Table 1

	Patients' current therapy at enrollment			
	CD (N=414)		UC (N=245)	
5ASA	88	21%	177	72%
ATB	20	5%	9	4%
AZA/6MP	208	50%	98	40%
Anti-TNF	98	24%	20	8%
MTX	48	12%	8	3%
CST	112	27%	87	36%
Other	75	18%	45	18%

prospective data in order to evaluate disease course using these therapies, as well as their appropriateness (http://www.epact.ch).

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STUDY OF THIOPURINE-METHYL-TRANSFERASE (TPMT) GENOTYPE AND PHENOTYPE IN AN ITALIAN POPULATION

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Background: Immunomodulating regimens, mainly based on thiopurines, are increasingly prescribed in inflammatory bowel disease (IBD). Thiopurine-methyl-transferase (TPMT) genotype and phenotype is expected to exhert notable effects on availability and toxicity of these drugs, but it may vary considerably in different populations.
Aim of the study: Aim of this study was to analyse TPMT genotype and phenotype in a Northern Italian population of healthy controls and IBD patients.
Patients and methods: The study protocol was accepted by local Ethical Committee. All consecutive IBD out-patients (n=169) and 533 healthy controls (HC) were recruited. A blood sample was drawn, DNA was extracted for genetic analyses and erytrocytes were separated. Major TPMT genetic variants (TPMT*2, *3A, *3B, *3C) were analysed by means of standardised PCR techniques, while intra-erythrocyte TMPT activity was analysed using a standardised high pressure liquid chromatography (HPLC) technique. Clinical data were recorded for IBD patients.
Results: Regarding TPMT genotype, TPMT was mutated in 8/169 (4.7%; 95%CI 1.5-8%) IBD cases (7 heterozigous for *3A and 1 for *3C, respectively) and in 34/533 (6.4%; 95%CI 4-8%) HC (28 heterozigous for *3A, 3 for *3B and 3 for *3C, respectively); the difference was not statistically significant (p=0.549). Hardy Weinberg distribution was respected, no mutant homozygous was observed among the cases or controls. No significant differences were noted for any subgroup analysis.
As for TPMT activity, the distribution of TPMT activity was sharply different in IBD cases, with distribution not-normal ($p<0.001$) and significantly lower than among HC (however not-normally distributed, p=0.014). TPMT activity figures are reported in Table 1.

Table 1

	TPMT		P
	nMol/h/g Hgb	IQR	
HC (n=533)	33.23	26.61-42.04	comparator
IBD cases (n=169)	27.16	21.45-46.85	0.0016
Crohn's disease (n=79)	27.82	20.80-45.11	0.0175
Ulcerative/indeterminate colitis (n=90)	27.02	21.65-29.89	0.0168

No significant TPMT phenotype difference was noted based on IBD subgroups based on TPMT genotype or on other clinical characteristic, but we found that IBD patients on systemic steroids (n=16) had higher median TPMT activity (46.54 nMol/h/g Hgb) compared to those not on steroid treatment (n=153, 26.98 nMol/h/g Hgb), p=0.0023.
Discussion: This is the first report on TPMT genotypic and phenotypic characteristics of a Northern Italian population. No difference was observed for TPMT genotype when comparing cases and controls, however allelic frequency was lower than expected and no homozygous mutant subject was observed (at very high risk of adverse event if on thiopurine treatment). TPMT activity was lower in IBD patients compared to healthy controls.
Larger groups of patients are under investigation in order to confirm these observations.
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CARD15 GENE EXPRESSION AND ACTIVITY AND PHENOTYPE OF CROHN'S DISEASE

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Aim: Familial aggregations of Crohn's disease (CD) have suggested the influence of genetic factors in the disease development. CARD15 gene has been the first susceptibility gene known for CD. Aim of our study was evaluation of CARD15 gene expression in different subgroups of CD patients living in Lower Silesia, Poland.
Material and method: CARD15 gene expression was assessed in 90 individuals: 60 patients with CD (30 females, 30 males) hospitalized in Department of Gastroenterology and Hepatology, Wroclaw Medical University and in the control group (30 healthy volunteers). CARD15 gene expression was compared to: phenotype of CD (according to the Montreal Classification for Crohn's disease (age of diagnosis, location, behavior)), presence of extraintestinal symptoms and disease activity basing on lab tests and CDAI. CARD15 gene expression was measured in peripheral mononuclear cells using real-time RT-PCR. Total RNA was extracted using E.Z.N.A. Total RNA Kit (Omega Bio-Tek). Total RNA was reverse transcribed by the TaqMan Reverse Transcription Reagents (Roche). All quantitative real-time PCR (TaqManTM) primers, probes and Universal master mix were obtained from Applied Biosystems (USA). All PCRs were performed utilizing 5 μl cDNA per reaction in triplicates of 25 μl volume on a ABI Prism 7900HT Sequence Detection System (TaqMan) using a 2-step PCR protocol after the initial denaturing of the cDNA (10 min at 95°C) with 45 cycles of 95°C for 15 s and 60°C for 1 min. cDNA aliquots were quantified for target genes using the threshold cycle (Ct) method normalized for the house keeping gene GAPDH.
Results: CARD15 expression in peripheral mononuclear cells in CD patients was significantly higher than in controls. The highest CARD15 expression was found in CD patients in forth decade of life. Additionally, we observed higher CARD15 expression in patients with disease duration between 12 and 60 months. There was no relation between CD phenotype and CARD15 expression. However, we found positive correlation of both, CDAI and classical inflammatory markers values (ESR, CRP) and CARD15 expression.
Conclusion: Expression of gene CARD15 in Crohn's disease is higher than in healthy individuals. Disease activity, and not disease phenotype, seems to be the most important factor influencing CARD15 expression.

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CLINICAL FEATURES AND PATHOGENESIS OF INFLAMMATORY BOWEL DISEASE-ASSOCIATED INTESTINAL CANCER

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Patients with inflammatory bowel disease (IBD) [ulcerative colitis (UC) and Crohn's disease (CD)] are at an increased risk of developing gastrointestinal, and mainly colorectal cancer (CRC). The main risk factors are the long-standing duration of the disease, extensive colitis, chronic stenosis, primary sclerosing cholangitis and a family history of CRC. The estimated prevalence, the risk factors, the clinical features and the molecular pathogenetic alterations of IBD-associated intestinal carcinoma were examined retrospectively in a cohort of patients with IBD from a well-defined East-Hungarian geographical area.
Methods: 14 patients with IBD-associated intestinal cancer (10 with UC and 4 with CD; 13 with CRC and 1 with small bowel cancer) were evaluated at the University of Szeged between 1987 and 2005. Medical records were reviewed retrospectively for endoscopic and histologic characteristics and for detailed clinical phenotypes. The following parameters were investigated by a tissue microarray technique in the tumor and in auto control healthy tissues: the grade of differentiation, the lymphovascular invasion, the perineural spread, peritumoral inflammation, necrosis, the desmoplastic reaction and the gene expression profiles of p53, MLH1, MSH2, β-catenin, PTEN, APC, K-ras, Cox-2, NOS, NF?B and VEGF.
Results: The estimated prevalence of IBD-associated intestinal cancer among all CD and UC patients during the examined period was 0.015%. The median age at diagnosis of the IBD-related intestinal cancer cases was 56 years; the median duration was 23.7 years. The main risk factors were chronic activity, extension of the disease and a positive familial anamnesis for CRC. 8/14 of the IBD-related tumors were distal to the splenic flexure. The inflammatory markers were positive in all tumors. The molecular alterations found in IBD-associated cancers involved the same targets as in sporadic CRC.
Conclusion: Chronic uncontrolled inflammation leads to neoplastic transformation on the main molecular pathways of colon carcinogenesis in IBD patients. Thus, the central question of carcinoma prevention in IBD is the continuous control of inflammation throughout the gastrointestinal tract.