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GSTP1, GSTM1, AND GSTT1 POLYMORPHISMS IN TIBETAN MOUNTAINEERS

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Abstract. Exposure to high altitude can increase the level of reactive oxygen species (ROS) in human beings. Physical exercises or working can exacerbate the effects of high altitude and further cause free radical mediated oxidative tissue injury. The glutathione S-transferases (GSTs) can scavenge ROS. Variant genotypes of GSTs lead to the inactive or decreased form of the enzymes. We hypothesized that the polymorphisms within these genes may explain the interindividual variation in response to high altitude hypoxia. We have evaluated the polymorphisms of GSTP1, GSTM1 and GSTT1 genes in 86 excellent Tibetan mountaineers and in 90 sea-level Han Chinese were used as the control group. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) were performed to genotype GSTP1 polymorphism in exon 5 (Ile105Val). Both GSTM1 and GSTT1 genotypes were determined by multiplex PCR. The result showed that GSTM1 null genotype was found in 60.5% of Tibetan mountaineers and in 54.4% of sea-level Han Chinese. No difference was observed in the frequency of polymorphic genotype for GSTM1 ($\times^2$=0.65, p=0.26), (OR=0.78; 95% CI:0.43-1.42). The frequency of GSTT1 null genotype was 36.1% among Tibetan mountaineers, 51.1% among Han Chinese. The difference was statistically significant ($\times^2$=4.06, p=0.031), (OR=1.86; 95% CI: 1.01-3.39). The proportion of $GSTP_{1\text{-}105}$ mutant homozygote was significantly lower in the Tibetan mountaineers than in the control subjects (26.7% vs 44.4%) ($\times^2$=5.99, p=0.011).The OR for $GSTP_{1\text{-}105}$ mutant genotype versus wild genotype was 2.19 (95% CI=1.16-4.13). These results suggest that GSTT1 and $GSTP_{1\text{-}105}$ genotypes may be associated with the interindividual variation in response to high altitude hypoxia, and may be two new markers in gene screening for human adaptation to high altitude. ***(Biol.Sport 23:303-311, 2006)***

Key words: High altitude - GSTM1 - GSTT1 - GSTP1 - Reactive oxygen species

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Introduction

Exposure to high altitude can increase the level of reactive oxygen species (ROS) production of human beings and lead to oxidative damage to macromolecules. Physical exercises can exacerbate the effects of high altitude and further increase the related oxidative stress burden. ROS are involved in acute mountain sickness (AMS) and are even a causative factor of AMS [3,14]. It is generally acknowledged that intensive muscular contraction can result in oxidative stress [12], and that muscle cell protein oxidation and lipid peroxides reduce muscle force production [11]. Antioxidant enzymes have an important mechanism by which cells limit the damage caused by ROS. Glutathione *S*-transferases (GSTs) is a family of genes with a critical function in the protection against electrophiles and the products of oxidative stress [6]. GSTs catalyze the conjugation of glutathione to a wide range of electrophilic compounds, thereby allowing them to be excreted in the bile and urine [9]. Many of these enzymes are polymorphic, and the existence of isoenzymes within each gene family may be involved in differential responses to oxidative stress. Functional polymorphisms GST pi (GSTP1), GST mu (GSTM1) and GST theta (GSTT1) in GSTs genes have been shown to be associated with catalytic activity of enzymes and scavenging capacity of ROS [2,4]. They are critical in the protection of cells from ROS damage because they can utilize as substrates a wide variety of products of oxidative stress [7]. GSTM1 enzymes may offer protection against DNA damage induced by free radicals as well as electrophilic metabolites of polycyclic aromatic hydrocarbons (PAH) [13]. The lack of the GSTM1 activity is a result of a homozygous deletion (null genotype) of the GSTM1 gene. Similar a deletion polymorphism has been observed for GSTT1 gene. The enzyme encoded by GSTT1 (present) gene detoxifies oxidized lipid and DNA [2]. Two genetic polymorphisms at the GSTP1 locus result from a single base pair substitution in exon 5 (Ile 105 Val) and exon 6 (Ala 114 Val) [5]. The affected codon is in the electrophile binding site of the GSTP1 enzyme [1]. The GSTP1 variants may have different conjugation abilities towards different electrophilic compounds [8]. In vitro cDNA expression studies suggest that the Ile 105 Val substitution results in an enzyme with reduced activity [15]. GSTP1 enzyme catalyzes the detoxification of (base) propenals that are major metabolites of lipid peroxides [2]. The deleted or variant genotypes lead to the inactive or decreased forms of the enzymes. Defects in detoxifying ROS may influence the development and the severity of diseases related to ROS. We hypothesized that polymorphisms within these genes may explain the interindividual variation in response to high altitude hypoxia and perhaps indicate

adaptation to hypobaric hypoxia and enhance or reduced performance at altitude. To test these hypothesis, we studied the distribution of GSTP1, GSTM1 and GSTT1 genotypes among excellent Tibetan mountaineers and compared to GSTs gene polymorphism frequencies in a group of Han Chinese living at sea-level to clarify whether certain profiles of GSTs genotypes might be associated with the tolerance or the susceptibility of high altitude hypoxia. An effective detoxifier genotype with the presence of the GSTM1 or /and GSTT1 present genotypes or/and the homozygous wild type for GSTP1 (Ile/Ile) would be considered tolerant or beneficial genotype. A poor detoxifier genotype with GSTM1 or/and GSTT1 null genotypes or/and a heterozygous or homozygous variant allele (GSTP1 Ile/Val or GSTP1 Val/Val) would be considered susceptible genotype.

Materials and Methods

Study population: The study group consisted of total of 176 people. Han Chinese people comprised of 90 individuals (mean age=26±5 years) as the control population. They were students of Physical Education College of PLA of Guangzhou in China. Tibetan mountaineers consisted of 86 people (mean age=28±6 years). They came from Tibetian mountaineering school and mountaineering team. They were interviewed on the subjects of their ethnic people (also about their parents' and grandparents' race). Related healthy blood donor volunteers for the study were selected by doctors.

Study protocol: Peripheral blood samples were collected into EDTA (2 ml) and stored at 4°C ice box and then taken back to Da-An Gene Diagnostic center of Sun Yat-Sen University for genotype analysis. DNA was isolated from these samples and stored at -20°C until use. The GSTM1 and GSTT1 polymorphisms were studied using multiplex polymerase chain reaction (PCR) with the GSTM1, GSTT1 and an internal control CYP1A1 primers as described by Gattás [4]. The PCR and restriction fragment length polymorphism (RFLP) were used to detect the polymorphism of GSTP1 in exon 5 using the $GSTP_{1\text{-}105}$ primers described by Mary A. Watson [10]. DNA (20 ng) was amplified in a 50 μl multiples reaction mixture containing 5 pmol of each of the following primers: GSTM1 (F and R), GSTT1 (F and R), CYP1A1 (F and R) and $GSTP_{1\text{-}105}$ (F and R) respectively (Table 1). Taq polymerase 3U, 5×PCR buffer (containing 10 mM Tris-HCI, pH 8.0, 50 mM KCI, 2 mM $MgCI_2$) and 1 μl of dNTP mix. All amplifications were performed by using microamp tubes in a Perkin-Elmer GeneAmp PCR System 9600 thermocycler (Singapore). Cycling conditions were: 93°C for 5 min, 40 cycles of 93°C for 1 min, 55°C for 1 min, 72°C for 1 min, and a final extension of

72°C for 7 min. The final PCR products from co-amplification of GSTM1 (215 bp), GSTT1 (480 bp) and CYP1A1 (312 bp) were visualized on an ethidium bromide-stained 1.5% agarose gel. The actual bands electrophoresed are showed in Fig. 1. 9 μl of PCR product of $GSTP_{1\text{-}105}$ (433 bp) was digested for three hours at 37°C with 5u of Alw26I in a total volume of 20 μl. The digested fragments were 328 bp, 222 bp, 106bp and 105 bp for heterozygote variant alleles, 222 bp, 106 bp and 105 bp for homozygous variant alleles, 328 bp 106 bp and 105 bp for homozygous wild alleles. The digested fragments were separated on a 2% agarose gel stained with ethidium bromide to visualize the bands. As shown in the Fig. 2.

Table 1
Oligonucleotide primers sequences for GSTs genetic polymorphism detection

Gene		Sequence	PCR product (bp)
GSTM1	Left primer	5'-GAACTCCCTGAAAAGCTAAAGC -3'	312
	Right primer	5'-GTTGGGCTCAAATATACGGTGG -3'	
GSTT1	Left primer	5'-TTCCTTACTGGTCCTCACATCTC-3'	480
	Right primer	5'-TCACCGGATCATGGCCAGCA-3'	
GSTP1	Left primer	5'- GTA GTT TGC CCA AGG TCA AG-3'	433
	Right primer	5'-AGC CAC CTG AGG GGT AAG-3'	
CYP1A1	Left primer	5'- GAACTGCCACTTCAGCTGTCT -3'	215
	Right primer	5'- CAGCTGCATTTGGAAGTGCTC -3'	

Statistical analysis: The subjects were classified as high altitude exposure group (Tibetan mountaineers) and unexposure one (sea-level Han Chinese). The association between the genotype distributions was assessed by Odds Ratios (OR) and Confidence Intervals (CI). The OR and CI were calculated by unconditional logistic regression. All the P values are one-sided and P values <0.05 were considered statistically significant. All statistical analysis was performed using the statistical software SSPS 11.5.

Results

The GSTM1 null genotype was found in 49 of 90 Han Chinese and 52 of 86 Tibetan mountaineers. The frequency of GSTM1 null genotype was in Tibetan mountaineers (60.5%) and sea-level Han Chinese (54.4%). Difference was not

statistically significant ($\times^2$=0.65, p=0.26). The OR of GSTM1 null genotype versus present one was 0.78 (95% CI=0.43-1.42). In the test of GSTT1, 36.1% of Tibetan mountaineers and 51.1% of Han Chinese were null genotype. This difference in the frequency of the GSTT1 null genotype between two groups was statistically significant ($\times^2$=4.06, p=0.031). The OR of GSTT1 null genotype versus present one was 1.86 (95% CI=1.01-3.39). For GSTP1, The calculation was done for mutant homozygote and heterozygote versus wild homozygote. The proportion of $GSTP_{1\text{-}105}$ mutant genotype was significantly lower in Tibetan mountaineers than in sea-level Han Chinese (26.7% versus 44.4%), ($\times^2$=5.99, p=0.011). The OR of $GSTP_{1\text{-}105}$ mutant genotype versus wild genotype was 2.19 (95%CI=1.16-4.13) see Table 2. The "worst" detoxifier genotype (the combined genotype of GSTM1 null,GSTT1 null and $GSTP_{1\text{-}105}$ mutant) was found in 6 of 86 Tibetan mountaineers and 15 of 90 sea-level subjects. The difference was statistically significant ($\times^2$=4.03, p=0.038). The OR of "worst" detoxifier genotype versus all other genotypes was 2.72 (95% CI=1.00-7.45).

Table 2
GSPT1, GSTM1, and GSTT1 genotype frequencies n(%) in Tibetan mountaineers and Han Chinese at sea level.

	GSTM1		GSTT1		$GSTP_{1\text{-}105}$	
	Present	null	present	null	wild	mutant
Han Chinese	41(45.6)	49(54.4)	42(48.9)	48(51.1)	50(55.6)	40(44.4)
Tibetan mountaineers	34(39.5)	52(60.5)	53(63.9)	33(36.1)	63(73.3)	23(26.7)

Wild refers to wild homozygote genotype, mutant refers to mutant heterozygote and mutant homozygote genotype.

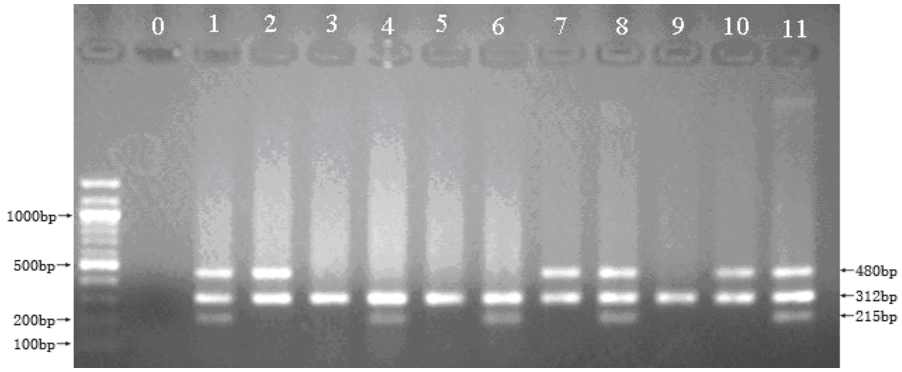

Fig. 1

Agarose gel electrophoresis of the GSTT1, GSTM1 and CYP1A1 PCR amplification products

(+) refers to present, (-) refers to null;

Lanes 0: negative control; Lanes 1, 8 and 11: GSTM1(+) and GSTT1(+) genotype; Lanes 2, 7 and 10: GSTT1(+)and GSTM1(-) genotype; Lanes 3, 5 and 9: GSTM1(-) and GSTT1(-) genotype; Lanes 4 and 6: GSTM1(+) and GSTT1(-) genotype

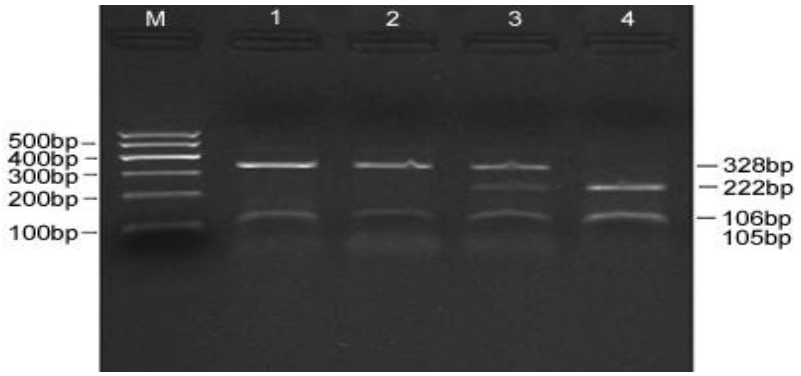

Fig. 2

Agarose gel of the GSTP1 PCR products after digestion with Alw26I for the detection of $GSTP_{1\text{-}105}$ polymorphisms

Line 1 and 2: Homozygous for the wild-type GSTP1 gene (GSTP1 Ile/Ile); Lane 3: Heterozygous for the mutated GSTP1 gene (GSTP1 Ile/Val); Line 4: Homozygous for the mutated GSTP1 gene (GSTP1 Val/Val)

Discussion

Tibetans, longtime residents at high altitude, have a remarkable tolerance or they are less susceptible to high altitude hypoxia. They have evolved mechanisms to more closely relate oxygen supply and metabolism and subsequent energy generation and conferred selective advantage in the hypoxic challenge. They have a greater physical capacity than Han Chinese when they exercise or work at altitude. This is very significant for humans live or recreation practicing sports at extreme altitude. Tibetans mountaineers, a special group of Tibetans, are more tolerant to altitude hypoxia, and they have higher physical capacity than any other Tibetans because they have excellent mountaineering performance. It is possible to select sportsmen by screening their gene. In our study, we focused on the GSTP1 (Ile 105 Val) genetic polymorphism because the GSTP1 polymorphism in exon 6 is less common than that in exon 5. So far, there is no report about glutathione S-transferase genetic polymorphisms in high altitude groups (Quechua, Aymara, Sherpa, Tibetan and Kenyan). In our study, the frequency of GSTT1 null genotype was lower in Tibetan mountaineers (36.1%) than in sea-level residents Han Chinese (51.1%) and higher in that of black (28.6%) [4]. The proportion of $GSTP_{1\text{-}105}$ mutant genotype was significantly lower in Tibetan mountaineers than those of in sea-level Han Chinese (26.7% versus 44.4%). These results imply that individuals with GSTT1 null genotype or $GSTP_{1\text{-}105}$ mutant genotype may be more susceptible to altitude hypoxia than those with GSTT1 present genotype or $GSTP_{1\text{-}105}$ wild genotype. Individuals having GSTT1 present genotype or $GSTP_{1\text{-}105}$ wild-type genotype may be more tolerant to altitude hypoxia than those having GSTT1 null genotype or $GSTP_{1\text{-}105}$ mutant genotype because Tibetan mountaineers, a remarkable tolerance residents to altitude hypoxia, have higher frequencies of GSTT1 present genotype and $GSTP_{1\text{-}105}$ wild-type genotype than those of sea-level residents, Han Chinese. The susceptibility of individuals with GSTT1 null genotype and with $GSTP_{1\text{-}105}$ mutant genotype to high altitude hypoxia revealed 0.86-fold and 1.19-fold increase than those of with GSTT1 present genotype and with GSTP1 wild genotype respectively. Likewise, For the 'worst' genotype (the combined genotype of GSTM1 null, GSTT1 null and GSTP1 mutant) showed 1.72-fold increase than those of with other genotypes. Different isoenzymes of GSTs are known to exhibit overlapping substrate species [5]. Deficiencies of GST isoenzymes studied may be compensated by other ones. Individuals having a defective genotype for more than one of these genes can thus be expected to have a lower catalytic activity of enzymes and scavenging capacity of ROS than those having a defective genotype for only one gene.

We hypothesize that different genotypes may explain interindividual variation in response to high altitude hypoxia and perhaps imply adaptation to hypobaric hypoxia and enhance or reduce the performance at altitude. Our results were consistent with this hypothesis, because Tibetan mountaineers who lived in high altitude for centuries, have higher frequency of beneficial genotypes than those of sea-level population. GSTP1 and GSTT1 genotypes may be two new markers of screening genes in human adaptation to high altitude. It is, however, unclear that GSTM1 genotype is not associated with the selection pressure of high altitude residence.

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