

## Peninsular Malaysia's Negrito Orang Asli and Its Theory of African Origin (Orang Asli Negrito di Semenanjung Malaysia dan Teori Asal Usul Afrika)

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### ABSTRACT

*Negritos of Peninsular Malaysia have physical features which strongly resemble the African pygmies rather than any of the other main South East Asian ethnic groups. In addition, their features are also completely different from the two other large sub-groups of the Peninsular Malaysia Orang Asli, i.e. Senoi and Proto-Malay. In this study, we genetically screened three African-specific markers, Glucose-6 Phosphate Dehydrogenase (G6PD) gene PvuII Type 2 polymorphism and A- mutation; and Sickle Cell trait in 103 unrelated individuals with G6PD deficiency. None of the Negritos' samples carried A- and Sickle cell mutations but all males and females have the PvuII Type 2 polymorphism. The same results were seen in all DNA samples of the Malaysian's Malay, Chinese and Indians. Additionally, all females in this study were homozygous for PvuII Type 2 polymorphism. Thus, we concluded that this polymorphism is widespread in all Malaysian population and is not unique to just Africans. However, these findings indicated that the polymorphism was widely conserved and can be used to study the African descendant in any world population hitherto supporting the 'Out of Africa' theory.*

*Keywords: G6PD; Malaysia; Negrito; Orang Asli*

### ABSTRAK

*Negrito di Semenanjung Malaysia mempunyai ciri-ciri fizikal yang sangat menyerupai orang Pigmi di Afrika berbanding kumpulan etnik lain di Asia Tenggara. Sebagai tambahan, ciri-ciri mereka juga secara keseluruhannya berbeza berbanding dua lagi sub-kumpulan Orang Asli di Semenanjung Malaysia iaitu Senoi dan Melayu-Proto. Dalam kajian ini, kami membuat penyaringan genetik menggunakan tiga penanda khas populasi Afrika, polimorfisme PvuII Type 2 dan mutasi A- untuk G6PD gen serta mutasi sel sabit ke atas 103 individu tak bersaudara yang mengalami kekurangan G6PD. Kesemua sampel Negrito tidak membawa mutasi A- dan sel sabit tetapi kesemua lelaki dan perempuan dalam kajian ini mempunyai polimorfisme PvuII Type 2. Hasil yang sama dicerap dalam semua sampel DNA Melayu, Cina dan India di Malaysia. Tambahan lagi, semua perempuan dalam kajian ini homozigus bagi polimorfisme PvuII Type 2. Oleh itu, kami menyimpulkan bahawa polimorfisme ini tersebar luas dalam kesemua populasi Malaysia dan tidak khusus untuk orang Afrika sahaja. Walau bagaimanapun, penemuan kajian ini memberi petunjuk bahawa polimorfisme ini terpelihara secara meluas dan boleh digunakan untuk mengkaji keturunan Afrika bagi mana-mana populasi dunia, seterusnya menyokong teori 'Out of Africa'.*

*Kata kunci: G6PD; Malaysia; Negrito; Orang Asli*

### INTRODUCTION

Various studies conducted on Glucose-6 phosphate dehydrogenase (G6PD) deficiency and hemoglobinopathies involving the molecular mutational analysis have provided valuable information on the characterisation of a population's specific pattern of common mutation (Beutler & Vulliamy 2002). Thus, the distribution of common mutation is always used to predict population admixture and genetic diversity for any isolated populations (Balgir 2010). Normal activity of G6PD enzyme plays a role as a provider of NADPH in red blood cells and detoxification of any harmful oxidative components from human circulation. Additionally, normal hemoglobin is vital to ensure a healthy function of human organs. Most importantly, previous studies have shown a parallel association between

malaria with G6PD deficiency and Sickle cell trait in human survival, resulting in a similar world distribution of malaria with G6PD deficiency and sickle cell condition. Recently, the role of polymorphisms such as single nucleotide polymorphism (SNP) has been reported. Collaborative work between scientists in Asia through the Pan-Asia Single Nucleotide Polymorphism Initiative (PASNPI) have reported on the human migration pattern from Africa into Asia (Abdulla et al. 2009), suggesting that South East Asia (SEA) as the entry point into Asia. Negritos, being an isolated population in SEA, could have been the remnant of the original population that first migrated into Asia.

Peninsular Malaysia Orang Asli represents a small proportion (0.5%) of the Malaysian populations (Figure 1). As one of the three sub-tribes of Orang Asli, Negritos

are easily recognisable by their black woolly hair, dark skin, short stature, rounded face and wide nose that largely resemble the people in Papua New Guinea, Australian or African aborigines in general. However, documented evidence from research studies to support Negrito as a branch origin of Africans is still unavailable. They are concentrated in just a few villages. Besides Jahai and Bateq; Kensiu, Kintak, Lanoh and Mendriq can nowadays be found in one village. The shy, endogamous and traditionally lived Negrito can be found in remote location such as the deeply isolated jungles of Perak and Kelantan (Amini et al. 2011). Only Kensiu sub-ethnic group lived near the main road not far from a popular hot spring recreational spot in Baling, Kedah. Overall, the population suffers recurrent malaria endemics and blood screening is done frequently for the past three decades.

PvuII Type 2 is a single nucleotide polymorphism (SNP) positioned at 612nt in intron V or at 13017nt, 60bp downstream of exon 6 of G6PD gene with C allele presentation. G allele presentation is likewise called the PvuII Type 1. Two reports were published in Human Genetics journal. Firstly, Fey et al. (1990) declared that PvuII Type 2 polymorphism belongs to African originated population present in 0.32-0.40 percent of X chromosomes of Kenyans, Nigerians, Zambians and West Indians but not found in the British, Filipino, Icelander and Saudi populations. Secondly, Beutler and Kuhl (1990) found PvuII Type 2 in 26 of the 43 males of various races or ethnic origins such as Africans, Puerto Ricans, Mexican, US Caucasians and Spanish. In their study, strong linkage disequilibrium was shown by PvuII Type 2 with G6PD deficient males carrying A- mutation of 202A/376G haplotype. Beutler et al. (1991) later concluded that A- is an ancient source from Africa. Since then, many

researchers used PvuII Type 2 as a marker for confirming African origin in the respective populations (Coetzee et al. 1992; Manco et al. 2007; Saad et al. 1997). Both A- and PvuII Type 2 were not reported in any Asian population except by Nuchprayoon et al. (2008) in one G6PD-deficient individual, but only as novel mutation not polymorphism (94 C to G).

Sickle cell trait is a genetic condition caused by a single mutation or single nucleotide polymorphism (SNP) causing a replacement of glutamic acid with valine at position 70614 in beta globin gene. This change will cause the production of unstable hemoglobin easily seen as a sickle red blood cell in blood smear. Thus, sickle red blood cell will affect the body supply of oxygen into organs and tissues.

Due to the lack of molecular information on the origin of Negritos and their close resemblance to Africans, as well as the contribution of genetic discoveries to a better health care delivery and minimization of any disparities between minority populations (Meilleur et al. 2011), we conducted this study to identify any possible relationship between the two populations using African- specific G6PD mutations.

#### MATERIALS AND METHODS

Population screening was performed on 600 Negritos in three states of Malaysia including Kedah, Kelantan and Perak from November 2005 until August 2009. The Negrito samples were collected from five of its six sub-ethnicities due to high sub-group marriages in Mendriq. Samples from the Malaysian Malay, Chinese and Indian were collected and went through the same procedure as Negritos. At least, a *three-generation pedigree* is obtained to be sure that there are no mixed marriages. Informed consent was obtained



FIGURE 1. Negritos distribution in Peninsular Malaysia

and 5-10 mL of whole blood was taken for molecular analysis. Fluorescent spot test and quantitative analysis were performed for G6PD screening purposes. DNA was extracted from blood using Salting out method (Miller & Dykes 1988). Peripheral DNA from 189 individuals is kept in our collection. A total of 103 DNA samples of unrelated G6PD deficient Negritos, Malay, Chinese and Indian were screened for A- and Sickle cell mutation as well as PvuII Type 2 polymorphism. This study was approved by the Ethics Committee, Universiti Kebangsaan Malaysia Medical Centre (UKMMC - FF-200-2008).

## RESULTS AND DISCUSSION

Out of the 600 subjects that were screened, 68 individuals were identified as deficient (no fluorescent) by qualitative analysis (40 males and 31 females), showing a prevalence

of 11.8% (Figure 2 and Table 1). However, quantitative analysis of G6PD enzyme activity found 46.6% of the population is deficient (Table 2).

We believed inbreeding plus malaria epidemic have caused a high concentration of recessive G6PD alleles in Orang Asli genomic pool compared to the main races i.e. Malay, Chinese and Indian. The prevalence ranged from 4.9% to 3% (Ainoon et al. 1999, 2003, 2004). This is the highest prevalence ever reported in comparison to other Southeast Asian countries (Table 3). However, A- and Sickle cell mutation was absent in all the studied populations. On the other hand, PvuII Type 2 polymorphism was detected in all samples regardless of G6PD level (Figure 3).

It is believed, unreported PvuII Type 2 polymorphism in Asia is probably caused by its non-involvement in G6PD deficient phenotype and its intronic location. Therefore, we suspected that almost all Asian population will carry

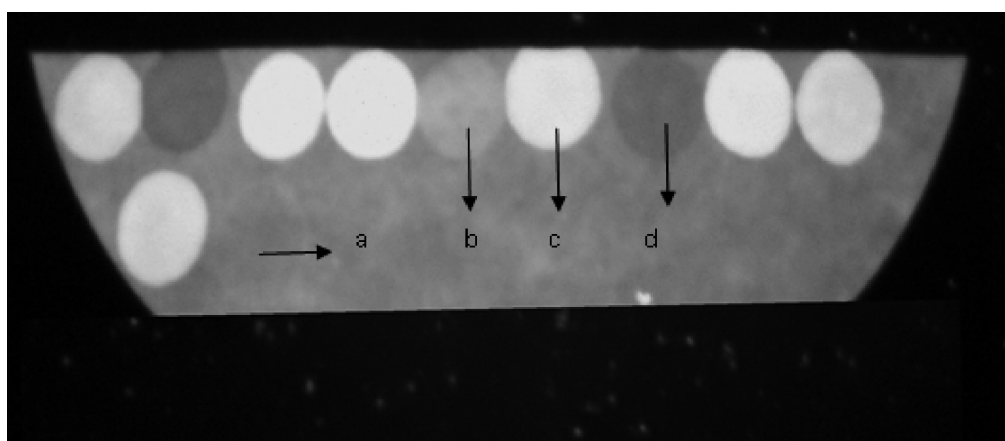


FIGURE 2. Normal RBC containing G6PD showed bright fluorescence (c) and deficient RBC lacking G6PD or less G6PD showed weak (b) or no fluorescence (d). One negative control (water) was run with each set of samples (a)

TABLE 1. Fluorescence spot test among males and females

Sub-tribe	No. of study population	Deficient male/ overall male (Prevalence %)	Deficient female/overall female (Prevalence %)	Total deficiency (%)
Bateq	67	8/44 (18.2)	2/23 (8.7)	10/67 (14.9)
Jahai	170	4/71 (5.6)	2/99 (2.0)	6/170 (3.5)
Kensiu	118	0/62 (0)	0/56 (0)	0/118 (0)
Kintak	81	12/44 (27.3)	13/37 (35.1)	25/81 (30.8)
Lanoh	164	16/80 (20.0)	14/84 (17.0)	30/164 (18.3)
Total	600	40/300 (13.3)	31/300 (10.3)	71/600 (11.8)

TABLE 2. Results of G6PD quantitative analysis

Sub-tribe	No. of family (males+females)	No. of males with low activity	No. of females with low activity	Total deficiency (%)
Bateq	22 (11+11)	6	10	16 (72%)
Kintak	22 (10+12)	4	6	10 (45.5%)
Jahai	28 (12+16)	4	5	9 (32.1%)
Lanoh	31 (15+16)	3	10	13 (41.9%)
Total	103 (48+55)	17	31	48 (46.6%)

TABLE 3. Prevalence of G6PD deficiency in Southeast Asian countries compared with Malaysian Negritos

Country	Overall G6PD deficiency (%)	Reference
Malaysia (Negrito)	46.6	Present Study
Cambodia	13.4	(Louicharoen & Nuchprayoon 2005)
Vietnam (South)	11.3	(Hue et al. 2009)
Myanmar	11.0	(Matsuoka et al. 2004)
Laos	7.1	(Iwai et al. 2001)
Thailand	3 – 15.0	(Nuchprayoon et al. 2002; Poon et al. 1988)
Indonesia	4.0	(Matsuoka et al. 2003)
Malaysia (Malay, Chinese, Indians)	3.4	(Ainoon et al. 2002)
Papua New Guinea	3.0	(Chockalingam & Board 1980)
Philippines	3.9	(Padilla et al. 2003)
Singapore	1.6	(Joseph et al. 1999)

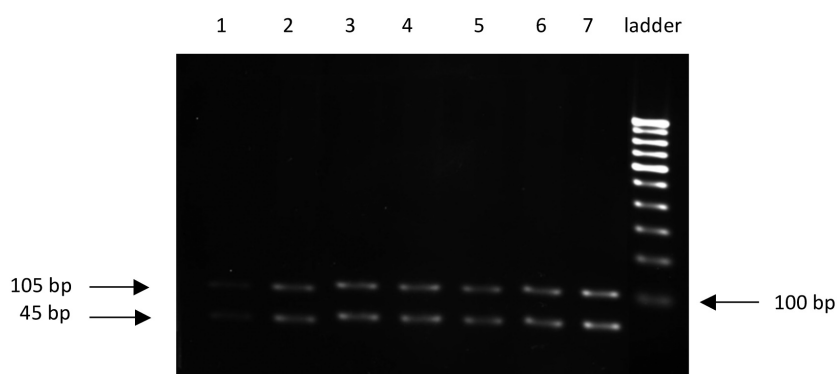


FIGURE 3. Restricted digestion of 155 bp amplified product of G6PD gene using PvuII. Lanes 1, 2, 3, 4, 5, 6 and 7 showing bands 105 and 45 bp. Lane 8: 100 bp DNA ladder

this polymorphism. If PvuII Type 2 was indeed a specific marker to reflect an African origin and the theory of East African migration of *Homo sapiens* is true, our findings have shown PvuII Type 2 as conserved African originated marker. Since Tishkoff et al. (2001) dated A- as a young mutation aged between 3840 and 11760 years ago and Shi et al. (2005) concluded that South East Asian is inhabited in the last 25000-30000 years based on O3-M122 lineages, the absence of A- in Negrito is another prove on how recent this mutation is.

Based on similar endemicity of malaria as well as high hemoglobinopathies in Africa (sickle cell anemia) and in Negrito (G6PD deficiency), PvuII Type 2 could probably be the ancient polymorphism selected upon by its advantages in the fight of human towards the malaria parasite and later on retained in Negrito (Denic & Nicholls 2007). Therefore, people with African-origin living in countries or area not exposed to high risk of malaria could lose the PvuII Type 2 site due to its selective advantage and become insignificant survival factor as shown by Kay et al. (1992) whereby genotyping on 54 male African-Americans failed to find any PvuII Type 2 polymorphism with G6PD A (+) 376G, G6PDA(-)202A/376G and G6PDB genotypes.

## CONCLUSION

We concluded that the existence of PvuII Type 2 polymorphism in all Malaysians (Negritos Orang Asli, Malay, Chinese and Indian) supported the African origin of all races. This polymorphism is preserved and inherited from their African ancestor probably due to its survival advantage against malaria parasite in their endemic region. Being an old marker, the PvuII Type 2 polymorphism is also suitable for tracing the African origin plus malaria history in other world populations.

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