Short Report

Analysis of TPI Gene Promoter Variation in Three Sub-Saharan Africa Population Samples

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ABSTRACT   Population samples from Angola, Mozambique, and S. Tomé e Príncipe were screened for the TPI gene promoter variants -5A→G, -8G→A and -24T→G. Three haplotypes were identified in the three populations: the haplotype -5A-8G-24T (average frequency 65.3%) and two less common haplotypes -5G-8G-24T (average frequency 24.7%) and -5G-8A-24T (average frequency 10.0%). A population sample from Central Portugal showed the haplotype -5A-8G-24T in 139 chromosomes and one subject heterozygous for haplotype -5G-8A-24G. The exact test of sample differentiation among three groups of malaria-infected individuals classified according to the severity of the disease showed no significant differences. We confirmed TPI gene diversity in sub-Saharan Africa, but we could not detect any association between TPI promoter variation and a malarial protective effect. Larger scale epidemiological studies are thus required to clarify this putative mechanism of natural host defense against this worldwide public health problem. Am. J. Hum. Biol. 21:118–120, 2009.   © 2008 Wiley-Liss, Inc.

Triosephosphate isomerase (TPI, EC 5.3.1.1) catalyzes the reversible isomerisation of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G-3-P). TPI deficiency is the most severe autosomal recessive disorder of the glycolytic pathway, associated with neonatal jaundice, chronic hemolytic anemia, progressive neuromuscular dysfunction, and increase propensity to infection (Schneider, 2000). Almost all reported cases are of European origin (15/18) and lethality was common in early childhood.

TPI gene promoter mutations -5A→G and -8G→A, within the cap proximal element (CPE), and -24T→G, within TATA box, occurring essentially in haplotypes -5 alone, -5 -8, and -5 -8 -24, have been identified in populations with a wide geographical distribution (Humphries et al., 1999a; Schneider et al., 1998). However, the greatest diversity was found in sub-Saharan African populations with variant haplotypes present in more than 40% subjects (Humphries et al., 1999a; Schneider et al., 1998). The -5G allele reaches a high frequency in sub-Saharan African and Oriental populations, and phylogenetic analyses suggest that this may represent the ancestral promoter haplotype (Humphries et al., 1999a).

Functional studies of erythrocyte TPI enzyme activity showed that -5G mutation does not change TPI activity, whereas the -8A mutation alone or -8A-24G haplotype were associated with moderate reduction in the enzyme activity (Humphries et al., 1999b). Most probably, mutations -8A and -24G disable the binding of transcription factors to the conserved promoter regions CPE and TATA, affecting gene transcription (Humphries et al., 1999b).

Malaria was a major selective force in the recent evolutionary history of human genome. The best characterized protective polymorphisms of human host against malarial infection involve erythrocyte-specific enzymes among which G6PD deficient variants (Luzzatto, 2006) and HbS (Fleming et al., 1979). Other putative selective targets such as TPI may deserve investigation due to their variant promoter haplotypes reaching high frequencies in individuals with African origin associated with subtle reduction in enzyme activity (Humphries et al., 1999a; Schneider et al., 1998).

In this study, we examined for the TPI gene promoter variants -5A→G, -8G→A and -24T→G, three different sub-Saharan African populations and a European Portuguese sample. As the precise biological significance of TPI promoter sequence variation remains to be elucidated, as a preliminary study, we tested the TPI haplotype frequencies in three groups of malaria-infected individuals classified according to the severity of the disease.

MATERIALS AND METHODS
Samples

Whole-blood samples were collected from three African geographic regions, Mozambique ($N = 13$), Angola ($N = 50$), S. Tomé e Príncipe ($N = 65$), and from Central Portu-

TABLE 1. TPI gene promoter genotype distribution and frequencies found in the population samples from Angola (ANG), Mozambique (MOZ), and S. Tomé e Príncipe (STP)

TPI genotypes	ANG	Frequency (%)	MOZ	Frequency (%)	STP	FREQ (%)
-5A/-5A-8G/-8G	20	40.0	3	23.1	36	55.4
-5A/-5G-8G/-8G	17	34.0	7	53.8	14	21.5
-5G/-5G-8G/-8G	3	6.0	1	7.7	0	0
-5A/-5G-8G/-8A	7	14.0	2	15.4	10	15.4
-5G /-5G-8G/-8A	3	6.0	0	0.0	4	6.2
-5G/-5G-8A/-8A	0	0.0	0	0.0	1	1.5
Total	50	100.0	13	100.0	65	100.0

Locus-24 was monomorphic for allele T.

TABLE 2. TPI gene promoter polymorphic sites -5 -8 and -24 allelic and haplotype frequencies in the population samples from Angola (ANG), Mozambique (MOZ), and S. Tomé e Príncipe (STP)

Variant	n	-5A > G		-8G > A		-24 T > G		Haplotype 5-8-24	Haplotype 5-8-24	Haplotype 5-8-24
		A	G	G	A	T	G	AGT	GGT	GAT
ANG	50	0.640	0.360	0.900	0.100	1.00	0.00	0.640	0.260	0.100
P		1.000		1.000						
Ho		0.480		0.200						
He		0.478		0.199						
MOZ	13	0.577	0.423	0.923	0.077	1.00	0.00	0.577	0.346	0.077
P		0.274		1.000						
Ho		0.69		0.15						
He		0.50		0.14						
STP	65	0.738	0.262	0.877	0.123	1.00	0.00	0.738	0.138	0.124
P		0.750		1.000						
Ho		0.369		0.215						
He		0.389		0.231						

Ho, Observed Heterozygosity; He, Expected Heterozygosity; P, Hardy-Weinberg Equilibrium Probability Value.

gal ($N = 70$). Collection was approved by the local Ethics Committees and informed consent was obtained.

In Mozambique, sampling took place in the Manhiça district by active search (all individuals were asymptomatic at the time of collection) in the dry (August 2001) and wet (February 2002) seasons as described by Marques et al. (2005). In Angola, samples were collected from malaria-patients: malaria outcome were defined as (1) severe malaria (SM, 25 children) and (2) uncomplicated malaria (UM, 25 children) according to WHO criteria (WHO, 2000)

DNA analysis

DNA was obtained from samples of dried blood spots (Angola and Mozambique) or leukocytes (S. Tomé e Príncipe and Central Portugal) by standard methods. The promoter region of TPI gene was amplified by PCR with primers 5′-GGCCATGGCGGAGGACGGCG-3′ (forward) and 5′-GCCAGACCCCTCCTCGGCGA-3′ (reverse) (Schneider et al., 1998). PCR products were digested with restriction enzymes *Tse*I, *Msc*I, and *Sfc*I to examine -5A→G, -8G→A and -24T→G variants, respectively, as reported by Schneider et al. (1998).

Detection of Plasmodium

Detection of malaria infection and identification of *Plasmodium* species was carried out by nested-PCR amplification of the small subunit ribosomal RNA genes (Snounou et al., 1993).

Statistical analysis

Allele frequencies were estimated by gene counting. Hardy-Weinberg equilibrium probability value, heterozygosity, and exact *P* values for linkage disequilibrium (Fisher exact probability test) and for sample differentiation (Raymond and Rousset, 1995) were obtained using the software package Arlequin, version 3.01 (Excoffier et al., 2005; http://cmpg.unibe.ch/software/arlequin3/), as well as the linkage phase from diploid data, by statistical inference via ELB algorithm.

RESULTS AND DISCUSSION

TPI gene promoter nucleotide substitutions -5A→G, -8G→A and -24T→G were analyzed on three sub-Saharan population samples from Angola, Mozambique, and S. Tomé e Príncipe. Genotype distribution is shown in Table 1 and individual allele and haplotype frequencies are shown in Table 2. The observed genotype distributions on the two polymorphic loci -5 and -8 showed no deviations from the Hardy-Weinberg equilibrium ($P > 0.05$).

The most common A allele for the -5 locus shows an average frequency of 0.65 allowing for the three population samples from Angola, Mozambique, and S. Tomé e Príncipe; for -8 variant, the most common G allele reach a mean frequency of 0.90. Only T allele was found at locus -24. Three haplotypes were identified across the three sub-Saharan population samples: the haplotype -5A-8G-24T (average frequency 65.3%) and two less common haplotypes, -5G-8G-24T (average frequency 24.7%) and -5G-8A-24T (average frequency 10.0%). Individual allele and haplotype frequencies were similar in the three population samples (Table 2) and with other sub-Saharan populations

previously studied (Humphries et al., 1999a; Schneider et al., 1998). Linkage disequilibrium was observed between loci -5 and -8 in Angola (exact $P = 0.0003 \pm 0.0001$) and S. Tomé e Príncipe (exact $P = 0.000$), but in Mozambique a P value not statistically significant (exact $P = 0.70 \pm 0.004$) was found, which may be related with the small population sample under study ($N = 13$). The exact test of differentiation based on TPI gene promoter haplotype frequencies showed no significant differences among the three sub-Saharan populations.

A population sample of 70 individuals from Central Portugal showed the haplotype -5A-8G-24T in 139 chromosomes. One individual was found heterozygous with genotype -5A/G -8G/A -24T/G, a similar result to Humphries et al. (1999a) in Mediterranean ($N = 55$), Asian Indian ($N = 48$) and Caribbean ($N = 26$) population samples where one heterozygous subject for all the three alleles was found in each group.

To tentatively provide results concerning the biological significance of TPI promoter variation, three groups of malaria-infected individuals from Angola and Mozambique, classified according to the severity of disease with severe malaria (SM) (25 chromosomes), uncomplicated malaria (UM) (25 chromosomes), and asymptomatic (AS) (13 chromosomes) were tested for TPI gene promoter mutations. Sample differentiation across the three groups based on -5-8-24 haplotype frequencies showed no statistical significant differences (exact P values: SM vs. UM = 0.864 ± 0.004; SM vs. AS = 0.713 ± 0.005, and UM vs. AS = 0.805 ± 0.005). In the same way, using individual locus -5 and -8 allele frequencies to testing sample differentiation, no significant differences were found (exact P values between 0.622 and 1.000). These results seems to indicate that TPI gene promoter variation observed in sub-Saharan Africa human populations is not associated with malarial selective influences, validating Humphries et al. (1999a) results showing that TPI gene diversity predates the evolutionary period within the 10,000 years of the malarial selective impact on human populations. Therefore, the different pattern of TPI gene promoter variation between African and non-African populations could be related to random demographic events.

In the same way, the exact test of sample differentiation between two groups from the population sample of S. Tomé e Príncipe, classified as infected and noninfected by the detection of *Plasmodium* DNA obtained from human peripheral blood samples, showed to be nonsignificant (exact P value 0.434 ± 0.012).

In conclusion, we have confirmed through the analysis of three population samples from different geographic regions the diversity of TPI promoter region in sub-Saharan Africa. Analyzing three groups of *Plasmodium* infected individuals classified according to the clinical phenotype of malaria, we could not detect any association between the observed TPI promoter variation and a malarial protective effect. However, this still is a preliminary study and larger scale epidemiological studies are thus required to clarify if this effect does exist indeed but is too weak to be detected in samples of modest size. Malaria is nowadays a serious worldwide public health problem, and new tools and approaches to control and combat this disease are urgently needed. These studies may provide insight into naturally occurring mechanisms of host defense, which could be used to develop new therapeutic agents.

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