Acute pancreatitis in adults and in children

Ph.D. Thesis

Andrea Párniczky, M.D.

Heim Pál Children's Hospital

Institute for Translational Medicine, University of Pécs

Supervisors:

Prof. Péter Hegyi, M.D., Ph.D., D.Sc.[1,2,3]

István Hritz, M.D., Ph.D.[4]

[1]First Department of Medicine Faculty of Medicine University of Szeged, Szeged, Hungary

[2]MTA-SZTE Lendület Translational Gastroenterology Research Group, Szeged, Hungary

[3]Institute for Translational Medicine, University of Pécs, Hungary

[4]First Department of Surgery, Endoscopic Unit Semmelweis University, Budapest, Hungary

Szeged

2017

TABLE OF CONTENTS

Publications related to the subject of the thesis

1. **Párniczky A**, Kui B, Szentesi A, Balázs A, Szűcs Á, Mosztbacher D, Czimmer J, Sarlós P, Bajor J, Gódi S, Vincze Á, Illés A, Szabó I, Pár G, Takács T, Czakó L, Szepes Z, Rakonczay Z, Izbéki F, Gervain J, Halász A, Novák J, Crai S, Hritz I, Góg C, Sümegi J, Golovics P, Varga M, Bod B, Hamvas J, Varga-Müller M, Papp Z, Sahin-Tóth M, Hegyi P; Hungarian Pancreatic Study Group. Prospective, Multicentre, Nationwide Clinical Data from 600 Cases of Acute Pancreatitis.
PLoS One. 2016 31;11(10):e0165309.
IF (2015/2016): 3.54

2. **Párniczky A**, Mosztbacher D, Zsoldos F, Tóth A, Lásztity N, Hegyi P; Hungarian Pancreatic Study Group and the International Association of Pancreatology.
Analysis of Pediatric Pancreatitis (APPLE Trial): Pre-Study Protocol of a Multinational Prospective Clinical Trial
Digestion. 2016 93(2):105-10.
IF (2015/2016): 1.06

3. Hegyi E, Geisz A, Sahin-Tóth M, Derikx MH, Németh BC, Balázs A, Hritz I, Izbéki F, Halász A, **Párniczky A**, Takács T, Kelemen D, Sarlós P, Hegyi P, Czakó L; Hungarian Pancreatic Study Group. SPINK1 Promoter Variants in Chronic Pancreatitis.
Pancreas. 2016 45(1):148-53.
IF (2015/2016): 2.738

Publications not related to the subject of the thesis

1. **Párniczky A**, Hegyi E, Tóth AZ, Szücs Á, Szentesi A, Vincze Á, Izbéki F, Németh BC, Hegyi P, Sahin-Tóth M.
Genetic Analysis of Human Chymotrypsin-Like Elastases 3A and 3B (CELA3A and CELA3B) to Assess the Role of Complex Formation between Proelastases and Procarboxypeptidases in Chronic Pancreatitis.
Int J Mol Sci. 2016 Dec 20;17(12). pii: E2148.
IF (2015/2016): 4.01

2. **Párniczky A**, Czakó L, Dubravcsik Z, Farkas G, Hegyi P, Hritz I, Kelemen D, Morvay Z, Oláh A, Pap Á, Sahin-Tóth M, Szabó F, Szentkereszti Z, Szmola R, Takács T, Tiszlavicz L, Veres G, Szücs Á, Lásztity N; Magyar Hasnyálmirigy Munkacsoport, Hungarian Pancreatic Study Group.
Pediatric pancreatitis. Evidence based management guidelines of the Hungarian Pancreatic Study Group.
Orv Hetil. 2015 Feb 22;156(8):308-25. Review. Hungarian.
IF (2015): 0.291

3. Mosztbacher D, Farkas N, Solymár M, Pár G, Bajor J, Szűcs Á, Czimmer J, Márta K, Mikó A, Rumbus Z, Varjú P, Hegyi P, **Párniczky A**.
Restoration of energy level in the early phase of acute pediatric pancreatitis.
World J Gastroenterol. 2017 Feb 14;23(6):957-963.
IF (2015/2016): 3.8

4. Szücs Á, Marjai T, Szentesi A, Farkas N, **Párniczky A**, Nagy G, Kui B, Takács T, Czakó L, Szepes Z, Németh BC, Vincze Á, Pár G, Szabó I, Sarlós P, Illés A, Gódi S, Izbéki F, Gervain J, Halász A, Farkas G, Leindler L, Kelemen D, Papp R, Szmola R, Varga M, Hamvas J, Novák J, Bod B, Sahin-Tóth M, Hegyi P; Hungarian Pancreatic Study Group.
Chronic pancreatitis: Multicentre prospective data collection and analysis by the Hungarian Pancreatic Study Group.
PLoS One. 2017 Feb 16;12(2):e0171420.
IF (2015/2016): 3.54

5. Rumbus Z, Matics R, Hegyi P, Zsiboras C, Szabo I, Illes A, Petervari E, Balasko M, Marta K, Miko A, **Parniczky A**, Tenk J, Rostas I, Solymar M, Garami A.
Fever Is Associated with Reduced, Hypothermia with Increased Mortality in Septic Patients: A Meta-Analysis of Clinical Trials.
PLoS One. 2017 Jan 12;12(1):e0170152.
IF (2015/2016): 3.54

6. Tenk J, Mátrai P, Hegyi P, Rostás I, Garami A, Szabó I, Solymár M, Pétervári E, Czimmer J, Márta K, Mikó A, Füredi N, **Párniczky A**, Zsiborás C, Balaskó M.
In Obesity, HPA Axis Activity Does Not Increase with BMI, but Declines with Aging: A Meta-Analysis of Clinical Studies.
PLoS One. 2016 Nov 21;11(11):e0166842.
IF (2015/2016): 3.54

7. Alfaro Cruz L, **Parniczky A**, Mayhew A, Hornung LN, Lin TK, Palermo JJ, Jackson K, Abu-El-Haija M.
Utility of Direct Pancreatic Function Testing in Children.
Pancreas. 2017 Feb;46(2):177-182.
IF (2015/2016): 2.738

8. Szentesi A, Tóth E, Bálint E, Fanczal J, Madácsy T, Laczkó D, Ignáth I, Balázs A, Pallagi P, Maléth J, Rakonczay Z Jr, Kui B, Illés D, Márta K, Blaskó Á, Demcsák A, **Párniczky A**, Pár G, Gódi S, Mosztbacher D, Szücs Á, Halász A, Izbéki F, Farkas N, Hegyi P; Hungarian Pancreatic Study Group..
Analysis of Research Activity in Gastroenterology: Pancreatitis Is in Real Danger.
PLoS One. 2016 Oct 24;11(10):e0165244.
IF (2015/2016): 3.54

9. Márta K, Farkas N, Szabó I, Illés A, Vincze Á, Pár G, Sarlós P, Bajor J, Szűcs Á, Czimmer J, Mosztbacher D, **Párniczky A**, Szemes K, Pécsi D, Hegyi P.
Meta-Analysis of Early Nutrition: The Benefits of Enteral Feeding Compared to a Nil Per Os Diet Not Only in Severe, but Also in Mild and Moderate Acute Pancreatitis.
Int J Mol Sci. 2016 Oct 20;17(10).
IF (2015/2016): 4.01

10. Balázs A, Németh BC, Ördög B, Hegyi E, Hritz I, Czakó L, Czimmer J, Gódi S, Csiszkó A, Rakonczay Z Jr, **Párniczky A**, Izbéki F, Halász A, Kahán Z, Hegyi P, Sahin-Tóth M; Hungarian Pancreatic Study Group.
A Common CCK-B Receptor Intronic Variant in Pancreatic Adenocarcinoma in a Hungarian Cohort.

Pancreas. 2016 Apr;45(4):541-5.
IF (2015/2016): 2.738

11. Zsoldos F, **Párniczky A**, Mosztbacher D, Tóth A, Lásztity N, Hegyi P; Hungarian Pancreatic Study Group and the International Association of Pancreatology.
Pain in the Early Phase of Pediatric Pancreatitis (PINEAPPLE Trial): Pre-Study Protocol of a Multinational Prospective Clinical Trial.
Digestion. 2016;93(2):121-6.
IF (2015/2016): 1.06

12. Balázs A, Ruffert C, Hegyi E, Hritz I, Czakó L, Takács T, Szepes Z, Németh BC, Gervain J, Izbéki F, Halász A, Kelemen D, Szmola R, Novák J, Crai S, Illés A, Vincze Á, Molnár Z, Varga M, Bod B, Farkas G Jr, Sümegi J, Szepes A, Dubravcsik Z, Lásztity N, **Párniczky A**, Hamvas J, Andorka C, Veres G, Szentkereszty Z, Rakonczay Z Jr, Maléth J, Sahin-Tóth M, Rosendahl J, Hegyi P; Hungarian Pancreatic Study Group.
Genetic analysis of the bicarbonate secreting anion exchanger SLC26A6 in chronic pancreatitis.
Pancreatology. 2015 Sep-Oct;15(5):508-13.
IF (2015): 2.406

13. Szmola R, Farkas G, Hegyi P, Czakó L, Dubravcsik Z, Hritz I, Kelemen D, Lásztity N, Morvay Z, Oláh A, **Párniczky A**, Rubovszky G, Sahin-Tóth M, Szentkereszti Z, Szücs Á, Takács T, Tiszlavicz L, Pap Á; Magyar Hasnyálmirigy Munkacsoport, Hungarian Pancreatic Study Group..
Pancreatic cancer. Evidence based management guidelines of the Hungarian Pancreatic Study Group.
Orv Hetil. 2015 Feb 22;156(8):326-39. Review. Hungarian.
IF (2015): 0.291

14. Dubravcsik Z, Farkas G, Hegyi P, Hritz I, Kelemen D, Lásztity N, Morvay Z, Oláh A, Pap Á, **Párniczky A**, Sahin-Tóth M, Szentkereszti Z, Szmola R, Takács T, Tiszlavicz L, Szücs Á, Czakó L; Magyar Hasnyálmirigy Munkacsoport, Hungarian Pancreatic Study Group.
Autoimmune pancreatitis. Evidence based management guidelines of the Hungarian Pancreatic Study Group.
Orv Hetil. 2015 Feb 22;156(8):292-307. Review. Hungarian.
IF (2015): 0.291

15. Takács T, Czakó L, Dubravcsik Z, Farkas G, Hegyi P, Hritz I, Kelemen D, Lásztity N, Morvay Z, Oláh A, Pap Á, **Párniczky A**, Patai Á, Sahin-Tóth M, Szentkereszti Z, Szmola R, Tiszlavicz L, Szücs Á; Magyar Hasnyálmirigy Munkacsoport. Hungarian Pancreatic Study Group.
Chronic pancreatitis. Evidence based management guidelines of the Hungarian Pancreatic Study Group.
Orv Hetil. 2015 Feb 15;156(7):262-88. Review. Hungarian.
IF (2015): 0.291

16. Hritz I, Czakó L, Dubravcsik Z, Farkas G, Kelemen D, Lásztity N, Morvay Z, Oláh A, Pap Á, **Párniczky A**, Sahin-Tóth M, Szentkereszti Z, Szmola R, Szücs Á, Takács T, Tiszlavicz L, Hegyi P; Magyar Hasnyálmirigy Munkacsoport,
Acute pancreatitis. Evidence-based practice guidelines, prepared by the Hungarian Pancreatic Study Group.

Orv Hetil. 2015 Feb 15;156(7):244-61. Review. Hungarian.
IF (2015): 0.291

17. Balaskó M, Soós S, **Párniczky A**, Koncsecskó-Gáspár M, Székely M, Pétervári E.
Anorexic effect of peripheral cholecystokinin (CCK) varies with age and body composition (short communication).
Acta Physiol Hung. 2012 Jun;99(2):166-72.
IF (2012): 0.43

18. E. Pétervári, M. Balaskó, M. Solymár, **A. Párniczky**, M. Székely, Z. Szelényi
CCK-8 induces fever-like regulated hyperthermia and symptoms of sickness behavior in mice: A biotelemetric study
J Therm Biol, 2012 July;37(4):297–301.
IF (2012): 1.84

19. Faluhelyi N, Matkovits A, **Párniczky A**, Csernus V.
The in vitro and in ovo effects of environmental illumination and temperature on the melatonin secretion from the embryonic chicken pineal gland.
Ann N Y Acad Sci. 2009 Apr;1163:383-5.
IF (2009): 1.07

Number of full publications:	**22**	**(4 first author)**	**IF: 8.9**
Cumulative impact factor:	**47.055**		

LIST OF ABBREVIATION

AP	acute pancreatitis
ACP	alcoholic chronic pancreatitis
ALP	alkaline phosphatase
APA	American Pancreatic Association
APPLE	Analysis of Pediatric Pancreatitis
BISAP	Bedside Index of Severity in Acute Pancreatitis
BMI	body mass index
BUN	blood urea nitrogen
CP	chronic pancreatitis
CFTR	cystic fibrosis transmembrane conductance regulator
CPA1	carboxypaptidase A1
CRF	clinical research form
CRP	C-reactive protein
CT	computertomography
CTRC	chymotrypsin C
EBM	evidence based medicine
EDTA	ethylenediamine tetraacetic acid
ERCP	endoscopic retrograde cholangiopancreatography
ESGE	European Society of Gastointestinal Endoscopy
EST	endoscopic sphinchterotomy
GGT	gamma-glutamyl transferase
HPSG	Hungarian Pancreatic Study Group
IAP	International Association of Pancreatology
ICMJE	International Committee of Medical Journal Editors
INSPPIRE	International Study Group of Pediatric Pancreatitis: In Search for a Cure
K	potassium
LDH	lactate dehydrogenase
LOH	length of hospitalization
Na	sodium
OR	odds ratio
PCR	polymerase chain reaction
PCT	procalcitonin
PP	pediatric panreatitis
PPI	proton pump inhibitor
PRSS1	protease serine 1
SGOT	glutamic oxaloacetic transaminase
SGPT	glutamic pyruvic transaminase
SPINK1	serine peptidase inhibitor Kazal type 1
US	ultrasonography
WBC	white blood cells
WHO	World Health Organization

1. INTRODUCTION

Acute pancreatitis (AP) is a serious disease with high mortality (1). The reported incidence is variable in different countries (10–100/100,000 people) (2), and AP is a leading cause of acute hospitalization for gastrointestinal disorders (3). Therefore, large, nationwide, prospectively collected cohorts are needed. Adherence to treatment guidelines has been documented to reduce mortality and/or severity of AP (1). Inspite of the rising incidence of pediatirc pancreatitis in the past few years (4–11), international guideline for the management of childhood onset pancreatitis is still missing.

Therefore research on pancreatitis both in adult and pediatruc field are crucially important.

2. AIMS

2.1. Specific aim 1.

The main goals of our study were to analyse the course of AP in a prospectively collected cohort of patients from Hungarian centres and to validate the major recommendations in the IAP/APA evidence-based guidelines for the management of AP.

2.2. Specific aim 2.

To organize an international, observational clinical trial for pediatric pancreatitis to collect a critical mass of clinical data and biomedical research samples in uniform, prospective manner (Analysis of Pediatric Pancreatitis-APPLE).

2.3. Specific aim 3.

Identify potential pathogenic promoter variants of SPINK1 in CP patients, determine their possible linkage with SPINK1 coding region variants, and assess the relevance of the novel promoter variants in relation to disease.

3. PATIENTS AND METHODS (P&M)

3.1. P&M for Aim 1: Prospective, Multicentre, Nationwide Clinical Data from 600 Cases of Acute Pancreatitis

3.1.1. Subjects and study design

The study was approved by the Scientific and Research Ethics Committee of the Medical Research Council (22254-1/2012/EKU). All the participants provided written informed consent

to participate in this study. For this HPSG study cohort, 600 patients in Hungary were prospectively enrolled for two years between 1 January 2013 and 1 January 2015. Eighty-six different parameters were collected (http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0165309 S2Fig). Overall, 77% of the requested data were provided by investigators.

3.2. P&M for Aim 2: Analysis of Pediatric Pancreatitis (APPLE Trial): Pre-Study Protocol of a Multinational Prospective Clinical Trial

The study protocols have been discussed in our international meeting held in Szeged in November 2014, where expert pediatric pancreatologists attended. The study has received the relevant ethical approval (No. ad.52499-3/2014) issued by the National Hungarian Ethical Authority (ETT TUKEB). The trial has been registered at the ISRCTN registry (ISRCTN89664974) which is a primary clinical trial registry recognized by Word Health Organization (WHO) and International Committee of Medical Journal Editors (ICMJE). Electronic clinical research forms have been developed (http://www.pancreas.hu/studies/apple).

3.3. P&M for Aim 3: Variants in Chronic Pancreatitis

3.3.1. Nomenclature

Nucleotide numbering reflects coding DNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the SPINK1 reference sequence (RefSeq: NG_008356.1). Promoter variants were numbered relative to the first nucleotide 5′ of the ATG initiation codon, designated −1.

3.3.2. Patients

Patients were recruited through the Hungarian National Pancreas Registry (www.pancreas.hu). All patients enrolled were Hungarian and gave their informed consent according to the ethical guidelines of the Declaration of Helsinki. A total of 100 unrelated patients with CP (cases) and 100 subjects with no pancreatic disease (controls) were enrolled.

3.3.3. DNA Extraction

Whole blood was collected in EDTA tubes and stored at −80°C. Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany).

3.3.4. Mutational Analysis

The p.N34S mutation was detected by polymerase chain reaction (PCR)–restriction fragment length polymorphism.

3.3.5. Construction of Luciferase Reporter Plasmids With SPINK1 Promoter

A DNA fragment corresponding to the region between c.-541 and c.35 of the SPINK1 genewas cloned into the pGL3-Basic vector (Promega, Madison, Wis) upstream of a firefly luciferase reporter gene (pGL3-SPINK1 plasmid) using restriction sites KpnI and HindIII. SPINK1 promoter variants were introduced into this construct by overlap extension PCR mutagenesis.

3.3.6. Dual Luciferase Reporter Gene Assay

Luciferase expression was measured using the Dual-Glo Luciferase Assay System (Promega). Relative luciferase activity was determined by dividing the firefly and renilla luciferase luminescence results and expressing this firefly/renilla ratio as the percentage of the wild-type pGL3-SPINK1 value. Results were obtained from 3 to 8 independent transfection experiments.

3.4. Statistical methods for studies 3.1-3

Several statistical models have been used to see association between the investigated variables (for details see doctoral thesis and the articles).

4. RESULTS

4.1. Pancreatitis in adults

4.1.1. Epidemiology and aetiology

In our cohort, 56% (n=335) of the patients were male, and 44% (n=265) were female. AP incidence in the males increased between 33 and 38 years and remained high until 68 years, after which it sharply declined. In the females, the highest incidence was between 53 and 78 years. The majority (61.2%) of the cases were mild, 30% were moderate, and 8.8% were severe, according to the revised Atlanta classification (66). Mortality was higher in severe AP (28.3%; $p<0.001$) versus moderate (0.6%) and mild AP (0.3%). The length of hospitalization showed significant differences between these groups (mild: 8.3±0.2 days; moderate: 14.6±0.5 days; severe: 26.2±3.1 days; $p<0.001$). The cost of treatment (calculating only the costs of medications, examinations and interventions) for mild AP was HUF 99,006 (approximately 330 euros); however, it increased to HUF 1,725,135 (approximately 5,750 euros) for severe AP, based on an average of 10 patients per group.

The most common aetiology of AP was biliary disease (43.8%) and alcohol abuse (26.5%). Relative to other aetiologies, the frequency of hyperlipidaemia was significantly lower in mild AP, whereas it was higher in moderate and severe AP, indicating that hyperlipidaemia is a risk factor for severity.

Anamnestic data collected at admission revealed that 21.2% of AP was recurrent and 4.9% had a family history of AP. History of alcohol consumption or smoking was associated with higher mortality in severe AP.

4.1.2. Diagnosis, anamnestic data and symptoms at admission

The majority of AP patients usually presented at emergency departments 1–6 hours or 19–24 hours after the onset of abdominal pain. Not seeking medical attention during the first four days of AP strongly heightened the risk for severe AP and mortality.

In 44.9% of the cases, all three diagnostic criteria (abdominal pain, serum pancreatic enzyme elevation, imaging alterations) were present. Importantly, the lack of an increase in enzyme was a risk factor for severe pancreatitis, whereas the lack of abdominal pain demonstrated a risk for mortality. The absence of imaging alterations in the pancreas significantly decreased the risk of severe AP.

4.1.3. Physical examination at admission

Although a tendency for association was observed between the rise in BMI and severity of AP, a significant difference was not found. With regard to the physical examination, abdominal tenderness developed in all the patients suffering from severe AP; however, the lack of this symptom was favourable for mortality. Abdominal guarding developed in 6.4% of the patients during AP, and this symptom was associated with increased mortality in severe AP. Systolic blood pressure above 180 Hgmm or elevation of heart rate above 100 was significantly connected with severe AP.

4.1.4. Imaging at admission

All the patients had either abdominal US or CT at admission. During abdominal US, only 74.3% of the investigators described the status of the lungs. Pleural fluid and/or pulmonary infiltrates were found in 6.7% of the US examinations, 26.6% of the X-rays and 75.6% of the CT scans. Importantly, mortality was not observed in the absence of lung injury at admission.

4.1.5. Laboratory parameters at admission

A white blood cell (WBC) count above 23,000/μL was associated with severe AP (OR 3.2; 95% CI 1.1–9.2). Furthermore, the average WBC counts differed significantly between the mild vs. moderate and the mild vs. severe AP groups. The level of C-reactive protein (CRP) above 200 mg/L was associated with severe AP (OR 2.8; 95% CI 1.3–6.2). The average CRP levels differed significantly between the mild vs. moderate and the mild vs. severe AP groups. Procalcitonin (PCT) levels above 10 U/L were associated with severe AP (OR 20.6; 95% CI 3.7–115.4). Calcium levels below 2 mmol/L were associated with severe AP (OR 5.2; 95% CI 1.5–17.7). Triglyceride (Tg) levels above 40 mmol/L were associated with severe AP (OR 4.1; 95% CI 1.3–13.6). The average glucose levels differed significantly between mild vs. moderate and mild vs. severe AP cases.

4.1.6. Complications

The most common organ complication in severe AP was of pancreatic origin (87.5%), followed by lung (68.1%), cardiac (47.7%), kidney (36.4%) and brain (11.1%) injury. Mortality in severe AP without respiratory failure was 6.7%, whereas it increased to 50% if lung injury persisted for more than 24 hours. Mortality in severe AP with no cardiac failure was 8.7%,

while it was elevated to 57.1% if cardiac failure lasted for more than 24 hours. Importantly, local complications during AP had no effect on the risk of mortality.

4.1.7. Conservative therapy

Statistical analyses of fluid resuscitation practices in the first 24 hours showed that both severity and mortality are affected by the amount of fluid administered. The optimal fluid amount was between 1500 and 3500 mL. Enteral feeding was employed in 28.7% of the patients. The majority (85.4%) of the patients with severe AP received enteral feeding. In severe AP, the mortality rate rose from 27% to 57% when enteral feeding was not administered.

The practice of antibiotic therapy was widespread in our cohort (77.1% of the cases). In two-thirds of the cases, the indication was for the prevention of infectious complications. There were no relevant differences in mortality or severity between patients who received antibiotics for prevention and those who were treated with antibiotics for infection.

4.1.8. Endoscopic therapy

In 80.6% of the patients with biliary aetiology, ERCP was performed. In 91.9% of the cases, the endoscopic intervention was carried out within the first 48 hours after the diagnosis of AP. In 33.1% of the patients, cholangitis was present, 81.3% had obstruction, and 35.3% had severe or moderate AP. Endoscopic biliary sphincterotomy was carried out in 71.8% of the cases, while endoscopic pancreatic sphincterotomy was conducted in only 7%. A biliary stent was placed in 11.2% of the cases, and 11.5% of the patients received a pancreatic stent.

4.1.9. Interventions

Interventions were performed in 26 cases (4.3%) in our cohort, of which 62% were surgical operations, 19% endoscopic procedures and 19% radiological interventions.

4.2. Pediatric pancreatitis

Our APPLE trial is currently running. Until now we have enrolled 75 acute, 32 recurrent acute and 14 chronic pancreatitis cases to the APPLE-R study: Concerning the etiology, biliary and drug-induced 9-9%, trauma, acohol2-2%, post-ERCP and anatomic 5-5%, other 14% were identified however 54% of the cases still remained idiopathic. In 121 cases, genetic analyses of *PRSS1*, *SPINK1*, *CFTR* and *CTRC* genes have been completed. 48,8% (59/121) of the patients

have pathogenic variants. Genetic alterations in *PRSS1* were found in 4 cases, *SPINK1* in 13 cases, *CPA1* in 2 cases, *CFTR* in 15 case and *CTRC* in 51 cases. Pathogenic variants in two genes were observed: 2 *PRSS1-CTRC*, 1 *PRSS1-SPINK1*, 6 *SPINK1-CTRC*, 1 *SPINK1-CFTR*, 7 *CTRC-CFTR*, 1 *CPA1-CFTR*. There were no pathogenic variants in 62 cases.

APPLE-P: We have already enrolled 18 patients with AP. More data is necessary for detailed analysis (12).

4.3. SPINK1 Promoter Variants in Chronic Pancreatitis

4.3.1. Sequence Analysis of the SPINK1 Promoter Region

In the approximately 1.2-kb SPINK1 promoter region, 6 different variants were detected. Two common polymorphisms (c.-253T>C and c.-807C>T) were present in cases and controls. The C-allele of the c.-253T>C polymorphism was significantly overrepresented in CP patients (19%) compared with controls (10%) (odds ratio [OR], 2.1; 95% confidence interval [95% CI], 1.2-3.8; P = 0.015). In subgroup analysis, the variant was observed in similar frequency in ACP patients (13.3%) and controls (10%) (OR, 1.4; 95% CI, 0.7–2.9; P = 0.4) but occurred significantly more frequently in ICP patients (24.5%) (OR, 2.9; 95% CI, 1.5–5.6; P = 0.002). The distribution of the c.-807C>T polymorphism in cases (21%) and controls (17.5%) did not differ significantly (P = 0.45). Three novel heterozygous variants were identified in single cases in patients with ACP, c.-14G>A, c.-108G>T, and c.-246A>G, whereas the heterozygous c.-215G>A variant occurred in 3 patients (2 ACP and 1 ICP). None of these 4 promoter variants was detected in controls.

4.3.2. Sequence Analysis of the SPINK1 Coding Region

The c.-14G>A variant was not associated with other SPINK1 variants. In the case with the c.-108G>T variant, 3 common polymorphisms were detected, the intron 3 variant c.195–323C>T in the heterozygous state and the 3 prime region variants c.*318A>T and c.*407C>G in the homozygous state. In all 3 patients carrying the c.-215G>A variant, the c.194+2T>C mutation in intron 3 was confirmed. Two of these also had the c.*318A>T and c.*407C>G polymorphisms in the heterozygous state. The patient with the c.-246A>G variant carried also a heterozygous variant c.88–23A>T of unknown significance in intron 2.

4.3.3. Functional Analysis of Promoter Variants Using Luciferase Reporter Gene Assay

To determine their functional significance, promoter variants c.-14G>A, c.-108G>T, c.-215G>A, c.-246A>G, and c.-253T>C were introduced into a luciferase reporter plasmid carrying the SPINK1 promoter and AR42J cells were transfected with the constructs. Compared with the wild type, 3 variants (c.-14G>A, c.-108G>T, and c.-246A>G) showed a statistically significant decrease in luciferase expression, with residual activities of 80%, 31%, and 47%, respectively. Variant c.-253T>C had no appreciable effect on the promoter activity, whereas variant c.-215G>A increased luciferase activity to 201%.

5. DISCUSSION

Our study in adults contain among the largest cohorts of any publication on prospectively collected clinical data in AP to date. With regard to epidemiology, our study confirmed findings from other cohorts showing that AP in women is more likely related to gallstones, alcohol-related pancreatitis is more common in men (13,14), and age is a risk factor for AP (15). Cigarette smoking has been linked to AP (16,17); we observed that smoking was connected with greater mortality in severe AP.

Hypertriglyceridaemia was an etiological factor of AP in our cohort, as described for other cohorts (18-21). A rise in a serum triglyceride level above 11.3 mmol/L in a patient with AP is considered diagnostic, whereas above 5.6 mmol/L considered suspicion for hypertriglyceridemia-induced AP (19). In our study, an increase in serum triglycerides above 5.6 mmol/L was considered as causative whenever no other obvious aetiology of AP was apparent. One of the most important new observations of this study is that hypertriglyceridaemia is associated with severe AP. We could see a tendency between the rise in BMI and risk of severe AP.

With regard to laboratory parameters, a WBC count above 23,000/µL, CRP evels above 200 mg/L, PCT levels above 10 U/L, calcium levels below 2 mmol/L and triglyceride levels above 40 mmol/L were all associated with severe AP. Average glucose levels differed significantly between the three AP severity groups.

Our study support the recommendations in the IAP/APA guidelines. The first 24 hours were found to be the “golden hours” in AP (22, 23). For example, recommendations D9–10 highlight the importance of fluid resuscitation at admission. In our cohort, fluid volume below

1500 mL or above 3500 mL raised the risk of mortality and AP severity. In the current study, 71.7% of the patients received the recommended amount of fluid. In this group, the severity rate was only 7.8%, whereas it increased to 14.1% in patients who were given less than 1500 mL or more than 3500 mL. Moreover, the mortality rate was also elevated from 2.3% to 5.1%. If we consider that around 7,000 new AP cases are diagnosed in Hungary in a year, hypothetically, we would prevent 124 patients from developing severe AP if fluid therapy were administered according to the IAP/APA guidelines. This would result in cost savings of HUF 200 million (about 672,000 euros) and could save 57 lives in a country of 10 million people. Projection of these calculations to the entire Eastern European region, with a population of about 200 million people, indicates that implementation of the EBM guidelines could save over a thousand lives.

Secondly, we wanted to investigate the situation is children. Although the incidence of AP in children is only 2–3 times less than in adults, our knowledge in pediatric AP is very limited. The current situation is difficult for the following reasons: (i) there is a limited number of scientists working in the field, genetic tests for pancreatic patients are unavailable or testing is not organized at the national level in most of the countries, (ii) there is a big and unacceptable high difference in disease recognition and outcome among the countries, (iii) limited number of biobanks or patient registers are available; those that exist are mostly organized on national levels with no links to international consortia and (iv) funding is often refused because of the low importance. Therefore, multinational efforts are crucially needed (24).

In summary, we propose a multinational observational clinical trial (APPLE) to collect a critical mass of clinical data from children suffering from AP in an uniform prospective manner to help fill the knowledge gap and provide simple, pediatric-specific, clinical scoring system that can stratify patients with AP on presentation. It is important to mention that after the APPLE study, we plan to design a follow-up longitudinal study for better understanding of PP.

Thirdly, In the present thesis we choose a gene which alterations can be found in both adult and children. Sequencing of the SPINK1 promoter region in a Hungarian cohort of CP patients revealed 6 different variants. Of these, the c.-807C>T variant was found in both cases and controls with comparable frequencies and could be considered as a common polymorphism with no clinical significance. The c.-253T>C variant occurred significantly more frequently in ICP patients than in controls; however, a similar enrichment was not observed in ACP patients. We conducted functional characterization of this variant by luciferase reporter gene assay. This variant had no considerable effect on promoter activity, suggesting that c.-253T>C is a harmless variant.

6. NEW DISCOVERIES

6.1. Prospective, Multicentre, Nationwide Clinical Data from 600 Cases of Acute Pancreatitis

The first report about the epidemiology, risk factors, management of AP from Hungary on a prospectively collected cohort. The article highlights the importance of evidence based guidelines. Following results have not been published before:

- Hyperlipidaemia is a risk factor for severity.
- Lack of serum enzyme elevation posed a risk for severe AP.
- Lack of abdominal pain at admission demonstrated a risk for mortality.
- Deviation from the recommendations in the IAP/APA evidence-based guidelines on fluid replacement, enteral nutrition and timing of interventions increased severity and mortality.

6.2. Analysis of Pediatric Pancreatitis (APPLE Trial)

This is the first worldwide study on the field of childhood onset pancreatitis for earlier (APPLE-R) and ongoing episodes (APPLE-P) of pancreatitis to collect a critical mass of clinical data and biomedical research samples in uniform prospective manner.

6.3. SPINK1 Promoter Variants in Chronic Pancreatitis

The common SPINK1 promoter variant c.-253T>C was associated with CP in this cohort. Two of 3 newly identified SPINK1 promoter variants seem to exhibit significant functional defects and should be considered potential risk factors for CP.

7. ACKNOWLEDGEMENTS

I would like to express my most sincere gratitude to my mentor and supervisor, **Professor Péter Hegyi**, at the First Department of Medicine, University of Szeged and Institute for Translational Medicine, University of Pécs who supports my improvement with his guidance, criticism day by day and trusts in my ability unshakeably. Without his outstanding supervision, this Ph.D thesis would not have been possible.

I am grateful to **Professor Miklós Sahin-Tóth** who guided me in the labirinth of the basic science and gave me the opportunity to have international experience.
These great scientist have served as a role model for me.

I would like to express my gratitude also to my other supervisor, **Dr. István Hritz** at the Endoscopic Unit, First Department of Surgery, University of Semmelweis, who supported me with his valuable advices and comments.

I am grateful to the leadership of the Heim Pál Children's Hospital, especially **Dr. Anikó Nagy, Dr. Ferenc Fekete and Dr. András Kiss**, who made me possible to work on this project. Special thanks to **Dr. Judit B. Kovács, Dr. Margit Lőrincz,** to attract my attention to the pediatric gastroenterology and to **Dr. Natália Lásztity**, who encourage me to get deeper knowledge on field of pancreatology.

This work would not have been possible to accomplish without the support and active participation of **all the members of the Hungarian Pancreatic Study Group** and the **Pancreas Laboratory** at the University of Szeged, especially **Zsuzsa Miklósné Árva, Márta Bába, Erika Darvasi, Dr. Csilla Andorka, Dr. Anita Balázs, Dr. Renáta Bor, Dr. László Czakó, Dr. Judit Czelecz, Dr. Alexandra Demcsák, Dr. László Gárdos, Dr. Szilárd Gódi, Dr. Ildikó Guthy, Dr. Adrienn Halász, Dr. Eszter Hegyi, Dr. Emese Horváth, Dr. Judit Gervain, Dr. Imre Ignáth, Dr. Veronika Ila, Dr. Dóra Illés, Dr. Nelli Farkas, Dr. Ferenc Izbéki, Dr. Balázs Kui, Dr. Gábor Lakatos, Dr. József Maléth, Dr. Katalin Márta, Dr. Tímea Molnár, Dr. Dóra Mosztbacher, Dr. Balázs Németh, Dr. Petra Pallagi, Dr. Gabriella Pár, Dr. Dániel Pécsi, Dr. Zoltán Rakonczay, Dr. Anikó Nóra Szabó, Dr. Zoltán Szepes, Dr. Ákos Szücs, Dr. Tamás Takács, Dr. István Tokodi, Dr. Erika Tomsits, Dr. Anna Tóth, Dr. Péter Varjú, Dr. Ibolya Vass, Dr. Viktória Venglovecz, Dr. Fanni Zsoldos, Rea Fritz, Erzsébet Zoltánné Fuksz, Krisztina Harth, Ágnes Kocsisné Halas, Enikő Horváth, Kinga Kaán, Klaudia Kárász, Balázs Koncz, Félix Márk Juhász, Tünde Pritz, Zsuzsanna Répásy, Lajos Szakó, Andrea Szentesi** and all the contributors to the Registry for Pancreatic Patients, a very special thanks to them.

I am greatful to **Andrea Talabér, Fabiola Zsuppán and Dr. Zsolt Bognár** for their help.

Thanks to the international professional advisory team**: Maisam Abu-el Haija, Aliye Uc, Mark Lowe, Flóra Szabó, Micheal Wilschanski, Heiko Witt, Grzegorz Oracz, David Withcomb.**

Last but not least, I owe warm thanks to **my parents, Csilla** and **Péter Párniczky**, who thaught me to be brave and tenacious. Thank you, **my brother Peti** for your patience.

8. FINANCIAL SUPPORT

The studies in this thesis were supported by the Hungarian Academy of Sciences – University of Szeged, Momentum Gastroenterology Multidisciplinary Research Group (LP2014-10/2014 to Péter Hegyi), the National Research, Development and Innovation Office (K116634 to Péter Hegyi) and the Economic Development and Innovation Operational Program (GINOP-2.3.2-15, University of Pécs). Functional studies in the laboratory of Miklós Sahin-Tóth were supported by NIH grants R01DK058088, R01DK082412, and R01DK095753**.**

9. REFERENCES

1. Gompertz M, Lara I, Fernandez L, Miranda JP, Mancilla C, Watkins G, et al. Mortality of acute pancreatitis in a 20 years period]. Rev Med Chil. 2013 May;141(5):562-7.
2. Roberts SE, Akbari A, Thorne K, Atkinson M, Evans PA. The incidence of acute pancreatitis: impact of social deprivation, alcohol consumption, seasonal and demographic factors. Aliment Pharmacol Ther. 2013 Sep;38(5):539-48.
3. Peery AF, Dellon ES, Lund J, Crockett SD, McGowan CE, Bulsiewicz WJ, et al. Burden of gastrointestinal disease in the United States: 2012 update. Gastroenterology. 2012 Nov;143(5):1179-87 e1-3.
4. Lopez MJ: The changing incidence of acute pancreatitis in children: a single-institution perspective. J Pediatr 2002; 140: 622–624.
5. Morinville VD, Barmada MM, Lowe ME: Increasing incidence of acute pancreatitis at an American pediatric tertiary care center: is greater awareness among physicians responsible? Pancreas 2010; 39: 5–8.
6. Nydegger A, Heine RG, Ranuh R, Gegati- Levy R, Crameri J, Oliver MR: Changing incidence of acute pancreatitis: 10-year experience at the Royal Children's Hospital, Melbourne. J Gastroenterol Hepatol 2007; 22: 1313–1316.
7. Park A, Latif SU, Shah AU, Tian J, Werlin S, Hsiao A, Pashankar D, Bhandari V, Nagar A, Husain SZ: Changing referral trends of acute pancreatitis in children: a 12-year single-center analysis. J Pediatr Gastroenterol Nutr 2009; 49: 316–322.
8. Sánchez-Ramírez CA, Larrosa-Haro A, Flores-Martínez S, Sánchez-Corona J, Villa-Gómez A, Macías Rosales R: Acute and recurrent pancreatitis in children: etiological factors. Acta Paediatr 2007; 96:534–537.
9. Werlin SL, Kugathasan S, Frautschy BC: Pancreatitis in children. J Pediatr Gastroenterol Nutr 2003; 37: 591–595.
10. Pant C, Deshpande A, Olyaee M, Anderson MP, Bitar A, Steele MI, Bass PF 3rd, Sferra TJ: Epidemiology of acute pancreatitis in hospitalized children in the United States from 2000–2009. PLoS One 2014; 9:e95552.
11. Meyer A, Coffey MJ, Oliver MR, Ooi CY: Contrasts and comparisons between childhood and adult onset acute pancreatitis. Pancreatology 2013; 13: 429–435.
12. A. Párniczky, D. Mosztbacher, A Tóth, B Cs Németh, A Demcsak, N Lásztity, et al. Protocols and early results of an internatinal clinical trial on peditaric pancreatitis (APPLE). Pancreatology. 2016 June; 3S1(16):S117
13. Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology. 2013 Jun;144(6):1252-61.
14. Lankisch PG, Lowenfels AB, Maisonneuve P. What is the risk of alcoholic pancreatitis in heavy drinkers? Pancreas. 2002 Nov;25(4):411-2.
15. Wu BU, Johannes RS, Sun X, Tabak Y, Conwell DL, Banks PA. The early prediction of mortality in acute pancreatitis: a large population-based study. Gut. 2008 Dec;57(12):1698-703.
16. Alexandre M, Pandol SJ, Gorelick FS, Thrower EC. The emerging role of smoking in the development of pancreatitis. Pancreatology. 2011;11(5):469-74.
17. Yuhara H, Ogawa M, Kawaguchi Y, Igarashi M, Mine T. Smoking and risk for acute pancreatitis: a systematic review and meta-analysis. Pancreas. 2014 Nov;43(8):1201-7.
18. Click B, Ketchum AM, Turner R, Whitcomb DC, Papachristou GI, Yadav D. The role of apheresis in hypertriglyceridemia-induced acute pancreatitis: A systematic review. Pancreatology. 2015 Jul-Aug;15(4):313-20.
19. Scherer J, Singh VP, Pitchumoni CS, Yadav D. Issues in hypertriglyceridemic pancreatitis: an update. J Clin Gastroenterol. 2014 Mar;48(3):195-203.
20. Valdivielso P, Ramirez-Bueno A, Ewald N. Current knowledge of hypertriglyceridemic pancreatitis. Eur J Intern Med. 2014 Oct;25(8):689-94.
21. Czako L, Szabolcs A, Vajda A, Csati S, Venglovecz V, Rakonczay Z, Jr., et al. Hyperlipidemia induced by a cholesterol-rich diet aggravates necrotizing pancreatitis in rats. Eur J Pharmacol. 2007 Oct 15;572(1):74-81.

22. Fisher JM, Gardner TB. The "golden hours" of management in acute pancreatitis. Am J Gastroenterol. 2012 Aug;107(8):1146-50.
23. Schepers NJ, Besselink MG, van Santvoort HC, Bakker OJ, Bruno MJ. Early management of acute pancreatitis. Best Pract Res Clin Gastroenterol. 2013 Oct;27(5):727-43.
24. Abu-El-Haija M, Lin TK, Palermo J: Update to the management of pediatric acute pancreatitis: highlighting areas in need of research. J Pediatr Gastroenterol Nutr 2014; 58: 689–693.