GENETIC VARIATION OF G6PD AND ADAPTIVE PHENOMENA IN MALARIAL INFECTION

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ABSTRACT

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-chromosome-linked hereditary abnormality with a high degree of clinical, biological and genetic variations. Some people are born with a mutation of the G6PD gene, which, when reacting to certain triggers, such as infection, some drugs like anti-malarials drug or some foods such as fava beans, can cause anemia. But those same people also seem more able to resist the worst effects of malaria. More than 300 G6PD variants have been defined, most common are those variants associated with acute intermittent hemolytic anemia. The geographic distribution of populations with a high gene frequency of deficient variants overlaps closely with the prevalence of malaria, suggesting that G6PD deficiency might be protective against malaria. Evidence for a protective affect of G6PD deficiency against Plasmodium falciparum comes from both epidemiological correlation and in vitro studies. Malaria is a major killer worldwide with high mortality rates among children. Of the four common malarial parasites in humans, P. falciparum is the deadliest. There are hundreds of million cases due to other parasite species: P. vivax, P. malariae and P. ovale. The risk of malaria transmission in endemic countries is increased in rural areas and varies seasonally in many locations.

Keywords: *Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency, Malaria, G6PD variant, Indonesia*

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INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a grup of hereditary abnormalities in which the activity of the erythrocyte enzyme G6PD is markedly diminished (Beutler 1994). It is one of the most common inherited hemolytic disorders reported and studied among humans, affecting around 400 million people worldwide (WHO 1989). The G6PD enzyme disorder was discovered in the 1950s when it was found that in some people administration of an anti-malarial drug like primaquine results in hemolytic anemia. Most of these individuals are otherwise asymptomatic.

Similar sort of responses had been reported in cases of a few other drugs, favism and in case of some infections. Generally asymptomatic, G6PD-deficient individuals show the symptoms in response to one or more oxidative stresses (Tripathy et al 2007).

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-chromosome-linked hereditary abnormality with a high degree of clinical, biological and genetic variations. The abnormal gene responsible for this inherited enzyme deficiency is located on the X-chromosome. Therefore, the illnesses associated with G6PD deficiency occur more frequently in males than females, since males only have one X-chromosome. There are different degress of G6PD deficiency, which vary according to the magnitude of the missing enzyme (Beutler 1994; Luzzatto 1995). In G6PD deficient females, the red cells are mosaic with two populations: one G6PD normal level and the other G6PD deficient level. This was postulated to be due to the random inactivation of one of the two X chromosomes in the females. When this occurs in the multicellular stage of human embryo development, the haematopoietic self-renewal stem cells are variably X-inactivated, hence accounting for the mosacism of the progeny cells in adult life. In fact the same phenonema was proposed by Mary Leon who studied X-linked coat colour in rodents. In practice, this information explains the finding of severe enzyme deficiency in some heterozygote females due to extreme degree of X inactivation of the normal chromosome. Furthermore, even in females with 50% activity, half of the mosaic red cells with G6PD deficiency are prone to haemolysis from drugs similar to their male counterpart. Hence, females with G6PD deficiency are clinically important although the degree of haemolysis is generally milder (Beutler 1962; Luzzatto 1979).

G6PD is a housekeeping enzyme which catalyzes the first step in the pentose phosphate pathway (PPP). The PPP also produces NADPH molecules which function as an electron donor and thus provides the reducing energy of the cell by maintaining the reduced glutathione in the cell. Reduced glutathione functions as an antioxidant and protects the cells against oxidative damage (Beutler 1994). In G6PD-deficient erythrocytes (RBC), production of NADPH (nicotinamide adenine dinucleotide phosphate) is decreased and this may lead to reduction of glutathione (GSH) levels, its levels in G6PD-deficient RBC are reported to be a half of normal RBC. Thus lack of G6PD enzyme in the red blood cells is lethal and deficiency in the enzyme in case of oxidative stress is deleterious to the cell. Any oxidative stress in the red blood cells with deficient G6PD enzyme may result in hemolytic anemia. This condition also has been proposed as an inhibitory mechanism of parasite growth in these RBC (Cappadoro et al 1998; Roth et al 1983; Tripathy et al 2007). Hemolytic anemias have been found to be associated with G6PD deficiency for the following oxidative stresses:

1. Anti-malarial drugs like primaquine and many other drugs
2. Fava beans (components like divicine and isouramil have been found responsible)
3. Chemicals like nepthalene, antifungal sprays
4. Herbs like Coptis sinesis and Calculus bovis
5. Infectious diseases
6. Neonatal jaundice (Beutler 1994; Luzzato 1995).

The hemolytic crisis may manifest within hours of exposure to oxidant stress. In severe cases, hemoglobinuria and peripheral circulatory collapse can occur. Since only older red cells are affected most, the problem is usually self-limiting. There will be a rapid drop in hematocrit, rise in plasma hemoglobin and unconjugated bilirubin. Heinz bodies can be seen on crystal violet staining. These are removed in the spleen in a day or two and 'bite cells', with loss of a portion of the periphery of the red cell, may be seen (Kakkilaya 2006).

MOLECULAR BIOLOGY OF G6PD DEFICIENCY

G6PD is composed of 515 amino acid subunits with a calculated molecular weight of 59,256 daltons. Aggregiation of the inactive monomers into catalytically active dimmers and higher forms requires the presence of NADP. The gene consists of 13 exons, which are the regions of the DNA that code for the enzyme, and 12 introns, which are intervening sequences. The enzyme's function is determined, however, by the sequence and size of the G6PD gene and the mRNA encoded by the gene. G6PD deficiency is one of the most prevalent disease-causing mutations worldwide. However, most of the G6PD isoenzymes with decreased activity are associated with only moderate health risks without a significant effect on longevity (Beutler 1994; Scriver et al 1995).

All humans have the G6PD gene, it's a general housecleaning agent, helping with glucose metabolism. Some people are born with a mutation of the G6PD gene, which, when reacting to certain triggers, such as infection or some foods such as fava beans, can cause anemia, itself a serious and even deadly condition. But those same people also seem more able to resist the worst effects of malaria (Beutler 1994; Tishkoff et al 2001). More than 300 G6PD variants have been defined, most are sporadic but some occur at a high frequency. G6PD variants can be divided into three categories based on the type of hemolysis they cause. Most common are those variants associated with acute intermittent hemolytic anemia, some of these variants are endemic. In contrast, the variants associated with chronic hemolytic anemia are very rare and the severity of hemolysis is highly variable, ranging from mild to transfusion dependent. The third type of variant is associated with no obvious risk of hemolysis (Beutler 2001; Prchal 2005).

G6PD deficiency is known to have over 400 variant alleles, or different forms of the same gene. A mutant G6PD enzyme may be different from person to person; mutations can be in the form of point mutations or can range from one to several base pair deletions as well as replacements in the DNA . Different populations have different types of mutations, but within a specific population, common mutations are usually shared. For example, in Egypt there exists only one type of allele, called the "Mediterranean" variant, among the population, whereas in Japan there is a different variant with a different type of mutation prevalent within that population, this one called the "Japan" variant. With regards to the demographics of G6PD deficiency, shows that most of the affected individuals reside in Africa, the Middle East, and Southeast Asia. African Americans and some isolated tribes in Africa and Southeast Asia exhibit the highest frequency of incidence for any given population, a defective enzyme can be found in as many as one in four people among these populations (Beutler 1994; Scriver 1995).

G6PD DEFICIENCY AND NATURAL SELECTION

Various processes such as selection, mutation, migration and genetic drift are known to determine the frequency

of genetic disease in human populations, but so far it has proved almost impossible to decide to what extent each is responsible for the presence of a particular genetic disease. Most red cell genetic defects in human populations are due to the exposure to malaria, a disease estimated to have arisen about 3000 years ago with the emergence of agriculture. Humans exhibit variable susceptibility to malaria infection, most of the resistance to this infection is either genetic or based upon previous exposure. The high gene frequency of G6PD deficiency in some ethnic groups implies that this gene confers or in the past conferred a selective advantage to those who inherited it. The distribution of the gene in tropical area in which *Plasmodium falciparum* malaria was common led to the suggestion that the advantage of G6PD deficiency might be that it provided resistance to infection with malaria (Beutler 1994; Flint 1993,).

Plasmodium falciparum malaria, the deadly form of malaria, has a life cycle that includes two subcycles: a vector cycle in an Anopheles mosquito and a human cycle that includes a liver stage and an obligatory erythrocytic stage. Genetic resistance appears to be limited the erythrocytic stage, there are no data concerning possible resistance during the hepatic stage of infection. Genetic resistance to *P. falciparum* malaria at the erythrocytic stage may involve one or more of the following mechanisms:

1. Inhibition of merozoite entry into the red cell
2. Impairment in intracellular growth of the parasite
3. Prevention of the erythrocyte lysis that occurs at the end of parasite maturation, which leads to release of merozoites into the bloodstream
4. Enhanced phagocytosis of parasite-infected red cells (Tiffert 2005; Yuthavong 1988).

Although all of the four types of human malaria *P. falciparum, ovale, vivax, and malariae* can cause selective pressure on the human population, this effect is strongest with *P. falciparum*, which is the only lethal variety, particularly in children under the age of five. This resistance is due to the fact that the parasite selectively infects red blood cells. In G6PD deficient red blood cells, an essential metabolite for the survival of the parasite is present in insufficient quantities. This is due to decreased activity of G6PD within these cells which ultimately leads to the death of the parasite. The most likely mechanism of malarial protection may be increased phagocytosis of G6PD-deficient erythrocytes containing the early ring-stage parasites. In the ring-stage parasite-infected cells, the level of reduced glutathione was lower in the G6PD-deficient cells compared with normal red cells, leading to membrane damage of deficient cells containing parasites that may be preferentially targeted for destruction (Cappadoro 1998; Luzzatto et al 1979; Scriver et al 1995).

The gene mutation that gives humans natural resistance to malaria is a striking example of how infectious disease can shape the human genome. Although mutations in the glucose-6-phosphate dehydrogenase (G6PD) gene result in several blood-related diseases in humans, they also confer resistance to malarial infection. This association between G6PD and malaria was supported by population genetic analyses of the G6PD locus, which indicated that these mutations may have recently risen in frequency in certain geographic regions as a result of positive selection. Different variations of the gene appear in the different areas and seem to have evolved independently of each other, likely as a response to selection resulting from malarial infection (Tishkoff et al 2001; Tripathy 2007).

ABOUT MALARIA PARASITE

Malaria is probably one of the oldest diseases known to mankind that has had profound impact on our history. It has been responsible for the decline of nations and crushing military defeats. For centuries it prevented any economic development in vast regions of the earth. It continues to be a huge social, economical and health problem, particularly in the tropical countries (Kakkilaya 2006).

EPIDEMIOLOGY

Although malaria was once widespread in North America and other temperate regions, the last major outbreak of malaria in North America occurred in the 1880s. The disease today occurs mostly in tropical and subtropical countries, particularly sub-Saharan Africa and Southeast Asia. According to the World Health Organization, malaria is prevalent in over 100 countries. Approximately 300 to 500 million cases of malaria and 700,000 to 2.7 million deaths occur annually worldwide, especially in children in tropical developing countries. *P. falciparum* predominates in tropical Africa, Southeast Asia, Oceania, Haiti, the Amazon basin of South America, and the Dominican Republic, while *P. vivax* is most prevalent in Central America, the Middle East, and India (Adam 2005; WHO 1989).

Malaria is an infectious disease caused by the parasite called Plasmodia. There are four identified species of this parasite causing human malaria, namely, *Plasmodium vivax, P. falciparum, P. ovale* and *P. malariae*. *Plasmodium falciparum* is the most common species in tropical areas and is transmitted primarily during the rainy season. This species is the most dangerous, accounting for half of all clinical cases of malaria and 90 percent of deaths from the disease.

Plasmodium vivax is the most widely distributed parasite, existing in temperate as well as tropical climates. *Plasmodium malariae* can also be found in temperate and tropical climates but is less common than *Plasmodium vivax*. *Plasmodium ovale* is a relatively rare parasite, restricted to tropical climates and found primarily in eastern Africa (Adam 2005; Greenwood 2005).

The majority of malaria infection is caused by either *P. falciparum* or *P. vivax*, and most malaria-associated deaths are due to *P. falciparum*. Mixed infection due to more than one malarial species occurs in five to seven percent of infections. Malaria infection has been increasing over recent years due to a combination of factors including:

1. Increasing resistance of malarial parasites to chemotherapy
2. Increasing resistance of the Anopheles mosquito vector to insecticides
3. Ecologic and climate changes
4. Increased international travel to malaria-endemic areas (Greenwood 2005).

MALARIA TRANSMISSION

The female anopheles mosquito is the vector for human malaria. Malaria transmission begins when a female mosquito bites a human already infected with the malaria parasite. The mosquito ingests blood containing immature male and female gametes of the malaria parasite. The mosquito phase of the malaria parasite's life cycle is normally completed in 10 to 14 days. This development process occurs more slowly in areas with cooler temperatures. The female anopheles mosquito bites man between 5 pm and 7 am, with maximum intensity at midnight. The risk of malaria transmission in endemic countries is increased in rural areas and varies seasonally in many locations, transmission is highest at the end of the rainy season. Transmission decreases at altitudes about 2,000 meters. Humans are the only important malarial reservoir, and there are no free-living plasmodia. Some 60 species of this mosquito have been identified as vectors for malaria, and their distribution varies from country to country (Adam 2005; Strickland 1991).

Other modes of transmission

Rarely malaria can spread by the inoculation of blood from an infected person to a healthy person. In this type of malaria, asexual forms are directly inoculated into the blood and pre-erythrocytic development of the parasite in the liver does not occur. Therefore, this type of malaria has a shorter incubation period and relapses do not occur.

1. Blood transfusion (Transfusion malaria): This is fairly common in endemic areas. Most infections occur in cases of transfusion of blood stored for less than 5 days and it is rare in transfusions of blood stored for more than 2 weeks. Frozen plasma is not known to transmit malaria. Donor blood can be tested with indirect fluorescent antibody test or ELISA, and direct examination of the blood for the parasite may not
2. Mother to the growing fetus (Congenital malaria): Intrauterine transmission of infection from mother to child is well documented. Placenta becomes heavily infested with the parasites. Congenital malaria is more common in first pregnancy, among non - immune populations.
3. Needle stick injury: Accidental transmission can occur among drug addicts who share syringes and needles. The defect is known to provide partial protection against malaria, by providing defective environment in the affected red cells (Kakkilaya 2006; Strickland 1991).

MALARIA SYMPTOM

The fever that characterizes malaria develops when merozoites invade and destroy red blood cells.The body responds by producing fever, an immune response that speeds up other immune defenses to fight the foreign invaders in the blood. The fever usually occurs in intermittent episodes. An episode begins with sudden, violent chills, soon followed by an intense fever and then profuse sweating that brings the patient's temperature down again. Upon initial infection with the malaria parasite, the episodes of fever frequently last 12 hours and usually leave an individual exhausted and bedridden. Repeated infections with the malaria parasite can lead to severe anemia, a decrease in the concentration of red blood cells in the bloodstream. The malaria parasite consumes or renders unusable the proteins and other vital components of the patient's red cells. The pattern of intermittent fever and other symptoms in malaria varies depending on which species of *Plasmodium* is responsible for the infection. Infections caused by *Plasmodium falciparum*, *Plasmodium vivax*, and *Plasmodium ovale* typically produce fever approximately every 48 hours, or every first and third day. Infections caused by *Plasmodium malariae* produce fever every 72 hours, or every fourth day (Adam 2005; Greenwood 2005; Strickland 1991).

Infections caused by *Plasmodium falciparum* are marked by their severity and high fatality rate. This type of malaria can also cause severe headaches,

convulsions, and delirium. The infection sometimes develops into cerebral malaria, in which red blood cells infected with parasites attach to tiny blood vessels in the brain, causing inflammation and blocking the flow of blood and oxygen. In *Plasmodium vivax* and *Plasmodium ovale* infections, some merozoites can remain dormant in the liver for three months to five years. These merozoites periodically enter the bloodstream, triggering malaria relapses (Adam 2005; Strickland 1991).

TREATMENT OF MALARIA

Treatment of malaria involves supportive measures as well as specific antimalarial drugs. Patients with *P. vivax*, *P. ovale* or *P. malariae* infection can often be treated as outpatients. It is important that patients with *P. falciparum* malaria have their treatment commenced without delay, and they should generally be admitted to the hospital so that they can be observed for any evidence of complications. This is particularly true for patients diagnosed with falciparum malaria in non-endemic countries such as the United States (Strickland 1991). Individuals who live in a malaria-endemic country with frequent exposure to the disease develop partial immunity to infection, but this immunity wanes. Thus, these semi-immune individuals who have migrated to non-endemic countries are at similar risk of complications and death due to malaria as short-term travelers and expatriates if they travel back to endemic regions (Fernandez 2006; Greenwood 2005).

In non-endemic western countries, the diagnosis of malaria should be confirmed before treatment is commenced because the non-specific symptoms can mimic many other types of infections. In endemic countries where diagnostic facilities are not always readily available, empiric therapy for malaria may be necessary. Studies have shown that delays in the recognition and treatment of malaria are directly associated with increases in morbidity and mortality. The case fatality rate of imported *P. falciparum* malaria varies from 0.6 to 3.8 percent. Progression from asymptomatic infection to death can occur in 36 to 48 hours Among United States civilians who die of malaria, the diagnosis is initially missed in approximately 40 percent (Fernandez 2006; Strickland 1991).

PROTECTION AGAINST MALARIA IN THE G6PD DEFICIENCY

Evidence for a protective affect of G6PD deficiency against *Plasmodium falciparum* comes from both epidemiological correlation and in vitro studies. The studies showed that G6PD-deficient cells are protective against malaria is come from Ruwende et al, based on two large case-control studies of over 2,000 African children, concluded that the common African form of G6PD deficiency (G6PD A-) is associated with a 46% reduction in risk of severe malaria for female heterozygous and 58% for male hemizygous individuals. Thus both homozygous female and hemizygous males should also be protected (Ruwende et al 1995). Luzzatto et al found that parasitization was 2-80 times greater in non-deficient than in deficient cells (Luzzatto et al 1979). Protection against severe malaria conferred by G6PD deficiency has been demonstrated in children (Ruwende et al 1995) and in heterozygous pregnant women (Mockenhaupt et al 2003).

Early studies of females heterozygous for G6PD deficiency showed higher levels of malaria parasites in normal compared to G6PD-deficient red cells. Although malaria invasion of the cells was similar, the growth of the parasites in the G6PD-deficient cells was inhibited (Roth et al 1983). In vitro studies comparing the growth of parasites in severe G6PD-deficient red blood cells with growth in normal cells showed that inhibition of parasite growth in G6PD deficient cells could be detected by the third day, although malaria invasion of the cells was similar (Tantular 1997). Different results were found, however, in studies of the Mediterranean variant, where no differences in malaria invasion and growth of G6PD-deficient and non-deficient cells. Similarly, conflicting results have been reported about whether hemizygous G6PD-deficient males have protection against malaria (Ruwende et al 1995; Tishkoff et al 2001).

Tantular et al examined the effects of two chemicals, Bathocuproine disulphonate and cysteine on the in vitro growth of *Plasmodium falciparum* in G6PD-deficient red blood cells. Bathocuproine disulphonate (BCS), a copper chelator, as well as cysteine have been shown to synergistically stimulate the in vitro growth of various mammalian cells and Trypanosoma under oxygenated conditions. Based on this study showed that addition of BCS and cysteine synergistically enhanced the growth of the parasites in G6PD deficient red blood cells to the same level as in normal red blood cells. However, BCS or cysteine alone had no stimulatory effect (Tantular et al 2003).

The mechanism of adaptation of the parasite against G6PD deficiency has been demonstrated by Roth and Schulman. The parasites produce their own G6PD to adapt against the G6PD deficiency of the host red blood cells. They reported that adaptation of the parasite to the

G6PD Mediterranean deficient red cells is minimal compared to those with G6PD A- deficiency (Roth 1988). Luzzatto et al reported that though the G6PD-normal and G6PD Mediterranean deficient were infected at the same rate by falciparum, parasite growth was reduced by 40% in the deficient cells by the second schizogonic cycle .They further reported that the falciparum parasite which have undergone several cycles in the G6PD-deficient cells infected both the normal and deficient cells at similar rates, thus providing evidence for adaptation of the parasite against G6PD deficiency in the host cells (Luzzatto et al 1983). Based on a series of experiments they further concluded that the parasite adapts to the G6PD deficiency in hemizygous males and homozygous females after a few cycles and thus the initial protection enjoyed by the G6PD-deficient cell against the parasite is negated by the parasite (Usanga et al 1985).

The geographic distribution of populations with a high gene frequency of deficient suggest that G6PD deficient also one of the polymorphisms that confers resistance to infection with falciparum malaria. However, the mechanism of protection is unknown. Epidemiologic investigations indicate that the highest gene frequencies are present among population living in low lying areas in which the incidence of malaria is high (Beutler 1994). Tantular et al reported based on their field survey on G6PD deficiency by single-step screening method for detection of glucose-6-phosphate dehydrogenase deficiency in combination with a rapid diagnosis of malaria by an Acridine Orange staining (AO) method at some villages in Halmahera island, Maluku Province, Indonesia. The results showed that there was no significant difference in the prevalence *of P. falciparum* and *P. vivax* infections between G6PD normal and severe deficient group. There seemed to be no correlation between malaria protection and severe G6PD deficiency (Tantular et al 1999). In the population-based studies at Maumere, Flores island, Jalloh et al explored the possibility of such protection by comparing malaria prevalence in G6PD-normal subjects to those with G6PD deficiency, and no significant association between G6PD deficiency and malaria prevalence was detected (Jalloh et al 2004).

G6PD VARIANTS

Information about the prevalence of specific variants is lacking in many populations. Such information is necessary for the implementation of anti-malaria program, especially in malarial endemic areas. Therefore, comprehensive studies of G6PD gene are recommended among the populations in the malarial endemic areas. In Indonesia, numerous ethnic groups are distributing, and thus many G6PD variants might be included. From G6PD screening results showed that the frequency of G6PD deficiency in Indonesia was highly variable. Results on molecular analysis suggested that the distribution of the G6PD mutations vary with geographical areas and/or ethnic groups. Genetic analysis of G6PD deficiency may help to clarify the genetic relationship among populations and to infer their historical traces (Iwai et al 2001; Scriver 1995,).

Prevalence of G6PD deficiency in Indonesia

Prevalence of G6PD deficiency in the Indonesia community was reported from North Sumatra, at Nias island , at Asahan and at Medan with prevalence of 3,9% (Matsuoka et al 1986). Sofro reported the prevalence rate of G6PD deficiency at Sasak 18,4%, at Bima 12% and at Irian Barat 8% (Sofro 1986). The Javanese population at Middle of Java showed prevalence of 14% (Soemantri et al 1995). We have reported the frequency of G6PD deficiency at some villages in the Halmahera island was varies between 1,1-14,1 % in different villages. Most of residents living at some villages had transmigrated from other small islands after natural disasters such as volcano's exposure or scanty of drinking water. It may be probable that the frequency of G6PD deficient alleles is higher at nearly isolated environment of these small islands (Tantular et al 1999). From G6PD mass screening in General Hospital at Surabaya, East of Java showed G6PD prevalence 3% (Damanik 2001). We have also documented prevalence rates of G6PD deficiency among Sikkanese students from eight primary schools at Sikka district, Flores island was values for the schools ranged from 0% to 14.8% (Jalloh et al 2004).

Novel variants reported from Indonesia

A number of G6PD variants novel to Indonesia populations have been reported based on biochemical characterization of G6PD. We have reported five mixed variants (G6PD Gaohe, G6PD Chatham, G6PD Surabaya, G6PD Canton and G6PD Kaiping) in the ethnic Chinese at Surabaya ,East Java. We have found a new variant, a novel mutation, 1291 G-A from a 18 year-old Chinese male, who visited a hospital in Surabaya due to an episode of acute hemolytic attack without chronic hemolysis.We name it G6PD Surabaya (Iwai et al 2001).

Previous survey at Halmahera and Buru islands (Amboinese) at Maluku, we also have found 11 cases of G6PD Vanua Lava (383T>C), which was previously found in Vanuatu in the Western Pacific. This suggests the genetic relationship between the populations in Maluku and the islanders in Vanuatu. Most of the inhabitants in Vanuatu are Melanesians. The dominant

G6PD mutation in Melanesians is reported to be G6PD Union (Hirono et al 1995). It is somewhat surprising that we found no G6PD Union in Amboinese despite their possible connection with Melanesians. A possible reason for the lack of G6PD Union in Maluku population is the genetic drift caused by their long-term isolation (Iwai et al 2001).

Another studies at Flores Island, which was a part of the Sunda Archipelago, we have genetically analyzed the G6PD genome in randomly, selected 11 persons including hemi & heterozygous deficient persons in the Sikka population on Flores Island. From eleven G6PD-deficient persons variant has been found five different G6PD variants; one case of G6PD Vanua Lava , five cases of G6PD Coimbra ,one case of G6PD Viangchan , one case of G6PD Chatham and three cases of G6PD Kaiping Distribution of G6PD variants was quite unique in this small population. This suggests that people of Flores Island are derived from various ancestries (Matsuoka et al 2003).

Further field surveys for malaria and G6PD deficiency were conducted at Sikka district and at Ende district, in the eastern part of Flores island. From 3 ethnic groups (Sikka, Ende, Bajo) were detected 55 G6PD deficient and we have identified 7 G6PD variants. In the Sikka, G6PD Kaiping was the highest followed by G6PD Chatham, G6PD Coimbra and G6PD Vanua Lava, whereas in the Ende, G6PD Vanua Lava, G6PD Kaiping, G6PD Chinese-5 and G6PD Chatham were identified. Three deficient cases was detected from a Bajo family, and we have found a novel variation from a 7-year-old boy at a Bajo village near Maumere. This new type was categorized as Class II (mild type), and given a name of G6PD Bajo Maumere (844G>T). n the Bajo population, G6PD Viangchan was the most common as seen in continent Southeast Asian population, followed by the new variant, G6PD Vanua Lava and G6PD Coimbra (Kawamoto et al 2006).

REFERENCES

Beutler, E, Yeh, M, Fairbanks, VF 1962, 'The normal human female as a mosaic of X-chromosome activity, Studies using the gene for G6PD deficiency as a marker', *Proc Natl Acad Sci USA*, vol. 48, pp. 9-16.

Beutler, E 1994, 'G6PD deficiency', *Blood*, vol. 84, pp. 3613-3636.

Beutler, E 2001, 'Glucose-6-phosphate dehydrogenase deficiency and other red cell enzyme abnormalities', in E Beutler, MA, Lichtman, BS Coller, et al. (eds), *Williams Hematology*, 6th ed, pp. 527–547.

Cappadoro, M, Giribaldi, G, O'Brien, E et al, 1998, 'Early phagocytosis of glucose-6-phosphate dehydrogenase (G6PD)-deficient erythrocytes parasitized by Plasmodium falciparum may explain malaria protection in G6PD deficiency', *Blood* , vol. 92, pp. 2527- 2534.

Damanik, SM, Ugrasena, IDG, Harianto, A et al. 2001, 'Mass screening of G6PD in Dr Soetomo General Hospital, Folia Medica Indonesiana, vol. 27, no. 2, pp. 63-65.

Fernandez, MC, Bobb, BS, Malaria 2006. Retrieved October 8, 2007, from http://www.emedicine.com/ emerg/topic305.htm

Greenwood, BM, Bojang, K, Whitty, CJ, Targett, GA 2005, 'Malaria', *Lancet*, vol. 365 (9469), pp. 1487-98.

Hirono, A, Ishii, A, Kere, N, Fujii, H et al, 1995, 'Molecular analysis of glucose-6-phosphate dehydrogenase variants in the Solomon Islands', *Am J Hum Genet*, vol. 56, pp.1243-1245.

Iwai, K, Hirono, A, Matsuoka, H, Tantular, IS et al, 2001, 'Distribution of glucose-6-hosphate dehydrogenase mutations in Southeast Asia', *Human Genetics*, vol. 108, pp. 445-449.

Jalloh, A, Tantular, IS, Pusarawati, S, Kawamoto, F et al. 2004, 'Rapid epidemiologic assessment of glucose-6-phosphate dehydrogenase deficiency in malaria-endemic areas in Southeast Asia using a novel diagnostic kit', *Trop Med Int Health*, vol. 9, pp. 615-623.

Kakkilaya, B S, 2006, *G6PD Def*, Malaria Web Site. Retrieved September 18, 2007, from http://www.malariasite.com/malaria/G6PD.htm.

Kawamoto, F, Matsuoka, T, Kanbe, Tantular, IS et al. 2006, 'Futher investigations of Glucose-6-phosphate dehydrogenase variants in Flores island, eastern Indonesia', J Human Genet, vol. 51, no. 11, pp. 952-957.

Luzzatto, L 1979, 'Genetic of red cells and susceptibility to malaria', *Blood*, vol. 54, no. 5, pp. 961-976.

Luzzatto, L, Sodeinde, O, Martini, G 1983, 'Genetic variation in the host and adaptive phenomena in *Plasmodium falciparum* infection', in *Malaria and the Red Cell*, Ciba Found Symp, vol. 94, pp. 159–173.

Luzzatto & Mehta 1995, 'Glucose 6-Phosphate dehydrogenase deficiency', *The Metabolic and Molecular Bases of Inherited Disease*, pp. 3367-3388.

Matsuoka, H, Ishii, A, Panjaitan, W, Sudiranto, R 1986, 'Malaria and glucose-6-phosphate dehydrogenase deficiency in North Sumatra, Indonesia', *Southeast Asian Journal of Tropical Medicine and Public Health*, vol. 17, pp. 530-536.

Matsuoka, H, Arai, M, Tantular IS, Kawamoto, F et al, 2003, 'Five different glucose-6-phosphate dehydrogenase (G6PD) variants found amoung 11 G6PD-deficient persons in Flores Island, Indonesia', *J Hum Genet*, vol. 48, pp. 541-544.

Mockenhaupt, F, Mandelkow, J, Till, H et al. 2003, 'Reduced prevalence of plasmodium falciparum infection and of concomitant anaemia in pregnant women with herozygous G6PD deficency', *Tropical Medicine and International Health*, vol. 8, pp**.** 118-124.

Prchal, JT and Gregg, XT 2005, 'Red cell enzymopathies', in R Hoffman, E Benz (eds), *Hematology: Basic Principles and Practice*, 4th edn, pp. 653–659.

Roth, EF, Raventos, SC, Rinaldi, A, Nagel RL, 1983, 'Glucose-6-phosphate dehydrogenase deficiency inhibits in vitro growth of *Plasmodium falciparum*', *Proc Natl Acad Sci USA*, vol. 80, pp. 298–299.

Roth, EF Jr and Schulman, S 1988, 'The adaptation of Plasmodium. falciparum to oxidative stress in G6PD deficient human erythrocytes', *Br J Haematol*, vol. 70, pp. 363-367.

Ruwende, C, Khoo, S, Snow, R et al. 1995, 'Natural selection of hemi- and heterozygotes for G6PD deficiency in Africa by resistance to severe malaria', *Nature*, vol. 376, pp. 246–249.

Scriver, CR et al. 1995, 'Glucose-6-Phosphate Dehydrogenase deficiency', in *The Metabolic and Molecular Bases of Inherited Disease*, 7th edn, McGraw- Hill Inc., pp. 3367-3398.

Soemantri, AG, Saha, S, Saha, N, Tay, JSH 1995, 'Molecular variants of red cell Glucose-6-phosphate dehydrogenase deficiency in Central Java, Indonesia', *Hum Hered*, vol. 45, pp. 346-350.

Sofro, ASM, 1986, 'Ovalocytosis in Indonesia: distribution and its relation to the malarial-hypothesis', *Medika*, vol. 12, pp. 954-958.

Strickland, GT 1991, 'Malaria', in GT Strickland (ed), *Hunter's Tropical Medicine*, 7th edn, WB Saunders Company, Philadelphia, World Health Organization, pp. 586-617.

Tantular, I, 1997, An in vitro study of *Plasmodium falciparum* growth within the red blood cell with severe glucosa – 6 – Phosphate dehydrogenase deficiency, National Congress of Biochemistry and Molecular Biology association, Surabaya, July 5-7.

Tantular, IS, Iwai,K, Matsuoka,H, Kawamoto,F et al, 1999, 'Field trials of rapid test for G6PD deficiency in combination with a rapid diagnosis of malaria', *Trop Med and International Health*, vol. 4, no. 4, pp. 245-250.

Tantular, IS, Jalloh, A, Dachlan,YP, Kawamoto, F ,et al. 2003, 'Synergistic enhancement of a copper chelator, BCS and cysteine on invitro growth of *P.falciparum* in G6PD deficient erytrocytes', *Southeast Asian J Trop Med Public Health*, vol. 34, no. 2, pp. 301-309.

Tiffert, T, Lew, VL, Ginsburg, H, Krugliak, M et al, 2005, 'The hydration state of human red blood cells and their susceptibility to invasion by Plasmodium falciparum', *Blood*, vol. 15, no. 105(12), pp. 4853-4860.

Tishkoff, ET, 2001, *The Archaeology and Genetics of Malaria Resistance*, retrieved September 18, 2007, from http://www.unisci.com/stories/20012/0626011.htm.

Tripathy, V and Reddy, BM 2007, 'Present status of understanding on the G6PD deficiency and natural selection', *J Postgrad Med*, vol. 53, pp. 193-202.

Usanga, EA, Luzzatto, L, 1985, 'Adaptation of Plasmodium falciparum to glucose 6-phosphate dehydrogenase-deficient host red cells by production of parasite-encoded enzyme', *Nature*, vol. 313, pp. 793-795.

WHO, 1989, 'Working Group Glucose-6-phosphate dehydrogenase deficiency', *Bull WHO*, vol. 67, pp. 601.

Yuthavong, Y, Butthep, P, Bunyaratvej, A, Fucharoen, S et al. 1988, 'Impaired parasite growth and increased susceptibility to phagocytosis of Plasmodium falciparum infected alpha-thalassemia or hemoglobin constant spring red blood cells', *Am J Clin Pathol*, vol. 89, no. 4, pp. 521-525.