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## SHORT REPORT: SEVERE *PLASMODIUM FALCIPARUM* MALARIA IN CAMEROON: ASSOCIATED WITH THE GLUTATHIONE S-TRANSFERASE M1 NULL GENOTYPE

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**Abstract.** Glutathione S-transferases (GST) are a family of enzymes involved in phase-II detoxification of endogenous and xenobiotic compounds. Polymorphisms in GST genes have been associated with susceptibility to different diseases. In this study we determined the frequencies of polymorphisms in *GSTM1*, *GSTT1*, and *GSTP1* in DNA of 138 children from Cameroon, presenting with uncomplicated malaria ( $N = 19$ ), malaria with minor complications ( $N = 81$ ), or severe malaria ( $N = 38$ ). Analyses of *GSTM1* and *GSTT1* were performed using PCR-multiplex procedure, while *GSTP1* was done by PCR-RFLP. Subjects presenting with malaria with complications were found more often of the *GSTM1*-null genotype (58–64%) as compared with those with uncomplicated malaria (32%), a difference that was statistically significant. We conclude that the *GSTM1*-null genotype is associated with malaria with complications.

In severe malaria, release of oxygen free radicals and a variety of pro-inflammatory cytokines can lead to increased vascular permeability, leakage of colloid from intravascular space, pathologic vasodilation, and myocardial depression with ultimate hypovolemia and impaired organ perfusion. In children with malaria, lipid peroxidation in both blood plasma and erythrocytes is increased, while erythrocytic antioxidants such as glutathione (GSH), catalase, and tocopherol were shown to be lower in patients with malaria than in control subjects.<sup>1</sup> The intracellular parasite digests hemoglobin, thereby releasing free ferroprotoporphyrin or heme, which in the presence of oxygen oxidizes to ferriprotoporphyrin or hemin. This process produces superoxide, which decomposes into  $H_2O_2$  and  $O_2$ . Antimalarials like chloroquine and amodiaquine inhibit this reaction, thus building up a toxic concentration of ferro/ferriprotoporphyrin (FP) leading to parasite death.<sup>2,3</sup> Indeed, several studies have suggested that an increase in oxidative stress in parasitized red blood cells (RBCs) offers an advantage to parasite clearance rather than exacerbating the pathology.<sup>2-4</sup> Glucose-6-phosphate dehydrogenase (G6PD)-deficient individuals for instance, are partially protected against malaria. G6PD is essential for NADPH production, which reduces oxidized glutathione.

Glutathione as an antioxidant is required for growth of the parasite<sup>2,5</sup> but also for maintenance of the redox state of the host cell. Altered redox metabolism of the host cells, particularly the endothelial cells, may exacerbate disease complications, for example by enhancing endothelial damage, brain inflammation, and development of cerebral malaria. Several studies document escape of FP from the parasite's food vacuole with consequent damage to erythrocyte membrane through oxidation of proteins and lipids with eventual lysis of the host cell.<sup>6-8</sup> To maintain the integrity of the erythrocyte membrane, an efficient redox system is required. A number of mechanisms are involved in the removal of reactive oxygen species and maintenance of cellular redox state, among which

are the glutathione S-transferases (GSTs). GSTs are intracellular proteins that detoxify electrophilic compounds of endogenous and exogenous origin by conjugating them with GSH, predisposing them to export out of the cell by multi-drug resistance proteins. In addition GSTs are also known to exhibit glutathione peroxidase activity.

Human GSTs comprise a family of proteins, classified into 4 major classes: GST-alpha (*GSTA*), GST-mu (*GSTM*), GST-theta (*GSTT*), and GST-pi (*GSTP*). Many studies in both humans and laboratory animals have documented strong associations between impaired or deficient GST activity and various diseases connected to oxidative stress injury. Such diseases include lung cancer, acute leukemia, and many others.<sup>9-11</sup> However, there is no documented study on the association between genetic polymorphisms in GSTs associated with loss of GST enzyme activities and malaria. Since malaria pathology progresses with increasing oxidative stress, we hypothesize that impaired GST activity would increase severity of malaria pathology.

In this study we investigated the role of polymorphisms in *GSTM*, *GSTT*, and *GSTP* in DNA of 138 hospitalized children with mild, severe, and very severe malaria, which was collected in Yaoundé, Cameroon at the Department of Pediatrics of the Central Hospital and in the outpatient clinic of the Messa Dispensary. The patients studied were enrolled between February 1994 and October 1996.<sup>12</sup> Blood of children with symptomatic clinical malaria according to World Health Organization (WHO) standards, aged between 8 months and 14 years attending the outpatient clinic or admitted to the Central Hospital of Yaoundé, was collected after informed consent was obtained. Children with clinical malaria were admitted to the emergency room of the Department of Pediatrics and were examined by the clinical staff. All children underwent a complete physical examination and a thick smear was made. Then the severity of malaria was assessed according to WHO standards as mild/uncomplicated and severe malaria. The severe malaria group was subdivided into severe and very severe. Patients with coma (Blantyre score  $< 3$ <sup>13</sup>,  $N = 30$ ), respiratory distress (Marsh et al.<sup>14</sup>,  $N = 9$ ), repeated convulsions ( $> 1$ ,  $N = 10$ ), and/or severe anemia (Hb  $< 5$  g/dL,  $N = 1$ ) were categorized as very severe,

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TABLE 1  
Distribution of GST genotypes among malarial groups

|   | <i>GSTM1</i> |         | <i>GSTT1</i> |         | <i>GSTP1</i> |         |         |
|---|--------------|---------|--------------|---------|--------------|---------|---------|
|   | (+) (%)      | (-) (%) | (+) (%)      | (-) (%) | WT (%)       | HT (%)  | HV (%)  |
| Total number of subjects (n = 138)        | 58 (42)      | 80 (58) | 89 (64)      | 49 (36) | 42 (31)      | 64 (46) | 32 (23) |
| Uncomplicated malaria (n = 19)            | 13 (68)      | 6 (32)  | 15 (79)      | 4 (21)  | 4 (21)       | 11 (58) | 4 (21)  |
| Malaria with minor complications (n = 81) | 29 (36)      | 52 (64) | 47 (58)      | 34 (42) | 28 (35)      | 35 (43) | 18 (22) |
| Severe malaria (n = 38)                   | 16 (42)      | 22 (58) | 27 (71)      | 11 (29) | 10 (26)      | 18 (48) | 10 (26) |

WT = homozygous wild type, HT = heterozygous, HV = homozygous variant.

whereas those only suffering from impaired consciousness (Blantyre 3–4,  $N = 72$ ), hyperparasitemia ( $> 5\%$ ,  $N = 1$ ) and/or hyperpyrexia ( $> 40^\circ\text{C}$ ,  $N = 18$ ) were categorized as severe cases. The number of patients categorized in the groups of mild/uncomplicated, severe, and very severe malaria was 19 (average age 5.0; 10 female and 9 males), 81 (average age 4.8; 34 females, 44 males and 3 not recorded), and 38 (average age 3.5; 16 females, 20 males and 2 not recorded), respectively.

For *GSTM1* and *GSTT1* null polymorphisms, no gene products are detected. For *GSTM1*, primers were used as described by Bröckmoller et al.<sup>15</sup> Beta-globin primers were included in the reaction mixture as an internal positive control. A 650-bp PCR product was expected for *GSTM1* and absence of this fragment indicated 2 null alleles. For *GSTT1*, primers and methods were used following description by Pemble et al.<sup>16</sup>, yielding a 480-bp fragment, absence of which indicated the null genotype. The PCR was repeated for negative samples to confirm the results. Three *GSTP1* genotypes occur, resulting from a single point mutation that changes Ile-105 to Val, yielding wild-type, heterozygous, and homozygous mutant genotypes. The heterozygous form has reduced enzyme activity while the homozygous mutant enzyme showed even lower activity.<sup>17</sup> The polymorphism detection is based on an *Alw261* PCR-RFLP and PCR primers were designed as described by Watson et al.<sup>18</sup>

Results of the GST genotyping are depicted in Table 1. Overall, 42% of the patients were of the *GSTM1* (+) genotype, while 58% possessed the *GSTM1*-null (-) genotype. For *GSTT1*, 64% of the patients possessed a functioning allele (*GSTT1* (+)) while 36% had the *GSTT1*-null (-) genotype. For *GSTP1*, 31% of the samples were of the homozygous wild-type genotype, 46% heterozygous, and 23% of the homozygous mutant genotype. There was a significant difference in the distribution of the *GSTM1*-null genotypes between uncomplicated patients and those with malaria with minor complications and severe malaria ( $\chi^2 = 6.7$ ,  $P = 0.005$ ;  $\chi^2 = 3.5$ ,  $P = 0.031$ ). The *GSTT1* genotype in uncomplicated malaria and malaria with minor complications was just significantly different ( $\chi^2 = 2.9$ ,  $P = 0.045$ ), but no significant difference was observed for *GSTT1* in uncomplicated malaria versus severe malaria ( $\chi^2 = 0.4$ ,  $P = 0.26$ ). Also in the *GSTP1* polymorphisms no significant differences were detected. This study does not show any interaction between the GST polymorphisms and the different malaria groups.

These findings indicate that subjects lacking the *GSTM1* enzyme are significantly over-represented in the malaria with minor complications/severe malaria groups as compared with the uncomplicated falciparum malaria group, thus supporting our hypothesis of involvement of GSTs in malaria pathogenesis. Because *GSTM1* catalyzes conjugation of GSH to a wide

range of electrophiles, we suggest that *GSTM1* is important for prevention of malaria with minor complications by reduction of oxidative stress in malaria. Increase in oxidative stress within the food vacuole of *Plasmodium berghei* helps to kill this parasite as has been demonstrated by the action of chloroquine and amodiaquine.<sup>19</sup> On the other hand, oxidative stress may increase oxidative damage of erythrocyte membranes, reducing the deformability of the cells and ultimately inducing removal and massive destruction of erythrocytes by macrophages, leading to increase in severe anemia, occlusion of peripheral microvasculature, cerebral pathology (hypoxia), and cardiac injury observed in severe falciparum malaria.<sup>1</sup>

In a future study the need for measuring the allele frequency in a control group is necessary. When comparing the general distribution of the *GSTM1* null genotypes in our patient group, however, with the documented distribution in African populations, the percentage of *GSTM1*-null is very high (58%). Other studies document 22–38% of *GSTM1*-null individuals,<sup>20–22</sup> which is in line with our results in the uncomplicated malaria group (32%), indicating that individuals with the null phenotype might be more likely hospitalized when suffering from malaria. In Caucasians, however, *GSTM1* is lacking in 50–63% of the population.<sup>20,22</sup>

In conclusion, we found an association between the *GSTM1*-null genotype and malaria with minor complications/severe malaria. It is important to perform additional large studies in Africa, to further investigate the connection of GSTs and malaria.

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