Original Article

Association of glutathione S-transferases M1 and T1 gene polymorphisms with the risk of metabolic syndrome in an Iranian population

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Abstract

Background: Glutathione S-transferases (GSTs) are important factors in cell sensitivity to oxidative stress and susceptibility to cardiometabolic disorders. We aimed to investigate the GSTM1 and T1 gene polymorphisms, as well as their interactions in metabolic syndrome (MetS) patients and healthy individuals in an Iranian population.

Materials and Methods: The study sample comprised of 220 healthy individuals (mean age: 41.9 – 15.1 years) and 165 MetS patients (mean age: 49.7 – 11.5 years). The diagnostic criteria for MetS were defined following the criteria provided by the modified National Cholesterol Education Program Adult Treatment Panel III. Genotyping of GSTM1 and T1 genes were performed using polymerase chain reaction.

Results: Our analyses have shown that neither GSTM1 (odds ratio [OR] =0.89, 95% confidence interval [CI]: 0.59 – 1.33, $P = 0.57$) nor GSTT1 (OR = 1.26, 95% CI: 0.76 – 2.02, $P = 0.38$) null genotypes were associated with increased risk. Moreover, no significant differences were observed between various combinations of GST genotypes.

Conclusion: Contrary to our primary hypothesis, what we found disaffirms any kind of association between GSTM1 and T1 polymorphisms and the risk of MetS. However, being the first polymorphism study of GSTs in MetS patients, further studies are required to confirm our results in other populations.

Key Words: Gene deletion, genetic polymorphism, glutathione transferase, metabolic syndrome X

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Received: 20.12.2014, **Accepted:** 23.02.2015

INTRODUCTION

Redox state of cells is the balance between oxidation/reduction reactions that is determined by the rate of reactive oxygen species production and antioxidant capacity of cells. Impairment of this balance in favor of oxidative processes is called oxidative stress (OS). While reactive oxygen species play crucial roles in multiple physiologic systems, under the conditions of OS, they can damage redox-sensitive molecules and consequently, interfere with cell signaling and gene regulation systems.[1] The contribution of OS in the pathophysiology of several human diseases such as diabetes and atherosclerosis is well-documented.[2]

The glutathione (GSH) buffer system along with superoxide dismutase is the main intracellular

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.179185

How to cite this article: Rafiee L, Shokouh P, Roohafza H, Mansourian M, Javanmard SH. Association of glutathione S-transferases M1 and T1 gene polymorphisms with the risk of metabolic syndrome in an Iranian population. Adv Biomed Res 2016;5:63.

antioxidant.[3] Apart from its antioxidant activities, GSH has an essential role in the detoxification of xenobiotics through the action of glutathione S-transferases (GSTs).[4] GSTs are a multi-gene super family of dimeric phase II enzymes that catalyze the production of thioether bond between the cysteine on the GSH and an electrophilic compound. Eight classes of cytosolic GSTs are recognized in mammalian cells consisted of alpha, kappa, mu, omega, pi, sigma, theta, and zeta; each are divided into subclasses.[5]

Polymorphisms of GSTM1 (a member of class mu; MIM: 138350) and GSTT1 (a member of class theta; MIM: 600436) exist in all human populations.[6] Different alleles are found at the locus of GSTM1 (1P13.3) including gene deletion (GSTM1-0) and functional mutations (GSTM1a and GSTM1b).[7] The same is the case for GSTT1 at 22q11.2, for which GSTT1-0 allele represents deletions of the gene.[8] GSTM1 and T1 genes deletions result in a loss of enzymatic activity.[9] It has been demonstrated that GST null-genotypes are important factors in cell sensitivity to OS and susceptibility to cardiovascular and metabolic disorders.[10,11]

A growing body of evidence indicates a link between OS and all individual components of the metabolic syndrome (MetS).[12-14] MetS is a cluster of metabolic and cardiovascular risk factors assumed to be caused by two major etiologies: Abdominal adiposity and insulin resistance.[15] It is believed that genetic and environmental factors both play roles in the development of the syndrome. Although the heritability of MetS is not thoroughly investigated, it is clear that all components of the syndrome are strongly heritable.[16] The association of polymorphisms of alpha-1 (A1), mu-1 (M1), P1, and T1 subclasses with some of the MetS components and complications have already been studied.[11,17-19]

To the best of our knowledge, there is no published study investigating the distribution of GSTM1 and T1 null-genotypes in MetS patients to date. Therefore, we made an effort to assess GSTM1 and T1 polymorphisms and their interactions in Iranian MetS patients and to compare them with non-MetS controls.

MATERIALS AND METHODS

Study population

The present study was performed in Physiology Research Center at the Isfahan University of Medical Sciences, between December 2010 and May 2011. Study subjects were randomly chosen among the participants of Isfahan Cohort Study (ICS).[20] The ICS participants aged between 18 and 65 years were all randomly selected from the community of three counties in central Iran in the first stage of Isfahan Healthy Heart Program (IHHP). Methodological details of IHHP could be found elsewhere.[21]

Enrolled subjects who were identified as having MetS were regarded as cases and others as controls. MetS was defined following the criteria provided by the modified National Cholesterol Education Program Adult Treatment Panel III adjusted to indices of obesity for Asians.[22] Participants with any of the followings were not eligible for the study: Diabetes mellitus, neoplastic disorders, any debilitating medical condition, and pregnancy or lactation. The study protocol was in compliance with the declaration of Helsinki and approved by the Ethics Committee of Isfahan University of Medical Sciences. An informed written consent was obtained from all participants.

Demographic and laboratory assays

Venous blood samples were obtained after at least 12 h of overnight fasting and collected into ethylene diamine tetra acetic acid tubes. Immediately after collection, whole blood was stored at-20°C until use. Serum total, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), and triglycerides levels were measured by Hitachi 902 auto-analyzer (Japan) using Pars Azmoon (Iran) analytical kits. Plasma fasting glucose measurement was done using the same machine and Biosystem (France) kits. All the measurements were carried out in Isfahan Cardiovascular Research Institute laboratory, which is continuously controlled by the National Reference Lab of Iran (Tehran-Iran) and INSTAND e.V. Laboratory (Düsseldorf-Germany). Anthropometric variables were measured according to standard methods by a trained nurse by means of a calibrated scale and an anthropometer and a calibrated scale. Blood pressure was measured in the right arm at sitting position after a 10-min rest using a standard mercury sphygmomanometer. The body mass index (BMI) was calculated using the formula: Weight (kg)/height^2 (m^2).

Genotyping

Genomic deoxyribonucleic acid (DNA) for polymerase chain reaction (PCR) was isolated from whole blood using blood mini kit (PrimePrep Genomic DNA Isolation Kit, Genet Bio Inc.,). According to the manufacture protocol, Lysis buffer was added to 100 μl of whole blood and incubated in 56^{0}c, after the addition of ethanol, supernatant was transferred to spin columns then washed twice; at the end the DNA was eluted.

To determine the GSTT1 and GSTM1 genotypes of the subjects, PCR amplification was performed using the following primers: GSTT1 forward primer 5′-TTC CTT ACT GGT CCT CAC ATC TC-3′, and reverse primer 5′-TCA CCG GAT CAT GGC CAG CA-3′; GSTM1 forward primer 5' AGA CAG AAG AGG AGA AGA TTC 3', and reverse primer 5' TCC AAG TAC TTT GGC TTC AGT 3'. Albumin gene sequence amplification was used as an internal control in the PCR reactions, for which the primers were: Forward primer 5′-GCC CTC TGC TAA CAA GTC CTA C-3′, and reverse primer 5′-GCC CTA AAA AGA AAA TCG CCA ATC-3′. Each 25 µl of PCR reaction contained 50-100 ng of genomic DNA, 0.25 mM of each dNTP, 10 pmol of each primer, 1 U Taq DNA polymerase, 1.5 mM $MgCl_2$, and 10X PCR buffer (Cinagen Co., Iran). The PCR reaction conditions were explained previously.[23] The absence of amplified product was considered as the homozygous null genotype of GSTM1 and T1. It is noteworthy that this technique cannot distinguish between heterozygote and homozygote carriers of the positive genotypes. Successful amplification by albumin specific primer confirmed the proper function of the PCR reaction. Samples with ambiguous results were re-tested, and a random 15% of all tests were repeated, no discrepancy was discovered upon replicate testing.

Statistical analysis

The data were coded and stored in a computer database. Statistical analysis was performed using the Statistical Package for Social Sciences software version 15.0 (SPSS Inc., Chicago, Illinois, USA). Comparisons of selected quantitative characteristics between cases and controls were done by independent *t*-test. The relative associations between GST genotypes and MetS risk were assessed using multiple logistic regression method to calculate odds ratios (ORs) and 95% of confidence intervals (CIs). For all analyses, statistical significance was assessed at a level of 0.05 (2-tailed).

RESULTS

A total of 385 individuals (165 MetS patients and 220 controls) were genotyped for the two GST subclasses. Comparison of the two groups considering age, sex, BMI, waist circumference, serum triglyceride, total cholesterol, HDL-C, LDL-C, fasting plasma glucose, and systolic and diastolic blood pressures showed statistically significant differences [Table 1].

In order to show the influence of the absence of GSTM1 and T1 expression on the assessed variables, the differences between individuals with various GST genotypes were compared. As could be seen in Table 2, no pronounced differences were found between groups.

Detailed genotype distributions are shown in Table 3. The prevalence of GSTM1 null genotype was 46.7% in the patients and 52.3% in the control group. As shown, the differences were not significant (OR = 0.89, 95% CI: 0.59–1.33, P = 0.57). GSTT1 null genotype was detected in 75.5% of the controls and 75.8% of the patients. In the same manner to the M1 subclass, the difference between two groups never reached a significant level (OR = 1.26, 95% CI: 0.76–2.02, P = 0.38).

To determine whether different combinations of genotypes from the GST genes are involved in the development of the syndrome, we analyzed the association between the combinations of genotypes and the risk of MetS which is presented in Table 4. According to the table, there were no associations between the combinations of genotypes and the risk of MetS.

DISCUSSION

The prevalence of null genotypes of GSTM1 and T1 among the subjects of the present study was in accordance with our previous report from another Iranian population.[23] Unexpectedly, we failed to track any anthropometric and/or metabolic influences caused by the polymorphism of two subclasses of GST genes. Furthermore, in our multivariate analyses, no association was found between GSTM1 and T1 polymorphisms and the risk of MetS regardless of age and sex. Whether this

Table 1: Comparison of characteristics of individuals with and without MetS[a,b]

Variables	Cases (n=165)	Controls (n=220)	P[c]
Age (years)	49.7±11.5	41.9±15.1	<0.001
Sex			
Female	99 (60)	106 (48.2)	0.023
Male	66 (40)	114 (51.8)	
Body weight (kg)	79.4±14.0	71.6±13.7	<0.001
BMI (kg/m^2)	30.3±3.9	25.5±4.3	<0.001
Waist circumference (cm)	100.9±11.2	80.5±8.9	<0.001
FPG (mg/dl)	90.6±10.5	85.7±8.0	<0.001
Total cholesterol (mg/dl)	194.6±35.3	168.8±31.0	<0.001
Triglyceride (mg/dl)	152.40±54.6	91.39±43.4	<0.001
HDL-C (mg/dl)	46.1±9.3	50.9±8.8	<0.001
LDL-C (mg/dl)	118.1±29.0	99.5±27.7	<0.001
SBP (mmHg)	110.8±19.3	103.2±13.6	<0.001
DBS (mmHg)	78.3±10.3	70±8.9	<0.001

[a]HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, FPG: Fasting plasma glucose, SBP: Systolic blood pressure. [b]Data are expressed as mean±SD for continuous variables and the number (%) of participants for categorical variables. [c]P value associated with either Student's *t*-test or Chi-square test. DBS: Deep brain stimulation, SD: Standard deviation, BMI: Body mass index, MetS: Metabolic syndrome

Table 2: Differences in selected characteristics of participants based on GST genotype

Variables (mean±SD)	GSTT1 genotypes			GSTM1 genotypes		
	Present (*n*=291)	Null (*n*=94)	*P*	Present (*n*=193)	Null (*n*=192)	*P*
Body weight (kg)	75.3±14.9	73.9±12.41	0.41	74.7±14.9	75.2±13.9	0.77
BMI (kg/m^2)	27.5±4.7	27.8±4.7	0.57	27.5±4.8	27.6±4.6	0.85
Waist circumference (cm)	89.3±14.3	88.8±13.7	0.77	89.0±14.7	89.4±13.7	0.84
FPG (mg/dl)	87.6±9.2	88.5±10.5	0.44	87.0±9.0	88.6±9.9	0.10
Total cholesterol (mg/dl)	179.1±34.9	178.3±37.3	0.44	180.9±31.9	176.6±38.7	0.87
Triglyceride (mg/dl)	116.1±55.9	121.9±62.0	0.39	113.8±56.3	121.2±57.8	0.20
HDL-C (mg/dl)	49.0±9.4	48.4±9.2	0.59	48.99±9.2	48.8±9.4	0.82
LDL-C (mg/dl)	106.84±29.0	106.5±31.9	0.45	108.4±28.0	106.5±31.3	0.54
SBP (mmHg)	110.0±17.7	109.4±18.9	0.77	110.8±17.5	109.0±18.5	0.34
DBS (mmHg)	73.5±9.9	73.6±11.5	0.91	73.6±10.5	73.5±10.3	0.95

BMI: Body mass index, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, FPG: Fasting plasma glucose, SBP: Systolic blood pressure, DBS: Deep brain stimulation, GST: Glutathione S-transferases, GSTT1: Glutathione S-transferases theta 1, GSTM1: Glutathione S-transferases mu-1, SD: Standard deviation

Table 3: Association between polymorphisms of GSTM1 and T1 and risk of MetS

Genotype (number (%))	Cases (165)	Controls (220)	OR (95% CI)[a]	*P*
GSTT1 polymorphism				
Present	40 (24.2)	54 (24.5)	1	0.38
Null	125 (75.8)	166 (75.5)	1.26 (0.76-2.02)	
GSTM1 polymorphism				
Present	88 (53.3)	105 (47.7)	1	0.57
Null	77 (46.7)	115 (52.3)	0.89 (0.59-1.33)	

[a]OR (95% CI) adjusted for age and sex. OR: Odds ratio, CI: Confidence interval, GSTT1: Glutathione S-transferases theta 1, GSTM1: Glutathione S-transferases mu-1, MetS: Metabolic syndrome

Table 4: Association between GST genotype profiles and risk of MetS

GSTM1	GSTT1	Cases (165)	Controls (220)	OR (95% CI)[a]	*P*
Present	Present	60 (36.4)	82 (37.3)	1	1
Present	Null	17 (10.3)	33 (15.0)	1.80 (0.91–3.56)	0.09
Null	Present	65 (39.4)	84 (38.2)	1.08 (0.68–1.72)	0.75
Null	Null	23 (13.9)	21 (9.5)	0.84 (0.42–1.68)	0.63

[a]OR (95% CI) adjusted for age and sex. OR: Odds ratio, CI: Confidence interval, GST: Glutathione S-transferases, GSTT1: Glutathione S-transferases theta 1, GSTM1: Glutathione S-transferases mu-1, MetS: Metabolic syndrome

failure was a consequence of our sample size or not is a matter of question, which awaits elucidation by future larger-sample studies.

The essential role of OS in dysregulation of adipocytokines and the development of insulin resistance as the first level, and MetS as the second level of metabolic abnormalities is previously demonstrated.[24] GSTs as the main enzymes of glutathione buffer system play crucial roles in maintaining the redox balance of cells. Therefore, their enzymatic inactivity caused by gene deletions should be associated with the consequences of OS. Pursuant to this justification, the investigation of the association of GST polymorphisms with the risk of the components and construct of the MetS sounds reasonable.

Up to this point, no more than a handful of studies have implemented to investigate the relationship between GST polymorphisms and some MetS components. Oniki *et al*. have reported that the risk of hypertension is increased in carriers of nonfunctional alleles of GSTA1, GSTM1, and GSTT1.[18] Similarly, double deletion genotypes of the GSTM1 and T1 were reported to have a significant association with hypertriglyceridemia and low HDL-C levels.[17] Another study by Yalin *et al*. has concluded that GSTM1 null-genotype is significantly associated with diabetes mellitus in a Turkish population.[11] It should be noted that even in this limited number of relevant studies, some extent of discrepancy exists. As Delles *et al*. could not find any association between GSTM1 null-genotype and hypertension.[25]

Contrary to our supposition, our findings have shown no relationship between MetS and GSTM1 and/or GSTT1 null-genotypes. These findings might be explained by the multifactorial and polygenic nature of this syndrome. As known, MetS is a constellation of metabolic abnormalities; each dealing with multiple genetic variations. Accordingly, each individual genetic variant generally has only a modest effect. Besides, environmental factors and life-style, which were not assessed in this study, may play important roles in the expression of syndrome traits. This fact along with the genetic heterogeneity of human populations, make it possible to obtain disparate results in similar polymorphism studies in different populations.

Here, another point that has the merit to be mentioned is the existence of a fundamental disagreement about the concept of the MetS. Some authors believe that the cardiovascular risk associated with the MetS do not exceed the risk explained by the sum of its components. This notion was supported by the results of three studies[26-28] when adjusted for the specific components of the syndrome.[29] Considering of these

considerable pieces of evidence, these questions may arise: Are the components of the syndrome genetically and pathologically related? Could polymorphism of a gene be related to some components but not with the whole syndrome? More investigations are surely, required before these questions could be answered. Clarification of these types of questions is essential before getting any conclusion from the studies of similar design.

The current study is subject to some limitations. First is our limited sample size. Maybe significant associations could be found in greater samples. Second is that the life-style factors were not assessed in this study. As mentioned before, these factors have substantial effects on the severity and age of onset of phenotypic traits of the MetS.

To our knowledge, this is the first polymorphism study of GSTs in MetS patients. Hence, it could be considered as an outset of future studies. Replicated results in other populations would help in better understanding of the genetic basis of MetS and identifying new targets for pharmacologic therapy.

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Source of Support: The present study was financially supported by grants from Physiology Research Centr of the Isfahan University of Medical Sciences,
Conflict of Interest: None declared.