TWENTY-SEVEN BASE PAIR DELETION OF *BAND 3* GENE: ITS PREVALENCE AMONG INDIGENOUS PEOPLE OF CROCKER RANGE SABAH, MALAYSIA.

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ABSTRACT

Southeast Asia Ovalocytosis (SAO) is a hereditaty hemolytic anemia caused by a 27 base-pairs deletion in band 3 *gene of the patient. The EPB3 gene encodes* band 3 *of the red cell membrane and ovalocytic erythrocytes have been shown to be rigid and resistant to invasion* in vitro *by* Plasmodium falciparum *and* Plasmodium knowlesi. *A survey was undertaken to determine the prevalence of this specific mutation in a group of indigenous Kadazandusun population living in the vicinities of the Crocker Range National Park Sabah, Malaysia. The study took advantage of the fact that PCR product of the normal gene fractionates at 175-bp while the mutant gene yields a product size of 148-bp. The study failed to detect the 27-bp deletion in the* band 3 *gene in all of the samples processed to date. Possible reasons for this detection failure was discussed.*

INTRODUCTION

Hereditary Hemolytic Anemias includes genetic disorders such as hereditary spherocytosis, hereditary ellliptocytosis or poikilocytocis and Southeast Asia Ovalocytosis (SAO). Of these hereditary hemolytic anemias, SAO is an asymptomatic trait characterized by rigid, poorly formed erythrocytes that resist invasion (in vitro) by several strains of malaria parasites, including *Plasmodium falciparum* and *Plasmodium knowlesi* (Castelino et al. 1981 and Kidson et at. 1981). SAO is widespread in certain ethnic groups of Malaysia, Papua New Guinea, the Philippines and Indonesia (Lie-Injo 1965 and Serjeanson et al. 1997). Moreover, in areas of endemic malaria, the ovalocytic subjects contain reduced number of intracellular parasites *in vivo* (Cattani et al. 1987).

The genetic etiology of SAO has been a matter of many speculations in the past. Moriyama et. al. has concluded that the 27-hp deletion mutation is responsible for the functional abnormality in SAO (Moriyama et al. 1992). The underlying molecular genetic defect for the abnormal red cell phenotype in SAO is attributed to the deletion of amino acids 400 through to 408 on the anion exchange protein AE-1 (syn. Band 3 protein) (Jarolim et al. 1991 and Salhany et al. 1996).

The fascinating fact about the SAO is that the heterozygosity for 27-bp deletion on *band 3* gene mutation provides protection against cerebral malaria in children (Genton et al. 1995) and homozygosity is absent in the population, indicating that Band 3 is crucial for survival.

MATERIALS AND METHODS

Study site and population

The study was undertaken in the month of October 1999. Three villages at Crocker range,

Sabah were targeted as the sampling area and they are Kampung Tikolod of Tambunan, Kampung Sunsuran of Tambunan and Kampung Bandukan of Keningau.

Genomic DNA extraction

Venous blood was drawn from each sample and stored in EDTA containing vacutainers. 1mL of the blood was blotted onto a 3MM Whatman paper (to ease in transport and facilitate in DNA extraction at convenience). From the blood spots immobilized on the filter paper, genomic DNA extraction was isolated using the NaI method (Wang et al. 1994). These DNA samples were then stored at —20ºC. Concentration and quality of DNA were verified by spectrophotometry and/or fractionation in a low percentage agarose.

Polymerase chain reaction (PCR)

Amplification of genomic DNA was performed with specific primers p198 and p199 that encompasses the 27-bp deletion (Jarotim et al. 1991). An initial denaturation at 95^0C for 5 min was followed by 30 cycles of denaturation at 94^0C for 1min annealing and extension at 70^0C for 1min with a final extension at 70^0C for 5 min. The Taq used was either from Bohringer Manheim or FERMENTAS product. The PCR product was size-fractionated in 2.5% agarose get (Gene-Lab) or 2.7% Nuseive gel (FMC product) and visualized by ethidium bromide (BioRad) stainning. The expected sized of the PCR products are 175-bp and 148-bp for the normal and mutant gene, respectively. The gel was then photographed using the Syngene, Synoptics digital imaging system. Digital images of the gel are kept in a database.

RESULTS AND DISCUSSION

Table 1. Groups studied with the locality and gene frequencies of band 3 *27-bp deletion*

Group (majority)	Locality	n	Sampling number	Frequency of 27-bp deletion
Kadazan-Dusun	Tikolod, Tambunan	57	1-57	0.0
Kadazan-Dusun	Sunsuran, Tambunan	51	1-57	0.0
Kadazan-Dusun	Bandukan, Keningau	11	109-118	0.0
Total		119		0.0

Detail study of the prevalence of 27-bp deletion in *band 3* gene has not been undertaken in this part of the world. There are literatures indicating that, 27-bp deletion mutation is found in Southeast Asia, together with another linked mutation (memphis polymorphism) (Jarolim et al. 1991) and these mutations might be associated with resistance to cerebral malarial disease (Genton et al. 1995) .

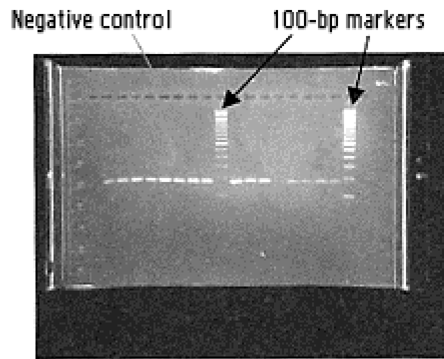

Figure 1. PCR amplification of genomic DNA with primers p198 and p199. Samples runs from 47 through to 62.

The study failed to detect the 27-bp deletion in the *band 3* gene in alt of the samples processed tilt to date. Table 1 depicts the locality and the ethnic background of the sample group. This data is in accordance with the data collected by Masako *et al* (verbal communication). This Japanese group discovered that the gene frequency for this deletion is between 0.0 and 0.012 in various region of Southeast Asia. The highest frequency was in Southern Sulawesi and the Dayaks of Southern Borneo. The absence of 27-bp deletion in the Crocker Range survey suggests the presence of molecular heterogeneity of SAO (morphologically defined SAO).

The population under study here is probably too small to exclude the possible presence of the 27-bp deletion in the Crocker Range area. A more comprehensive and wider scale molecular epidemiotogical survey will be carried out to include other parts of the East Malaysian population in future. This is to elucidate the probable origin or distribution of the 27-bp deletion of *band 3* and the mutation's role in genetic adaptation to malaria.

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