INFLAMMATORY BOWEL DISEASE

Early development of stricturing or penetrating pattern in Crohn's disease is influenced by disease location, number of flares, and smoking but not by NOD2/CARD15 genotype

E Louis, V Michel, J P Hugot, C Reenaers, F Fontaine, M Delforge, F El Yafi, J F Colombel, J Belaiche

Gut 2003;**52**:552–557

See end of article for authors' affiliations

Correspondence to: E Louis, Service de Gastroentérologie, CHU de Liège, 4000 Liège, Belgium; edouard.louis@ulg.ac.be

Accepted for publication 27 November 2002

Background: Crohn's disease is a heterogeneous entity. Disease behaviour, characterised as stricturing, penetrating, or non-stricturing non-penetrating, is a clinically important phenotype as it is associated with complications and need for surgery. It has recently been showed that the behaviour of Crohn's disease changes over the course of the disease.
Aim: To assess the association between rapid development of a penetrating or stricturing pattern of Crohn's disease and demographic and clinical characteristics as well as NOD2/CARD15 genotype.
Patients and methods: A total of 163 patients with a firm diagnosis of Crohn's disease and who had non-penetrating non-stricturing disease at diagnosis were studied. Various demographic and clinical characteristics as well as antisaccharomyces cerevisiae antibody status and NOD2/CARD15 genotype were documented in these patients. These characteristics were compared in subgroups of patients according to evolution of disease behaviour five years after diagnosis.
Results: Five years after diagnosis there were 110 (67.5%) patients with non- structuring non-penetrating disease, 18 (11%) with stricturing disease, and 35 (21.5%) with penetrating disease. In multivariate analysis, only disease location and number of flares per year were significantly discriminant between the three subgroups (p=0.0009 and 0.0001, respectively). Ileal location of the disease was associated with a stricturing pattern while a high number of flares was associated with a penetrating pattern. Active smoking was also associated with a penetrating pattern compared with a non-stricturing non-penetrating pattern only.
Conclusions: Early development of stricturing or penetrating behaviour in Crohn's disease is influenced by disease location, clinical activity of the disease, and smoking habit, but not by NOD2/CARD15 genotype.

Crohn's disease (CD) is a multifactorial polygenic disease characterised by chronic inflammation of the gastrointestinal tract.[1] Several diagnostic criteria have been proposed, usually relying on clinical, endoscopic, radiological, or histological criteria.[2–4] So defined however, CD is still a heterogeneous entity. The disease can present in various forms, for example depending on its location, its inclination to develop anatomical complications, or response to treatment.[5] Recently, NOD2/CARD15 has been disclosed as the first gene associated with CD by two independent groups[6 7] and rapidly confirmed by others.[8] Three independent mutations within NOD2/CARD15 (one frameshift mutation (1007fs) and two missense mutations (R702W and G908R)) have been found to be associated with CD in Caucasians. However, from recent data, it appears that only 25–43% of CD patients carry at least one of the identified NOD2/CARD15 mutations.[9–11] These data confirm and emphasise the heterogeneity of CD. Among the multiple subphenotypes proposed for CD, age at diagnosis, location, and pathological behaviour (including development of penetrating or stricturing lesions) have recently been considered by a panel of experts as the most relevant and potentially genetically determined, and were therefore included in the newly proposed "Vienna classification" of CD (table 1).[12] NOD2/CARD15 variants have been recently associated both with young age at onset[9 10 13 14] and small bowel location.[9–11 13 15]

The behaviour of CD is clinically relevant as it is associated with the development of complications and need for surgery.

Table 1 Vienna classification of Crohn's disease

Age at diagnosis	A1, <40 years
	A2, >40 years
Location*	L1, terminal ileum†
	L2, colon
	L3, ileocolon
	L4, upper gastrointestinal tract‡
Behaviour	B1, non-stricturing non penetrating
	B2, stricturing¶
	B3, penetrating§

*Maximum extent at any time before first resection.
†Limited to the terminal ileum with or without spill over into the caecum.
‡Any disease location proximal to the terminal ileum regardless of additional involvement.
¶Occurrence of constant luminal narrowing (radiological, endoscopic, or surgical) with prestenotic dilatation or obstructive signs, without penetrating disease, at any time in the course of the disease.
§Occurrence of intra-abdominal or perianal fistulas, inflammatory masses, and/or abscesses at any time in the course of the disease. Perianal ulcers, but not skintags, are also included.

Abbreviations: CD, Crohn's disease; ASCA, antisaccharomyces cerevisiae antibodies; PCR, polymerase chain reaction.

We have recently shown that the behaviour of CD changes over the course of the disease.[16] The majority of patients present with non- stricturing non-penetrating disease at diagnosis while after 25 years the majority harbour either a stricturing or penetrating pattern. This changing behaviour may still be determined by both genetic and environmental factors that may influence its inclination and speed of evolution. An association between a stricturing pattern and NOD2/CARD15 variants has recently been suggested[9 17 18] but the NOD2/CARD15 genotype was not uniformly found as an independent factor.[10 13 19] The only environmental factor clearly associated with CD is active smoking. Smoking is significantly more frequent in CD than in healthy controls.[20] Smoking has also been associated with more aggressive disease[21 22] but not with other clinical or biological characteristics, particularly CD behaviour. Besides smoking, the demographic and clinical characteristics of the disease, including treatments used, may influence its changing behaviour. Antisaccharomyces cerevisiae antibodies (ASCA) represent the main serological marker associated with CD.[23 24] ASCA have not been definitively associated with particular features of CD although an increased prevalence has been described in small bowel CD[25 26] and higher levels have been suggested in disease with early onset, as well as fibrostenosing and internal penetrating disease behaviours.[26] Overall, these data suggesting an association between NOD2/CARD15 or ASCA and disease behaviour are flawed by different definitions of disease behaviour and by the absence of consideration of evolution of this behaviour over time.

The aim of our work was to look for an association between evolution of CD behaviour, defined according to the validated Vienna classification, over the first five years after diagnosis on the one hand and NOD2/CARD15 genotype, smoking habit, ASCA, and various clinical and demographic characteristics on the other.

PATIENTS AND METHODS

Patients

A total of 163 patients with a firm diagnosis of CD according to the criteria of Lennard- Jones,[2] classified as non-stricturing non-penetrating disease (B1) at diagnosis based on the Vienna classification,[12] and regularly followed at our institution (CHU Liège, Clinique St Joseph Liège) were studied. Patients gave informed consent for the study. These patients formed part of the population in which we previously studied evolution of CD characteristics over time according to the Vienna classification.[16] For all of these patients, we recorded age at diagnosis, location and behaviour of the disease at diagnosis and after five years of evolution. After five years, according to the Vienna classification, CD was classified as: B3 in the case of intra-abdominal fistulising lesions or the existence of perianal disease (including abscess, fistula, or Crohn's fissure); B2 when significant strictures were present in the absence of penetrating disease; and B1 in the absence of stricturing or penetrating lesions.

Patients were defined as smokers if they smoked at least seven cigarettes/week and non- smokers if they had never smoked or had stopped smoking before diagnosis of CD. Information on smoking was available in 127 patients. Three of these had stopped smoking after the onset of CD and were excluded from the analysis.

A familial form of the disease was defined as the presence of an inflammatory bowel disease in a relative, whatever the parental degree. This information was collected, asking the patient whether he had a relative with CD or ulcerative colitis. If possible, the relative was then personally contacted to confirm these data. In case of doubt, the general practitioner or gastroenterologist in charge of the patient was also contacted. This information was regularly updated during follow up.

ASCA status was defined in 90 patients for whom we had a serum sample dating back to the time of diagnosis, by commercial ELISA (Medipan Diagnostica, Selchow, Germany), according to the manufacturer's instructions. Investigators who determined ASCA were blinded to the behaviour of the disease. Patients were considered as ASCA+ when either ASCA IgA or IgG was present.

The number of flares per year was evaluated retrospectively based on patient medical notes. A flare was considered when the physician in charge of the patient (EL, FF, MD, JB) documented it in the notes and changed the treatment accordingly. As patients had exclusively non-stricturing non-penetrating disease, these flares were characterised by a combination of digestive and general symptoms, including diarrhoea, abdominal pain, fever, weight loss, worsening of the general physical condition, or systemic manifestations. The number of flares was calculated until the development of stricturing or penetrating disease or over the first five years of the disease for patients who remained non-stricturing non- penetrating. A mean number of flares per year was then calculated for every patient. The information in the medical notes was available and judged to be reliable for 126 patients.

The number of steroid courses was also determined retrospectively from patient medical notes. As for flares, the number of steroid courses was calculated until the development of stricturing or penetrating lesions or over the first five years of the disease for patients remaining non-stricturing non-penetrating. A mean number of steroid courses per year was then calculated for every patient. The information in the medical notes was available and judged to be reliable for 126 patients.

Reliable information on immunosuppressive treatment was available in 145 patients. Only 24 patients had taken azathioprine during the first five years of the disease. Sixteen had started azathioprine after the development of penetrating disease. The remaining eight patients received azathioprine because of steroid dependent or chronic active disease. In these patients, the number of months of azathioprine treatment before the development of a penetrating or stricturing disease or over the first five years of the disease for those who remained non-penetrating non-stricturing was calculated.

Demographic and clinical characteristics of the patients are shown in table 2.

NOD2/CARD15 genotyping

In 98 patients, DNA was collected and genotyped for the three main variants of NOD2/CARD15 that are associated with CD (OMIM 605956), as previously defined.[6] In this initial report, the nomenclature of mutations was derived from the sequence of IBD1. This sequence is identical to the smaller open reading frame of NOD2/CARD15 described by Ogura and colleagues.[7] However, an alternative open reading frame, characterised by a translation initiation site located 81 nucleotides above the smaller one, was also reported by Ogura *et al*. Others have recently proposed this sequence as the sequence-reference for mutation annotation.[8] In order to avoid confusion, we therefore used this new method of annotation in the 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, GenBank accession No 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. Genotypes were directly deduced from the migration profiles. The missense mutation G908R (ex G881R) (SNP12,

Table 2 Characteristics of the patients

Age at diagnosis (%) (n=163)	
<40 years (A1)	85.4
>40 years (A2)	14.6
Location of disease (%) (n=163)	
Ileal (L1)	45.4
Colonic (L2)	27.6
Ileocolonic (L3)	22.1
Upper gastrointestinal tract (L4)	4.9
Sex (%) (n=163)	
Male	33.7
Female	66.3
Smoking habit (%) (n=124)	
Yes	52.4
No	47.6
Familial disease (%) (n= 156)	
Yes	16.0
No	84.0
ASCA (%) (n=90)	
Positive	62.2
Negative	37.8
NOD2/CARD15 (%) (n=101)	
Wild-type	54.5
Variant	45.5
Steroid courses (No/year) (n=126)	0.4 (0.53)*
Flares of CD (No/year) (n=126)	0.89 (0.8)*
Azathioprine treatment (months/year) (n=145)	0.25 (0.1)*

CD, Crohn's disease; ASCA, antisaccharomyces cerevisiae antibodies.
*Mean (SD).

GenBank accession No 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) and electrophoresed on a 2% agarose gel. The profile of the G908R variant was characterised by two bands (138 bp and 242 bp, respectively). For the frameshift mutation 1007fs (SNP13, GenBank accession No G67955), PCR products using 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. Genotypes were deduced from the sizes of the PCR products: 230 bp (wild-type) and 231bp (1007fs, ex 980fs). Investigators who determined NOD2/CARD15 genotypes were blinded to the behaviour of the disease.

Statistical analysis

Statistical analyses were performed using SAS 6.12 software. We initially used univariate analysis to study the association between evolution of disease behaviour (determined by disease behaviour five years after diagnosis—B1, B2, or B3) and each of the described factors, using either a χ^2 or Kruskal-Wallis test, as required. In addition, a subgroup analysis was performed for patients who became B3 (penetrating disease): these patients were subdivided into intra-abdominal penetrating disease and perianal penetrating disease.

A multivariate analysis was then performed using stepwise discriminant analysis. This multivariate analysis was performed on a subgroup of 83 patients for whom we had complete data. A p value <0.05 was considered significant.

RESULTS

Disease behaviour evolution

After five years of evolution, the behaviour of CD was classified as still non-stricturing non-penetrating (B1) in 110 patients (67.5%), stricturing (B2) in 18 patients (11.0%), and penetrating (B3) in 35 patients (21.5%). Demographic and clinical characteristics, as well as NOD2/CARD15 genotype and ASCA status in these subgroups are showed in table 3.

Association between evolution of CD behaviour (B1, B2, or B3) and demographic, clinical, and biological characteristics: univariate analysis

In univariate analysis, disease location, number of flares per year, number of steroid courses per year, and ASCA status were significantly different between the three groups, while smoking and familial form were borderline for significance (table 3). There was no association between NOD2/CARD15 genotype (defined as variant carriers or not, or as wild- type, simple heterozygotes, compound heterozygotes, and homozygotes) and evolution of CD behaviour (table 3). There was no association between various NOD2/CARD15 variants and evolution of CD behaviour.

Association between evolution to subtypes of penetrating disease (B3) and demographic, clinical, and biological characteristics: univariate analysis

When intra-abdominal and perianal penetrating disease were analysed separately, significant differences were found. Location at diagnosis differed significantly between these groups, with proportions of L1, L2, L3, and L4 being 83.3%, 8.3%, 8.3%, and 0%, and 13.3%, 40%, 40%, and 6.7% in intra-abdominal and perianal penetrating disease, respectively ($p=0.004$). When perianal penetrating diseases were excluded from the group of penetrating disease (B3), the association between location of disease and disease behaviour was still significant ($p<0.0001$). Smoking was significantly more frequent in perianal (86.7%) than in intra-abdominal (45.5%) penetrating disease ($p=0.03$). However, the association between smoking and evolution of disease behaviour was still borderline significant when perianal penetrating disease was excluded from the group of penetrating disease ($p=0.057$). No other significant difference was found between intra-abdominal and perianal penetrating disease. As regards the association between evolution of disease behaviour and clinical, demographic, or biological characteristics, when those with perianal penetrating disease were excluded from the group of penetrating disease, apart from location of disease and smoking habit, the association also remained significant for the number of flares per year (still higher in penetrating disease ($p=0.001$)) while there was no significant association for the other parameters.

Association between evolution of disease behaviour (B1, B2, B3) and demographic, clinical, and biological characteristics: multivariate analysis

In multivariate analysis, only location of disease and number of flares per year were selected when comparing the three groups (p values for these parameters in the model were 0.0009 and 0.0001, respectively; the p value of the model was 0.0001). When patients who became B2 were compared with those who remained B1, only disease location was selected (p value of the model 0.0001). When patients who became B3 were compared with those who remained B1, both the number of flares per year and smoking were selected (p values of these parameters in the model were 0.0001 and 0.02, respectively; the p value of the model was 0.0001). Finally, when patients who became B2 were compared with those who became B3, the number of flares per year, location, and existence of a familial form of the disease were selected (p values of these parameters in the model were 0.0004, 0.001, and 0.04, respectively; the p value of the model was 0.0001).

DISCUSSION

We previously showed that CD behaviour changed over the course of the disease.[16] Theoretically, both genetic and environmental factors could influence this changing behaviour. The present data indicate that evolution of disease behaviour over the first five years is influenced by disease

Table 3 Demographic, clinical, and biological parameters depending on Crohn's disease behaviour*

	B1	B2	B3	p Value
Age at diagnosis (%) (n=163)	n=110	n=18	n=35	
<40 years (A1)	82.0	88.9	94.3	0.18
>40 years (A2)	18.0	11.1	5.7	
Location of the disease (%) (n=163)	n=110	n=18	n=35	
Ileal (L1)	38.2	89.9	45.7	0.0003
Colonic (L2)	33.6	0	22.9	
Ileocolonic (L3)	23.6	0	28.6	
Upper gastrointestinal tract (L4)	4.6	11.1	2.9	
Sex (%) (n=163)	n=110	n=18	n=35	
Male	34.5	22.2	37.1	0.53
Female	65.5	77.8	62.9	
Smoking habit (%) (n=124)	n=79	n=16	n=29	
Yes	41.8	62.5	65.5	0.052
No	58.2	37.5	34.5	
Familial disease (%) (n= 156)	n=106	n=16	n=34	
Yes	17.0	31.3	5.9	0.07
No	83.0	68.7	94.1	
ASCA (%) (n=90)	n=54	n=14	n=22	
Positive	64.8	35.7	72.7	0.044
Negative	35.2	64.3	27.3	
NOD2/CARD15 (%) (n=101)	n=48	n=18	n=35	
Wild-type	47.9	44.4	68.6	0.34
Heterozygotes	39.6	38.9	22.8	
Homozygotes and compound heterozygotes	12.5	16.7	8.6	
Steroid courses (No/year) (n=126)	n=83	n=15	n=28	
	0.35 (0.48)*	0.23 (0.26)*	0.69 (0.71)*	0.026
Flares of CD (No/year) (n=126)	n=83	n=15	n=28	
	0.74 (0.77)*	0.68 (0.22)*	1.53 (0.82)*	<0.0001
Azathioprine treatment (months/year) (n=145)	n=99	n=16	n=30	
	0.25 (0.12)*	0.4 (0.4)*	0.16 (0.16)*	0.84

*B1, non-stricturing non-penetrating; B2, stricturing; B3, penetrating, according to the Vienna classification, five years after diagnosis.
p values correspond to univariate analysis for each parameter using either χ^2 or Kruskal Wallis tests, as required.
CD, Crohn's disease; ASCA, antisaccharomyces cerevisiae antibodies.
*Mean (SD).

location, number of flares per year, and smoking habit, but not by NOD2/CAD15 genotype.

The behaviour of CD represents a major characteristic of the disease. Penetrating and stricturing phenotypes are associated with the development of complications and need for surgery. An attempt to identify markers or factors that are associated with these phenotypes is motivated by the need to predict this behaviour to be able to select patients for appropriate early management. Such an association between phenotype and clinical, demographic, or genetic factors may also help to understand the mechanisms underlying phenotype development. Studies on disease behaviour have been hampered by the variability of classifications used[27–29] and by their unsatisfactory degree of inter-rater agreement.[30] More recently, the Vienna classification has been proposed which aims to stratify patients on the basis of widely accepted and reproducible criteria.[12] Its reproducibility has recently been confirmed to be good.[31] A certain degree of constancy of disease behaviour has been suggested by some authors on the basis of surgical indications.[27 32 33] The significantly conserved behaviour in multiply affected families has also suggested that this behaviour could be at least partly genetically determined.[34–36] However, using the Vienna classification, and carefully reviewing the medical history of individual patients, we have demonstrated a dramatic change in disease behaviour over the course of the disease.[16] This is not necessarily in contradiction with previous studies but indicates that the disease behaviour of CD cannot be analysed without taking into account the duration of disease. After a certain duration of disease however, some characteristics of disease behaviour may be more stable or even fixed.[16] To analyse the factors potentially involved in disease behaviour determinism, we studied a homogeneous population of uncomplicated disease (B1) at diagnosis and over a predefined duration of follow up. We looked at rapid development of penetrating or stricturing disease (within five years) to identify factors strongly influencing the development of such complications.

The two main factors independently associated with change in disease behaviour within five years after diagnosis were ileal location of the disease and number of flares per year. Ileal location was essentially associated with the development of stricturing disease and was not a discriminant factor between penetrating disease and non-stricturing non-penetrating disease when only these two phenotypes were compared. However, when penetrating disease was divided into intra-abdominal and perianal disease, ileal location was significantly more frequent in intra-abdominal than perianal penetrating disease and it reached, in the first subgroup, a frequency similar to that observed in stricturing disease. This association between stricturing disease or intra-abdominal penetrating disease and ileal location has been described in studies of disease behaviour based on surgical series, irrespective of the duration of disease.[27 37] One explanation for the association with stricturing disease may be the smaller diameter of the gut lumen in the ileum than in the colon, making functionally significant strictures more likely to occur. Another explanation could be the nature of the immuno-inflammatory reaction in the ileum compared with the colon, but such a difference has not yet been clearly demonstrated, although the number of Peyer's patches is greater in the ileum than in the colon and may influence the mucosal immuno-inflammatory reaction.[38] The development of intra-abdominal penetrating lesions may in itself be favoured by downstream strictures in a subset of patients.[39] A high number of flares of the disease per year was essentially associated with the development of penetrating disease (intra-abdominal as well as perianal penetrating disease) and was not a discriminant factor between stricturing and non-stricturing non-penetrating disease when only these two phenotypes were compared. Again, this is not unexpected as penetrating disease is often a

clinically more aggressive disease. The development of penetrating lesions could be a consequence of the severity of the inflammatory reaction at the mucosal level. In contrast, stricturing disease can often be silent for a long time, manifesting only when the stricture has developed.

Active smoking was more frequent in patients who developed penetrating but also stricturing disease. It was independently associated with the development of penetrating disease when patients who developed penetrating disease and patients who remained non- stricturing non-penetrating were compared. This association between smoking and penetrating disease was particularly pronounced for perianal penetrating disease. Smoking has previously been associated with more aggressive CD[21 22] but not specifically with penetrating or perianal complications. The effect of smoking could have been related to an effect on disease severity and number of flares. This association was however independent in the present study. The mechanism by which smoking may favour penetrating lesions, as well as more aggressive disease, remains to be elucidated.

Globally, as previously discussed, factors associated with intra-abdominal or perianal penetrating disease are not the same. Indeed, if a more aggressive disease (defined by the annual number of flares) seems to be associated with both subtypes of penetrating disease, it is not the case for ileal location, essentially associated with intra-abdominal penetrating disease, or for active smoking, more associated with perianal disease. These data suggest mechanisms at least partly different for the development of these two types of penetrating disease. Furthermore, in our series, only a small minority of patients harboured both intra- abdominal and perianal penetrating disease. Therefore, they maybe represent distinct entities and should perhaps be studied independently.

When patients who developed stricturing and those who developed penetrating lesions were compared, independent discriminating factors were the number of flares (higher in penetrating disease, as already discussed) and ileal location, as well as the familial form of the disease (more frequent in patients who developed stricturing lesions). The association between stricturing disease and familial disease has already been described but was thought to be mainly linked to ileal location and early age at onset.[40] Interestingly, a familial history of the disease was associated with stricturing behaviour in our study independent of ileal location or age at onset.

In the univariate analysis, ASCA were significantly more frequent in patients with penetrating lesions. An association had already been suggested between ASCA and the penetrating phenotype.[26] In the present study however, this association was not independent, indicating that ASCA status does not represent a significant marker associated with disease behaviour. A similar conclusion can be drawn for steroid use, associated in the univariate analysis with penetrating behaviour but no longer present after multivariate analysis. This is probably due to the correlation between the number of flares independently associated with disease behaviour and steroid use. With regard to treatment, we found no association between immunosuppressive treatment and disease behaviour. Very few patients were receiving immunosuppressive treatments however, mainly because we studied the early phase of the disease up to five years after diagnosis and because immunosuppressive treatments are often started after the development of penetrating lesions. The influence of immunosuppressive treatments or biotherapies on disease behaviour should be an area of study in a larger sample of treated patients.

Importantly, in this study we found no association between NOD2/CARD15 variants and disease behaviour. This is in contrast with reported studies.[9 10 17–19] However, in those previous studies, results were partly discordant, criteria used to classify disease behaviour were inhomogeneous, and duration of disease before definition of disease behaviour was not taken into account. It must also be emphasised that the recently documented association between NOD2/CARD15 mutations and ileal location may induce secondary association with stricturing behaviour.[10] Therefore, we believe that these previous data are inconclusive. Our study is the first to have carefully analysed this point, with a validated classification, and we can conclude that there is no major influence of NOD2/CARD15 genotype on CD behaviour. However, we cannot exclude a small influence, acting slowly and having an impact on disease behaviour after a longer period of time. This should be tested in an adequately designed study.

In conclusion, we have shown that ileal location of CD and aggressive disease with a high number of flares were independently associated with rapid development of stricturing and penetrating disease, respectively. Smoking habit was also significantly associated with the development of penetrating behaviour while there was no association between NOD2/CARD15 genotype and evolution of disease behaviour. When studying potential associations between genetic markers and disease behaviour in CD, patients should be stratified according to disease location, disease aggressiveness, and also probably smoking habit.

ACKNOWLEDGEMENTS

E Louis is a Research Associate at the FNRS Belgium

Authors' affiliations
E Louis, V Michel, C Reenaers, F El Yafi, J Belaiche, Department of Gastroenterology, University Hospital of Liège, Belgium
J P Hugot, Fondation Jean Dausset CEPH, Paris, France
F Fontaine, M Delforge, Department of Gastroenterology, Clinique St Joseph Liège, Belgium
J F Colombel, Department of Gastroenterology, CHU Lille, France

REFERENCES

1 **Fiocchi C**. Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology* 1998;**115**:182–205.
2 **Lennard-Jones JE**. Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1989;**24**:2–6.
3 **Gower-Rousseau C**, Salomez JL, Dupas JL, *et al*. Incidence of inflammatory bowel disease in Northern France (1988–1990). *Gut* 1994;**35**:1433–8.
4 **Jenkins D**, Balsitis M, Gallivan S, *et al*. Guidelines for the initial biopsy diagnosis of suspected chronic idiopathic inflammatory bowel disease. The British Society of Gastroenterology Initiative. *J Clin Pathol* 1997;**50**:93–105.
5 **Sutherland LR**. Different patterns of Crohn's disease. In: Prantera C, Korelitz B, eds. *Crohn's Disease*. New York: Marcel Dekker Inc, 1996:201–15.
6 **Hugot JP**, Chamaillard M, Zouali H, *et al*. Association of NOD2 leucin-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001;**411**:599–603.
7 **Ogura Y**, Bonen DK, Inohara N, *et al*. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001;**411**:603–6.
8 **Hampe J**, Cuthbert A, Crouche PJP, *et al*. Association between insertion mutation in NOD2 gene and Crohn's disease in German and British population. *Lancet* 2001;**357**:1925–8.
9 **Lesage S**, Zouali H, Cezard JP, *et al*. CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *Am J Hum Genet* 2002;**70**:845–57.
10 **Ahmad T**, Armuzzi A, Bunce M, *et al*. The molecular classification of the manifestations of Crohn's disease. *Gastroenterology* 2002;**122**:854–66.
11 **Cuthbert AP**, Fisher SA, Mirza MM, *et al*. The contribution of NOD2 gene mutations to the risk and site of disease in inflammatory bowel disease. *Gastroenterology* 2002;**122**:867–74.
12 **Gasche C**, Scholmerich J, Brynskov J, *et al*. A simple classification of Crohn's disease: report of the working party of the world congresses of gastroenterology, Vienna 1998. *Inflamm Bowel Dis* 2000;**6**:8–15.
13 **Louis E**, Hugot JP, Colombel JF, *et al*. NOD2/CARD15 mutations in Crohn's disease are associated with young age at diagnosis and ileal location. *Gastroenterology* 2002;**122**(suppl):M1405.
14 **Eri RD**, Pandeya N, Purdie D, *et al*. Frequency and association of the 3020INSC insertion in the NOD2 Crohn disease susceptibility gene in a well-characterized patient cohort. *Gastroenterology* 2002;**122**(suppl):M1410.
15 **Vermeire S**, Esters N, Pierik M, *et al*. Detailed study on phenotypical associations of NOD2/CARD15 mutations in Crohn's disease. *Gastroenterology* 2002;**122**(suppl):M1413.

16 **Louis E**, Collard A, Oger AF, *et al*. Behaviour of Crohn's disease according to the Vienna classification: changing pattern over the course of the disease. *Gut* 2001;**49**:777–82.
17 **Abreu MT**, Taylor KD, Lin YC, *et al*. Mutations in NOD2 are associated with fibrostenosing disease in patients with Crohn's disease. *Gastroenterology* 2002;**123**:679–88.
18 **Radlmayr M**, Török HP, Martin K, *et al*. The c-insertion mutation of the NOD2 gene is associated with fistulizing and fibrostenotic phenotypes in Crohn's disease. *Gastroenterology* 2002;**122**:2091–2.
19 **Hampe J**, Grebe J, Nikolaus S, *et al*. Association of NOD2 (CARD15) genotype with clinical course of Crohn's disease cohort study. *Lancet* 2002;**359**:1661–5.
20 **Calkins BM**. A meta-analysis of the role of smoking in inflammatory bowel disease. *Dig Dis Sci* 1989;**34**:1841–54.
21 **Sutherland LR**, Ramcharan S, Bryant H, *et al*. Effect of cigarette smoking on recurrence of Crohn's disease. *Gastroenterology* 1990;**98**:1123–8.
22 **Cosnes J**, Carbonnel F, Beaugerie L, *et al*. Effects of smoking on the long term course of Crohn's disease. *Gastroenterology* 1996;**110**:424–31.
23 **Main J**, McKenzie H, Yeaman GR, *et al*. Antibody to Saccharomyces cerevisiae (bakers' yeast) in Crohn's disease. *BMJ* 1988;**297**:1105–6.
24 **Sendid B**, Colombel JF, Jacquinot PM, *et al*. Specific antibody response to oligomannosidic epitopes in Crohn's disease. *Clin Diagn Lab Immunol* 1996;**3**:219–26.
25 **Quinton JF**, Sendid B, Reumaux D, *et al*. Anti-Saccharomyces cerevisiae mannan antibodies combined with anti-neutrophil cytoplasmic antibodies in inflammatory bowel disease: prevalence and diagnostic role. *Gut* 1998;**42**:788–91.
26 **Vasiliauskas EA**, Kam LY, Karp LC, *et al*. Marker antibody expression stratifies Crohn's disease into immunologically homogeneous subgroups with distinct clinical characteristics. *Gut* 2000;**47**:487–96.
27 **Greenstein AJ**, Lachman P, Sachar DB, *et al*. Perforating and non-perforating indications for repeated operations in Crohn's disease: evidence for two clinical forms. *Gut* 1988;**29**:588–92.
28 **Sachar DB**, Andrews HA, Farmer RG, *et al*. Proposed classification of patient subgroups in Crohn's disease. *Gastroenterol Int* 1992;**5**:141–54.
29 **Greenway SE**, Buckmire MA, Marroquin C, *et al*. Clinical subtypes of Crohn's disease according to surgical outcome. *J Gastrointest Surg* 1999;**3**:145–51.
30 **Steinhart AH**, Girgrah N, McLeod RS. Reliability of a Crohn's disease clinical classification scheme based on disease behavior. *Inflamm Bowel Dis* 1998;**4**:228–34.
31 **Achkar JP**, Brzezinski A. Interobserver agreement for disease behaviour phenotype in Crohn's disease. *Gastroenterology* 2002;**122**(suppl):W1293.
32 **Lautenbach E**, Berlin JA, Lichtenstein GR. Risk factors for early postoperative recurrence of Crohn's disease. *Gastroenterology* 1998;**115**:259–67.
33 **Yamamoto T**, Allan RN, Keighley MR. Perforating ileocaecal Crohn's disease does not carry a high risk of recurrence but usually re-presents as perforating disease. *Dis Colon Rectum* 1999;**42**:519–24.
34 **Peeters M**, Nevens H, Baert F, *et al*. Familial aggregation in Crohn's disease: increased age-adjusted risk and concordance in clinical characteristics. *Gastroenterology* 1996;**111**:597–603.
35 **Bayless TM**, Tokayer AZ, Polito JM II, *et al*. Crohn's disease: concordance for site and clinical type in affected family-members-potential hereditary influences. *Gastroenterology* 1996;**111**:573–9.
36 **Satsangi J**, Grootscholten C, Holt H, *et al*. Clinical patterns of familial inflammatory bowel disease. *Gut* 1996;**38**:738–41.
37 **Farmer RG**, Hawk WA, Turnbull RB Jr. Indications for surgery in Crohn's disease. *Gastroenterology* 1976;**71**:245–50.
38 **Van Kruiningen HJ**, Ganley LM, Freda BJ. The role of Peyer's patches in the age- related incidence of Crohn's disease. *J Clin Gastroenterol* 1997;**24**:470–5.
39 **Kelly JK**, Preshaw RM. Origin of fistulas in Crohn's disease. *J Clin Gastroenterol* 1989;**11**:193–6.
40 **Colombel JF**, Grandbastien B, Gower-Rousseau C, *et al*. Clinical characteristics of Crohn's disease in 72 families. *Gastroenterology* 1996;**111**:604–7.