THE GAMMA-GLUTAMYLCYCLOTRANSFERASE GENE VARIANTS INTERACT WITH SMOKING AND VEGETABLE/FRUIT INTAKE TO INFLUENCE THE RISK OF TYPE 2 DIABETES

КУРСКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ
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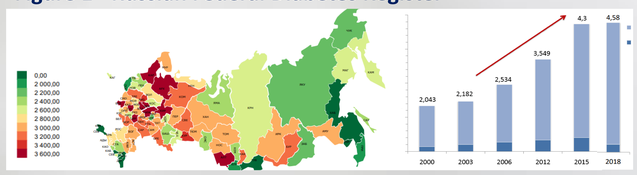
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Introduction

According to the International Diabetes Federation, 425 million people suffer from diabetes in the world [1]. At the end of 2018, there were 4.58 million patients with diabetes in Russia, more than 90% of whom suffer from type 2 diabetes mellitus (T2D), Figure 1 [2].

Imbalance in the system of redox homeostasis is an important link in the pathogenesis of T2D. Gamma-glutamyl cyclotransferase is an antioxidant defense enzyme directly involved in the metabolism of glutathione, an endogenous antioxidant.

Figure 1 - Russian Federal Diabetes Register



Aim

The aim of the study was to examine the association of polymorphisms rs38420 (G>A), rs4270 (T>C), rs6462210 (C>T) and rs28679 (G>A) in *GGCT* gene with the risk of developing T2D.

Materials and Methods

The study included 1022 T2D patients and 1064 healthy volunteers. Genotyping of *GGCT* gene polymorphisms was performed using iPLEX technology on a MassArray Analyzer 4 genome time-of-flight mass spectrometer (Agena Bioscience).

Clinical and laboratory characteristics of the study participants are given in Table 1:

Table 1. Clinical and laboratory characteristics of the study participants

Baseline characteristics	Controls, n=1064	T2D patients, n=1022	P-value*
Age, mean ± Std.Dv.	61,00±7,82	61,57±10,44	0,16
Males, n (%)	392 (36,8)	358 (35,0)	0,41
Females, n (%)	672 (63,2)	664 (65,0)	
Body mass index (kg/m²), mean ± Std.Dv.	27,04±3,55	32,13±6,60	**0,001**
Consumers of fresh fruits and vegetables, n (%)	887 (83,4)	500 (48,9)	**<0,0001**
Smokers, (ever/never), n (%)	308 (28,9)	230 (22,5)	**0,0009**
Duration of diabetes, Me [Q1; Q3]	-	10,01 [4; 14]	-
Positive family history of diabetes, n (%)	-	355 (34,7)	-
HbA_{1c} (%), Me [Q1; Q3]	4,58 [4,11; 4,87]	9,10 [7,90; 11,00]	**<0,0001**
Fasting blood glucose (mmol/L), Me [Q1; Q3]	4,71 [4,39; 4,84]	12,00 [9,49; 14,90]	**<0,0001**
Total cholesterol (mmol/L), Me [Q1; Q3]	3,06 [2,86; 3,12]	4,93 [4,14; 5,90]	**<0,0001**
LDL (mmol/L), Me [Q1; Q3]	1,74 [1,60; 1,79]	3,10 [2,50; 4,05]	**<0,0001**
HDL (mmol/L), Me [Q1; Q3]	1,47 [1,36; 1,62]	0,84 [0,73; 1,00]	**<0,0001**
Triacylglycerols (mmol/L), Me [Q1; Q3]	1,15 [0,98; 1,23]	2,17 [1,55; 2,93]	**<0,0001**

Notes: LDL – low-density lipoprotein; HDL – high-density lipoproteins; Bold values P<0,05

Results

Polymorphism rs4270 of the *GGCT* gene is associated with reduced risk of T2D in the Russian population.

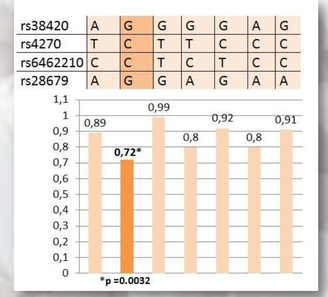
Table 2. Frequencies of the *GGCT* genotypes and alleles in type 2 diabetic patients and controls

SNP	Genotype, allele	Controls, n=1064	T2D patients, n=1022	OR (95 CI)	P-value	OR (95 CI)*	P*-value
rs38420	G/G	671 (63,5)	657 (64,3)	1,00	0,41R	1,00	0,31R
	G/A	337 (31,9)	325 (31,8)				
	A/A	48 (4,5)	39 (3,8)	0,83 (0,54-1,28)		0,78 (0,48-1,27)	
	A	20,5	19,7	0,95 (0,82-1,11)	0,538		
rs4270	T/T	604 (57,5)	639 (62,8)	1,00	**0,014D**	1,00	**0,013D**
	T/C	381 (36,2)	330 (32,4)	**0,80 (0,67-0,96)**		**0,80 (0,67-0,95)**	
	C/C	66 (6,3)	49 (4,8)				
	C	24,4	21,0	**0,82 (0,71-0,95)**	**0,009**		
rs6462210	C/C	782 (74,3)	755 (73,9)	1,00	0,39R	1,00	0,29R
	C/T	250 (23,8)	242 (23,7)				
	T/T	20 (1,9)	25 (2,5)	1,29 (0,71-2,34)		1,41 (0,74-2,70)	
	T	13,8	14,3	1,04 (0,87-1,24)	0,641		
rs28679	G/G	533 (51)	531 (52,2)	1,00	0,12R	1,00 R	0,053R
	G/A	414 (39,6)	410 (40,3)				
	A/A	98 (9,4)	76 (7,5)	0,78 (0,57-1,07)		0,71 (0,49-1,01)	
	A	29,2	27,6	0,93 (0,81-1,06)	0,268		

Notes:*- the calculations are corrected for gender, age and body mass index (R - recessive model, AD - log-additive model)

Analysis of the haplotype frequencies in patients and healthy (Figure 2) found that the G-C-C-G haplotype (including the minor alleles of the studied SNPs) is associated with a reduced risk of disease (OR 0.72, 95CI 0.58-0.90, P = 0.0032).

Figure 2 - Analysis of haplotype frequencies in patients and healthy in the general sample



Since smoking is a powerful risk factor for T2D, analysis in subgroups of study participants with different attitude to vegetables and fruits was carried out taking into account the status of smoking. The T/C-C/C rs4270 genotypes were significantly associated with a reduced risk of developing T2D only in the subgroup of non-smoking patients who consume enough fruits and vegetables daily (Figure 3, 4).

Figure 3 - Consuming enough vegetables and fruits rs4270

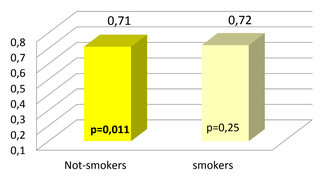
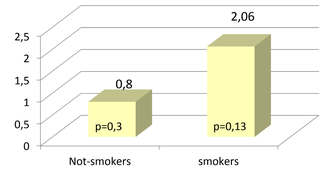


Figure 4 - Not consuming enough fruits and Vegetables rs4270



Assessment of the redox status of study participants showed that the level of hydrogen peroxide H2O2 in the plasma of patients was significantly higher than that in the plasma of healthy patients. The content of reduced GSH glutathione was also higher in the group of patients with T2DM compared with the control (Figures 5 and 6).

Figure 5 - The content of reduced GSH glutathione in plasma in the total sample

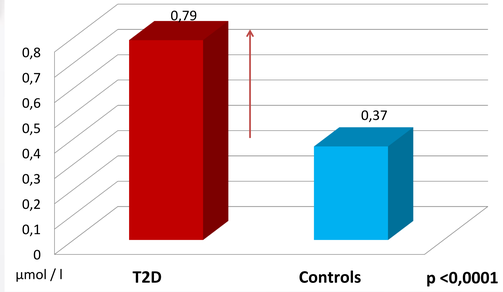
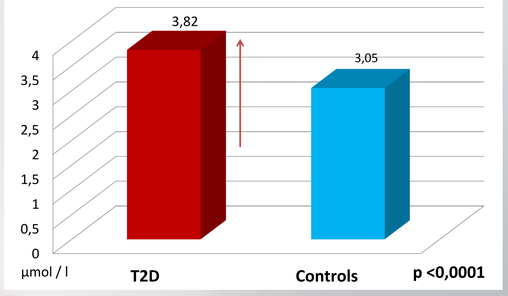
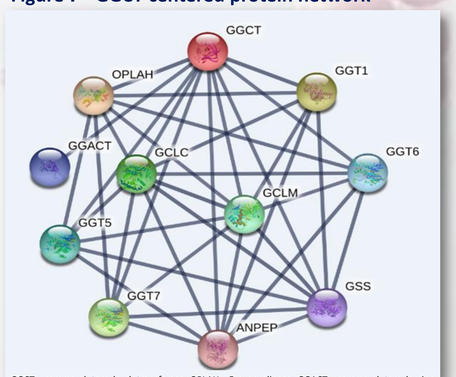


Figure 6 - The content of hydrogen peroxide plasma in the total sample



An analysis of the interactions of GGCT with other genes at the level of protein products, performed using the STRING tool [3], showed that gamma-glutamylcyclotransferase forms a network of 10 proteins (Figure 7).

Figure 7 - GGCT centered protein network



GGCT - gamma-glutamylcyclotransferase; OPLAH - 5-oxoprolinase; GGACT - gamma-glutamylamine cyclotransferase; GCLC - glutamate cysteine ligase, catalytic subunit; GCLM - glutamate cysteine ligase modifying subunit; GSS - glutathione synthetase; ANPEP - aminopeptidase N; GGT1 - gamma-glutamyltransferase 1; GGT5 - gamma-glutamyltransferase 5; GGT6 - gamma-glutamyltransferase 6; GGT7 - gamma-glutamyltransferase 7.

Gene Ontology [4] found that eight enzymes: 5-oxoprolinase (OPLAH), glutamate cysteine ligase catalytic subunit (GCLC), glutamate cysteine ligase modifying subunit (GCLM), glutathione synthetase (GSS); gamma-glutamyltransferase 1 (GGT1); gamma-glutamyl transferase 5 (GGT5); gamma-glutamyltransferase 6 (GGT6); gamma-glutamyltransferase 7 (GGT7), together with GGCT, are responsible for glutathione biosynthesis (FDR = 1.47 * 10-23), while GCLC, GCLM and GGCT are involved in apoptotic changes in mitochondria (FDR = 4.67 * 10-5).

Conclusion

Polymorphic variant rs4270 in the *GGCT* gene is associated with a predisposition to T2D, but its relationship with the disease is modulated by smoking and fresh plant foods consumption.

References

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