Different intestinal permeability patterns in relatives and spouses of patients with Crohn’s disease: an inherited defect in mucosal defence?

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Abstract
Background—A familial defect in intestinal barrier function has been found in Crohn’s disease.
Aim—To investigate possible genetic and environmental influences on this barrier defect by studying intestinal permeability in both relatives and spouses of patients with Crohn’s disease.
Subjects—The study included 39 patients with Crohn’s disease, 34 healthy first degree relatives, and 22 spouses. Twenty nine healthy volunteers served as controls.
Methods—Intestinal permeability was assessed as the lactulose:mannitol ratio in five hour urinary excretion after oral load, both before (baseline) and after ingestion of acetylsalicylic acid. The permeability response represents the difference between the two tests. A ratio above the 95th percentile for controls was classified as abnormal.
Results—Baseline permeability was higher in patients and spouses than in controls. An abnormal baseline permeability was seen in 36% of the patients, 23% of the spouses, 18% of the relatives, and 3% of the controls. After ingestion of acetylsalicylic acid, permeability increased significantly in all groups. Relatives were similar to patients with regard to permeability after exposure to acetylsalicylic acid, whereas spouses were similar to controls. The proportions with an abnormal permeability response to acetylsalicylic acid were 32% in patients, 14% in spouses, 41% in relatives, and 3% in controls.
Conclusion—The findings suggest that baseline permeability is determined by environmental factors, whereas permeability provoked by acetylsalicylic acid is a function of the genetically determined state of the mucosal barrier, and support the notion that environmental and hereditary factors interact in the pathogenesis of Crohn’s disease.

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Keywords: acetylsalicylic acid; environment; genetics; inflammatory bowel disease; lactulose; mannitol

Crohn’s disease is associated with increased intestinal permeability, but it is unclear whether this is of importance in the induction of intestinal inflammation or if it is a result of the inflammatory process itself. A deranged intestinal barrier is, however, thought to be an early event in the inflammatory process, and knockout mice with a breached intestinal barrier have been found to develop intestinal inflammation resembling that in Crohn’s disease.

A genetic predisposition seems to be important in the pathogenesis of Crohn’s disease. First degree relatives have a 15- to 30-fold increased risk of contracting the disease, and studies on twins have shown a high degree of concordance of disease in monozygotic, as opposed to dizygotic, twin pairs with Crohn’s disease. It was recently suggested that Crohn’s disease is a polygenic disorder, in which susceptibility genes are present on several chromosomes. The finding of a disrupted intestinal barrier in relatives of patients with Crohn’s disease led to the hypothesis of a genetic permeability disorder of aetiological importance. The exciting initial observations could not be reproduced, and subsequent studies have produced conflicting results. One reason for the discrepancies could be the use of different marker molecules. Several studies have, however, suggested that a subgroup of the relatives of patients with Crohn’s disease have a disturbed intestinal barrier. Recent results on familial aggregation of increased intestinal permeability in Crohn’s disease have differed somewhat: Hilsden et al9 and Pironi et al10 have shown a high degree of concordance of disease in monozygotic, as opposed to dizygotic, twin pairs with Crohn’s disease.6

Background—Crohn’s disease is a polygenic disorder, in which susceptibility genes are present on several chromosomes.9 The finding of a disrupted intestinal barrier in relatives of patients with Crohn’s disease led to the hypothesis of a genetic permeability disorder of aetiological importance.10 The exciting initial observations could not be reproduced,11 12 and subsequent studies have produced conflicting results. One reason for the discrepancies could be the use of different marker molecules.13 14 Several studies have, however, suggested that a subgroup of the relatives of patients with Crohn’s disease have a disturbed intestinal barrier.11 15 16 Recent results on familial aggregation of increased intestinal permeability in Crohn’s disease have differed somewhat: Hilsden et al9 and Pironi et al10 have shown a high degree of concordance of disease in monozygotic, as opposed to dizygotic, twin pairs with Crohn’s disease.6

Materials and methods
STUDY GROUPS
The study groups comprised 39 Crohn’s disease patients with a median (range) disease duration

Abbreviations used in this paper: ASA, acetylsalicylic acid; CDAI, Crohn’s disease activity index; L/M ratio, lactulose:mannitol ratio; NSAID, non-steroidal anti-inflammatory drug.
Table 1  Demographic characteristics of the study groups

<table>
<thead>
<tr>
<th>Age (median range)</th>
<th>Patients (n=39)</th>
<th>Relatives (n=34)</th>
<th>Spouses (n=22)</th>
<th>Controls (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>44 (26–63)</td>
<td>46 (18–70)</td>
<td>47 (25–61)</td>
<td>38 (22–60)</td>
<td></td>
</tr>
<tr>
<td>Sex M/F</td>
<td>18/21</td>
<td>16/18</td>
<td>11/11</td>
<td>12/17</td>
</tr>
<tr>
<td>Smokers/non-smokers</td>
<td>19/20</td>
<td>8/26</td>
<td>4/18</td>
<td>2/27</td>
</tr>
</tbody>
</table>

Table 2  Characteristics of the patients with Crohn’s disease

- **Patients (n=39):**
  - Disease activity:
    - Simplified CDAI: 120 (0–460)
    - Active/inactive disease: 17/22
  - Medication:
    - Azathioprine ± corticosteroids: 5
    - Mesalazine only: 6
    - No anti-inflammatory: 11
  - Bowel resection: Yes/no: 25/14
  - Location of disease:
    - Ileocecal or ileal: 30
    - Ileum + extensive colon: 5
    - Colitis: 4

- **Relatives (n=34):**
  - Disease activity:
    - Simplified CDAI: 120 (0–460)
    - Active/inactive disease: 17/22
  - Medication:
    - Azathioprine ± corticosteroids: 5
    - Mesalazine only: 6
    - No anti-inflammatory: 11
  - Bowel resection: Yes/no: 25/14
  - Location of disease:
    - Ileocecal or ileal: 30
    - Ileum + extensive colon: 5
    - Colitis: 4

- **Spouses (n=22):**
  - Disease activity:
    - Simplified CDAI: 120 (0–460)
    - Active/inactive disease: 17/22
  - Medication:
    - Azathioprine ± corticosteroids: 5
    - Mesalazine only: 6
    - No anti-inflammatory: 11
  - Bowel resection: Yes/no: 25/14
  - Location of disease:
    - Ileocecal or ileal: 30
    - Ileum + extensive colon: 5
    - Colitis: 4

- **Controls (n=29):**
  - Disease activity:
    - Simplified CDAI: 120 (0–460)
    - Active/inactive disease: 17/22
  - Medication:
    - Azathioprine ± corticosteroids: 5
    - Mesalazine only: 6
    - No anti-inflammatory: 11
  - Bowel resection: Yes/no: 25/14
  - Location of disease:
    - Ileocecal or ileal: 30
    - Ileum + extensive colon: 5
    - Colitis: 4

*The disease was considered to be active when the simplified CDAI was above 150.

CDAI, Crohn's disease activity index.
with a pulsed amperometric detector (Dionex Corporation, Sunnyvale, California, USA). The carbohydrates were separated isocratically at a flow rate of 1.0 ml/min at 22°C, with an eluent consisting of 90 mmol/l NaOH and 0.375 mmol/l zinc acetate. Like Kynaston et al., we obtained a clear separation between lactose and lactulose. Five standard solutions containing lactulose (0.025–1.0 mmol/l) and mannitol (0.5–20 mmol/l) were used for quantification by internal standardisation with peak:area ratios. The coefficients of variation were 4.2 and 4.9% for mannitol and lactulose respectively.

**CALCULATIONS AND STATISTICS**

The lactulose:mannitol (L/M) ratio in the urinary output was calculated and used as a permeability index to minimise the influence of differences in gastrointestinal motility, absorptive capacity, urinary volume, and renal function. The difference in L/M ratio between baseline and after ASA provocation was calculated in each individual as a measure of the permeability response to ASA. The 95th percentile in the controls was defined as the upper limit of normality with regard to baseline permeability and response to ASA. Data showed a skewed distribution and are presented as median (interquartile range), and scattergrams. The Kruskal-Wallis and Mann-Whitney tests were used for comparison between groups, and the paired sign test for within-group comparisons. Analysis of covariance was used to assess the influence of various patient factors on permeability. Differences with p<0.05 were considered to be significant.

**ETHICS**

The study protocol was approved by the ethics committee of the Faculty of Health Sciences, Linköping University and was conducted according to the Declaration of Helsinki.

**RESULTS**

The groups did not differ with regard to sex distribution and age. No relation was seen between permeability and age, gender or smoking habits, either at baseline or after ASA ingestion (analysis of covariance).

**BASELINE PERMEABILITY**

Table 3 gives the baseline L/M ratios. The 95th percentile for controls (0.0195) was used as the upper limit of normality (95th percentile in the control group) at baseline (0.0195) is indicated by the dotted line and the upper limit after ASA (0.032) is indicated by the dashed line. At baseline the median L/M ratio was increased in patients and spouses as compared with controls (p<0.05, Kruskal-Wallis). After intake of ASA the L/M ratio was higher in both patients and relatives than in controls (p<0.05, Kruskal-Wallis), but not in spouses. Note the marked increase in permeability in a large subgroup of the relatives after ingestion of ASA.
upper limit for normal permeability. Baseline permeability was increased in the patient group as compared with the controls (table 3; fig 1), with a baseline permeability above normal in 14/39 (36%) of the patients. Permeability was not related to the location or activity of the disease (analysis of covariance), but higher permeability was seen in patients on medication than in untreated patients (median 0.017 and 0.012 respectively; p<0.05). The baseline permeability of the relatives was equal to that of the controls (table 3; fig 1), although in a subgroup of the relatives (6/34; 18%) the permeability was increased. The spouses formed an intermediate group with increased permeability compared with controls (table 3; fig 1), and a raised baseline permeability in 5/22 (23%). In the group denoted “solely relatives”, a raised baseline permeability was found in 1/11 (9%), whereas the corresponding value for “spouses at diagnosis” was 3/9 (33%; table 3).

PERMEABILITY AFTER INGESTION OF ASA
ASA ingestion increased the L/M ratio significantly in all study groups (p<0.005; paired sign test; table 3), with an increase in the 95th percentile for controls to 0.032. After exposure to ASA, the ratio in the group of relatives was increased to that of the patient group (table 3; fig 1). L/M ratios for spouses did not show a similar rise, and did not differ from the controls after ingestion of ASA (table 3; fig 1).

RESPONSIVENESS TO ASA
The median permeability response to ASA—that is, the increase in L/M ratio from baseline—was 0.009 (0.004–0.011) in the controls, with the 95th percentile being 0.018. There were no differences in the median permeability responses between the various groups, being 0.009, 0.010, and 0.007 in the patients, relatives, and spouses respectively.

An abnormal permeability response to ASA—that is, a rise exceeding that of the 95th percentile for controls—and the local e discovered in our study is absorbed mainly in the proximal small bowel,31 32 although disputed30) did not differ from that for the controls (table 3). In the group of “solely relatives” the proportion of hyperresponders was 3/11 (27%), whereas no hyperresponders (0/9) were found among “spouses at diagnosis” (table 3).

MONOZYGOTIC TWINS
Baseline L/M ratios in the twin groups were 0.014 (0.010–0.016) and 0.015 (0.009–0.019) for twins with Crohn’s disease and healthy twins respectively. After ingestion of ASA the permeability increased to 0.022 (0.019–0.035) and 0.023 (0.018–0.023) respectively. None of the healthy twins (0/4) had an augmented permeability response to ASA, but an abnormal response was seen in four (28%) of the 14 twins with Crohn’s disease (p = 0.03 compared with controls; Fisher’s exact test).

Discussion
This is the first study to compare baseline and ASA-provoked L/M ratios in both relatives and spouses of patients with Crohn’s disease. This approach allowed us to investigate the importance of both hereditary and environmental factors in ASA-provoked intestinal permeability in Crohn’s disease. Our most important finding is that relatives and spouses differed with regard to changes in permeability patterns on administration of ASA. The relatives showed significant increase in permeability in subgroups of patients, relatives, and spouses, which substantiates the data of Peeters and co-workers, and we observed an exaggerated response to ASA in relatives, which was also noted by Hilsden and colleagues. Taken together, our findings suggest that baseline permeability is dependent on environmental factors, whereas the responsiveness to ASA is controlled by hereditary factors.

However, only one third of the patients and relatives and none of the healthy twins were hyperresponders, suggesting that hyperresponsiveness is a feature of a subgroup of patients and relatives with a genetic defect in the mucosal defence system. This is well in line with the genetic heterogeneity and polygenic inheritance suggested in recent studies.

Certain early life events have been suggested to be relevant to Crohn’s disease,27 28 and our findings do not rule out a common childhood factor as the cause of a barrier defect. It should also be kept in mind that only a subgroup of the first degree relatives were studied, raising the possibility of a selection bias interfering with the results, and caution is needed when interpreting our data.

Our study confirms that ASA in clinically used doses increases intestinal permeability in man, as opposed to animal studies.27 28 The mechanisms behind the increased L/M ratios obtained after exposure to ASA are, however, unclear. Hilsden and co-workers49 showed that, in contrast with the L/M ratio (which reflects total small bowel permeability46), the ASA induced increase in sucrose permeability (suggested to reflect gastroduodenal mucosal permeability, although disputed50) did not differ between controls and relatives of patients with Crohn’s disease. The formulation of ASA used in our study is absorbed mainly in the proximal small bowel,31 32 and the local effect during absorption is thought to be important in NSAID induced damage of the small bowel.51

Taken together, the cited data imply that an exaggerated increase in the L/M ratio after
Ingestion of ASA is probably an indication of a defect in the mucosal defence system in the jejunum. This is in line with previous studies showing that Crohn's disease encompasses a number of defects in jejunal function, including permeability, and suggests that the entire gastrointestinal tract is involved in the disorder. In rat intestine, NSAIDs such as ASA have been found to induce a rapid effect on the mitochondria which includes uncoupling of oxidative phosphorylation. That effect should lead to a quick drop in the ATP content in enterocytes, and thereby elicit contraction of the cytoskeleton and increase tight junction permeability. In the light of our results, this is a very attractive model for the mechanism of the effects of ASA, as Crohn's disease has been suggested to be a disorder of the tight junctions. Nonetheless, a possible link between ASA and tight junction permeability remains to be proved, and consideration must also be given to other plausible mechanisms, such as effects on mucins and prostaglandins. An aggravating effect of NSAIDs on colitis has been observed, and inhibition of prostaglandin synthesis seems to be the most likely mechanism. If this is also the case in the small bowel, the increase in intestinal permeability in the relatives of patients with Crohn's disease could be a reflection of a subclinical inflammatory or immunological abnormality, rather than a primary disturbance of the epithelial cells.

Our findings support the notion of an interaction of environmental and hereditary factors in the pathogenesis of Crohn's disease, and they are well in line with recent reports indicating polygenic inheritance. Increased knowledge of the mechanisms underlying the effects of ASA on small bowel permeability could provide important clues to the causes of Crohn's disease.

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