Evaluation of Glutathione S-transferase T1 (GSTT1) deletion polymorphism on type 2 diabetes mellitus risk in a sample of Yazdian females in Yazd, Iran

Saeedhossein Khalilzadeh¹, Mohammadhosain Afrand ², Seyed Khalil Froozan-Nia³, Mohammad Hasan Sheikhha ⁴

¹Assistant Professor, Department of Endocrinology, Yazd Diabetes Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
²M.D, Medical Scientific Association, Ali-Ebne Abitaleb Faculty of Medicine, Islamic Azad University, Yazd Branch, Yazd, Iran
³Professor, Department of Cardiac Surgery, Tehran Heart Center, Tehran University of Medical Sciences, Tehran, Iran
⁴Ph.D. in Medical Genetics, Department of Medical Genetics, Yazd Diabetes Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Corresponding Author:
Mohammadhosain Afrand, Medical Scientific Association, Islamic Azad University, Safaiyeh Boulevard Daneshgah, Ali-Ebne Abitaleb Faculty of Medicine, Yazd, Iran. Postal code: 8917145438, Tel: +98.3517237280, Fax: +98.3515231421, Email: hosain.afrand@yahoo.com

Abstract
Background: There has been much interest in the role of free radicals and oxidative stress in the pathogenesis of diabetes mellitus (DM). The aim of this study was to assess the possible association between genetic polymorphisms of the glutathione S-transferase-mu (GSTT1) and the risk of the development of DM in a sample of Yazdian females in Yazd, Iran.
Methods: This was a case-control study in which GSTT1 polymorphism was genotyped in 51 randomly selected DM patients and 50 randomly selected healthy controls among Yazdian females whose ages ranged from 40 to 70.
Results: The frequencies of GSTT1 null genotype and GSTT1 present were 8% and 92%, respectively, in the control samples. In patients with type 2 diabetes (T2DM), the frequencies of GSTT1 null genotype and GSTT1 present were 14% and 86%, respectively. There were higher levels of triglycerides (TG), fasting blood sugar (FBS), total cholesterol (TC), low density lipoprotein (LDL), body mass index (BMI), and high density lipoprotein (HDL) in patients with GSTT1 null genotype than in patients with the GSTT1 present genotype.
Conclusions: Our results indicated that the GSTT1 deletion polymorphism is a risk factor for T2DM. We did not determine any significant association between the GSTT1 null genotype and T2DM.

Keywords: glutathione S-transferase T1, genetic polymorphism, type 2 diabetes, female, Iran

1. Introduction
1.1. Background
It is estimated that 347 million adults in the world suffer from diabetes mellitus (DM) (1); DM has become an important cause of mortality and morbidity worldwide resulting from its direct clinical sequelae and increased mortality due to associated cardiovascular and kidney diseases (2-5). DM results from the body's ineffective use of insulin, which is determined by several different genes and environmental factors. The causes of DM are both various and complex, and one of these causes is oxidative stress, which arises as a result of an imbalance between
free radicals and antioxidant defenses (6). Since β-cells are very sensitive to cytotoxic stress because of their little expression of the antioxidant enzymes, they are susceptible to the oxidative stress, and the dysfunction of β-cells after undergoing oxidative stress may result in the development of DM (7). Glutathione S-transferases (GSTs) are the most important family of phase II isoenzymes known to detoxify a variety of electrophilic compounds, including carcinogens, chemotherapeutic drugs, environmental toxins, and DNA products generated by the damage caused to intracellular molecules by reactive oxygen species, chiefly by conjugating them with glutathione (8). GSTs play a major role in cellular antimutagen and antioxidant defense mechanisms (9). The glutathione S-transferase T1 (GSTT1) gene is polymorphic in humans, and the null genotype result in the absence of enzyme function contributes to the interpersonal differences in response to xenobiotics.

1.2. Statement of the problem and objectives

The prevalence and incidence of type 2 diabetes mellitus (T2DM) are high in the Middle Eastern countries (10), and it has been estimated that these countries will have the largest increases in the prevalence of diabetes by 2030 (11). The prevalence of T2DM was determined to be 14.52% in Yazd, Iran (12). Environmental factors, such as urbanization and subsequent westernization of lifestyles, and genetic susceptibility are considered as possible etiologies for the T2DM epidemic in Asia (13). In recent years, many studies have assessed the associations between DM and GSTT1 polymorphism (14-19). Ramprasath and colleagues demonstrated significant associations between GSTM1/GSTT1 null genotypes and DM risk (18), and similar results also were reported in other studies (14). However, some studies reported different conclusions and showed that there were no obvious associations between GSTT1 null genotype and DM risk (15-19). In Yazd, Dadbinpour examined the deletion of GSTT1 and GSTM1 genes in 57 diabetic patients with retinopathy and 58 diabetic patients without retinopathy. They indicated that there was a significant relationship between GSTM1 null genotype with the retinopathy side effect of T2DM. However, there was no significant relationship between GSTT1 null genotypes with retinopathy in T2DM (20). Thus, it remains unclear whether there are significant associations between GSTT1 polymorphism and the risk of DM. Recently, the GSTT1 null genotype interacting with current-smoking status was shown in two different studies to be a genetic risk factor for the development of T2DM and its cardiovascular complications (17, 18). In the Sinai area of Egypt, in a study of 100 T2DM patients and 100 healthy controls, matched for age, gender, and origin, the proportion of the GSTT1 null genotype was significantly greater in the diabetic patients than in the controls. It was reported that the risk of having T2DM increased by a factor of 3.17 in patients who had null polymorphism compared to those with the normal genotype of this gene (P = 0.009) (19). The rationale for the study was that the contribution of GSTT1 polymorphism to the risk of the development of T2DM is currently unknown. The aim of this study was to determine the association between the GSTT1 genotype and T2DM among the sample of the female population of Yazd, Iran.

2. Material and Methods

2.1. Study setting and design

In this case-control study conducted at the Yazd Diabetes Research Center, 51 women with T2DM were selected from the subjects who participated in a community-based, cross-sectional study of the Yazdian population of Yazd, Iran. In that cross-sectional study 51, women met the criteria established by the American Diabetes Association (ADA) for a diagnosis of diabetes, and all of them enrolled in the current study. To facilitate equal sampling, a control group of 50 healthy women who did not meet ADA’s criteria for a diagnosis of diabetes were selected randomly from the same geographic region. The size of the sample was determined by the following formula for a one-sided hypothesis test:

\[ n = \frac{(Z_{\alpha})^2 2\bar{p}(1-\bar{p}) + Z_{\beta}^2 \sqrt{p_1(1-p_1) + p_2(1-p_2)}}{(p_1 - p_2)^2} \]

\[ \bar{p} = \frac{p_1 + p_2}{2} \]

Where \( n \) is the size of the sample, \( Z = 1.64 \), \( \alpha \) is the significance level of the test (0.05), \( \beta \) is the probability of failing to detect a shift of one standard deviation (0.20), \( p_1 \) is the proportion for control group (0.12), and \( p_2 \) is the proportion for the cases (0.29). The values of \( p_1 \) and \( p_2 \) were hypothesized based on past studies.
2.2. Anthropometric variables and biochemical assays of blood
The subjects’ weights were measured to the nearest 0.1 kg using a calibrated scale (Seca 220, Seca GmbH & Co. KG., Hamburg, Germany) with the subjects wearing light clothing and standing in an upright position. The subjects’ heights were measured to the nearest 0.5 cm using a standard stadiometer (Seca 220, Seca GmbH & Co. KG., Hamburg, Germany) while the subjects were not wearing shoes. BMI was calculated by dividing the subjects’ weights (kg) by their height squared (m²). After a 10-minute rest, the subjects’ blood pressures (BPs) were measured twice (on a single occasion) by a standard mercury sphygmomanometer. The measurements were made to an accuracy of the nearest 2 mmHg with the subjects in a seated position. After 12-14 hours of overnight fasting, venous blood samples were taken from the subjects and analyzed in the laboratory of the Yazd Diabetes Research Center. An oral glucose tolerance test (OGTT) was conducted using a 75-gm oral dosage of glucose powder. Blood levels of glucose, triglycerides (TGs), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), urea, creatinine (Cr), and uric acid were measured by an autoanalyzer (AMS Autolab, Italy) using pertinent Pars Azmun kits (Pars Azmun Co., Tehran, Iran), i.e., GOD-PAP for glucose, CHOD-PAP for TC, GPO-PAP for TG, ENZYMATIC for LDL, and PERCIPITANT for HDL. The latest criteria established by the ADA were used for the diagnosis of DM in the subjects (21).

2.3. DNA extraction and genotyping
Blood was collected into EDTA-containing tubes, and DNA was extracted from the lymphocytes using a high-purity template preparation kit (Roche Diagnostics, GmbH, Mannheim, Germany). The characterization of GSTT1 polymorphism was performed using a real-time polymerase chain reaction (PCR) with a Light Cycler instrument and hybridization probes in combination with the Light Cycler DNA master hybridization probes kit (Roche Diagnostics). Both the PCR primers and hybridization probes were synthesized by TIB MOLBIOL (Berlin, Germany). The PCR conditions were essentially the same as those described by Ko and colleagues (22) and included 4 mmol L⁻¹ of MgCl₂ (magnesium chloride), 0.2 mmolL⁻¹ of each hybridization probe, 10 pmol of each PCR primer, 2 µl of the Light Cycler DNA master hybridization mix, and 50 ng of genomic DNA in a final volume of 20 µl. The fluorescence signal was plotted against temperature to give melting curves for each sample.

2.4. Ethical considerations
The study’s protocol was approved by the Medical Ethics Committee of Yazd Islamic Azad University of Medical Sciences. Written informed consent forms were obtained from all participants.

2.5 Statistical analyses
Allele distributions were compared using chi-squared tests. The Student’s t-test was used to determine differences in the means of age. P values < 0.05 were considered statistically significant. The associations of the GSTT1 polymorphism in the study group and in the control subjects were modeled using binary logistic regression analysis. Odds ratios (ORs) and confidence intervals (CIs) were used to analyze the relationship of the GSTT1 genotype in patients with T2DM compared to the control groups. SPSS version 17 (SPSS Inc., Chicago, Illinois, United States) was used to analyze the data.

3. Results
A total of 101 individuals (51 patients with T2DM and 50 controls) were genotyped for the GSTT1. The frequency distribution of GSTT1 genotype in healthy subjects and patients was determined by using real-time PCR. The mean ages of the patients and controls were 52.4±11.2 and 59.4±9.9, respectively.

3.1. Association between GSTT1 genotype profile and the development of T2DM
In the control samples, the frequencies of GSTT1 null genotype and GSTT1 present were 8 and 92%, respectively, while in the patients with T2DM, the frequencies were 14 and 86%, respectively (OR = 1.97, 95% CI = 0.51-7.52, P = 0.31) (Table 1).

3.2. Anthropometric and clinical variables according to GSTT1 genotype
We investigated the clinical parameters that accompanied the high risk genotype (GSTT1 null genotype) compared to non-risk genotype (GSTT1 present) in patients and controls (Table 2). The participants who had the GSTT1 present genotype had higher levels of fasting blood sugar (FBS), TC, Urea, Cr, BMI, and HDL than the GSTT1 null genotype in the controls. In the patients, there were higher levels of TC, TG, FBS, HDL, and LDL in the GSTT1
null genotype than in the GSTT1 present genotype. We also showed that there were higher levels of TG, LDL, FBS, Urea, Cr, and BMI in the controls with GSTT1 null genotype than in the GSTT1 null genotype in the patients.

**Table 1.** Association between GST genotype profile and the development of metabolic syndrome

| Locus | genotype | Patients (n = 51) (%) | Controls (n = 50) (%) | Odds ratio (95% CI) | P-value |
|-------|----------|---------------------|----------------------|---------------------|---------|
| GSTT1 | Present  | 43 (86)             | 46 (92)              | 1.00 (Ref)          |         |
|       | Null     | 7 (14)              | 4 (8)                | 1.97 (0.51-7.52)    | 0.31    |

n: number of sample

**Table 2.** Anthropometric and metabolic variables according to GSTM1 genotype

| Clinical parameters | Case | control |
|---------------------|------|---------|
|                     | Present (n = 43) | Null (n = 7) | P-value | Present (n = 46) | Null (n = 4) | P-value |
| Age (years)         | 52±11.1 | 55±12.5 | 0.519 | 51.5±10.6 | 58.7±6.8 | 0.884 |
| FBS (mg/dl)         | 98.1±15.8 | 101.5±20.7 | 0.613 | 103.3±9 | 103.2±13.8 | 0.816 |
| TG (mg/dl)          | 154±52.8 | 167.5±57.3 | 0.539 | 185.7±43.8 | 201.2±58.8 | 0.511 |
| TC (mg/dl)          | 204.7±38.9 | 219±50 | 0.392 | 123.6±22.6 | 108.2±36.7 | 0.220 |
| LDL (mg/dl)         | 131.2±22.1 | 137.2±29.7 | 0.529 | 171.2±76 | 187±42.7 | 0.686 |
| HDL (mg/dl)         | 40.7±7.8 | 42.7±9.8 | 0.419 | 38.7±9.8 | 34.7±5.9 | 0.433 |
| Urea (mg/dl)        | 32±7.01 | 30±7.7 | 0.176 | 33.4±6.3 | 30.2±4 | 0.197 |
| Cr (mg/dl)          | 0.93±0.16 | 0.88±0.06 | 0.40 | 0.98±0.2 | 0.90±0.02 | 0.689 |
| BMI (kg/m²)         | 25.7±3.1 | 22.5±5.1 | 0.026 | 26.7±4.2 | 25.3±3.6 | 0.527 |

Data are reported as means ± S.D.; n: number of samples; FBS: fasting blood glucose; TG: triglyceride; TC: total cholesterol; LDL: low density lipoprotein; HDL: high density lipoprotein; Cr: creatinine; BMI: body mass index

**4. Discussion**

**4.1. Association between GSTT1 genotype profile and the development of T2DM**

In this case-controlled study, GSTT1 deletion polymorphism was evaluated for its association with susceptibility to T2DM. We demonstrated an association of the GSTT1 null genotype with an increased risk of T2DM. The distributions of the GSTT1 null genotypes were not significantly different for the patients and the control group. The deletion frequency of GSTT1 in the control group (8%) was lower than the frequencies obtained in a study conducted by Arruda and colleagues in Brazil, i.e., 18 to 20%, which might have been due to ethnic differences among regions of the Brazilian population (23). In addition, diabetic patients had a higher frequency of the GSTT1 null genotype (29.2%) than healthy subjects (12.2%). Our study showed that the GSTT1 null genotype resulted in almost a two-fold increase in the risk T2DM. Thus, individuals may have decreased antioxidant defenses when this genotype was deleted. Furthermore, it has been well documented that a GSTT1 present genotype can provide protection against the development of T2DM (14, 24, and 25). These results suggest that the GSTT1 deletion polymorphism may play a role in the pathogenesis of T2DM. It also was found that there was no association of GSTT1 with susceptibility to T2DM. There are studies that have reported significant association between both null genotypes of GST and T2DM (14, 18), but others have indicated that there is no association between GSTT1 and GSTM1 polymorphisms and T2DM (17, 25). In addition, others studies have shown that only the GSTM1 null genotype may play a significant role in the aetopathogenesis of T2DM (16, 19). In a study of the Turkish population (19), the authors suggested that the GSTM1 gene may be a useful marker in the prediction of T2DM susceptibility. The OR obtained for the GSTM1 null genotype was 3.7, indicating an association between the incidence of diabetes and the GSTM1 deletion polymorphism, and a study of the population in India reported a significant association of the GSTM1 null genotype (OR = 2.042) with T2DM and no significant association with GSTT1 (16).

Despite some divergence in the data in the literature, the GSTT1 null genotype and the GSTT1 null/GSTM1 null genotypes consistently have been considered risk factors for the development of T2DM, as reported by a meta-analysis study (26). In a study conducted by Amer and colleagues (14), the authors found significant differences between the double present genotype (+ / +) and either or both null genotypes of diabetics (P = 0.002 and P = 0.009, respectively) when compared to the control subjects. They confirmed that GSTT1 and GSTM1 cooperatively play a protective role against the development of T2DM. Furthermore, in the Indian study (18), the results implied that the risk for T2DM was almost doubled with the combination of either null genotypes of GSTM1/GSTT1 (+ /2 or 2/ +).
4.2. Anthropometric and clinical variables according to GSTT1 genotype
The evaluation of the association of clinical variables with GST polymorphism in diabetic patients showed that the GSTT1 null genotype relates to significantly higher levels of FBS, TC, TG, LDL, and HDL than the present genotype. This allows us to infer that the absence of GSTT1 may contribute to type 2 diabetes-related complications, such as dyslipidemia. These results are consistent with studies conducted on the Chinese population (24), the Egyptian population (14), and the Indian population (18), in which a GSTT1 null genotype association with lipid alterations also was observed. Thus, the GSTT1 gene could be added to a set of potential genetic markers to identify individuals at increased risk for developing T2DM and complications associated with dyslipidemia in diabetic patients. While a significant relationship between the GSTT1 deletion polymorphism and susceptibility to disease was not verified, it was possible to observe the influence of this polymorphism on clinical parameters related to TG and HDL. Therefore, the deletion of GSTT1 can have relevance in the clinical course of diabetic patients, since those two variables, along with lipid profile, are focal points for disease monitoring to prevent T2DM complications. The mechanisms underlying the results of association obtained in this and other studies still should be investigated in future research.

4.3. Study limitations
This study had various limitations. First, the small number of subjects was a major limitation. Therefore, the study may not have had enough power to clarify whether the GSTT1 polymorphism is related with risk of acquiring DM, and future studies with larger patient samples that include both genders and use a longitudinal design are necessary. These findings may not be generalizable to other populations, given that differences in racial attitudes toward lifestyle may influence these results. One strength of the study is that, to the best of our knowledge, it is the first study to investigate the association between GSTT1 polymorphism and DM in a sample of Yazdian females in Yazd, Iran.

5. Conclusions
The most obvious finding to emerge from this study was that the GSTT1 deletion polymorphism is associated with a greater risk of acquiring diabetes. However, we observed no significant association between the GSTT1 null genotype and DM, suggesting that the GSTT1 gene may not play a significant role in the aetiopathogeneses of DM. It is recommended that future studies investigate the role of the GSTT1 and its combination with other GST genotypes in the pathogenesis of DM and its associated complications in large-scale cohorts in a different population.

Acknowledgments
The authors are sincerely grateful for all participants in the study. Special thanks to Mohammad Hossein Ahmadieh (Department of Epidemiology, Shahid Sadoughi University of Medical Sciences) for his perfect assistance in statistical analysis.

Conflict of Interest:
There is no conflict of interest to be declared.

Authors' contributions:
All of authors contributed to this project and article equally. All authors read and approved the final manuscript.

References:
1. Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, et al. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. Lancet. 2011 Jul 2; 378(9785):31-40. doi: http://dx.doi.org/10.1016/S0140-6736(11)60679-X , PMID: 21705069
2. Danaei G, Lawes CM, Vander Hoorn S, Murray CJ, Ezzati M. Global and regional mortality from ischaemic heart disease and stroke attributable to higher-than-optimum blood glucose concentration: comparative risk assessment. Lancet. 2006 Nov 11; 368(9548):1651-9. doi: http://dx.doi.org/10.1016/S0140-6736(06)69700-6 , PMID: 17098083
3. Khaw KT, Wareham N, Bingham S, Luben R, Welch A, Day N. Association of hemoglobin A1c with cardiovascular disease and mortality in adults: the European prospective investigation into cancer in Norfolk. Ann Intern Med. 2004 Sep 21; 141(6):413-20. doi: http://dx.doi.org/10.7326/0003-4819-141-6-200409210-00006 . PMID: 15381514

4. Lawes CM, Parag V, Bennett DA, Suh I, Lam TH, Whitlock G, et al. Blood glucose and risk of cardiovascular disease in the Asia Pacific region. Diabetes care. 2004 Dec; 27(12):2836-42. doi: http://dx.doi.org/10.2337/diacare.27.12.2836 . PMID: 15652194

5. Nakagami T. Hyperglycaemia and mortality from all causes and from cardiovascular disease in five populations of Asian origin. Diabetologia. 2004 Mar; 47(3):385-94. doi: http://dx.doi.org/10.1007/s00125-004-1334-6 . PMID: 14985967

6. West IC. Radicals and oxidative stress in diabetes. Diabet Med. 2000 Mar; 17(3):171-80. doi: http://dx.doi.org/10.1046/j.1464-5491.2000.00259.x . PMID: 10784220

7. Tiedge M, Lortz S, Drinkgern J, Lenzen S. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. Diabetes. 1997 Nov; 46(11):1733-42. doi: http://dx.doi.org/10.2337/diabeha.46.11.1733 . PMID: 9356019

8. Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annu Rev Pharmacol Toxicol. 2005; 45:51-88. doi: http://dx.doi.org/10.1146/annurev.pharmtox.45.120403.095857 . PMID: 15822171

9. Baiocco P, Gourlay LJ, Angelucci F, Fontaine J, Herve M, Miele AE, et al. Probing the mechanism of GSH activation in Schistosoma haematobium glutathione-S-transferase by site-directed mutagenesis and X-ray crystallography. J Mol Biol. 2006 Jul 14; 360(3):678-89. doi: http://dx.doi.org/10.1016/j.jmb.2006.05.040 . PMID: 16777141

10. Harati H, Hadaegh F, Saadat N, Azizi F. Population-based incidence of Type 2 diabetes and its associated risk factors: results from a six-year cohort study in Iran. BMC public health. 2009;9:186. doi: http://dx.doi.org/10.1186/1471-2458-9-186 . PubMed PMID: 19531260. Pubmed Central PMCID: PMC2708154. Epub 2009/06/18. eng.

11. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest. 2006 Jul; 116(7):1784-92. doi: http://dx.doi.org/10.1172/JCI29126 . PubMed PMID: 16823476. Pubmed Central PMCID: PMC1483172. Epub 2006/07/11. eng.

12. Geisler SA, Olshan AF. GSTM1, GSTT1, and the risk of squamous cell carcinoma of the head and neck: a mini-HuGE review. Am J Epidemiol. 2001 Jul 15; 154(2):95-105. doi: http://dx.doi.org/10.1093/aje/154.2.95 . Pubmed PMID: 11447041. Epub 2001/07/12. eng.

13. Spencer-Jones NJ, Wang X, Snieder H, Spector TD, Carter ND, O'Dell SD. Protein tyrosine phosphatase-1B gene PTPN1: selection of tagging single nucleotide polymorphisms and association with body fat, insulin sensitivity, and the metabolic syndrome in a normal female population. Diabetes. 2005 Nov; 54(11):3296-304. doi: http://dx.doi.org/10.2337/diabetes.54.11.3296 . PubMed PMID: 16249458. Epub 2005/10/27. eng.

14. Amer MA, Ghattas MH, Abo-Elmatty DM, Abou-El-Ela SH. Influence of glutathione S-transferase polymorphisms on type-2 diabetes mellitus risk. Genet Mol Res. 2011; 10(4):3722-30. doi: http://dx.doi.org/10.4238/2011.October.31.14 . PMID: 22058002

15. Bekris LM, Shephard C, Peterson M, Hoehna J, Van Yserloo B, Rutledge E, et al. Glutathione-s-transferase M1 and T1 polymorphisms and associations with type 1 diabetes age-at-onset. Autoimmunity. 2005 Dec; 38(8):567-75. doi: http://dx.doi.org/10.1080/0896930500407238 . PMID: 16390810

16. Bid HK, Konwar R, Saxena M, Chaudhari P, Agrawal CG, Banerjee M. Association of glutathione S-transferase (GSTM1, T1 and P1) gene polymorphisms with type 2 diabetes mellitus in north Indian population. J Postgrad Med. 2010 Jul-Sep; 56(3):176-81. doi: http://dx.doi.org/10.4103/0022-3859.68633 . PMID: 20739761

17. Datta SK, Kumar V, Pathak R, Tripathi AK, Ahmed RS, Kalra OP, et al. Association of glutathione S-transferase M1 and T1 gene polymorphism with oxidative stress in diabetic and non-diabetic chronic kidney disease. Ren Fail. 2010; 32(10):1189-95. doi: http://dx.doi.org/10.3109/0886022X.2010.517348 . PMID: 20954980

18. Ramprath S, Senthil Murugan P, Prabakaran AD, Gomathi P, Rathinavel A, Selvam GS. Potential risk modifications of GSTT1, GSTM1 and GSTP1 (glutathione-S-transf erase) variants and their association to CAD in patients with type-2 diabetes. Biochem Biophys Res Commun. 2011 Apr 1; 407(1):49-53. doi: http://dx.doi.org/10.1016/j.bbrc.2011.02.097 . PMID: 21352813
19. Yalin S, Hatungil R, Tamer L, Ates NA, Dogruer N, Yildirim H, et al. Glutathione S-transferase gene polymorphisms in Turkish patients with diabetes mellitus. Cell Biochem Funct. 2007 Sep-Oct; 25(5):509-13. doi: http://dx.doi.org/10.1002/cbf.1339 . PMID: 16927413
20. Dadbinpour A, Sheikhha MH, Darbouy M, Afkhami-Ardekani M. Investigating GSTT1 and GSTM1 null genotype as the risk factor of diabetes type 2 retinopathy. J Diabetes Metab Disord. 2013; 12(1):48. doi: http://dx.doi.org/10.1186/2251-6581-12-48 . PubMed PMID: 24355557. Pubmed Central PMCID: PMC3937192. Epub 2013/12/21. eng.
21. Diagnosis and classification of diabetes mellitus. Diabetes care 2013; 36 Suppl 1:S67-74. doi: http://dx.doi.org/10.2337/dc13-S067 . PMID: 23264425. PMCID: PMC3537273
22. Ko Y, Koch B, Harth V, Sachinidis A, Thier R, Vetter H, et al. Rapid analysis of GSTM1, GSTT1 and GSTP1 polymorphisms using real-time polymerase chain reaction. Pharmacogenetics. 2000 Apr;10(3):271-4. doi: http://dx.doi.org/10.1097/00008571-200004000-00009 . PubMed PMID: 10803684. Epub 2000/05/10. eng.
23. Arruda VR, Grignolli CE, Goncalves MS, Soares MC, Menezes R, Saad ST, et al. Prevalence of homozygosity for the deleted alleles of glutathione S-transferase mu (GSTM1) and theta (GSTT1) among distinct ethnic groups from Brazil: relevance to environmental carcinogenesis? Clin Genet. 1998 Sep; 54(3):210-4. doi: http://dx.doi.org/10.1111/j.1399-0004.1998.tb04286.x . PMID: 9788723
24. Wang G, Zhang L, Li Q. Genetic polymorphisms of GSTT1, GSTM1, and NQO1 genes and diabetes mellitus risk in Chinese population. Biochem Biophys Res Commun. 2006 Mar 10; 341(2):310-3. doi: http://dx.doi.org/10.1016/j.bbrc.2005.12.195 . PMID: 16413497
25. Hori M, Oniki K, Ueda K, Goto S, Mihara S, Marubayashi T, et al. Combined glutathione S-transferase T1 and M1 positive genotypes afford protection against type 2 diabetes in Japanese. Pharmacogenomics. 2007 Oct; 8(10):1307-14. doi: http://dx.doi.org/10.2217/14622416.8.10.1307 . PMID: 17979505
26. Zhang J, Liu H, Yan H, Huang G, Wang B. Null genotypes of GSTM1 and GSTT1 contribute to increased risk of diabetes mellitus: a meta-analysis. Gene. 2013 Apr 15; 518(2):405-11. doi: http://dx.doi.org/10.1016/j.gene.2012.12.086 . PMID: 23296061