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BRIEF ARTICLE

Crohn's disease genotypes of patients in remission *vs* relapses after infliximab discontinuation

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Abstract

AIM: To investigate genetic differences between Crohn's disease (CD) patients with a sustained remission *vs* relapsers after discontinuing infliximab while in corticosteroid-free remission.

METHODS: Forty-eight CD patients received infliximab and were in full corticosteroid-free clinical remission but then discontinued infliximab for reasons other than a loss of response, were identified by review of an electronic database and charts. Infliximab-associated remission was defined as corticosteroid-free plus normalization of clinical disease activity [CD activity index (CDAI) < 150] during follow-up visits based on physician global assessments. A CD relapse (loss of infliximab-induced remission) was clinically defined as a physician visit for symptoms of disease activity (CDAI > 220) and a therapeutic intervention with CD medication(s), or a hospitalization with complications related to active CD. Genetic analyses were performed on samples from 14 patients (n = 6 who had a sustained long term remission after stopping infliximab, n = 8 who rapidly relapsed after stopping infliximab). Nucleotide-binding oligomerization domain 2 (NOD2)/caspase activation recruitment domain 15 (CARD15) polymorphisms (R702W, G908R and L1007fs) and the inflammatory bowel disease 5 (IBD5) polymorphisms (IGR2060a1 and IGR3081a1) were analyzed in each group.

RESULTS: Five single nucleotide polymorphisms of IBD5 and NOD2/CARD15 genes were successfully analyzed for all 14 subjects. There was no significant increase in frequency of the NOD2/CARD15 polymorphisms (R702W, G908R and L1007fs) and the IBD5 polymorphisms (IGR2060a1 and IGR3081a1) in either group of patients; those whose disease relapsed rapidly or those who remained in sustained long term remission following the discontinuation of infliximab. Nearly a third of patients in full clinical remission who stopped infliximab for reasons other than loss of response remained in sustained clinical remission, while two-thirds relapsed rapidly. There was a marked difference in the duration of clinical remission following discontinuance of infliximab between the two groups. The patients who lost remission did so after 1.0 years ± 0.6 years, while those still in remission were at the time of this study, 8.1 years ± 2.6 years post-discontinuation of infliximab, P < 0.001. The 8 patients who had lost remission after discontinuing infliximab had a

mean number of 5 infusions (range 3-7), with a mean treatment time of 7.2 mo (range 1.5 mo-15 mo). The mean duration of time from the last infusion of infliximab to the time of loss of remission was 382 d (range 20 d-701 d). The 6 patients who remained in remission after discontinuing infliximab had a mean number of 6 infusions (range 3-12), with a mean treatment duration of 12 mo (range 3.6 mo-32 mo) (P = 0.45 relative to those who lost remission).

CONCLUSION: There are no IBD5 or NOD2/CARD15 mutations that predict which patients might have sustained remission and which will relapse rapidly after stopping infliximab.

Key words: Infliximab; Anti-tumor necrosis factor alpha; Crohn's disease; Inflammatory bowel disease; Genotype

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INTRODUCTION

Crohn's disease (CD), a type of inflammatory bowel disease (IBD), is a complex chronic condition characterized by relapsing transmural inflammation throughout the gastrointestinal tract. The etiology of CD remains unclear and as with most complex-trait diseases, the development of IBD involves a combination of genetic and environmental risk factors. Epidemiological data and family studies do suggest a genetic basis for CD[1,2]. For instance, the single greatest risk factor for developing IBD is having an affected family member, as demonstrated by twin studies in which a monozygotic twin of a CD proband has approximately a 1 in 3 lifetime risk of developing IBD[3]. Genome-wide association studies have found approximately 100 genetic loci to be significantly associated with IBD[4].

In terms of CD itself, a potent proinflammatory cytokine, tumor necrosis factor alpha (TNF-α), is a well-known mediator of inflammation[5]. Clinical trials have shown anti-TNF-α agents such as infliximab to be highly efficacious in terms of induction and maintenance responses (ACCENT 1 and 2)[6,7]. However, anti-TNF-α agents are costly, and the long-term safety risks such as development of autoantibodies, drug-induced lupus, infection, and neoplasia can be concerning in the long term[5,8-10]. Furthermore, the appropriate duration of anti-TNF-α therapy for CD patients who are in remission is as yet undefined; specifically, the factors which affect the duration of sustained clinical benefit after discontinuation of infliximab remain poorly understood.

As part of a project to achieve such an understanding, a longitudinal cohort study at our institution of over 7 years duration, identified CD patients who were in full corticosteroid-free clinical remission and then discontinued infliximab for reasons unrelated to loss of response[11]. This study demonstrated that one third of these CD patients remained in a sustained clinical remission for up to 7 years after the infliximab was discontinued, and that concomitant drug therapy, number of infliximab doses, age, gender, and CD location were not predictive of this sustained clinical benefit. This finding lends support to the hypothesis that a patient-unique genotype of CD may play a role in determining the duration of long-term remission after the discontinuation of anti-TNF-α agent therapy.

Research on CD genotypes associated with anti-TNF-α therapy has generally studied infliximab responders and compared the results to infliximab non-responders. Caspase activation recruitment domain 15 (*CARD15*) was the first gene to be associated with CD[12,13]. The nucleotide-binding oligomerization domain 2 (*NOD2*)/*CARD15* product is an intracellular protein involved in recognizing bacterial lipopolysaccharides in the nuclear factor kappa B-TNF-α pathway[14]. This *NOD2*/*CARD15* gene is known to be associated with susceptibility to CD[12,15]. Previous studies have identified certain TNF-α polymorphisms as predictive of a lack of response to infliximab[16], while other studies focusing on *NOD2*/*CARD15* mutations (R702W, G908R, 1007fs)[17,18] have not confirmed a relationship between such mutations and the response to anti-TNF-α agents. Urcelay *et al*[19] studied 40 Spanish CD patients who received infliximab, 25 responders and 15 non-responders, and identified a homozygous mutant in the IBD5 region on chromosome 5q31 that was significantly associated with a lack of response to infliximab[19]. However, no studies have reported correlations of CD genotypes for patients who relapse rapidly versus patients who do not relapse after discontinuing anti-TNF-α therapy.

This study thus examined a cohort of CD patients who had discontinued infliximab for reasons other than non-response while in full corticosteroid-free remission, to determine if genotypic differences might contribute to the varying lengths of post-infliximab remission times observed.

MATERIALS AND METHODS

Selection of infliximab-treated patients in remission who then discontinued infliximab

This study was reviewed and approved by the ethics

board at the University of Alberta prior to study initiation and subjects were provided informed consent before study participation. Forty-eight Caucasian CD patients who received infliximab between July 2001 and July 2007, and who were in full corticosteroid-free clinical remission but then discontinued infliximab for reasons other than a loss of response, were identified by review of an electronic database and chart reviews. Reasons for infliximab discontinuance included physician choice, loss of insurance coverage, fear of side-effects, adverse events, and pregnancy. These patients were referred from the IBD practices of thirteen gastroenterologists associated with the University of Alberta. Long-term follow-up data for each patient was available through to July 6, 2011.

Patients with CD were eligible for study inclusion based on the following criteria: (1) clinical response to infliximab (5 mg/kg) induction dosing administered at weeks 0, 2 and 6; (2) advancement to scheduled maintenance infliximab therapy every 8 wk; (3) maintenance of a stable corticosteroid-free clinical benefit for at least 6 mo; (4) discontinuation of infliximab therapy for reasons other than loss of response; and (5) sufficient follow-up that allowed assessment of ongoing wellness and/or disease relapse. A subject was excluded from the study if he/she was under the age of 18 years. Eligible patients were contacted via telephone by a research associate with a request for participation in the current study.

Standardized infliximab administration

Each patient included in the study had received her or his infliximab in accordance with the Canadian Association of Gastroenterology's anti-TNF-α treatment guidelines for CD patients[20]. The "step-up" approach specified by these guidelines requires that both corticosteroids and immunosuppressive (azathioprine or methotrexate) treatments have failed the patient due to a lack of response or intolerance before infliximab therapy is begun.

Definition of infliximab-induced remission

Infliximab-associated induction and maintenance remission were defined as corticosteroid-free plus normalization of clinical disease activity [CD activity index (CDAI) < 150] during follow-up based on physician global assessments at each successive visit. The dates of each initial and final infliximab infusion were determined using an infliximab infusion database.

Definition of loss of remission after discontinuing infliximab

A CD relapse, and thus loss of infliximab-induced remission (CDAI > 220), for those patients who had discontinued infliximab was defined as a physician or hospital visit for documented symptoms of disease activity and a therapeutic intervention with CD medication(s), or a hospitalization with complications related to active CD. The date of a CD relapse after discontinuation of infliximab therapy was identified through review of patient medical records. Mucosal healing, laboratory investigations such as CRP and radiological evaluation were analyzed for each patient but not reported in this paper.

Genotyping

Informed consent for participation in the study and genotyping was obtained from those participants who met the inclusion criteria and who were willing to join the study. Urban patients attended an appointment at the University of Alberta outpatient gastroenterology clinic, where an 8 mL blood sample was obtained via arm venipuncture. For patients residing in rural areas, the blood sample was obtained by the patient's local laboratory and was couriered to the University of Alberta hospital for genetic processing.

The laboratory scientists who analyzed the disease loci from these samples were blinded to the study groups as they performed the following steps. Genomic DNA was purified from peripheral blood leukocytes. All sam-ples were amplified from an individual FTA card. Five single nucleotide polymorphisms (SNPs) (R702W, G908R, L1007fs, IGR2060a1, IGR3081a1) were detected. R702W and G908R were analyzed using the SNaPshot method (Single base pair extension). L1007fs, IGR2060a1, and IGR3081a1 were analyzed using the Sanger sequencing method. All samples were run on a capillary instrument. Previously sequenced samples were used as controls. The polymerase chain reaction primers are listed in Table 1.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 software (Statistical Package for the Social Sciences Inc., Chicago, IL). Association between genotype mutation and CD remission status was estimated using the odds ratio with a 95% CI. Fisher's exact test was utilized to calculate *P* values. A *P* value of 0.05 was considered to indicate statistical significance.

RESULTS

Patient recruitment

A total of 48 CD patients whose disease was in an infliximab-induced corticosteroid-free clinical remission, and who had then discontinued infliximab for reasons other than non-response (see Methods above) were identified from an electronic database and chart reviews. Six patients were excluded from the study for the following reasons: 2 patients were excluded due to non-Caucasian ancestry and its possible confounding effects on genetics and 4 patients were not interested in participating. Of the 42 patients who met the inclusion criteria, 3 had changed their telephone number and were unable to be contacted, 5 had moved out of province, 10 rural patients agreed to participate but did not have blood samples drawn, 3 patients missed venipuncture appointments, and 7 could not be reached by telephone despite two messages being left. The remaining fourteen CD patients were stratified into a group of those still in full clinical remission (n = 6) and a group of those who had

Table 1 Polymerase chain reaction primers used for genotyping

Genetic variant	Sense primers	Antisense primers
Arg702Trp	5′-CAT CTG AGA AGG CCC TGC TC(C/T)-3′	5′-CAG ACA CCA GCG GGC ACA-3′
Gly908Arg	5′-TTG GCC TTT TCA GAT TCT GG(G/C)-3′	5′-CCC CTC GTC ACC CAC TCT G-3′
Leu1007fs insC	Not available	Not available
IGR2060a1	5′-CTC ATT ACA TCC TTG CAA CCC T(G/C)-3′	5′-GAC ACA TGG TGT GAG CTC AGT CA-3′
IGR3081a1	5′-TCG CGT GAG TCC TAT TCT TTC T(T/G)-3′	5′-TTC ATA CTT CCA GCA GCG GG-3′

Table 2 Characteristics for Crohn's disease patients still in remission and those who lost remission after discontinuing infliximab

Characteristic	Remaining in remission following discontinuing infliximab (n = 6)	Lost remission following discontinuing infliximab (n = 8)
Male:female (n)	4:2	4:4
Age of Crohn's disease onset (yr), mean (range)	30 (14-47)	26 (15-38)
Current age (yr), mean (range)	53 (41-60)	41 (25-57)
Duration of disease prior to first infliximab infusion (yr), mean ± SD	15 ± 8.0	10.1 ± 11.3
Age at first infliximab infusion (yr), mean ± SD	45 ± 7.6	36 ± 11.1
Number of infusions, mean ± SD	5.7 ± 3.5	4.7 ± 1.7
Duration of infliximab treatment (mo), mean (range)	12 (3.6-36.2)	7.2 (1.5-15)
Duration of remission after infliximab stopped (yr), mean ± SD	8.1 ± 2.6[1]	1.0 ± 0.6[2]
Disease distribution, n (%)		
Ileum	2 (33)	2 (25)
Ileo-colonic	3 (50)	4 (50)
Colon	1 (17)	2 (25)
Current medications in patients who lost remission, n		
Adalimumab		4
Infliximab		2
Azathioprine alone		1
No Biological or immunosuppressive therapy[3]		1

[1]All patients still remain in remission at the time of this study; [2]Follow-up time from last dose of infliximab to either date of flare or last day of study (July 6, 2011), $P < 0.001$; [3]This patient developed both basal cell and renal cell carcinoma.

lost remission (n = 8).

Characteristics of patients still in remission and those who lost remission after discontinuing infliximab

There were no statistical significant differences in terms of the mean age, gender, disease duration, disease location, duration of disease prior to first infliximab infusion, age at first infliximab infusion, mean number of infliximab infusions, or mean duration of infliximab treatment between the CD patients who were still in remission after discontinuing infliximab (n = 6) and those who had lost remission after discontinuing their infliximab (n = 8) (Table 2).

The 8 patients who had lost remission after discontinuing infliximab had a mean number of 5 infusions (range 3-7), with a mean treatment time of 7.2 mo (range 1.5-15 mo). The mean duration of time from the last infusion of infliximab to the time of loss of remission was 382 d (range 20-701 d). The 6 patients who remained in remission after discontinuing infliximab had a mean number of 6 infusions (range 3-12), with a mean treatment duration of 12 mo (range 3.6-32 mo) (P = 0.45 relative to those who lost remission). Of the 8 patients who lost remission, 4 patients are currently on adalimumab, 2 patients are again on infliximab, 1 patient remains on azathioprine alone, and 1 patient is currently on no biological or immunomodulating agents due to a malignancy.

In contrast, there was a marked difference in the duration of clinical remission following discontinuance of infliximab between the two groups. The patients who lost remission did so after just 1.0 years ± 0.6 years, whereas those who were still in remission were at the time of this study, 8.1 years ± 2.6 years post-discontinuation of infliximab, $P < 0.001$ (Table 2). This marked difference confirms a group of patients that remained in remission for an extended interval of time after infliximab was discontinued while another group rapidly relapsed. We proceeded to genetically type these two groups.

Patient genotypes

Five SNPs of IBD5 and NOD2/CARD15 genes were successfully analyzed for all 14 subjects. There were no significant differences in SNPs between the group of patients who remained in remission for an extended interval of time after infliximab was discontinued, and the group of those who relapsed rapidly after infliximab was discontinued (Table 3).

DISCUSSION

Anti-TNF-α agents are well known to be effective for both induction and maintenance of remission in CD. However, no phenotypic features, environmental fac-

Table 3 5q31 and nucleotide-binding oligomerization domain 2/caspase activation recruitment domain 15 variants in crohn's disease patients still in remission and those who lost remission after discontinuing infliximab

Variant	Remaining in remission following discontinuing infliximab (n = 6)	Lost remission following discontinuing infliximab (n = 8)	Odds ratio	P value
Arg702Trp			1.50	1.00
Homozygous wild type, CC (%)	4 (67)	6 (75)		
Heterozygous, CT (%)	2 (33)	2 (25)		
Homozygous mutant, TT (%)	0	0		
Gly908Arg			-	-
Homozygous wild type, GG (%)	6 (100)	8 (100)		
Leu1007fs insC	2 (33)	2 (25)	1.50	1.00
IGR2060a1			1.67	1.00
Homozygous wild type, GG (%)	1 (17)	2 (25)		
Heterozygous, GC (%)	3 (50)	2 (25)		
Homozygous mutant, CC (%)	2 (33)	4 (50)		
IGR3081a1			-	0.47
Homozygous wild type, TT (%)	0	2 (25)		
Heterozygous, GT (%)	4 (66)	2 (25)		
Homozygous mutant, GG (%)	2 (33)	4 (50)		

tors, or clinical markers have been definitively shown to predict the duration of sustained clinical benefit after discontinuation of infliximab.

The lack of response to infliximab in patients with CD appears to be a stable occurrence over time, thus suggesting that infliximab response/non-response is related to a genetic predisposition[5]. Though over 100 genetic loci have been associated with IBD thus far, only two genes, *IBD5* and *CARD15*, have reliably been linked to CD susceptibility, and few genes have been proven to influence response to infliximab[12,19,21]. Although less than 20 percent of patients are homozygous for *CARD15* and the relative risk of developing CD for these individuals ranges from 10 to 40 percent, genetic markers remain important to understanding the complex pathogenesis of this disorder[22]. In comparison to biochemical markers such as C-reactive protein, genetic elements theoretically offer ideal markers for predicting disease outcomes, as genes are not affected by disease activity and are stable over time. As we more fully understand genetic variants and the roles they play in the development of CD, it is very plausible that certain genetic polymorphisms may be predictors of response to or relapse from anti-TNF-α agents such as infliximab.

Utilizing a well-defined cohort of patients with CD who were in an infliximab-induced corticosteroid-free clinical remission and then discontinued infliximab for reasons other than non-response[11], we studied two genetic variants of the IBD5 locus on chromosome 5q31 (IGR 2060a1 and IGR3081a1) along with three *NOD2/CARD15* polymorphisms (Arg702Trp, Gly908Arg, Leu1007fs insC), in those patients who had relapsed rapidly and compared them to those patients who remained in a long-term sustained remission.

Our study results show no significant increase in frequency of the *NOD2/CARD15* and IBD5 polymorphisms, for either the CD patients whose disease relapsed rapidly or for the CD patients who remained in a long-term sustained remission following the discontinuation of infliximab.

Urcelay *et al*[19] reported that the homozygous mutants in the 5q31 locus were related to a significant lack of response to infliximab and the frequency of these mutants was significantly higher in the non-responder group. Our study did not identify such findings, likely due to the fact that we were examining those infliximab-treated patients who had initially all responded to infliximab but after discontinuing infliximab fell into two groups: those that had a long-term sustained remission and those that rapidly relapsed.

Other studies have also shown no association between *NOD2/CARD15* genotype and response to infliximab[17,18]. In a large study of two CD patient cohorts with 534 participants (ACCENTI), Mascheretti *et al*[18] found no association between SNPs 8, 12 and 13 of the *CARD15* gene and infliximab response. SNP 8 includes the variant R702W and SNP12 includes G908R, which were also analyzed in our study. Genes encoding for TNF-α and TNF-α receptors have also been an area of some study, given the primary role of TNF-α in the inflammation of CD. A large cohort study by Louis *et al*[23] in 2002 did not show a CD response to infliximab amongst patients with the -308 polymorphism, a TNF-α promoter region mutation thought to influence TNF-α transcription. In another study focusing on the receptors TNFR1 and TNFR2, where TNF-α binds and exerts its effects, no clear effect of the polymorphisms on infliximab response was observed[24]. Again, the results from these previous investigations may not be comparable to those of the current study, in that we examined those infliximab-treated patients who initially responded to infliximab but who after discontinuing infliximab, fell into two groups: those that had a long-sustained response and those whose disease rapidly relapsed.

Of our 8 patients who experienced a flare of their disease after the discontinuation of infliximab, 4 are on adalimumab, 2 are on infliximab, 1 is only on azathioprine, and the remaining patient is on no therapy due

to active malignancy. Interestingly, of the 48 initially identified CD patients in the longitudinal cohort who were in an infliximab-induced clinical remission and then discontinued infliximab, approximately 16 patients (34%) remained in clinical remission a median of 477 d (95% CI; range of 290 to 1339 d; data from Waugh *et al*[11] as of July 2009. However, as time progressed, of these 16 patients who had remained in remission, 6 additional patients have now flared and are on alternative therapy as of July 2011 and only 10/48 (21%) remained in remission. This implies that even those patients who are in a long term sustained remission after discontinuing infliximab may ultimately relapse.

As we have seen with infliximab primary response, no genetic markers have been predictive of CD response to adalimumab. In a recent study assessing such a response to adalimumab, the same three NOD2 polymorphisms (Arg702Trp, Gly908Arg, Leu1007fs insC), in addition to TLR4 and CD14 mutations, were analyzed for 24 Spanish CD patients who had lost response to, or were unable to tolerate infliximab[25]. No association with any of these polymorphisms for the adalimumab response was noted.

There is a complex and not yet well-understood relationship between gene expression and disease behavior; indeed many genes remain to be identified in the case of CD. Of the genes identified so far, no variants have adequate sensitivity and specificity to be used in clinical decision making with biologic agent response[26]. Perhaps attention should be turned to pharmacogenetic studies, as despite being given optimal doses and duration of medications, 20 to 30 percent of IBD patients find their disease refractory to any given medication[26]. Currently, the only genetic test available in guiding clinical treatment decisions is that used for assessing risk of hematopoeitic toxicity from metabolism of thiopurine analogues by the enzyme thiopurine methyl transferase, which is under genetic control[27]. Polymorphisms of proteins targeted by drugs or enzymes involved in drug metabolism may be potential areas of useful study. However, limitations related to heterogeneity in IBD, such as disease severity, behaviour, and environmental factors affecting drug response, also make this a challenging area.

In the case of other autoimmune conditions such as rheumatoid arthritis, advancements have been made in predicting response to infliximab, specifically using whole-genome gene expression analysis with microarrays[27]. Julià *et al*[28] have presented an 8-gene model obtained from whole blood RNA samples with an 85.7% prediction accuracy of treatment response. However, like CD, no studies in rheumatoid arthritis have examined the genotypes responsible for relapse or remission after anti-TNF-α therapy has been stopped.

In conclusion, this study found that, in a cohort of infliximab-treated CD patients who had a sustained corticosteroid-free clinical remission and then discontinued infliximab, there are two groups; those who relapsed early usually within a year, and those who have a sustained clinical remission of up to a decade. Furthermore, this study was the first to attempt to identify genetic polymorphisms in these two subgroups, though the findings suggest no specific correlations for the polymorphisms tested.

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COMMENTS

Background

The etiology of Crohn's disease (CD) remains unclear and as with most complex-trait diseases, it involves a combination of genetic and environmental risk factors. Although over 100 genetic loci have been associated with inflammatory bowel disease (IBD) thus far, only two genes, *IBD5* and caspase activation recruitment domain 15 (*CARD15*), have reliably been linked to CD susceptibility. In terms of CD itself, a potent proinflammatory cytokine, tumor necrosis factor alpha (TNF-α), is a well-known mediator of inflammation. Furthermore, the appropriate duration of anti-TNF-α therapy for CD patients who are in remission is as yet undefined; specifically, the factors which affect the duration of sustained clinical benefit after discontinuation of infliximab remain poorly understood. This lends support to the possibility that a patient-unique genotype of CD may play a role in determining the duration of long-term remission after the discontinuation of anti-TNF-α agent therapy. Previous studies have analyzed genetic differences in infliximab responders and compared the results to infliximab non-responders, but the patient population does not include patients who have all initially responded to infliximab such as in this study.

Research frontiers

In the area of identifying genetic factors responsible for infliximab responses, the research hotspot has mainly focused on nucleotide-binding oligomerization domain 2 (*NOD2*)/*CARD15* genotypes and few studies have analyzed TNF-α receptor and promoter region polymorphisms. No genetic markers have been definitively predictive of CD response to infliximab.

Innovations and breakthroughs

A breakthrough in the area has been that over 100 genetic loci have been associated with IBD and thus far, two genes, *IBD5* and *CARD15*, have reliably been linked to CD susceptibility. However, few genes have been proven to influence response to infliximab. Utilizing a well-defined unique CD patient population who were in an infliximab-induced corticosteroid-free clinical remission and then discontinued infliximab for reasons other than non-response, the authors studied two genetic variants of the IBD5 locus on chromosome 5q31 (IGR 2060a1 and IGR3081a1) along with three *NOD2*/*CARD15* polymorphisms (Arg702Trp, Gly908Arg, Leu1007fs insC), in those patients who had relapsed rapidly and compared them to those patients who remained in a long-term sustained remission. These patients experienced either a sustained remission up to a decade or have relapsed rapidly often within a year. The study results show no significant increase in frequency of the *NOD2*/*CARD15* and *IBD5* polymorphisms for either group of CD patients. This study was the first to attempt to identify genetic differences in these two unique subgroups.

Applications

There is a complex and not yet well-understood relationship between gene expression and disease behavior. Anti-TNF-α agents such as infliximab are costly, and the long-term safety risks can be concerning in the long term. Furthermore, the appropriate duration of anti-TNF-α therapy for CD patients who are in remission is as yet undefined; specifically, no genes have been definitively identified to predict which CD patients who discontinued their infliximab while in full corticosteroid-free clinical remission might either have a sustained remission or will relapse rapidly after stopping infliximab.

Terminology

CARD15 was the first gene to be associated with CD; The *NOD2*/*CARD15* product is an intracellular protein involved in recognizing bacterial lipopolysaccharides in the nuclear factor kappa B-TNF-α pathway. This *NOD2*/*CARD15* gene is known to be associated with susceptibility to CD. IBD5 is a 250 kb haplotype in the 5q31 gene cluster associated with increased risk of CD.

Peer review

This is an interesting study comparing genetic differences between Crohn's disease patients who after discontinuing their infliximab while in full corticosteroid-free clinical remission, experienced a sustained remission versus those patients whose disease relapsed rapidly.

REFERENCES

1 **Bouma G**, Strober W. The immunological and genetic basis of inflammatory bowel disease. *Nat Rev Immunol* 2003; **3**: 521-533
2 **Satsangi J**, Morecroft J, Shah NB, Nimmo E. Genetics of inflammatory bowel disease: scientific and clinical implications. *Best Pract Res Clin Gastroenterol* 2003; **17**: 3-18
3 **Russell RK**, Satsangi J. IBD: a family affair. *Best Pract Res Clin Gastroenterol* 2004; **18**: 525-539
4 **Cho JH**, Brant SR. Recent insights into the genetics of inflammatory bowel disease. *Gastroenterology* 2011; **140**: 1704-1712
5 **Su C**, Lichtenstein GR. Are there predictors of Remicade treatment success or failure? *Adv Drug Deliv Rev* 2005; **57**: 237-245
6 **Hanauer SB**, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, Rachmilewitz D, Wolf DC, Olson A, Bao W, Rutgeerts P. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* 2002; **359**: 1541-1549
7 **Sands BE**, Anderson FH, Bernstein CN, Chey WY, Feagan BG, Fedorak RN, Kamm MA, Korzenik JR, Lashner BA, Onken JE, Rachmilewitz D, Rutgeerts P, Wild G, Wolf DC, Marsters PA, Travers SB, Blank MA, van Deventer SJ. Infliximab maintenance therapy for fistulizing Crohn's disease. *N Engl J Med* 2004; **350**: 876-885
8 **Afif W**, Loftus EV, Faubion WA, Kane SV, Bruining DH, Hanson KA, Sandborn WJ. Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. *Am J Gastroenterol* 2010; **105**: 1133-1139
9 **Colombel JF**, Loftus EV, Tremaine WJ, Egan LJ, Harmsen WS, Schleck CD, Zinsmeister AR, Sandborn WJ. The safety profile of infliximab in patients with Crohn's disease: the Mayo clinic experience in 500 patients. *Gastroenterology* 2004; **126**: 19-31
10 **Hamzaoglu H**, Cooper J, Alsahli M, Falchuk KR, Peppercorn MA, Farrell RJ. Safety of infliximab in Crohn's disease: a large single-center experience. *Inflamm Bowel Dis* 2010; **16**: 2109-2116
11 **Waugh AW**, Garg S, Matic K, Gramlich L, Wong C, Sadowski DC, Millan M, Bailey R, Todoruk D, Cherry R, Teshima CW, Dieleman L, Fedorak RN. Maintenance of clinical benefit in Crohn's disease patients after discontinuation of infliximab: long-term follow-up of a single centre cohort. *Aliment Pharmacol Ther* 2010; **32**: 1129-1134
12 **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, Nuñez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; **411**: 603-606
13 **Mederos A**, Lamberg-Karlovsky CC. Converting currencies in the Old World. *Nature* 2001; **411**: 437
14 **Van Limbergen J**, Wilson DC, Satsangi J. The genetics of Crohn's disease. *Annu Rev Genomics Hum Genet* 2009; **10**: 89-116
15 **Inohara N**, Ogura Y, Chen FF, Muto A, Nuñez G. Human Nod1 confers responsiveness to bacterial lipopolysaccharides. *J Biol Chem* 2001; **276**: 2551-2554
16 **Taylor KD**, Plevy SE, Yang H, Landers CJ, Barry MJ, Rotter JI, 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
17 **Vermeire S**, Louis E, Rutgeerts P, De Vos M, Van Gossum A, Belaiche J, Pescatore P, Fiasse R, Pelckmans P, Vlietinck R, Merlin F, Zouali H, Thomas G, Colombel JF, Hugot JP. NOD2/CARD15 does not influence response to infliximab in Crohn's disease. *Gastroenterology* 2002; **123**: 106-111
18 **Mascheretti S**, Hampe J, Croucher PJ, Nikolaus S, Andus T, Schubert S, Olson A, Bao W, Fölsch UR, Schreiber S. Response to infliximab treatment in Crohn's disease is not associated with mutations in the CARD15 (NOD2) gene: an analysis in 534 patients from two multicenter, prospective GCP-level trials. *Pharmacogenetics* 2002; **12**: 509-515
19 **Urcelay E**, Mendoza JL, Martinez A, Fernandez L, Taxonera C, Diaz-Rubio M, de la Concha EG. IBD5 polymorphisms in inflammatory bowel disease: association with response to infliximab. *World J Gastroenterol* 2005; **11**: 1187-1192
20 **Sadowski DC**, Bernstein CN, Bitton A, Croitoru K, Fedorak RN, Griffiths A. Canadian Association of Gastroenterology Clinical Practice Guidelines: The use of tumour necrosis factor-alpha antagonist therapy in Crohn's disease. *Can J Gastroenterol* 2009; **23**: 185-202
21 **Rioux JD**, Daly MJ, Silverberg MS, Lindblad K, Steinhart H, Cohen Z, Delmonte T, Kocher K, Miller K, Guschwan S, Kulbokas EJ, O'Leary S, Winchester E, Dewar K, Green T, Stone V, Chow C, Cohen A, Langelier D, Lapointe G, Gaudet D, Faith J, Branco N, Bull SB, McLeod RS, Griffiths AM, Bitton A, Greenberg GR, Lander ES, Siminovitch KA, Hudson TJ. Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. *Nat Genet* 2001; **29**: 223-228
22 **Waschke KA**, Villani AC, Vermeire S, Dufresne L, Chen TC, Bitton A, Cohen A, Thomson AB, Wild GE. Tumor necrosis factor receptor gene polymorphisms in Crohn's disease: association with clinical phenotypes. *Am J Gastroenterol* 2005; **100**: 1126-1133
23 **Louis E**, Vermeire S, Rutgeerts P, De Vos M, Van Gossum A, Pescatore P, Fiasse R, Pelckmans P, Reynaert H, D'Haens G, Malaise M, Belaiche J. A positive response to infliximab in Crohn disease: association with a higher systemic inflammation before treatment but not with -308 TNF gene polymorphism. *Scand J Gastroenterol* 2002; **37**: 818-824
24 **Pierik M**, Vermeire S, Steen KV, Joossens S, Claessens G, Vlietinck R, Rutgeerts P. Tumour necrosis factor-alpha receptor 1 and 2 polymorphisms in inflammatory bowel disease and their association with response to infliximab. *Aliment Pharmacol Ther* 2004; **20**: 303-310
25 **Barreiro-de Acosta M**, Ouburg S, Morré SA, Crusius JB, Lorenzo A, Potel J, Salvador-Peña A, Domínguez-Muñoz JE. NOD2, CD14 and TLR4 mutations do not influence response to adalimumab in patients with Crohn's disease: a preliminary report. *Rev Esp Enferm Dig* 2010; **102**: 591-595
26 **Vermeire S**, Van Assche G, Rutgeerts P. Role of genetics in prediction of disease course and response to therapy. *World J Gastroenterol* 2010; **16**: 2609-2615
27 **Weinshilboum RM**, Sladek SL. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *Am J Hum Genet* 1980; **32**: 651-662
28 **Julià A**, Erra A, Palacio C, Tomas C, Sans X, Barceló P, Marsal S. An eight-gene blood expression profile predicts the response to infliximab in rheumatoid arthritis. *PLoS One* 2009; **4**: e7556

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