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Glutathione S-transferases gene polymorphism influence on the age of diabetes type 2 onset

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ABSTRACT

Introduction Type 2 diabetes (T2D) is a multifactorial disease affecting mostly adults older than 40 years. The aim of the study was to examine *GST* gene polymorphism influence on the risk of T2D, especially in young adults.

Research design and methods 200 diabetic patients and 221 healthy controls participated in this study. Three *GST* gene polymorphism have been analyzed: *GSTP1* (single-nucleotide polymorphism $Ile_{105}Val$), homozygous deletion of *GSTT1* (null/null) and *GSTM1* (null/null), using TaqMan real-time quantitative PCR.

Results The distribution of examined polymorphisms was similar in patient group and control group. Statistically significant differences were demonstrated for the combination of *GSTP1 Val/Val* and *GSTT1 null/null* genotypes between patients diagnosed before 40 years of age and healthy people (12.5% vs 0.9%, p=0.016). Moreover, all three examined gene polymorphism together (*GSTP1 Val/Val*, *GSTM1nul/null* and *GSTT1 null/null* genotype) was observed in 12.5% of patients diagnosed before 40 years of age and in 0.5% of healthy individuals (p=0.013).

Conclusion In conclusion, the results suggest that *GST* polymorphism may be one of the risk factors for developing T2D at a younger age than the T2D population average.

Significance of this study

What is already known about this subject?

- Most type 2 diabetes (T2D) cases are diagnosed after the age of 40 years.
- *GST* gene family polymorphism results in decreased cell protection against environmental pollutants, carcinogens, oxidative stress products and a wide spectrum of xenobiotics.
- Studies focusing on *GST* gene family polymorphism as a risk factor of T2D published in the last few years demonstrated ambiguous results.

What are the new findings?

- Our study identified association between *GST* gene family polymorphism and early onset of T2D.
- It was the first study of *GSTM1, GSTT1* and *GSTP1* gene polymorphisms in the Polish population of patients with diabetes.

How might these results change the focus of research or clinical practice?

- Genetic tests might be used to predict the risk of developing T2D in adults younger than 40 years if our results are confirmed on a larger cohort.

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INTRODUCTION

Type 2 diabetes (T2D) is a global public health problem that is evolving with the increasing prevalence of obesity, unhealthy lifestyle (especially western-style diet and low physical activity) and the population's aging problem. The disease affects mostly adults, but also children and adolescents, especially in high-income countries.[1] According to the International Diabetes Federation (IDF), T2D is recognized in over 90% of cases with diabetes mellitus.[2] In Poland in 2017, there were over 2 million adults suffering from T2D (IDF statistics)[3] and by 2030, the number will have increased to 2.5 million.[4] It is characterized by chronic hyperglycemia and other metabolic alterations resulting from a lack of insulin in the body and insulin resistance of tissues.[1 5] Pathogenesis of the disease is complex and involves not only environmental factors but also genetic vulnerability. Most of T2D cases are diagnosed after 40 years of age, especially in subjects with obesity or overweight following a western diet lifestyle,[4 6] but there is also a group of younger patients. The development of molecular technologies in the twenty-first century has allowed researchers to focus on individual genetic predispositions to T2D. It is considered that antioxidant and detoxification gene polymorphism play an important role in the risk of T2D.[7 8] Some of them are *GST* genes coding glutathione S-transferases which are phase II key detoxifying enzymes. These enzymes are involved in the glutathione-coupling reactions of a broad range of electrophilic substances, thus facilitating their detoxification, metabolism and excretion. That is why they play an important role in cell protection against environmental

pollutants, carcinogens, chemotherapeutics, oxidative stress products and a wide spectrum of xenobiotics. *GSTP1*, *GSTM1* and *GSTT1* gene polymorphisms result in low enzyme activity as reported for the *GST* gene family. Loss or reduction of enzyme activity leads to the reduction of the ability to neutralize toxins.[6 7] There have been many studies focused on *GST* gene polymorphism as a risk factor of T2D in last few years, but the results are ambiguous.[1 2 6–12] It is worth mentioning that only a few of them were conducted in Europe.[1 11 12] The aim of the study was to examine how an individual's genetic makeup can have an impact on the risk of T2D, especially in young adults.

RESEARCH DESIGN AND METHODS

Population sample

This study was conducted in Kielce (Poland). Sampling was done from June to December 2019. Patients were recruited from the diabetic outpatient center, the control was unrelated volunteers aged over 18 years with no diabetic symptoms, no history of fasting glucose level exceeded and no family history of glucose metabolism disorders. Patients were enrolled following the inclusion criteria: age over 18 years and diagnosis of T2D, the exclusion criteria being history of endocrine disorders, known malignancies, alcoholism, patients with diabetes secondary to chronic pancreatitis, Cushing's disease with treatment that can induce hyperglycemia, type 1 diabetes, pregnant and lactating women. The T2D diagnosis was determined by a diabetologist according to the (revised) criteria of the American Association of Diabetology.[13] All procedures of the study were conducted according to the principles of the Declaration of Helsinki.

After the subjects of both groups signed written consent forms for the genetic testing of *GST* genes, clinical data and blood samples were taken (test tubes with EDTA provided by Sarstedt) by a qualified nurse. Appropriately coded samples were frozen at −20°C until the time of genetic testing.

Genotyping

Peripheral blood leukocytes were the material for genetic testing. The genomic DNA was extracted from blood samples using the Genomic Micro AX Blood Gravity kit from A&A Biotechnology (Gdynia, Poland). The purity and concentration of the isolated DNA were evaluated spectrophotometrically at 260 nm and 280 nm (NanoDrop 2000, Thermo Fisher Scientific). Analysis of the single nucleotide polymorphism (rs1695) of the *GSTP1* gene was conducted using the TaqMan quantitative PCR (qPCR) method—endpoint genotyping (Assay ID C_3237198_20). The deletion of copies of genes *GSTT1* (Assay ID Hs00010004_cn) and *GSTM1* (Assay ID Hs02575461_cn) was analyzed using the qPCR relative quantification method with the TERT control gene. In all cases, the LightCycler 96 instrument and TaqMan primer/probe kit (produced by Life Technologies) were used. PCR amplification using ≈10 ng of genomic DNA was performed with an initial step of 95°C for 10 min followed by 50 cycles of 95°C for 15 s (denaturation step) and 60°C for 90 s (annealing and elongation steps).

Statistical analysis

Quantitative data are described by means, SD, medians, quartiles and range (minimum and maximum). Categorical data were summarized by frequencies and percentages. Group comparisons were performed using the χ^2 or Fisher's exact test for categorical variables, the t-test for quantitative, normally distributed variables or the Mann-Whitney test for quantitative, non-normally distributed variables (normality of distribution was checked with the Shapiro-Wilk test). Statistical tests were two-tailed and p-value of less than 0.05 was considered significant. The Bonferroni correction was applied in case of multiple comparisons. Departure in the distribution of genotypes from Hardy-Weinberg equilibrium was assessed through the χ^2 test. All statistical analyses were performed using R (V.3.1.2; The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

In our case–control study, we included a total of 421 participants: 200 unrelated patients with T2D and control group consisting of 221 unrelated individuals without T2D. Demographic characteristics of both group is presented in table 1. *GST* gene polymorphism frequency in the examined population does not differ statistically from the epidemiological data for the Caucasian race (table 2).[14]

The distribution of examined polymorphisms was similar in patient group and control group. Moreover, there were no statistical differences between *GST* genotypes distribution between groups when the analysis was conducted for each sex separately (table 3).

The analysis of the gene polymorphism was also performed in the groups of patients differing in the age of T2D diagnosis.

Patients were divided into two groups: group A (n=16) patients with T2D diagnosed before 40 years; group B (n=184) patients with T2D diagnosed after and at the age of 40 years.

The rationale for this division is that T2D is mostly diagnosed after 40 years of age.[4] Moreover, the US Preventive Services Task Force recommends screening for T2D, individuals 40 to 70 years of age who are overweight or obese.[5] The control group were people with no T2D aged ≥40 years (group C, n=212). The results of this analysis are shown in table 4.

When analyzing each of the *GST* genes separately, the difference in frequency of their polymorphism was observed but statistically insignificant. For example, *GSTP1 Val/Val* genotype was found in 18.8% of group A and in 10.3% of group B (p=0.39) and in 9.9% of group C (p=1.0). *GSTT1 null/null* genotype was found in 31.2%

Table 1 Demographic characteristic of patients and controls

	Patients (n=200)	Controls (n=221)	P value
Age			<0.001
Missing data	0	2	
Mean (SD)	66.8 (8.07)	53.9 (10.22)	
Age*			0.8131
Missing data	0	2	
Mean (SD)	54.1 (10.74)	53.9 (10.22)	
Sex			<0.001
Female	97 (48.5%)	167 (75.6%)	
Male	103 (51.5%)	54 (24.4%)	
BMI			<0.001
Missing data	1	2	
Mean (SD)	30.9 (4.8)	27.2 (4.7)	
Median (Q1, Q3)	30.7 (27.8 to 33.8)	26.8 (23.6 to 29.9)	
Range	19.2–46.5	17.3–41.5	

*In patient group age at a diagnosis of type 2 diabetes.
BMI, body mass index.

of group A and in 24.5% of group B (p=0.55) and in 18.9% of group C (p=0.18).

In the combined analysis of *GST* gene polymorphism, the differences were more extensive (table 4). *GSTP1 Val/Val* combined with *GSTT1 null/null* was found in 12.5% of group A and in 2.2% of group B (p=0.13) and in 0.9% of group C. This result was statistically significant (p=0.016). *GSTP1 Val/Val* combined with *GSTM1 null/null* was found in 18.8% of group A and in 6.5% of group B (p=0.14) and in 4.2% of group C (p=0.082). *GSTT1 null/null* combined with *GSTM1 null/null* was found in 18.8% of group A and in 12.5% of group B (p=0.21) and in 8.5% of group C (p=0.14). All three examined gene polymorphism together (*GSTP1 Val/Val*, *GSTM1 null/null* and *GSTT1 null/null* genotype) was observed in 12.5% of group A, 1.6% of group B (p=0.052) and 0.5% of group C (p=0.013).

Patients with T2D diagnosed before 40 years did not differ from patients with T2D diagnosed after and at the age of 40 years in terms of body mass index (BMI): 29.9 (SD 6.2) versus 31.0 (SD 4.6); p=0.4.

It suggests that other causes may be implicated in the disease development in younger age, for example, genetic vulnerability. However, the size of each group with the age division is small and obtained results require confirmation on a larger cohort.

DISCUSSION

Most current studies concerning genetic factors of T2D are conducted in Asia, because it is a major area of the rapidly emerging T2D global epidemic. Some of these studies confirm a relationship between particular *GST* gene polymorphism and the risk of the disease.[7 10 15–18] For

Table 2 *GST* polymorphism frequency in patient group with type 2 diabetes and in control group

	Total	Patients	Control group	
	N=421	n=200	n=221	P value
*GSTP1**				0.91
Wild type (Ile/Ile)	191 (45.4%)	89 (44.5%)	102 (46.2%)	
Heterozygous (Ile/Val)	186 (44.2%)	89 (44.5%)	97 (43.9%)	
Homozygous (Val/Val)	44 (10.5%)	22 (11.0%)	22 (10.0%)	
GSTT1				0.26
Wild type	327 (77.7%)	150 (75.0%)	177 (80.1%)	
Null/null	94 (22.3%)	50 (25.0%)	44 (19.9%)	
GSTM1				0.47
Wild type	232 (55.1%)	106 (53.0%)	126 (57.0%)	
Null/null	189 (44.9%)	94 (47.0%)	95 (43.0%)	

*Hardy-Weinberg equilibrium is not disturbed (patients: χ^2=0.001, p=0.97; control group: χ^2=0.023, p=0.88).

Table 3 *GST* polymorphism frequency in patient group with type 2 diabetes and in control group with sex division

	Women					Men				
	Patients (n=97)	Controls (n=167)	OR	95% CI	P value	Patients (n=103)	Controls (n=54)	OR	95% CI	P value
GSTP1										
Ile/Ile	45 (46.4%)	75 (44.9%)	Ref. level			44 (42.7%)	27 (50.0%)	Ref. level		
Ile/Val	46 (47.4%)	75 (44.9%)	1.02	0.61 to 1.72	0.93	43 (41.7%)	22 (40.7%)	1.20	0.59 to 2.42	0.61
Val/Val	6 (6.2%)	17 (10.2%)	0.59	0.22 to 1.60	0.30	16 (15.5%)	5 (9.3%)	1.96	0.65 to 5.97	0.23
GSTT1										
Wild type	71 (73.2%)	136 (81.4%)	Ref. level			79 (76.7%)	41 (75.9%)	Ref. level		
Null/null	26 (26.8%)	31 (18.6%)	1.61	0.89 to 2.91	0.12	24 (23.3%)	13 (24.1%)	0.96	0.44 to 2.08	0.91
GSTM1										
Wild type	52 (53.6%)	94 (56.3%)	Ref. level			54 (52.4%)	32 (59.3%)	Ref. level		
Null/null	45 (46.4%)	73 (43.7%)	1.11	0.67 to 1.84	0.67	49 (47.6%)	22 (40.7%)	1.32	0.68 to 2.57	0.41

example, according to Banerjee *et al*, the null/null allele combination of *GSTM1* and *GSTT1* increases the disease risk up to 1.7-fold.[15] Other studies, both conducted in the north India, showed the combined effect of *GSTM1*, *GSTT1* and *GSTP1* polymorphism on T2D risk.[17 18] A systematic review of 19 studies has shown that individually or a combination of *GSTT1null/null* and *GSTM1null/null* genotypes are associated with T2D.[16] Another study, conducted in Iran, revealed that *GSTP1Ile105Val* polymorphism is associated with an increased risk of new-onset diabetes mellitus after liver transplantation.[10] On the other hand, a meta-analysis of 18 studies has shown no significant association between *GSTP1* polymorphism and the risk of T2D.[19] Results of our study has shown only slight differences in *GST* gene polymorphism frequency between patients with T2D and healthy controls, statistically not significant (table 2). The differences in the results may result from ethnic, and therefore genetic differences between the Asian and Caucasian races, as well as from a small size of the analyzed population. However, by analyzing the distribution of polymorphisms in groups that take into account the age at which the participant contracted T2D, the differences were much more pronounced. For example, the *GSTP1 Val/Val* genotype resulting in the lack of the active enzyme glutathione S-transferase is two times more common in patients diagnosed with diabetes before the age of 40 years, compared with healthy people after the age of 40 (18.8% vs 9.9%; table 4). In turn, the frequency of this genotype does not differ between patients with subsequent diagnosis of diabetes and healthy people (10.3% vs 9.9%; table 4); therefore, it can be assumed that factors other than the *GSTP1* genotype prevail in the development of T2D after the age of 40. In a study carried out in Romania, Stoian *et al* demonstrated significant differences in the frequency of *GSTP1* polymorphism between older patients with T2D (mean age 63 years) and the control group.[1] Nevertheless, the author does not provide us with the age at which diabetes was diagnosed, which is the basis for the stratification of patients in our study. Analyzing together the homozygous deletion of the *GSTT1* and *GSTM1* genes, we noticed that the *GSTT1 null/null and GSTM1 null/null* genotype is much more common in the group of patients diagnosed before the age of 40 (18.8%) in relation to patients with diabetes diagnosed after 40 years of age (12.5%) and in comparison to the control group (8.5%). However, since these polymorphisms occur together relatively rarely in the population, the number of subjects in the compared subgroups was very low. The observed trend is also confirmed by other studies from around the world.[20 21] Banerjee *et al*, in a study on a much larger population (558 with T2D and 410 in the control group), showed a significantly higher frequency of *GSTT1* and *GSTM1* polymorphisms in patients with T2D than in healthy people.[15] In addition, our results are in line with the meta-analysis of 25 studies conducted in Asian and Caucasian populations. According to this meta-analysis, combined *GSTT1 null/null and GSTM1 null/null* genotypes increases the risk of developing diabetes more than twofold. An increase in risk was observed regardless of ethnicity.[21] For all three genes analyzed, it was noted that as the number of gene combinations increases, there is a rise in the disease risk, especially in the subjects younger than 40, which suggest that a gene–gene interaction plays an important role in T2D susceptibility. Statistically significant differences were demonstrated for the combination of *GSTP1 Val/Val* and *GSTT1null/null* genotypes between patients diagnosed before 40 years of age and healthy people (12.5% in group A vs 0.9% in group C, p=0.016; table 4), also for the combination of all three genes: *GSTP1 Val/Val*, *GSTT1null/null* and *GSTM1 null/null* (12.5% in group A vs 0.5% in group C, p=0.013; table 4). Although the small number of subjects in the groups requires careful conclusions, other authors confirm these results, pointing to even 13.5 times higher risk of developing T2D in the case of such a genotype.[15]

Groups of patients diagnosed with diabetes before the age of 40 (group A; table 4) and patients diagnosed with diabetes after 40 years of age (group B; table 4) did not differ significantly in mean BMI, which suggests that

Table 4 *GST* genotype distribution in patients and controls in terms of age of diabetes onset

	T2D diagnosed <40 years old	T2D diagnosed ≥40 years old	Control group (at age ≥40 years old)	P value*		
				A vs B	A vs C	B vs C
	n=16	n=184	n=212			
GSTP1				0.60	0.52	0.97
Wild type (Ile/Ile)	6 (37.5%)	83 (45.1%)	98 (46.2%)			
Heterozygous (Ile/Val)	7 (43.8%)	82 (44.6%)	93 (43.9%)			
Homozygous (Val/Val)	3 (18.8%)	19 (10.3%)	21 (9.9%)			
GSTP1				0.39	0.23	1.0
Wild type or heterozygous	13 (81.2%)	165 (89.7%)	191 (90.1%)			
Homozygous (Val/Val)	3 (18.8%)	19 (10.3%)	21 (9.9%)			
GSTT1				0.55	0.32	0.18
Wild type	11 (68.8%)	139 (75.5%)	172 (81.1%)			
Null/null	5 (31.2%)	45 (24.5%)	40 (18.9%)			
GSTM1				0.21	0.44	0.36
Wild type	11 (68.8%)	95 (51.6%)	120 (56.6%)			
Null/null	5 (31.2%)	89 (48.4%)	92 (43.4%)			
GST combination						
P1=wild, T1=wild, M1=null/null	0 (0.0%)	32 (17.4%)	35 (16.5%)			
P1=wild, T1=null/null, M1=wild	2 (12.5%)	8 (4.3%)	11 (5.2%)			
P1=wild, T1=null/null, M1=null/null	1 (6.2%)	14 (7.6%)	5 (2.4%)			
P1=heterozygous, T1=wild, M1=wild	6 (37.5%)	38 (20.7%)	40 (18.9%)			
P1=heterozygous, T1=wild, M1=null/null	1 (6.2%)	25 (13.6%)	31 (14.6%)			
P1=heterozygous, T1=null/null, M1=wild	0 (0.0%)	13 (7.1%)	10 (4.7%)			
P1=heterozygous, T1=null/null, M1=null/null	0 (0.0%)	6 (3.3%)	12 (5.7%)			
P1=homozygous, T1=wild, M1=wild	0 (0.0%)	6 (3.3%)	11 (5.2%)			
P1=homozygous, T1=wild, M1=null/null	1 (6.2%)	9 (4.9%)	8 (3.8%)			
P1=homozygous, T1=null/null, M1=wild	0 (0.0%)	1 (0.5%)	1 (0.5%)			
P1=homozygous, T1=null/null, M1=null/null	2 (12.5%)	3 (1.6%)	1 (0.5%)	0.052	0.013	0.341
P1 and T1				0.13	0.016	0.57
P1=wild, T1=wild	3 (18.8%)	61 (33.2%)	82 (38.7%)			
P1=wild, T1=null/null	3 (18.8%)	22 (12.0%)	16 (7.5%)			
P1=heterozygous, T1=wild	7 (43.8%)	63 (34.2%)	71 (33.5%)			
P1=heterozygous, T1=null/null	0 (0.0%)	19 (10.3%)	22 (10.4%)			
P1=homozygous, T1=wild	1 (6.2%)	15 (8.2%)	19 (9.0%)			
P1=homozygous, T1=null/null	2 (12.5%)	4 (2.2%)	2 (0.9%)			
P1 and M1				0.14	0.082	0.23
P1=wild, M1=wild	5 (31.2%)	37 (20.1%)	58 (27.4%)			
P1=wild, M1=null/null	1 (6.2%)	46 (25.0%)	40 (18.9%)			
P1=heterozygous, M1=wild	6 (37.5%)	51 (27.7%)	50 (23.6%)			
P1=heterozygous, M1=null/null	1 (6.2%)	31 (16.8%)	43 (20.3%)			
P1=homozygous, M1=wild	0 (0.0%)	7 (3.8%)	12 (5.7%)			
P1=homozygous, M1=null/null	3 (18.8%)	12 (6.5%)	9 (4.2%)			

Continued

Table 4 Continued

	T2D diagnosed <40 years old	T2D diagnosed ≥40 years old	Control group (at age ≥40 years old)	P value*		
				A vs B	A vs C	B vs C
T1 and M1				0.21	0.14	0.43
T1=wild, M1=wild	9 (56.2%)	73 (39.7%)	98 (46.2%)			
T1=wild, M1=null/null	2 (12.5%)	66 (35.9%)	74 (34.9%)			
T1=null/null, M1=wild	2 (12.5%)	22 (12.0%)	22 (10.4%)			
T1=null/null, M1=null/null	3 (18.8%)	23 (12.5%)	18 (8.5%)			

*According to the Bonferroni correction, a p-value less than 0.017 (=0.05/3) is considered as statistically significant.
T2D, type 2 diabetes.

body weight had no effect on the onset of diabetes in the studied patient population.

A lack of data regarding diet, which is a weakness of our study, does not allow us to determine possible correlations of eating habits and genotype in the risk of developing T2D, which would be a valuable aspect in discovering the complex mechanisms of the development of this lifestyle disease. Nevertheless, an objective description of patients' eating habits which could lead, over many years, to the development of a metabolic disease such as T2D is very difficult due to the need for long-term retrospective assessment of diet.

A certain limitation is also the small pool of young patients (in whom diabetes appeared before the age of 40 years). However, this confirms the fact that T2D is a disease most commonly associated with adulthood, diagnosed mainly after 40 years of age.[4 6]

The strength of this study is definitely in the selection of the control group for the age of diabetes diagnosis. This approach is crucial when stratifying groups by age and increases the value of this study in the light of most of the results discussed, where the control group is age-matched to the age of patients with diabetes at the time of entry into the study. In addition, it should be emphasized that this study is one of the few in Europe conducted in this subject and the first in Poland.

CONCLUSIONS

To sum up, the distribution of *GST* gene polymorphisms assessed in the study does not differ from the published data for the Caucasian race. A higher frequency of *GSTP1 Val /Val*, *GSTT1 null/null* and *GSTM1 null/null* genotypes has been demonstrated in patients diagnosed with T2D before 40 years of age than in patients who became ill later and healthy subjects from the control group. These differences increase as the number of gene combinations increases. Our results suggest that *GST* polymorphism may be one of the risk factors for developing T2D at a younger age than the population average, but it need to be confirmed in a larger cohort of young adults.

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