Original Article

The complex interplay of Selenoprotein P, NO metabolites, and pancreatic enzymes in chronic pancreatitis and hypothyroidism is partially orchestrated by the SEPP1 gene's rs7579 polymorphism: focus on gender aspect

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ABSTRACT

Objective

To establish the association of the *SEPP1* gene's (rs7579) polymorphism with enzymatic, metabolic and hormonal activity in patients with chronic pancreatitis (CP) and primary hypothyroidism.

Materials and methods

Eighty CP patients (40 with comorbid hypothyroidism) and 30 healthy controls participated in the case-control study. Pancreatic enzymes, Selenoprotein P, NO metabolites (NO_2^-+NO_3^-), glucose, total cholesterol (TC), triglycerides (TG) and low/high density lipoprotein cholesterol (LDL-, HDL-C), Atherogenicity Index (AI), thyroid-stimulating hormone (TSH), Thyroxine (T4), glomerular filtration rate (GFR) were studied. *SEPP1* (rs7579) genotyping performed by PCR (FlexCycler BU).

Results and discussion

SEPP1 (rs7579) gene's *A*-allele increases the risk of hypothyroidism twice in CP [OR=2.0; OR 95%CI:1.09-3.66; p=0.023]. Pancreatic patients with hypothyroidism and *SEPP1* gene's (rs7579) *AA*-genotype had 60.0% (p_{AA}=0.013) lower elastase and 13.78% (p=0.003) higher α-amylase as well as TSH by 10.81% (p_{AA}=0.009) and 15.64% (p_{AA}=0.009), especially in women 14.55-45.18% (p<0.001) at a lower value of Selenoprotein P – by 28.65-48.08% (p≤0.048) regardless of the *SEPP1* gene (rs7579) variants. Women have 17.43-18.14% (p_W≤0.032) higher NO metabolites than men; *A*-allele women have free T4 value 2.62 times lower (p_{GG}=0.01) than *GG*-women and 2.97 times lower (p_W=0.011) than men. Comorbid patients with *A*-allele had elevated TC and LDL-C, by 11.77-26.45% (p_{AA}≤0.01) and 18.06-26.21% (p_{AA}≤0.019), respectively. Women have 54.50% (p_W=0.002) higher AI with 39.30% lower (p_W=0.034) Selenoprotein P and 21.96-24.56% (p_W≤0.033) GFR than men.

Conclusion

SEPP1 (rs7579) gene polymorphism influences the risk of hypothyroidism in CP patients, changes of pancreatic enzymes, dyslipidaemia particular in *AA*-genotype carriers, mainly women. There was no dependence of *SEPP1* and total NO metabolites values on the *SEPP1* gene (rs7579) polymorphism.

Keywords

pancreatitis; hypothyroidism; *SEPP1* gene (rs7579) polymorphism; Selenoprotein P; NO metabolites; metabolism, lipids.

INTRODUCTION

It is generally accepted that chronic pancreatitis (CP) is responsible for significant impact on one's quality of life and may lead to severe negative long-term consequences[1]. The incidence of CP in European countries ranges from 5 to 10 cases per 100,000 individuals in the population. It is estimated that approximately 120 out of 100,000 inhabitants have CP with a median survival of 20 years. Over the last two decades, there has been a notable doubling in the number of CP cases, accompanied by increased levels of disability and mortality, primarily attributed to oncological factors[2].

The development and progression of CP, alongside with the emergence of complications, are predominantly influenced by a combination of lifestyle choices and genetic factors. Global clinical guidelines, such as those established by HaPanEU and the Japan Pancreas Society, have already incorporated several genetic factors into their evidence-based recommendations

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for CP diagnosis and treatment[3-6]. Eventually, the role of mutations in the cationic trypsinogen gene (*PRSS1*) in hereditary pancreatitis has been well-established[7] and there is confirmed linkage between the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) and pancreatitis [8-11]. Furthermore, the influence of the serine peptidase inhibitor Kazal type 1 gene (*SPINK1*) on pancreatic secretory function has been elucidated[10,12], as has the role of chymotrypsin C (*CTRC*)[13], carboxypeptidase A1 (*CPA1*)[14], and selective transient calcium ion channels (*TRPV6*)[4]. In fact, mutations in *PRSS1* and *SPINK1* genes are now included in the 2019 clinical diagnostic criteria for early-stage CP diagnosis[2,4,6]. However, it's worth noting that genetic testing for chronic pancreatitis has not yet become standard practice in a global aspect.

Furthermore, the precise roles and positions of other genes in the development of chronic pancreatitis, which govern glutathione antioxidant protection activity[15], or are linked to alterations in lipid-carbohydrate metabolism, endocrine gland secretion, or influence signaling pathways and the transport of crucial nutrients within the immune, endocrine, and nervous systems, such as zinc, selenium, and iodine, have yet to be definitively elucidated[16,17]. Selenium (Se) holds a prominent position among these factors[18]. Selenium is incorporated as the amino acid selenocysteine (Sec) into 25 selenoproteins, which, as per extensive research, are known to provide defense against reactive oxygen species (ROS), encompassing functions related to redox processes and oxidative stress. Subsequently, they play a pivotal role in the development of oncopathology[19,20], bridging CP and neoplastic process. Among the well-known families of selenoproteins are selenoprotein P (*SEPP*) and selenoprotein S (*SEPS*)[21,22].

OBJECTIVE

Hence, the primary objective of our study was to establish the correlation between the selenoprotein P gene *SEPP1* (rs7579) and clinical as well as metabolic-hormonal parameters in individuals diagnosed with CP, both in isolation and in conjunction with hypothyroidism.

MATERIALS AND METHODS

Ethical clearance. Current Study fully adhered to European Convention on Human Rights and Biomedicine, GCP, GLP, EU Commission directive #609 and other European and International legislations on bioethics. The Research Design and Research Protocol were approved by the Ethics' Committee of the Bukovinian State Medical University, Protocol #3, 19.11.2020. Each participant signed an Informed Consent to willingly participate in the study, having been adequately informed about the research details. The Study is defined as prospective, case-control study.

Diagnosis. Inclusion/Exclusion criteria. *Chronic pancreatitis* was defined according to International consensus statements on early chronic pancreatitis as a continuing inflammatory disease of the pancreas, characterized by irreversible morphological changes, and typically causing pain and/or permanent loss of function (Recommendations from the working group for the international consensus guidelines for chronic pancreatitis in collaboration with the International Association of Pancreatology, American Pancreatic Association, Japan Pancreas Society, PancreasFest Working Group and European Pancreatic Club, 2018-2020)[2,4,6,7]. The clinical diagnosis was linked to clinical scenario (complaints and history, signs and symptoms, clinical course), disease biomarkers (pancreatic elastase, α-amylase, biochemical data), risk factors and individual variables within a lifetime evaluation, progressive features analysis, abdominal ultrasound and computer tomography or MRI assessment.

Primary Hypothyroidism due to autoimmune thyroiditis was diagnosed after clinical symptoms (tiredness, facial edema, weight gain, hair loss) with Thyroid-stimulating hormone (TSH) blood level increase above 4.0 mIU/mL accompanied by free Thyroxine (T4) value decrease ≤0.8 ng/dL or within normal range (0.8-1.8 ng/dL) and respective ultrasonography changes.

Inclusion criteria. Age over 18 years and confirmed CP (clinically, laboratory, instrumentally), and combination of CP and primary hypothyroidism were considered inclusion criteria.

Exclusion criteria. We excluded patients with complicated course of CP, acute pancreatitis and exacerbation of CP; cystic fibrosis; patients with autoimmune hepatitis, viral hepatitis, liver cirrhosis, non-alcoholic fatty liver disease or any other sub- and decompensated liver diseases (triple growth over the normal values of aspartate aminotransferase, alanine aminotransferase); the presence of ulcers in any part of the gastrointestinal tract; diabetes mellitus

(DM); chronic kidney disease (CKD) with estimated glomerular filtration rate (eGFR) decline <60 ml/min/1,73m^2); malignancies of any location; infectious diseases of any location or during unstable remission; exacerbated systemic connective tissue diseases or during unstable remission; less than 3 months after acute disorders of local blood circulation (acute coronary syndrome, stroke) or coronary heart disease exacerbation; bronchial asthma; chronic obstructive pulmonary disease III-IV stage with C or D risk value (GOLD 2021); administration of oral corticosteroids or contraceptives or any hormone replacement therapy; pregnancy or lactation; age of less than 18 yo; alcohol/drug addiction; mental disorders. Eighty screened patients were selected (Table 1) for further examination (52 women, 28 men, mean age 60.37±4.57 yo). Among them 40 CP individuals were comorbid with hypothyroidism condition due to autoimmune thyroiditis. The genetic examination was performed for 49 patients (34 women, 15 men). The control group consisted of 30 practically healthy individuals with no evidence of CP or hypothyroidism who were not related to the patients (18 women, 12 men, mean age 25.86±4.37 y.o.). Gender division based on anamnestic data, all enrolled subjects were from the same residential area (Northern Bukovina, Western Ukraine).

Laboratory and clinical data collection. All enrolled patients underwent a complex of examinations: clinical anamnesis recording, general clinical examinations, body mass index (BMI, kg/m^2), Waist-to-Hip ratio (WHR), office measurement of SBP, DBP, heart rate (HR), complete blood count, glucose, total cholesterol (TC) level, triglycerides (TG) and low/high density level cholesterol (LDL, HDL-C), Atherogenicity Index (AI = (TC – HDL-C) / HDL-C, Unit), blood pancreatic elastase, α-amylase by amyloclastic method (in mg/sec×L), total bilirubin and its fractions, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), serum creatinine, GFR calculation (according to Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation with Creatinine level), serum thyroid hormones (TSH, free T4), abdominal ultrasound and pancreas computer tomography or MRI, 12 leads ECG, consultations of gastroenterologist.

The *Human Selenoprotein P* (*SEPP1*) serum level was determined by Enzyme Linked-Immuno-Sorbent Assay (ELISA) according to the Manufacturer's Guidelines based on sandwich enzyme-linked immune-sorbent assay technology with highly sensitive Human *SEPP1* (Selenoprotein P) ELISA Kit® (Wuhan Fine Biotech Co., Ltd, China) on a "StatFax 303" equipment (USA). The *SEPP1* assay has a sensitivity of 0.469 ng/ml.

Table 1 Baseline characteristics of patients and controls subjects

Variables		Control group, n=30 (%)	Study group, n=80 (%)	p
Age (years), mean±SD		25.86±4.37	60.37±4.57	<0.001*
Gender, n	Female	18 (60.0)	52 (65.0)	0.624
	Male	12 (40.0)	28 (35.0)	
Smokers, n		12 (40.0)	30 (37.5)	0.806
Family anamnesis aggravated by pancreatitis, n		14 (46.67)	59 (73.75)	0.007
BMI, n	≥25.0 kg/m^2	14 (46.67)	54 (67.5)	0.045*
	<25.0 kg/m^2	16 (53.33)	26 (32.5)	
BMI (kg/m^2), mean±SD		24.71±2.55	26.12±2.43	0.07

*Significant level of less than 0.05. SD: Standard deviation. BMI: Body mass index.

The *Monoxide Nitrogen metabolites* (NO_2^-/NO_3^-) concentration was evaluated in blood, stabilized with EDTA (1 mg/ml), by colorimetric method with Total $NO/NO_2^-/NO_3^-$ Assay Kit (RDS, UK) on a 550 nm Spectrophotometer (TS8210, China) as it was described in previous publications[23-25].

Genotyping assay. Venous blood was collected in a sterile vacutainer, stabilized by K2-EDTA. DNA was isolated from the whole venous blood lymphocytes' nuclei and purified according to "Quick-DNA Mini Prep Plus Kit" (Zymo Research, США) Manufacturer's Guidance. DNA fragments of analyzed gene *SEPP1 25191G/A* (rs7579) amplified by Quantitative Real-Time PCR (qRT-PCR) with specific (Table 2) primers (Metabion, Bayern, Germany) and genotyping with "DreamTaq Green PCR Master Mix" (Thermo Scientific, США) on thermocycler "FlexCycler BU" (Analytik Jena, Germany).

Table 2 Primer sequences used for *SEPP1 25191G/A* (rs7579) genotypes evaluation

Primers	Primer sequences (5` – 3`)
Forward (inner):	5`-TGACCTTCAAACTAAATATTTAAAATCGG -3`
Reverse (inner):	5`-TGTGTCTAGACTAAATTGGGGAGTATTTT -3`
Forward (outer):	5`-GAGGAGAACATAACTGAATCTTGTCAGT-3`
Reverse (outer):	5`-CTCCATCATAAAAAATATGGTTTGAGTC-3`

The PCR thermal profile was as follows: pre-melting at 95°C for 3 min, 35 cycles of melting (at 94°C for 30 sec), annealing (at 58°C for 30 sec), synthesis (at 72°C for 30 sec) and finally – synthesis prolongation at 72°C for 5 min. After PCR running, the amplified fragments of *SEPP1 25191G/A* (rs7579) gene were electrophoresed on 3% agarose gel ("TopVision Agarose", Thermo Scientific, USA; "10xTVE Electrophoresis Buffer", Thermo Scientific, USA) stained by ethidium bromide. Amplicons were visualized by UV Transilluminator Clear View by Micro DOC (Cleaver Scientific Ltd, Great Britain) System (Figure 1).

Statistical analysis. Statistical analysis performed using StatSoft Statistica v.7.0 software (StatSoft Inc., USA). To verify the differences between groups we applied the Student's t-test (two-tail distribution and equal

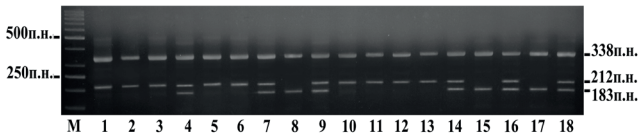

Figure 1 Electrophoregram of the *SEPP1* (rs7579) gene polymorphism restriction fragments distribution. Samples 1, 2, 3, 5, 6, 10-13 – genotype *GG*; samples 4, 7, 9, 14, 16, 18 – genotype *GA*; samples 8, 15, 17 – genotype *AA*; M – DNA ladder, 50 base pair.

variances between the two samples), ANOVA, Pearson's χ^2 test, or the Wilcoxon-Mann-Whitney U-test (in case of uneven data distribution according to W-Shapiro-Wilk or Kolmogorov-Smirnov test results). Logistic regression analysis was accomplished to calculate the risk of pathology using relative risk (RelR), risk ratio (RR), odds ratio (OR) with 95% confidence interval [95% CI]. Differences were regarded as significant at P<0.05 values.

RESULTS AND DISCUSSION

The *SEPP1* gene's (rs7579) polymorphic variants distribution in patients with isolated CP and comorbid CP with hypothyroidism is shown in Figure 2. There were no subjects with mutated *AA*-genotype among the patients with CP, in contrast to the comorbid course of CP: the *A*-allele in pancreatitis patients with hypothyroidism was found 15% more often and *G*-allele on the contrary is less common than in isolated CP individuals (χ^2=5.13; p=0.02). The *A*-allele increases the risk of hypothyroidism twice in CP patients [OR=2.0; OR 95%CI:1.09-3.66; p=0.023]. The clinical course of CP was milder compared to comorbidity condition in terms of the course severity, grievousness of complaints and laboratory-diagnostic markers.

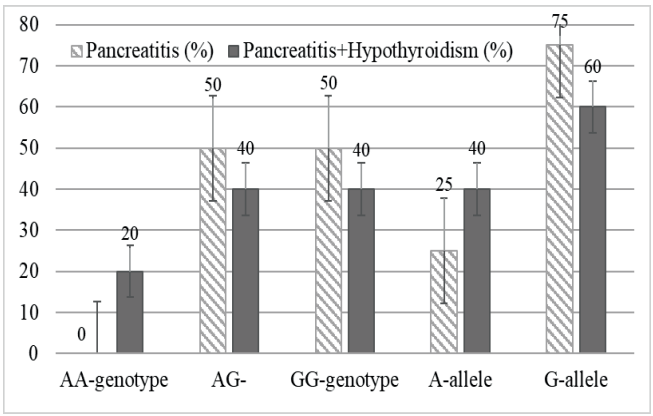

Figure 2 Genotypes and alleles distribution of the *SEPP1* (rs7579) gene depending on patient's groups (chronic pancreatitis vs chronic pancreatitis + hypothyroidism).

Pancreatitis patients with hypothyroidism and *AA*-genotype had lower concentration of pancreatic elastase (Table 3) by 60.0% (p_{AA}=0.013) and higher by 13.78% (p=0.003) level of pancreatic α-amylase, as well as TSH – by 10.81% (p_{AA}=0.009) and 15.64% (p_{AA}=0.009), respectively; at a lower value of Selenoprotein P – by 28.65-48.08% (p≤0.048) regardless of the *SEPP1* gene (rs7579) polymorphic variants. In case of CP comorbid course the TSH concentration was 2.20-2.44 times higher (p<0.001) as well as total NO metabolites content – by 29.49-37.74% (p<0.001) and contrary the level of free T4 on was 3.68-3.97 times lower (p<0.001) than in isolated CP patients.

Table 3 Enzymatic and hormonal activity markers and NO metabolites levels in patients with chronic pancreatitis and hypothyroidism depending on *SEPP1* (rs7579) gene's polymorphism, mean±SD

Parameters	*SEPP1* gene (rs7579) genotypes	Chronic pancreatitis patients	Chronic pancreatitis with hypothyroidism patients
Pancreatic elastase, μg/g	AA-	-	100.0
	AG-	158.33±26.92	140.0±25.45
	GG-	150.0±25.90	160.0±27.27 p_{AA}=0.013
Pancreatic α-amylase according to Caraway, mg/(sec*l)	AA-	-	11.70±0.35
	AG-	10.52±0.49	11.97±0.33 p=0.003
	GG-	10.65±0.56	11.03±0.43 p_{AG}=0.041
Selenoprotein P, ng/mL	AA-	-	2.60±0.31
	AG-	4.43±0.35	2.30±0.32 p<0.001
	GG-	3.77±0.55	2.69±0.47 p=0.048
TSH, mIU/mL	AA-	-	7.69±0.21
	AG-	3.15±0.28	6.94±0.28 p<0.001 p_{AA}=0.04
	GG-	2.85±0.34	6.65±0.32 p<0.001 p_{AA}=0.009
Free T4, ng/dL	AA-	-	0.34±0.05
	AG-	1.27±0.09	0.32±0.08 p<0.001
	GG-	1.51±0.10 p_{AG}=0.026	0.41±0.05 p<0.001
Total NO metabolites (NO_2^-/NO_3^-), μmol/l	AA-	-	38.06±0.31
	AG-	29.33±0.52	37.98±0.80 p<0.001
	GG-	28.80±1.18	39.67±1.02 p<0.001

P – significance of differences with Chronic pancreatitis patients; P_{AA}, P_{AG} - significance of differences with *AA*-genotype or *AG*-genotype carriers in corresponding group; TSH - Thyroid-stimulating hormone; T4 - free Thyroxine.

In comorbid CP patients with hypothyroidism BMI, Glucose, TG, LDL-C values and AI were higher than in CP patients by 11.83-57.98% (p≤0.024) with lower GFR (CKD-EPI after creatinine value) – by 18.30% (p=0.024) (Table 4). But depending on polymorphic variants of *SEPP1* (rs7579) gene in comorbid *GG*-genotype carriers there was a surprisingly higher blood glucose level than in those with the *AA*-genotype – by 13.72% (p_{AA}=0.012). On the other hand, pancreatic comorbid patients with *A*-allele (particular *AA*-genotype) had higher concentration of TC and LDL-C than those with the *GG*-genotype – by 11.77-26.45% (p_{AA}≤0.01) and 18.06-26.21% (p_{AA}≤0.019), respectively.

Table 4 Metabolic activity in patients with chronic pancreatitis and hypothyroidism depending on *SEPP1* (rs7579) gene's polymorphism, mean±SD

Parameters	*SEPP1* gene (rs7579) genotypes	Chronic pancreatitis patients	Patients with chronic pancreatitis and hypothyroidism
BMI, kg/m²	*AA*-	-	27.50±0.28
	AG-	24.69±1.80	27.61±0.94 p=0.006
	GG-	24.39±1.24	27.75±0.93 p=0.01
Glucose, mmol/l	*AA*-	-	4.52±0.12
	AG-	4.65±0.17	4.73±0.17
	GG-	4.46±0.21	5.14±0.17 p=0.006 p_{AA}=0.012
TC, mmol/l	*AA*-	-	6.74±0.10
	AG-	5.14±0.20	6.03±0.25 p=0.002 p_{AA}=0.01
	GG-	4.92±0.26	5.33±0.27 p_{AA}<0.001 p_{AG}=0.028
TG, mmol/l	*AA*-	-	2.04±0.07
	AG-	1.52±0.17	1.99±0.17 p=0.021
	GG-	1.67±0.19	2.30±0.20 p=0.016
HDL-C, mmol/l	*AA*-	-	1.0±0.13
	AG-	1.19±0.12	0.98±0.09
	GG-	1.0±0.13	0.92±0.09
LDL-C, mmol/l	*AA*-	-	3.66±0.07
	AG-	2.64±0.25	2.90±0.21 p_{AA}=0.001
	GG-	2.37±0.30	3.10±0.25 p=0.015 p_{AA}=0.019
AI, U	*AA*-	-	6.45±0.81
	AG-	3.57±0.39	5.64±0.71 p=0.01
	GG-	4.56±0.80	5.20±0.45
GFR (CKD-EPI after creatinine value), mL/min/1.73m²	*AA*-	-	68.58±3.82
	AG-	79.45±7.27	64.91±3.63 p=0.024
	GG-	83.53±6.54	72.47±5.53

P – significance of differences with Chronic pancreatitis patients; P_{AA}, P_{AG} - significance of differences with *AA*-genotype or *AG*-genotype carriers in corresponding group; TC – total cholesterol; TG – triglycerides; HDL-C, LDL-C – high-, low density level cholesterol; AI – Atherogenicity Index; GFR CKD-EPI – glomerular filtration rate (GFR) is estimated by an equation developed by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI).

Gender distribution of pancreatitis patients depending on polymorphic variants of *SEPP1* (rs7579) gene showed that female *A*-allele carriers have a higher pancreatic α-amylase level and TSH blood concentration by 14.55% (p_{GG}<0.001) and 45.18% (p_{GG}<0.001), but lower than in *GG*-men by 9.50% (p_W<0.001) and 60.31% (p_W=0.034), respectively (Table 5). On the other hand, the *A*-allele women have free T4 value 2.62 times lower (p_{GG}=0.01) than *GG*-women and 2.97 times less (p_W=0.011) than men. Moreover, women generally sowed higher levels of total NO metabolites by 17.43-18.14% (p_W≤0.032) and 39.30% lower of Selenoprotein P content (p_W=0.034) than men.

Table 5 Enzymatic and hormonal activity markers and NO metabolites level in observed patients with chronic pancreatitis depending on gender, mean±SD

Parameters	*SEPP1* gene (rs7579) genotypes	Observed patients with Chronic pancreatitis	
		Women	Men
Pancreatic elastase, μg/g	*AA-, AG-*	123.08±18.13	166.67±18.13
	GG-	153.85±26.92	155.55±27.77
Pancreatic α-amylase according to Caraway, mg/(sec*l)	*AA-, AG-*	11.89±0.40	11.43±0.38
	GG-	10.38±0.37 $p_{AA,AG}$<0.001	11.47±0.49 p_W<0.001
Selenoprotein P, ng/mL	*AA-, AG-*	2.44±0.27	4.02±0.60 p_W=0.034
	GG-	3.12±0.54	3.51±0.55
TSH, mIU/mL	*AA-, AG-*	7.23±0.29	4.51±0.69 p_W=0.005
	GG-	4.98±0.77 $p_{AA,AG}$<0.001	3.99±0.78
Free T4, ng/dL	*AA-, AG-*	0.32±0.07	0.95±0.17 p_W=0.011
	GG-	0.84±0.27 $p_{AA,AG}$=0.01	1.25±0.16
Total NO metabolites (NO_2^-/NO_3^-), μmol/l	*AA-, AG-*	37.99±0.61	32.35±1.64 p_W=0.012
	GG-	36.02±2.01	30.49±2.25 p_W=0.032

P_W – significance of differences with women; P_{AA}, P_{AG} - significance of differences with *AA*-genotype or *AG*-genotype carriers in corresponding group; TSH - Thyroid-stimulating hormone; T4 - free Thyroxine.

Women with pancreatitis and *A*-allele of the *SEPP1* gene (rs7579) had higher concentration of TC than *GG*-genotype women and men by 26.57% (p_{GG}<0.001) and 23.65% (p_W<0.001), respectively (Table 6). Therewithal, women generally have higher LDL-C and AI values by 35.87-54.50% (p_W≤0.017) with a lower GFR – by 21.96-24.56% (p_W≤0.033) than men, respectively.

Table 6 Metabolic activity in observed patients with chronic pancreatitis depending on gender, mean±SD

Parameters	*SEPP1* gene (rs7579) genotypes	Observed patients with Chronic pancreatitis	
		Women	Men
BMI, kg/m²	*AA-, AG-*	27.28±0.70	26.92±0.85
	GG-	26.45±1.55	25.14±1.04
Glucose, mmol/l	*AA-, AG-*	4.70±0.16	4.50±0.20
	GG-	4.76±0.28	4.78±0.19
TC, mmol/l	*AA-, AG-*	6.43±0.23	5.20±0.13 p_W<0.001
	GG-	5.08±0.33 $p_{AA,AG}$<0.001	5.16±0.16
TG, mmol/l	*AA-, AG-*	1.98±0.10	1.58±0.21
	GG-	2.05±0.26	1.82±0.19
HDL-C, mmol/l	*AA-, AG-*	0.94±0.10	1.05±0.07
	GG-	0.95±0.14	0.99±0.08
LDL-C, mmol/l	*AA-, AG-*	3.17±0.23	2.62±0.24
	GG-	3.03±0.18	2.23±0.32 p_W=0.017
AI, U	*AA-, AG-*	6.35±0.80	4.11±0.30 p_W=0.002
	GG-	5.06±0.68	4.55±0.58
GFR (CKD-EPI after creatinine value), mL/min/1.73m²	*AA-, AG-*	64.98±4.0	86.13±7.80 p_W=0.033
	GG-	70.40±4.90	90.21±6.20 p_W=0.005

P_W – significance of differences with women; P_{AA}, P_{AG} - significance of differences with *AA*-genotype or *AG*-genotype carriers in corresponding group; TC – total cholesterol; TG – triglycerides; HDL-C, LDL-C – high-, low density level cholesterol; AI – atherogenity index; GFR CKD-EPI – glomerular filtration rate (GFR) is estimated by an equation developed by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI).

Genetic factors play a significant role in determining the risk of recurrent acute pancreatitis (RAP) progressing to chronic pancreatitis[26]. European Clinical Guidelines recommend genetic testing in cases where the cause of CP is unclear, there is a family history of pancreatic diseases, the disease persists following

therapeutic interventions (such as RAP after biliary system clearance), or the patient is below 35 years of age[7]. Additionally, genetic testing is advised in cases of differential diagnosis for CP, including autoimmune inflammation affecting the pancreas, inflammation and fibrosis related to pancreatic islet cells associated with diabetes mellitus (DM), conditions or medications that affect the immune system, or renal diseases causing secondary effects on the pancreas[7]. Several studies have suggested a link between CP development and selenium deficiency, leading to metabolic disorders such as hyperglycemia and dyslipidemia, which can result in nutritional pancreatic atrophy due to the down-regulation of selenoprotein-encoding and insulin-signaling genes[27,28]. Selenocysteine, incorporating selenium, is a key component of numerous selenoproteins, with selenoprotein P containing a substantial portion of selenium (10 atoms per molecule). These selenoproteins are thought to play a crucial role in protecting against reactive oxygen species and are linked to various health benefits, including supporting a healthy immune response[17,29], cancer prevention, improved male fertility, reduced cardiovascular disease mortality, and regulation of inflammatory mediators in asthma[21]. We suggest that these effects of Selenoproteins are due to their catalytic functions in numerous chemical processes and are expressed in various tissues. This family includes glutathione peroxidase enzymes (GPx), such as classical, gastrointestinal, plasmatic, and phospholipid hydroperoxide forms, as well as thioredoxin reductase (TR) enzymes, which regulate metabolic activity through NADPH-dependent reduction of thioredoxin. Another important group of selenoproteins influences thyroid hormone production and activity by catalyzing the conversion of thyroxine (T4) into the active thyroid hormone, triiodothyronine (T3), through iodothyronine deiodinase enzymes[21].

In our research, we investigated the comorbid condition of CP and hypothyroidism in relation to a deficit in Selenoprotein P, resulting in a decrease of 28.65-48.08% ($p \leq 0.048$). We also explored the reverse scenario, irrespective of *SEPP1* rs7579 gene's polymorphism, alongside accompanying dysmetabolic changes, including hyperglycemia, elevated total cholesterol, triglycerides, and low-density lipoprotein cholesterol, and a decrease in glomerular filtration rate.

Chronic inflammation contributes to the overproduction of reactive oxygen species (ROS) and nitrogen species (RNS), including superoxide radicals, hydrogen peroxide (H_2O_2), nitric oxide (NO), and peroxynitrite. These elements result in reactive oxidative and nitrosative stress (ROS/RNS), potentially worsening pancreatic dysfunction and destruction, as well as inflammation in CP. Hyperglycemia[30] and adipose tissue in insulin-resistant individuals lead to increased production of proinflammatory cytokines, superoxide generation, and enhanced NO levels through the stimulation of NOx and inducible NO synthase[31,32]. The excessive NO production, when combined with superoxide, generates toxic NO metabolite peroxynitrite, which can contribute to inflamed pancreatic matrix destruction, cell apoptosis, endocrine and exocrine pancreatic dysfunction, damage to the intestinal microbiome, and other related issues[33], forcing cellular apoptosis, progression of both endocrine and exocrine pancreatic dysfunction[2], damage to the intestinal microbiome, etc. Thyroid dysfunction can exacerbate the underlying pathology through metabolic pathways, regardless of its location[34-37]. In our study, we observed that lower levels of the antioxidant protein *SEPP1*, particularly in women and in patients with comorbid CP and hypothyroidism, were associated with increased serum concentrations of total NO metabolites (NO_{2-}/NO_{3-}) by 29.49-37.74% ($p<0.001$). We hypothesized that the heightened production of NO metabolites might worsen exocrine and endocrine pancreatic dysfunction.

While the *SEPP1* gene (rs7579) polymorphism has been previously studied in various oncological pathologies, including prostate cancer, gastric cancer, breast cancer, colorectal cancer, and advanced distal colorectal adenoma, no research has explored its relation to CP[22,27,38-40]. Therefore, our study represents the first investigation into the association between the *SEPP1* gene (rs7579) polymorphism, pancreas enzymatic activity, thyroid hormone production, and metabolic changes in CP patients. Several systemic similarities with other genetically based clinical studies may be observed, including bronchial asthma[41], coronary artery disease[42], and arterial hypertension[43]. Our findings suggest that downregulation of *SEPP1*, particularly in cases of hyperglycemia, dyslipidemia, and thyroid dysfunction (especially in women and

A-allele carriers of the *SEPP1* rs7579 gene's single nucleotide polymorphism), may contribute to pancreatic insufficiency and hypothyroidism in the pathogenesis of CP, potentially playing a role in the antioxidant protection of pancreatic cells. This statement is well consistent with other studies related to metabolic disorders[44,45].

CONCLUSIONS

The *A*-allele two-fold increases the risk of hypothyroidism in CP patients. Pancreatic patients with hypothyroidism and *AA*-genotype of the *SEPP1* gene (rs7579) have lower concentration of pancreatic elastase by 60.0% and higher level of pancreatic α-amylase – by 13.78% as well as TSH – by 10.81-15.64%, respectively, especially in women – 14.55-45.18% ($p<0.001$) at lower value of Selenoprotein P – by 28.65-48.08% ($p\leq0.048$) regardless of the *SEPP1* gene's (rs7579) polymorphic variants.

Besides, women generally have higher level of total NO metabolites by 17.43-18.14% and 39.30% lower Selenoprotein P content ($p_W\leq0.034$) than men; *A*-allele women have free T4 value 2.62 times lower than *GG*-women and 2.97 times less than men. Moreover, pancreatic comorbid patients with *A*-allele (particularly *AA*-genotype, mainly women) had elevated content of TC and LDL-C than those with the *GG*-genotype – by 11.77-26.45% and 18.06-26.21%, respectively. In addition, women generally have higher AI by 54.50% with a lower GFR – by 21.96-24.56% than men.

There was no dependence of *SEPP1* level and Total NO metabolites values on the *SEPP1* gene (rs7579) polymorphism discerned.

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