Original Article

Frequency of Representative Single Nucleotide Polymorphisms Associated with Inflammatory Bowel Disease in the Czech Republic and Slovak Republic

(gene polymorphism / Crohn's disease / ulcerative colitis / *NOD2 / ICAM-1 / CCR5 /* PCR-RFLP)

J. HOŠEK[1], L. BARTOŠOVÁ[2*], P. GREGOR[3], M. KOLORZ[2], P. DÍTĚ[4], M. BÁTOVSKÝ[5], M. BARTOŠ[1]

[1]Department of Nature Pharmaceuticals, [2]Department of Human Pharmacology and Toxicology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic
[3]Department of Ecology and Environmental Sciences, Palacký University, Olomouc, Czech Republic
[4]Internal/Hepatogastroenterology Department, University Hospital Brno, Brno, Czech Republic
[5]Third Medical Clinic, Dérer's Faculty Hospital with Policlinic, Bratislava, Slovak Republic

Abstract. Involvement of genetic factors in the aetiology of inflammatory bowel disease (IBD) has been known for a long time. Our aim was to investigate the prevalence of polymorphisms in *NOD2*, *ICAM-1* and *CCR5* genes in Czech and Slovak patients with IBD in comparison with healthy controls. The frequency of well-known mutations (R702W, G908W and 1007fs in the *NOD2* gene; K469E in the *ICAM-1* gene, and Δ32 in the *CCR5* gene) involved in IBD was tested in 45 patients with CD and 22 patients with UC. The allele frequency of these mutations was determined and genotype-phenotype correlation was specified. Isolated DNA was genotyped, and allele frequency was counted and statistically verified. Significant differences between the healthy control group and CD patients were observed in mutation 1007fs of the *NOD2* gene (P = 0.0203). We also associated allele E469 of the *ICAM-1* gene with CD (P = 0.0024). No significant association between other alleles and CD was found, and no gene variation was linked to UC. The number of mutations and mutated genes was higher among patients with CD than among patients with UC. Our results support previous findings about participation of mutations of *NOD2* and *ICAM-1* genes in IBD. We confirmed that both CD and UC are polygenic diseases with a gene-dosage effect. This observation strengthens the opinion that genetic factors play a more important role in CD than in UC.

Introduction

Two main diseases belonging to the group of idiopathic bowel inflammation are ulcerative colitis (UC) and Crohn's disease (CD). Ulcerative colitis is a non-specific haemorrhagic-catarrhal or ulcerative inflammation of the rectum and adjacent areas or the entire colon with chronic spasmodic exacerbate behaviour. Crohn's disease is a chronic non-specific inflammation of the small or large intestine, or both of them, or another part of the gastrointestinal tract. It is segmental or plurisegmental, transmural, typically granulomatous. Both diseases (CD and UC) can be distinguished according to the differences in the clinical-pathological features (Podolsky, 2002; Mathew and Lewis, 2004; Bamias et al., 2005). The environmental factors and genetic predispositions participate in their emergence (Podolsky, 2002). Familiar accumulation of the disease, monozygotic twins' concordance, or ethnic differences give evidence about the influence of genetic factors involved in the disease development (Podolsky, 2002; Braat et al., 2005). These genetic predispositions are more important in CD than in UC (Podolsky, 2002; Halme et al, 2006). The risk of an outbreak of inflammatory bowel disease (IBD) is 10 times higher among first-degree relatives of

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*Corresponding author: Ladislava Bartošová, Department of Pharmacology and Toxicology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1-3, 612 42 Brno, Czech Republic; Phone: +420 541 562 891; Fax: +420 541 240 605; e-mail: bartosoval@vfu.cz

Abbreviations: CD – Crohn's disease; IBD – inflammatory bowel disease; LRR – leucine-rich repeat; PAMP – pathogen-associated molecular pattern; PCR-RFLP – polymerase chain reaction – restriction fragment length polymorphism; PRR – pattern recognition receptor; SNP – single nucleotide polymorphism; UC – ulcerative colitis.

patients with CD or UC than in normal population (Orholm et al., 1991). No simple mendelian heritability exists in IBD, which means that more products of more genes are involved in the disease development (Podolsky, 2002).

In 1996 an *IBD1* locus was found by linkage analysis (Hugot et al., 1996), which is related to susceptibility to CD. However, a specific predisposing gene – *NOD2* – was identified more recently (Hugot et al., 2001). The protein coded by this gene belongs to a group of pattern recognition receptors (PRRs) of the innate immune system and recognizes a pathogen-associated molecular pattern (PAMP) (Braat et al., 2005; Zelinkova et al., 2005). Its leucine-rich repeats (LRRs) domain serves as an intracellular receptor for bacterial muramyl dipeptide and activates the immune response via NF-κB (Ogura et al., 2001a; Pauleau and Murray, 2003). Three main single nucleotide polymorphisms (SNPs) near or in the LRRs domain have been revealed. Two of them are missense [SNP 8 (R702W), SNP 12 (G908R)] and one is a frame-shift mutation [SNP 13 (1007fs)] (Hugot et al., 2001; Ogura et al., 2001b), which causes protein truncation by 33 amino acids. These three mutations present 81% mutations of *NOD2* in CD (Lesage et al., 2002). Carriers of one mutant allele have 2–4 times higher risk of CD outbreak and recessive homozygotes 20–40 times higher (Bonen and Cho, 2003).

ICAM-1 plays a key role in the neutrophil migration to the inflammatory tissue, which may prove connection with some inflammatory diseases (Matsuzawa et al., 2003). This protein is expressed on the surface of activated endothelial cells, where it regulates rolling of lymphocytes. When neutrophiles bind ICAM-1, they start penetrating from capillaries to the tissue (Springer, 1990). Two SNPs were identified in the coding region of the *ICAM-1* gene. Mutation G241R is not as common as K469E (Vora et al., 1994; Özen et al., 2006).

Chemokines and their membrane receptors participate in the pathogenesis of various inflammatory diseases, where they play an important role in migration and activation of leukocytes (Sallusto et al., 2000). Herfarth et al. (2001) suggest the hypothesis that polymorphisms in the CCR5 receptor may participate in the course and localization of CD. Carriers of the Δ32 mutation have less frequent CD finding in the upper part of the gastrointestinal tract and are probably protected against an aggressive form of CD. Oki et al. (2005) discovered higher occurrence of $CCR5^+$ cells around CD granulomas. These findings led to the idea that non-functionality of CCR5 caused by deletion mutation Δ32 results in a decrease of migration of pro-inflammatory lymphocytes to inflammatory tissue followed by a decrease of inflammatory demonstration.

In the Czech Republic there is a prevalence of CD 18-22/100,000 and UC 40-45/100,000 (Lukáš, 2003). However, data related to the frequency of risk alleles in IBD-susceptible genes are absent. We focused on the determination of frequencies of three mutations in the *NOD2* gene (R702W, G908R, 1007fs), one in the *ICAM-1* gene (K469E) and one in the *CCR5* gene (Δ32) in a small sample of the Czech and Slovak population and set it in all-European context. We also tried to define the genotype-phenotype correlation between these mutations and CD or UC, respectively.

Material and Methods

Patients and healthy controls

Forty-five patients with CD, 22 patients with UC, and 59 healthy (without diagnosed IBD and with negative familiar case history) unrelated individuals were selected in this study (Table 1). All investigated persons came from the Czech Republic or Slovak Republic. Because of the similar historical and geographical origin, we examined all samples together. Diagnosis was made at the Internal/Hepatogastroenterology Department of the University Hospital Brno (GAEK FN Brno, the Czech Republic) and at the Dérer's Faculty Hospital with Policlinic in Bratislava-Kramare (Slovakia) using standard examination methods. Volunteers, such as students and employees of the Faculty of Pharmacy, were enrolled as healthy controls. All participants signed an informed consent.

Although there is a discrepancy between the age of healthy controls and patients, it has no influence on statistical evaluation. With respect to the facts that about 30 % of patients will take IBD between 10 and 30 years of age (Bamias et al, 2005) and that healthy volunteers were without IBD-positive family history, we assumed that nobody from the control group would have positive diagnosis in the future.

Also, the sex ratio in the control group differed from the patients. However, according to other authors, there is no relation of IBD to gender (Prikazska et al., 1998). It is the reason why we did not take the gender into regard and evaluated both, men and women, together.

Table 1. Characteristic of the CD patients, UC patients and healthy control groups

Number	**Patients with CD** **45**	**Patients with UC** **22**	**Healthy control** **59**
Sex	Males 29 (64.4 %) Females 16 (35.6 %)	Males 12 (54.5 %) Females 10 (45.5 %)	Males 12 (20.3 %) Females 47 (79.7 %)
Age in years (± S.D.)	Males 33.7 (± 10.8) Females 39 (± 12.1)	Males 42.8 (± 13.8) Females 43.8 (± 13.8)	Males 24.6 (± 1.3) Females 29.3 (± 11.4)

DNA isolation and genotype determination

Patients' and healthy controls' peripheral blood was collected into Na-citrate tubes. DNA was isolated from whole blood by the QIAamp DNA Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer's manual. Isolated DNA was stored at 4°C during the experimental span and for longer storage it was frozen at -20°C.

The PCR-RFLP method was used for detection of selected polymorphisms. Briefly, DNA was amplified by PCR and subsequently digested with a suitable restriction endonuclease (New England Biolabs, Ipswich, MA) (Table 2). Products of the digestion were separated by electrophoresis in 2% agarose gel containing ethidium bromide (0.15 mg/ml). Visualization was performed on a UV transilluminator using 312 nm light.

The allele W702 in the *NOD2* R702W polymorphism, the allele R908 in the *NOD2* G908R polymorphism and the allele 1007fs in the *NOD2* 1007fs polymorphism are called "mutant alleles" in the following text. The allele R702 in the *NOD2* R702W polymorphism and the allele G908 in the *NOD2* G908R polymorphism are called "standard alleles".

Positive control preparation

Genotypes for all studied mutations were determined for a few individuals in a pilot experiment. PCR products of both standard and mutant alleles were cloned into the plasmid vector pCR2.1 (Invitrogen, Carlsbad, CA) and these constructs were transformed into chemically competent cells of *Escherichia coli* TOP10F' according to the manufacturer's manual. Bacterial colonies containing the insert, determined by blue-white test with X-gal, were tested by PCR-RFLP for the presence of standard or mutant alleles. The accuracy of the test was verified by sequencing (MGW, Erbersberg, Germany). Selected bacterial colonies were cultivated and pure plasmids were isolated by the QIAprep Spin Kit (Qiagen), which served as a positive control to the PCR and for the restriction analysis.

Statistical methods

Our investigation was made as a case-control study where we compared the patients' genotypes with healthy controls' genotypes.

Statistical tests were done by statistical software R version 2.4.1. To test our hypothesis three different statistical tests were used. The first one, the χ^2 test, is commonly used to test whether the observed distribution differs significantly from the expected distribution and also whether two random variables are independent. For the same reason Fisher's exact test is used, which is the best in the analysis where sample sizes are small. By contrast, to test differences among more independent groups, ANOVA was used. The significance level of $\alpha = 0.05$ was chosen for all tests.

Results

The genotype of the R702W, G908R and 1007fs mutations in the *NOD2* gene, one mutation in the *ICAM-1* gene (K469E) and one mutation in the *CCR5* gene (Δ32) were determined for 45 CD patients, 22 UC patients and 59 healthy controls. The presence of separate genotypes and allele frequencies is summarized in Table 3. Frequencies of all focused mutations of healthy volunteers, patients with CD and UC are shown in Fig. 1.

NOD2 gene variation

The polymorphism R702W of the mutant allele W702 was present in 10 % of healthy controls, 13 % of CD patients, and 2 % of UC patients. All individuals with the mutant allele were heterozygotes; no homozygote constitution was found. No statistical significance between the healthy control and CD patients and between the healthy control and UC patients was noticed, but UC patients had less frequent occurrence of this mutation than CD patients ($P = 0.0315$).

Gene variation with the G908R mutation was observed in only three cases of CD patients and only in the heterozygote constitution. There was no statistically significant linkage to the CD or UC ($P = 0.1221$).

Table 2. Used primers and restriction endonucleases

Polymorphism	Used primers	Restrictase	Wild-type allele	Mutant allele
ICAM1 K469E	5'- GGA ACC CAT TGC CCG AGC -3' 5'- GGT GAG GAT TGC ATT AGG TC -3'	*Bst*U I	223 bp	136 bp + 87 bp
NOD2 1007fs	5'- CCT GCA GTC TCT TTA ACT GG -3' 5'- CTT ACC AGA CTT CCA GGA TG -3'	*Nla* IV	168 bp	128 bp + 40 bp
NOD2 G908R	5'- AAG TCT GTA ATG TAA AGC CAC -3' 5'- CCC AGC TCC TCC CTC TTC -3'	*Hha* I	380 bp	242 bp + 138 bp
NOD2 R702W	5'- CTT CCT GGC AGG GCT GTT GTC -3' 5'- CAT GCA CGC TCT TGG CCT CAC -3'	*Msp* I	76 bp + 54 bp + + 24 bp + 22 bp	130 bp + 24 bp + + 22 bp
CCR5 Δ 32	5'- TGG TGG CTG TGT TTG CGT CTC -3' 5'- AGC GGC AGG ACC AGC CCC AAG -3'	–	162 bp	130 bp

Table 3. Results of genotyping

		Healthy control N = 59	CD patients N = 45	UC patients N = 22
NOD2 gene (R702W)				
genotype	+/+ (R/R)	47 (79.66%)	33 (73.33%)	21 (95.45%)
	+/- (R/W)	12 (20.34%)	12 (26.67%)	1 (4.55%)
	-/- (W/W)	0 (0%)	0 (0%)	0 (0%)
allele frequency	+ (R)	0.90	p = 0.87	p = 0.98
	- (W)	0.10	q = 0.13	q = 0.02
NOD2 gene (G908R)				
genotype	+/+ (G/G)	59 (100%)	42 (93.33%)	22 (100%)
	+/- (G/R)	0 (0%)	3 (6.67%)	0 (0%)
	-/- (R/R)	0 (0%)	0 (0%)	0 (0%)
allele frequency	+ (G)	1	0.97	1
	- (R)	0	0.03	0
NOD2 gene (1007fs)				
genotype	wt/wt	53 (89.83%)	30 (66.67%)	17 (77.27%)
	wt/fs	5 (8.47%)	10 (22.22%)	5 (22.73%)
	fs/fs	1 (1.69%)	5 (11.11%)	0 (0%)
allele frequency	(wt)	0.94	0.78	0.89
	(fs)	0.06	0.22	0.11
ICAM-1 gene (K469E)				
genotype	K/K	36 (61.02%)	14 (31.11%)	7 (31.82%)
	K/E	21 (35.59%)	21 (46.67%)	13 (59.09%)
	E/E	2 (3.39%)	10 (22.22%)	2 (9.09%)
allele frequency	K	0.79	0.54	0.61
	E	0.21	0.46	0.39
CCR5 gene				
genotype	wt/wt	41 (69.49%)	34 (75.56%)	15 (68.18%)
	wt/Δ32	18 (30.51%)	11 (24.44%)	6 (27.27%)
	Δ32/ Δ32	0 (0%)	0 (0%)	1 (4.55%)
allele frequency	wt	0.85	0.88	0.82
	Δ32	0.15	0.12	0.18

wt = wild-type
+ = standard allele, - = mutant allele
fs = frameshift
R, W, Q, K, E = details in text

The most frequent mutation in the *NOD2* gene was 1007fs. Its frequency was 6% for healthy volunteers, 22% for the CD patients and 11% for the UC patients. The linkage to CD was found (P = 0.0203). Our sample included five mutant homozygotes among the CD patients and one among healthy controls. We did not note any association of the *NOD2* gene mutations to the UC.

Only 46.7% patients with CD were without mutation in *NOD2*; the frequency of mutation in the healthy control and the UC patients was similar, 71.2% and 72.7%, respectively.

One mutation in this gene was carried by a similar number of individuals of all examined groups, from 25.4% to 28.9 %.

Each person carrying two mutations was either a homozygote or a heterozygote in two indenpendent loci (double heterozygote). We observed only two individuals among healthy controls who had two mutations in *NOD2*, one double heterozygote (R702W + 1007fs) and one homozygote for the 1007fs mutation. On the other hand, six CD patients were double heterozygotes (five for R702W+1007fs and one for G908R+1007fs) and five CD patients were homozygotes for the 1007fs mutant allele only. No patient with UC had two mutations in the *NOD2* gene.

No one in the examined group had three or more mutations in both alleles.

The CD patients carried significantly more mutations than the healthy control group (P = 0.0003). A trend of decreasing *NOD2* mutation number was observed in the UC patients compared to CD patients (P = 0.0812). This situation is presented in Fig. 2.

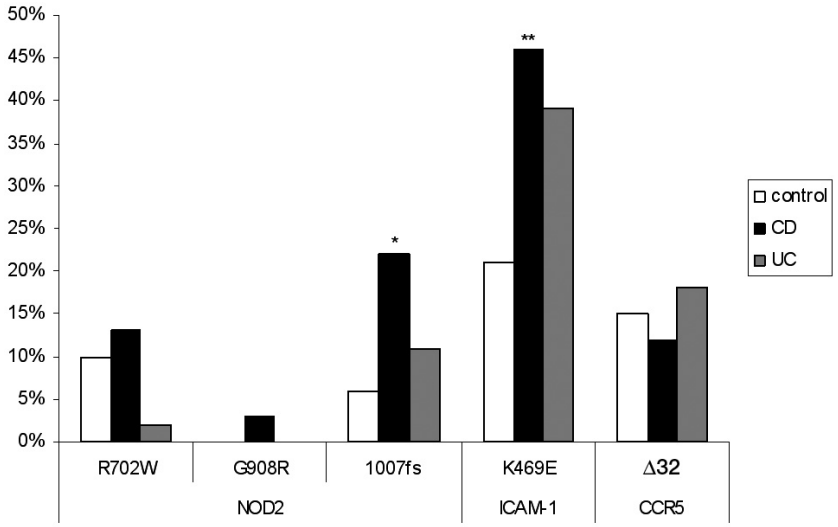

Fig. 1. The allele frequency of mutant alleles in the CD patients, UC patients and healthy control groups.
Allele frequencies of these variants in CD and UC populations were compared with those in the control group, and the level of significance (P value) is denoted here by asterisks: *, $P < 0.05$; **, $P < 0.005$

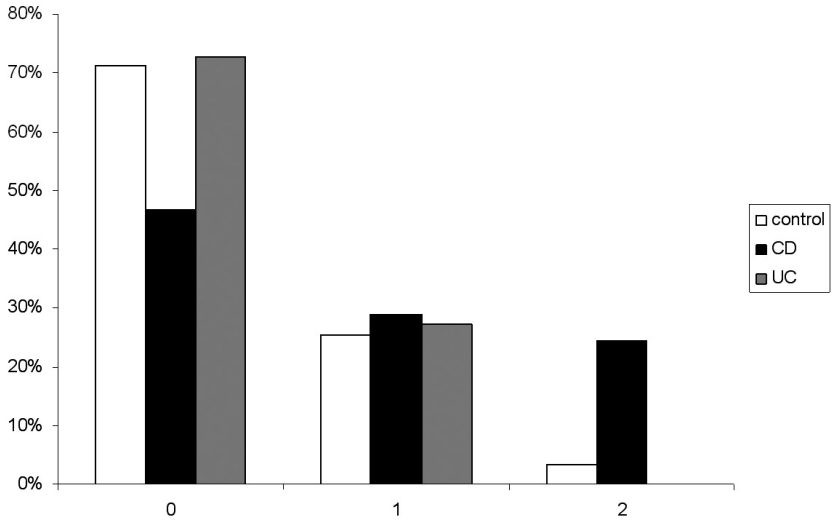

Fig. 2. Number of NOD2 mutation distributed in the CD patients, UC patients and healthy control groups.

ICAM-1 gene variation

The allele E469 of the *ICAM-1* gene had a frequency of 21% in the healthy control group, 46% among the CD patients, and 39% among the UC patients. We found two homozygotes in the healthy group and the same number in the UC group, whereas 10 homozygotes were present in the CD group. A strong association between the presence of this mutation and CD was proved ($P = 0.0024$). Simultaneously, a weak relationship between this mutation and UC was found ($P < 0.06$).

CCR5 gene variation

All examined groups had a similar (12% to 18%) allele frequency of the Δ32 mutation in the *CCR5* gene. No relationship between this mutation and CD or UC was observed. Only one deletion homozygote was found in the entire complex and it was among the UC patients.

Multiplex analysis

In the monitored group of healthy volunteers, 71.2% individuals carried at least one mutation in the monitored genes (*NOD2*, *ICAM-1*, and *CCR5*), in the CD or UC patients it was 93.3% or 81.8%, respectively. The mutations in all groups are summarized in Fig. 3. The difference between the healthy group and the CD group is statistically significant ($P = 0.0003$). Comparison of the healthy group with the UC patients did not show any statistically significant difference ($P = 0.205$).

Analysis of the number of all mutated genes was done as well. A mutated gene was considered when the individual carried at least one mutation in a given gene (mutant homozygote, heterozygote, double heterozygote in the *NOD2* gene case). Due to the analysis of three genes the maximum number of the mutated genes was three. Fig. 4 presents distribution of these genes in the examined

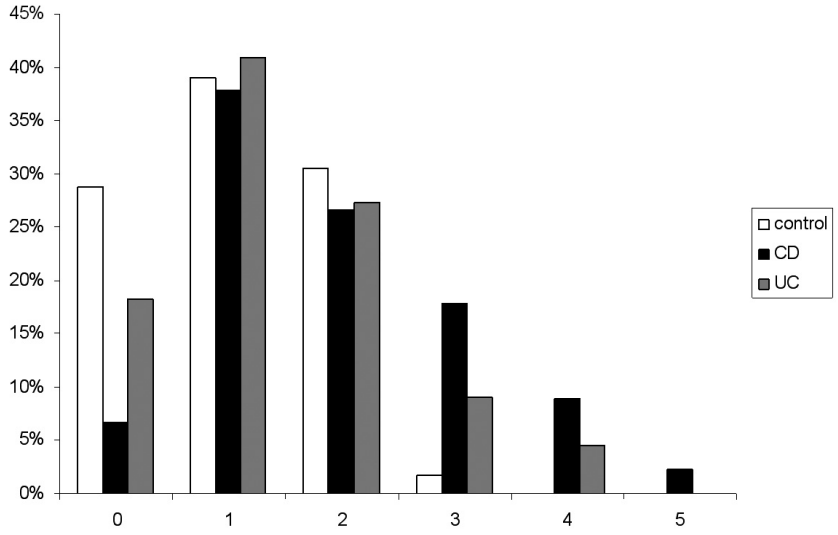

Fig. 3. Number of all mutations in the CD patients, UC patients and healthy control groups.

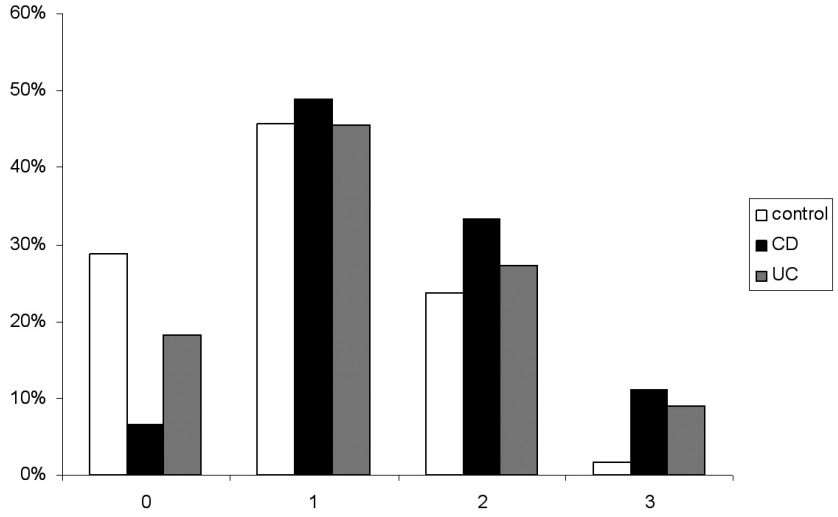

Fig. 4. Number of all mutated genes (with at least one mutation) in the CD patients, UC patients and healthy control groups.

samples. Patients with CD had significantly more mutated genes than the healthy control group (P = 0.0101).

Discussion

In this article we analysed five SNPs involved in IBD susceptibility of a small part of Czech and Slovak population. Although the number of samples was small, it brought valuable information about the situation in the Middle-European region compared to the entire Europe and prepared ground for further investigation.

The first studied gene was *NOD2*. Lesage et al. (2002) discovered 67 sequence variants in the *NOD2* gene, but part of them were called polymorphisms (allele frequency >5 %), not mutations. The R702W, G908R and 1007fs mutations, which are independently associated with CD (Braat et al., 2005; Lesage et al, 2002; Özen et al., 2006), were studied in this work. In the first of them – R702W in the *NOD2* gene – a relatively high frequency of the W702 allele was found in both the control group (10.2 %) and the CD patients group (13.3 %). On the other hand, the patients with UC had a very low frequency of this mutant allele (2.3 %). This value is significantly lower than in the CD patients group; however, it can be given by the small sample number of UC patients and results in statistical distortion. A similarly high frequency of the mutant allele (17.6 %) was revealed in the Spanish CD patients by Alvarez-Lobos et al. (2005) and this mutation occurred in 10 % of the Italian patients observed by Laghi et al. (2005). In an all-European study the frequency was determined to be 11 % among the CD patients (Lesage et al., 2002). Noteworthy is the absence of recessive homozygotes, which are expected at this allele frequency. At present, we cannot explain this fact, but we assume that it might be caused by the small number of samples. Only the high frequency of the mu-

tant allele in the healthy group is astonishing. Although the mutation R702W was previously associated with CD (Lesage et al., 2002), it cannot be excluded that this mutation participates in the disease aetiopathology only in a small part. It may be the reason why it occurs in the population in such a high degree. The outbreak of the disease would then be dependent on the presence of further factors, both environmental and genetic.

The mutation G908R in the *NOD2* gene seems to be very rare. We found only three R908 alleles in the CD group, which equals allele frequency of 3.3 %. Such a low frequency is not uncommon in Europe. In Spain it is 4.7% (Alvarez-Lobos et al., 2005), in Italy 4.6% (Laghi et al., 2005) and in Turkey only 2.1% (Özen et al., 2006). However, the all-Europe average in the CD population is 6% (Lesage et al., 2002).

Unambiguously associated mutation to CD is the 1007fs of *NOD2*. As a sole mutation in *NOD2*, it was found in a homozygous state. The CD patients displayed its frequency of 22.2%, which is the highest value detected in Europe and corresponds to double of European mean (11%) (Lesage et al., 2002). Even in the control group, where the frequency was 5.9%, this number is very high. We discovered one individual (female) in the control group who was a homozygote for the 1007fs allele. Although she was not classified as CD or UC, she had similar problems – diarrhoea, bad food intake, and she even underwent an operation of a large intestine. Originally, irritable colon was diagnosed, and we recommended a new gastroenterological examination for possibly wrong diagnosis.

Complete analysis of the *NOD2* mutations demonstrates that patients with CD carry two mutations more frequently than the healthy control group (24.4% in CD and 3.4% in healthy control). Only 46.7% of the CD patients had no mutation, in comparison with 71.2% in the healthy population. The healthy control group result is the same as in the study by Braat et al. (2005), who determined 2.2% of healthy individuals as carriers of two mutant alleles. However, they found only 7.4% of CD patients who were double heterozygotes or homozygotes for the mutant alleles. On the other hand, Lesage et al. (2002) discovered that 50% of patients with CD carried at least one mutation, including 17% who had a double mutation. These values correspond to our results, where 53.3% of the CD patients had one or two mutations and 24.4% had two mutations.

We found one double heterozygote in the control healthy group. We assume that both mutations were on one allele, which means that one allele was completely functional. On the contrary, the patients with the double heterozygote constitution carried one mutation on each allele. It could be the reason of the CD outbreak.

One of the goals of this work was to determine the susceptible allele of gene polymorphism K469E in the *ICAM-1* gene in the Czech and Slovak population. The achieved results show the influence of allele E469 on the CD outbreak. Association of IBD with this allele is supported by other European studies (Braun et al., 2001; Papa et al., 2004). In the Turkish population no association between the K469E polymorphism and IBD was noted, but a higher frequency of the E469 allele is apparent among patients with CD (Özen et al., 2006). On the other hand, in Japanese population, significant association of the second allele – K469 – to CD (65.2% in the diseased and 49.5% in the control) was observed (Matsuzawa et al. 2003). Surprisingly, a similar result was achieved among British patients (Low et al, 2004). Both Japan and the United Kingdom are island states, so a relatively higher isolation could lead to setting different Hardy-Weinberg equilibrium than on the continent.

According to our study, the allele E469 frequency is 21.2% in the healthy population in comparison with 45.6% in CD or 38.6% in UC. The occurrence of homozygote constitution E/E in the control is 3.4%, which is very low. Braun et al. (2001) found this genotype in 9.5% of the healthy population in Germany and Papa et al. (2004) noted 11.8% in Italy. According to our observation, a similar genotype structure of healthy population was described by Özen et al. (2006) in Turkey, where genotype E/E was found in 2.8% cases. However, the heterozygote constitution K/E was found in 63.2% of healthy population in comparison with 35.6% in the Czech and Slovak population. E/E frequency was 17.9% in the IBD patients (CD and UC together) (CD 22.2% and UC 9.1%). In previous studies (Braun et. al., 2001; Papa et al., 2004) this genotype was found in 26% of CD patients and 21.2% of UC patients, i.e. in 24.9% of the IBD patients.

A strong association of the E469 allele to CD was found in the K469E polymorphism of the *ICAM-1* gene. Higher frequency of the E469 allele was also noted in the UC patients, but without statistical evidence. These results support the fact that mutations in the *ICAM-1* gene influence the development of many chronic inflammatory diseases, including IBD (Vora et al., 1994; Low et al., 2004). Part of healthy volunteers who carried the E469 allele suffered from a chronic inflammatory disease, e.g. asthma, atopic eczema or rheumatoid arthritis (data not shown).

The third investigated gene was *CCR5* and its polymorphisms Δ32. All three examined groups had a similar allele frequency of Δ32 (12.2% – 18.2%). In the study by Herfarth et al. (2001) the frequency of this allele was similar in healthy volunteers and CD patients (9.2% or 9.8%, respectively) as well. We observed a higher representation of the Δ32 allele in the Czech and Slovak population, but still in Hardy-Weinberg equilibrium.

Generally, the Δ32 allele frequency is 14.7 %. This value approximately agrees with the north-south gradient of distribution in the entire Europe (Novembre et al., 2005).

Finally, all IBD susceptible polymorphisms were examined together. A significantly higher number of mutated genes ($P = 0.0101$) resulting in higher mutation number ($P = 0.0003$) was observed in the CD patients group. This fact supports the hypothesis that CD is

a polygenic disorder with a gene-dose effect. No statistically higher number of mutations or risk alleles was noted in the UC patients, but the trend of these increasing parameters is obvious from the Fig. 3 and Fig. 4**.** The number of mutations and risk alleles of UC patients ranges between the CD patients and the healthy control group. It confirms the opinion that genetic predispositions play a less important role in UC than in CD.

Conclusion

This work confirmed that mutations in *NOD2* and *ICAM-1* genes participate in the CD outbreak. No influence of deletion mutation Δ32 in the *CCR5* gene on Crohn's disease development was observed. We also confirmed that genetic predispositions are more important in CD than in UC.

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