Published in: Gastroenterology (2002), vol. 123, pp. 106-111
Status: Postprint (Author's version)

NOD2/CARD15 Does Not Influence Response to Infliximab in Crohn's Disease

Severine Vermeire,* Edouard Louis,[†] Paul Rutgeerts,* Martine De Vos,[§] Andre Van Gossum,[‖] Jacques Belaiche,[†] Paul Pescatore,[¶] Rene Fiasse,[#] Paul Pelckmans,** Robert Vlietinck,[††] Françoise Merlin,[§§] Habib Zouali,[§§] Gilles Thomas,[§§,‖‖] Jean-Frederic Colombel,[¶¶] And Jean-Pierre Hugot[§§,##] for the Belgian Group of Infliximab Expanded Access Program and Fondation Jean Dausset CEPH, Paris, France

**Department of Gastroenterology, UZ Gasthuisberg, Leuven, Belgium; [†]Department of Gastroenterology, CHU of Liège, Liège, Belgium; [§]Department of Gastroenterology, UZ Gent, Gent, Belgium; [‖]Department of Gastroenterology, Erasme University Hospital, Brussels, Belgium; [¶]Department of Gastroenterology, CHU, Luxembourg, Luxembourg; [#]Department of Gastroenterology, St. Luc University Hospital, Brussels, Belgium; **Department of Gastroenterology, AZ Antwerpen, Antwerpen, Belgium; [††]Genetic Epidemiology, KU Leuven, Leuven, Belgium; [§§]Fondation Jean Dausset CEPH, Paris, France; [‖‖]Hôpital Saint Antoine, Paris, France; [¶¶]Department of Gastroenterology, CHU Lille, Lille, France; [##]Hôpital Robert Debré, Paris, France*

Abstract

Background & Aims: NOD2/CARD15 was recently identified as the first gene underlying Crohn's disease (CD) susceptibility. Monoclonal antibodies to tumor necrosis factor (TNF)-α (infliximab) are a potent treatment for CD, with about 70% of patients responding. It is not clear which factors influence treatment outcome. We assessed whether variants in N0D2/CARD15 are predictive for differences in clinical response. Methods: Two hundred forty-five CD patients (86 fistulizing, 159 luminal) receiving infliximab in an expanded access program were genotyped for the 3 main associated variants of N0D2/CARD15, without knowledge of the treatment response. Short-term clinical response was assessed at 4 weeks (refractory) or 10 weeks (fistulizing) after first infliximab infusion, and the mean duration of response was calculated. In a subgroup of patients, production of TNF in response to lipopolysaccharide (LPS) in mucosal biopsy tissue was also determined by means of immunoassay, and results were related to the different N0D2/ CARD15 genotypes. *Results:* In total, 32.6% of patients carried mutations in N0D2/CARD15 (18.8% R702W, 8.6% G908R, and 10.2% 1007fs) compared with 15% in controls ($P < 0.001$). Despite observed differences In TNF production in mucosal biopsy tissue, there was no relationship between the overall presence of a mutation in N0D2/CARD15 or of any of the mutations separately and short-term infliximab response or response duration. Furthermore, multivariate analysis could not identify clinical characteristics that, in combination with N0D2/CARD15 mutations, were associated with response to infliximab. *Conclusions:* In this cohort of CD patients, the frequency of NOD2/CARD15 mutations was significantly greater than that of healthy controls. However, NOD2/CARD15 was not predictive of treatment outcome with infliximab in CD.

Crohn's disease (CD) is a chronic inflammatory bowel disease (IBD) affecting 1 of 1000 (mostly young) people in Western countries.[1,2] The disease has a multifactorial etiology, including a strong genetic susceptibility. Recently, the first gene for CD was identified and encodes a protein called Nod2/Card15, a member of the Apaf-1/Ced-4 family of apoptosis regulators with homology to plant disease resistance gene products.[3,4] Nod2/ Card15 is an intracellular protein expressed in monocytes and macrophages and has binding affinities for bacterial lipopolysaccharides (LPS) through its leucine-rich repeat (LRR) domain, located in the carboxy-terminal region of the gene, leading to nuclear factor-κB (NF-κB) activation and apoptosis under normal circumstances.[5] Three independent mutations within NOD2/CARD15 (1 frameshift mutation [1007fs] and 2 missense mutations [R702W and G908R]) have been found to be associated with CD.[3] The identified mutations would modify the structure of the LRR domain of the protein or the adjacent region, inducing an altered response to LPS, as has been shown for the frameshift mutation.[4] Hence, NOD2/CARD15 likely confers susceptibility to CD by altering recognition of bacterial sequences and/or by inappropriate activation of NF-κB in monocytes. By extrapolation from the study by Hugot et al.,[3] 43% of CD patients would carry at least 1 of the identified NOD2/CARD15 mutations.

Abbreviations used in this paper: CD, Crohn's disease; IBD, inflammatory bowel disease; IL, interleukin; LPS, lipopolysaccharide; LRR, leuclne-rich repeat; NF-κB, nuclear factor-κB; PCR, polymerase chain reaction; TNF, tumor necrosis factor.

Monoclonal antibodies against the proinflammatory cytokine tumor necrosis factor (TNF)-α (infliximab) are a potent treatment for CD. Response rates of 70% are obtained with this drug.[6-8] A subgroup of patients, therefore, does not benefit for reasons as yet unknown. The lack of response to infliximab seen in some patients is stable over time, which makes the response potentially genetically determined. Several polymorphisms in the gene encoding TNF and the TNF receptor have been examined, but most studies have been without success.[9] A recent study in a small number of treated CD patients showed that homozygosity for the lymphocytotoxin LTA NcoI-TNFc-aa13L-aa26 haplotype and a pANCA fluorescence pattern are associated with poor response to infliximab.[10]

NF-κB may play a central role by controlling the transcription of genes encoding proinflammatory cytokines, such as TNF-α. It has already been shown that the deletion of the LRR region of NOD2/CARD15 is associated with increased NF-κB activity in lamina propria mononuclear cells.[4] Therefore, mutations in NOD2/ CARD15 might well be involved in the differences in response seen in clinical practice with infliximab, based on overactivation of NF-κB and subsequently alteced production of TNF. For this reason, we studied the identified mutations in NOD2/CARD15 in a large cohort of infliximab-treated CD patients. We also studied the relationship between NOD2/CARD15 genotypes and TNF production in response to LPS in mucosal biopsy specimens of these patients.

Materials and Methods

Patients and Treatment

Over the last 2 years, 245 CD patients receiving infliximab through the Belgian Infliximab Expanded Access Program (Schering-Plough NV/SA, study P01246-1) for severe refractory luminal (n = 159) or fistulizing (n = 86) CD were prospectively scored for response to treatment. Eligible patients had to belong to 1 of the following 3 groups: (1) single or multiple enterocutaneous draining fistula(s) as a complication of CD resistant to conventional treatment for at least 3 months ("severe" fistula patients); (2) moderately to severely active CD of at least 6-months' duration, with colitis, ileitis, or ileocolitis, confirmed by radiography or endoscopy, and refractory or dependent on oral corticosteroid therapy (>8 mg/day prednisone equivalent); and (3) patients refractory or inrolerant to methotrexate, azathioprine, 6-mercaptopurine, or cyclosporine. According to the protocol of the Expanded Access Program, patients with refractory luminal disease received a single infusion at week 0. For fistulizing disease, 3 consecutive infusions at weeks 0, 2, and 6 were administered. Infliximab (Remicade; Centocor Inc., Malvern, PA) was given as an IV infusion of 5 mg/kg in all patients. Detailed demographic and clinical information on all patients was obtained through review of the clinical charts (Table 1). All patients were white. A group of 95 healthy individuals, again all white, served as controls. The study was approved by the ethical committee of each institution, and all patients gave informed written consent before inclusion.

Table 1: *Demographic and Clinical Characteristics of the Study Population*

	Total study population (n = 245)
Mean CDAI	265
Female/male (%)	152/93 (62/38)
Fistulizing/refractory (%)	86/159 (35/65)
Median current age (yr ± IQR)	35 (28-44)
Mean age at diagnosis, yr (%)	25.1
Disease localization	
Ileitis only	39 (16.4)
Ileocolitis	118 (47.5)
Colitis only	88 (36.1)
Anal involvement	106 (43.4%)
Upper Gl involvement	20 (8.2%)
Concomitant treatment	
5-Aminosalicylates	110 (43.8%)
Corticosteroids	106 (42.2%)
Azathioprine/6-mercaptopurine	122 (48.6%)
Methotrexate	11 (4.4%)
Total immunosuppressives	132 (52.6%)

Genotyping

From all participants, DNA was collected and genotyped for the 3 main variants of NOD2/CARD15 that are associaced with CD (OMIM 605956) as previously defined.[3] In this initial report, the nomenclature of mutations was derived from the sequence of IBD1.[3] This sequence is identical to the smaller open reading frame of NOD2/CARD15 described by Ogura et al.[4] However, an alternative open reading frame, characterized by a translation initiation site located 81 nucleotides upstream of the smaller one was also reported by Ogura et al. Other authors have recently proposed this sequence as the sequence reference for mutation annotation.[11] To avoid confusion, we therefore used this new method of annotation in this study, which is easily deduced from the initial one by adding 27 to the initial number of the mutated amino acid.

The missense mutation R702W (ex R675W; SNP8; Gen-Bank accession number G67950) was genotyped by an allele-specific polymerase chain reaction (PCR) procedure. After amplification (primers: 5'-ATC TGA GAA GGC CCT GCT CC-3' [wild-type, forward], 5'-ATC TGA GAA GGC CCT GCT CT-3' [mutated, forward], and 5'-CCC ACA CTT AGC CTT GAT G-3' [reverse], annealing temperature 58°C, 30 cycles), the PCR products were loaded on a 2% agarose gel with internal controls. The genotypes were directly deduced from the migration profiles. The missense mutation G908R (ex G881R; SNP12; GenBank accession number G67951) was genotyped by a PCR-restriction fragment length polymorphism procedure. In brief, after PCR (primers: 5'-CCCAGCTCCTCCCTCTTC-3' and 5'-AAGTCTGTAATGTAAAGCCAC-3' annealing temperature 55°C, 30 cycles), the 380-bp products were digested by the restriction enzyme Hha1 (Gibco BRL, Gaithersburg, MD) and electrophoresed on a 2% agarose gel. The profile of the G908R variant was characterized by 2 bands (138 bp and 242 bp). For the frameshift mutation 1007fs (SNP13; GenBank accession number G67955), PCR products with fluorescently labelled primers (5'-GAATGTCAGAATCAGAAGGG-3' and 5'-CTCACCATTGTATCTTCTTTTC-3', annealing temperature 55°C, 30 cycles) were loaded on a 377 ABI Prism automatic sequencer. The genotypes were deduced from the sizes of the PCR products: 230 bp (wild-type) and 231 bp (1007fs, ex 980fs). The investigators performing the NOD2/CARD15 genotyping were blinded for the clinical response of each patient.

Treatment Response

Short-term response to infliximab was determined at 4 or 10 weeks after first infusion, for refractory luminal or fistulizing CD, respectively. For patients with refractory luminal disease, response was defined as a drop of at least 70 points on the CD activity index (CDAI; with a CDAI > 150 defined as clinical remission).[6] For patients with fistulizing CD, response was defined as at least a 50% decrease in the number of draining fistulae from baseline at 2 consecutive visits.[7] The median duration of response was calculated for all patients from the mean interval (in weeks) between subsequent infusions.

Intestinal Biopsies

A subgroup of 21 patients, all requiring colonoscopy for medical reasons, were included in the functional study of TNF production. During colonoscopy, colonic biopsy specimens were obtained from macroscopically and microscopically unaffected or affected areas. In each patient, a set of 5 biopsy specimens in affected and/or unaffected regions was taken. At each time, 2 specimens were fixed in formalin for histological assessment and 3 were placed at 4°C in a medium consisting of Ca^{2+}- and Mg^{2+}-free (CMF) Hank's solution (Gibco BRL) supplemented by 100 IU/mL penicillin and 100 µg/mL streptomycin.

Tissue Culture

After collection, biopsy specimens were transferred to the laboratory. Within a maximal lag time of 3 hours after biopsy, tissue was gently washed 3 times in CMF Hank's medium supplemented with antibiotics, blotted carefully, weighed, and individually placed in 24-well tissue culture plates in triplicate (1 mL culture medium/well). The culture medium consisted of RPMI-1640 (Gibco BRL) supplemented with 10% heat-inactivated fetal calf serum (Gibco BRL), 2 mmol/L L-glutamine (Gibco BRL), penicillin (100 IU/mL), and streptomycin (100 µg/mL), to which 1 ng/mL of LPS (*Salmonella enteritidis* from Sigma, St. Louis, MO) was added. After 18 hours of culture at 37°C in a humidified 95% air/5% CO_2 atmosphere, medium was removed, centrifuged, and stored at -80°C until required for cytokine assays. The structural integrity was assessed by standard histology and by measurement of lactate dehydrogenase release according to Wardle et al.[12] After 18 hours of culture, no histological changes were noted compared with precultured tissue. Furthermore, the release of lactate dehydrogenase in cultured tissues was significantly lower than in uncultured tissues, i.e., tissues processed in the same way as cultured tissues but placed in saline instead of culture medium (data not shown).

Immunoassay for TNF-α

TNF-α was measured with specific immunoassays, performed according to the manufacturer's instructions (EASIA from Biosources Europe, Fleurus, Belgium). The detection limit was 3 pg/mL, TNF-α production was expressed by its concentration per milliliter of culture medium and per milligram of tissue in culture. The final result for each patient corresponds to the mean of the 3 individual biopsy cultures. The mean coefficient of variation between the 3 individual biopsy specimens was 35.7% ± 13-2% for TNF-α.

Expression of TNF-α Production by Tissue Cultures

The production of TNF-α at the mucosal level may be influenced by the degree of inflammation. This may interfere with a possible genetic influence. We thus expressed TNF production by the ratio between the TNF-α concentration in the supernatants of colonic biopsy specimens and the histological score of inflammation. The overall inflammation score was adapted as previously described.[13] Because it has been shown that the histological inflammation correlates well with the production of interleukin (IL)-6 by tissue culture,[14] we also expressed TNF-α production as a ratio between TNF-α and IL-6 concentrations (units of TNF-α production) in the supernatants of biopsy cultures, to take into account more accurately the variations in the degree of inflammation in the assessment of individual colonic TNF-α production. IL-6 was measured by a commercial enzyme-linked immunosorbent assay (ELISA; Biosources Europe), according to the manufacturer's instruction. The detection limit was 2 pg/mL. The final result for each patient corresponds to the mean of 3 individual biopsy cultures in inflamed and/or uninflamed regions. Production of TNF-α was compared among CD patients according to their NOD2/ CARD15 genotype.

Statistical Analysis

Allele frequencies and NOD2/CARD15 genotypes were compared by means of the χ^2 test or Fisher exact test where appropriate. When data showed a Gaussian distribution, results were expressed as means and standard deviations. If a non-Gaussian distribution was observed, the results were expressed as medians and interquartile ranges (IQR). Comparison of TNF production was made using the Mann-Whitney *U* test. Multivariate analysis (SAS/STAT Release 2.01; SAS Institute, Raleigh, NC) was performed to assess whether NOD2/ CARD15 mutations were associated with clinical response in a particular subgroup of patients (with $\alpha = 0.05$).

Results

NOD2/CARD15 Genotyping and Treatment Response

The overall prevalence of NOD2/CARD15 mutations in CD patients (32.6%, 80 of 245) was significantly higher than that in healthy controls (15%, 14 of 95; $P < 0.001$). This observation was linked to a higher frequency of all 3 studied mutations in patients compared with controls (R702W 18.8% vs. 4%, $P < 0.001$; G908R 8.6% vs. 1%, $P = 0.011$; 1007fs 10.2% vs. 2%, $P = 0.013$). With this cohort, we observed fewer homozygotes (2.4%, 6 of 245) compared with the cohort used in the *Nature* paper[3] (6.0%, 28 of 468; $\chi^2 = 4.42$, df = 1, $P = 0.03$). The prevalence of compound heterozygotes in the present study (4.9%, 12 of 245), however, was not significantly different from the prevalence reported in the *Nature* paper[3] (8.5%, 40 of 468; $\chi^2 = 3.17$, df = 1, $P = 0.07$).

There were 77.6% (190 of 245) responders (with 53.9% of patients entering clinical remission) and 22.4% (55 of 245) nonresponders to infliximab in the study group. This is in accordance with the published clinical trials. Table 2 and Figure 1 show the NOD2/CARD15 genotypes stratified for response (both remission and partial response) to infliximab. There was no difference in prevalence of the allelic variants nor of the different NOD2/CARD15 genotypes between the 2 response groups. Along the same line were the response and failure rates of the compound heterozygotes (n = 12) in this cohort: Nine of them (75%) showed a response (with 6, or 50%, entering remission), and 3 of 12 (25%) did not show a response. Also, when remission (rather than response) was considered, and compared with nonresponse, results lacked significance.

In the group of the responders, the median duration of response was 12 weeks (IQR, 9-20 weeks). This was not different between those patients carrying NOD2/ CARD15 variants (median response duration 12 weeks, IQR, 8-20 weeks) and those without NOD2/CARD15 variants (median response duration, 12 weeks; IQR, 9-18 weeks). Along the same lines was the median response duration of homozygous mutant and compound heterozygous patients (14 weeks [IQR, 10-20 weeks] and 12 weeks [IQR, 12-16 weeks], respectively).

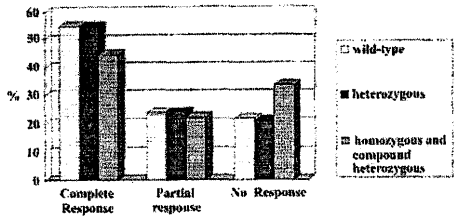
The indication for infliximab treatment (i.e., refractory luminal or fistulizing disease) was not associated with

NOD2/CARD15 genotypes. Similarly, a multivariate analysis could not confirm a subpopulation of patients with NOD2/CARD15 mutations in conjunction with certain clinical parameters to be associated with response or lack of response to infliximab (data not shown).

Table 2: *Clinical Response to Infliximab According to N0D2/CARD15 Genotypes*

	Responders (n = 190) (%)		
Genotype	**Remission (n = 132)**	**Partial Response (n = 58)**	**Nonresponders (n = 55) (%)**
R702W			
Heterozygous (n = 43)	28 (65.1)	9 (20.9)	6 (14)
Homozygous (n = 3)	1 (33.3)	1 (33.3)	1 (33.3)
G908R			
Heterozygous (n = 19)	9 (47.4)	4(21)	6 (31.6)
Homozygous (n = 2)	1 (50)	0	1 (50)
1007fs			
Heterozygous (n = 24)	9 (37.5)	8 (33.3)	7 (29.2)
Homozygous (n = 1)	0	0	1 (100)
No mutation (n = 165)	90 (54.6)	39 (23.6)	36 (21.8)
One mutation (n = 62)	34 (54.8)	15 (24.2)	13 (21.0)
Two mutations (n = 18)	8 (44.4)	4 (22.2)	6 (33.4)

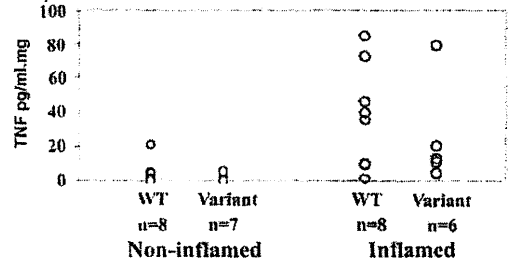
Figure 1: *Percentage of patients carrying 0,1, or 2 doses of NOD2/ CARD15 mutations according to their infliximab responder status.*



TNF Production According to N0D2/CARD15 Genotypes

Among the subgroup of 21 patients included in the functional analysis, 10 were wild-type homozygotes, 6 were simple heterozygotes, 3 were compound heterozygotes, and 2 were homozygotes mutants. In the noninflamed colonic biopsies, a difference in TNF production was observed between patients with at least 1 NOD2/ CARD15 variant and wild-type homozygotes [0.6 pg/ ml · mg (0-6.0) vs. 4.0 pg/mL · mg (0.1-21.4), respectively], although the difference did not reach significance (Figure 2). The same conclusion was drawn when comparing TNF production from inflamed colonic biopsy specimens (12.4 pg/mL · mg [4.2-79.3] vs. 37.5 pg/mL · mg [1.4-85] in patients carrying NOD2/CARD15 variants and wild-type homozygotes, respectively). When TNF production was expressed as a ratio between the TNF concentration in the culture supernatants and the histological score, again a difference was seen (0.49 pg/ mL · mg [0-6.6] vs. 0.78 pg/mL · mg [0.02-7.1] in mutation carriers and wild-type homozygotes, respectively). Finally, when TNF production was expressed as a ratio between the TNF and the IL-6 concentrations in the culture supernatants, the trend toward a lower production of TNF in mutation carriers than in wild-type homozygotes was confirmed (0.0295 [0-0.178] vs. 0.047 [0.002-0.248]; $P = 0.07$).

Figure 2: *TNF production (pg/ml · mg) according to the NOD2/ CARD15 genotypes: wild-type (WT) and N0D2/CARD15 mutation carriers (variant) in uninflamed and inflamed colonic biopsy specimens. Decreased TNF production was observed in mutation carriers, but significance was not reached.*



Discussion

The identification of the first gene underlying CD susceptibility is a breakthrough in the search for the cause of this incapacitating disease. Not more than 5 years after the initial publication of a susceptibility locus for CD on the pericentromeric region of chromosome 16,[15] this IBD1 locus has now been identified as NOD2/ CARD15. The recent article by Hugot et al.[15] reported a prevalence of the 3 main NOD2/CARD15 variants in 43% of CD patients compared with 15% of UC and healthy controls.[8]

The present study, performed with an independent Belgian group of CD patients, confirms that the previously reported 3 main variants of NOD2/CARD15 (10076 [ex 980ft], R702W [ex R675W], and G908R [ex G881R]) are associated with CD. Whereas the association of the 1007fs frame shift mutation with CD was clearly demonstrated, not only in a large European population' but also in North American patients[4] and in a German-British cohort of CD patients,[11] the association between the R702W and G908R mutations and CD has not been studied by independent investigators. The present replication provides a strong argument in favor of their role in CD. However, we observed fewer homozygotes (2.4%, 6 of 245) compared with the cohort used in the study by Hugot et al. (6.0%, 28 of 468; $\chi^2 = 4.42$, df = 1, $P = 0.03$). Because this is the only study available as yet, much additional information is necessary from other cohorts before firm statements can be made. The prevalence of compound heterozygotes in the present study (4.9%, 12 of 245) was not significantly different from the prevalence reported by Hugot et al.[3] (8.5%, 40 of 468; $\chi^2 = 3.17$, df = 1, $P = 0.07$).

Little is as yet known about the phenotypic expression of NOD2/CARD15 variants or about its implication in disease or treatment outcome. Current treatment of CD with corticosteroids and sulphasalazine is based on inhibition of NF-κB. Newer therapies, such as infliximab, also depend on the suppression of the NF-κB system. NOD2/CARD15 activates NF-κB and potentially interacts with TNF production. Mutations in NOD2/CARD15, causing inappropriate activation of NF-κB, could therefore well be involved in the differences in response seen in clinical practice with infliximab.

Functional data on TNF production in mucosal biopsy specimens from CD patients could not show a significant difference in the capacity of TNF production according to the NOD2/CARD15 genotypes. However, there is a tendency toward a lower production of TNF in mutation carriers than in wild-type homozygotes. The lack of significance may result from the small number of studied patients, and these preliminary observations must be confirmed by additional data (work in progress). However, the finding supports the hypothesis that NOD2/ CARD15 may indirectly interfere with TNF production.

In this study, we examined whether NOD2/CARD15 variants influence response to infliximab treatment but could not find a significant association. Despite the fact that the number of patients genotyped was high, we cannot exclude with certitude an influence of NOD2/ CARD15 on infliximab outcome. However, if such an association exists, it would be a weak one, and its clinical relevance would be questionable.

Although this study was not performed in the setting of a controlled trial, inclusion criteria were very strict and well defined, and clinical response to treatment was rigorously and prospectively assessed. All patients received infliximab for severe active CD, but according to the behavior of the disease (fistulizing or refractory luminal CD), 2 different therapy regimens were applied following the study protocol. Although these patients might represent 2 different groups, multivariate analysis could not indicate an association of NOD2/CARD15 variants with clinical response in either of the subgroups, and similar results were obtained for the fistulizing and luminal

subgroups.

In conclusion, we have studied mutations in NOD2/ CARD15, the first identified gene conferring susceptibility to CD, with an eye to its value in predicting response to infliximab treatment. No association was found between any particular mutations, or between the overall presence of NOD2/CARD15 mutations and treatment outcome. Other genetic polymorphisms and immunological or serological markers should be studied co unravel the differences in response seen with this biological treatment in clinical practice.

References

1. Calkins BM, Mendelhoff AI. The epidemiology of idiopathic inflammatory bowel disease. In: Kirsner JB, Shorter RG, eds. Inflammatory bowel disease. 4th ed. Baltimore: Williams & Wilkins, 1995: 31-68.

2. Binder V. Genetic epidemiology in inflammatory bowel disease. Dig Dis 1998;16:351-355.

3. Hugot JP, Chamaiilard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Aimer S, Tysk C, O'Morain C, 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/ CARD15 leucin-rich repeat variants with susceptibility to Crohn disease. Nature 2001;411:599-603.

4. 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/CARD15 associated with susceptibility to Crohn disease. Nature 2001;411:603-606.

5. Ogura Y, Inohara N, Benito A, Chen FF, Yamaoka S, Nunez G. NOD2/CARD15, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-κB. J Biol Chem 2001:276:4812-4818.

6. Targan SR, Hanauer SB, van Deventer SJH, Mayer L, Present DH, Braakman TAJ, DeWoody KL, Schaible TF, Rutgeerts P, and the Crohn Disease cA2 Study Group. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor *α* for Crohn disease. N Engl J Med 1997;337:1029-1035.

7. Present DH. Rutgeerts P, Targan S, Hanauer SB, Mayer L, van Hogezand RA, Podolsky DK, Sands BE, Braakman T, DeWoody KL, Schaible TF, van Deventer SJ. Infliximab for the treatment of fistulas in patients with Crohn's disease. N Engl J Med 1999; 340:1398-1405.

8. Rutgeerts P, D'Haens G, Targan S, Vasiliauskas E, Hanauer SB, Present DH, Mayer L, Van Hogezand RA, Braakman T, DeWoody KL, Schaible TF, Van Deventer SJH. Efficacy and safety of retreat-ment with anti-tumor necrosis factor antibody (infliximab) to maintain remission in Crohn's disease. Gastroenterology 1999;117: 761-769.

9. Mascheretti S, Hampe J, Sfikas N, Kuhbacher T, Andus T, Her-farth H, Foelsch U, Scheiber S. Pharmcacogenetic association between the TNF receptor 2 genotype and response to infliximab treatment in Crohn's disease (aostr). Gastroenterology 2001; 120:A362.

10. Taylor KD, Plevy SE, Yang H, Landers CJ, Barry MJ, Rotter Jl, Targan SR. ANCA pattern and LTA haplotype relationship to clinical responses to anti-TNF antibody treatment in Crohn's disease. Gastroenterology 2001;120:1347-1355.

11. Hampe J, Cuthbert A, Croucher PJP, Mirza MM, Mascheretti S, Fisher S, Frenzel H, King K, Hasselmeyer A, MacPherson AJS, Bridger S, van Deventer S, Forbers A, Nikolaus S, Lennard-Jones JE, Foelsch UR, Krawczak M, Lewis C, Schreiber S, Mathew CG. Association between insertion mutation in NOD2/CARD15 gene and Crohn's disease in German and British populations. Lancet 2001;357:1925-1928.

12. Wardie TD, Hall L, Turnberg LA. Use of coculture of colonic biopsies to investigate the release of eicosanoids by inflamed and unimflamed mucosa from patients with inflammatory bowel disease. Gut 1992;33:1644-1651.

13. Riley SA, Mani V, Goodman MJ, Dutt S, Herd ME. Microscopic activity in ulcerative colitis: what does it mean? Gut 199l;32: 174-178.

14. Reimund JM, Wittersheim C, Dumont S, Muller CD, Kennet JS, Baumann R, Poindron P, Duclos B. Increased production of tumor necrosis factor-α, interleukin-1β, and interleukin-6 by morphologically normal intestinal biopsies from patients with Crohn's disease. Gut 1996;39:684-689.

15. Hugot JP, Laurent-Puig P, Gower-Rousseau C, Olson JM, Lee JC, Beaugerie L, Naom I, Dupas JL, Van Gossum A, Orhoim M, Bonaiti-Pellie C, Weissenbach J, Mathew CG, Lennard-Jones JE, Cortot A, Colombel JF, Thomas G. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. Nature 1996; 379:821-823.