doi: 10.1111/j.1744-313X.2006.00572.x

TNF-857 polymorphism in Israeli Jewish patients with inflammatory bowel disease

Herma H. Fidder,* Roel Heijmans,† Yehuda Chowers,* Simon Bar-Meir,* Benjamin Avidan,* A. Salvador Pena†‡ & J. Bart A. Crusius†

Summary

Tumour necrosis factor (TNF)-α is an important pro-inflammatory cytokine that has been implicated in the pathogenesis of inflammatory bowel disease (IBD). The promoter *TNF*-857 C→T single nucleotide polymorphism (SNP) is functional through the binding to the transcription factor octamer transcription factor-1 (OCT-1). In order to investigate the frequency of this SNP in Israeli Jewish IBD patients, we analysed a cohort of well-characterized patients, 153 with Crohn's disease (CD) and 78 with ulcerative colitis (UC) and 188 healthy controls individually matched for age, sex and ethnicity. Forty-one per cent of the patients were of Ashkenazi and 48% were of non-Ashkenazi background. The remaining 11% were of mixed Ashkenazi–non-Ashkenazi background. Patients and controls were genotyped for the *TNF*-857 SNP by Taqman technology. Stratification for the *CARD15* Arg702Trp, Gly908Arg and Leu1007fsinsC mutations took place in 136 CD patients.

Carrier frequency of *TNF*-857T between CD and controls (36% vs. 40%; $P = 0.556$; OR: 1.18, 95% CI 0.74–1.88), or between UC and controls (41% vs. 37%; $P = 0.743$; OR: 0.85, 95% CI 0.45–1.62) did not differ significantly. Neither did stratifying for the presence of at least one of the common *CARD15* mutations result in a significant difference between CD and controls. No associations were found between *TNF*-857T and CD phenotype as defined by the Vienna classification, perianal disease or extra-intestinal disease irrespective of *CARD15* carrier status. In conclusion, it appears that *TNF*-857 SNP does not contribute to susceptibility of IBD, neither does it define the phenotype of CD in Israeli Jewish IBD patients.

* Department of Gastroenterology, Chaim Sheba Medical Center, Tel Hashomer, Israel, † Laboratory of Immunogenetics, Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands and ‡ Department of Gastroenterology, VU University Medical Center, Amsterdam, The Netherlands

Received 19 September 2005; accepted 17 November 2005

Correspondence: Herma H. Fidder, M.D., Ph.D., Department of Gastroenterology, Chaim Sheba Medical Center, 52621 Tel Hashomer, Israel. Tel: +972-3-530-2679; Fax: +972-3-530-3070; E-mail: hermafidder@hotmail.com

Introduction

Crohn's disease (CD) and ulcerative colitis (UC), the two major forms of inflammatory bowel disease (IBD), are heterogeneous, multifactorial diseases hallmarked by chronic inflammation of the gastrointestinal tract. The incidence and prevalence of IBD vary considerably depending on the racial and ethnic background and geographic location. Although the exact aetiology remains elusive, family clustering and twin studies suggest that genetic predisposition is an important factor in the aetiology of both diseases (Tysk *et al*., 1988; Thompson *et al*., 1996). Genetic linkage studies have identified a region on chromosome 6p21, *IBD3* (MIM604519), as a susceptibility locus for CD (Yang *et al*., 1999) and IBD (Hampe *et al*., 1999; Rioux *et al*., 2000). The major histocompatibility complex (MHC) contains the classical human leucocyte antigen (HLA)-presenting genes and non-HLA genes such as the tumour necrosis factor (*TNF*) gene. Both sets of genes are possibly involved in the susceptibility to suffer from IBD and in determining the clinical phenotype (Satsangi *et al*., 1996; Stokkers *et al*., 1999). TNF-α is an important pro-inflammatory cytokine with mRNA and proteins found in increased concentrations in the mucosa of CD patients (Reinecker *et al*., 1993; Dionne *et al*., 1997), and therapy directed against TNF-α is a highly successful treatment modality for CD (Targan *et al*., 1997). Recent evidence has proven the value of this therapy in severe pancolitis in reducing the number of colectomies (Jarnerot *et al*., 2005).

TNF-α production in healthy individuals shows wide variation and appears to be genetically determined (Westendorp *et al*., 1997). The source of this genetically determined variation is unknown, and extensive research has been focused on the promoter region of the *TNFI* gene. The distal promoter *TNF*-308 single nucleotide polymorphism (SNP) did receive much attention, but different methodologies did not result in unequivocally demonstrating a functional effect probably resulting from cell type, context and stimulus dependency of *TNF* gene regulation (Bayley *et al*., 2004). The *TNF*-857 and *TNF*-863 influence the binding of the transcription factors OCT-1 and NF-κB, respectively, to their putative consensus binding sites, thus modulating the expression of the

TNF gene indirectly (Higuchi *et al.*, 1998; Hohjoh *et al.*, 1999; van Heel *et al.*, 2002). The effects of these polymorphisms on TNF-α transcription and production, however, are contradictory (Higuchi *et al.*, 1998; Skoog *et al.*, 1999; van Heel *et al.*, 2002).

Several studies have reported an association between the *TNF*-857 SNP and UC, CD and IBD overall in the Caucasian population (van Heel *et al.*, 2002; O'Callaghan *et al.*, 2003). In a small case-control study in the Japanese population, allele *TNF*-857T was shown to be associated with CD (Negoro *et al.*, 1999), a finding that could not be substantiated in a larger cohort (Kawasaki *et al.*, 2000). CD has been reported to be more prevalent among Ashkenazi Jews as compared to the non-Jewish population living in the same geographic area (Monk *et al.*, 1969). The Jewish population is comprised of two major genetically distinct subgroups, Ashkenazi Jews of European descent and non-Ashkenazi Jews primarily of Asian African extraction. In Israel, most studies report higher rates of CD in the Ashkenazi than in the non-Ashkenazi Jewish population (Fireman *et al.*, 1989; Odes *et al.*, 1989). In this study, we investigated the *TNF*-857 SNP for association with IBD and disease characteristics in Israeli Jewish patients.

Patients and methods

Subjects

Blood samples were obtained from 153 patients with CD, 78 patients with UC and a total of 188 controls individually matched for age, sex and ethnicity. Patients of mixed ethnic background could not be age matched. All subjects were unrelated Jews followed in the Chaim Sheba Medical Centre in Israel. The origin of non-Ashkenazi Jews was either Asian, North African, Yemenite or Balkan. Healthy members of the staff of departments at the centre and individuals attending the gastroenterology unit for other reasons than IBD served as controls.

The diagnosis of CD and UC was determined according to conventional endoscopic, radiological and histological criteria (Lennard-Jones, 1989). Patients with undetermined colitis were not included. Data obtained from each patient included demographic characteristics (e.g. age at diagnosis, sex, ethnic background and family history), disease location and behaviour (i.e. penetrating, stricturing or inflammatory), the presence of perianal disease, and the presence of extra-intestinal manifestations. Extra-intestinal disease included musculoskeletal, dermatological, ophthalmological and hepatobiliary manifestations of either UC or CD. Information on age at diagnosis, disease location and behaviour was used to group patients according to the Vienna classification (Gasche *et al.*, 2000). The data were stored in a computerized database. The study was approved by the institutional review board, and each participant signed a written informed consent.

DNA isolation and *TNF*-857 SNP genotyping

Genomic DNA was prepared from anticoagulated venous blood samples using standard techniques, employing the Gentra Kit (Gentra Inc., Minneapolis, MN, USA) and using the manufacturer's recommended protocol.

For genotyping the *TNF*-857 C→T SNP [the Single Nucleotide Polymorphism database (dbSNP ID): rs1799724], we used the *Taq*man allele discrimination assay on demand (C_11918223_10; Applied Biosystems, Foster City, CA). In the context sequence (GTCGAGTATGGGGACCCCC(C→A)CTTAA[C→T]GAAGACAGGGCCATGTAGAGGGCCC), the polymorphic site at position −857 is given between square brackets and the assay does not interfere with the polymorphic site at position −863 (between round brackets). Polymerase chain reaction amplification was performed in ABI buffer, and cycling conditions included initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and annealing/extension at 60 °C for 1 min. Reactions were performed and read on an ABI PRISM 7000 Sequence Detection System. *TNF*-857 genotypes determined by the *Taq*Man method are concordant with those determined by PCR-single-strand conformation polymorphism or DNA sequencing methods (Ferreira *et al.*, 2005).

Statistical analysis

Multiple Chi-square test or Fisher's exact test, where appropriate, were used for comparison between categorical variables. Odds ratio and their corresponding 95% confidence intervals were calculated. A P level of 0.05 or less was considered significant.

Results

Table 1 shows the demographic characteristics of patients with UC or CD. The average duration of follow-up was 7.5 ± 8.3 years (range 2–27 years).

Allele frequencies in CD and UC patients vs. healthy controls

The results of genotyping the *TNF*-857 SNP in CD patients and matched controls is shown in Table 2(a), in UC patients and matched controls in Table 2(b). The

Table 1. Demographic features of Israeli Jewish patients with UC and CD

	UC	CD
Total number (*n*)	78	153
Sex ratio (M/F)	50/28	85/68
Ethnic background		
Ashkenazi	27 (35%)	68 (44%)
Sephardic	44 (56%)	66 (43%)
Mixed	7 (9%)	19 (12%)
Age at diagnosis (mean ± SD)	28.09 ± 13.62	28.06 ± 13.61

Table 2a. The *TNF*-857 SNP in CD and healthy controls (HC)

		Genotype frequency (%)			Allele frequency	Carrier frequency
Phenotype	*n*	CC	CT	TT	*TNF*-857T (%)	*TNF*-857T (%)
CD	153	98 (64)	47 (31)	8 (5)	63/306 (21)[a]	55/153 (36)[b]
HC	153	92 (60)	56 (37)	5 (3)	66/306 (22)	61/153 (40)

[a] The frequency of allele *TNF*-857T in CD vs. HC: $P = 0.929$; OR: 0.98 (95% CI 0.67–1.44).
[b] Carrier frequency of *TNF*-857T in CD vs. HC: $P = 0.556$; OR: 1.18 (95% CI 0.74–1.88).

Table 2b. The *TNF*-857 SNP in UC and healthy controls (HC)

		Genotype frequency (%)			Allele frequency	Carrier frequency
Phenotype	*n*	CC	CT	TT	*TNF*-857T (%)	*TNF*-857T (%)
UC	78	46 (59)	28 (36)	4 (5)	36/156 (23)[a]	32/78 (41)[b]
HC	78	49 (63)	27 (35)	2 (3)	31/156 (20)	29/78 (37)

[a] The frequency of allele *TNF*-857T in UC vs. HC: $P = 0.581$; OR: 0.83 (95% CI 0.48–1.42).
[b] Carrier frequency of *TNF*-857T in UC vs. HC: $P = 0.743$; OR: 0.85 (95% CI 0.45–1.62).

control groups did not deviate from the Hardy–Weinberg equilibrium. The allele and carrier frequencies of the minor allele *TNF*-857T were not statistically significantly different between either CD patients and matched controls or between UC patients and matched controls. Analysis of Ashkenazi, Separdic and mixed Israeli Jewish subgroups was only performed in CD patients because of the low numbers of non-Ashkenazi patients in the UC group. Frequencies of allele *TNF*-857T were especially high in Ashkenazi CD patients (24%) and healthy controls (22%). The differences between CD patients and controls were larger in the non-Ashkenazi groups of Asian (21% vs. 14%) and North African (23% vs. 19%) extraction, but these differences did not reach statistical significance. The presence of two missense mutations Arg702Trp and Gly908Arg and one frameshift mutation Leu1007fsinsC (also known as R675W, G881R and 980FS981X, respectively) in *CARD15* had been previously determined in 136 CD patients (Fidder *et al*., 2003). The *CARD15* status did not affect the frequencies of *TNF*-857 alleles: the frequency of allele *TNF*-857T was 17% in *CARD15* mutation carriers vs. 18% in non-carriers ($P = 0.981$; OR: 1.08, 95% CI 0.52–2.23).

Genotype–phenotype analyses

Table 3 shows the disease characteristics according to the Vienna classification in CD patients and the presence of perianal disease or extra-intestinal manifestations in relation to *TNF*-857 genotypes in CD patients. *TNF*-857 genotype did not affect disease distribution along the gastrointestinal tract, nor disease behaviour, i.e. penetrating or stricturing disease pattern. Age of onset, classified in below and above 40 years, was not associated either. No association was found between perianal disease or extra-intestinal involvement and *TNF*-857 variants. Because extra-intestinal disease has been reported to be more frequent in non-Ashkenazi Israeli Jewish CD

Table 3. Disease characteristics of patients with CD according to the Vienna classification, perianal disease and extra intestinal manifestations in relation to *TNF*-857 genotypes

	Genotype frequency (%)[a]		
	TT (*n* = 8)	CT (*n* = 47)	CC (*n* = 98)
Age at onset			
A1 < 40 years	7 (88%)	40 (85%)	80 (82%)
A2 ≥ 40 years	1 (12%)	7 (15%)	18 (18%)
Location[b]			
L1 Ileum	3 (43%)	17 (40%)	43 (47%)
L2 Colon	3 (43%)	8 (19%)	22 (24%)
L3 Ileocolon	1 (14%)	12 (29%)	22 (24%)
L4 Upper GI	0	5 (12%)	5 (5%)
Disease behaviour[c]			
B1 Inflammatory	4 (66%)	17 (41%)	43 (48%)
B2 Stricturing	1 (17%)	11 (27%)	26 (29%)
B3 Penetrating	1 (17%)	13 (32%)	21 (23%)
Perianal disease[d]			
Yes	0	15 (39%)	38 (40%)
No	7 (100%)	23 (61%)	58 (60%)
Extra-intestinal manifestations[e]			
Yes	1 (14%)	11 (28%)	31 (33%)
No	6 (86%)	28 (72%)	63 (67%)

[a] Frequencies pertaining all variables were not significant. [b] Data on disease location were available on 141 patients. [c] Data on disease behaviour on 137 patients. [d] Data on perianal disease on 141 patients. [e] Data on extra-intestinal manifestations on 140 patients.

patients (Fidder *et al*., 2003), patients were stratified for ethnic background for analysis of this disease feature. In both the non-Ashkenazi and Ashkenazi subgroups, no association was observed between the *TNF*-857 genotypes or frequencies of allele *TNF*-857T and extra-intestinal manifestations (data not shown).

We did not find an association between UC and extent of disease. Subgroup analysis of Ashkenazi patients did yield similar results (data not shown).

Discussion

Associations between IBD and the *TNF*-857 SNP were first reported in a Japanese study, with increased frequencies of the allele *TNF*-857T in CD patients (Negoro *et al*., 1999). In contrast, two studies in Caucasian cohorts from Great Britain and Australia detected positive associations between allele *TNF*-857C and IBD (van Heel *et al*., 2002; O'Callaghan *et al*., 2003). In the present study, we determined *TNF*-857 genotypes in Israeli Jewish patients with CD, UC and matched healthy controls. Allele and carrier frequencies of *TNF*-857T did not differ between IBD patients and controls. This is in concert with studies from Newfoundland and Portugal that also failed to show an association between *TNF*-857 and CD (Ferreira *et al*., 2005; Zipperlen *et al*., 2005). The frequencies of allele *TNF*-857T in the healthy Ashkenazi was remarkably high: 24% as compared to 6.3–8.6% in other Caucasian populations (Grutters *et al*., 2002; van Heel *et al*., 2002; Waller *et al*., 2004). *TNF*-857T allele frequencies in Asian populations also appear to be higher, ranging from 12.2% to 17.7%, resembling the 16% we observed in our non-Ashkenazi Jewish healthy controls (Negoro *et al*., 1999). These discrepancies between the different populations emphasize the importance of scrutinous ethnic matching to prevent population stratification (Freedman *et al*., 2004).

Nucleotide-binding oligomerization domain protein 2 (NOD2) is an intracellular receptor for bacterial peptidoglycan and muramyl dipeptide (MDP) and can induce activation of the NF-κB pathway, resulting in the production of pro-inflammatory cytokines, such as TNF-α (Inohara & Nunez, 2003). A recent study showed that the three *TNF*-863/*TNF*-857 haplotypes detected in the Swedish population respond differently to NOD2 activation by MDP (Linderson *et al*., 2005). It has been suggested that the presence of *NOD2/CARD15* mutations may influence the association between the *TNF*-857 variant in patients with CD. Although a British study only found an association in CD patients without mutations in *NOD2/CARD15* (van Heel *et al*., 2002), another study reported the strongest association of *TNF*-857C with CD in *NOD2/CARD15* mutation carriers (O'Callaghan *et al*., 2003). In our patient population and in that reported at the American Gastroenterological Association of 2004 by Waller *et al*. (2004) in the UK, stratifying for *NOD2/CARD15* mutations did not reveal an association with the *TNF*-857 SNP.

Families with multiple IBD patients have shown a high degree of concordance for site and the behaviour of the disease, suggesting that disease phenotype may have an inherited component (Bayless *et al*., 1996). Fowler *et al*. (2005) reported that Australian patients carrying the allele *TNF*-857C have an increased risk for stricturing or penetrating disease behaviour. In Japan, alleles of the *TNF*-857 SNP were not associated with location and extent of the disease. However, the allele *TNF*-857T was more frequent in CD patients without steroid therapy (Negoro *et al*., 1999). In our patient population, however, no such associations with CD patient characteristics, age at diagnosis, disease behaviour, location, perianal disease or extra-intestinal manifestations were detected. Lack of an association with the clinical phenotype has also been reported in a previous study from the UK (van Heel *et al*., 2002).

Previous studies on various TNF promoter SNPs have yielded conflicting results. The relatively small numbers of patients, disease heterogeneity, variation between ethnic groups and poorly matched controls are some of the reasons that may explain the little consistency in findings. Although we attempted to ethnically match our cases and controls as optimally as possible, our study is hampered by an ethnically heterogenous study population, resulting in small ethnic subgroups that prevent us from drawing firm conclusions. It remains to be determined if the differences between carrier frequencies in the small non-Ashkenazi subgroups reach statistical significance in a larger study cohort. However, the very similar results in both the UC and CD cases and their carefully matched controls in the relatively large Ashkenazi Jewish Israeli patient group does suggest that the *TNF*-857 SNP in Ashkenazi Jewish patients, neither determines the susceptibility, nor influences the clinical phenotype of CD or UC. However, studies on larger cohorts of non-Ashkenazi Jewish patients are warranted to be able to establish the role of this SNP in this ethnic group.

References

Bayless, T.M., Tokayer, A.Z., Polito, J.M. 2nd, Quaskey, S.A., Mellits, E.D. & Harris, M.L. (1996) Crohn's disease: concordance for site and clinical type in affected family members: potential hereditary influences. *Gastroenterology*, **111**, 573.

Bayley, J.P., Ottenhoff, T.H. & Verweij, C.L. (2004) Is there a future for TNF promoter polymorphisms? *Genes and Immunity*, **5**, 315.

Dionne, S., Hiscott, J., D'Agata, I., Duhaime, A. & Seidman, E.G. (1997) Quantitative PCR analysis of TNF-alpha and IL-1 beta mRNA levels in pediatric IBD mucosal biopsies. *Digestive Diseases and Sciences*, **42**, 1557.

Ferreira, A.C., Almeida, S., Tavares, M., Canedo, P., Pereira, F., Regalo, G. *et al*. (2005) NOD2/CARD15 and TNFA, but not IL1B and IL1RN, are associated with Crohn's disease. *Inflammatory Bowel Diseases*, **11**, 331.

Fidder, H.H., Olschwang, S., Avidan, B., Zouali, H., Lang, A., Bardan, E., Picard, O., Bar-Meir, S., Colombel, J.F. & Chowers, Y. (2003) Association between mutations in the CARD15 (NOD2) gene and Crohn's disease in Israeli Jewish patients. *American Journal of Medical Genetics. Part A*, **121**, 240.

Fireman, Z., Grossman, A., Lilos, P., Eshchar, Y., Theodor, E. & Gilat, T. (1989) Epidemiology of Crohn's disease in the Jewish

population of central Israel, 1970. *American Journal of Gastroenterology*, **84**, 255.

Fowler, E.V., Eri, R., Hume, G., Johnstone, S., Pandeya, N., Lincoln, D., Templetonand, D. & Radford-Smith, G.L. (2005) TNF-α and IL10 SNPs act together to predict disease behaviour in Crohn's disease. *Journal of Medical Genetics*, **42**, 523.

Freedman, M.L., Reich, D., Penney, K.L., McDonald, G.J., Mignault, A.A., Patterson, N. *et al.* (2004) Assessing the impact of population stratification on genetic association studies. *Nature Genetics*, **36**, 388.

Gasche, C., Scholmerich, J., Brynskov, J., D'Haens, G., Hanauer, S.B., Irvine, E.J. *et al.* (2000) A simple classification of Crohn's disease: report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998. *Inflammatory Bowel Diseases*, **6**, 8.

Grutters, J.C., Sato, H., Pantelidis, P., Lagan, A.L., McGrath, D.S., Lammers, J.W., van den Bosch, J.M., Wells, A.U., du Bois, R.M. & Welsh, K.I. (2002) Increased frequency of the uncommon tumor necrosis factor-857T allele in British and Dutch patients with sarcoidosis. *American Journal of Respiratory and Critical Care Medicine*, **165**, 1119.

Hampe, J., Shaw, S.H., Saiz, R., Leysens, N., Lantermann, A., Mascheretti, S. *et al.* (1999) Linkage of inflammatory bowel disease to human chromosome 6p. *American Journal of Human Genetics*, **65**, 1647.

van Heel, D.A., Udalova, I.A., De Silva, A.P., McGovern, D.P., Kinouchi, Y., Hull, J. *et al.* (2002) Inflammatory bowel disease is associated with a TNF polymorphism that affects an interaction between the OCT1 and NF-κB transcription factors. *Human Molecular Genetics*, **11**, 1281.

Higuchi, T., Seki, N., Kamizono, S., Yamada, A., Kimura, A., Kato, H. & Itoh, K. (1998) Polymorphism of the 5′-flanking region of the human tumor necrosis factor (TNF)-alpha gene in Japanese. *Tissue Antigens*, **51**, 605.

Hohjoh, H., Nakayama, T., Ohashi, J., Miyagawa, T., Tanaka, H., Akaza, T., Honda, Y., Juji, T. & Tokunaga, K. (1999) Significant association of a single nucleotide polymorphism in the tumor necrosis factor-alpha (TNF-alpha) gene promoter with human narcolepsy. *Tissue Antigens*, **54**, 138.

Inohara, N. & Nunez, G. (2003) Cell death and immunity: NODs: intracellular proteins involved in inflammation and apoptosis. *Nature Reviews Immunology*, **3**, 371.

Jarnerot, G., Hertervig, E., Friis-Liby, I., Blomquist, L., Karlen, P., Granno, C. *et al.* (2005) Infliximab as rescue therapy in severe to moderately severe ulcerative colitis: a randomized, placebo-controlled study. *Gastroenterology*, **128**, 1805.

Kawasaki, A., Tsuchiya, N., Hagiwara, K., Takazoe, M. & Tokunaga, K. (2000) Independent contribution of HLA-DRB1 and TNF alpha promoter polymorphisms to the susceptibility to Crohn's disease. *Genes and Immunity*, **1**, 351.

Lennard-Jones, J.E. (1989) Classification of inflammatory bowel disease. *Scandinavian Journal of Gastroenterology. Supplement*, **170**, 2.

Linderson, Y., Bresso, F., Buentke, E., Pettersson, S. & D'Amato, M. (2005) Functional interaction of CARD15/NOD2 and Crohn's disease-associated TNFalpha polymorphisms. *International Journal of Colorectal Disease*, **20**, 305.

Monk, M., Mendeloff, A.I., Siegel, C.I. & Lilienfeld, A. (1969) An epidemiological study of ulcerative colitis and regional enteritis among adults in Baltimore. II. Social and demographic factors. *Gastroenterology*, **56**, 847.

Negoro, K., Kinouchi, Y., Hiwatashi, N., Takahashi, S., Takagi, S., Satoh, J., Shimosegawa, T. & Toyota, T. (1999) Crohn's disease is associated with novel polymorphisms in the 5′-flanking region of the tumor necrosis factor gene. *Gastroenterology*, **117**, 1062.

O'Callaghan, N.J., Adams, K.E., van Heel, D.A. & Cavanaugh, J.A. (2003) Association of TNF-alpha-857C with inflammatory bowel disease in the Australian population. *Scandinavian Journal of Gastroenterology*, **38**, 533.

Odes, H.S., Fraser, D. & Krawiec, J. (1989) Inflammatory bowel disease in migrant and native Jewish populations of southern Israel. *Scandinavian Journal of Gastroenterological. Supplement*, **170**, 36.

Reinecker, H.C., Steffen, M., Witthoeft, T., Pflueger, I., Schreiber, S., MacDermott, R.P. & Raedler, A. (1993) Enhanced secretion of tumour necrosis factor-alpha, IL-6, and IL-1 beta by isolated lamina propria mononuclear cells from patients with ulcerative colitis and Crohn's disease. *Clinical and Experimental Immunology*, **94**, 174.

Rioux, J.D., Silverberg, M.S., Daly, M.J., Steinhart, A.H., McLeod, R.S., Griffiths, A.M. *et al.* (2000) Genomewide search in Canadian families with inflammatory bowel disease reveals two novel susceptibility loci. *American Journal of Human Genetics*, **66**, 1863.

Satsangi, J., Welsh, K.I., Bunce, M., Julier, C., Farrant, J.M., Bell, J.I. & Jewell, D.P. (1996) Contribution of genes of the major histocompatibility complex to susceptibility and disease phenotype in inflammatory bowel disease. *Lancet*, **347**, 1212.

Skoog, T., van't Hooft, F.M., Kallin, B., Jovinge, S., Boquist, S., Nilsson, J., Eriksson, P. & Hamsten, A. (1999) A common functional polymorphism (C→A substitution at position –863) in the promoter region of the tumour necrosis factor-alpha (TNF-alpha) gene associated with reduced circulating levels of TNF-alpha. *Human Molecular Genetics*, **8**, 1443.

Stokkers, P.C., Reitsma, P.H., Tytgat, G.N. & van Deventer, S.J. (1999) HLA-DR and -DQ phenotypes in inflammatory bowel disease: a meta-analysis. *Gut*, **45**, 395.

Targan, S.R., Hanauer, S.B., van Deventer, S.J., Mayer, L., Present, D.H., Braakman, T., DeWoody, K.L., Schaible, T.F. & Rutgeerts, P.J. (1997) A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. *New England Journal of Medicine*, **337**, 1029.

Thompson, N.P., Driscoll, R., Pounder, R.E. & Wakefield, A.J. (1996) Genetics versus environment in inflammatory bowel disease: results of a British twin study. *British Medical Journal*, **312**, 95.

Tysk, C., Lindberg, E., Jarnerot, G. & Floderus-Myrhed, B. (1988) Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. *Gut*, **29**, 990.

Waller, S., Tremelling, M., Bredin, F., Woodward, J., Middleton, S. & Parkes, M. (2004) NOD2/CARD15 and TNF promoter polymorphisms in a UK IBD population. *Gastroenterology*, **126** (**Suppl 2**), A356.

Westendorp, R.G., Langermans, J.A., Huizinga, T.W.J., Elouali, A.H., Verweij, C.L., Boomsma, D.I., Vandenbroucke, J.P. & Vandenbrouke, J.P. (1997) Genetic influence on cytokine production and fatal meningococcal disease. *Lancet*, **349**, 170.

Yang, H., Plevy, S.E., Taylor, K., Tyan, D., Fischel-Ghodsian, N., McElree, C., Targan, S.R. & Rotter, J.I. (1999) Linkage of Crohn's disease to the major histocompatibility complex region is detected by multiple non-parametric analyses. *Gut*, **44**, 519.

Zipperlen, K., Peddle, L., Melay, B., Hefferton, D. & Rahman, P. (2005) Association of TNF-alpha polymorphisms in Crohn disease. *Human Immunology*, **66**, 56.

