Efficacy and strategy of pneumatic dilatation in achalasia

We read with interest the article by Eckardt et al. regarding the long term results of pneumatic dilatation in achalasia (Gut 2004;53:629–33). Fifty four patients were followed up for a median of 14 years after a single pneumatic dilatation using the Brown-McHardy dilator. Five and 10 year remission rates were 40% and 36%, respectively, and repeated dilatations only mildly improved the clinical response. Most of the relapses occurred within one year of dilatation. Patients with post-dilatation lower oesophageal sphincter pressures of <10 mm Hg had a significantly better outcome. The authors suggest that failure to respond to the first dilatation should lead to consideration of alternative therapy.

We disagree with this conclusion and we would like to bring to your attention a recent prospective study on the long term effects of pneumatic dilatation in 11 patients with achalasia. A different approach was chosen—that is, treatment consisted of one or more pneumatic dilatations under conscious sedation in order to achieve stable clinical remission, defined as persisting one year after dilatation. To this end, closer follow up was performed in the first year after dilatation (scheduled assessments at three and 12 months). Thereafter, clinical and manometric assessments were performed yearly for six years. The clinical score had improved the clinical response. Most of the relapses occurred within one year of dilatation. The clinical score was 40% and 36%, respectively, for control and oral route injections of 5×10⁷ live microorganisms, 24 hours prior to induction of colitis. Surprisingly, protection by the LAB model allowed us to discriminate “protective” strains, showing between 30% and 70% reduction of inflammatory score, from strains which did not significantly attenuate experimental colitis. We could select highly performing strains of Lactobacillus salivarius and Lactobacillus rhamnosus that consistently lowered colitis. In a recent study, in which 56 patients were treated with the Rigiflex dilator and observed for more than 10 years, the long term success rate was 55%. Thus it is my impression that differences in treatment results are not so much related to differences in technique and operator experience but rather to the number of patients investigated, duration of follow up, and quality of the study design. It has been shown that careful design of randomised studies, which are now in progress, will tell us whether we should continue to offer pneumatic dilatation to the great majority of patients with achalasia or whether we should advise them to undergo surgery instead.

References


Probiotics in IBD: mucosal and systemic routes of administration may promote similar effects

We read with considerable interest the paper by Sheil et al. (Gut 2004;53:694–700) who reported the successful application of the subcutaneous route for probiotic attenuation of colitis.

We agree with the corresponding commentary of Ghosh et al. (Gut 2004;53:620–2) regarding the need to study mechanisms underlying probiotic interactions. Recently, we further standardised a method to compare the anti-inflammatory potential of orally administered lactic acid bacteria (LAB) in a murine model of acute 2,4,6-trinitrobenzene sulphonic acid (TNBS) induced colitis. This model allowed us to discriminate “protective” strains, showing between 30% and 70% reduction of inflammatory score, from strains which did not significantly attenuate experimental colitis. We could select highly performing strains of Lactobacillus salivarius and Lactobacillus rhamnosus that consistently lowered colitis. In a recent study, in which 56 patients were treated with the Rigiflex dilator and observed for more than 10 years, the long term success rate was 55%. Thus it is my impression that differences in treatment results are not so much related to differences in technique and operator experience but rather to the number of patients investigated, duration of follow up, and quality of the study design. It has been shown that careful design of randomised studies, which are now in progress, will tell us whether we should continue to offer pneumatic dilatation to the great majority of patients with achalasia or whether we should advise them to undergo surgery instead.

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Authors’ reply

Penagini and Cantù should be congratulated for the remarkable results they were able to obtain in 11 patients with achalasia treated by pneumatic dilatation. To my knowledge, not a single study has so far produced similar results. A review of prospective studies in a single study has so far produced similar results. A review of prospective studies in achalasia: comparison and efficacy. Am J Gastroenterol 1998;93:21–35.

References


Conflict of interest: None declared.

PostScript

LETTERS

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Conflicts of interest: None declared.
observed that heat treatment of an orally administered probiotic cocktail abolished the colitis protection in a DSS model, while irradiation improved it. Consequently, cellular integrity appears to be necessary to explain at least some part of the effect, although cell walls and peptidoglycans of killed bacteria cannot be considered as passive. Possibly both “good” and “bad” signals are given out by LAB, and the immune system is integrating all of them. Those “mixed” signals will no doubt be specific for each strain as well as dose dependent. Differences in physicochemical status could explain the mortality seen by Shell et al., especially when using heat treated bacteria. Pereyra and colleagues established that the maximal non-lethal quantity of injected live LAB was 5 × 10^7 but it can be hypothesised that toxicity may also differ with strain and viability status. Both pro- and anti-inflammatory components of probiotics have been reported to interact with systemic immune cells, showing effects comparable with other well known anti-inflammatory or therapeutic molecules. It is therefore most probable that systemic delivery of specific live or killed probiotics may influence the delicate balance between Th1 and Th2 immunity, and subsequently have an impact on local immunity. Clear relationships, however, are not obvious. A first example is the case of subcutaneous CpG DNA that promoted a Th1 response and was able to alleviate some symptoms of DSS colitis but caused inflammation when administered after the onset of colitis. Secondly, different experimental models of colitis support a potential benefit of probiotic DNA, although it seems very premature to restrict this probiotic effect to nucleic acids only.

As emphasised by Gosh and colleagues (Gut 2004;53:620–2), approaches involving fractional studies are essential tools to complete the knowledge obtained from in vitro and ex vivo models and assist in understanding the interactions between LAB and the immune system. These studies may reveal common mechanisms active in inflammation, tolerance, and allergy models. Even if this study confirms the importance of the systemic route for certain probiotic activity, we cannot neglect the possible influence of local and innate immunity, the general state of the gut flora, and the role of epithelial cells in the cross talk between both.

Acknowledgements
We are indebted to Danisco France for financial support.

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References

Mutations in anionic trypsinogen gene are not associated with tropical calcific pancreatitis

Pancreatitis is considered to be an autodigestive disease due to premature activation of trypsinogen inside the pancreas. Its genetic basis has recently been established with the identification of causal mutations in cationic trypsinogen gene (PRSS1) in patients with hereditary and non-hereditary pancreatitis. Mutations in other genes such as SPINK1 (encoding pancreatic secretory trypsin inhibitor) and cystic fibrosis transmembrane conductance regulator (CFTR) genes have also been associated with the disease. Tropical calcific pancreatitis is a type of idiopathic pancreatitis, reported particularly in the tropics. Recently, we and others demonstrated absence of PRSS1 mutations but significant prevalence of the N34S mutation in the SPINK1 gene in these patients. However, our study raised two important questions: firstly, the exact role of SPINK1 mutations in disease causation as cationic trypsinogen is normal with an intact autolysis site; and secondly, the cause of the disease in the remaining patients negative for both PRSS1 and SPINK1 mutations.

Of the nine members of the human trypsinogen gene family, only PRSS1, PRSS2, and PRSS3 are functional genes coding for cationic, anionic, and meso-trypsinogen isoforms, respectively. The cationic form accounts for about one third of the total trypsins in pancreatic juice. We investigated whether mutations in the anionic trypsinogen gene may contribute to the pathogenesis of tropical calcific pancreatitis. Of 20 mutations reported to date in the PRSS1 gene, 17 are clustered in exons 2 and 3 only. The remaining three are in the promoter region but reported in isolated patients. Hence we initially screened exons 2 and 3 of the anionic trypsinogen gene in 68 well characterised Indian patients with tropical calcific pancreatitis. Subsequently, we also sequenced the promoter, complete coding region, and the flanking region in an attempt to look for any novel mutation.

Owing to the extremely high sequence homology between PRSS1 and PRSS2, a nested polymerase chain reaction (PCR) was used to ensure specificity. The primers were selected from the published study of Chen and colleagues and all of the exons of PRSS2 were PCR amplified, purified, and sequenced in both alleles using internal sequencing primers and the Big Dye terminator cycle sequencing approach. However, we did not find any of the reported or any novel mutations in the coding region or in the splice site junctions, except for one polymorphism A90A (GCA-GGC) in exon 3 of the anionic trypsinogen gene. This variation was observed in both the heterozygous...
and homoyzous states with a mutant allele frequency of 0.58 (9 AA, 20 GG, and 39 AG) and was comparable with 0.61 in 50 controls (7 AA, 18 GG and 25 AG) analysed.

Our results thus exclude any association of mutations in the anionic trypsinogen gene in tropical calcific pancreatitis and suggest a role for other genetic or non-genetic factors in the pathogenesis of the disease. Screening of genes such as CPT2 may explain the disease in the remaining patients. It also affirms the importance of the N34S mutation in SPINK1 as the major genetic factor for this type of pancreatitis.

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Conflict of interest: None declared.

References


Elevated plasma protein C levels correlate with the presence of fatty liver (NASH and NAFLD)

The clinical implications of non-alcoholic fatty liver disease (NAFLD) are derived mostly from its common occurrence in the general population and the potential of the condition to progress to fibrosis and cirrhosis. Markers that help in making an early diagnosis and treatment are warranted. Protein C is a vitamin K-dependent glycoprotein that functions as a circulating anticoagulant through proteolytic cleavage and inactivation of the coagulation factors Va and VIIIa. Whether protein C levels increase in patients with NAFLD has not been assessed.

We measured protein C levels in 44 patients (28 men and 16 women; mean ages 45 (11) and 49 (12) years, respectively); 15 patients with fatty liver (FL), 15 with non-alcoholic steatohepatitis (NASH), and 14 with chronic viral hepatitis B+H+C (CH). All were diagnosed by histology and liver technetium scan or ultrasound. Ten healthy subjects were used as controls. Obesity, hyperlipidaemia, and diabetes were present in 60%, 73%, and 23% of cases, respectively; 64% of patients had elevated liver enzyme tests (alanine aminotransferase 45 (21) IU/l in FL and 43 (18) IU/l in NASH). Mean protein C levels were significantly increased in patients with NAFLD (n = 30) compared with those with chronic viral hepatitis (140 (36)% vs 101 (24)%; p = 0.0009) and healthy individuals (140 (36)% v 120 (12); p = 0.04).

* No significant difference in protein C levels was noted between simple fatty liver and NASH. (149 (34)% and 130 (37)% respectively; p = 0.071).

* A significant correlation was found between protein C and extent of fatty infiltration (r = 0.63; p = 0.001) (fig 1), insulin resistance index (r = 0.3; p = 0.03), and triglyceride levels (r = 0.45; p < 0.001). Protein C could discriminate correctly between NAFLDs and chronic viral hepatitis in 82% of cases. No significant association was found between protein C and aminotransferase levels.

In conclusion, protein C was elevated in patients with NAFLD. The underlying mechanism remains unknown. Agewall et al suggested an increase in hepatic synthesis of protein C due to increased hepatic insulin resistance. Increased levels have been reported in patients with diabetes, hypertriglyceridaemia, and nephrotic syndrome, with the use of anabolic steroids, oral contraceptives, and alcohol, and with increasing age. Diabetes and hypertriglyceridaemia are predisposing conditions to fatty liver and were present in 23% and 73% of cases, respectively. The remaining conditions were excluded by clinical and biochemical findings. Although more studies are needed, these preliminary findings suggest that elevated protein C levels together with elevated liver enzymes may be used as markers for NAFLD and may obviate the need for liver biopsy.

Coexistent chronic idiopathic intestinal pseudo obstruction and inflammatory bowel disease

Chronic idiopathic intestinal pseudo obstruction (CIPI) is a severe condition presenting with abdominal pain and dysmotility. Inflammatory or degenerative changes of the autonomic nervous system or of the muscles of the bowel have been observed in CIPI. As patients with inflammatory bowel disease (IBD) may show clinical and histological signs of autonomic neuropathy and dysmotility, the aim of this study was to examine whether there is an association between CIPI and IBD.

Six patients at our hospital presenting with signs and symptoms of intestinal dysmotility were diagnosed with CIPI based on clinical features, antroduodenal manometry, and full thickness biopsies (table 1). Patient No 1 had an acute erosive colitis some years previously with bloody diarrhea and an enhanced sedimentation rate, which was treated with steroids, and patient No 2 had relapsing proctitis treated with 5-amino-salicylic acid (5-ASA). Patient No 3 was diagnosed with Crohn’s disease, patient No 4 had idiopathic inflammatory bowel disease, and patients No 5 and No 6 had idiopathic chronic constipation of neurogenic origin with rectal sensory deficiency and paradoxical anal sphincter relaxation.
totally and patient No 4 partially colectomised because of slow transit constipation. Patient No 6 was proctocolectomised due to dysmotility developed after proctocolectomy. Ganglionitis in patient No 3 could be caused by Crohn’s disease before other symptoms of the disease developed. Treatment with 5-ASA has reduced her abdominal pain. The normal histology of the small intestine in ulcerative colitis. Proliferation of and injury to smooth muscle and autonomic nerve function in patients with ulcerative colitis. Gastroenterology 1987;93:934–40.


Cannabinoid hyperemesis: not just a problem in Adelaide Hills

We read the article by Allen and colleagues (Gut 2004;53:1566–70) with interest and would like to report a case of probable cannabinoid hyperemesis seen in a district general hospital in the UK.

A 21 year old chef was admitted to our hospital on seven occasions over a two year period (April 2001 to December 2002) with profuse vomiting. Apart from a history of migraine as a child, he was fit and well. He smoked cannabis. Physical examination was unremarkable. The observation that the patient wanted to take regular baths because he had found that bathing eased the sickness was documented in the nursing notes but its significance was not appreciated. Investigations during attacks disclosed neutrophilia but blood urea, electrolytes, liver biochemistry, and serum amylase were normal. Abdominal x ray was also normal. Upper gastrointestinal endoscopy showed grade I oesophagitis and gastritis. Gastric biopsies were histologically normal. An abdominal ultrasound scan and small bowel barium follow through examination were normal. Additional normal or negative investigations included: autoantibodies and immunoglobulins, C reactive protein, and urinary porphyrin screen. Computed tomography scan of the brain was also normal.

During his last admission, the patient’s girlfriend showed us an article published in an Australian newsletter which she had obtained via the internet, in which Dr JH Allen had raised the possibility of a link between recurrent vomiting and cannabis abuse. With the aid of the internet we traced and contacted Dr Allen who shared his experience of this condition with us.

Reviewing the patient’s history, he freely admitted to smoking cannabis and experiencing the compulsive desire to bathe during bouts of vomiting. Following his last admission in December 2002, our patient stopped smoking cannabis and has remained free of symptoms. The clinical presentation with vomiting is almost identical to the cases described by Allen et al, together with the response to cessation of smoking cannabis, supports the view that our patient was suffering from cannabinoid hyperemesis and that this condition is international.

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Conflict of interest: None declared.

Inflammatory bowel disease stimulates formation of carcinogenic N-nitroso compounds

In patients with inflammatory bowel disease (IBD), increased incidence of colorectal cancer is observed.1 Although severe inflammatory conditions per se represent a risk factor for neoplasia, we would like to draw attention to the possible role of increased activity of inducible nitric oxide synthase (iNOS), as found in IBD patients,2 in the endogenous formation of carcinogenic N-nitroso compounds (NOC). In healthy individuals, relatively small amounts of NOC are formed by the interaction between NOC precursors (NOCP), present in dietary items such as meat and fish, and nitrosating agents derived from dietary nitrate. It has been proposed that endogenous formation of NOC may explain the link between meat consumption and colon cancer risk found in epidemiological studies.2 We hypothesised that as a result of chronic inflammatory conditions in the large intestine, increased colonic iNOS activity may produce an excess of NO, nitrogen oxides, and nitrite, which in turn react with NOCP present in the colon to produce relatively high levels of NOC. Increased formation of NOC in IBD patients may thus contribute to the relatively high incidence of colorectal cancer associated with this disease.

A recent population based case control study showed that in cases with a history of IBD, increased exposure to drinking water nitrate was associated with an increased risk of colon cancer whereas no such association was found in the overall population.3 This clearly indicates that the risk of colon cancer in IBD patients is not only determined by the disease itself but dietary factors known to influence the endogenous formation of NOC are also associated with an increased risk in these patients. Although both the increased formation of NOC found in mice with chemically induced colitis4 and increased levels of NO and nitrite found in the colonic lumen of patients with ulcerative colitis5 support this hypothesis, the NOCP levels have never been investigated in IBD patients.

Therefore, we collected faecal samples from 17 patients diagnosed with ulcerative colitis and 17 healthy controls, and determined levels of N-nitrosodimethylamine (NDMA), a predominant carcinogenic NOC, using gas chromatography-mass spectrometry, as previously described. The study was approved by the medical ethics committee of the Maasland Hospital, Sittard, the Netherlands, and all patients gave their consent. In 41% of patients, we found levels of NDMA above the detection limit of 1 ng/g faeces, compared with 35% of controls. Comparison of concentrations in NDMA positive patients to controls showed that the average concentrations in patients was significantly higher than that in the control group (table 1). When IBD patients were subdivided into hospitalised and non-hospitalised cases, the difference between the non-hospitalised group and controls was even more pronounced, whereas NDMA concentrations in hospitalised patients and controls were comparable. All hospitalised patients received only liquid nutrition (Nutrison; Nutricia, UK) without additional treatment with omeprazole.

Most research on endogenous NOC exposure has focused predominantly on the intragastric formation of these compounds in relation to the gastric cancer risk. However, we now report that faecal NDMA levels in IBD patients are considerably higher than those we reported previously in gastric juice (0.25 (0.3) ng/g),6 which indicates that NOC exposure may be even more relevant in colon carcinogenesis.

Based on these results, we conclude that the colon of IBD patients is exposed to relatively high concentrations of this carcinogenic compound, probably as a direct consequence of continuous NO production by the inflammatory process. As this exposure may strongly contribute to the increased colon cancer risk associated with IBD, dietary recommendations for IBD patients, avoiding high NOC intake, seem warranted.

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doi: 10.1136/gut.2004.057471
Conflict of interest: None declared.

Table 1 Faecal N-nitrosodimethylamine (NDMA) concentrations in patients with inflammatory bowel disease (IBD) and in healthy controls

<table>
<thead>
<tr>
<th>NDMA (ng/g)</th>
<th>All IBD cases (n = 17)</th>
<th>Non-hospitalised cases (n = 10)</th>
<th>Hospitalised cases (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDMA positive</td>
<td>35</td>
<td>41</td>
<td>56</td>
</tr>
<tr>
<td>NDMA (ng/g)</td>
<td>1.4</td>
<td>10.9†</td>
<td>14.3††</td>
</tr>
</tbody>
</table>

*Average concentration of NDMA positive samples.
†p<0.05, ††p<0.01: significantly higher compared with the control group (Mann-Whitney U test).
‡p<0.05: significantly lower compared with non hospitalised cases (Mann-Whitney U test).

References
3 Bingham SA, Pigottelli B, Pollack JB, et al. Does increased endogenous formation of N-nitroso compounds in the human colon explain the association between red meat and colon cancer? Carcinogenesis 1996;17:615–23
5 Mirvish SS, Haorah J, Zhou L, et al. N-Nitroso compounds in the gastrointestinal tract of rats and in the feces of mice with induced colitis or fed hot dogs or beef. Carcinogenesis 2003;24:595–600

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Hepatocellular carcinoma occurring after successful treatment of childhood cancer with high dose chemotherapy and radiation

Hepatocellular carcinoma (HCC) is one of the world’s most common malignancies and accounts for more than 90% of all primary liver cancers. A number of different risk factors have been identified for the development of HCC. Hepatitis B carrier state, environmental toxins, chronic hepatitis C virus infection, hereditary haemochromatosis, and liver cirrhosis of almost any cause are well known risk factors for HCC. In addition, environmental toxins such as aflatoxins and contaminated drinking water may contribute to the pathogenesis of HCC, especially in Asia and underdeveloped countries. Finally, a number of HCC cases have occurred after the use of thorotrast for diagnostic procedures, and survivors of the atomic bomb of Hiroshima were also at higher risk for HCC development, indicating that radiation might also induce the development of HCC. Within our series we describe a rare case of HCC occurring in a patient 17 years after successful treatment of peripheral neuroectodermal tumour (PNET).

A 32 year old female presented with pain in the right upper quadrant of her abdomen. Seventeen years prior to presentation in our hospital this patient was treated for a PNET with a combination of high dose chemotherapy (vincristine, adriamycin, ifosfamide, and actinomycin D) and surgical removal of the 10-5 cm tumour from her right chest followed by combined radiation (60 Gy) and chemotherapy. There were no signs of any recurrence of the tumour observed on her last check up 12 months earlier. Physical examination of the patient in our clinic showed typical signs of late radiation damage (erythema of the skin and an underdeveloped right breast) (fig. 1). A firm 3-5 cm mass was palpable at the lower edge of the liver. Laboratory tests showed elevated a-fetoprotein (41881 m\(^3\)) and hepatitis B surface antigen positive. EBER, EBNA2, and cell markers as well as the anaplastic large cell lymphoma kinase (ALK) and Epstein-Barr virus (EBV) associated proteins. A multiplex polymerase chain reaction approach revealed a clonal T cell population and an oligoclonal B cell population. Based on these results, the diagnosis was ALK negative anaplastic large cell lymphoma with null/T cell phenotype. Clinical stage was IAE. CHOP-chemotherapy resulted in complete clinical and histological remission, which was evidenced by computer tomography, positron emission tomography, and negative rectal histology. Polymerase chain reaction analysis of the rectal biopsies revealed no T cell receptor rearrangement.

Three months later, the patient presented with postobstructive pneumonia. Bronchial biopsies showed a diffuse large B cell lymphoma. In contrast with the preceding rectal biopsies, bronchial tumour cells were positive for CD20, EBER, EBNA2, and LMP-1, indicating EBV infection of latency type III, were detected in tumour cells.

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Biologics in inflammatory disease: infliximab associated risk of lymphoma development

In their excellent overview of currently available biologic compounds that are in use or under investigation for Crohn’s disease (CD), Sandborn and Faubion (Gut 2004;53:1366–66) reconfirm the unique standing of infliximab. They also note the ongoing discussion concerning the increased occurrence of lymphoproliferative disorders in patients who received infliximab. Recently, we followed a 61 year old patient with a 31 year history of relapsing CD. Initial treatment was with steroids but after 10 years with ulcerations and multiple rectal fistulas. Biopsies showed a polymorphous tumour infiltrate. Tumour cells were positive for CD30 and negative for T and B cell markers as well as the anaplastic large cell lymphoma kinase (ALK) and Epstein-Barr virus (EBV) associated proteins. A multiplex polymerase chain reaction approach revealed a clonal T cell population and an oligoclonal B cell population. Based on these results, the diagnosis was ALK negative anaplastic large cell lymphoma with null/T cell phenotype. Clinical stage was IAE. CHOP-chemotherapy resulted in complete clinical and histological remission, which was evidenced by computer tomography, positron emission tomography, and negative rectal histology. Polymerase chain reaction analysis of the rectal biopsies revealed no T cell receptor rearrangement.

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Table 1 Details of five cases of secondary hepatocellular carcinoma

<table>
<thead>
<tr>
<th>First malignancy</th>
<th>Age (y)</th>
<th>Treatment</th>
<th>Age when HCC was diagnosed (y)</th>
<th>Time from first to second malignancy (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>4</td>
<td>CTx*</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>4</td>
<td>CTx, RTx</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>ALL</td>
<td>4</td>
<td>na</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>PNET</td>
<td>15</td>
<td>CTx, RTx</td>
<td>33</td>
<td>18</td>
</tr>
<tr>
<td>Teratoma</td>
<td>2</td>
<td>na</td>
<td>19</td>
<td>5</td>
</tr>
</tbody>
</table>

ALL, acute lymphocytic leukaemia; PNET, peripheral neuroectodermal tumour; CTx, chemotherapy; RTx, radiation therapy.

*This patient was hepatitis B surface antigen positive.


Conflict of interest: None declared.

References


However, tumour cells were negative for CD30 and ALK protein. Molecular analysis demonstrated a monoclonal immunoglobulin heavy chain rearrangement in the absence of a T cell receptor rearrangement, confirming a heavy chain rearrangement in the absence of light chain rearrangement.

The tumour was neither CD30-positive nor to the CD56, indicating a lymphoid origin. The finding of a monoclonal immunoglobulin light chain suggests a proliferation of B lymphocytes.

Genotypes 677TT and 677CT+1298AC of methylenetetrahydrofolate reductase are associated with the severity of ulcerative colitis in central China

Increased blood levels of homocysteine have been found to be associated with inflammatory bowel disease (IBD) in several studies. The main genetic determinant associated with elevated plasma levels of homocysteine (t-Hcys) is the *MTHFR* 677CT→T gene polymorphism of methylenetetrahydrofolate reductase, a critical enzyme involved in the remethylation pathway of homocysteine. An association of the *MTHFR* 677TT allele with IBD has been reported in Northern Europe, but not in three other populations from Central Europe, Asia, and the United States.

Methylenetetrahydrofolate reductase (MTHFR) C677T or T1298A

### Table 1 Patients with infliximab therapy and development of lymphoma

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (y), sex, CD phenotype</th>
<th>Dose of infliximab (mg/kg)</th>
<th>Lymphoma</th>
<th>EBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77, M, NR</td>
<td>NR</td>
<td>Burkitt lymphoma</td>
<td>NR</td>
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<td>2</td>
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<td>Hodgkin lymphoma</td>
<td>NR</td>
</tr>
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<td>NR</td>
<td>Hodgkin lymphoma</td>
<td>NR</td>
</tr>
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<td>4</td>
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<td>NR</td>
<td>DUBCL</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>70, M, NR</td>
<td>NR</td>
<td>DUBCL</td>
<td>Positive</td>
</tr>
<tr>
<td>6</td>
<td>29, M, CD</td>
<td>5 mg/kg, 3</td>
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<td>NR</td>
</tr>
<tr>
<td>7</td>
<td>68, F, NR</td>
<td>NR</td>
<td>B cell NHL</td>
<td>NR</td>
</tr>
<tr>
<td>8</td>
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<td>DUBCL</td>
<td>Positive</td>
</tr>
<tr>
<td>9</td>
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<td>NR, multiple</td>
<td>Mantle cell lymphoma</td>
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</tr>
<tr>
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<td>10 mg/kg, 8</td>
<td>B cell NHL</td>
<td>NR</td>
</tr>
<tr>
<td>11</td>
<td>48, M, RA</td>
<td>10 mg/kg, 2</td>
<td>B cell NHL</td>
<td>NR</td>
</tr>
<tr>
<td>12</td>
<td>59, F, RA</td>
<td>3 mg/kg, 5</td>
<td>Multiple myeloma</td>
<td>NR</td>
</tr>
<tr>
<td>13</td>
<td>61, M, RA</td>
<td>NR</td>
<td>Hodgkin lymphoma</td>
<td>NR</td>
</tr>
<tr>
<td>14</td>
<td>36, M, CD, HIV</td>
<td>10 mg/kg, NR</td>
<td>B cell NHL</td>
<td>NR</td>
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<tr>
<td>15</td>
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<td>10 mg/kg, 1</td>
<td>Intravascular B-NHL</td>
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<tr>
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<td>48, F, DM</td>
<td>5 mg/kg, 3</td>
<td>DUBCL</td>
<td>Positive</td>
</tr>
<tr>
<td>17</td>
<td>47, M</td>
<td>6 mg/kg, 3</td>
<td>CD30+ T-cell lymphoma</td>
<td>Negative</td>
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Psoriasis

<table>
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<tr>
<th>Age (y), sex, CD phenotype</th>
<th>Dose of infliximab (mg/kg)</th>
<th>Lymphoma</th>
<th>EBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>70, M, CD</td>
<td>5 mg/kg, 3</td>
<td>Follicular lymphoma</td>
</tr>
<tr>
<td>19</td>
<td>51, M, CD</td>
<td>5 mg/kg, 4</td>
<td>Hodgkin lymphoma</td>
</tr>
<tr>
<td>20</td>
<td>25, M, CD</td>
<td>5 mg/kg, 1</td>
<td>NK cell lymphoma</td>
</tr>
<tr>
<td>21</td>
<td>79, M, CD</td>
<td>5 mg/kg, 1</td>
<td>B cell NHL</td>
</tr>
<tr>
<td>22</td>
<td>24, F, CD</td>
<td>5 mg/kg, NR</td>
<td>B cell NHL</td>
</tr>
<tr>
<td>23</td>
<td>65, M, RA</td>
<td>NR</td>
<td>DUBCL</td>
</tr>
<tr>
<td>24</td>
<td>24, NR, RA</td>
<td>NR</td>
<td>Mixed cell NHL</td>
</tr>
<tr>
<td>25</td>
<td>25, NR, RA</td>
<td>NR</td>
<td>B cell NHL</td>
</tr>
<tr>
<td>26</td>
<td>26, NR, RA</td>
<td>NR</td>
<td>B cell NHL</td>
</tr>
<tr>
<td>27</td>
<td>27, NR, RA</td>
<td>NR</td>
<td>B cell NHL</td>
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<tr>
<td>28</td>
<td>88, M, RA</td>
<td>NR</td>
<td>DUBCL</td>
</tr>
<tr>
<td>29</td>
<td>89, NR, RA</td>
<td>B cell NHL</td>
<td>NR</td>
</tr>
<tr>
<td>30</td>
<td>89, M, NR</td>
<td>Low grade NHL</td>
<td>NR</td>
</tr>
<tr>
<td>31</td>
<td>89, M, RA</td>
<td>NR</td>
<td>Mixed cell NHL</td>
</tr>
<tr>
<td>32</td>
<td>33, NR, CD</td>
<td>5 mg/kg, 1</td>
<td>NK cell lymphoma</td>
</tr>
<tr>
<td>33</td>
<td>34, M, CD, NR</td>
<td>10 mg/kg, 3</td>
<td>Metachronous lymphoma (ALCL, DUBCL)</td>
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### References


Genotypes 677TT and 677CT+1298AC of methylenetetrahydrofolate reductase are associated with the severity of ulcerative colitis in central China

Increased blood levels of homocysteine have been found to be associated with inflammatory bowel disease (IBD) in several studies. The main genetic determinant associated with elevated plasma levels of homocysteine (t-Hcys) is the *MTHFR* 677CT→T gene polymorphism of methylenetetrahydrofolate reductase, a critical enzyme involved in the remethylation pathway of homocysteine. An association of the *MTHFR* 677TT allele with IBD has been reported in Northern Europe, but not in three other populations from Italy and France. Double heterozygosity with the *MTHFR* C677T allele has also been detected, with a frequency of 5–7% in Central Europe, Asia, and the United States.

The relationship between the *MTHFR* gene and IBD has been studied in several populations. A study in a population-based cohort from Stockholm County, Sweden, found an increased risk of IBD with the *MTHFR* 677TT genotype. The relationship between the *MTHFR* gene and IBD has been studied in several populations. A study in a population-based cohort from Stockholm County, Sweden, found an increased risk of IBD with the *MTHFR* 677TT genotype. The relationship between the *MTHFR* gene and IBD has been studied in several populations. A study in a population-based cohort from Stockholm County, Sweden, found an increased risk of IBD with the *MTHFR* 677TT genotype.

In this study, we have evaluated the association of ulcerative colitis (UC) with the *MTHFR* 677CT→T, *MTHFR* 1298A→C, and *TCN1* 776C→T genotypes. The research protocol was approved by the local appointed committee. Extraction of DNA and determination of genotypes were performed as described previously by us. A continuity corrected t-test and an ANOVA test were performed, respectively, to assess differences in categorical and continuous variables between groups. Odds ratios of independent categorical variables were calculated.
Table 1 Clinical characteristics and methyltetrahydrofolate reductase (MTHFR) and transcobalamin (TCN) polymorphisms in 72 patients with ulcerative colitis (UC) and 111 controls from central China

<table>
<thead>
<tr>
<th>Genotypic polymorphisms</th>
<th>Allele</th>
<th>% (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR 677T allele</td>
<td>50 (34.7) [27.3–42.7]</td>
<td></td>
</tr>
<tr>
<td>MTHFR 677T/CT allele</td>
<td>14 (10.0) [8.8–20.7]</td>
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<tr>
<td>MTHFR 677CT allele</td>
<td>42 (85.7) [74.3–93.6]</td>
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<tr>
<td>TCN 776G allele</td>
<td>62 (63.3) [53.6–72.3]</td>
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</tr>
<tr>
<td>TCN 776G/GG allele</td>
<td>50 (50.0) [37.3–62.3]</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotypic polymorphisms</th>
<th>Allele</th>
<th>% (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR 677TT/CT/1298AC</td>
<td>7 (4.2) [1.7–12.4]</td>
<td></td>
</tr>
<tr>
<td>MTHFR 677TT/T1298AC</td>
<td>10 (6.1) [3.7–16.0]</td>
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</tr>
<tr>
<td>MTHFR 677TT/1298AC</td>
<td>73 (46.9) [37.0–57.9]</td>
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</tr>
<tr>
<td>TCN 776G allele</td>
<td>67 (67.7) [58.0–78.2]</td>
<td></td>
</tr>
<tr>
<td>TCN 776G/GG allele</td>
<td>33 (33.3) [23.3–46.3]</td>
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</table>

<table>
<thead>
<tr>
<th>Extent of UC (n (%))</th>
<th>Current smoker (n (%))</th>
<th>Sex (F/M)</th>
<th>Age (y) (mean (SD))</th>
<th>Age of onset (y) (mean (SD))</th>
<th>Current smoker (n (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total colon</td>
<td>72 (44.0) [34.8–53.1]</td>
<td>72 (44.0) [34.8–53.1]</td>
<td>35 (37) [58–60]</td>
<td>41 (15) [38–14]</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>Rectum</td>
<td>40 (55.6) [35.4–71.0]</td>
<td>40 (55.6) [35.4–71.0]</td>
<td>38 (18) [34–58]</td>
<td>40 (13) [38–14]</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>Right colon</td>
<td>1 (1.4) [0.6–2.4]</td>
<td>1 (1.4) [0.6–2.4]</td>
<td>1 (1.4) [0.6–2.4]</td>
<td>1 (1.4) [0.6–2.4]</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>Total colon</td>
<td>17 (23.6) [15.2–35.5]</td>
<td>17 (23.6) [15.2–35.5]</td>
<td>17 (23.6) [15.2–35.5]</td>
<td>17 (23.6) [15.2–35.5]</td>
<td>1 (1.4)</td>
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</tbody>
</table>

In conclusion, our study showed that the genotypes of MTHFR, associated with a decrease in enzyme activity, seemed to be more significantly associated with extension of disease than with the primary risk, at least in central China.

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Conflict of interest: None declared.

Figure 2

- 78-82 (n=34)
- 88-92 (n=102)
- 93-97 (n=176)
- 98-02 (n=207)

p=0.81

References