PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

ELSEVIER

World J Gastroenterol 2005;11(5):681-685
World Journal of Gastroenterology ISSN 1007-9327

• CLINICAL RESEARCH •

Association between polymorphisms in the Toll-like receptor 4, CD14, and *CARD15/NOD2* and inflammatory bowel disease in the Greek population

Maria Gazouli, Gerassimos Mantzaris, Athanassios Kotsinas, Panayotis Zacharatos, Efstathios Papalambros, Athanassios Archimandritis, John Ikonomopoulos, Vassilis G Gorgoulis

Maria Gazouli, Athanassios Kotsinas, Panayotis Zacharatos, Vassilis G Gorgoulis, Department of Histology-Embryology, School of Medicine, University of Athens, 11527 Athens, Greece
Gerassimos Mantzaris, Department of Gastroenterology, "Evagelismos" Hospital, 11521 Athens, Greece
Efstathios Papalambros, First Department of Surgery, Laikon Hospital, School of Medicine, University of Athens, 11527 Athens, Greece
Athanassios Archimandritis, Department of Pathophysiology, Gastroenterology Section, School of Medicine, University of Athens, "Hippocration" Hospital, 11527 Athens, Greece
John Ikonomopoulos, Agricultural University of Athens, Department of Anatomy-Physiology, Faculty of Animal Science, 11855 Athens, Greece
Supported by the EU Project "Sacrohn" N. QLK2-CT-2000-00928
Correspondence to: Dr. Vassilis G Gorgoulis, Department of Histology-Embryology, 53 Antaiou St. Ano Patisia, 11146 Athens, Greece. histoclub@ath.forthnet.gr
Fax: +302106535894
Received: 2004-05-25 **Accepted:** 2004-07-27

Abstract

AIM: Crohn's disease (CD) and ulcerative colitis (UC) are multifactorial diseases with a significant genetic background. Apart from *CARD15/NOD2* gene, evidence is accumulating that molecules related to the innate immune response such as CD14 or Toll-like receptor 4 (TLR4), are involved in their pathogenesis. In further exploring the genetic background of these diseases, we investigated the variations in the *CARD15/NOD2* gene (Arg702Trp, Gly908Arg and Leu1007fsinsC), and polymorphisms in the TLR4 gene (Asp299Gly and Thr399Ile) as well as in the promoter of the CD14 gene (T/C at position -159) in Greek patients with CD and UC.

METHODS: DNA was obtained from 120 patients with CD, 85 with UC and 100 healthy individuals. Genotyping was performed by allele specific PCR or by PCR-RFLP analysis.

RESULTS: The 299Gly allele frequency of the TLR4 gene and the T allele and TT genotype frequencies of the CD14 promoter were significantly higher in CD patients only compared to healthy individuals ($P = 0.026<0.05$; $P = 0.0048<0.01$ and $P = 0.047<0.05$ respectively). Concerning the *NOD2/CARD15* mutations the overall presence in CD patients was significantly higher than that in UC patients or in controls. Additionally, 51.67% of the CD patients were carriers of a TLR4 and/or CD14 polymorphic allele and at least one variant of the *NOD2/CARD15*, compared to 27% of the UC patients. It should be pointed out that both frequencies significantly increased as compared with the 10% frequency of multiple carriers found in healthy controls. A possible interaction of the *NOD2/CARD15* with TLR4 and especially CD14, increased the risk of developing inflammatory bowel disease (IBD).

CONCLUSION: Our results indicate that co-existence of a mutation in either the TLR4 or CD14 gene, and in *NOD2/CARD15* is associated with an increased susceptibility to developing CD compared to UC, and to developing either CD or UC compared to healthy individuals.

Key words: Inflammatory bowel disease; CARD15/NOD2 gene; Toll-like receptor 4; CD14 Antigen

Gazouli M, Mantzaris G, Kotsinas A, Zacharatos P, Papalambros E, Archimandritis A, Ikonomopoulos J, Gorgoulis VG. Association between polymorphisms in the Toll-like receptor 4, CD14, and *CARD15/NOD2* and inflammatory bowel disease in the Greek population. *World J Gastroenterol* 2005; 11(5): 681-685
http://www.wjgnet.com/1007-9327/11/681.asp

INTRODUCTION

Inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC) are multifactorial disorders characterized by failure to limit the inflammatory response to luminal antigens. Genetic predisposition to IBD has been well established through epidemiological studies and genome wide linkage analyses, but little is known about the accountable genes[1]. Animal models have demonstrated that genes involved in the regulation of the immune response are likely to play a crucial role in the genetic predisposition to IBD[2].

The innate immune response represents the first defense line in preventing systemic infection with bacteria. Several host receptors interact with endotoxins and mediate cytokine production of macrophages. Lipopolysaccharides (LPS) are the main endotoxins derived from Gram-negative bacteria, and their pivotal role in the pathogenesis of a variety of infectious and allergic diseases has been suggested[3,4]. *CARD15/NOD2*, a cytosolic protein expressed in monocytes, is involved in the innate immune response to LPS and peptidoglycans (PGN)[5].

The association between mutations in the *CARD15/NOD2* gene and CD has been described recently[6,7]. The 3 major variants Gly908Arg, Arg702Trp, and Leu1007fsinsC are associated with a deficit in NF-κB activation in response to bacterial components, providing a unifying mechanism for the major CD-associated *CARD15/NOD2* variants[5].

The question arises as to how *CARD15/NOD2* mutations and impaired NF-κB activation confer susceptibility to CD. It has been suggested that the answer most likely lies within the leucine-rich repeats (LRR) of the *CARD15/NOD2* gene and the family of Toll-like receptors. These receptors recognize pathogen-associated molecular patterns and activate signal transduction pathways of the innate immune response genes including inflammatory cytokines and the NF-κB signaling

pathway[8]. Therefore, one could speculate that extracellular Toll-like receptors and intracellular *CARD15/NOD2* participate as pattern-recognition receptors in the regulation of mucosal innate immune responses to intestinal microbes. Among the Toll-like receptors, Toll-like receptor 4 (TLR4) was found to be strongly up-regulated in both UC and CD[9]. TLR4 binds to LPS together with CD14 and by internalization prevents inappropriate NF-κB activation[10].

Very recently, Arbour *et al*[11] reported that the Asp299Gly and Thr399Ile polymorphisms of human TLR4 determine, in concert with other genetic changes, the airway responsiveness to inhaled LPS in humans. In addition, Klein *et al*[12] have demonstrated an association of CD with a functional relevant single nucleotide polymorphism in the promoter of the CD14 gene (T/C at position -159) and suggested that the interaction of the *CARD15/NOD2* and CD14 genes increases the risk for developing CD[13].

In order to evaluate whether the above mentioned polymorphisms in TLR4 and CD14 genes contributed to the predisposition to IBD, as well as whether the interaction of *CARD15/NOD2*, TLR4 and CD14 genes could increase the risk for IBD in a Greek population, we genotyped 120 patients with CD, 85 patients with UC and 100 healthy controls for the Asp299Gly and Thr399Ile polymorphisms of the TLR4 gene and the promoter of the CD14 gene (T/C at position -159).

MATERIALS AND METHODS

Materials

Blood samples from 120 patients with CD, 85 patients with UC and 100 age and sex-matched healthy individuals were collected at the IBD Outpatient Clinic between September 2002 and February 2003. The diagnosis of either CD or UC was based on standard clinical, endoscopic, radiological, and histological criteria[14]. Before commencement of the study, the Ethics Committee at the participating centers approved the recruitment protocols. All participants were informed of the study. DNA was isolated from blood with the NucleoSpin Blood Kit (Macherey-Nagel, Germany).

Methods

Genotyping for the TLR4 Asp299Gly and TLR4 Thr399Ile polymorphisms was performed using PCR-RFLP as previously described[15]. Specifically, primers for TLR4 Asp299Gly were forward (5' GATTAGCATACTTAGACTACTACCTCCATG 3') and reverse (5' GATCAACTTCTGAAAAAGCATTCCCAC 3'). Primers for TLR4 Thr399Ile were forward (5' GGTTGCTGTTCT CAAAGTGATTTTGGGAGAA 3') and reverse (5' CCTGAAGA CTGGAGAGTGAGTTAAATGCT 3'). The underlined bases in both forward primers indicate the altered nucleotide to create a *Nco*I (TLR4 Asp299Gly) and a *Hinf*I (TLR4 Thr399Ile) restriction site, respectively. PCR reactions were run at 95 ℃ for 5 min followed by 35 cycles at 95 ℃ 30 s, at 55 ℃ for 30 s, at 72 ℃ for 30 s, and a final incubation at 72 ℃ for 5 min. A 15-μL aliquot of the product was digested with the appropriate restriction enzyme and electrophoresed in a 3% agarose gel to identify the TLR4 alleles on the basis of the respective allele size. After digestion, fragment sizes for carriers of the polymorphic allele decreased from 249 bp (wild-type) to 223 bp for the 299 residue, and from 406 bp (wild-type) to 377 bp for the 399 residue.

Genotyping for -159(C/T) of the CD14 gene was performed using the method described by Hubacek *et al*[16] In brief, the promoter of the CD14 receptor gene was amplified by the primers CDP-1 (5' TTGGTGCCAACAGATGAGGTTCAC 3'), and CDP-2 (5' TTCTTTCCTACACAGCGGCACCC 3') under the following conditions: an initial denaturation at 95 ℃ for 5 min, followed by 35 cycles at 92 ℃ for 40 s, at 62 ℃ for 35 s, and at 72 ℃ for 50 s. The final extension step was prolonged to 5 min. The 561 bp PCR product was digested with the restriction enzyme *Hae*III, into the fragments of 204, 201 and 156 bp in length in the presence of the wild-type allele. The variant allele showed a loss of one *Hae*III cleavage site, resulting in the presence of fragments 360 and 201 bp in length.

The cytosine insertion mutation was genotyped by a PCR amplification of specific allele assay using two allele-specific forward primers L1007fsinsCWTF: 5' CAGAAGCCCTCCTGCA GGCCCT 3' for the wild-type allele and L1007fsinsCMUTF: 5' CAGAAGCCCTCCTGCAGGCCCCT 3' for the L1007fsinsC mutant allele, in combination with a common primer L1007fsinsCR: 5' TCTTCAACCACATCCCCATT 3', in two separate PCR reactions. The 3'-ends of the forward primers, were able to anneal to regions that differed between the two alleles. The PCR profile was as follows: initial denaturation at 95 ℃ for 5 min, followed by 35 cycles of denaturing at 94 ℃ for 45 s, annealing at 65 ℃ for 40 s and extension at 72 ℃ for 30 s and a final incubation at 72 ℃ for 10 min. The missense mutation R702W was genotyped by a PCR amplification of specific allele assay using two allele-specific forward primers R702WWTF: 5' ATCTGAGAAGGCCCTGCTCC 3' for the wild-type allele and R702WMUTF: 5' ATCTGAGAAGGCCCTGCTCT 3' for the R702W mutant allele, in combination with a common primer R702WR: 5' CCCACACTTAGCCTTGATG 3', in two separate PCR reactions. The 3'-ends of the forward primers, were able to anneal to regions that differed between the two alleles. The PCR profile was as follows: initial denaturation at 95 ℃ for 5 min, followed by 35 cycles of denaturing at 94 ℃ for 45 s, annealing at 53 ℃ for 40 s and extension at 72 ℃ for 30 s and a final incubation at 72 ℃ for 10 min. The missense mutation G908R created a restriction site for *Hha*I and was genotyped by a PCR-RFLP method (5' CCCAGCTCCTCCCTCTTC 3' and 5' AAGTCTGTAATGTAAAGCCAC 3'). The presence of a wild-type allele resulted in an intact 380 bp band, whereas the RFLP profile of the G908R variant was characterized by two bands of 138 bp and 242 bp. The PCR conditions were as follows: initial denaturation at 95 ℃ for 5 min, followed by 35 cycles of denaturing at 94 ℃ for 45 s, annealing at 53 ℃ for 40 s, extension at 72 ℃ for 30 s, and a final incubation at 72 ℃ for 10 min. All PCR assays were performed in a 50 μL volume reaction containing 10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 2 mmol/L $MgCl_2$, 250 μmol/L dNTPs, 0.20 μmol/L concentration of each primer, 200 ng of genomic DNA and 2.5 U of Taq DNA polymerase (Promega). PCR products were electrophoresed on an agarose gel and visualized by ethidium bromide staining.

Statistical analysis

Odds ratios (OR) were calculated with the corresponding 95% confidence intervals ($CI_{95\%}$). Frequencies and susceptibilities of mutations among CD, UC and controls were compared based on χ^2 distribution. All tests were 2-tailed with significance at $P<0.05$. Inference was aided by GraphPad InStat (version 3.00, GraphPad Software Inc., San Diego, CA).

RESULTS

TLR4 Asp299Gly and Thr399Ile genotype carrier frequencies are summarized in Table 1. The 299Gly allele frequencies were 7.92%, 3.53%, and 3% in CD, UC and healthy controls, respectively. The frequency of the 299Gly allele was significantly higher in CD patients than in controls ($P = 0.026<0.05$, OR = 2.78 $CI_{95\%}$: 1.088-7.103). The 299Gly allele was not found to be significantly associated with UC ($P = 0.77$, OR = 1.18 $CI_{95\%}$: 0.37-3.74). No significant difference was found in the frequencies of the 399Ile polymorphism among CD or UC

patients and controls.

Allele and genotype frequencies of the polymorphism -159 (C/T) of the CD14 gene are presented in Table 2. T allele and TT genotype frequencies were increased in CD patients only compared to controls ($P = 0.0048<0.01$, OR = 1.73, $CI_{95\%}$: 1.18-2.54 and $P = 0.047<0.05$, OR = 1.93, $CI_{95\%}$: 1.00-3.72, respectively).

Concerning the *NOD2/CARD15* mutations, the overall presence in CD patients (81.7%; 98/120) was significantly higher than that in UC patients (47%; 40/85) ($P < 0.0001<0.01$, OR = 5.01, $CI_{95\%}$: 2.67-9.38) or in healthy control individuals (21%; 21/100) ($P < 0.0001<0.01$, OR = 16.76 $CI_{95\%}$: 8.60- 32.67) (Table 3). A significant association was found between ileal disease and possession of one or more variant alleles. For each *NOD2/CARD15* variant, allele frequencies for overall ileal involvement (ileal disease and ileocolitis) were significantly different from non-ileal diseases (R702W ileal 8.3%, non-ileal 1.7%, $P = 0.014<0.05$; G908R ileal 12.1%, non-ileal 2%, $P < 0.0001<0.01$; and L007fsinsC ileal 17.1%, non-ileal 0.83% $P < 0.0001<0.01$).

Co-existence of the TLR4 polymorphic allele and the T allele of the polymorphism -159 (C/T) of the CD14 gene was observed in 12 CD patients (10%), in 5 UC patients (5.88%) and in 4 healthy controls (4%). Notably, there was a higher percentage of mutated allele coexistence in CD patients compared to UC or healthy subjects, suggesting that coexistence might increase the susceptibility to CD. However the χ^2 of CD *versus* the controls was marginal ($P = 0.08$) and that of UC *versus* the controls was not significant.

Among the 98 CD patients harboring *NOD2/CARD15* variants, 9 (9.2%) were found to carry also a TLR4 polymorphic allele, whereas among the 40 UC patients harboring *NOD2/CARD15* variants, 5 (12.5%) were found to carry also a TLR4 polymorphic allele. None of the 21 healthy controls harboring *NOD2/CARD15* variants was found carrying a TLR4 polymorphic allele. This indicated that coexistence of mutations in these genes could also increase the risk for IBD. However, there was no significantly increased risk of association with the disease.

As indicated in Table 4, the TT genotype and T allele frequencies of the -159(C/T) polymorphism in the CD14 gene increased in CD patients harboring at least one variant of the *NOD2/CARD15,* compared to controls. The odds of developing CD significantly increased in either case ($P = 0.012<0.05$, OR = 9.4 $CI_{95\%}$: 1.18-74.40, and $P = 0.003<0.01$, OR = 1.45 $CI_{95\%}$: 1.48-6.81, for the T genotype and TT allele respectively).

Four of the CD patients (3.3%), 3 of the UC patients (3.5%) and none of the healthy controls were found to carry simultaneously a polymorphic allele of all the genes tested.

Additionally, 62 out of 120 (51.67%) of the CD patients were carriers of a TLR4 and/or CD14 polymorphic allele and at least one variant of the *NOD2/CARD15*, compared to 23 out of 85 (27%) of the UC patients ($P = 0.0004<0.01$, OR= 2.88 $CI_{95\%}$:

Table 1 TLR4 Asp299Gly and Thr399Ile genotype carriers in CD and UC patients and healthy individuals

Group	TLR4 Asp299Gly genotype					TLR4 Thr399Ile genotype				
	Asp/Asp	Asp/Gly	Gly/Gly	299Gly allele frequencies (%)	OR	Thr/Thr	Thr/Ile	Ile/Ile	399Ile allele frequencies (%)	OR
CD	103	15	2	7.92	2.78[a]	119	1	0	0.42	0.41[a]
UC	79	6	0	3.53	1.18	82	3	0	1.76	1.78
Controls	95	4	1	3	98	2	0	1		

[a]$P<0.05$ *vs* control group.

Table 2 Allele and genotype frequencies of the promoter polymorphism at position -159 of the CD14 gene in CD and UC patients and healthy controls

Group	Alleles				Genotypes				
	C	T	T allele frequencies (%)	OR	CC	CT	TT	TT genotype frequencies (%)	OR
CD	119	121	50.42	1.73[b]	33	53	34	28.33	1.93[a]
UC	102	68	40	1.13	32	38	15	17.65	1.046
Controls	126	74	37		43	40	17	17	

[a]$P<0.05$, [b]$P<0.01$, *vs* control group.

Table 3 *NOD2/CARD15* mutant allele frequencies in Crohn's disease (CD) patients, in ulcerative colitis (UC) patients and controls

Samples		Genotype			Allele frequency (%)	OR
		1	2	3		
R702W	CD	96	24	0	10	11.05[b]
	UC	73	12	0	7.1	7.52[b]
	Control	98	2	0	1	
G908R	CD	87	32	1	14.2	4.51[b]
	UC	63	21	1	13.5	4.31[b]
	Control	93	7	0	3.5	
L1007fsincC	CD	79	39	2	17.9	3.42[b]
	UC	79	6	0	3.5	0.57
	Control	88	12	0	6	

1: homozygous wild-type; 2: heterozygous; 3: homozygous mutant, [b]$P<0.01$ *vs* UC group and control group.

Table 4 Allele and genotype frequencies of the promoter polymorphism at position -159 of the CD14 gene

CD14 genotypes	Genotyped for the *CARD15/NOD2* gene					
	CD		UC		Controls	
	No variant (n = 47)	At least one variant (n = 73)	No variant (n = 49)	At least one variant (n = 36)	No variant (n = 81)	At least one variant (n = 19)
CC	17	16	18	14	34	9
CT	21	32	23	15	31	9
TT, n (%)	9 (19.1)	25 (34.2) (P = 0.012)[a]	8 (16.3)	7 (19.4) (P = 0.156)	16 (19.7)	1 (5.3)
CD14 Alleles						
C, n (%)	55 (58.5)	64 (43.8)	59 (60.2)	43 (59.7)	99 (61.1)	27 (71)
T, n (%)	39 (41.5)	82 (56.2) (P = 0.003)[b]	39 (39.8)	29 (40.3) (P = 0.24)	63 (38.8)	11 (28.9)

[a]P<0.05, [b]P<0.01 *vs* control group.

1.88-5.24). It should be pointed out that both frequencies significantly increased in CD and UC as compared to the 10% frequency of multiple carriers found in healthy controls (P<0.0001<0.01, OR=9.62 $CI_{95\%}$: 4.56-20.27 and P=0.002<0.01, OR= 3.34, $CI_{95\%}$: 1.48-7.50, for CD and UC respectively). Consequently, the co-existence of a mutation in either the TLR4 or CD14 gene and in *NOD2/CARD15* increased the risk for developing CD.

DISCUSSION

Crohn's disease and ulcerative colitis are multifactorial diseases with a polygenic nature. Despite both being chronic disorders of the gastrointestinal tract with unknown etiology, an abnormal inflammatory response directed against the enteric microflora in a genetically susceptible host has been postulated as a possible explanation[17]. In human system, the TLR4.MD2.CD14 complex has been demonstrated to serve as a surface receptor for LPS[18]. In addition to the cell surface TLR4 complex, there is evidence that mammalian cells have an intracellular receptor that could detect LPS in the cytoplasm of infected cells[19]. These data suggest that TLRs and members of the NOD family represent another innate immune system for the recognition of a wide array of pathogen products[20].

Our study dealt with the relationship between the major mutations in TLR4, CD14 and *CARD15/NOD2* genes singularly and in combination, and IBD in a Greek population.

Regarding the TLR4, Cario and Podolsky[9] recently showed that TLR4 was strongly up-regulated in CD and UC, which may be caused by an exaggerated host defense reaction of the intestinal epithelium to endogenous luminal bacterial flora. It was intriguing to hypothesize that the Asp299Gly allele of the TLR4 gene could be related to such an imbalanced reaction. Our findings indicate that the frequency of the 299Gly allele was significantly higher in CD patients compared to UC patients and controls and support the hypothesis that innate immunity may play a role in Crohn's disease pathogenesis. Our results differ from those of a recent study in 86 UC patients of Japanese population in whom this mutation could not be detected[21]; however, our results are in accordance with those of recent studies in European patients[22,23]. Nevertheless, it is well known that the frequency of the mutations varies in different populations[24,25].

Concerning the CD14 gene, functional relevance between T allele and increased expression of CD14, has been demonstrated previously[26]. A significant increase of the T allele and the TT genotype was found exclusively among CD patients. Our results are in agreement with those from a recent study by Klein *et al*[12] but differ from those of a cohort in a Japanese population[27]. However, they demonstrated a significant association of the T allele and TT genotype frequencies with UC[27].

Regarding *NOD2/CARD15*, all the three risk alleles were more common in patients with IBD than in the control Greek population. The frequencies of the R702W and L1007fsinsC mutations were significantly higher in CD patients compared to UC patients and controls, whereas the frequency of the G908R mutation was similar in CD and UC patients but significantly higher compared to controls. Our results concerning the presence of L1007fsinsC in Greek population differ from those of a recent study in a Cretan population whose incidence was only 5.3%[28]. This inconsistency may be attributed to the fact that Crete is an isolated geographic region with a homogenous population dispersed over a small geographic area where this mutation does not seem to predispose to the disease or to the relatively small size of the examined sample[28]. Collectively our study confirmed previous studies, which reported, increased mutation carrier frequencies of one of the three variant alleles in CD patients compared to UC patients or healthy controls[6,29]. However, in contrary to the previously mentioned European studies, which reported that mutation frequencies in UC patients are comparable with those found in healthy controls. The allelic frequencies of R702W and G908R appeared to be higher in our UC patients than those found in healthy individuals. Interestingly, very recently Andriuilli *et al*[30] reported a significant association between the L1007fsincC mutation and UC, although at a lower frequency in comparison with that observed in CD patients, suggesting a possible involvement of the *NOD2/CARD15* also in UC patients[30]. Our findings may indicate the contribution of *NOD2/CARD15* variants to the pathogenesis of UC in our population as well, or may reflect the well known difficulty to classify correctly from the onset in all the patients with inflammatory bowel disease.

Co-existence of TLR4 and CD14 mutated alleles was higher in CD patients compared to UC or healthy subjects, but this association was not was significant. Additionally, there was no significantly increased risk of IBD related to the coexistence of mutations in TLR4 and in *CARD15/NOD2*. On the contrary, it is of interest that the T allele in the TT genotype appeared to increase the relative risk for developing CD in combination with at least one variation in the *CARD15/NOD2* gene. These results, are in accordance with a recent study by Klein *et al*[13], suggesting that as both CD14 and *CARD15/NOD2* genes are involved in the recognition of LPS and subsequent activation of NFκ-B, disturbed activation of the innate immune system by bacterial antigens may be crucial in some patients with CD.

Co-existence of mutations in all the three genes was found in only a small fraction of the CD or UC patients. But as mentioned earlier, the extracellular complex TLR4.MD2.CD14

and the intracellular *CARD15/NOD2* might participate in the regulation of innate immune responses to intestinal microflora. This seemed to point to the danger of IBD development in patients with at least one mutated allele in TLR4 and/or CD14 and at least one variant of *CARD15/NOD2*. Our results indicate that, co-existence of a mutation in either the TLR4 or CD14 gene and in *NOD2/CARD15* is associated with an increased susceptibility to CD or UC.

In conclusion, the Greek population suffering from IBD most likely carry polymorphisms in one or more of the genes related to the innate immune system. However, further research in larger and diverse populations is needed to elucidate the biological mechanism behind IBD susceptibility. Understanding the influence of predisposing genes can lead to a more precise diagnosis and permit the development of personalized medicines.

REFERENCES

1 **Hugot JP**, Thomas G. Genome-wide scanning in inflammatory bowel diseases. *Dig Dis* 1998; **16**: 364-369
2 **Strober W**, Ludviksson BR, Fuss IJ. The pathogenesis of mucosal inflammation in murine models of inflammatory bowel disease and Crohn disease. *Ann Intern Med* 1998; **128**: 848-856
3 **Heumann D**, Glauser MP, Calandra T. Molecular basis of host-pathogen interaction in septic shock. *Curr Opin Microbiol* 1998; **1**: 49-55
4 **Herz U**, Lacy P, Renz H, Erb K. The influence of infections on the development and severity of allergic disorders. *Curr Opin Immunol* 2000; **12**: 632-640
5 **Bonen DK**, Ogura Y, Nicolae DL, Inohara N, Saab L, Tanabe T, Chen FF, Foster SJ, Duerr RH, Brant SR, Cho JH, Nunez G. Crohn's disease-associated NOD2 variants share a signaling defect in response to lipopolysaccharide and peptidoglycan. *Gastroenterology* 2003; **124:** 140-146
6 **Hugot JP**, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411**: 599-603
7 **Ogura Y**, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nunez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; **411**: 603-606
8 **Medzhitov R**, Janeway CA. Innate immunity: impact on the adaptive immune response. *Curr Opin Immunol* 1997; **9**: 4-9
9 **Cario E**, Podolsky DK. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. *Infect Immun* 2000; **68**: 7010-7017
10 **Kopp EB**, Medzhitov R. The Toll-receptor family and control of innate-immunity. *Curr Opin Immunol* 1999; **11**: 13-18
11 **Arbour NC**, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, Frees K, Watt JL, Schwartz DA. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 2000; **25**: 187-191
12 **Klein W**, Tromm A, Griga T, Fricke H, Folwaczny C, Hocke M, Eitner K, Marx M, Duerig N, Epplen JT. A polymorphism in the CD14 gene is associated with Crohn disease. *Scand J Gastroenterol* 2002; **37**: 189-191
13 **Klein W**, Tromm A, Griga T, Folwaczny C, Hocke M, Eitner K, Marx M, Duerig N, Epplen JT. Interaction of polymorphisms in the CARD15 and CD14 genes in patients with Crohn disease. *Scand J Gastroenterol* 2003; **38**: 834-836
14 **Podolsky DK**. Inflammatory bowel disease (1). *N Engl J Med* 1991; **325**: 928-937
15 **Lorenz E**, Hallman M, Marttila R, Haataja R, Schwartz DA. Association between the Asp299Gly polymorphisms in the Toll-like receptor 4 and premature births in the Finnish population. *Pediatr Res* 2002; **52**: 373-376
16 **Hubacek JA**, Rothe G, Pit'ha J, Skodova Z, Stanek V, Poledne R, Schmitz G. C(-260)-->T polymorphism in the promoter of the CD14 monocyte receptor gene as a risk factor for myocardial infarction. *Circulation* 1999; **99**: 3218-3220
17 **Fiocchi C**. Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology* 1998; **115**: 182-205
18 **Aderem A**, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. *Nature* 2000; **406**: 782-787
19 **Philpott DJ**, Yamaoka S, Israel A, Sansonetti PJ. Invasive *Shigella flexneri* activates NF-kappa B through a lipopolysaccharide-dependent innate intracellular response and leads to IL-8 expression in epithelial cells. *J Immunol* 2000; **165**: 903-914
20 **Inohara N**, Ogura Y, Chen FF, Muto A, Nunez G. Human Human Nod1 confers responsiveness to bacterial lipopolysaccharides. *J Biol Chem* 2001; **276**: 2551-2554
21 **Okayama N**, Fujimura K, Suehiro Y, Hamanaka Y, Fujiwara M, Matsubara T, Maekawa T, Hazama S, Oka M, Nohara H, Kayano K, Okita K, Hinoda Y. Simple genotype analysis of the Asp299Gly polymorphism of the Toll-like receptor-4 gene that is associated with lipopolysaccharide hyporesponsiveness. *J Clin Lab Anal* 2002; **16**: 56-58
22 **Franchimont D**, Vermeire S, El Housni H, Pierik M, Van Steen K, Gustot T, Quertinmont E, Abramowicz M, Van Gossum A, Deviere J, Rutgeerts P. Deficient host-bacteria interactions in inflammatory bowel disease? The toll-like receptor (TLR)-4 Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis. *Gut* 2004; **53**: 987-992
23 **Torok HP**, Glas J, Tonenchi L, Mussack T, Folwaczny C. Polymorphisms of the lipopolysaccharide-signaling complex in inflammatory bowel disease: association of a mutation in the Toll-like receptor 4 gene with ulcerative colitis. *Clin Immunol* 2004; **112**: 85-91
24 **Pena AS**. Genetics of inflammatory bowel diseases--past, present, and future. *Dig Dis* 2003; **21**: 85-90
25 **Arnott ID**, Nimmo ER, Drummond HE, Fennell J, Smith BR, MacKinlay E, Morecroft J, Anderson N, Kelleher D, O'Sullivan M, McManus R, Satsangi J. NOD2/CARD15, TLR4 and CD14 mutations in Scottish and Irish Crohn's disease patients: evidence for genetic heterogeneity within Europe? *Genes Immun* 2004; **5**: 417-425
26 **Baldini M**, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A Polymorphism* in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am J Respir Cell Mol Biol* 1999; **20**: 976-983
27 **Obana N**, Takahashi S, Kinouchi Y, Negoro K, Takagi S, Hiwatashi N, Shimosegawa T. Ulcerative colitis is associated with a promoter polymorphism of lipopolysaccharide receptor gene, CD14. *Scand J Gastroenterol* 2002; **37**: 699-704
28 **Roussomoustakaki M**, Koutroubakis I, Vardas EM, Dimoulios P, Kouroumalis EA, Baritaki S, Koutsoudakis G, Krambovitis E. NOD2 insertion mutation in a Cretan Crohn's disease population. *Gastroenterology* 2003; **124**: 272-273; author reply 273-274
29 **Ahmad T**, Armuzzi A, Bunce M, Mulcahy-Hawes K, Marshall SE, Orchard TR, Crawshaw J, Large O, de Silva A, Cook JT, Barnardo M, Cullen S, Welsh KI, Jewell DP. The molecular classification of the clinical manifestations of Crohn's disease. *Gastroenterology* 2002; **122**: 854-866
30 **Andriulli A**, Annese V, Latiano A, Palmieri O, Fortina P, Ardizzone S, Cottone M, D'Inca R, Riegler G. The frame-shift mutation of the NOD2/CARD15 gene is significantly increased in ulcerative colitis: an *IG-IBD study. *Gastroenterology* 2004; **126**: 625-627

Edited by Wang XL