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

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 shown that the behaviour of Crohn’s disease changes over the course of the disease.

Aim: To assess the association between rapid development of a stricturing or penetrating 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-stricturing non-penetrating 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-stricturing 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.

Table 1 Vienna classification of Crohn’s disease

<table>
<thead>
<tr>
<th>Location*</th>
<th>Age at diagnosis</th>
<th>Behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>A1, &lt;40 years</td>
<td>B1, non-stricturing non penetrating</td>
</tr>
<tr>
<td>L2</td>
<td>A2, &gt;40 years</td>
<td>B2, stricturing†</td>
</tr>
<tr>
<td>L3</td>
<td>L, ileocolon</td>
<td>B3, penetrating§</td>
</tr>
<tr>
<td>L4</td>
<td>L, upper gastrointestinal tract§</td>
<td></td>
</tr>
</tbody>
</table>

*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.
Patients and Methods

Patients

A total of 163 patients with a firm diagnosis of CD according to the criteria of Lennard-Jones, classified as non-strictureing non-penetrating disease (B1) at diagnosis based on the Vienna classification, 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 the association between a stricturing pattern and NOD2/CARD15 genotype, smoking habit, ASCA status, and other clinical and demographic characteristics of the disease. Patients were included in the study if the following criteria were met:

- Diagnosis of CD before the age of 50 years;
- Duration of disease at diagnosis of at least 6 months;
- 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.

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. In this initial report, the nomenclature of mutations was derived from the sequence of the initial number of the mutated amino acid. 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 strictureing 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.

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.

ASCAs

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 strictureing 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.

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.
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 L4), PCR products using fluorescently labelled primers (5′- GAATGTCAGAATCAGAAGGG-3′ and 5′- CTCACCATTGTATCTTCTTT-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 χ² 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 shown 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 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=0.004)) 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. 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...
location, number of flares per year, and smoking habit, but not by NOD2/CARD15 genotype.

The behaviour of CD represents a major characteristic of the disease. Penetrating and strictureing 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. Recent studies on disease behaviour have been hampered by the variability of classifications used and by their unsatisfactory degree of inter-rater agreement. More recently, the Vienna classification has been proposed which aims to stratify disease by a factory degree of inter-rater agreement.

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

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

*p values correspond to univariate analysis for each parameter using either χ² or Kruskal Wallis tests, as required.

CD, Crohn’s disease; ASCA, antiasaccharomyces cerevisiae antibodies.

*Mean (SD).

Factors influencing early development of stricturing or penetrating Crohn’s disease

Three 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. One explanation for the association with stricturing disease may be the smaller diameters 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 immunoinflammatory 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 immunoinflammatory reaction. The development of intra-abdominal penetrating lesions may in itself be favoured by downstream strictures in a subset of patients. 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, strictureing 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 strictureing disease. It was independently associated with the development of penetrating disease when patients who developed penetrating disease and patients who remained non-strictureting 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, 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.

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 may represent distinct entities and should perhaps be studied independently.

When patients who developed strictureing and those who developed penetrating lesions were compared, independent, 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 strictureting lesions). The association between strictureting disease and familial disease has already been described but was thought to be mainly linked to ileal location and early age at onset. Interestingly, a familial history of the disease was associated with strictureting 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. In the present study however, this association was not independent but also stritching the 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. However, in those previous studies, results were partly discordant, criteria used to classify disease behaviour were inhogeneous, 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 strictureting behaviour. 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 strictureting 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.

ACKNOWLEDGMENTS
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 Liege, Belgium
J P Hugot, Fondation Jean Dausset CEPH, Paris, France
F Fontaine, M Delforge, Department of Gastroenterology, Clinique St Joseph Liege, Belgium
F Colombel, Department of Gastroenterology, CHU Ulle, France

REFERENCES

www.gutjnl.com

10 Ahmad T
11 Culbert AP
12 Gasche C
13 Louis E
14 Eri RD
15 Vermeire S
Factors influencing early development of stricturing or penetrating Crohn’s 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 and J Belaiche

Gut 2003 52: 552-557
doi: 10.1136/gut.52.4.552

Updated information and services can be found at:
http://gut.bmj.com/content/52/4/552

References
This article cites 37 articles, 8 of which you can access for free at:
http://gut.bmj.com/content/52/4/552#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/