DOI: 10.15386/cjmed-436

COMBINED GLUTATHIONE S TRANSFERASE M1/T1 NULL GENOTYPES IS ASSOCIATED WITH TYPE 2 DIABETES MELLITUS

MIHAI D. POROJAN[1], CORNELIA BALA[2], ROXANA ILIES[3], ANDREEA CATANA[3], RADU A. POPP[3], DAN L. DUMITRASCU[1]

[1]Department of Internal Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[2]Department of Diabetes, Nutrition and Metabolic Diseases, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[3]Department of Molecular Sciences, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

Abstract

Background. *Due to new genetic insights, a considerably large number of genes and polymorphic gene variants are screened and linked with the complex pathogenesis of type 2 diabetes (DM).*

Our study aimed to investigate the association between the two isoforms of the glutathione S-transferase genes (Glutathione S transferase isoemzyme type M1-GSTM1 and Glutathione S transferase isoemzyme type T1-GSTT1) and the prevalence of DM in the Northern Romanian population.

Methods. *We conducted a cross-sectional, randomized, case-control study evaluating the frequency of GSTM1 and GSTT1 null alleles in patients diagnosed with DM. A total of 106 patients diagnosed with DM and 124 healthy controls were included in the study. GSTM1 and GSTT1 null alleles genotyping was carried out using Multiplex PCR amplification of relevant gene fragments, followed by gel electrophoresis analysis of the resulting amplicons.*

Results. *Molecular analysis did not reveal an increased frequency of the null GSTM1 and GSTT1 alleles (mutant genotypes) respectively in the DM group compared to controls (p=0.171, OR=1.444 CI=0.852-2.447; p=0.647, OR=0.854, CI=0.436-1.673). Nevertheless, the combined GSTM1/GSTT1 null genotypes were statistically significantly higher in DM patients compared to control subjects (p=0.0021, OR=0.313, CI=0.149-0.655)*

Conclusions. *The main finding of our study is that the combined, double GSTM1/GSTT1 null genotypes are to be considered among the polymorphic genetic risk factors for type 2 DM.*

Keywords*: G*STT1, GSTM1 null alleles, diabetes mellitus type 2, Romanian population.

Introduction

Type 2 diabetes mellitus (DM), is a multifactorial, chronic adult-onset condition characterized by insufficient insulin secretion and inefficient insulin action, thus presenting the common manifestation of hyperglycemia [1].

According to WHO (2015), 374 million people around the world suffer from a form of diabetes, of which 90% have DM and estimates that diabetes related deaths will double between 2005 and 2030, therefore this condition and all its comorbidities are an important global health issue [2]. In 2014 Romania reported 9.3 prevalence for type

Manuscript received: 24.03.2015
Accepted: 07.04.2015
Address for correspondence: catanaandreea@gmail.com

2 DM, with a total of 1.5 million diagnosed individuals [3].

Several studies have shown that individuals with a decreased antioxidant capacity are at higher risk of developing DM [4,5].

Glutathione S-transferases (GSTs) are the primary cellular defense mechanism against reactive oxygen species as phase II detoxifying isoenzymes known to metabolize a variety of electrophilic compounds, including drugs, carcinogens, toxins and ROS generated DNA products. Eight members of soluble GSTs have been identified in humans, named Mu (M), Kappa (K), Alpha (A), Pi, accounting for 4% of the total cytosolic proteins in the liver [6,7]. Among the numerous candidate genes involved in DM pathogenesis, the GST isoemzyme family has been intensely studied in all types of diabetes due to its potential modulating roles in individual susceptibility to multifactorial, environmentally induced disorders. Among these, glutathione S transferase isoemzyme type M1 (GSTM1) and glutathione S transferase isoemzyme type T1 (GSTT1) genes are extremely polymorphic in humans and the null genotypes resulting from loss of function mutations are accompanied by lack of enzyme activity [8,9].

Several studies investigated the possible relation between GSTM1 and GSTT1 null alleles and T2DM; Turkish, North Indian and Southern Iran populations highlighted an association between GSTM1 deletion and DM whereas Egyptian, Chinese, and Brazilian population reported significant association between GSTM1/GSTT1 null genotypes and type 2 diabetes [10-15].

In a recent studies and meta-analysis, GSTM1 and GSTT1 null genotypes have been associated with a higher risk of developing DM in Japanese and South Indian and African population [16-19].

The aim of the present study is to determine the possible association between the incidence of type 2 DM and gene polymorphisms of glutathione S-transferase GSTM1 and GSTT1 in a population group of Northern Romania and to see if GSTM1/GSTT1 null genotypes may be considered a genetic risk factor for DM.

Materials and methods

Patients and controls

This is a molecular epidemiologic case-controlled study, in which we included a group of 106 patients diagnosed with type 2 DM (patients of Diabetes Clinic of the Emergency County Hospital of Cluj) and 124 healthy controls (non-diabetic patients recruited from the Internal Medicine Department of the Emergency County Hospital Cluj). In order to obtain homogeneity regarding proportion and the range of ages and gender we selected and approximate similar age/gender matched subject for each of the subject, respectively control groups.

Ethical issues. The current study was approved by the Local Ethics Committee. The study was conducted according to the Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association Declaration of Helsinki. Written informed consent was obtained from each participant after a brief and clarified explanation about the survey.

Molecular analysis of GSTM1 and GSTT1 genes *(Protocol adapted after Bid HK et al.)* [20]

Genotyping for GSTM1 and GSTT1 alleles was carried out using a Multiplex PCR protocol (18).

A total volume of 25 µl reaction commercial mixture containing 12.5 µl 2xPCR Master Mix *(Thermo Fischer Scientific Inc., MA, USA),* 25 µl of free nuclease water and 10 µm of each specific forward and reverse primers.

GSTM1 and GSTT1 null allele identification required the use of 3 pairs of primers

(5'-GAACTCCCTGAAAAGCTAAAGC-3'; 5'-GTTGGGCTCAAATATAGGGTGG-3' and

5'-TTCCTTACTGGTCCTCACATCTC-3'; 5'-TCACCGGATCATGGCCAGCA-3' (Eurogentec Bg.)

As an internal standard amplification marker, β Globin was co-amplified using another pair of primers (5'-*CAACTTCATCCACGTTCACC-3'* and *5'-GAAGAGCCAAGGACAGGTAC-3' (Eurogentec, Bg.)* Termocycling conditions consisted of an initial denaturation at 94°C for 5 minutes followed by 35 repetitive cycles each with a DNA denaturation at 94°C for 1 minute, primers annealing for 1 minute at 58°C, polymerization at 72°C for 1 minute and a final elongation for 10 minutes at 72°C. *(Mastercycler Gradient®, Eppendorf, Germany).* The resulted amplified DNA products were then analyzed by agarose 2% gel electrophoresis *(MetaPhor® Agarose, Cambrex Bio Science Inc.),* obtaining 3 different fragments, one of 215 bp (for GSTM1gene), one of 480 bp (for GSTT1 gene) and a 268 bp fragment (for the β Globin gene control fragment). The absence of the amplification specific products revealed the presence of the mutant, null genotypes. We must specify that the current protocol easily identifies the GSTT1 and GSTM1 homozygous null genotypes but cannot distinguish between GSTM1 and GSTT1 homozygous and heterozygous positive genotypes (Fig. 1).

Statistical analysis

Statistical analysis used a licensed Graphpad software. Odds ration (OR) with 95% confidence limits calculated by logistic regression. GSTM1 and GSTT1 genotypes were classified as either null (homozygous deletion) or wild type, non-deleted. For a more accurate risk evaluation, Z and P values were also calculated.

Results

A total of 106 subjects with DM and 124 controls were investigated in the current study. The proportions of genders in case and control group proved not significantly different (Z-statistics=1.515, p-value=0.1298). The mean age of subjects with DM was 64.43±9.72 years old and

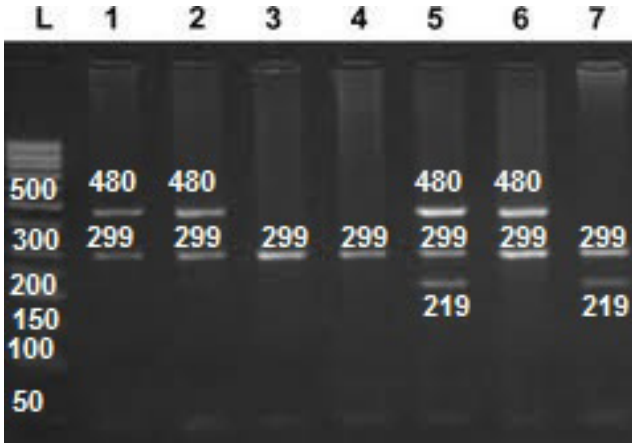

Figure 1. Electrophoretic analysis for GSTM1 and GSTT1 null genotypes (Multiplex PCR).
Lanes 1, 2, 6: GSTT1 positive genotype, GSTM1 null genotype, **Lanes 3, 4:** GSTT1/GSTM1 null genotypes; **Lane 5:** GSTM1/GSTT1 positive genotypes **Lane 7:** GSTM1 positive, GSTT1 null genotype, **Lane L:** 50 bp DNA ladder marker.

of 65.31±5.92 years old for controls with no statistically significant differences (t-statistics=-0.80, p-value=0.4220) (Table 1). The studied genotype followed Hardy-Weinberg equilibrium of genetic distribution (Chi-square test p=0.08).

Statistical analysis did not reveal an increased frequency of GSTM1, respectively GSTT1 null genotypes (mutant alleles) in the DM group compared to controls (p=0.171, OR=1.444 CI=0.852-2.447; p=0.647, OR=0.854, CI=0.436-1.673). Nevertheless, the combined GSTM1/GSTT1 null genotypes were statistically significant higher in DM patients compared to control subjects (p=0.0021, OR=0.313, CI=0.149-0.655) (Table II).

The genotype-gender associated statistical data revealed a higher frequency for the combined GSTM1/GSTT1 null genotypes in women with diabetes compared to male subjects (p=0.037, OR=0.245, CI=0.065-0.918). There was also an increased frequency of the GSTT1 null genotypes in males diabetics compared to controls (p=0.021, OR=0.343. CI=0.138-0.851).

In cases of the GSTM1-null genotype and combined GSTM1/GSTT1 null genotypes there were higher levels of HbA1c compared to GSTM1/GSTT1 positive genotypes. (p=0.028, OR=0.271; CI=0.124- 0.742) (Table III).

Table I. Parameters of study groups.

	Type 2 DM Group	Control Group	p-value
Number (M/F)	106 (52/54)	124 (62/62)	0.1298
Age (years)	64.43±9.72	65.31±5.92	0.4220
Weight (kg)	85.4 ±13.2	81.72± 12.7	0.2456
Body mass index (kg/m2)	27.84±2.1	25.1 ± 5.1	0.3561
HbA1c (%)	7.6±1.3	NA	NA

Table II. Distribution of single GSTM1, GSTT1 and combined GSTM1/GSTT1 null genotypes among patients with type 2 DM and controls.

Genotype	p	Odd ratios	95% IC	
			Inferior limit	Superior limit
GSTM1 null	0.171	1.444	0.852	2.447
GSTT1 null	0.647	0.854	0.436	1.673
GSTM1/GSTT1 null	0.0021*	0.313	0.149	0.655

* Significant statistical association for p<0.050.

Discussion

Type 2 DM is one of the most common chronic diseases in modern countries and represents a health problem and a major epidemic during the past decades. [21,22]

Many studies have evaluated the association between GST polymorphisms and to our knowledge this is the first study evaluating the association of GSTM1 and GSTT1 null genotypes and type 2 DM in the Romanian population.

We hypothesized that the genetic variability of GST detoxifying enzymes regulating oxidative stress could be involved in development of DM.

The GSTT1 and GSTM1 combined deletion polymorphisms (null alleles), which associate abolished

Table III. Significant Gender and HbA1C, single GSTM1, GSTT1 and combined GSTM1/GSTT1 null genotypes associations.

GENOTYPE T2DM	Gender		HbA1C >7%	
	Male	Female	Male	Female
GSTM1null	-	-	*P=0.028** *OR=0.271* *CI=0.124-0.742*	-
GSTT1 null	*P=0.021** *OR=0.343* *IC=0.138-0.851*	-	-	-
GSTM1/GSTT1 null	-	*P=0.037** *OR=0.245* *IC=0.065-0.918*	*P= 0.013** *OR=0.436* *IC=0.279-0.862*	

* Significant statistical association for p<0.050.
HbA1C – Glycosylated haemoglobin
DM- Type 2 Diabetes Mellitus

enzyme activity, have been associated with type 2 DM when compared to control subjects. However, single GSTM1 and GSTT1 mutant variants did not have a higher frequency in diabetic patients compared to controls [23].

Our results come in agreement with other studies that highlighted the same results, placing combined GSTM1/GSTT1 deleted genotypes among the inheritable risk factor for DM development [19-22,24,25].

Another finding of the current study is that a genotype-gender statistical association revealed a higher frequency for the combined GSTM1/GSTT1 null genotypes in women with diabetes compared to male subjects. Also, there was an increased frequency of the GSTT1 null genotypes in males diabetics compared to controls. There are only a few studies that evaluate the GST mutant genotype – gender in DM.

In conclusion, GSTM1 and GSTT1 mutant variants individually are not associated with DM; however, combined GSTM1/GSTT1 null genotypes could represent independent risk factor for DM. Additionally, GSTT1 null genotypes and combined GSTM1/GSTT1 null genotypes could be considered genetic risk factor in type 2 DM in males and women respectively. Testing for genetic markers in genes encoding antioxidant enzymes like GSTs, either individually or in combination with other polymorphic genetic variants possibly involved in DM pathogen, could be added to a genetic panel for identifying patients at higher risk for developing diabetes in clinical practice.

Our study has a series of limitations. First, the small number of subjects represents a major limitation, therefore the resulting data of the study may not be representative for the general population. For this purpose, future studies with larger number of subjects with different population ethnicity are required and a longitudinal design is also necessary. Another limitation is the lack of correlation with clinical parameters associated with type 2 DM (lifestyle, medication, associated pathology, complications, etc.).

Acknowledgements

This paper was published under the frame of European Social Found, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/138776.

References

1. Glamočlija U, Jevrić-Čaušević A. Genetic polymorphisms in diabetes: Influence on therapy with oral antidiabetics. Acta Pharmaceutica. 2010;60(4):387–406.
2. http://www.who.int/nmh/publications/ncd_report_chapter1.pdf
3. http://www.idf.org/worlddiabetesday/toolkit/gp/facts-figures
4. Gallou G, Ruelland A, Legras B, Maugendre D, Allannic H, Cloarec L. Plasma malondialdehyde in type 1 and type 2 diabetic patients. Clin Chim Acta. 1993;28(214):227–234.
5. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes. 1999;48:1–9.
6. Oakley A. Glutathione transferases: a structural perspective. Drug Metab Rev. 2011;43:138–151.
7. Wilce MC, Parker MW. Structure and function of glutathione S-transferases. Biochim Biophys Acta. 1994;1205:1–18.
8. Pemble S, Schroeder KR, Spencer SR, Meyer DJ, Hallier E, Bolt HM, et al. Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. Biochem J. 1994;300:271–276.
9. Seidegard J, Vorachek WR, Pero RW, Pearson WR. Hereditary differences in the expression of the human glutathione transferase active on trans-stilbene oxide are due to a gene deletion. Proc Natl Acad Sci USA. 1988;85:7293–7297.
10. Gönül N, Kadioglu E, Kocabaş NA, Ozkaya M, Karakaya AE, Karahalil B. The role of GSTM1, GSTT1, GSTP1, and OGG1 polymorphisms in type 2 diabetes mellitus risk: a case-control study in a Turkish population. Gene. 2012;505:121–7.
11. 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;56: 176–181.
12. Moasser E, Kazemi-Nezhad SR, Saadat M, Azarpira N. Study ofthe association between glutathione S-transferase (GSTM1, GSTT1, GSTP1) polymorphisms with type II diabetes mellitus in southern of Iran. Mol Biol Rep. 2012;39:10187–10192.
13. 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;10:3722–3730.
14. 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;341:310–313.
15. Pinheiro DS, Rocha Filho CR, Mundim CA, Júnior Pde M, Ulhoa CJ, Reis AA, et al. Evaluation of glutathione S-transferase (GSTM1 and GSTT1) deletion polymorphisms on type-2 diabetes mellitus risk. PLoS One. 2013;8:e76262.
16. Tang ST, Wang CJ, Tang HQ, Zhang Q, Wang Y. Evaluation of glutathione S-transferase genetic variants affecting type 2 diabetes susceptibility: a meta-analysis. Gene. 2013;530:301–308.
17. 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;8:1307–1314.
18. Ramprasath T, Murugan PS, Prabakaran AD, Gomathi P, Rathinavel A, Selvam GS. Potential risk modifications of GSTT1, GSTM1 and GSTP1 (glutathione S-transferases) variants and their association to CAD in patients with type 2 diabetes. Biochem Biophys Res Commun. 2011;47:49–53.
19. Amer MA, Ghattas MH, Abo-Elmatty DM, Abou-El-Ela SH. Evaluation of glutathione S-transferase P1 genetic variants affecting type-2 diabetes susceptibility and glycemic control. Arch Med Sci. 2012;8:631–636.
20. 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;56:176–181.
21. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27:1047-1053.
22. Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: a review of current trends. Oman Med J. 2012;27(4):269–273.
23. London SJ, Yuan JM, Chung FL, Gao YT, Coetzee GA, Ross RK, et al. Isothiocyanates, glutathione S-transferase M1 and T1

polymorphisms, and lung-cancer risk: a prospective study of men in Shanghai, China. Lancet. 2000;356: 724-729.
24. Elham Moasser. Influence of glutathione S-transferase gene polymorphism on the risk of type 2 diabetes mellitus retinopathy in south Iranian population. J Diabetes Metab. 2013,4:6
25. Vats P, Chandra H, Banerjee M. Glutathione S-transferase and Catalase gene polymorphisms with Type 2 diabetes mellitus. Dis Mol Med. 2013;1(3):46-53.