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Original Research

Does *TP53* gene polymorphism increase the risk of obesity and chronic pancreatitis comorbidities in type 2 diabetic patients?

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Abstract

Background and aims: To establish the prevalence of genotype frequencies for *TP53* (rs1042522) alleles in type 2 diabetic patients with comorbid overweight/obesity and chronic pancreatitis (CP). **Materials and methods**: The study involved 34 type 2 diabetic patients and 10 healthy individuals. Genomic DNA was extracted from peripheral blood leukocytes using a commercially available DNA isolation kit. The *TP53* gene rs1042522 C>G polymorphism was genotyped using the Taq Man real-time PCR method. **Results**: We found a significant association between the C/C and C/G genotypes of *TP53* rs1042522 SNP polymorphism in type 2 diabetic patients with normal body weight without CP and in type 2 diabetic patients with overweight/obesity. However, no significant difference was found between genotypes C/C, C/G, and G/G of rs1042522 SNP in *TP53* gene in type 2 diabetic patients with obesity and CP vs control group. **Conclusions**: The presence of the C allele of *TP53* SNP rs1042522 polymorphism in both homozygous and heterozygous states may indicate the increased risk of comorbid obesity development in the type 2 diabetic population of Ternopil region, Ukraine. A small number of patients used in this study warrants further research.

Keywords: type 2 diabetes mellitus, comorbidities, *TP53* gene, polymorphism.

Background and aims

Diabetes mellitus (DM) is among the major challenges to human health and wellbeing, as this disorder reached pandemic levels [1, 2]. More importantly, DM patient outcomes, available treatment and management means, and associated healthcare expenses are altogether affected by the presence of comorbidities [3, 4]. Commonly, type 2 diabetes mellitus (T2DM) is linked to dyslipidemia, obesity, in addition to insulin resistance (IR). Consequently, excessive abdominal fat, adipose tissue dysfunction and inflammation are characterized by increased secretion of diabetogenic adipocytokines. This group of cytokines links obesity and dyslipidemia to T2DM since they disrupt insulin action in body organs, including skeletal muscle, brain, and liver [5, 6]. Chronic pancreatitis (CP) is also associated with obesity, IR, and T2DM [7].

Despite the fact that genetic predisposition to T2DM leaves no doubts nowadays and was supported by large-scale genome and clinical studies [8], many genetic factors, as well as their interaction, leading to the development of T2DM, remain unclear [9]. A special interest is paid to the "multi-talented" "guardian of the genome", namely, tumor protein p53 (TP53), which is fundamental for preventing tumor development through the regulation of important cellular processes such as DNA replication repair, cell cycle arrest, senescence, and apoptosis. It is encoded by the TP53 gene (OMIM no. 191170), which is located on chromosome 17p13.1 [10]. It has been shown that TP53 expression in adipose tissue is

crucially involved in the development of IR [11]. Moreover, associations were shown between TP53 and diabetes-related complications: diabetic cardiomyopathy [12], retinopathy [13], neuropathy [14], nephropathy [15], vasculopathy [16], peripheral arterial disease [17] and defective wound healing [18].

Therefore, various mutations and variations of the TP53 gene may be of interest to clinical researchers as possible influencing factors on the development and progression of metabolic diseases, such as T2DM. Moreover, there have been no previous studies of TP53 gene polymorphisms among T2DM patients in Ukrainian population, neither with only T2DM nor with comorbid obesity and/or CP. Identification of genes polymorphisms contributing to T2DM pathogenesis and treatment response is the first step towards the development of personalized medicine corresponding to the patient's unique diabetes pathogenesis.

For this reason, the aim of our study was to establish the prevalence of genotype frequencies for TP53 (rs1042522) alleles in type-2 diabetic patients with overweight/obesity and chronic pancreatitis.

Materials and methods

Characteristics of the participants

The study involved 33 type-2 diabetic patients hospitalized to the Endocrinology Department of Ternopil University Hospital (Ternopil, Ukraine) in 2019-2020 who were divided into the following groups: group 1 – patients with normal body weight and without CP (n=9); group 2 – patients with overweight/obesity without CP (n=14); group 3 – patients with overweight/obesity with CP (n=10); and group 4 – healthy individuals as control (n=10).

There were no significant age and sex differences between the groups in this study.

Inclusion criteria: clinical, laboratory, and instrumental signs of T2DM, CP, and overweight/obesity, no sharp increase (exceeding normal activity not more than 3 times) of blood serum alpha-amylase, lipase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyltransferase.

Exclusion criteria from the study: signs of clinically significant neurological, mental, renal, hepatic, immune, gastrointestinal, urogenital disorder; injuries of the musculoskeletal system, skin, sense organs, endocrine system (except T2DM); or uncontrolled hematologic diseases; acute pancreatitis, unstable or life-threatening heart disease; patients with malignant neoplasms who have not been in complete remission for at least 5 years, medication (drug) and alcohol dependence.

T2DM diagnoses were confirmed according to the 2019 Recommendations of the American Diabetes Association (ADA) [19]. The diagnosis criteria use the level of glycated hemoglobin (HbA_{1c}) (≥6.5%), which was determined using an automatic biochemical analyzer COBAS 6000 (Roche Hitachi, Germany) and plasma glucose level, which was determined on an automatic biochemical analyzer BAS INTEGRA® 400 (Roche Diagnostics) using a standard set. CP diagnoses were following the recommendations of the American Pancreatic Association [20]. Body mass index (BMI) was calculated using the formula: body weight (kg) / height (m^2). Data were interpreted according to the WHO guidelines: normal weight in the range of 20.0–24.9 kg/m^2; overweight (pre-obesity), 25.0–29.9 kg/m^2; class 1 obesity, 30.0–34.9 kg/m^2; class 2 obesity, 35.0–39.9 kg/m^2 and class 3 obesity, >40 kg/m^2.

Sample preparation

Genomic DNA was extracted from peripheral blood leukocytes using a commercially available DNA isolation kit (QIAamp Blood DNA Mini Kit, QIAGEN, Germany).

Genetic analyses

The TP53 gene rs1042522 C>G polymorphism was genotyped using the TaqMan real-time PCR method (Applied Biosystems, Foster City, CA, USA) [21]. Quality control was performed with eight negative control and positive control samples

in each 96-well plate. In addition, approximately 10% of the samples were randomly selected for further quality control, and the concordance rate was 100%. Amplification of the 540-bp TP53 sequence including rs1042522 was performed by using PCR with 5′-AACCCCAGCCCCCTAGCAGAGACC-3′ as the forward primer and 5′-GGGGATACGG CCAGGCATTGAAGT-3′ as the reverse primer. Three genotypes of the rs1042522 TP53 polymorphism were detected (C/C, C/G, and G/G).

Ethics

The ethical principles included in the Declaration of Human Rights adopted in Helsinki, in 1975, and revised in 2008, were fully respected in our study. The enrolled subjects participated in this study voluntarily, completed and signed written informed consent. The study protocol was approved by the Ethics Committee of I. Horbachevsky Ternopil National Medical University.

Statistics

Statistical analysis of the data was performed using the software STATISTICA 7.0. Hardy-Weinberg equation was applied to verify the conformity of genotype distribution in the sample to expected distribution in the general population. Observed and expected frequencies, calculated using the formula $p^2 + 2pq + q^2 = 1$ (Hardy-Weinberg equation), were compared using Pearson's chi-squared test (χ^2). Significance values of $p>0.05$ were calculated assuming the null hypothesis of the sample equality, namely the correspondence of frequencies distribution in the selected sample to the general population. Comparative analysis of frequency tables was performed using Pearson's chi-squared test and two-tailed p-value for Fisher's exact test (p_F – in cases where the values of expected frequencies of individual indicators did not exceed 5). To assess the effect of a factor (the presence of a certain genotype or allele) on the occurrence of disease, the odds ratio (OR), its 95% confidence interval (CI), and significance coefficient p-value were determined.

Results

The frequency distribution of the rs1042522-associated TP53 gene genotypes and the assessment of their compliance with the Hardy-Weinberg population equilibrium were performed in the study and control groups. It was found that the frequencies of the genotypes associated with C/G rs1042522 polymorphism of the TP53 gene in only T2DM, T2DM with comorbid overweight/obesity, and in the combined triple pathology (T2DM, obesity, CP), as well as in the control group, did not deviate significantly from Hardy-Weinberg equilibrium ($p>0.05$). Therefore, it was assumed that the selected population samples corresponded to the general population (Table 1).

The corresponding frequencies for the genotypes of the TP53 gene (rs1042522) were as follows: 100.0% for C/C in only T2DM; 64.3% for C/C and 35.7% for C/G in T2DM + overweight/obesity; 40.0% for C/C and 60.0% for C/G in T2DM + overweight/obesity + CP. As for control group (Group 4), 40.0% for C/C, 20.0% for C/G, and 40.0% for G/G were observed (Table 1).

The frequencies of rs1042522-associated alleles in the study patients' cohorts are shown in Table 2. In T2DM patients with normal body weight without CP, as well as in the T2DM + overweight/obesity group, the C allele dominated, while both the C allele and G allele were equally present in the control group. It should be noted that the C allele was twice more likely to occur in T2DM patients with normal body weight without CP and 1.6 times more common in T2DM patients with overweight/obesity, compared to control ($p_F<0.03$).

The results presented in Table 3 indicated a statistically significant relationship between investigated factor (rs1042522 C/G allele polymorphism) and the occurrence of T2DM in combination with overweight/obesity ($p<0.05$). Investigation of the association between the rs1042522 C/G allele polymorphism and disease risk showed that the risk of developing only T2DM, as well as T2DM with comorbid overweight/obesity, increased in carriers of the C allele.

Estimation of the reliability coefficient in the analysis of the odds ratio showed a probable influence of the C/C rs1042522 genotype on

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Table 1: SNP rs1042522 polymorphism of TP53 gene in study groups and conformity to Hardy-Weinberg equilibrium.

Genotype Expected		Only T2DM (Group 1)		T2DM + overweight/ obesity (Group 2)		T2DM + overweight/obesity + CP (Group 3)		Control (Group 4)	
		Present	Expected	Present	Expected	Present	Expected	Present	Expected
Homozygotes, common	C/C	9	9	9.4	9	4.9	4	2.5	4
Heterozygotes	C/G	0	0	4.1	5	4.2	6	5	2
Homozygotes, rare	G/G	0	0	0.5	0	0.9	0	2.5	4
χ^2, p		χ^2=0; p>0.05		χ^2=0.66; p>0.05		χ^2=1.84; p>0.05		χ^2=3.6; p>0.05	

T2DM – type 2 diabetes mellitus, CP – chronic pancreatitis, SNP – single nucleotide polymorphism, TP53 – tumor protein p53.

Table 2: Allele frequency (rs1042522) of TP53 gene in control individuals, T2DM patients, and T2DM patients with comorbidities.

Allele frequency	Only T2DM (Group 1)		T2DM + overweight/ obesity (Group 2)		T2DM + overweight/ obesity + CP (Group 3)		Control (Group 4)	
	n	%	n	%	n	%	n	%
Allele C	18	100.00	23	82.14	14	70.00	10	50.00
Allele G	0	0	5	17.86	6	30.00	10	50.00
p_F (patients/control)	p_F<0.001*		p_F=0.027*		p_F=0.333		-	

p_F: Fisher test. *Statistically significant differences.
T2DM – type 2 diabetes mellitus, CP – chronic pancreatitis, TP53 – tumor protein p53.

Table 3: The odds ratio for the rs1042522 alleles in T2DM patients without and with comorbidities.

Experimental group	Gene TP53 (rs1042522)					
	Allele C			Allele G		
	OR	95% CI	p	OR	95% CI	p
Only T2DM (Group 1)	37.00*	1.96–697.39	<0.05	0.03*	0.01–0.51	<0.05
T2DM + overweight/obesity (Group 2)	4.60*	1.25–16.97	<0.05	0.22*	0.06–0.80	<0.05
T2DM + overweight/obesity + CP (Group 3)	2.33	0.64–8.54	>0.05	0.43	0.12–1.57	>0.05

*Statistically significant differences.
T2DM – type 2 diabetes mellitus, CP – chronic pancreatitis, TP53 – tumor protein p53, OR – odds ratio, CI – confidence interval.

the T2DM development (p<0.05) (Table 4). This is confirmed by a probable difference in the dominant model of rs1042522 genotype inheritance only in T2DM patients with normal body weight without CP, compared with the control group (pF=0.01). Thus, the presence of the C allele in the homozygous state as a C/C genotype may increase the risk of T2DM development (Table 5).

It should be noted that in a recessive model of rs1042522 inheritance in T2DM patients

Table 4: The odds ratio for the rs1042522 genotypes in T2DM patients without and with comorbidities.

Experimental group	Genotype					
	C/C		C/G		G/G	
	OR	95% CI	OR	95% CI	OR	95% CI
Only T2DM (Group 1)	27.44*	1.25–601.61	0.18	0.01–4.28	0.08	0.01–1.67
T2DM + overweight/obesity (Group 2)	2.70	0.51–14.37	2.22	0.33–14.80	0.05	0.01–1.07
T2DM + overweight/obesity + CP (Group 3)	1.00	0.17–5.98	6.00	0.82–44.35	0.07	0.01–1.50

*p<0.05.
T2DM – type 2 diabetes mellitus, CP – chronic pancreatitis, TP53 – tumor protein p53, OR – odds ratio, CI – confidence interval.

Table 5: Dominant model of TP53 (rs1042522) heredity in patients with only T2DM and T2DM patients with comorbidities.

Genotypes	Disease group %	Control %	pF	OR	95% CI	p-Value
Only T2DM (Group 1)						
C/C	100.00	40.00	0.01*	27.44*	1.25–601.61	<0.05
C/G+G/G	0	60.00		0.04*	0.001–0.80	<0.05
T2DM + overweight/obesity (Group 2)						
C/C	64.29	40.00	0.408	2.70	0.51–14.37	>0.05
C/G+G/G	35.71	60.00		0.37	0.07–1.97	>0.05
T2DM + overweight/obesity + CP (Group 3)						
C/C	40.00	40.00	1.000	1.00	0.17–5.98	>0.05
C/G+G/G	60.00	60.00				

*Statistically significant differences.
T2DM – type 2 diabetes mellitus, CP – chronic pancreatitis, TP53 – tumor protein p53, OR – odds ratio, CI – confidence interval.

with normal body weight without CP, T2DM patients with overweight/obesity, and T2DM patients with overweight/obesity and CP, significant differences were found in T2DM patients with overweight/obesity compared with the control group, indicating an increase in the likelihood of comorbid obesity in the presence of C allele in either homozygous (C/C genotype) or heterozygous (C/G genotype) states (Table 6).

Discussion

Nowadays, approximately 85 polymorphisms and 27580 somatic mutations are described in the TP53 gene [10, 22]. In addition to somatic mutations that occur during cancer, the TP53 gene also contains many single nucleotide polymorphisms (SNPs) that alter the amino acid sequence of the protein [23]. The most studied TP53 SNP occurs at amino acid codon 72, where the nucleotide sequence CCC or CGC encodes either proline (P72) or arginine (R72), known as rs1042522 P72R SNP [24, 25].

The first evidence linking TP53 to the development of T2DM came in 2008 when Gaulton, K. J. et al., reported the involvement of the P72R variant of TP53 in T2DM susceptibility [26]. In 2009, Minamino et al. demonstrated that diet-induced IR in Ay transgenic mice, which

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Table 6: Recessive model of TP53 (rs1042522) heredity in T2DM patients without and with comorbidities.

Genotype	Disease group %	Control %	pF	OR	95% CI	p-Value
Only T2DM (Group 1)						
C/C+C/G	100.00	60.00	0.09	13.15	0.60–288.34	>0.05
G/G	0	40.00		0.08	0.01–1.67	>0.05
T2DM + overweight/obesity (Group 2)						
C/C+C/G	100.00	60.00	0.02*	20.08	0.94–430.24	>0.05
G/G	0	40.00		0.05	0.01–1.07	>0.05
T2DM + overweight/obesity + CP (Group 3)						
C/C+C/G	100.00	60.00	0.09	14.54	0.67–316.71	>0.05
G/G	0	40.00		0.07	0.01–1.50	>0.05

T2DM – type 2 diabetes mellitus, CP – chronic pancreatitis, TP53 – tumor protein p53, OR – odds ratio, CI – confidence interval, pF – Fisher test.

are susceptible to diabetes, is mediated by TP53 [11]. They showed that inhibition of TP53 activity, both by siRNA knockdown in cells or by TP53 gene knockout in mice, alleviated senescence and caused decreased inflammatory cytokine expression in the adipose tissue of mice, ultimately preventing them from developing IR. A year later, in a study focusing on the connection between non-homologous end-joining (NHEJ) DNA repair mechanisms and TP53, Tavana et al., discovered another unexpected connection between TP53 and diabetes [27]. Specifically, knockout of Lig4 in mice resulted in NHEJ deficiency and embryonic lethality; not surprisingly, this embryonic lethality was rescued by TP53 deficiency. The authors found that Lig4−/−; p53−/− mice developed B-cell lymphoma, but that introduction of a hypomorphic TP53 mutant that fails to induce apoptosis but retains the ability to induce growth arrest and senescence (p53R172P) prevented the lymphomagenesis. However, the result was severe diabetes and early fatality in these mice; these were attributed to senescence of the pancreatic beta cells in these Lig4−/−; p53R172P mice. In 2011, using clinical data from over 55,000 Europeans, a subsequent meta-analysis study confirmed that the R72 variant of TP53 (less frequent allele G of SNP rs1042522), was linked to T2DM [28]. Similar findings were also confirmed among the Chinese population, indicating that the R72 variant increased susceptibility to T2DM was not a race-specific phenomenon [29]. There are some data indicating that the combination of two polymorphisms, specifically, of TP53 gene with hepatocyte nuclear factor-1 A (HNF1A) gene, increases the risk for T2DM development by three-fold [30]. To summarize, these studies implicated TP53-mediated senescence of adipocytes and pancreatic beta cells, respectively, in the development of IR and diabetes [24].

Contrary, Sorokina et al., evaluated p53 SNP (Pro72Arg rs1042522 C215G) in eighty-nine patients, firstly diagnosed with T2DM before the beginning of pharmacotherapy, and 80 individuals without carbohydrate metabolism deficiencies. They found no significant connection between frequencies of CC, GG, and GC genotypes between investigated groups [31]. Also, this group of researchers did not find a significant difference between representatives of haplotypes of single nucleotide polymorphism of the p53 protein gene between genders, age, body mass index, and other comorbidities.

Our results indicate a significant association between the C/C and C/G genotypes of TP53 SNP (rs1042522) polymorphism in type-2 diabetic patients with normal body weight without CP and in type-2 diabetic patients with overweight/obesity without CP ($p<0.05$). This was confirmed by a significant difference in the TP53 gene dominant inheritance model in only T2DM and in the TP53 gene recessive model in T2DM + overweight/

obesity compared to the control group ($p<0.05$). Additionally, no significant difference was found between the genotypes C/C, C/G, and G/G of the SNP rs1042522 of TP53 gene in type-2 diabetic patients with overweight/obesity and CP compared to the control group.

Obesity is characterized by chronic inflammation and hypoxia of adipose tissue, resulting in abnormal production of cytokines, growth factors, non-esterified fatty acids, activation of pro-inflammatory pathways and IR [32]. Speliotes et al. have found significant associations between the P72R variant of TP53 and increased BMI [33]. Consistent with this report, a separate study showed that the association between BMI and DM incidence is much stronger in individuals of the R72 genotype [34]. A cohort study of over 2500 Dutch and Finnish subjects found that the R72 variant is associated with increased waist circumference [35]. These data suggested that the R72 variant of TP53 SNP (rs1042522) polymorphism might predispose individuals to adiposity/obesity.

Study limitations

This study involved a small sample size; because of it, significant relationships between study factors were difficult to establish. The patients included in T2DM + overweight/obesity and T2DM + overweight/obesity + CP groups were not randomly selected, potentially resulting in selection bias. While we cannot support the assumption that study participants represent the population of type 2 diabetic patients with comorbid obesity and CP in the Ternopil region, but obtained results give grounds for further studies with larger sample sizes reflecting a more inclusive population.

Conclusions

We found a significant association between the C/C and C/G genotypes of TP53 SNP (rs1042522) polymorphism in type-2 diabetic patients with normal body weight without CP and in type-2 diabetic patients with overweight/obesity ($p<0.05$). Thus, the presence of the C allele of TP53 SNP rs1042522 polymorphism in both homozygous and heterozygous states may indicate the increased risk of comorbid obesity development in type 2 diabetic population of the Ternopil region, Ukraine. A small number of patients used in this study warrants further research.

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Competing Interests

The authors declare that this manuscript was approved by all authors in its form and that no competing interest exists.

Conflict of interest

The authors declare no conflict of interest.

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