genetic disorders and malaria in Indo-China region

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### Abstract

High prevalence of malaria in Southeast Asia including Thailand is believed to be a major public health problem to the population in this area since time immemorial. Adaptation of the population in this area following the principle of natural selection coupled with genetic disorders can be expected. Some good examples for natural selection of malaria are the co-existence of high prevalence of thalassaemia as well as glucose-6-phosphate dehydrogenase deficiency. In this report, general aspects of some important genetic disorders and malaria in Indo-China area (Thailand, Laos, Cambodia, Myanmar, Vietnam, Yunnan and Manipur) are summarized and discussed.

**Key words** Glucose-6-phosphate dehydrogenase – Indo-China – malaria – natural selection – sickle-cell – thalassaemia

### Malaria in Southeast Asia

Tropical Asia is the well-known endemic area of malaria. In other Indo-China countries, high prevalence of malaria is reported. Singhasivanon<sup>1</sup> emphasized that there was a great diversity in disease patterns in the Indo-China region and at sub-national administrative unit area level in each country, so that in the region as a whole there was a marked asymmetry in disease distribution, with many areas of high endemicity. Singhasivanon<sup>1</sup> noted that focal expansion of maps in the vicinity of international border areas delineated the differential trans-border malaria distribution that presented a challenge for disease control. Singhasivanon<sup>1</sup> also noted that the malaria pattern was also depicted in environmental context against regional elevation and forest cover profiles, which affected mosquito breeding site distribution and agricultural activity. Concerning Cambodia, Denis and Meek<sup>2</sup> noted that there were around half a million cases of malaria with 5–10,000 deaths per year. Concerning Laos, Pholsena<sup>3</sup> reported that malaria was endemic in all the 17 provinces of Laos and the trans-

mission was perennial with a ‘seasonal peak’ coinciding with the rainy season. Malaria is still the most common infectious cause of morbidity and mortality in Vietnam as it is in many developing countries in the tropics<sup>4</sup>. These reports can confirm the importance and high prevalence of malaria in Thailand and nearby Indo-China countries.

### Natural selection: what is it?

Natural selection is an important way of evolution process. It is the process by which individual organisms with favourable traits are more likely to survive and reproduce<sup>5</sup>. Natural selection works on the whole individual, but only the heritable component of a trait will be passed on to the offspring, with the result that favourable, heritable traits become more common in the next generation<sup>5</sup>. Given enough time, this passive process can result in adaptations and speciation<sup>5</sup>. Evolution of the human genome under selective pressure from malaria is a good example of natural selection<sup>6</sup>. Of several disorders, the most well-known condition is sickle-cell disorder<sup>7</sup>. It is accepted that

sickle-cell disorder is a good model for natural selection in medicine. This disorder is believed to be a result of natural selection process in response to the high prevalence of malaria in African ancestors. However, in addition to natural selection on human genome, the natural selection on malarial genome also leads to the problem of drug resistant malaria at present<sup>8</sup>.

### Important genetic disorders and malaria in Indo-China

Similar to the correlation between sickle-cell disorder and malaria in Africa, a condition resembling to natural selection process in malaria can be seen in Indo-China. Several haematological abnormalities in this area are mentioned for their correlation to the natural selection process of malaria. Some good examples for natural selection of malaria in Indo-China are the co-existence of high prevalence of thalassaemia as well as glucose-6-phosphate dehydrogenase deficiency (Fig. 1).

*Haemoglobin E disorder:* A well-known haemoglobinopathy, haemoglobin E is peak endemic in this area, in northeastern of Thailand and Laos<sup>9,10</sup>. According to the recent study of Dode *et al*<sup>11</sup>, high prevalence of HbE and  $\alpha$ -thalassaemia were found among the Southeast Asian refugees. However, there are some other haemoglobinopathies, such as Hb Tak, Hb Suandok and Hb Mahidol in Southeast Asia as well. Heterozygotes and homozygotes for HbE ( $\beta$ -26, GAG-AAG, Glu-Lys) are microcytic, minimally anaemic, and asymptomatic<sup>12</sup>. Haemoglobin E has the same electrophoretic mobility on alkaline cellulose acetate as haemoglobin A2 and haemoglobin C, however, the mobility of these haemoglobins differs on agar gel electrophoresis (pH 6.2) and they can be distinguished by this method<sup>9-12</sup>. The synthesis of haemoglobin E in reticulocytes of A/E heterozygotes and E/E homozygotes appears to be significantly impaired, seems to be in the production of  $\beta$ E chains, therefore, the HbE structural gene may be viewed as a  $\beta$ -thalassaemia-like gene<sup>9-12</sup>. Rees *et al*<sup>13</sup>

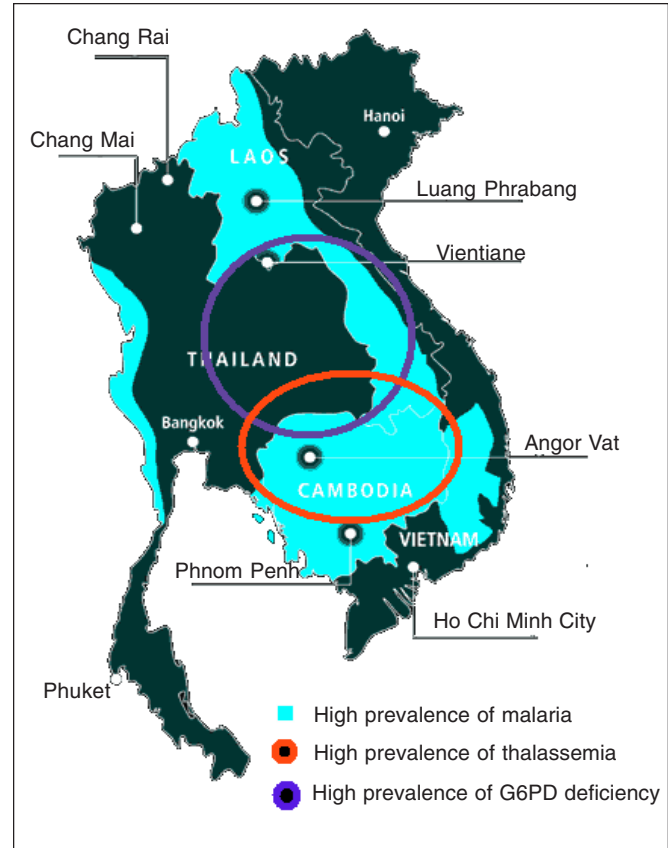


Fig. 1: Indo-China map demonstrating the frequencies of genetic disorders and malaria endemicity

reported that the microcytosis is attributed to the  $\beta$ -thalassaemic nature of the  $\beta$ E gene, whereas the *in vitro* instability of HbE does not contribute to the phenotype, however, the compound heterozygote state HbE/ $\beta$ -thalassaemia results in a variable, and often severe anaemia, with the phenotype ranging from transfusion dependence to a complete lack of symptoms<sup>13</sup>. The question of single or multiple origins for HbE in Southeast Asia is unresolved. Recombination events producing  $\alpha$ -thalassaemia deletions are frequent, whereas  $\alpha$ -thalassaemia is produced by a variety of large deletions, each of which has had a single origin<sup>14</sup>. The evidence favouring natural selection by *Plasmodium falciparum* malaria as the primary cause of high frequencies of the thalassaemias throughout the tropics and subtropics is documented<sup>14</sup>.

In Thailand, Wasi *et al*<sup>15</sup> studied HbA2 and HbE quantities by DEAE-Sephadex chromatography in 89 patients with *P. falciparum* malaria and concluded that *P. falciparum* malaria did not increase the levels of HbA2 and HbE. They also noted that the finding of increased HbA2 concerns *P. vivax* and still remains very important and should be tested in other parts of the world. This study led to the conclusion that HbE disorder might be due to the natural selection of malaria. Of interest, the trend of lower Hb in the malaria presented with unknown status of Hb electrophoresis pattern is documented. Pongyingpis<sup>16</sup> found that the value of platelet count, Hb level which tested by the Median test in both sexes were significantly lower in malarial patients than in non-malarial patients. However, this finding might be due to the underlying inherited anaemic disease or the malaria-induced anaemia. Similar to Thailand, HbE disorder is common in Laos. In 2004, Flatz *et al*<sup>17</sup> studied the  $\beta$ -globin anomalies in the Lao Theung population of southern Laos. The HbE frequencies were 0.426 in the So of Khammuan Province, 0.433 in the Alak/Ngeh of Sekong Province and 0.253 in the Oy of Attapeu Province<sup>17</sup>. They also reported that the HbE frequencies in the So and Alak/Ngeh were the highest observed in Southeast Asia in representative population samples. According to this work, the results were discussed with respect to natural selection by malaria, selection time, effects of  $\beta$ -thalassaemia and the ethnic history of the population of Southeast Asia<sup>17</sup>. Ebisawa and Muto<sup>18</sup> noted that the primaquine sensitivity of the Laotians might also relate to the haemoglobin disorder.

The situation of haemoglobin disorder in Myanmar is less reported, which might be due to the limitation in scientific research from this country. However, it is accepted that both malaria and HbE are endemic in Myanmar, although the actual prevalence of these disorders in the different indigenous races is not yet reported<sup>19,20</sup>. Win *et al*<sup>19</sup> studied the haemoglobin E prevalence in malaria-endemic villages in Myanmar by performing haemoglobin electrophoresis in four

malaria endemic villages of Myanmar, each having a different predominating indigenous race. The overall prevalence of HbE was 11.4%, ranging from 2–6% in the Kayin-predominant villages to 13.1–24.4% in the Bamar-predominant villages<sup>20</sup>. Oo *et al*<sup>21</sup> performed an interesting study on genetic red cell disorders and severity of falciparum malaria in Myanmar. In this work, a hospital-based survey was undertaken to investigate the relationship between the incidence and severity of malaria infection and various red cell disorders in Myanmar and found that the mean parasitaemia levels of patients with  $\alpha$ - or  $\beta$ -thalassaemia trait or with severe glucose-6-phosphate dehydrogenase (G6PD) deficiency were lower than those with normal haemoglobin AA or with heterozygous HbE. Than *et al*<sup>22</sup> collected samples from 916 members of various ethnic groups from malaria-endemic southern Shan State, Myanmar and analyzed for 3-thalassaemia (3-thal), abnormal haemoglobin variants and glucose-6-phosphate dehydrogenase deficiency. According to this work, the overall frequencies for the various red cell genetic disorders were as follows: 3-thal (37.5%); haemoglobin E (20.3%); glucose-6-phosphate dehydrogenase-Mahidol (17.5%); and 3-thal (0.3%)<sup>22</sup>. The frequencies of combined disorders were 6.9% for 3-thal/ haemoglobin E, 5.7% for 3-thal/glucose-6-phosphate dehydrogenase-Mahidol, 2.8% for haemoglobin E/glucose-6-phosphate dehydrogenase-Mahidol, 1.1% for 3-thal/haemoglobin E/glucose-6-phosphate dehydrogenase-Mahidol and 0.1% for 3-thal/3-thal/glucose-6-phosphate dehydrogenase-Mahidol<sup>22</sup>. They also mentioned that race was the dominant factor affecting the frequencies of red cell genetic disorders in malaria-endemic areas of Myanmar<sup>22</sup>.

Similar to Thailand, haemoglobin E disorder is also common in Cambodia<sup>23</sup>. Everett<sup>24</sup> said that malaria and beriberi are two unresolved military medical problems contributing to the fall of Cambodia. Concerning Vietnam, Le Hung *et al*<sup>25</sup> studied anaemia, malaria and hookworm infections in a Vietnamese ethnic minority with an aim to determine the preva-

lence of anaemia and evaluate the relationship of malaria and helminth infections on anaemia status in Phan Tien village, a mountainous ethnic minority community in southern Vietnam and reported that malaria was significantly associated with anaemia. In Yunnan, it is accepted that both malaria and HbE are endemic, although the actual prevalence of these disorders in the different indigenous races is not yet known. There are limited reports on haemoglobinopathy and malaria in Yunnan. Finally, it is accepted that both malaria and HbE are endemic in Manipur, although the actual prevalence of these disorders in the different indigenous races is not yet known. There are limited reports on haemoglobinopathy and malaria in Manipur. Chishti *et al*<sup>26</sup> confirmed the presence of severe and complicated falciparum malaria in this part of India and documented for the necessity of malarial research.

*Glucose-6-phosphate dehydrogenase (G6PD) deficiency:* G6PD (EC1.1.1.49) is an enzyme expressed in all tissues, where it catalyses the first step in the pentose phosphate pathway<sup>27–29</sup>. This first reaction in the pathway leads to the production of pentose phosphates and reducing power in the form of NADPH for reductive biosynthesis and maintenance of the cellular redox state. The defect of this enzyme namely G6PD deficiency is the most common sex-linked inherited enzymatic defect, affecting over 400 million persons worldwide<sup>27–29</sup>. This disorder can cause haemolytic anaemia<sup>27</sup>. The prevalence of G6PD deficiency in the Southeast Asia is high. This region is the endemic area of malaria, therefore, it is no doubt for this finding. In this region, the prevalence of G6PD deficiency has been continuously studied. In 1999, Tanphaichitr<sup>30</sup> performed a study in the Thais and found that the prevalence of G6PD deficiency in Thai males ranged from 3–18% depending upon the geographic region and G6PD-Mahidol (163 Gly —> Ser) was the most common variant found in the Thai population. A similar study was performed in the Thai neonates by Nuchprayoon *et al*<sup>31</sup>. They found the prevalence of G6PD deficiency as 22.1% in males and 10.1%

in females. However, they proposed that G6PD-Viangchan (871G>A), not G6PD-Mahidol, as the most common deficiency variant in the Thai population.

Data from *in vitro* studies demonstrate impaired growth of *P. falciparum* parasites in G6PD-deficient erythrocytes<sup>32</sup>. Attempts to confirm that G6PD deficiency is protective in field studies of malaria have yielded conflicting results, but recent results from large case control studies conducted in East and West Africa provide strong evidence that the most common African G6PD deficiency variant, G6PD A<sup>-</sup>, is associated with a significant reduction in the risk of severe malaria for both G6PD female heterozygotes and male haemizygotes<sup>32</sup>. In Thailand, the high prevalence of G6PD deficiency is believed to be due to the natural selection of malaria. Forty years ago, Kruatrachue *et al*<sup>33</sup> indicated that lower parasite count in G6PD deficient children was due to the effect of anaemia which would be considered as disadvantage of this abnormal gene in the presence of *P. falciparum* infection when it was compared with normal individuals. However, according to a recent study by Insiripong *et al*<sup>34</sup>, haemoglobin typing and methemoglobin reduction test performed on 115 malaria patients and matched controls revealed that the number of thalassaemia/haemoglobinopathies in the malaria group and in the control group were not significantly different and also occurrence of G6PD deficiency in the malaria group was not different from that of the controls. They concluded that there is no protective effect against malaria in G6PD deficiency<sup>34</sup>. Further, verification on the correlation between G6PD deficiency, especially in the molecular level, and malaria in Thailand is still needed.

In Laos, both G6PD deficiency and HbE are prevalent<sup>35–37</sup>. However, there are limited reports on G6PD deficiency and malaria in Laos. Aung-Than-Batu<sup>38</sup> said that the double genetic defect of thalassaemia trait and severe G6PD deficiency appeared to confer some degree of protection against malaria in studies conducted in Myanmar. In 1994, Myat-Phone-Kyaw

*et al*<sup>39</sup> studied the use of primaquine in malaria infected patients with red cell G6PD deficiency in Myanmar in 32 subjects with *P. falciparum* gametocytes, and 31 cases with *P. vivax* infection from two hospitals treated with quinine 600 mg three times a day for seven days followed by primaquine 45 mg single dose for gametocytes and 45 mg weekly for eight weeks for vivax malaria. The authors reported that a single dose of primaquine 45 mg and/or weekly for eight weeks is adequate for the treatment of patients with *P. falciparum* gametocytes and/or *P. vivax* malaria ignoring these red cell G6PD enzyme deficient variants in Myanmar<sup>39</sup>.

Everett *et al*<sup>40</sup> studied haemoglobin E and G6PD in the Khmer Air Force in Cambodia on 106 male Khmer Air Force and found that primaquine induced a significant, but not a dangerous, haemolysis in G6PD-deficient Khmer troops and the G6PD deficiency seen in Khmer Air Force subjects was G6PD-Mahidol. He also noted that G6PD deficiency-Mahidol was linked to haemoglobin E. In 2005, Matsuoka *et al*<sup>41</sup> conducted a survey of malaria diagnosis and G6PD testing in remote areas of Cambodia. Blood specimens from 670 people were collected by the finger-prick method<sup>41</sup>. Of these people, 24.9% were found to have malaria, and 7% of people were G6PD deficient<sup>41</sup>, and reported that the Cambodian population was derived from homogeneous ancestries and is different from the Myanmar population<sup>41</sup>. They noted that all G6PD-Viangchan cases were linked to two other mutations of 1311C>T and IVS-11 nt93T>C in the G6PD gene<sup>41</sup>.

G6PD deficiency is common among Vietnamese<sup>42</sup>. Matsuoka *et al*<sup>42</sup> conducted a survey for G6PD deficiency using blood samples from male outpatients of a local hospital in southern Vietnam. Most of the samples were from the Kinh (88.9%), the largest ethnic group in Vietnam, with a small number (11.1%) coming from the K'Ho, Chauma, Nung, and Tay minorities. They detected 25 G6PD-deficient cases among 1104 (2.3%) samples. Verlé *et al*<sup>43</sup> performed

another interesting study on G6PD deficiency in 1676 schoolboys in northern Vietnam and reported that the trait was nearly absent in boys of the Kinh (0.5%) and the Mong (0.7%) ethnic groups that traditionally have lived outside malaria transmission areas. The authors also reported that prevalence among ethnic groups living in the foothills, the breeding area of the main malaria vector *Anopheles minimus* (Diptera: Culicidae), ranged from 9.7 to 31%<sup>43</sup>. These findings support the hypothesis of a selective advantage of the trait in *P. falciparum*-endemic areas<sup>43</sup>. In 1973, Butler<sup>44</sup> also reported interesting natural selection process for G6PD deficiency and malaria in Black Americans in Vietnam, which was similar to haemoglobin E disorder. It is concluded that haemoglobin and red cell enzyme variation in some populations of Vietnam matches with the malaria hypothesis<sup>45</sup>.

For Yunnan, there are some reports on G6PD deficiency and malaria. In order to understand the molecular evolution, race origin and the relationship between the G6PD gene structure and clinical symptoms, Jiang *et al*<sup>46</sup> identified the molecular characterization of G6PD and determined the G6PD gene frequency in four ethnic groups in Yunnan province of China. According to this work, the gene frequency of G6PD in Bai population in Dali City is 0.0113 and the incidence is 1.19% which are different from those in Dai population. They also said that G6PD- G1388A, G1376T, A95G and C1024T were the mutations in national minorities as well as in the Han people and suggested that different national minorities of China might have the same ancestor and concluded that the distribution of G6PD deficiency in Yunnan was associated with the distribution of malaria epidemic in that province<sup>46</sup>. Finally, the knowledge on the prevalence of G6PD deficiency in Manipur is limited. There are limited reports on G6PD deficiency and malaria in Manipur.

### Conclusion

Malaria, haemoglobinopathy and G6PD deficiency

are common in Indo-China. The interrelation between the three disorders is widely documented. It is no doubt that the natural selection process of malaria can be seen in cases with either haemoglobinopathy or G6PD deficiency. However, the research on this area is still needed, especially for the areas with limited reports such as Laos, Myanmar, Yunnan and Manipur.

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