PhD Thesis

POSSIBLE PATHOGENETIC ROLE OF VASCULAR, IMMUNOLOGICAL AND GENETIC FACTORS IN CERTAIN GASTOENTEROLOGICAL DISORDERS

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DEBRECEN
2008

1. Introduction

Celiac disease is a genetically determined T lymphocyte-mediated chronic inflammatory disorder with an autoimmune component induced by the ingestion of wheat gliadin or related rye and barley proteins. The disease may present either in childhood or during adult life, and may involve multiple organ systems. According to our current understanding of disease susceptibility, the presence of human leukocyte antigen (HLA) DQ2 or DQ8 alleles is necessary but not sufficient for the disease development and other, non-HLA associated genes can also play a role. Recent epidemiologic studies revealed that celiac disease is common and affects approximately 1% of European and North American populations. The clinical presentation of the disease is highly variable and little is known about the factors that determine the type of symptoms but remains widely under diagnosed.

Inflammatory bowel diseases (IBD) are chronic disorders with unknown origin involving the gastrointestinal tract, which are also associated with various extraintestinal symptoms. The diseases primarily affect the young adult population. The pathogenetic processes of both Crohn's disease and ulcerative colitis are multifactorial, with dual environmental and genetic components. The incidence and prevalence of IBD continue to rise especially in low-incidence areas such as Eastern European countries like Hungary. Moreover, the previous ratio of 4-5:1 between ulcerative colitis and Crohn's disease is gradually decreasing. In our country, the incidence rate ranges from 4 to 5 cases per 100 000 person-years for Crohn's disease and 11 cases per 100 000 person-years for ulcerative colitis. Extensive heterogeneity is observed in terms of presentation, extraintestinal manifestations, and location in Crohn's disease, while behaviour and response to treatment are heterogeneous in both Crohn's disease and ulcerative colitis.

Haptoglobin (Hp) is a potent antioxidant and a positive acute phase reaction protein with the main function to scavenge free haemoglobin which is cytotoxic. Hp also has direct angiogenic, anti-inflammatory, and immunomodulatory properties exerted in extravascular tissues and body fluids. It is able to sieve through vessel walls and is expressed in different tissues in response to certain stimuli. Furthermore, Hp can also be released from neutrophil granulocytes at sites of injury or inflammation and dampens tissue damage locally. Hp's receptors include CD163 expressed on the monocyte-macrophage system and CD11b (CR3) found on granulocytes, natural killer cells, and small subpopulations of lymphocytes. Hp has also been shown to bind to the majority of CD4+ and CD8+ T lymphocytes, directly inhibiting their proliferation and modifying the T helper (Th) 1/Th2 balance. Hp's polymorphism is related to two co-dominant allele variants (Hp1 and Hp2) on chromosome 16q22, encoding the Hp α-chain, and resulting in three major Hp phenotypes (Hp*1-1*, Hp*2-1* and Hp*2-2*). Homozygous Hp*1-1* individuals express

Hp*1-1* at the protein level, which is a single α1β homodimer. Homozygous Hp*2-2* individuals express the Hp*2-2* phenotype, which consists of cyclic Hp polymers containing three or more α2β subunits. The haptoglobin molecules synthesized in Hp*2-1* heterozygous people are assembled into linear homodimers and multimers from various amounts of α2β subunits flanked by one α1β subunits at each terminus, and thus the Hp*2-1* phenotype is distinctly different from those produced by the homozygotes for the Hp2 or Hp1 gene. Phenotype-dependent functional differences exist in the antioxidant, scavenging and immunoregulatory properties of Hp, and the genetic polymorphism of Hp was shown to influence the course of a number of inflammatory and autoimmune diseases.

Serologic response to various microbial and autoantigens can develop in IBD. Although anti-Saccharomyces cerevisiae antibodies (ASCA) and atypical perinuclear antineutrophil cytoplasmic antibodies (P-ANCA) remain the most widely investigated, an increasing amount of experimental data is available on newly discovered antibodies directed against various microbial antigens, e.g anti-OmpC (outer membrane porin C transport protein of the *E. coli*), anti-I2 against *Pseudomonas fluorescens*, anti-flagellin (anti-CBir1) or various anti-glycan antibodies. The real importance and formation of the antibodies produced against various microbial and autoantigens is still unclear. A fundamental question that remains to be answered is whether these antibodies play a role in the immunopathogenesis of IBD, or their appearance is merely a consequence of the inflamed, leaky bowel mucosa.

The role of host genetic regulation of the innate immune response in the pathogenesis of CD has been brought into sharp focus by the identification of the *NOD2/CARD15* gene mutations in IBD1 region, which were consistently replicated among different populations, including in Hungary. The gene product is a cytosolic pattern recognition receptor responsible for intracellular recognition of bacterial products such as lipopolysaccharide (LPS) and peptidoglycan (PGN) derived from Gram-negative and -positive bacteria, respectively. Another important family of receptors involved in sensing bacterial products by the innate immune system is the cell-surface toll-like receptors (TLRs). Among these, *TLR4* is required for the recognition of LPS. A possible correlation between genetic and serological markers is supported by the finding that variant *NOD2/ CARD15* alleles lead to increased intestinal permeability, while elevated serum levels of serological markers showed a positive relationship with intestinal permeability. Still, studies investigating the relationship between ASCA and *NOD2/ CARD15* status in CD have produced conflicting results, which may reflect, at least partially, clinical and genetic variations among patient populations.

2. Aims

Haptoglobin (Hp) α-chain alleles 1 and 2 account for three phenotypes which have biologically important differences in their antioxidant, scavenging and immunomodulatory properties, and may thereby influence the course of inflammatory diseases.

- Celiac disease is a common, genetically determined chronic inflammatory disorder involving multiple organ systems. The clinical presentation of the disease is highly variable and little is known about the factors that determine the type of symptoms. To our knowledge, no data are available in the current literature reporting on the role of Hp genetic polymorphism in relation to disease susceptibility and clinical presentation in a large cohort of celiac patients. The aim of this study was to investigate the distribution of Hp polymorphisms in a large cohort of unrelated Hungarian celiac disease patients comprising the whole spectrum of the disease as well as evaluate possible associations with clinical presentation.

- Parallel to the increasing incidence of Crohn's disease, extensive heterogeneity is observed in terms of presentation, extraintestinal manifestations, and location in Crohn's disease which can be mainly explained with various genetic background. There are no large scale studies examining the correlation between Hp polymorphism and Crohn's disease found in the literature. The aim of our study was to investigate the distribution of Hp polymorphisms in a large number of Crohn's disease patients and the possible association with clinical presentation, response to treatment, and extraintestinal manifestations. Furthermore, we studied the relationship of Hp to other genetic factors such as the NOD2/CARD15 mutations and the TLR4 D299G polymorphism.

Serologic response to various microbial and autoantigens can develop in inflammatory bowel diseases (IBD) which may be useful in the differential diagnosis of the disease. At the same time, the real importance and formation of these antibodies is still unclear. The presence of serological markers was associated with a more aggressive disease phenotype and a risk for surgery in recent studies. However, an important geographic heterogeneity was observed in terms of antibody occurrences. Genetic heterogeneity may be responsible for the differences found in the serologic response. Literature is split regarding the possible correlation between antibody production in patients with CD and the NOD2/CARD 15 status.

- Since only limited data are available from Eastern Europe, the aim of our study was to investigate the prevalence of serological markers (ASCA, anti-Omp és atypical P-ANCA) in a large cohort of Hungarian IBD patients and to study the possible interaction with the clinical presentation, response to treatment, and extraintestinal manifestations. Additionally, we studied the relationship between serological response and genetic factors (NOD2/CARD15 és TLR4).

- Unlike the vasculitis-associated ANCAs, The goal of our study was to evaluate the reliability of the combined use of ethanol-fixed and formalin-fixed granulocyte substrates and the feasibility of the new microscopic criteria for the identification of atypical P-ANCA which have been proposed but not studied systematically in IBD. We used tests and equipments available for routine laboratories and conducted an inter-assay and inter-observer variability study, comparing results obtained by four different commercially available fluorescence substrates and by two geographically distinct laboratories. Besides of the evaluation of fluorescence patterns on ethanol- and formalin-fixed slides, our study included the investigation of the presence of anti-nuclear antibodies (ANA) and measuring the antibody levels against MPO, proteinase-3 (PR3), elastase, lactoferrin, cathepsin G, lysozyme and bactericidal/permeability increasing protein (BPI) by ELISA.

3. Methods

Haptoglobin phenotype/genotype analysis

Hp phenotypes are easily determined by gel electrophoresis and assigned to corresponding genotypes. Hp phenotypes were determined in a blinded fashion from serum samples of different patient groups and healthy controls using phenotyping method established by *Yang et al.* and modified in our laboratory. Briefly, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was run on 5-10% gradient gel followed by immunoblotting using Millipore polyvinyl-difluoride (PVDF) immobilion-P transfer membrane (Millipore, Bedford, MA, USA), polyclonal rabbit anti-human haptoglobin and goat anti-rabbit-horseradish peroxidase (HRP) antibodies (Dako, Glostrup, Denmark) in 1:1000 and 1:2000 dilution, respectively, as previously described. Genotypes were reconstructed by comparison to Hp*1-1* and Hp*2-2* manufacturer standards (Sigma-Aldrich, Schnelldorf, Germany) run in each assay. Different Hp phenotypes can be easily distinguished according to their electrophoretic mobility and banding patterns.

Statistical analysis

Variables were tested for normality with Shapiro Wilk's W test. T-test with separate variance estimates, $\chi 2$-test and Fisher's exact test were used to evaluate differences between different patients' group and controls. Within subgroups of given patient's group, the $\chi 2$-test and Fisher's exact test were used as appropriate. Logistic regression was used to compare genetic and clinical data and results were expressed as Odds Ratios (OR) with 95% confidence intervals (95% CI). A p value of <0.05 was considered as significant. The statistical analysis SPSS13.0 (SPSS Inc, Chicago, IL) was performed with the help of a statistician (Peter Vargha and Elek Dinya).

Anti-microbial antibody assays

Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to detect ASCA IgG, ASCA IgA and anti-Omp IgA (QUANTA Lite, INOVA Diagnostics, San Diego, CA) expression in sera. The results were presented as arbitrary units on the basis of an equation provided by the manufacturer with a cut-off for positivity of ≥25 units.

Antineutrophil Cytoplasmic Antibodies (ANCA) Assay

Determination of ANCA in sera was performed by the indirect immunofluorescence technique (IIF) on human peripheral blood neutrophil substrates (Nova Lite, INOVA Diagnostics). Ethanol- and formalin-fixed human peripheral blood neutrophils were examined in parallel for each patient.

For the *comparative ANCA examinations*, beside the INOVA ANCA assay, an additional three different commercially available immunofluorescent ANCA assays were used to test the serum samples (ImmuGlo, IMMCO Diagnostics, Inc., Buffalo, NY; Granulocyte Mosaic (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany and ANCA Test System Immunoconcepts N.A. Ltd., Sacramento, CA). Aliquots from the same blood samples were used to detect ANCA in inter-assay and inter-observer examinations.

Each assay includes ethanol-fixed and formalin-fixed human neutrophil granulocytes as substrate. Specimens were diluted to 1:40 (INOVA), 1: 20 (IMMCO and Immunoconcepts) and 1:10 (Euroimmun) in phosphate-buffered saline, and the assays were performed according to the manufacturers' instructions. Evaluation and classification of the patterns was performed under ultraviolet light (UV) using at 400x magnification. The Hungarian laboratory used Eurostar Plus microscope, Euroimmun Medizinische Labordiagnostika,

Luebeck, Germany; INOVA Diagnostics-ban: Nikon Eclipse E200 Labphot-2 indirect immunofluorescence microscope, San Diego, CA)

In the *inter-assay variability study* the patterns obtained from the above mentioned four immunfluorescent ANCA assays were analysed by a single examiner. On the contrary, during the *inter-observer variability* only the INOVA ANCA assay was used. The determinations were performed in two geographically distinct laboratories (Laboratory of Clinical Immunology, University of Debrecen, Debrecen, Hungary and INOVA Diagnostics, Inc., San Diego, CA), and were evaluated by observers experienced in the interpretation of ANCA IIF results.

The interpretation of the immunofluorescent results was based on the behavior of the specimens on ethanol- and formalin-fixed slides, and included the following patterns: *cytoplasmic (C)-ANCA, typical perinuclear (P)-ANCA and atypical P-ANCA. C-ANCA (atypical)*, the fourth characteristics staining pattern according to the Consensus Statement was not detected in this cohort of patients.

Antigen-specific ANCA Assays

Serum samples were tested for presence of IgG antibodies against MPO, PR3, lactoferrin, BPI, cathepsin G, elastase and lysozyme by enzyme-linked immunosorbent assay (ELISA) (Orgentec Diagnostika GmbH, Mainz, Germany). Serum levels of antibodies were evaluated separately according to the manufacturer's protocol. Quantitative results were obtained with the lactoferrin, BPI, cathepsin G, elastase and lysozyme assays, while qualitative values were determined in the case of anti-MPO and anti-PR3 (cut-off level ≥ 5 units).

Anti-Nuclear Antibodies (ANA) Assay

Serum samples were screened for the presence of anti-nuclear antibodies on HEp-2 cells (ANA-HEp-2) by IIF (ImmuGlo, IMMCO Diagnostics, Inc., Buffalo, NY). The assay was performed according to the manufacturer's instructions. Evaluation was performed under ultraviolet light (UV) using IIF microscope at a magnification of 400x.

Celiac-specific antibody determination (antibodies against to endomysium [EMA] and transglutaminase [anti-TG])

IgA and IgG class EMA were investigated on human umbilical cord substrate using IIF method. Anti-TG antibodies were measured by ELISA using human recombinant antigen expressed in *Eschericia coli*.

Genotyping methods

Genetic examinations were previously performed. Genomic DNA was isolated from whole blood. The *TLR4* D299G polymorphism was detected by polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP). Detection *of NOD2/CARD15* variants (single nucleotide polymorphism [SNP] 8, 12, 13 Mutations) was detected by denaturing high-performance liquid chromatography (DHPLC). Sequence variation, observed in the DHPLC profile, was sequenced on both strands to confirm the alteration. *HLA DQ alleles* were determined by using PCR method with sequence-specific primers.

Statistical Methods

Variables were tested for normality with Shapiro Wilk's *W*-test. *T*-test with separate variance estimates, $\chi 2$-test, and $\chi 2$-test with Yates correction were used to evaluate differences between IBD patients and controls, as well as within subgroups of IBD patients, as appropriate. Sensitivities, specificities, positive predictive values (PPV), and negative predictive values (NPV) values were calculated to determine the predictive power of P-ANCA, ASCA, and the combination of the 2 markers to distinguish among UC, CD, isolated colonic CD, and controls. Where appropriate, prevalence was estimated and used in these calculations. Logistic regression was used to compare genetic and clinical data and the results are expressed as OR with 95% confidence intervals (95% CI). A *P*-value <0.05 was considered significant.

Patient selection
Hp polymorphism evaluation

I. study: Seven-hundred-and-twelve consecutive, unrelated Hungarian patients with biopsy proven *celiac disease* (357 children and 355 adults, current median age: 17 years [range: 2-85], median age at diagnosis: 7 years [range: 1-85], male/female ratio: 233/479) were investigated. The diagnosis of celiac disease was based on small bowel biopsy showing severe villous atrophy with crypt hyperplasia (Marsh type III lesions) and elevated serum levels of antibodies to TG and/or EMA. In the absence of initial serology results, only patients with a diagnosis confirmed by traditional gluten challenge and the presence of HLA-DQ2 or DQ8 haplotypes were enrolled. Dermatitis herpetiformis was diagnosed by a skin direct immunofluorescent study showing granular IgA deposition in the dermal papillae. Patients were assigned to these major presentation types in a prospective manner, based on clinical, routine laboratory results [-2SD for age] and growth chart data [-2SD for age] at diagnosis. Of the 712 patients,

32.9% presented with severe malabsorption or growth failure, 22.8% with non-specific or minor gastrointestinal symptoms, 9.4% with iron deficiency anaemia, 15.6% with dermatitis herpetiformis and 12.1% with other symptoms. 7.2% of the patients were recognized through population screening.

II. study: 511 unrelated *IBD patients* (Crohn's disease, *n*=468; age, 36.5±12.7 years; male/female ratio, 233/235; duration, 8.2±6.7 years; and ulcerative colitis, *n*=43; age, 38.7±15.7 years; male/female ratio, 22/21; duration, 9.5±10.6 years) were investigated. The diagnosis was based on the Lennard-Jones criteria (clinical, endoscopic, radiological, and histopathologic). Age, age at onset, presence of EIMs (arthritis—peripheral and axial; ocular manifestations—conjunctivitis, uveitis, iridocyclitis; skin lesions—erythema nodosum, pyoderma gangrenosum; and hepatic manifestations—primary sclerosing cholangitis [PSC]), frequency of flare-ups (frequent flare-ups: >1/year), therapeutic effectiveness (e.g., need for steroid and/or immunosuppressive therapy, steroid resistance as defined by the ECCO [European Crohn's and Colitis Organisation] Consensus Report or short-term response to infliximab therapy), need for surgery (resections), the presence of familial IBD, and smoking habits were investigated by reviewing the medical charts by the physician and completing a questionnaire. In Crohn's disease, an additional parameter, perianal involvement, was also investigated. The disease phenotype (age at onset, duration, location, and behavior) was determined according to the Vienna classification (≤40 years [A1], >40 years [A2]; terminal ileum [L1], colon [L2], ilecolon [L3], upper gastrointestinal [L4] involvement; nonstricturing nonpenetrating [B1], stricturing [B2] or penetrating [B3] behavior). Only patients with a confirmed diagnosis for more than 1 year were enrolled. In UC the disease extent was defined by the maximum extent during follow-up.

The *control group* consisted of 384 healthy, ethnically similar and geographically matched individuals (median age: 38 years [range: 17-69], male/female ratio: 192/192) who had normal findings on a thorough medical examination, blood pressure measurements and routine laboratory tests to rule out any unknown underlying disease.

Serological evaluation

In all, 653 well-characterized, unrelated, consecutive *IBD patients* (Crohn's disease: 558, male/female ratio: 263/295, age: 36.3±12.6 years old, duration: 8.1±6.7 years; ulcerative colitis: 95, male/female ratio: 44/51, age: 39.7±14.5 years old, duration: 8.9±9.8 years) and 100 age- and gender-matched healthy subjects (male/female ratio: 47/53, age: 36.6±9.1 years) were investigated. For the comparative ANCA examinations 108 CD and 96 UC patients' sera were selected from the above mentioned IBD cohort.

4. Results

Haptoglobin polymorphism: a novel genetic risk factor for celiac disease development and its clinical manifestations

The frequency of Hp*2-1* was significantly higher (56.9%) in patients with celiac disease compared with the control population (46.1%, OR: 1.54 95%CI: 1.20-1.98; *p=0,0006*). Hp phenotype distribution of the healthy control group was similar to those found in previous surveys in the normal Hungarian population (n=2609) and Hp1 and Hp2 alleles were in Hardy-Weinberg equilibrium, and did not differ with age or gender.

All celiac patients with available HLA results carried at least one copy of DQ2 or DQ8, but the prevalence of the high-risk DQ genotypes (DQB1*0201/*0201 [DR3;DQ2/DR3;DQ2] or DQB1*0201/*0202 [DR3;DQ2/DR7;DQ2]) was similar in the three Hp groups (Hp*1-1*: 40.0%, *Hp2-1:* 40.7%, Hp*2-2*: 38.8%). This finding shows that HLA-related susceptibility markers were not overrepresented in the Hp*2-1* celiac group. HLA-DQ results were not available for the control group.

Clinical presentations of celiac disease were distributed differently in the three Hp genotype groups. Patients carrying with Hp*2-2* were at an increased risk for severe malabsorption or failure to thrive as the clinical presentation of celiac disease (OR: 2.21, 95%CI: 1.60-3.07), and accordingly, presented less often with only non-specific or minor gastrointestinal symptomps (OR: 0.38, 95%CI: 0.25-0.58) and remained less often clinically silent (OR: 0.35 95%CI: 0.16-0.76) compared to patients in other Hp groups. Of all celiac patients carrying Hp*2-2,* 44.9% were diagnosed because of malabsorption or failure to thrive, which was significantly higher than in celiac patients overall (32.9%, *p=0.0008*) or with other Hp genotypes (26.9%, *p<0.0001*). Likewise, 45.3% of all celiac cases that presented with severe malabsorption or failure to thrive carried Hp*2-2*, despite the under-representation of Hp*2-2* among celiac patients compared to the general population.

In patients who presented with non-specific or minor gastrointestinal symptoms and were discovered through screening, there was a stronger predominance of Hp2-*1* (69.1% and 72.5%) compared with the entire celiac group (56.9%) (*p=0.0004* and *p=0.0192*), while Hp2-*2* occurred less frequently.

Dermatitis herpetiformis tended to be more frequent in the Hp*1-1* group (22.5%) compared with other Hp groups (14.8%; *p=0.118*) or among celiac patients overall (15.6%; *p=0.131*), but this difference was not statistically significant. Only 14.4% of all dermatitis herpetiformis cases carried Hp*1-1*, while the majority of this group also had Hp*2-1*.

The rate of iron deficiency anemia and other clinical symptoms, for disease presentation, were similar among the three Hp phenotypes and their distribution was also not different as compared to the entire celiac population.

There was no correlation between Hp phenotypes and the age at diagnosis. In addition the male/female ratios were similar in all Hp groups.

Haptoglobin polymorphisms are associated with disease behavior and extraintestinal manifestations in Crohn's disease patients

Hp1 allele frequency was significantly higher in Crohn's disease than in healthy individuals (0.395 vs. 0.345; OR: 1.24, 95% CI: 1.02-1.51, *p=0.03*). However, Hp phenotype distribution did not differ in the IBD patient groups as compared to controls.

The polymorphism was not associated with either the age at onset, the duration of the disease or familial disease. Moreover, it had no effect on disease location or perianal manifestations. There was no difference in the frequency of relapses between the Hp phenotypes.

Hp polymorphism was associated with the disease's behavior: the *2-1* type was associated with the inflammatory (nonstricturing nonpenetrating) form [B1] (*2-1*: 44.9% vs. *1-1*: 36.6% and *2-2*: 34.3%; $OR_{B1Hp2\text{-}1 \text{ vs others}}$: 2.06, 95%CI: 1.29-3.28), while stricturing [B2] disease was less common (*2-1*: 20.3%, *1-1*: 32.4%, *2-2*: 32.5%; *p=0.04*).

In addition, Hp phenotypes were associated with PSC. Not a single *1-1* carrier was found among the 18 PSC patients. Our observations could be confirmed in large volume PSC (n=63) (Hp*1-1*: 0%, Hp*2-1*: 52.4%, Hp*2-2*: 47.6%, $OR_{Hp1\text{-}1 \text{ vs.}}$ others: 0.06, 95%CI: 0.003-0.99, *p= 0.0012*).

No association was found between the Hp phenotypes and the TLR4 D299G polymorphism or NOD2 variant alleles. Of interest, the presence of SNP 12 was significantly higher in the *1-1* phenotype (14.3% [9/63]) compared to other Hp phenotypes (*2-1*: 3.6% [7/186], *2-2*: 6.1% [9/138]) (p=0.025). No such association was found for either the SNP 8 or SNP13 mutations.

In UC, Hp phenotype was not associated with the disease, as well as no phenotype-genotype associations were found.

Seroreactivity to Microbial Components in Crohn's Disease Is Associated with Ileal Involvement, Noninflammatory Disease Behavior and NOD2/CARD15 Genotype, But Not with Risk for Surgery

Frequency and utility of serological markers in IBD

Anti-Omp, ASCA, and atypical P-ANCA antibodies were present in 31.2%, 59.3%, and 13.8% of CD, 24.2%, 13.7%, and 48.5% of UC patients, and in 20%, 16%, and 5.6% of controls, respectively. ASCA and anti-Omp positivity were associated with increased risk for Crohn's disease (OR_{ASCA}=7.65, 95%CI: 4.37–13.4; OR_{Omp} =1.81, 95% CI: 1.08 –3.05). In addition, atypical P-ANCA was associated with increased risk for ulcerative colitis ($OR_{P\text{-}ANCA}$=10.91, 95% CI: 4.34 –27.41) compared to the controls. Atypical P-ANCA and ASCA positivity was also significantly different between ulcerative colitis and Crohn's disease. 20.8% of patients with Crohn's disease were triple-positive for ASCA IgA, ASCA IgG, and anti-Omp antibodies, compared with only 3.2% of ulcerative colitis patients and 1% of control subjects ($p < 0.0001$ for both). However 35.1% of Crohn's disease patients were not reactive to either ASCA or Omp. A combination of serology testing (e.g., P-ANCA+/ ASCA-, P-ANCA+/ASCA- or both, IgA and IgG ASCA positivity) was able to differentiate between controls, ulcerative colitis, and Crohn's disease cases with acceptable specificity and PPV, even in CD patients with colon (L2) localization – which may most probably pose a differential diagnostic problem in the clinical routine. However, the overall sensitivity was low.

Association Between Antibody Response, Disease Phenotype, and Risk for Surgery

In Crohn's disease, using univariate analysis, anti-Omp was associated with younger age at onset ($p= 0.025$), longer disease duration ($p= 0.01$), noninflammatory ($p< 0.0001$, OR: 2.89, 95% CI: 1.93–4.33) disease behavior, ocular manifestations ($p= 0.019$, OR: 2.23, 95% CI: 1.16–4.30), azathioprine use ($p = 0.016$, OR:1.62, 95% CI: 1.10 –2.41), and need for surgery ($p< 0.0001$, OR: 2.26, 95% CI: 1.58 –3.27). ASCA was associated with younger age at onset ($p< 0.0001$), ileal involvement ($p<$ 0.0001, OR: 2.51, 95% CI: 1.70 –3.71), noninflammatory ($p< 0.0001$, OR: 2.02, 95% CI: 1.43–2.86) disease behavior, perianal disease ($p=0.002$, OR: 1.83, 95% CI: 1.25–2.70), and need for surgery ($p= 0.004$, OR: 1.69, 95% CI:–1.19-2.40).

The associations between serology and disease behavior, ocular manifestations and a need for surgery were also tested in a logistic regression analysis. Serological markers were independent risk factors for noninflammatory disease behavior, while Omp (B= 0.38, $p= 0.04$, OR: 1.47, 95% CI: 1.01–2.13) and female gender (B= 1.11, $p= 0.005$, OR: 3.05, 95% CI: 1.41–6.62) were independently associated with ocular manifestations in the same model, also including noninflammatory disease behavior as an independent variable. In contrast, longer disease duration, ileal involvement, and noninflammatory disease behavior, but not P-ANCA, ASCA, or anti-Omp statuses were independent predictors for surgery.

In our study patients with Crohn's disease, who were atypical P-ANCA-positive, had no particular clinical features compared with patients who were P-ANCA-negative. Furthermore, the serological profile did not predict disease phenotype in ulcerative colitis. No correlation was found between smoking habits and serology response.

Association between antibody response and NOD2/CARD15 genotypes

The presence of ASCA (*p<0.0001*, OR: 2.64, 95% CI: 1.71–4.10) and Omp (*p*=0.005, OR: 1.89, 95% CI:1.24–2.90) antibodies was associated with *NOD2/CARD15* genotype in univariate analysis. A gene dosage effect was also observed, the prevalence of antimicrobial antibodies increased significantly parallel to the number of mutations in NOD2/CARD15 gene.

ASCA positivity was found in 50.6%, 70.7%, and 80% in patients with 0, 1, or 2 variant *NOD2/CARD15* alleles (*p=0.0001*), while anti- Omp antibodies were found in 24.5%, 37.4% and 42.5% (*p=0.007*), respectively. In addition, the association between *NOD2/CARD15* and ASCA (B=0.406, *p=0.001*, OR: 1.50, 95% CI: 1.18–1.90), but not anti-Omp, still remained significant in the logistic regression models, including antibody positivity, gender, disease duration, ileal involvement or ileal-only disease, noninflammatory disease behavior, and smoking as independent variables.

Relationship between the quantity of serological marker positivity and disease phenotype

We found an association between the number of antibody responses (either ASCA, anti-Omp, or both) and ileal involvement (serology dosage effect), isolated colonic disease, noninflammatory behavior, and need for surgery in Crohn's disease. However, in a logistic regression analysis including disease duration, gender, ileal involvement, noninflammatory behavior and current smoking, we identified longer disease duration (*p<0.0001*, OR 3.01, 95% CI: 1.91– 4.72), noninflammatory behavior (*p<0.0001*, OR: 3.15, 95% CI: 2.46–4.03), and ileal involvement (*p<0.001*, OR: 2.38, 95% CI: 1.44 –3.93), but not serological positivity (*p<0.076*), as independent predictors for surgery.

Relationship between the magnitude of antibody response and disease phenotype

We also calculated the differences according to the levels of ASCA IgA, IgG, and anti-Omp as assessed by medians and quartiles. In a logistic regression analysis including the aforementioned variables, the levels of ASCA IgA, IgG, and anti-Omp assessed in quartiles were independently associated with

noninflammatory disease behavior (ASCA IgA: *p<0.0001*, OR: 1.39, 95% CI: 1.18–1.62; ASCA IgG: *p=0.03*, OR: 1.28, 95% CI: 1.09–1.15; anti-Omp: *p<0.0001*, OR: 1.44, 95% CI: 1.23–1.70), and need for surgery (ASCA IgA: *p= 0.004*, OR: 1.27, 95% CI: 1.08–1.49; ASCA IgG: *p=0.003*, OR: 1.30, 95% CI: 1.10–1.53; anti-Omp: *p<0.0001*, OR: 1.34, 95% CI: 1.14–1.58).

Evaluation of the combined application of ethanol-fixed and formaldehyde-fixed neutrophil substrates for identifying atypical P-ANCA in inflammatory bowel disease: Specificity and reproducibility

Inter-assay study – Fluorescence patterns

Sera from 108 patients with CD and 96 patients with UC were screened for the presence of ANCA by IIF microscopy using both ethanol- and formalin-fixed neutrophil substrates. The prevalence of all ANCA patterns as determined by four IF assays varied between 24.5% and 37.7% in the study population: 39.6-53.1% in patients with UC, and 11.1-24.1% in CD patient.

For all but one assay the ANCA patterns were mainly atypical P-ANCAs both in UC (68.4-76.3% of all ANCAs) and CD patients (71.4-90.0% of all ANCAs). With the IMMCO assay, a relatively low proportion of detected ANCAs showed atypical P-ANCA pattern in patients with UC and CD (17.6% and 38.5% of all ANCAs, respectively), instead, the prevalence of typical P-ANCAs was high (72.5% and 46.2% of all ANCAs, respectively). C-ANCA pattern was rarely detected with each assay.

Altogether, the agreement among the four different methods varied between 73.6-88.0%, 77.4-93.3%, and 93.3-98.1% for atypical P-ANCA, P-ANCA, and C-ANCA, respectively. However, the weighted Kappa coefficients (κ) suggested poor to fair concordance between the different assays; the κ value varied between 0.14-0.34 for atypical P-ANCA. The κ coefficient was not calculated for P-ANCA and C-ANCA because of the very low prevalence rates. Results were similar when UC and CD patients were evaluated separately.

To further examine and compare the results obtained by different assays, we established consensus results. The fluorescent pattern was considered true atypical P-ANCA, when three out of the four tests produced identical results. Consensus was reached only in 16 cases (7.8% of all patients), which clearly reflects the lack of agreement. However, when typical and atypical P-ANCA patterns were combined (according to the Consensus Statement), consensus was reached in 41 cases (20.1% of all patients). These data suggest that differentiation between typical and atypical P-ANCA using formalin-fixed slides is not effective, and the agreement can be improved if typical and atypical P-ANCA patterns are not differentiated. Out of the 41 consensus cases, the INOVA, IMMCO and Euroimmun assays detected similar percentage of

positivities (92.7, 90.2% and 82.9%, respectively), while the Immunoconcept assay underestimated the number of P-ANCA positive specimens (58.5%). Therefore, we excluded the results obtained by the Immunoconcept kit, and established a new consensus using results only from the three former assays. In this case, consensus (2 positive out of the three results) was reached in 28 cases regarding atypical P-ANCA (13.7% of all patients), and 57 cases regarding all P-ANCAs (27.9% of all patients), including 41 UC patients (42.7%) and 16 CD subjects (14.8%) (*p<0.0001* between UC and CD). Considering the overall rate of ANCA positivity (25.5-34.8% with the three above mentioned assays, which also includes a few C-ANCA patterns), these results suggest that by calculating all P-ANCAs together, consensus was reached for the vast majority of samples. The three different assays detected similar percentages of the consensus cases (89.5, 87.7 and 71.9%, respectively).

Antigen specificity

We assessed the presence of IgG antibodies reacting with different cytoplasmic components of the neutrophil, including MPO, PR3, lactoferrin, BPI, cathepsin G, elastase and lysozyme. Both the prevalence and the level of antibodies against MPO or PR3 were low. Anti-PR3 antibody was more frequently present than anti-MPO, furthermore, both anti-MPO and anti-PR3 antibodies were more prevalent in patients with UC than in those with CD (4.2% vs. 0.9%; n.s. and 16.7% vs. 3.7%; *p=0.002*). Considering only positive results, the anti-PR3 level was higher in UC than in CD (25.9 [IQR 15.8-38.1] vs. 9.6 [IQR 7.9-13.4]) n.s.*).* One UC patient had both anti-MPO and anti-PR3, and two CD and four UC patients had antibodies against lactoferrin and/or BPI besides of the anti-MPO and anti-PR3. Out of the 24 patients with different levels of anti-MPO or anti-PR3 antibodies, 21 (87.5%) showed some type of immunofluorescence on IMMCO slides, and 13-13 (54.2%) on INOVA, Euroimmun and Immunoconcept substrates. Excluding those specimens containing antibody levels lower than two times the cut-off, only 13 patients had considerable levels of anti-MPO or PR3 (6.4% of all patients), and 8 to 10 of them were positive with IIF by the various assays.

We could not demonstrate antibodies against cathepsin G or elastase in either groups, and detected anti-lysozyme antibodies in only one person in each patient group. The prevalence of anti-lactoferrin and anti-BPI antibodies was 10.4% vs. 4.6% and 10.4% vs. 7.4% in UC and CD, respectively (n.s.) (Table 4.). One CD and three UC subjects had both antibodies. Furthermore, concomitant anti-MPO or anti-PR3 was present in 6 patients. Altogether, out of the 31 cases (15.2% of all patients) with antibodies against lysozyme, lactoferrin and/or BPI, 27 (87.1%) showed positive IIF results on the IMMCO slides, and 20 (64.5%), 13 (41.9%) and 11 (35.5%) on INOVA, Euroimmun and Immunoconcept substrates. The distribution of the immunofluorescent patterns

varied between the methods, and included P-ANCA, atypical P-ANCA and C-ANCA as well.

The results obtained by ELISA were also analyzed with regards to clinical phenotype. No statistically significant differences concerning sex, age, age at onset, localization or behavior of the disease could be detected in patients with or without the above mentioned antibodies.

Antinuclear antibody (ANA) positivity by IIF on HEp-2 was observed in 17.7% of patients with UC and only in 6.5% of CD patients.

Inter-observer study

In the *inter-observer study*, the same test (Nova Lite ANCA, INOVA) was performed in two independent laboratories, and the results were evaluated by two different experienced readers. In patients with UC, the prevalence of reported ANCA patterns was higher in the San Diego lab than in the Debrecen lab (64.6% vs. 53.1%), which was mainly attributable to the more frequent atypical P-ANCA pattern (57.3% vs. 39.6%). Concurrently, the occurrence of P-ANCA (5.2% vs. 9.4%) and C-ANCA (2.1% vs. 4.2%) was somewhat lower. In patients with CD, the two observers found nearly identical prevalence rates for the different ANCA patterns: 17.6% vs. 16.7% for atypical P-ANCA, 2.8% vs. 1.9% for P-ANCA and 0.9% vs. for 0.0% C-ANCA (altogether 21.3 vs. 18.5%). The agreement rate in the whole study population for atypical P-ANCA was 75.5%, which corresponded to a κ value of 0.44, suggesting moderate inter-observer concordance. The κ coefficient was not calculated for P-ANCA and C-ANCA because of the very low prevalence rates.

When P-ANCA and atypical P-ANCA patterns were grouped together, the prevalence rate of P-ANCA in the UC and CD populations was 62.5% versus 20.4% ($p<0.0001$) and 49.0% versus 18.6% ($p<0.0001$) according to the San Diego and the Debrecen lab, respectively. This reflects an agreement rate of 77.9% and a corresponding κ value of 0.53. These results reflect 62.5% (52.0-72.0%) and 79.6% (70.6-86.5%) sensitivity and specificity (95% CI) of P-ANCA in UC according to the San Diego lab, and 49.0% (38.7-59.3%) and 81.5% (72.6-88.1%) sensitivity and specificity (95% CI) according to the Debrecen lab. The difference between the two labs regarding sensitivity and specificity in not significant.

5. Major scientific contributions

1. To our knowledge, this is the first report on the role of Hp genetic polymorphism in relation to disease susceptibility and clinical presentation in a large cohort of celiac patients. We found the frequency of the Hp2-*1* phenotype/genotype to be significantly higher in celiac patients than in control

subjects. At the same time, HLA related susceptibility markers were not overrepresented in the Hp*2-1* celiac group.

2. Clinical presentations of celiac disease were distributed differently in the three Hp genotype groups. Although the occurrence of Hp*2-2* was less frequent in celiac disease, patients with this phenotype face an increased risk for severe malabsorption or failure to thrive as clinical presentation forms. On the contrary, there was a stronger predominance of Hp2-*1* in patients who presented with non-specific or minor gastrointestinal symptoms and were discovered through screening compared with the entire celiac group. We could not, however, confirm the observation of the single study published so far which found a higher incidence of Hp*1-1* phenotype in a small cohort of dermatitis herpetiformis when compared to celiac disease patients.

3. The Hp1 allele frequency was significantly higher in our large cohort of Crohn's disease patients than control subjects. Opposing the data published in an Italian study carried out on a small number of subjects, in our large scale Crohn's disease cohort, the Hp phenotype distribution did not differ from the control group. Hp polymorphism was associated with disease behavior. In the case of Hp *2-1* type, the frequency of the inflammatory form of the disease – considered the milder form of the behaviors – was significantly higher while the stricturing disease was less common. In addition, Hp phenotypes were associated with the extraintestinal manifestations of the. Not a single *1-1* carrier was found among the patients with primary sclerosing cholangitis.

4. This is the first report investigating the complex associations between serology and genetic markers in a large cohort of IBD patients from Eastern Europe. The prevalence of ASCA and anti-Omp positivity was in accordance with previous studies performed in Caucasian patients in North America and Western Europe. The sensitivity, specificity, and PPV values for the markers were also comparable to those observed in earlier studies, further proving that the use of the markers alone is insufficient for diagnostic purposes. However, the high PPV supports their use in selected cases as additional diagnostic parameters.

5. The present study demonstrated that responses to microbial components are closely related to the clinical characteristics of Crohn's disease. Reactivity to these components was independently associated with younger age at onset, ileal disease and noninflammatory disease behavior, but was not useful in predicting response to medical therapy or the need for surgery. The number of antibodies produced against microbial antigens and their titers in Crohn's disease showed a positive correlation with the small bowel involvement and the severity of the disease course (serology dosage effect). Moreover, the extent of the serological

response correlated to the need for surgery. In contrast to the previously published data, in our study patients with Crohn's disease, who were atypical P-ANCA-positive, had no particular clinical features compared with patients who were P-ANCA-negative. Furthermore, the serological profile did not predict disease phenotype in ulcerative colitis.

6. Some studies investigating the relationship between serology markers and mutations of the innate immunity receptors in Crohn's disease have produced conflicting results. We found that the reactivity to microbial components was associated with NOD2/CARD15 genotype. Positive correlation was found between the number of mutations and the prevalence of antimicrobial antibodies (gene dosage effect). The association between serology and *NOD2/CARD15* genotype further supports the role of altered microbial sensing in the pathogenesis of Crohn's disease.

7. Our results suggest that differentiation between P-ANCA and atypical P-ANCA by using formalin-fixed slides is not reliable and not reproducible in routine laboratory circumstances using fluorescent microscopes. P-ANCAs (regardless of the typical or atypical appearance on ethanol-fixed substrates) should be considered together. Low level anti-MPO or anti-PR3 can be present in IBD. Without the knowledge of clinical symptoms the interpretation of the ANCA result (IIF+ELISA) is basically impossible. The determination of antibodies against lactoferrin, BPI, cathepsin G, elastase and lysozyme does not seem to provide useful information.

PUBLICATIONS

Papp M, Lakatos PL; Hungarian IBD Study Group; Palatka K, Foldi I, Udvardy M, Harsfalvi J, Tornai I, Vitalis Zs, Dinya T, Kovacs A, Molnar T, Demeter P, Papp J, Lakatos L, Altorjay I. Haptoglobin polymorphisms are associated with Crohn's disease, disease behaviour and extraintestinal manifestations in Hungarian patients. Digestive Disease and Sciences 2007; 52: 1279-84. IF: 1.448

Papp M, Foldi I, Nemes E, Udvardy, Harsfalvi J, Altorjay I, Mate I, Dinya T, Varvolgyi Cs, Barta Zs, Veres G, Lakatos PL, Tumpek J, Toth L, Szathmari E, Kapitany A, Gyetvai A, Korponay-Szabo IR. Haptoglobin polymorphism: a novel genetic risk factor for celiac disease development and its clinical manifestations. Accepted for publication 01/15/2008: Clinical Chemistry IIF: 5.454

Papp M, Lakatos PL; Hungarian IBD Study Group; Palatka K, Foldi I, Udvardy M, Harsfalvi J, Tornai I, Vitalis Z, Dinya T, Kovacs A, Molnar T, Demeter P, Papp J, Lakatos L, Altorjay I. Haptoglobin polymorphism in patients with inflammatory bowel diseases. Orvosi Hetilap 2006; 147: 1745-50. Hungarian

Papp M, Altorjay I, Lakatos PL. Relevance of serologic studies in inflammatory bowel diseases. Orvosi Hetilap 2007; 148: 887-896. Hungarian

Papp M, Norman GL, Altorjay I, Lakatos PL. The utility of serological markers in inflammatory bowel diseases: gadget or magic? World Journal of Gastroenterology 2007;13: 2028-36.

Papp M, Altorjay I, Norman GL, Shums Z, Palatka K, Vitalis Zs, Foldi I, Lakos G, Tumpek J, Udvardy ML, Harsfalvi J, Fischer S, Lakatos L, Kovacs A, Bene L, Molnar T, Tulassay Zs, Miheller P, Veres G, Papp J, the Hungarian IBD Study Group, Lakatos PL. Sero-reactivity to microbial components in Crohn's disease is associated with ileal involvement, non-inflammatory disease behaviour and NOD2/CARD15 genotype, but not with response to medical therapy or risk for surgery. Inflammatory Bowel Diseases 2007; 13: 984-92. IF: 3.912

Papp M, Udvardy M, Vitális Zs, Tornai I, Altorjay I. Gastroesophageal variceal hemorrhage – New achievements in pathophysiology. Orvosi Hetilap 2006;147: 309-314. Hungarian

Papp M, Farkas A, Udvardy M, Tornai I. Bacterial infections in cirrhosis. Orvosi Hetilap 2007;148: 387-395. Hungarian

Papp M, Mezei G, Udvardy M, Altorjay I. Changes in hematologic and hemostatic parameters after transjugular intrahepatic portosystemic shunt (TIPS) implantation. Orvosi Hetilap 2003; 144: 1341-5. Hungarian.

Vitalis Zs, **Papp M**, Tornai I, Altorjay I. Prevention and treatment of oesophageal variceal bleeding. Orvosi Hetilap 2006;147: 2455-63. Hungarian

Lakatos PL, Fischer S, Claes K, Kovacs A, Molnar T, Altorjay I, Demeter P, Tulassay Z, Palatka K, **Papp M**, Rutgeerts P, Szalay F, Papp J, Vermeire S, Lakatos L; Hungarian IBD Study Group. DLG5 R30Q is not associated with IBD in Hungarian IBD patients but predicts clinical response to steroids in Crohn's disease. Inflammatory Bowel Diseases 2006;12: 362-8. IF: 3.912

Fischer S, Lakatos LP; Hungarian IBD Study Group, Lakatos L, Kovacs A, Molnar T, Altorjay I, **Papp M**, Szilvasi A, Tulassay Zs, Osztovits J, Papp J, Demeter P, Schwab R, Tordai A, Andrikovics H. The ATP-binding Cassette Transporter ABCG2 (BCRP) and ABCB1 (MDR1) variants are not associated with disease susceptibility and disease phenotype in Hungarian patients with Inflammatory Bowel Diseases. Scandinavian Journal of Gastroenterology 2007; 42: 726-33. IF: 1.869

Molnar T, Hofner P, Nagy F, Lakatos PL and the Hungarian IBD Study Group, Fischer S, Lakatos L, Kovacs A, Altorjay I, **Papp M**, Palatka K, Demeter P, Tulassay Zs, Nyari T, Miheller P, Papp J, Mandi Y, Lonovics J: NOD1 gene E266K polymorphism is associated with disease susceptibility but not with disease phenotype or NOD2/CARD15 in Hungarian patients with Crohn's disease. Digestive Liver Diseases 2007; 39: 1064-1070. IF: 2.000

PRESENTATIONS

Papp M, Palatka K, Foldi I, Udvardy M, Harsfalvi J, Tornai I, Kovacs A, Molnar T, Demeter P, Papp J, Lakatos L, Lakatos P, Altorjay I: Haptoglobin polymorphisms are associated with Crohn's disease, disease behaviour and extraintestinal manifestations in Hungarian patients. Gut 2006; 38 Suppl 2: A113 (MON-G-164).

Papp M, Lakatos PL, Udvardy M, Foldi I, Harsfalvi J, Balogh I, Altorjay I, Dinya T, Habior A, Szalay F, Tornai I: Haptoglobin and myeloperoxidase promoter polymorphism in primary biliary cirrhosis. Falk Symposia 155 – 157, 2006 október (378).

Veres G, **Papp M**, Lakatos PL, Arato A, Molnár K, Tornai I, Dezsőfi A, Szőnyi L. Haptoglobin polymorphism in primary sclerosing cholangitis. J Ped Gastro Nutr 2007; 44 Suppl: 226.

Papp M, Altorjay I, Norman GL, Shums Z, Palatka K, Vitalis Zs, Foldi I, Lakos G, Tumpek J, Udvardy ML, Harsfalvi J, Fischer S, Lakatos L, Kovacs A, Bene L, Molnar T, Tulassay Zs, Miheller P, Veres G, Papp J, the Hungarian IBD Study Group, Lakatos PL: Sero-reactivity to microbial components in Crohn's disease is associated with ileal involvement, non-inflammatory disease behaviour and NOD2/CARD15 genotype, but not with response to medical therapy or risk for surgery. JCC 2007; 1 Suppl 1: P-002 és Gut 2007; 39 Suppl 1: A141 (MON-G-268).

Lakatos PL, Altorjay I, Dotan N, Tumpek J, Sipka S, Lakos G, Udvardy M, Palatka K, Lakatos L, Kovacs A, Molnar T, Tulassay Zs, Miheller P, Norman GL, Shums Z, Szamosi T, Papp J, **Papp M**: Comparing conventional and gASCA tests for the diagnosis and determination of clinical phenotype in Crohn's disease. Gut 2007; 39 Suppl 1: A119 (MON-G-162).

Papp M, Tumpek J, Norman GL, Shums Z, Lakos G, Sipka S, Altojay I, Lakatos PL: Detection of antineutrophil cytoplasmic antibodies in patients with inflammatory bowel diseases: interassay- and interobserver variability comparing four different commercially available assays. Gut 2007; 39 Suppl 1: A141 (MON-G-267).

Altorjay I, Palatka K, Mezei G, **Papp M**, Tornai I: Efficacy of mini-loop ligation in comparison to sclerotherapy in the treatment of esophageal varicosity. Gastrointestinal Endoscopy, 2002, 55: AB196-TI896

Altorjay I, **Papp M**, Palatka K, Mezei G, Tornai I: Additional, continuous proton pump inhibitor treatment prolonged survival in cirrhotic patients following sclerotherapy because of variceal bleeding. Gut, 2002, 51:A175.

Papp M, Udvardy M, Varga Zs, Káplár M, Sipka S, Alexa M, Palatka K, Balogh I, Hársfalvi J, Altorjay I: Inflammation and agressive proteolytic enzymes – Do they play a role in acute variceal bleeding? Gut 2004; 36 Suppl 1: A-259 (WED-G-137).

Palatka K, **Papp M**, Udvardy M, Altojay I: Assesement of rutine laboratory and clinical data for activity of Crohn's disease in correlation with myeloperoxidase and matrix metalloproteinase activity. Gut 2004; 36 Suppl 1: A-219 (TUE-G-336).

Kaplar M, Csongradi E, Paragh Gy, **Papp M**, Foldi I, Veszpremi A, Nagy B, Kappelmayer J: Changes in the level of soluble and thrombocyte surface P-selectin and the number of thrombocyte derived microparticles in obesity and in diabetes mellitus. Diabetologia, 2006; 49 Suppl 1.

Fischer S, Lakatos LP, Lakatos L, Kovacs A, Molnar T, Altorjay I, **Papp M**, Szilvasi A, Tulassay Zs, Osztovits J, Papp J, Demeter P, Schwab R, Tordai A, Andrikovics H: The ATP-binding Cassette Transporter ABCG2 (BCRP) V12M

and Q141K variants in Hungarian patients with inflammatory bowel diseases. Gut 2006; 38 Suppl 2: A112 (MON-G-160).

Lakatos PL, Fisher S, Claes K, Kovacs A, Molnar T, Altojey I, Demeter P, Palatka K, **Papp M**, Tulassay Zs, Rutgeerts P, Szalay F, Vermeire S, Papp J, Lakatos L: DLG5 R30Q is not associated with inflammatory bowel disease in Hungarian IBD patients, but predits clinical response to steroids in Crohn's disease. Gut 2006; 38 Suppl 2: A112 (MON-G-161).

Lakatos LP, Fischer S, Lakatos L, Kovacs A, Molnar T, Altorjay I, **Papp M**, Szilvasi A, Tulassay Zs, Osztovits J, Papp J, Demeter P, Schwab R, Tordai A, Andrikovics H: The ATP-binding Cassette Transporter ABCG2 (BCRP) and ABCB1 (MDR1) variants are not associated with disease susceptibility and disease phenotype in Hungarian patients with inflammatory bowel diseases. JCC 2007; 1 Suppl 1: P-120

Udvardy ML, Szekeres-Csiki K, **Papp M**, Harsfalvi J: Quantitation of von Willebrand factor multimer distribution by densitometry. J Thromb Haemost 2007; 5 Suppl 2: P-W-198.

Tornai I, **Papp M**, Udvardy ML, Harsfalvi J. VWF functions, ADAMTS-13 activity and antigen levels in patients with liver cirrhosis. J Thromb Haemost 2007; 5 Suppl 2: P-T-207.

Gacsal N, **Papp M**, Harsfalvi J, Udvardy M: Haptoglobin polymorphism in patients with chronic lympocytic leukemia. Blood 2007; 21 Suppl 1: P-037.

Papp M, Foldi I, Tumpek J, Varvolgyi Cs, Barta Zs, Sipka S, Dotan N, Korponay-Szabo IR, Nemes E, Veres G, Altorjay I, Lakatos PL: Anti-glycan antibodies in celiac disease before and after gluten-free diet. Gut 2007; 39 Suppl 1: A109 (MON-G-111).

Lakatos PL, Szamosi T, Lakatos L, Kovacs A, Molnar T, Altorjay I, **Papp M**, Szilvasi A, Szabo O, Tulassay Zs, Miheller P, Papp J, Demeter P, Tordai A, Andrikovics H: The 3'UTR NFKBIA variant is associated with extensive colitis in Hungarian patients. Gut 2007; 39 Suppl 1: A133 (MON-G-230).

Molnar T, Hofner P, Nagy F, Lakatos PL, Fisher S, Lakatos L, Kovacs A, Altojey I, **Papp M**, Palatka K, Demeter P, Tulassay Zs, Miheller P, Papp J, Mandi Y, Lonovics J: NOD1 gene E266K polymorphism is associated with disease susceptibility but not with disease phenotype or NOD2/CARD15 in Hungarian patients with Crohn's disease. Gut 2007; 39 Suppl 1: A134 (MON-G-233).