Severity of ulcerative colitis is associated with a polymorphism at diamine oxidase gene but not at histamine N-methyltransferase gene

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Abstract

AIM: To analyse the role of two common polymorphisms in genes coding for histamine metabolising enzymes as it relates to the risk to develop ulcerative colitis (UC) and the clinical course of these patients.

METHODS: A cohort of 229 unrelated patients with UC recruited from a single centre and 261 healthy volunteers were analysed for the presence of Thr105Ile and His645Asp amino acid substitutions at histamine N-methyltransferase (HNMT) and diamine oxidase (ABP1) enzymes, respectively, by amplification-restriction procedures. All patients were phenotyped and followed up for at least 2 years (mean time 11 years).

RESULTS: There were no significant differences in the distribution of ABP1 alleles between ulcerative colitis patients and healthy individuals [OR (95% CI) for variant alleles = 1.22 (0.91-1.61)]. However, mutated ABP1 alleles were present with higher frequency among the 58 patients that required immunosuppressive drugs [OR (95% CI) for carriers of mutated alleles 2.41 (1.21-4.83; \( P = 0.006 \)], with a significant gene-dose effect (\( P=0.0038 \)). In agreement with the predominant role of ABP1 versus HNMT on local histamine metabolism in human bowel, the frequencies for carriers of HNMT genotypes or mutated alleles were similar among patients, regardless clinical evolution, and control individuals.

CONCLUSION: The His645Asp polymorphism of the histamine metabolising enzyme ABP1 is related to severity of ulcerative colitis.

Key words: Ulcerative colitis; Pharmacogenetics; Histamine N-Methyltransferase; Diamine Oxidase

INTRODUCTION

Mast cells have been recognized as a key cell type involved in type I hypersensitivity. Several studies support a role of mast cells in the development of IBD. These studies indicate that among patients with IBD there is an increase in the number of mast cells and high levels of their degranulation products, such as histamine and tryptase, in the mucosa of colon and ileum.\[3,4\] In addition, it has been shown that the extent of N-methylhistamine urinary excretion is nearly twice in patients with IBD that in healthy subjects, thus indicating that histamine is involved in IBD.\[5,6\] Increased fecal levels of N-methylhistamine in patients with inflammatory bowel disease (IBD), as compared to control subjects, has also been reported.\[4,5\] Further evidences support that the use of mast cell stabilizers, such as ketotifen, attenuates the severity of IBD.\[6,7\]

Histamine is degraded through two enzymes, histamine N-methyltransferase (HNMT, EC. 2.1.1.8) and diamine oxidase (amiloride binding protein 1, ABP1; EC 1.4.3.6). The expression of ABP1 and HNMT in human bowel has been reported.\[8,9\] The genes coding for these enzymes are polymorphic and diverse nonsynonymous single nucleotide polymorphisms (SNPs) have been described.
on these genes. The HNMT gene, located in chromosome 1p32, shows eight SNPs, but only one of these, located in exon 4 C314T, causes amino acid substitution Thr105Ile. The variant allele is unambiguously related to decreased enzyme activity and immunoreactive protein and among Caucasian individuals corresponds to nearly 10% of alleles. Four nonsynonymous SNPs have been mapped to the ABP1 gene, located in chromosome 7q34-36. Among these, the SNP located in exon 3 C2029G codes for an altered protein with the amino acid substitution His645Asp and preliminary reports indicate that these occur with a high allele frequency, corresponding to roughly 50% alleles.

The role of histamine in IBD is sufficiently documented to allow the examination of the contribution of genetic polymorphisms in IBD. We hypothesize that individuals carrying variant alleles leading to a decreased inactivation of histamine may show altered susceptibility or a different evolution of IBD. A preliminary study involving 17 patients with ulcerative colitis (UC) and 34 patients with Crohn's disease indicated a similar frequency of HNMT C314T variant alleles among patients with IBD as compared to control subjects. In contrast, the same study reported an increased frequency of individuals carrying variant ABP1 alleles among patients with IBD. However, none of the ABP1 gene variations analyzed in such study is located within the coding region of the gene, and no evidences on a relevant effect of these mutations in ABP1 enzyme activity or expression have been published. The present study was undertaken to investigate whether common SNPs causing amino acid substitutions on HNMT and ABP1 enzymes are related to UC. For this we selected a large group of patients and controls, numerous enough to obtain conclusive evidences even if the allele frequencies differ only by 10% among cases and controls.

MATERIALS AND METHODS

Study population
We studied a cohort of 229 Caucasian unrelated consecutive patients with ulcerative colitis recruited in the Unit of Inflammatory Bowel Disease from a single tertiary referral center (Hospital Clínico San Carlos) in Madrid, Spain. Ninety-one were women (mean age ± SD at diagnosis 36.57±13.62), and 138 men (38.13±15.92), with a median duration of follow-up of 9 years (mean 11 years, range 2-41 years). Diagnosis of UC was based on standard clinical, radiological, endoscopic, and histologic criteria. Phenotypic details were obtained by review of clinical charts and personal interview with the patients. The same clinical questionnaire was completed for each patient. This questionnaire included: date of birth, sex, familial IBD (defined as the presence of disease in a first or second degree relative), age at diagnosis, duration of follow-up, smoking habits, history of previous surgery (tonsillectomy, appendectomy), extraintestinal clinical manifestations (articular, cutaneous, ocular, hepatic), and previous treatment as an indication of severity of disease (immunosuppressant and surgical intervention). The immunosuppressive therapy was defined by the use of azathioprine, 6-mercaptopurine and/or cyclosporine for a minimum of 3 mo continuous treatment at standard recommended doses to control disease activity. Surgical intervention was recorded when colectomy was indicated in patients who were intractable to medical therapy and for those with massive hemorrhage, colonic perforation and unresolving toxic megacolon. Disease distribution for UC patients was simplified into two groups: extensive UC, those with total or subtotal colitis (beyond the splenic flexure), while non-extensive disease was referred to disease limited to the left colon, including patients with left side colitis, proctosigmoiditis and proctitis. Distribution was assessed macroscopically and microscopically at the time of diagnosis and at subsequent colonoscopies. The maximum extent recorded was used in the classification for this study. All patient data were recorded by a gastroenterologist from the Unit of IBD who was blind to the genotype status of each patient.

A total of 261 normal healthy unrelated controls ethnically matched with patients and from the same geographic area were recruited. All individuals were Caucasian living in Madrid and surrounding area. Control subjects were selected among the staff of the Universities and Hospitals participating in the study. One hundred and four were women (mean age ± SD 45.42±12.91 years) and 157 men (43.51±10.62 years). Medical examination and history was obtained from every individual to exclude pre-existing disorders. Subjects with personal or familial (up to second degree relatives) antecedents of IBD, autoimmune or allergic diseases were excluded. The protocol was approved by the Ethics Committee of the Hospital Clínico San Carlos (Madrid), and all patients and controls were included in the study after informed consent. All UC patients and over 95% of the healthy subjects requested agreed to participate in the present study.

Genotyping
Genomic DNA was obtained from peripheral leukocytes and purified according to standard procedures. The presence of the SNPs was investigated by amplification-restriction and electrophoresis in agarose gels. The analysis of the HNMT C314T SNP was carried out after the amplification of a gene fragment comprising exon 4 using the following primers (based on the published human HNMT sequence Gene Bank Accession No. U44109): GAA AAA CGT TCT TTT TAT TGT GTA TAT AA and ATT TGG GCA GAT CAT GGT CAC TTTG T . After an initial step of 2 min at 94 °C, PCR amplification was carried out for 40 cycles of 25 s at 94 °C, 1 min at 52 °C, and 1 min at 72 °C, and a final extension period of 5 min at 72 °C. The 394 base pairs (bp) PCR product was incubated with the endonuclease EcoRV that digests the mutated allele in two fragments of 179 and 215 base pairs, leaving intact the wild-type sequence.

Regarding the C2029G SNP within the ABP1 gene, the following primers (based on the diamine oxidase gene sequence Gene Bank Accession No. X78212) were used: GGT CAC CTT AAC CCG GTT AAC and TTG TGG CCT CTT AAC TTG CCG. After an initial step of 2 min at 94 °C, PCR amplification was carried out for 40 cycles of 25 s at 94 °C, 1 min at 52 °C, and 1 min at 72 °C, and a final extension period of 5 min at 72 °C. The amplified
Table 1 HNMT genotypes and allele frequencies among overall UC patients and healthy controls

| Genotype | UC patients | Healthy controls | OR (95% CI) | p |
|----------|-------------|------------------|-------------|---|
| C/C      | 161 (82.6)  | 193 (78.8)       | 1.27 (0.79-2.05) | 0.33 |
| C/T      | 32 (16.4)   | 48 (19.6)        | 0.75 (0.45-1.23) | 0.34 |
| T/T      | 2 (1.0)     | 4 (1.6)          | 0.60 (0.31-1.28) | 0.50 |
| Total    | 195         | 245              |             |    |

C allele: 354 (90.8) (87.9-93.6) | 434 (88.6) (85.8-91.4) | 1.27 (0.80-2.02) | 0.29 |
T allele: 36 (9.2) (6.4-12.1) | 56 (11.4) (8.6-14.2) | 6.00 (1.32-26.8) | 0.50 |
Total alleles: 390 | 490 |

476 bp fragment contains a constitutive restriction site for the endonuclease AnaII, and a variant-allele specific restriction site. After endonuclease digestion the wild type gene yields fragments of 369 and 107 bp whereas the mutated gene is digested to fragments of 252, 117 and 107 bp.

**Statistical analysis**

The frequencies for the HNMT and ABP1 SNPs were estimated by counting genes and calculating sample proportions. Case-control analyses were performed with the $\chi^2$ statistics or Fisher exact test, each when adequate. The association between HNMT and ABP1 mutations and phenotypic characteristics of UC was estimated by the odds ratio (OR) with the 95% confidence interval (CI). To assess whether HNMT and ABP1 variants influence synergically upon the course of UC, subjects were classified as carriers and noncarriers of nonsynonymous SNPs. The above cited test was used for the comparison of carriers and noncarriers. Logistic regression analysis was performed to assess whether HNMT and ABP1 mutations were correlated with a particular clinical phenotype. Association was expressed as OR with 95% CI. A two-tailed $P$ value equal or less than 0.05 was considered as significant. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 10.07 for Windows (SPSS Inc., Chicago, Ill. USA).

**RESULTS**

The frequency analyses for HNMT and ABP1 polymorphisms are summarized in Tables 1 and 2. The HNMT genotype (Table 1) was analyzed in 195 patients and no differences with controls in genotype distribution were observed. The mutated HNMT allele frequencies were also similar among patients and controls, 9.2 and 11.4%, respectively. The OR (95% CI) value for the mutated allele was 0.79 (0.51-1.22).

In contrast, Table 2 shows that the distribution of ABP1 genotypes differs between UC patients and healthy controls, and that these differences are in the limit of statistical significance for individuals heterozygous for variant alleles (OR = 1.43; 95% CI = 0.98-2.08; $P$ = 0.050). The mutated ABP1 allele frequencies are increased at a non-significant level among patients (OR = 1.21; 95% CI = 0.92-1.60). Regarding the interaction of both genotypes, the frequencies for individuals with both mutated genotypes were similar among UC patients (12.2%) and healthy controls (10.8%). Among UC individuals with mutated HNMT genotypes, the frequencies for C/C, C/G and G/G ABP1 genotypes were 38.2%, 55.9% and 5.9%, respectively. Inversely, among UC individuals with mutated ABP1 genotypes, the frequencies for the HNMT genotypes C/C C/T and T/T were 80.6%, 18.4% and 1.0%, respectively. These allele frequencies are similar to those obtained in overall patients, thus indicating that no HNMT/ABP1 gene interaction is observed among UC patients.

In order to obtain further information on the effect of both studied polymorphisms on phenotypic and clinical characteristics of patients, these were grouped as carriers and non-carriers of the corresponding mutations, either in heterozygosity or homozygosity. ABP1 genotypes are similarly distributed when patients are subdivided according to gender, mean age at diagnosis, mean duration of the disease, family history of IBD, smoking habits, previous appendectomy or tonsillectomy, extent of UC, and extraintestinal clinical manifestations, including cutaneous, articular, ocular and hepatic manifestations of the disease (Table 3).

However, ABP1 genotypes were linked to parameters closely related to the severity of the disease, i.e. need of immunosuppressant therapy and/or need for surgery. Among the 58 patients that required immunosuppressive drugs, 18 of them ultimately needing colectomy due to unresponsiveness of the disease, 41 (70%) were carriers of ABP1 mutations, 6 of them in homozygosity, the proportion of carriers of the mutated allele being significantly higher than that found in the remaining patients [OR (95% CI) = 2.41 (1.21-4.83), $P$ = < 0.006] (Table 3). Moreover, the probability to need immunosuppressant therapy was higher in homo- (OR = 4.24) than in heterozygote (OR = 2.16) carriers of ABP1 mutated alleles (Chi-square for linear trend 8.36, $P$ = 0.0038).

The other clinical incidence related to severity of UC we have analyzed is the need for surgical intervention. Nineteen out of the 23 patients requiring intervention were also carriers of mutations at the ABP1 gene (OR = 2.50; 95% CI = 1.28-4.91; $P$ = 0.003). It should be noted, however, that 18 of these individuals are included in the group of patients needing immunosuppressant therapy.
In the remaining 5 patients colectomy was indicated by acute complications of the disease, as colonic perforation or toxic megacolon.

**HNMT** genotypes are similarly distributed when patients are subdivided according gender, mean age at diagnosis, mean duration of the disease, family history of IBD, smoking habits, previous appendectomy or tonsillectomy, extent of UC, and extraintestinal clinical manifestations, including cutaneous, articular, ocular and hepatic manifestations of the disease (Table 4). Moreover, no statistically significant differences were observed regarding HNMT allele frequencies among patients requiring either immunosuppressive drugs (OR = 1.03; 95% CI = 0.44-2.42) or surgical intervention (1.94; 0.72 - 5.24), respectively. Gene interactions for these subgroups of patients were calculated as described above and no differences were found in relation to the remaining patients.

### Table 3 Phenotypic characteristics among UC patients classified as carriers or non-carriers of mutations at the ABP1 gene

| Phenotypic characteristics | Carriers n = 128 | Non-carriers n = 101 | OR (95% CI) | p value |
|----------------------------|------------------|----------------------|-------------|---------|
| Male:Female                | 76:52            | 62:39                | 0.79        | 0.52-1.42 | 0.86   |
| Mean age at diagnosis, yr (range) | 37.9 (6.83) - 37.1 (17.81) | 22 (1.8) - 21 (20.8) | P = 0.04 | 0.62-2.39 | 0.54   |
| Mean duration of disease, yr (range) | 11.5 (2.39) - 11.0 (2.41) | 22 (1.8) - 21 (20.8) | P = 0.58 | 0.54   |
| Family history of IBD, n (%) | 31 (24.2)        | 21 (20.8)            | 1.22        | (0.62-2.39) | 0.54   |
| Smokers at diagnosis, n (%) | 28 (21.9)        | 22 (18.1)            | 0.99        | (0.51-1.98) | 1.01   |
| Previous appendectomy, n (%) | 5 (3.9)          | 6 (6.9)              | 0.54        | (0.16-2.47) | 0.58   |
| Previous tonsillectomy, n (%) | 11 (8.6)         | 14 (13.9)            | 0.29        | (0.02-1.55) | 1.58   |
| Extent of UC               |                 |                      |             |         |
| Extensive, n (%)           | 53 (41.4)        | 42 (41.6)            | 1.33        | (0.77-2.30) | 1.00   |
| Non-extensive, n (%)       | 75 (58.6)        | 59 (58.4)            |             |         |
| Extraintestinal manifestations |                 |                      |             |         |
| Cutaneous, n (%)           | 23 (17.9)        | 10 (9.9)             | 0.99        | (0.85-4.76) | 1.64   |
| Articular, n (%)           | 49 (38.3)        | 28 (27.7)            | 0.09        | (0.09-0.99) | 0.16   |
| Ocular, n (%)              | 3 (2.3)          | 4 (3.9)              | 0.58        | (0.01-0.39) | 0.12   |
| Hepatic, n (%)             | 31 (24.2)        | 14 (13.9)            | 0.07        | (0.99-4.42) | 0.77   |
| Therapy categories         |                 |                      |             |         |
| Immunosuppressants, n (%)  | 41 (70)          | 17 (30)              | 2.41        | (1.21-4.83) | 0.006  |
| Surgery, n (%)             | 19 (83)          | 4 (17)               | 2.50        | (1.28-4.91) | 0.003  |

1Eighteen of these patients are also included in the subgroup of patients receiving immunosuppressive therapy. Four of the remaining 5 patients in the surgery group were carriers of the variant ABP1 allele.

### Table 4 Phenotypic among UC patients classified as carriers or non-carriers of mutations at the HNMT gene

| Phenotypic characteristics | Carriers n = 34 | Non-carriers n = 161 | OR (95% CI) | p value |
|----------------------------|-----------------|----------------------|-------------|---------|
| Male:Female                | 20:14           | 91:70                | 1.10        | (0.49-2.49) | 0.81   |
| Mean age at diagnosis, yr (range) | 35 (6.83) - 37 (17.81) | 22 (1.8) - 21 (20.8) | P = 0.24 | 0.63   |
| Mean duration of disease, yr (range) | 12.4 (2.39) - 11.3 (2.41) | 22 (1.8) - 21 (20.8) | P = 0.11 | 0.63   |
| Family history of IBD, n (%) | 8 (23.5%)       | 35 (21.7%)           | 1.11        | (0.42-2.85) | 0.82   |
| Smokers at diagnosis, n (%) | 8 (25.3%)       | 31 (19.3%)           | 1.29        | (0.48-3.36) | 0.57   |
| Previous appendectomy, n (%) | 2 (5.9%)        | 6 (3.7%)             | 1.61        | (0.21-9.47) | 0.03   |
| Previous tonsillectomy, n (%) | 2 (5.9%)        | 17 (10.5%)           | 0.53        | (0.08-2.57) | 0.54   |
| Extent of UC               |                 |                      |             |         |
| Extensive, n (%)           | 15 (44.1%)      | 64 (39.8%)           | 1.20        | (0.53-2.69) | 0.63   |
| Non-extensive, n (%)       | 19 (55.9%)      | 97 (60.2%)           |             |         |
| Extraintestinal manifestations |                 |                      |             |         |
| Cutaneous, n (%)           | 6 (17.6%)       | 19 (11.8%)           | 1.60        | (0.52-4.66) | 0.4    |
| Articular, n (%)           | 15 (44.1%)      | 47 (29.2%)           | 0.91        | (0.84-4.35) | 0.39   |
| Ocular, n (%)              | 1 (2.9%)        | 5 (3.1%)             | 0.95        | (0.02-8.86) | 0.01   |
| Hepatic, n (%)             | 5 (14.7%)       | 27 (16.8%)           | 0.86        | (0.26-2.60) | 0.77   |
| Therapy categories         |                 |                      |             |         |
| Immunosuppressants, n (%)  | 8 (23.5%)       | 37 (23%)             | 1.03        | (0.39-2.65) | 0.94   |
| Surgery, n (%)             | 6 (17.6%)       | 16 (9.9%)            |             |         |

1As compared with the remaining patients.

### DISCUSSION

Nonrandom clustering of distinct diseases related to immunity supports that a common set of susceptibility genes may be involved in the etiology of immune diseases. A total of 18 clustered autoimmune loci have been reported, and among them, loci located at chromosomes 1, 3, 7, 12 and 16 have been reported to be linked to IBD. Among these, chromosome 7 accumulates the highest density of autoimmune loci in man. Five loci significantly related to UC were identified in chromosome 7, where **ABP1** gene is located, with **P** values ranging from 0.003 to 0.00001. Diverse gene polymorphisms have been reported to be linked to UC extension or severity, including **MDR1**, **HLA**, or **MUC3**.

It has been shown that **APBI** enzyme activity is decreased in patients with IBD, but the reason for such decrease remains unknown. Such alteration may be explained in part by polymorphisms on the **ABP1** gene that have been recently described. This is the first study analysing SNPs causing amino acid substitutions at ABP1.
enzyme in patients with UC and, with the exception of a preliminary study carried out in a small group of patients, this is also the first one that studies HNMT polymorphism in UC patients. It should be noted that the HNMT allele frequencies observed in this study are consistent with these described previously, and that the frequency for the His645Asp ABP1 polymorphism is only based on preliminary reports obtained in a limited number of individuals. In this study we have analysed a total of 456 patients and controls (912 alleles) and we have shown that in the analysed Caucasian population the mutated allele frequency observed was lower than initially expected, ranging from 25% to 30% (Table 2).

In this study we have shown that the severity of UC is influenced by a nonsynonomous ABP1 polymorphism. Previous findings indicate that ABP1 enzyme activity in bowel mucosa was about 50% decreased among patients with IBD, as compared to healthy individuals, thus supporting the hypothesis that individuals carrying mutations at ABP1 gene may show altered susceptibility to UC and greater activity of the disease. The lack of association of HNMT polymorphisms with UC may be related to the relative contribution of HNMT to histamine metabolism in bowel tissue, since HNMT activity is almost negligible in bowel tissue as compared to ABP1 activity. It has been reported a mean HNMT enzyme activity in human intestine of 0.47 nmol (min/mg), whereas ABP1 enzyme activity in human intestine is nearly 150 nmol (min/mg).

Histamine release into the bowel is increased in IBD patients compared with controls, and secretion of histamine is related to the disease activity. Markedly elevated mucosal histamine levels were observed in patients with UC, and increased levels of N-methylhistamine, a stable metabolite of histamine, were detected in the urine of patients with UC. In addition, the production of histamine and the urinary excretion of N-methylhistamine are coupled with the degree of inflammation in CD and with the extension of the mucosal surface involved in UC. N-methylhistamine urinary excretion rate was also related to the severity of mucosal inflammation, as it is higher in patients with moderate and high inflammatory mucosa than in patients with only mildly or non inflammatory mucosa. On the contrary, UC is confined to the mucosa of the colon. Extracellular histamine is metabolized to imidazole acetic acid primarily by ABP1 at the gut mucosal surface. Different polymorphisms at the ABP1 gene might affect in different degrees the catabolic rate of histamine in the gut mucosal surface; hence, we can speculate that the association we have found between the carrier state of the His645Asp substitution and the more frequent need to use immunosuppressant therapy or surgery to control the disease depends on a slower rate of histamine metabolic inactivation. Therefore, this specific mutation could be a marker of refractoriness to the standard therapies for UC (sulfasalazine, aminosalicylates and glucocorticoids).

Many symptoms of patients with IBD may be explained taking in consideration the known effects of histamine. Histamine is a key mediator responsible for diarrhea, contraction of smooth muscle, modulation of immune response, or mediation of pain in UC. Higher disease severity may result from a reduction of ABP1 activity in UC. In this subgroup of patients with a genetically reduced rate of ABP1 activity, the therapeutic use of inhibitors of mast cell degranulation could result particularly useful. This family of drugs has been evaluated, with conflicting results, in patients with IBD, but without considering the genetic-induced differences in histamine metabolism. The association between UC and allergic diseases could not be evaluated in the present study because the absence of personal or familial antecedents of allergy was a prerequisite to include subjects in the control group. Among UC patients, no significant differences in the frequency of allergy were identified when the HNMT of ABP1 polymorphisms were considered.

The results obtained in this study provide an additional support for a key role of histamine in UC. However, it is to be noted that the ABP1 gene is adjacent to NO33 gene for which there are variant alleles that seem to have differential effects on the inflammatory process. Thus it cannot be ruled out that linkage disequilibrium between ABP1 and NO33 variant alleles may be related to the findings obtained in the present study. In addition, it should be emphasized that polymorphisms on genes coding for histamine-metabolising enzymes may not be the only cause for increased histamine levels in patients with UC. In fact, it has been shown that intestinal mast cell secretion and release of histamine is increased in patients with IBD and this may be related to variations in histamine synthesis. Presently no variations affecting the histidine decarboxylase gene (HDC) have been reported. Once the analysis of such gene variations is completed, further studies should explore the role of variability in the HDC gene in IBD.

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