Prevalence and molecular study of G6PD deficiency in Malaysian Orang Asli

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Abstract

This study aims to define the prevalence and the molecular basis of G6PD deficiency in the Negrito tribe of the Malaysian Orang Asli. Four hundred and eighty seven consenting Negrito volunteers were screened for G6PD deficiency through the use of a fluorescent spot test. DNA from deficient individuals underwent PCR-RFLP analysis using thirteen recognized G6PD mutations. In the instances when the mutation could not be identified by PCR-RFLP, the entire coding region of the G6PD gene was subjected to DNA sequencing. In total, 9% (44/486) of the sample were found to be G6PD-deficient. However, only 25 samples were subjected to PCR-RFLP and DNA sequencing. Of these, three were found to carry Viangchan, one Coimbra and 16, a combination of C1311T in exon 11 and IVS11 T93C. Mutation(s) for the five remaining samples are unknown. The mean G6PD enzyme activity ranged 5.7 IU/gHb in deficient individuals. Our results demonstrate that the frequency of G6PD deficiency is higher among the Negrito Orang Asli than other Malaysian races. The dual presence of C1311T and IVS11 T93C in 64% of the deficient individuals (16/44) could well be a result of genetic drift within this isolated group.

The Orang Asli (OA), otherwise referred to as aboriginal people, have been living in different regions of the Malay Peninsula for an extensive period of time. The OA are divided into three main tribal groups: Negrito, Senoi and Proto-Malay. The Negrito is the smallest group of the OA with a population of just 4851. The Negrito group itself is comprised of six sub-ethnic groups: Kensiu, Kintak, Lanoh, Jahai, Bateq and Mendriq. An important point to note is that the OA suffer from several specific health problems besides malnutrition and hygiene issues, which include: malaria, tuberculosis, leprosy, filariasis, upper respiratory infections and skin problems.1 However, very little information is available on the genetic markers or background of the Malaysian OA and their origins due to the fact that little research has been undertaken on genetic associations.

G6PD deficiency is one of the most common human hereditary diseases.2 Over 400 million people worldwide are affected by some 140 different G6PD variants.3 G6PD deficiency is also highly prevalent in Asian countries, including Malaysia. While Malaysia is a multi-racial country, the only aboriginal people residing in the Malaysian Peninsula are the OA. Furthermore, although comprehensive studies on the G6PD deficiency have been undertaken, they have predominantly focused on the Malay and Chinese.4

The incidence rate of malaria in the OA area has been argued to be 20 times greater than in urban areas.5 Interestingly, complications associated with the drug treatment for malaria would largely be avoidable if the G6PD status was known6 by the local health provider. Consequently, the introduction of simple G6PD screening to this population is considered vital as a means to prevent future G6PD-associated death through increasing their awareness level.

As the majority of OA babies are delivered at home, there is very little information available on the OA neonatal jaundice status. It has been shown, however, that from a total of 65 OA admissions into the neonatal unit in one hospital, more than half (33/65) were jaundiced whereby 22 of them had G6PD deficiency.7 In addition, the OA community is recorded as having the highest mortality-rate for children under the age of five-years-old in Malaysia.8

Consequently, given the lack of information on the G6PD deficiency among the OA, the aim of this study was...
to determine the prevalence and the molecular basis of G6PD deficiency, in order to help improve the health-care delivery among the Negrito tribe of the OA.

This study was approved by the University Kebangsaan Malaysia (UKM) hospital’s ethics committee, whereby all subjects provided their written informed consent. A population screening was performed on 487 Negritos using a fluorescent spot test. This method is the most rapid and appropriate method for collecting samples in geographically isolated areas. The majority of the OA villages were visited using four-wheel-drive vehicles due to isolated locations within the remote jungle of the Malay Peninsula. Sample collection took place between November 2004 and February 2008. A G6PD quantification test was undertaken using the G6PD Kit from RANDOX Laboratory LTD (Antrim, UK) according to the manufacturer’s instructions and DNA was extracted using the Salting Out method. However, for the further molecular study, blood samples could only be collected from 25 individuals. PCR-RFLP was undertaken for 13 mutations, namely: Viangchan, Mediterranean, Mahidol, Canton, Gaohe, Coimbra, Andalus, Orissa, Union, Chatham, Kaiping, Vanua Lava and A- as described. These mutations were selected based on their high frequency among Asian populations. Assuming the African origin of the OA, the African A- mutation was selected for molecular mutational screening.

Samples which were negative for the aforementioned mutations were subjected to direct PCR sequencing for 12 exons of the G6PD gene and flanking introns. Primers sequence and PCR conditions were described elsewhere. Four hundred and eighty seven Negritos (245 males and 242 females) were screened for the G6PD deficiency. Of these, 28 males and 16 females were found to be G6PD-deficient. These results demonstrate that the overall prevalence of G6PD in the Negrito stood at 9% (44/486) (Table 1).

Table 2 shows the frequency of G6PD deficiency for each sub-tribe separately. The highest incidence was found among Lanoh (28%), while the lowest among Kensiu (0%). The mean G6PD enzyme activity ranged 5.7 IU/gHb in deficient individuals. Through the use of the PCR-RFLP method for detecting the 13 known G6PD mutations, the mutation types of four males were identified. Three of the males from Lanoh, two of who were siblings, carried the Viangchan mutation and one male from Jahai was found to carry the Coimbra mutation (Table 2).

By using DNA sequencing for all coding exons (12 exons) and flanking introns, 16 cases with the combined mutations of C1311T in exon 11 and IVS11 T93C were found. Three of the females were double heterozygotes for C1311T and IVS11 T93C; two others were heterozygotes for C1311T and homozygotes for IVS11 T93C; and lastly two females were homozygotes for both mutations. No mutation was found in the DNA of five deficient individuals.

Malaysia has implemented nationwide neonatal screening for G6PD deficiency since 1980; however, to date, no data relating to its incidence among Malaysian Orang Asli (OA) have come into existence. The reason for this is because OA children are predominantly delivered at home. However, the incident rates of G6PD deficiency among Malaysia males is cited as 5.3%. The current study has shown that the incidence rate of G6PD deficiency among Negrito is 11% for male and 7% for female. Nevertheless, due to the weakness of the fluorescent spot test to detect all the heterozygotes females, a higher frequency of G6PD deficiency is expected in female Negrito. While the frequency of G6PD deficiency in OA Negritos was higher than among other Malaysian races, this finding was expected as it was considered a likely result of the high epidemic of Malaria in the OA settlement area. Individuals with an inherited G6PD deficiency are at risk of developing anaemia if they are exposed to certain substances, such as anti-malaria drugs. The results from this study strongly suggest there is need to implement an appropriate screening method which would effectively detect G6PD-deficient OAs. This would be a pre-requisite to any successful health programme. Ainoon et al. have reported that 11 mutations are responsible for the G6PD deficiency in Malays and that 79% of Malay carry Viangchan, Mediterranean and Mahidol. However, an absence of these mutations in 87% of the study samples

### Table 1 Prevalence of G6PD in Negrito tribe

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<tr>
<th>Tribe</th>
<th>% G6PD (deficient/total)</th>
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<tr>
<td>Jahai</td>
<td>3% (5/170)</td>
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<tr>
<td>Kintak</td>
<td>18% (11/63)</td>
</tr>
<tr>
<td>Lanoh</td>
<td>28% (18/68)</td>
</tr>
<tr>
<td>Kensiu</td>
<td>0% (0/118)</td>
</tr>
<tr>
<td>Bateq</td>
<td>15% (10/67)</td>
</tr>
<tr>
<td>Total</td>
<td>9% (44/487)</td>
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</tbody>
</table>

### Table 2 Classification of molecular analysis of 25 G6PD-deficient Negrito

<table>
<thead>
<tr>
<th>G6PD variants</th>
<th>Number of deficient (%)</th>
<th>G6PD activity (U/gHb)</th>
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<tbody>
<tr>
<td>Viangchan</td>
<td>3 (12%)</td>
<td>1.2</td>
</tr>
<tr>
<td>Coimbra</td>
<td>1 (4%)</td>
<td>0.86</td>
</tr>
<tr>
<td>Combined C1311T and IVS11 T93C</td>
<td>16 (64%)</td>
<td>1.8–4.8</td>
</tr>
<tr>
<td>Unknown</td>
<td>5 (20%)</td>
<td>1.2–5.7</td>
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proves that the Negrito has been an isolated group for a period of time. On the other hand, the presence of Viangchan and Coimbra variants in a few of the OA samples could well be the result of interbreeding with other groups in the distant past. Unlike C1311T and IVS11 T93C, the majority of the known G6PD variants are single missense mutations. Individuals who carry this combination are deficient even though the G6PD protein was unchanged. A significant reduction in enzyme activity (and consequently its clinical implications) has been reported for this combined mutation. It has been assumed that there are further yet-to-be identified mutation(s) or SNP(s) causing this low enzyme activity. The presence of the combined mutation of C1311T and T93C in a large portion of the study samples is considered to be most likely the result of the genetic drift in this small isolated population over time. This result concurs with Hill et al. who analysed mitochondrial DNA control-region and coding-region markers in the OA and concluded that all OA groups have undergone high levels of genetic drift.

References


No A- variant has been observed in the OA which is in line with other reports which suggested that the A-mutation is 3840 to 11 760 years old and Negrito were the earliest inhabitants of the Malay Peninsula, having migrated from Africa over 50 000 years ago.

In summary, we conclude that the prevalence of G6PD is high in the Negrito and postulate that the molecular homogeneity of the G6PD mutation in this group may be a result of genetic drift. Further studies are required, however, to uncover specific mechanism(s) which correlate with the role of C1311T and IVS11 T93C in combination in the G6PD deficiency.

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