

GSTP1 ILE105VAL POLYMORPHISM IN SERBIAN PATIENTS WITH PANCREATIC DISEASES

POLIMORFIZAM GSTP1 ILE105VAL U SRPSKOJ POPULACIJI SA PANKREASNIM OBOLJENJIMA

Aleksandra Nikolić¹, Marija Stanković¹, Ivan Nišević¹, Snežana Lukić², Marina Anđelić-Jelić³, Dragan Popović², Dragica Radojković¹

¹Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia

²Department of Gastroenterology, Clinical Center of Serbia, Belgrade, Serbia

³Department of Endocrinology, University Clinical Center »Zvezdara«, Belgrade, Serbia

Summary: The aim of the current preliminary case-control study was to identify glutathione S-transferase P1 (GSTP1) Ile105Val allele and genotype frequency and to evaluate its impact on susceptibility to pancreatic diseases in a Serbian population. This study has encompassed 157 patients with three major types of chronic pancreatic pathology: 47 with pancreatic cancer, 50 with chronic pancreatitis and 60 with type 2 diabetes mellitus, as well as 107 healthy individuals. The presence of GSTP1 Ile105Val polymorphism was analyzed using a PCR-RFLP method. Allele 105Val was less frequent in patients with pancreatic cancer (24.5%) and chronic pancreatitis (24.0%) and slightly more frequent in patients with type 2 diabetes mellitus (31.7%) in comparison to healthy individuals (29.9%), but the differences were not statistically significant. Distribution of Ile105Val polymorphism genotypes differed between the analyzed groups, but differences were also not statistically significant. There are only a few studies regarding the role of GSTP1 Ile105Val polymorphism in pancreatic diseases and their results are inconsistent. The significance of GSTP1 Ile105Val polymorphism for pancreatic pathology remains unclear and further studies are needed in order to elucidate its role in pancreatic diseases.

Keywords: glutathione S-transferase P1 (GSTP1), Ile105Val polymorphism, pancreatic cancer, chronic pancreatitis, type 2 diabetes mellitus

Kratak sadržaj: Cilj ove studije bio je da se odredi učestalost polimorfizma Ile105Val u genu za glutathion S-transferazu P1 (GSTP1) i proceni njegov uticaj na rizik za obolavanje od pankreasnih bolesti u srpskoj populaciji. Studija je obuhvatila 157 pacijenata sa najčešćim bolestima pankreasa: 47 sa karcinomom pankreasa, 50 sa hroničnim pankreatitisom i 60 sa dijabetes melitusom tipa 2, kao i 107 zdravih ispitanika. Prisustvo polimorfizma Ile105Val u genu za GSTP1 je analizirano metodom PCR-RFLP (Polymerase Chain Reaction – Restriction Fragment Length Polymorphism). Učestalost alela 105Val je bila niža kod pacijenata sa karcinomom pankreasa (24,5%) i hroničnim pankreatitisom (24,0%) i neznatno povišena kod pacijenata sa dijabetes melitusom tipa 2 (31,7%) u odnosu na učestalost kod zdravih ispitanika (29,9%), ali razlike nisu bile statistički značajne. Učestalost pojedinih genotipova polimorfizma Ile105Val se razlikovala među analiziranim grupama, ali razlike takođe nisu bile statistički značajne. Samo nekoliko studija se do sada bavilo ulogom GSTP1 Ile105Val polimorfizma u bolestima pankreasa i uglavnom su dale oprečne rezultate. Značaj GSTP1 Ile105Val polimorfizma za bolesti pankreasa još uvek nije razjašnjen i potrebna su dalja istraživanja da bi se ispitala njegova uloga u pankreasnom tkivu.

Ključne reči: glutathion S-transferaza P1 (GSTP1), polimorfizam Ile105Val, karcinom pankreasa, hronični pankreatitis, dijabetes melitus tipa 2

Address for correspondence:

Aleksandra Nikolić
Institute of Molecular Genetics and Genetic Engineering
Vojvode Stepe 444A, PO Box 23
11010 Belgrade, Serbia
Tel: +381 11 3976658
Fax: +381 11 3975808
e-mail: aleksni@imgge.bg.ac.rs

Introduction

Three major types of chronic pancreatic pathology, pancreatic cancer, chronic pancreatitis and type 2 diabetes mellitus, have been under intensive investigation for many years, but their etiology, genetics and underlying molecular mechanisms still remain unclear (1, 2). Among numerous genetic and envi-

ronmental factors that might be associated with these diseases, special attention has lately been given to xenobiotic metabolizing enzymes, which play a crucial role in cell protection from reactive chemical intermediates and oxidative stress. It is proposed that normal activity or levels of xenobiotic metabolizing enzymes protect pancreatic tissue from increased production of reactive oxygen species and reduction in antioxidant defenses, which are associated with chronic pancreatic pathology (3–5).

Glutathione-S-transferases (GSTs) are a superfamily of phase II xenobiotic-metabolizing enzymes that catalyze the conjugation of reduced glutathione (GSH) to a wide variety of exogenous and endogenous electrophilic molecules. This process generally results in biologically less active compounds that are more water soluble, thereby facilitating their biliary or urinary excretion (6). Thus, the detoxification ability of GSTs plays a role in cellular protection from environmental and oxidative stress, yet it is also implicated in cellular resistance to drugs (7, 8). Numerous polymorphisms occur in the genes encoding GSTs, which are associated with a lack or an alteration of enzymatic activity toward several substrates (6, 7).

Glutathione-S-transferase P1 (GSTP1) is widely expressed in normal human epithelial tissue and it possesses unique enzymatic properties, including broad substrate specificity, glutathione peroxidase activity toward lipid peroxides, low sensitivity to organic anion inhibitors, high sensitivity to active oxygen species, and ligand-binding properties (6, 8). The GSTP1 is encoded by a single gene spanning approximately 3 kb and located on chromosome 11 (11q13). Two GSTP1 single nucleotide polymorphisms have been identified that are characterized by transitions at A1578G in exon 5 and C2293T in exon 6, resulting in amino acid substitutions Ile105Val and Ala114Val, respectively, which appear to be within the active site of the GSTP1 protein (9, 10).

The aim of the current preliminary case-control study was to determine the Ile105Val GSTP1 allele and genotype frequency and to evaluate the impact on susceptibility to pancreatic diseases in a Serbian population.

Materials and Methods

Human subjects

This study has encompassed 157 patients with three major types of chronic pancreatic pathology: 47 with pancreatic cancer, 50 with chronic pancreatitis and 60 with type 2 diabetes mellitus. The patients with pancreatic cancer and chronic pancreatitis were referred to the Clinic of Gastroenterohepatology and the First Surgical Clinic of the Institute for Digestive Diseases in the period 2004–2007. The patients with type 2 diabetes mellitus were referred to the Depart-

ment of Endocrinology of the University Clinical Center »Zvezdara« in the period 2003–2006. Written informed consent was obtained from all subjects. The study was approved by the ethics committees of the institutions involved in this research.

The group of patients with pancreatic cancer consisted of 47 individuals (28 male and 19 female, age 36–80 years). Diagnosis of pancreatic cancer was made based on radiological findings (abdominal ultrasound, computed tomography, nuclear magnetic resonance and endoscopic ultrasound) and laboratory findings (tumor marker CA 19-9 test). The diagnosis was confirmed by histopathological examination.

The group of patients with chronic pancreatitis consisted of 50 individuals (42 male and 8 female, age 15–72 years). Diagnosis of chronic pancreatitis was made based on the presence of exocrine and endocrine pancreatic insufficiency and the findings of morphological examinations: calcification in the pancreas detected by ultrasound, computed tomography or nuclear magnetic resonance of the abdomen, morphological changes of the pancreatic canalicular system detected by endoscopic retrograde cholangiopancreatography and morphological changes of the pancreas detected by endoscopic ultrasound.

The group of patients with type 2 diabetes mellitus consisted of 60 individuals (20 male and 40 female, age 36–81 years). Type 2 diabetes mellitus was diagnosed using criteria defined by the WHO. Microvascular complications of diabetes, polyneuropathy and retinopathy, were diagnosed by neurological examination (clinical examination, EMNG, monofilament testing and Neuro-pad testing) and FOU examination.

Control group consisted of 107 healthy individuals (77 male and 30 female, age 20–64 years) who were referred to the National Blood Transfusion Institute in the period 2000–2002. They were included in the study based on standard blood analyses, blood pressure measurement and absence of chronic diseases.

Analysis of GSTP1 Ile105Val polymorphism

The presence of GSTP1 Ile105Val polymorphism (A1578G) was analyzed using a previously described PCR-RFLP method (11). Genomic DNA was extracted from the whole blood, using the QIAamp DNA Blood Mini Kit (QIAGEN). The following primers were used to amplify a 433bp-long DNA fragment: 5'-GTAGTTTGGCCAAGTCAAG-3' and 5'-AGCCACCTGAGGGGTAAG-3'. Amplification was performed in a reaction mixture (total volume 25 µL) containing 250 ng of genomic DNA as a template, 5 pmol of each primer, 0.2 mmol/L each dNTP, 2.5 mmol/L MgCl₂, 1X Reaction buffer B (Solis BioDyne) and 1U of FIREPol DNA Polymerase (Solis

BioDyne). The amplification was performed under the following conditions: initial denaturation at 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 57 °C for 30 s and 72 °C for 30 s, with a final step of 72°C for 10 min for elongation. After amplification, 20 µL of PCR products was digested with 5U of BsmAI restriction enzyme (New England Biolabs) in a total volume of 25 µL overnight at 55 °C. The obtained fragments were separated by electrophoresis on an ethidium bromide stained 2% agarose gel. The amplified fragment contains one recognition site for BsmAI, while the presence of Ile105Val polymorphism introduces another. A 105bp and 328bp-long bands indicate the presence of a wild-type allele, while a 105bp, 108bp and 220bp-long bands indicate the presence of a mutant allele.

Statistical analysis

Deviations of genotypes' distributions from the Hardy-Weinberg equilibrium were assessed by χ^2 test for each cohort. Differences in allele and genotype frequencies between the patient groups and the control group were analyzed by SPSS software (version 10.0.1 for Windows) using Fisher's exact test. For every comparison of the groups the following statistical parameters were calculated: p-value, odds ratio (OR) and 95% confidence interval (95%CI), with $p \leq 0.05$ considered statistically significant.

Results

Analysis of GSTP1 Ile105Val polymorphism was performed in 157 cases of pancreatic pathology and 107 healthy individuals by the PCR-RFLP method.

Allele 105Val was less frequent in patients with pancreatic cancer (24.5%) and chronic pancreatitis (24.0%) and slightly more frequent in patients with type 2 diabetes mellitus (31.7%) in comparison to healthy individuals (29.9%), but the differences were not statistically significant. The obtained allele frequencies in patients with pancreatic diseases and healthy individuals are given in *Table I*.

Table I Allelic distribution of GSTP1 Ile105Val polymorphism in patients with pancreatic diseases and healthy individuals.

	No	N	%	M	%
Pancreatic cancer	94	71	75.5	23	24.5
Chronic pancreatitis	100	76	76.0	24	24.0
Type 2 diabetes mellitus	120	82	68.3	38	31.7
Control group	214	150	70.1	64	29.9

No – number of samples analyzed

N – allele Ile105

M – allele105Val

Table II GSTP1 Ile105Val genotypes in patients with pancreatic diseases and healthy individuals.

	No	N/N	%	M/N	%	M/M	%
Pancreatic cancer	47	25	53	21	45	1	2
Chronic pancreatitis	50	29	58	18	36	3	6
Type 2 diabetes mellitus	60	27	45	28	47	5	8
Control group	107	53	50	44	41	10	9

No – number of samples analyzed

N/N – homozygotes for normal allele

M/N – heterozygotes for GSTP1 Ile105Val polymorphism

M/M – homozygotes for GSTP1 Ile105Val polymorphism

Distribution of Ile105Val polymorphism genotypes differed between the analyzed groups, but the differences were also not statistically significant. The obtained genotype frequencies in patients with pancreatic diseases and healthy individuals are given in *Table II*. Genotype distributions were in Hardy-Weinberg equilibrium in each cohort ($\chi^2 < 3.84$, $df=1$, $p < 0.05$).

Discussion

Chronic pancreatic pathology is associated with an increased production of reactive oxygen species and a reduction in antioxidant defenses. Pancreatic tissue is highly sensitive to oxidative stress and it is therefore proposed that individuals with lowered antioxidant capacity are at increased risk of pancreatic disease development. Members of the GST superfamily are critical for protecting cells from oxidative stress because they can utilize a wide variety of products of oxidative stress as substrates. Enzyme GSTP1 catalyses the detoxification of products that arise from DNA oxidation and the lack of detoxification, which is genetically determined, may be a risk factor for disease development (12, 13). There are many studies dealing with GSTP1 gene polymorphisms in various diseases, but only a few studies have addressed the role of GSTP1 polymorphism in pancreatic diseases.

Analysis of GSTP1 Ile105Val polymorphism in Serbian subjects with pancreatic cancer has shown that 105Val allele is less frequent (24.5%) than in healthy individuals (29.9%). Also, the frequency of homozygotes for 105Val allele was decreased in pancreatic cancer patients (2% vs. 9% in the control group). A previous study conducted in a central European Slavonic population indicated an association between 105Val allele and elevated risk for pancreatic cancer (14). There is also evidence that 105Val may contribute to a possible protective effect against pancreatic cancer in older individuals and a survival advantage in patients treated with 5-fluorouracil (15).

Of the major pancreatic diseases, the role of GSTs, including GSTP1, has been most extensively studied in chronic pancreatitis. In Serbian patients with chronic pancreatitis the frequency of 105Val allele (24.0%) was slightly decreased in comparison to the control group (29.9%), as well as the frequency of homozygotes for 105Val allele (6% vs. 9%) and heterozygotes (36% vs. 41%). The association of homozygosity for 105Val allele with chronic pancreatitis was observed in one study, while three other studies showed no association between GSTP1 Ile105Val polymorphism and pancreatitis (15–18).

Analysis of Ile105Val polymorphism in patients with type 2 diabetes mellitus has shown increased frequency of 105Val allele (31.7% vs. 29.9%) and heterozygotes for Ile105Val polymorphism (47% vs. 41%) in comparison to the control group. The Ile105Val polymorphism alone or in combination with the smoking status was not found to affect the risk of type 2 diabetes mellitus or risk of cardiovascular

events in diabetic patients, but it was found to be associated with chronic renal insufficiency (19–22).

So far, there have been only a few studies regarding the involvement of GSTP1 Ile105Val polymorphism in pancreatic diseases and current data are insufficient and inconsistent. The significance of GSTP1 Ile105Val polymorphism for pancreatic pathology remains unclear and further studies are needed in order to elucidate its role in pancreatic diseases. Future studies should encompass a larger number of subjects and should take non-genetic factors into consideration as well.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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