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Glucose-6-phosphate dehydrogenase deficiency in northern Vietnam

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Summary Glucose-6-phosphate dehydrogenase (G6PD) deficiency was evaluated in 1676 schoolboys in northern Vietnam. 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. Prevalences among ethnic groups living in the foothills, the breeding area of the main malaria vector *Anopheles minimus*, ranged from 9.7% to 31%. These findings support the hypothesis of a selective advantage of the trait in *Plasmodium falciparum*-endemic areas.

keywords G6PD deficiency, malaria transmission, selective advantage, Vietnam

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Introduction

Deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD), a hereditary X-chromosome-linked defect, is considered the most common clinically significant enzyme defect in human biology (Beutler 1994). Over 200 million people are estimated to have this deficiency, but its distribution strongly differs from one ethnic group to another. Frequencies of G6PD deficiency up to 70% have been found among Kurdish Jews (Oppenheim *et al*. 1993). In East Asia too, high deficiency frequencies are encountered in some areas (Du *et al*. 1988; Beutler 1994). The main clinical manifestations are neonatal jaundice (sometimes leading to kernicterus) and haemolysis.

G6PD deficiency was first discovered following investigations of haemolytic anaemia after ingestion of the 8-aminoquinoline antimalarial drug primaquine (Beutler 1980). Meanwhile it has been shown that such induced haemolysis in G6PD-deficient individuals can be triggered by several other drugs, food substances and infections. The mechanism of haemolysis and the severity vary greatly.

Considering its geographical distribution, it was suggested that G6PD deficiency confers resistance to *falciparum* malaria (Allison 1960), since the highest frequencies were found among populations living in endemic areas. However, this relationship has also been questioned (Beutler 1994).

Knowledge of G6PD deficiency is still limited in Vietnam, but prevalences of up to 20% have been reported (Son *et al*. 1978). *Plasmodium falciparum* infection is considered a major health problem nationwide. In northern Vietnam, malaria is a problem in the foothills, where the main vector, *Anopheles minimus*, breeds in little streams under 1000 m of altitude (Harrison 1980; Meek 1995). These foothills are mainly populated by ethnic minorities who moved there from the plains after the Kinh (the Vietnamese) descended from China over a 1000-years ago. In the coastal plains and the delta of the Red River, the traditional habitat of the Kinh, malaria transmission is rare. At altitudes above 1000 m, the traditional habitat of the Mong group, malaria transmission has also been uncommon. Considering that certain ethnic groups have been living in malaria-endemic areas for a long time and others outside these areas, we explored the possible link between G6PD deficiency and malaria transmission in the past.

Materials and methods

The study was conducted between October 1996 and

February 1997. Two districts in each of four provinces of northern Vietnam (Thanh Hoa, Son La, Ha Giang, and Hoa Binh) were selected. In each district, 4 communes were chosen at random. In the primary schools of each selected commune, a minimum of 50 school boys were enrolled by systematic sampling. The ethnic origin of each subject was ascertained. Only one subject per household was recruited for the study. From each individual, a thick film was taken and 2 × 100 µl capillary blood collected in heparin.

A qualitative visual fluorescent test was used to detect G6PD deficient samples. Test kits (procedure no. 203) were obtained from Sigma Diagnostics®. Within 6 h from collection, 10 µl of blood was allowed to incubate with glucose-6-phosphate and nicotine adenine dinucleotide phosphate (NADP) in a 37 °C water bath. Drops of the mixture were removed at time zero, after 5 min and after 10 min, and placed on Whatman no. 1 filter paper. When dry, the spots were visually inspected under a long-wave ultraviolet light lamp. Samples were reassessed some days later, after being stored in a plastic bag with silica gel. Control samples with normal G6PD (G 6888) and with G6PD deficiency (G 5888) were included daily. Only grossly G6PD deficient samples were considered positive.

Blood collected in the second tube was spread on Whatman paper no. 3 and tested by Indirect Fluorescence Agglutination (IFAT) in Hanoi for *P. falciparum* antibodies.

Results

A total of 1676 schoolboys – between 5 and 15 years old (median 11 years) – were enrolled. They belonged to 16 ethnic groups, of which six (the Dao, the Kinh, the Mong, the Muong, the Thai and the Tho) provided sufficient data to be analysed separately.

All blood slides were negative for *Plasmodium* parasites. G6PD deficiency prevalences in these six ethnic groups ranged from 0.3% to 31% (Table 1). For the remaining 77 individuals belonging to 10 ethnic groups, a crude 16.9% G6PD deficiency prevalence was found. No statistically significant differences in G6PD deficiency prevalence were observed within any ethnic group for subpopulations presently living in different districts, except for the Muong.

Table 1 Prevalence of G6PD deficiency by ethnic group in schoolboys of 8 districts in northern Vietnam

Traditional habitat	Ethnic group	District	Province†	Current habitat	*n*	G6PD deficiency		IFAT > 80	
						%	(95% C.I.)	%	(95% C.I.)
Plains	Kinh	Nga Son	1	Plains	200	0.5		0	
		Kim Boi	4	Foothills	2	0		0	
		Total			202	0.5	(0–1.5)		
Foothills	Dao	Meo Vac	3	Foothills	5	0		0	
		Bac Me	3	Foothills	160	10.0		12.5	(9.8–15.2)
		Total			165	9.7	(5.2–14.2)		
	Muong	Kim Boi	4	Foothills	209	34.3	(28.0–40.8)	3.3	(1.9–4.8)
		Nhu Xuan	1	Foothills	52	17.3	(7.0–27.6)	0	
		Total			261	31.0*			
	Thai	Mai Chui	4	Foothills	211	20.4		0.5	(0–1.0)
		Mai Son	2	Foothills	165	15.2		3.0	(1.6–4.4)
		Muong La	2	Foothills	144	23.6		9.7	(7.3–12.1)
		Nhu Xuan	1	Foothills	55	16.4		1.8	(0.7–2.9)
		Total			575	19.3	(16.1–22.5)		
	Tho	Nhu Xuan	1	Foothills	106	22.6	(14.6–30.6)	0	
Mountain Top	Mong	Meo Vac	3	Mountain top	168	0.6		1.2	(0.3–2.1)
		Bac Me	3	Mountain top	25	0		16.0	(13.0–19.0)
		Mai Son	2	Mountain top	49	0		12.2	(9.6–14.9)
		Muong La	2	Mountain top	48	0		12.5	(9.8–15.2)
		Total			290	0.3	(0–1.0)		
		Total			1599	14.6		4.3	

†Provinces: 1, Thanh Hoa; 2, Son La; 3, Ha Giang; 4, Hoa Binh. *No summary C.I. calculated because significant difference between districts.

The prevalence detected among Muong schoolboys of Kim Boi district was 34% compared to 17% for the Muong schoolboys living in Nhu Xuan district ($\chi^2 = 4.94$, $P = 0.026$). Within the same district significant differences were observed between ethnic groups traditionally living in different habitats: between the Mong and the Thai in Muong La District (0% and 23.6%) and in Mai Son District (0% and 15%), and between the Mong and the Dao in Bac Me district (0% and 10%). An analysis of the G6PD deficiency contrasting ethnic groups living in previously highly malaria-endemic areas (foothills) with those living in areas of low endemicity (mountain top, plains) and stratified by current province of residence yielded a Mantel-Haenszel odds ratio of 52 (CI 95% 11.3–276).

Prevalence of IFAT titres > 1/80 against *P. falciparum* antigen ranged from 0% (Nga Son district) to 10.4% (Muong La district) and 13.0% (Bac Me district). No statistically significant differences in prevalences of IFAT titres were found among different ethnical groups living in the same districts, except for the district of Mai Son (Son La province) were titres among Mong boys were higher than among Thai boys ($\chi^2 = 5.21$, $P = 0.02$). Differences of prevalences of IFAT titres for subjects belonging to identical ethnical groups currently living in different districts were statistically significant for the Mong ($\chi^2 = 17.71$, $P = 0.00005$) and the Thai ($\chi^2 = 21.84$, $P = 0.00007$).

Discussion

Absence of parasitaemia reflects the success of the malaria control programme in northern Vietnam, which has been better resourced after a dramatic increase of malaria at the beginning of the 1990s (Verlé *et al*. 1999). IFAT titres reflecting contact with *P. falciparum* in the recent past were highest in the remote provinces Son La and Ha Giang, regardless of ethnic groups.

The recorded prevalences of G6PD deficiency are in line with the hypothesis that this trait has a selective advantage in areas with past prevalent *P. falciparum* (Allison 1960). Prevalence of G6PD deficiency was high among all ethnic groups that had traditionally lived in the foothills where malaria transmission used to be high. The highest prevalence of G6PD deficiency (34%) was observed among the Muong living in Kim Boi district, Hoa Binh province. This area features in an old Vietnamese proverb in relation to malaria: 'Take friends to a party to eat sticky rice, take your enemies to Kim Boi so that they get malaria'. The Thai arrived in Vietnam in the second millennium BC and settled in the foothills. The Muong are related to the Kinh but fled to the foothills around the first century AD after an invasion by the Chinese. The Tho are related to the Muong and the Kinh and fled from the plains to the foothills in the 17th century. The Dao arrived from China in Vietnam between the 13th and the 19th century and have lived both in the foothills and at higher altitudes (Van *et al*. 1993).

Hardly any G6PD deficiency was observed among the Kinh and Mong ethnic groups, who traditionally have been living outside the malaria transmission areas. Kinh are known to have been reluctant to reside in the foothill areas. The Mong people, who are found all over Indo-China and migrated to Vietnam less than 200 years ago (Van *et al*. 1993), tend to settle in mountainous areas at altitudes above 1000 m. In 1951, Norman Lewis observed that Mong people (also called Meo) fear to go down the mountain because they are afraid to get fever, and that if they descend, they prefer to sleep in their own villages at night (Lewis 1951). Strikingly, subjects of the Mong group were among those with the highest percentage of positive IFAT titres. Their residences remain at the mountain tops, so these results may indicate changing habits.

The test we used is now recommended for screening, but it is known that in subjects with anaemia and resulting reticulocytosis, with leucocytosis or with thrombocytosis, the assay may not be reliable and provide false-negative results. Therefore the true prevalence of hemizygous males is probably higher than observed in this study.

G6PD variants have been classified as Class 1, hereditary nonspherocytic haemolytic anaemia; Class 2, severe deficiency; Class 3, mild deficiency and Class 4, nondeficient variant (Betke *et al*. 1967). No information is available about the variant(s) of G6PD deficiency in Vietnam. Considering the variants in neighbouring countries (Beutler 1994), prevalence of a severe form can be expected. In Kim Boi district, were the highest prevalence of G6PD deficiency was found, the observation of black urine is not uncommon. Between 1993, and 1995, 16 patients were hospitalized in Kim Boi District hospital for Black Water Fever and several families are known for a history of black water in one or more of their members (Tinh, personal communication). Black Water Fever associated with G6PD deficiency has also been reported (Tran *et al*. 1996) in other areas of Vietnam.

Systematic use of drugs known to induce haemolysis in G6PD-deficient subjects should be questioned. The most obvious example is primaquine, widely used in northern Vietnam against malaria (World Health Organization 1992). While the G6PD variant remains unknown, primaquine should be administered only if follow-up can be assured.

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