Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology

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The discovery of a series of genetic and serological markers associated with disease susceptibility and phenotype in inflammatory bowel disease has led to the prospect of an integrated classification system involving clinical, serological and genetic parameters. The Working Party has reviewed current clinical classification systems in Crohn's disease, ulcerative colitis and indeterminate colitis, and provided recommendations for clinical classification in practice. Progress with respect to integrating serological and genetic markers has been examined in detail, and the implications are discussed. While an integrated system is not proposed for clinical use at present, the introduction of a widely acceptable clinical subclassification is strongly advocated, which would allow detailed correlations among serotype, genotype and clinical phenotype to be examined and confirmed in independent cohorts of patients and, thereby, provide a vital foundation for future work.

Key Words: *Anti-*Saccharomyces cerevisiae *antibodies; Crohn's disease; HLA complex; Indeterminate colitis; NOD2/CARD15; Ulcerative colitis*

Vers une classification clinique, moléculaire et sérologique intégrée des maladies inflammatoires de l'intestin : Rapport d'un groupe de travail du *World Congress of Gastroenterology* de 2005 à Montréal

La découverte d'une série de marqueurs génétiques sérologiques associés à la susceptibilité à la maladie et au phénotype dans les maladies inflammatoires de l'intestin a donné lieu à la perspective d'un système de classification intégré mettant en cause des paramètres cliniques, sérologiques et génétiques. Le groupe de travail a analysé les systèmes de classification clinique courants de la maladie de Crohn, de la colite ulcéreuse et de la colite indéterminée et a fourni des recommandations de classification clinique dans la pratique. Les progrès réalisés dans l'intégration des marqueurs sérologiques et génétiques ont été examinés à fond, et les conséquences sont exposées. Aucun système intégré n'est proposé en vue d'un usage clinique pour l'instant, mais l'adoption d'une sous-classification clinique acceptable est fortement recommandée, ce qui permettrait d'examiner et de confirmer les corrélations détaillées entre le sérotype, le génotype et le phénotype clinique dans des cohortes indépendantes de patients et, par conséquent, de fournir les fondements essentiels à de futurs travaux.

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Crohn's disease (CD) and ulcerative colitis (UC) afflict individuals in many parts of the world and have long been accepted as heterogeneous disorders with a wide variety of clinical presentations and manifestations. Falling under the general term idiopathic inflammatory bowel disease (IBD), these disorders clearly represent a constellation of diseases with both common and unique characteristics.

As a result of this significant clinical heterogeneity, efforts have been made to classify IBD using recognizable clinical and epidemiological features. Most recent attention has focused on CD. An international working team that issued its report in Rome in 1991 proposed a classification scheme in which CD is divided into numerous subgroups based upon anatomical distribution, operative history and predominant clinical 'behaviour', that is, inflammatory, fistulizing or stenotic. Subsequently, the international working group, commissioned for the 1998 World Congress of Gastroenterology in Vienna, Austria, refined the clinical classification of CD. The Vienna group classified CD according to three critical phenotypic characteristics (age at diagnosis, location and clinical behaviour), thereby developing a system that is more practicable for clinical use. These classification systems have since been applied to numerous clinical trials and studies of the pathogenesis and natural history of CD.

Since 1998, significant advances have been made in the discovery of molecular and serological markers related to IBD. Evaluation of these markers and of their importance to IBD diagnosis and phenotypic categorization is the subject of intense investigation. Moreover, limitations in the existing clinical CD classification scheme have become evident, as described by the original authors. It is also noteworthy that there remains a need for a similar reassessment of the classification of UC. The appropriate use of the term indeterminate colitis (IC) is another thorny issue in clinical and research settings. This Working Party has taken on the ambitious task of addressing these individual issues.

Individual teams have taken on the tasks of developing an updated CD classification system, proposing a clinical classification for UC and clarifying the clinical context in which the term IC should be used. The role of geography and ethnicity is discussed, but not of specific environmental influences. At the present time, the classification of IBD is based on predominantly clinical parameters, whereas molecular and serological markers largely remain in the research arena. The Working Party has evaluated currently available data with the goal of integrating clinical features of IBD with genetic and serological markers. Our findings are presented, together with recommendations for clinical classification systems. We hope that these data will catalyze progress over the next decade, with benefits for both clinicians and research investigators.

Crohn's disease clinical classification

Key points

Modification of the Vienna classification as follows:

- Introduction of an early age of onset category (diagnosis at 16 years or younger);
- Allow for the co-classification of location L4 (upper gastrointestinal [GI] involvement) with L1 to L3 (Table 1); and
- Inclusion of a modifier for perianal disease.

Significant references

Cosnes J, Cattan S, Blain A, et al. Long-term evolution of disease behavior of Crohn's disease. Inflamm Bowel Dis 2002;8:244-50.

Louis E, Collard A, Oger AF, Degroote E, Aboul Nasr El Yafi FA, Belaiche J. Behaviour of Crohn's disease according to the Vienna classification: Changing pattern over the course of the disease. Gut 2001;49:777-82.

Sachar DB, Bodian CS, Goldstein ES, et al. Is perianal Crohn's disease associated with intestinal fistulization? Am J Gastroenterol 2005;100:1547-9.

CD is a clinically heterogeneous disorder with a variety of demographic, clinical and phenotypic features. There have been attempts to use these characteristics to identify subgroups of CD patients who differ in their natural history, complications or response to therapy.

Existing classification systems

Greenstein et al (1) proposed classifying patients who require surgery for CD into those with perforating and nonperforating disease. They found that the indications for subsequent surgery tended to be the same as those for the first or previous operation. Moreover, patients with perforation tended to have earlier recurrence of CD following surgery, regardless of disease location. This categorization of CD into perforating and nonperforating subtypes has since been adapted to include patients managed with or without surgery and has been incorporated in the Vienna classification (2).

The Vienna classification of CD considers age of onset (A), disease location (L) and disease behaviour (B), resulting in 24 possible subgroups (2). However, because disease location and disease behaviour have been found to be highly correlated (3,4), the majority of patients fall into a much smaller number of categories. Furthermore, the distribution of patients within the subcategories is not even. In a review of 877 patients from a single practice, it was found that less than 1% had only upper GI involvement and only 15.6% were diagnosed after 40 years of age (5). In another series, 21.1% of patients were diagnosed after 40 years of age (6). This uneven distribution may be an accurate reflection of the relative rarity of some subtypes, but it can produce some practical problems when applying statistical analysis to patient datasets.

Despite some of these concerns, a number of studies have demonstrated correlations between the Vienna classification system and other aspects of disease presentation, genetic susceptibility and natural history. It has been shown that certain disease location and behaviour variables in the Vienna classification system are associated with anti-*Saccharomyces cerevisiae* antibody (ASCA) profiles (7,8), and with the presence of one of the nucleotide-binding oligomerization domain 2/caspase recruitment domain-containing protein 15 (NOD2/CARD15) gene mutations (8-10). The Vienna classification also appears to predict the need for immunosuppressants and surgery (11).

It has been well recognized that disease behaviour, however, has a tendency to progress from one category to another during prolonged follow-up (12-14). It has also been suggested that perianal disease should not be regarded as a manifestation of penetrating disease behaviour (B3) because its occurrence has been found to be independent of intestinal penetrating disease (15,16). In addition, the upper GI disease location

(L4) variable is said to be present when there is any disease proximal to the terminal ileum, even if the ileum and/or colon are involved. This potentially creates an artificial distinction that, although useful for its relative ease of use and simplicity, may obscure the importance of the occurrence of disease at two or more sites.

There are certain aspects of disease course and prognosis that are not included in existing classification systems. The severity and aggressiveness of the disease are features that are difficult to reliably define and evaluate, even though they are potentially relevant to studies of natural history and as prognostic variables for therapeutic trials. Potential corollaries of the severity and aggressiveness of disease are the responsiveness to different classes of medical therapies and the need for surgical therapy.

Age of onset

Age of onset is a variable that is likely to predict a greater genetic predisposition to disease or, alternatively, an earlier or heavier exposure to some, as of yet undetermined, environmental causative or permissive factor. There is evidence that the phenotype of disease at diagnosis varies according to the age of onset and may relate to some extent to genotype (17,18). Small intestinal and upper GI disease is more common in individuals diagnosed with CD before 20 years of age, whereas colonic disease is diagnosed more frequently in patients older than 60 years of age (19-24). Heyman et al (25) analyzed data from 1370 pediatric-onset IBD cases, with some striking findings. Colonic disease was present in almost all children diagnosed before the age of eight years, including those with CD, but in only 46% of those diagnosed thereafter. The older children were more likely to have small intestinal or upper GI disease (25-27). The male to female ratio also decreases with increasing age of onset of CD (25). Family history of IBD is also more likely to be elicited in patients with an earlier age of onset (22). Given the fact that children have different presentations and genotypes from those seen in adults, we propose the introduction of a new early-onset category. The revised age breakdown would now be A1, with age at diagnosis of 16 or younger; A2, with age of diagnosis 17 to 40 years; and A3, with age of diagnosis older than 40 years (Table 1).

Disease location

The major divisions of disease site that are agreed upon by most experts are ileum (distal small intestine), colon and ileocolonic. These are recognized and well-defined within the context of the Vienna classification system. As already discussed, however, the application of the definition of upper GI involvement essentially disregards any disease involvement distal to the jejunum. When the Vienna classification system was developed and existing databases were examined it appeared that the presence of disease distal to the jejunum was unusual in patients with upper GI involvement. Because these data were derived before the availability of sensitive techniques, such as wireless video capsule endoscopy, subtle disease in the small intestine might have been missed. We suggest that the issue of distal disease in patients with upper GI involvement should be re-examined using more sensitive techniques and, if necessary, modifications of the Vienna classification should be considered. To resolve this issue, we propose that, if proximal disease is found in conjunction with distal disease (categories L1 to L3), a category of L4 be added and not be considered a mutually exclusive category. For example, a patient with both distal ileal and jejunal disease would be given the designation L1 as well as L4. This would enable the analysis of patients with ileal and/or colonic involvement with concomitant proximal involvement (Table 1).

Although disease site tends to be relatively stable over time (16), there are patients in whom new sites of disease appear only years after the original diagnosis of CD. Regression of disease can also occur, so that previously affected areas can be completely healed by treatment. In some cases, surgical resection appears to have precipitated the extension of disease location. Although it would be virtually impossible to arbitrarily define a specific point in the disease course at which time disease location can be determined with reasonable validity, it would seem reasonable to continue to regard the maximum extent of disease before the first resection as the 'true' disease location for the purposes of the classification scheme.

TABLE 1
Summary of revised 'Montreal classification' of Crohn's disease

Age at diagnosis (A)			
A1	16 years or younger		
A2	17–40 years		
A3	Over 40 years		
Location (L)		**Upper GI modifier (L4)**	
L1	Terminal ileum	L1 + L4	Terminal ileum + Upper GI
L2	Colon	L2 + L4	Colon + Upper GI
L3	Ileocolon	L3 + L4	Ileocolon + Upper GI
L4	Upper GI	–	–
Behaviour (B)		**Perianal disease modifier (p)**	
B1*	Nonstricturing, nonpenetrating	B1p	Nonstricturing, nonpenetrating + perianal
B2	Stricturing	B2p	Stricturing + perianal
B3	Penetrating	B3p	Penetrating + perianal

**B1 category should be considered 'interim' until a prespecified time has elapsed from the time of diagnosis. Such a time period may vary from study to study (eg, 5-10 years is suggested) but should be defined in order for B1 behaviour to be considered 'definitive'. GI Gastrointestinal*

Disease behaviour

The classification of disease behaviour, as based on the definitions provided in the Vienna classification system, may be problematic for a number of reasons. First, disease categories are not necessarily independent. It has been shown, for example, that the relatively well-defined category of disease site tends to be correlated with disease behaviour (eg, ileal with fibrostenotic disease). Second, a number of factors might lead to interobserver disagreement in the assignment of a behaviour (28,29). Various disease behaviours can coexist, making it difficult to determine which feature is 'primary'. For example, internal fistulas almost always coexist with some degree of stenosis or obstruction distal to the origin of the fistula (30). Additionally, disease behaviour has been shown to change or progress over the course of the disease in some patients, typically from nonpenetrating, nonstricturing disease (B1) to stricturing (B2) or penetrating (B3) (5,16). Therefore, any future attempts at classifying disease behaviour should incorporate some aspect of time course or, at a

minimum, require a certain amount of time to have elapsed before assigning a disease behaviour designation. Data from several studies suggest five years as a reasonable interval because many, but not all, patients that are destined to develop stricturing or penetrating disease will have done so by that time (Table 1).

Although a five-year time period following diagnosis might seem somewhat arbitrary, it is based in part on the rate and incidence of disease progression reported by Louis et al (14). In that study, 26.3% of patients had stricturing or penetrating disease behaviour at diagnosis and, by five years after diagnosis, the percentage had increased to 48% and, by 10 years, to almost 70%. These individuals would be considered to have varying degrees of more progressive or active disease. In a population-based study from Olmsted County, development of a fistula (perianal or enteroenteric) was observed in 26% of patients by five years (31). Approximately one-half of these patients had one or more fistulas before, or within 30 days after, diagnosis. In that study, the initial disease behaviour at diagnosis for the remaining patients and the rates of progression to stricturing disease were not reported. Based upon the Louis data (14), if one were to use the 10-year point to determine the presence of progressive or aggressive disease, fully 70% of patients would fall into this category. This is probably an overestimate; therefore, the five-year cut-off was suggested as a reasonable compromise. It should be remembered that a requirement for a minimum five-year follow-up would mean that a significant proportion of patients could not be classified early in the course of their disease. This has important implications depending on the way in which the behaviour classification is used. For clinical trials, the disease behaviour at the time of intervention is probably more important than the possible disease behaviour at 10 or 20 years. In such cases, it might be reasonable to classify disease behaviour before the five-year point. On the other hand, for studies of genotype-phenotype correlations, it is important to have a well-defined and stable phenotype that does not change over time; thus, a minimum of 10 years or more follow-up might be more appropriate. It is suggested that investigators using the disease behaviour classification determine what is appropriate for their purposes, because it will almost certainly vary from study to study.

Another concern with the existing Vienna classification definition of penetrating disease behaviour (B3) is the inclusion of patients with only perianal fistula. Smith et al (14) studied the Vienna classification, disease progression and outcome, serology and genetic markers in 231 well-characterized CD patients and found that patients with perianal fistulas or abscesses differed from those with intestinal penetrating disease. The International Organization for Inflammatory Bowel Diseases Task Force on Disease Classification (16) examined the database records of 5491 CD patients from six centres and found that, in patients with colonic disease but not in those with ileal disease, there was an association between intestinal fistulas and perianal fistulas. In addition, data from a population-based research registry have shown that almost 80% of patients with perianal fistula have no enteric fistula (32). These observations suggest that the presence of perianal and enteric fistula describes two different phenotypes.

We suggest that the Vienna classification be modified so that perianal fistulas are no longer included in the penetrating disease category. The wording of the definition of this category would be changed to "the occurrence of intra-abdominal fistulas, inflammatory masses and/or abscesses at any time in the course of disease". We suggest the inclusion of perianal fistulas and abscesses as modifiers of the disease behaviour variable. This would be indicated by a 'p' (for perianal) appended to B1, B2 or B3, resulting in B1p, B2p or B3p (Table 1).

CONCLUSIONS

The recommendations outlined above are based, as much as possible, on existing clinical and natural history data, as well as expert opinions. The additions to the Vienna classification require further validation studies in the ongoing process of developing a robust scheme that incorporates the most recent clinical, serological and genetic information.

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Ulcerative colitis clinical classification

Key points

A classification system for UC is proposed that incorporates:

- Disease extent; and
- Disease severity of individual acute relapses.

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With an increasing understanding of the epidemiology and genetics of IBD, it has become evident that UC, like CD, may actually represent several forms of IBD. A formal clinical classification of UC similar to the Vienna classification of CD (1) does not exist. We sought to review the evidence that justifies the existence for a clinical classification of UC.

Theoretically, a clinical classification system for UC would be useful if it had implications for pathogenesis (ie, if it correlated with subclinical or genetic markers), therapy (eg, topical or oral therapy) or prognosis (eg, severity of disease, requirement for colectomy, colorectal cancer risk or mortality). The most commonly used classification systems in UC stratify patients by the extent of colonic involvement or by disease activity. This section reviews the rationale for these classification systems.

Classification by extent

UC can be defined by the extent of colorectal inflammation at a radiographic, endoscopic or histological level. For the purposes of simplification, we propose that the extent of UC be defined by endoscopic appearance and by maximal extent during follow-up. The three subgroups of UC defined by extent are:

1. Ulcerative proctitis (E1): involvement limited to the rectum (ie, proximal extent of inflammation is distal to the rectosigmoid junction).
2. Left-sided UC (E2) (also known as distal UC): involvement limited to the portion of the colorectum distal to the splenic flexure.
3. Extensive UC (E3) (also known as pancolitis): involvement extends proximal to the splenic flexure.

This three-tiered classification system appears to be useful in distinguishing patients by medical therapy and by prognosis. For example, hydrocortisone or mesalamine suppositories appear to be most useful as primary therapy for patients with ulcerative proctitis (2,3), while these same medications in foam or enema form are most useful as primary therapy in patients with left-sided UC (2,4,5). Oral delivery of sulfasalazine and the 5-aminosalicylate agents is, of course, possible in patients with proctitis and left-sided disease, but may not be necessary. On the other hand, use of topical therapy alone is usually insufficient for the primary therapy of extensive UC.

The extent of colitis has implications for the activity or severity of the condition, whether measured by rates of medication usage, hospitalization or colectomy. A population-based study from southeastern Norway (6) found that patients with extensive colitis were more likely to require 5-aminosalicylate agents or corticosteroids than patients with left-sided disease or proctitis. In a study of 269 recently diagnosed UC patients evaluated at St Mark's Hospital, United Kingdom, between 1966 and 1975 (7,8), crude hospitalization rates were significantly higher among those with extensive colitis (70%) than proctosigmoiditis (25%) or proctitis (13%). A review of selected referral centre-based (7,9) and population-based (6,10) cohorts shows that the actuarial risk of colectomy is influenced by the extent of UC (Table 2). The risk of colectomy among proctitis patients ranged from 2% to 9% after five years, while patients with extensive colitis had five-year colectomy rates of 30% to 44%.

It has been recognized for years that, in UC, the risk of colorectal cancer is correlated with disease extent. This correlation has been established in studies from referral centres (11,12) or population surveys (13-16) (Table 3). The cumulative risk of colorectal cancer in patients with ulcerative proctitis ranges from 0% to 12% after 30 years of disease, compared with a cumulative risk of 4% to 47% after 30 years in those with extensive or pancolonic disease.

TABLE 2
Risk of colectomy stratified by disease extent in selected cohorts of ulcerative colitis patients*

Setting (reference)	Proctitis	Proctosigmoiditis	Left-sided colitis	Extensive colitis	Pancolitis
St Mark's Hospital, UK (7,8)	2% at five years	6% at five years		21% at five years	30% at five years
Copenhagen County, Denmark (10)	9% at five years		19% at five years		35% at five years
Cleveland Clinic, USA (9)		14%†; 7% at five years	52%†		61%†; 44% at five years
Southeastern Norway (IBSEN) (6)	2% at one year		2% at one year	9% at one year	

**Rates are actuarial unless otherwise specified; †Crude rate, mean follow-up of 12.7 years (minimum five-year follow-up in 100%). IBSEN Inflammatory Bowel South-Eastern Norway Study Group; UK United Kingdom; USA United States of America*

TABLE 3
Risk of colorectal cancer stratified by disease extent in selected cohorts of ulcerative colitis patients*

Setting (reference)	Proctitis	Left-sided colitis	Extensive colitis	Pancolitis
Mayo Clinic, USA (11)	12% at 30 years	30% at 30 years	47% at 30 years	
Cleveland Clinic, USA (12)		4% at 30 years	25% at 30 years	
Stockholm County, Sweden (13)		<5% at 25 years		13% at 25 years
Central Israel (14)	0% at 20 years		4% at 20 years	14% at 20 years
Uppsala, Sweden (15)		<5% at 30 years	12–32% at 30 years	
Malmo, Sweden (16)	1.1%†	0.6%†		5.3%†

**Rates are actuarial unless otherwise specified; †Crude rate, mean follow-up of 13.9 years. USA United States of America*

TABLE 4
Standardized mortality ratio (SMR; observed deaths/expected deaths) and 95% CIs stratified by disease extent in selected cohorts of ulcerative colitis patients

Setting (reference)	Proctitis SMR (95% CI)	Proctosigmoiditis SMR (95% CI)	Left-sided colitis SMR (95% CI)	Extensive colitis SMR (95% CI)	Pancolitis SMR (95% CI)
Uppsala, Sweden (17)	1.4 (1.2–1.5)		1.2 (1.0–1.4)		1.9 (1.7–2.2)
Copenhagen County, Denmark (10)					1.68 (P<0.02)
Leicestershire, UK (19)	0.8 (0.52–1.17)		0.93 (0.55–1.47)	0.84 (0.42–1.49)	0.85 (0.54–1.27)
Wolverhampton, Salisbury and Swindon, UK (20)	0.64 (0.23–1.40)	0.9 (0.46–1.57)	1.56 (0.89–2.52)	1.01 (0.40–2.08)	
Copenhagen County, Denmark (1997 update) (18)	0.87 (0.72–1.07)			1.23 (1.04–1.44)	

UK United Kingdom

Population-based studies suggest that mortality in UC patients may also correlate with increasing extent of colitis (10,17,18) (Table 4). In the updated examination of mortality in the Copenhagen County, Denmark, cohort (18), extensive colonic involvement at diagnosis remained a significant predictor of mortality after adjusting for age, sex and calendar period of diagnosis. Other studies, however, have not shown a consistent relationship (19,20).

It is important to note that most of the aforementioned studies based the extent of colitis on radiographic or endoscopic, not histological, criteria. With the advent of total colonoscopy in the 1970s, it became evident the previous methods of evaluation for UC (ie, proctoscopy or sigmoidoscopy plus double contrast barium enema) were relatively insensitive and could frequently underestimate the extent of involvement or even miss a diagnosis (21). The significance of histological evidence of chronic colitis in an endoscopically normal proximal colon remains unclear in terms of the risk of colectomy, cancer, proximal progression of the disease, or mortality.

One drawback of the extent-based classification system is its instability over time, especially in the proctitis and left-sided colitis subgroups. Most longitudinal studies of UC cohorts that have specifically examined the problem report both progression and regression of the proximal extent of inflammation (9,22-25) (Table 5). The actuarial risk of proximal extension of proctitis after 10 years of disease is between 41% and 54%. For left-sided colitis, the likelihood of later progression to extensive colitis may be even higher. Although not as extensively studied, a diagnosis of extensive colitis may not necessarily be stable (9,23,26) (Table 5). The regression rate ranges from a crude rate of 1.6% (9) to an actuarial rate of 71% after 10 years (23). We therefore propose that the maximum extent of involvement be used in the clinical classification system.

It is not clear that additional terms that are occasionally used to classify UC by extent actually identify clinically important subgroups. For example, the term 'proctosigmoiditis' is sometimes used to describe patients with rectal and sigmoid colonic inflammation without descending colonic involvement. The clinical course and prognosis of these patients is quite similar to those deemed to have left-sided colitis. Another strategy occasionally used is to separate 'extensive UC' (involving the transverse colon but not the ascending

TABLE 5
Rate of disease extent progression and regression in selected cohorts of ulcerative colitis patients*

Setting (reference)	Proctitis to left-sided	Proctitis to extensive	Proctitis to any progression	Left-sided to extensive	Left-sided to proctitis	Extensive colitis to any regression	Pancolitis to any regression
St Mark's Hospital, UK (22)	12% at 10 years	7% at 10 years					
Cleveland Clinic, USA (9)	11.9%†	34.0%†	45.9%†	70.4%†	3.9%†		1.6%†
Copenhagen County, Denmark (23)			41% at 10 years			56% at 10 years	71% at 10 years
Birmingham, UK (24)			49% at 10 years	31% at 10 years			
Southeastern Norway (IBSEN) (26)			22% at 1 year	16% to 24% at 1 year	23% to 25% at 1 year	70% at 1 year	39% at 1 year
Northern Italy (25)	54% at 10 years	10% at 10 years					

**Rates are actuarial unless otherwise specified; †Crude rate, mean follow-up of 12.7 years (minimum of five years in 100%). IBSEN Inflammatory Bowel South-Eastern Norway Study Group; UK United Kingdom; USA United States of America*

colon or cecum) from 'pancolitis'. Again, the clinical courses and prognoses of these two groups are quite similar. There is little or no evidence that these terms enable us to differentiate UC patients by pathogenesis, therapy or prognosis any more precisely than is possible with the three-tiered classification system described above.

Classification by severity

UC can be classified broadly into four disease activity/severity categories:

1. UC in clinical remission (S0): No symptoms of UC.
2. Mild UC (S1): in the classic description of disease activity by Truelove and Witts (27), this was defined as four or fewer bloody stools daily, lack of fever, pulse of less than 90 beats/min, hemoglobin of 105 g/L or greater and erythrocyte sedimentation rate (ESR) of less than 30 mm/h. A similar definition was given in the practice guidelines for management of UC recently published by the American College of Gastroenterology (ACG) (28): four or fewer stools daily (with or without blood), no systemic signs of toxicity and a normal ESR.
3. Moderate UC (S2): Truelove and Witts (27) defined this as the state between mild and severe. The ACG guidelines defined moderate disease as more than four stools daily but with minimal signs of systemic toxicity (28).
4. Severe UC (S3): This was defined as the passage of at least six bloody stools daily, pulse of at least 90 beats/min, temperature of at least 37.5°C, hemoglobin of less than 105 g/L and ESR of at least 30 mm/h (27). The ACG guidelines defined severe colitis as at least six bloody stools daily and evidence of toxicity (fever, tachycardia, anemia or elevated ESR) (28). The latter guidelines separated 'fulminant colitis' from 'severe'. Fulminant patients were those with at least 10 stools daily, continuous bleeding, toxicity, abdominal tenderness and distension, requirement for blood transfusion and colonic dilation on plain abdominal films (28).

One of the limitations of the present disease activity classification systems is that they are useful only for predicting clinical course in the short term. As in CD, there are no longitudinal disease severity indices (ie, incorporating disease severity over time). One of the few studies to address this comes from Copenhagen County, where UC patients could be classified into those with prolonged remission, those with intermittent symptoms and those with continuous activity (29). Disease activity over the first three years of diagnosis predicted the clinical course over the next five years (29). Another issue that needs to be addressed is whether the term 'fulminant colitis' adds any additional value to a three-tiered activity classification system. In most series, fulminant colitis has typically been defined retrospectively to describe patients who failed medical therapy and required colectomy. A small subset of patients with fulminant colitis develop multiple organ dysfunction (30).

Should age at diagnosis be used to classify UC?

The possible existence of a bimodal distribution in age at onset of UC continues to be debated. Some studies found a unimodal distribution, with a peak incidence in the third or fourth decade of life (31,32), while others demonstrated a large peak in the third decade of life and a smaller peak in the elderly (33). Regardless of the pattern, age-related differences in incidence suggest that UC is a heterogeneous entity that varies by the age at diagnosis.

Early studies indicate that, compared with those diagnosed after 16 years of age, patients who are diagnosed in childhood present more often with sudden onset of severe symptoms rather than an insidious onset, experience more complications, are more likely to develop colorectal cancer and are more likely to die of disease-related complications (11,34). In the population-based Copenhagen County cohort, children diagnosed with UC before the age of 15 years had higher rates of extensive colitis, proximal progression of disease and mortality rates than those diagnosed in adulthood (35). On the other hand, that study found no difference in colectomy rates between the two groups. There are conflicting data about whether younger age at onset of UC is a risk factor for IBD-related colorectal cancer, independent of increased duration (15,36).

At the other end of the age spectrum, some investigators have reported that elderly patients with UC have a milder form of the disease. In a 1935 paper from the Mayo Clinic (37) describing 25 patients diagnosed with UC after the age of 60 years, most patients had mild, even intermittent, symptoms and responded promptly to treatment. Later reports (38,39) stated that the prognosis of UC in the elderly was significantly worse than that of the average patient, but were based on very small numbers of patients. Finally, other studies (40-42) suggest that elderly patients with IBD do no worse than expected for the average patient. Therefore, at this juncture, there is

insufficient information to warrant using age at diagnosis of UC in a clinical classification system for the disease. Nevertheless, it should be recorded as part of a minimal data set.

SPECIAL SITUATIONS

Primary sclerosing cholangitis-associated IBD

The characteristics of IBD associated with primary sclerosing cholangitis (PSC) deserve additional comment. While only 5% of UC patients have evidence of sclerosing cholangitis, approximately 70% to 80% of sclerosing cholangitis patients have IBD, with predominantly colonic involvement (43). The bowel disease is often quite mild and insidious in onset. A high proportion of newly diagnosed PSC patients, even those without GI complaints, prove to have chronic colitis when they undergo colonoscopy with biopsy (44). One such patient was even found to have colorectal dysplasia at the time of UC diagnosis, suggesting that some PSC patients may have had undiagnosed IBD for years. Several Mayo Clinic studies (45-47) found that PSC-IBD patients were more likely than patients with UC alone to have extensive colitis with rectal sparing and 'backwash ileitis' without other typical findings of CD, such as granulomas, skip areas, fistulas or strictures. PSC-IBD patients are less likely than matched UC controls to require colectomy (47). If they undergo proctocolectomy with ileal pouch-anal anastomosis (IPAA), however, PSC-IBD patients are more likely than UC patients without PSC to develop pouchitis (48). Furthermore, the risk of colorectal dysplasia and cancer seems to be higher in PSC-IBD patients than UC patients without PSC, even after accounting for differences in disease extent and duration (47,49,50). Because of this increased risk of neoplasia, some authorities have advocated initiating annual surveillance colonoscopy immediately after the diagnosis of PSC-IBD. The differences in clinicopathological features (rectal sparing and backwash ileitis) and prognosis (pouchitis and increased risk of colorectal neoplasia) suggest that PSC-IBD may be an IBD phenotype distinct from both UC and CD (47).

Right-sided colonic or periappendiceal inflammation in left-sided colitis

Several studies (51-55) in the past 15 years have revealed endoscopic evidence of right-sided colonic and/or periappendiceal inflammation in patients with left-sided UC. The prevalence of this finding ranges from 19% to 75%, but its clinical significance is debatable. There is no indication that these patients are more likely to later be diagnosed with CD. One recent pathological study of colon resection specimens suggested that patients with appendiceal inflammation were more likely to develop pouchitis (56), but this finding needs to be confirmed. At this time, there is insufficient evidence to incorporate periappendiceal inflammation into a clinical classification scheme.

CONCLUSIONS

UC can be classified by extent of disease into proctitis (E1), left-sided disease (E2) or extensive disease (E3), and can be classified by disease severity into mild (S1), moderate (S2) or severe (S3). We suggest avoidance of the terms 'proctosigmoiditis' and 'fulminant colitis' in classification systems, because they do not appear to further differentiate patients into clinically useful categories. PSC-IBD may represent a unique phenotype of bowel inflammation. Periappendiceal inflammation in the setting of left-sided colitis is not uncommon, but the clinical significance of this finding remains unclear.

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Indeterminate colitis

Key points

- The diagnosis of IC should be made only after colectomy.
- The term colonic IBD type unclassified (IBDU) should be used in all other cases where definitive features of CD and UC are absent.

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IC is a well-recognized term but there is a great deal of confusion about its exact meaning. In this section, we will discuss this problem and propose a new classification system for chronic inflammatory colitis. Population-based studies from Scandinavia (1-9) have shown that 5% to 20% of IBD patients with colonic inflammation only cannot be definitively diagnosed with CD or UC using available diagnostic tools, including clinical examination, radiology, endoscopy and histology. These patients have been placed in the category of IC, according to the original publication by Price (10). The incidence of

IC is estimated to be 1.6/100,000 to 2.4/100,000 in adults and at 0.2/100.000 in children (8,9).

Definition of IC: Evolution of diagnostic criteria

The term 'indeterminate colitis' was first introduced at St Mark's Hospital, United Kingdom, in 1978 by the pathologist Ashley Price and was based on examination of surgical specimens that displayed overlapping features of both CD and UC (10). Originally, IC was considered to be a temporary diagnosis because it was believed that the majority of patients would eventually prove to have either CD or UC during follow-up (2,3,11).

In the following years, the introduction of colonoscopy led to the development of an integrated diagnosis based on clinical features and endoscopy with biopsies. The disease is chronic and restricted to the colon. Typically, endoscopy is inconclusive and microscopic features of crypt architectural distortion (more than 10% of crypts) and patchy acute and chronic inflammation are described, with no particular diagnostic features of either CD or UC. Infectious colitis and other causes of colitis have to be excluded by stool cultures and histological examination (12-16). This change in definition supports the concept that IC is really a distinct disease instead of merely a temporary diagnosis.

CD, UC or IC: Does it matter?

One could argue that it does not really matter if chronic colitis is further classified as IC, CD or UC, because most treatments (5-aminosalicylic acid, corticosteroids, azathioprine and infliximab) are effective for all. But this is not entirely true. It has been shown that the clinical course and the prognosis of patients with IC is worse than that of UC, and several studies (17-22) have documented a worse outcome of surgery and greater risk of chronic pouchitis. However, these findings have not been confirmed by all investigators (22-24). A recent study from Toronto (22) found that patients with IC have a greater risk of pouchitis than those with UC (43% versus 22%; $P<0.05$), but the risk of pouch failure with excision was not significantly different (10% versus 6%). In the series of IC patients who underwent surgery at St Mark's Hospital in London between 1960 and 1983, patients who continued to have a diagnosis of IC after careful preoperative and postoperative assessment did well and were unlikely to later develop CD (23). So far, it is still unclear whether a total colectomy with IPAA should be recommended in these patients, and prospective studies will be required to answer this clinically important question.

Research agenda

- There is a need for prospective studies investigating the outcome of surgery and of IPAA in patients with IC.

Toward a molecular classification of IC – A role for serological markers?

A multicentre prospective study (25) from Leuven, Belgium, Vienna, Austria and Lille, France identified 97 patients who could not be classified as having either CD or UC. Serological markers ASCA and atypical antineutrophil cytoplasmic antibody with a perinuclear staining pattern at indirect immunofluoresence (pANCA) were determined in all patients. A definitive diagnosis of CD was made when there were characteristic small bowel lesions, fistulas or granulomas. A definitive diagnosis of UC was based on the finding in surgical specimens of diffuse involvement, starting distally, with a lack of transmural inflammation and, if applicable, more severe lesions distally. For the cases where only endoscopic samples were available, the diagnosis was based on examination of multiple biopsies, obtained during repeated endoscopies, that verified that the ileum was uninvolved and that colonic inflammation was more severe in the distal than the proximal colon. Further microscopic features included the presence of widespread and diffuse mucosal distortion, diffuse transmucosal lymphocytic inflammation, cryptitis and crypt abscesses. After a mean follow-up of six years, 31 of 97 patients (32%) acquired a definitive diagnosis of CD (17 cases) or UC (14 cases). Interestingly, almost one-half of the patients (48.5%) had neither ASCA nor pANCA, and the majority of these patients (85%) remained with the diagnosis of IC. In contrast, 61% of patients who eventually were given a definitive diagnosis of either CD or UC had one or both antibodies ($P<0.001$) (25). A follow-up study in 90 of the 97 patients in the original cohort (mean duration of follow-up 14.5 years, minimum of 2.5 years) confirmed that these seronegative patients more often continued to have a diagnosis of IC even after other antimicrobial antibodies (anti-OmpC and anti-I2) were sought (26). Using this panel of four antibodies, approximately one-quarter (26.4%) of the patients were seronegative and, in 74% of these patients, the exact diagnosis remained indeterminate, in contrast to only 50% of patients with positive antibodies ($P=0.04$).

It has been hypothesized that the higher incidence of chronic pouchitis in patients with IC and IPAA represent persistent immune reactivity to microbial antigens (27). In a prospective study, preoperative serological responses to ASCA, I2 and OmpC were assessed in 28 IC patients undergoing IPAA. With a median follow-up of 38 months (range three to 75 months), 61% of patients developed pouchitis, of whom 25% were acute and 75% chronic. Chronic pouchitis developed in 10 of 16 patients (63%) who had a positive antibody reactivity profile compared with only two of 12 patients (17%) with a negative profile ($P=0.015$). Therefore, IC patients who have a positive antibody reactivity profile before IPAA are at significantly higher risk of developing continuous pouch inflammation after surgery than are those with a negative profile.

Research agenda

- There is a need for studies on the value of novel (eg, Cbir) antimicrobial antibodies in patients with IC.
- There is a need for studies investigating which combination of serological markers provide the best specificity and positive predictive value for specific forms of IBD, including IC.

Toward a molecular classification of IC – A role for genetic markers?

At present, no full papers have been published examining the role of genetic markers in the further classification of patients with IC. DNA was available from a subgroup of the patients studied by Joossens et al (25) (68 of 97) and was genotyped for the three main CD-associated NOD2/CARD15 variants (28). Overall, 14 (20.6%) carried at least one NOD2/CARD15 variant and 15 patients were given a definitive diagnosis (10 CD

and five UC). Only one of the 10 patients with CD carried NOD2 mutations, compared with none of those with a final diagnosis of UC and 13 of the remaining 53 IC patients. There was one compound heterozygous patient, who is still currently categorized as having IC. These data do not suggest a role for NOD2 testing in the further classification of IC patients, but more studies of genetic markers are required.

Research agenda

- There is a need for genetic markers to help in the classification of IC patients.

Toward a novel classification of IC?

Advances in medical diagnostics as well as results from recent studies suggest that the concept of IC as a temporary diagnosis should be reconsidered. We believe that IC, as currently defined, is an ambiguous term that is applied to a heterogeneous group of patients with chronic inflammatory colitis. We therefore propose a revision of the concept, based on how the initial diagnosis is made (Figure 1).

In the cases in which the diagnosis is based on findings at surgical resection, in which features of both CD and UC are detected, we propose that the term 'IC' be retained, because this was also the setting in which the term was originally described by Price (10). Follow-up studies (24) have shown that, unless transmural lymphoid hyperplasia or granulomas are found, these patients usually prove not to have CD. The transmural polymorphous inflammation that is seen in these patients is a feature of severe colitis per se, and is not indicative of either CD or UC. On the other hand, we believe that studies investigating the serological markers ASCA, pANCA, antiflagellin, anti-OmpC and I2 might be helpful in further differentiating these conditions, especially when IPAA is envisaged (29-31).

The situation is different when the diagnosis is based on clinical features and endoscopy with biopsies. The clinical features consist of chronic IBD with inflammation restricted to the colon and without small bowel involvement. The endoscopy is inconclusive and histology reveals chronic inflammation with absence of diagnostic features of either CD or UC. In such cases, we propose that the term colonic IBDU be applied. A careful upper GI evaluation including gastroscopy and, if normal, novel endoscopic methods (such as video capsule endoscopy or double-balloon enteroscopy) may be useful. In general, we believe that more research, including the study of novel antimicrobial and genetic markers, is needed to further characterize this subgroup of patients. It is likely that international collaborations will be required to achieve this goal.

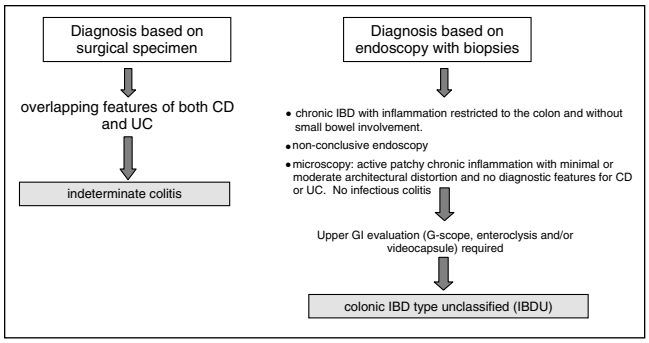

Figure 1) *Flow chart illustrating proposed classification for patients with chronic inflammatory colitis. CD Crohn's disease; GI Gastrointestinal; G-scope Gastroscope; IBD Inflammatory bowel disease; UC Ulcerative colitis*

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Geographic and ethnic factors associated with IBD

Key points

- Wide variations exist in the incidence and prevalence of IBD worldwide.
- A minimal dataset to be used in genetic and environmental research must involve details of ethnicity and residency.

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Current knowledge suggests that both genetic and environmental influences are important in the etiopathogenesis of IBD. In this section of the Working Party report, we assess the geographical and ethnic contributions to the patterns of IBD and make suggestions about how the disease should be classified to aid further research. Incidence rates are quoted as number of cases per 100,000 population.

Geographical variations in the incidence and prevalence of CD and UC

A global north-south variation in the incidence of IBD has been documented. Standardized incidence rates of 10.9 to 12.8 for UC and 6.0 to 7.0 for CD have been reported from northern California (1) and Scandinavia (2). Rates of 2.0 to 6.3 for UC and 0.9 to 3.1 for CD have been observed in the southern hemisphere (3). Direct comparisons can be misleading, because IBD is less prevalent in the developing world. For example, the incidence of UC and CD are 1.9 and 0.5, respectively, in Asia (4), 2.3 and 1.6, respectively in Africa (5), and 2.2 and 0.03, respectively, in Latin America (6). Studies from within Europe also suggest a north-south incidence gradient but, again, direct comparison of studies is confounded by differences in case ascertainment and data analysis. Raw data suggest that the incidence in northern areas is four to five times greater than those in southern areas (7-17).

Data from North America confirmed this observation in a single study (18) with higher hospitalization and mortality rates from IBD observed in the northern United States compared with southern states. This pattern was seen for African-Americans and Caucasians, males and females, and UC and CD. A prospective collaborative European study (EC-IBD) (19) found that UC was 40% more common in northern centres, and that CD was 80% more common. Differences were not as large as expected, which might be due to a rising incidence in southern Europe (14,19). More recent data examining the incidence of juvenile-onset CD in Scotland (20) found age-specific incidence rates for CD in northern Scotland (3.1) to be higher that those in the south (2.1). The absolute difference was relatively modest, with CD detected 47% more frequently in the north. This difference was not seen for UC.

There are a number of 'hot spots' for the incidence of IBD. The highest reported incidence and prevalence rates are from Manitoba, Canada (21,22); Scandinavia (2,13); Iceland (12); and the United Kingdom, especially Scotland (20,23,24). High incidence rates from other provinces in Canada, similar to that of Manitoba, support the notion of northern geographical areas having the highest incidence rates (25).

Variations according to ethnic background

Historical data suggest that African-Americans have a lower incidence of IBD than Caucasians. This pattern was seen in data from South Africa, but the blacks that did develop IBD were urbanized and had at least a partially Westernized diet (5). More recent data suggest that incidence rates in blacks may actually be closer to those in Caucasians. Kurata et al (26) found that hospitalization rates for CD were similar in African-Americans and Caucasians, and a tertiary referral centre study from Georgia, United States, identified a crude incidence rate of 5.3 for UC and 8.8 for CD in black children (27). A prospective United Kingdom survey of childhood IBD found that the incidence of both CD and UC in black children was similar to that in white populations (23). Furthermore, retrospective data from the United Kingdom found a nonsignificant difference in the incidence of CD in Afro-Caribbean and white populations in Derby (4.5 to 5.6 versus 7.0) (28). Southern Asian populations have been thought to have a low incidence of IBD but United Kingdom studies have found that Asians born in the United Kingdom have a higher incidence of UC and proctitis than white populations (29,30). A three-year prospective study from Leicester, United Kingdom found the incidence of UC to be higher in second-generation than first-generation Asians. Moreover, the incidence of UC in second-generation Asians exceeds that in Caucasians (31). Available data suggest that IBD remains uncommon in Hispanics, Asian Americans

TABLE 6
The prevalence of Crohn's disease in the Jewish Israeli population – stratification by birthplace of patients

			Prevalence*			
Reference	Population	Years	Total	Europe/ N Am	Asia/ Africa	Israel
42	Beer-Sheva	1961–1980	14.0	24.6	19.4	6.9
43	Central Israel	1970–1980	19.4	30.0	12.2	12.6
44	Kibbutz	1987	25.5	17.4	41.8	38.9
45	Kinneret	1960–1990	45.9	80.1	41.8	41.1
36	Beer-Sheva	1968–1992	50.6	58.7	55.0	46.3
46	Kibbutz	1997	65.1	79.2	11.9	78.3

**Figures are quoted per 100,000 population. N Am North America*

TABLE 7
Recommendations for geographical and ethnic data to be collected in a research minimal data set

Ethnicity	White/black/Asian/other
	Jewish: yes/no
Country of birth	Proband/parents/grandparents
Family history of IBD	First-degree relative (specify)
	Second-degree relative
	Multiple affected family members/other
Place of residence	Postal codes
Environment	Rural/urban

IBD Inflammatory bowel disease

and aboriginal North Americans (32). Aboriginal Canadians and First Nations persons from Manitoba are also less likely to develop IBD, especially CD (22).

Epidemiological data have consistently documented a higher incidence of IBD in Ashkenazi Jewish than in non-Jewish populations (33). One study suggested that the incidence of IBD within Jewish populations might be related to the country of origin (34), but more recent data from Israel found that the incidence (4.2) and prevalence (50.6) of CD were independent of country of origin, and comparable with those in many Caucasian populations (35,36) (Table 6). The incidence and prevalence of CD among non-Ashkenazi Jews approach those of Ashkenazi Jews in North America and Europe, and prevalence rates in Israel are lower than those reported from Manitoba and Rochester, Minnesota (21,37).

Variations in the incidence of IBD between rural and urban populations

Higher incidences of both CD and UC have been identified in urban than rural populations in the United States (18), Manitoba (22), Uppsala, Sweden (16), the Faroe Islands (38), Scotland (9) and Rochester, Minnesota (37,39). Data from Alberta, Canada identified an urban predominance for CD but not UC (40). Few studies have found no difference (41). The availability of health care resources is frequently cited as a confounding factor. Canada and Sweden (16,22) provide universal health care, thereby minimizing the ascertainment bias, and the observed differences for both CD and UC between urban and rural populations were confirmed.

Association between the incidence of IBD with socioeconomic status

Blanchard et al (22) found that a higher average household income was associated with a higher incidence of CD but not UC. This finding has been confirmed by recent data from Scotland, which showed an inverse association between the deprivation score and incidence of juvenile-onset CD (20).

CONCLUSIONS

There are a number of geographical and ethnic influences on the incidence of IBD. The most prominent are Ashkenazi Jewish ethnicity and living within an urban, high prevalence area (North America and western Europe, especially). Many of the other identified variables suggest a prominent environmental influence. Although many of these factors are too variable to be incorporated into a classification of IBD, it would be appropriate to collect data to enable population stratification for research purposes. We therefore suggest that a minimal data set include parameters shown in Table 7.

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Serological studies in IBD – Implications for classification

Key points

Serological markers:

- Serological markers should not be the only determinant for clinical decision making.
- Novel markers show promise and panels of markers may prove useful in differential diagnosis and prognosis.
- The IBD serological panel (Table 8).

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The two most widely studied serological tests are for the presence of pANCA (reviewed in [1,2]) and ASCA. The seroprevalence of these markers among different studies has been quite variable, likely in part due to the lack of standardization of the techniques used for these assays (3).

Immune responsiveness to several specific microbial antigens in patients with CD and UC has been described by Targan et al (4). OmpC is the outer membrane porin C of *Escherichia coli*. I2 is a fragment of bacterial DNA that has been cloned from lamina propria mononuclear cells in patients with active CD. This sequence has been shown to be associated with *Pseudomonas fluorescens*. In patients with CD, reported seroprevalence is 55% for anti-OmpC and 50% for anti-I2 (5). Linskens et al (6) used several strains of Gram-positive anaerobic coccoid rods in an agglutination test. The agglutinating antibodies to coccoid rods were mostly of immunoglobulin G isotype. A sensitivity of 52% for CD was reported. More recently, Lodes et al (7) used serological expression cloning in colitic C3H/HeJBir mice to identify commensal bacterial

TABLE 8
Prevalence of antibodies in inflammatory bowel disease

	pANCA	ASCA	PAB	Anti-OmpC	Anti-I2	Anti-CBir1 flagellin
CD	2–28	48–69	27–39	55	50	50
UC	45–82	5–15	3–23	2	10	6
Healthy controls	2.5	5	0	1.3	10	8

*Data from references 1,2,4,7,23 and 41. ASCA Anti-*Saccharomyces cerevisiae *antibody; CD Crohn's disease; OmpC Outer membrane porin C of* Escherichia coli*; PAB Antipancreatic antibody; pANCA Antineutrophil cytoplasmic antibody with a perinuclear staining pattern at indirect immunofluoresence; UC Ulcerative colitis*

TABLE 9
Results of anti-*Saccharomyces cerevisiae* antibody (ASCA) and antineutrophil cytoplasmic antibody (ANCA) in a prospective study of patients with indeterminate colitis

	n (%)	Ulcerative colitis n (%)	Crohn's disease n (%)	Indeterminate colitis n (%)
ASCA+/ANCA–	26 (26.8)	8 (30.8)	2 (7.7)	16 (61.5)
ASCA–/ANCA+	20 (20.6)	4 (20)	7 (35)	9 (45)
ASCA+/ANCA+	4 (4.1)	2 (50)	1 (25)	1 (25)
ASCA–/ANCA–	47 (48.5)	3 (6.4)	4 (8.5)	40 (85.1)
Total	97 (100)	17 (17.5)	14 (14.4)	66 (68.1)

Data from reference 15

proteins that could contribute to the pathogenesis of IBD. The dominant antigens identified were flagellins. A T cell line specific for one of those flagellins (CBir1) induced colitis when transferred into naive mice with severe combined immunodeficiency. A serum response to flagellin CBir1 was detected in 50% of CD patients, 6% of UC patients and 8% of controls (8).

Serological markers and IBD diagnosis

The clinical value of pANCA or ASCA testing in patients presenting with nonspecific GI symptoms is limited because of inadequate sensitivity. ANCA positivity has been observed in other inflammatory disorders of the colon, such as eosinophilic and collagenous colitis. The specificity of ASCA seems to be higher for CD, but ASCA positivity has been observed in patients with Behcet's disease, primary biliary cirrhosis, autoimmune hepatitis and celiac disease, in which positive results have been reported in up to 43% of patients (9). Although the use of serological markers in routine screening is not recommended in adults (10), the situation may be different in children (11). Two recent studies (12,13) in children came to the conclusion that, as in adults, the low sensitivity of serological markers limits their value in screening and evaluation of patients with suspected IBD.

Indeterminate colitis

The most specific serological test to distinguish CD from UC is the combination of ASCA and pANCA. The CD-associated serological pattern is ASCA+/pANCA–; conversely, the UC-associated pattern is pANCA+/ASCA–. Several independent studies have found that these combinations had positive predictive values of 77% to 96% for differentiating CD from UC (1,2). Using likelihood ratios, patients who are pANCA+ and ASCA– are 19 times more likely to have UC, whereas patients who are ASCA+ and pANCA– are 16 times more likely to have CD (14). It should be remembered, however, that these estimates are based on retrospective studies performed mostly in referral centre populations.

The results of the only prospective study that assessed the usefulness of serological markers in IC provided further evidence for the theory that IC, rather than being merely undiagnosed UC or CD, is rather a distinct clinical entity (15). ASCA and pANCA tests were performed for 97 patients with an initial diagnosis of IC. After a mean of one-year follow-up, a definitive diagnosis was reached in 31 of 97 patients (32%) (Table 9). ASCA+/ANCA– results predicted CD in 80% of IC patients, whereas ASCA–/ANCA+ results were predictive of UC in 64% (Table 10). Nevertheless, 48.5% of IC patients did not have antibodies against either ASCA or ANCA, perhaps limiting the clinical utility of serological testing. In summary, the serological tests may serve as an adjunct to the clinical workup in IC. Although it may be too early to suggest they be used alone to determine the appropriateness of restorative proctocolectomy in patients needing surgery (16), the addition of new markers may improve overall diagnostic accuracy.

TABLE 10
Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the combination of anti-*Saccharomyces cerevisiae* antibody (ASCA) and antineutrophil cytoplasmic antibody (ANCA) in a prospective study of patients with indeterminate colitis

	Diagnosis	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
ASCA+/ANCA–	CD	8/12 (66.7)	7/9 (77.8)	8/10 (80)	7/11 (63.6)
ASCA–/ANCA+	UC	7/9 (77.8)	8/12 (66.7)	7/11 (63.6)	8/10 (80)

Data from reference 15. CD Crohn's disease; UC Ulcerative colitis

In a study incorporating additional serological markers, the addition of anti-OmpC and anti-I2 to ASCA and pANCA was of no value in the diagnosis of IC (17); whereas the ASCA2 test allowed the reclassification of three CD patients (18). The new anti-CBir1 flagellin antibodies could be relevant in IC because they are associated with colonic CD, independent of ASCA, and may allow differentiation of CD from UC in patients who are positive for pANCA (4).

Serological markers and IBD stratification

Serological markers have been further used to categorize subgroups of patients with IBD. In patients with CD, pANCA positivity has been associated with a UC-like phenotype (19,20), and a recent analysis found that it is associated with late-onset disease and inflammatory disease type according to the Vienna classification (21). In general, these types of studies are very difficult to compare, and seemingly contradictory results could depend on the specific technique employed. For example, the use of indirect immunofluorescence alone allows only the detection of the antibody, but the ELISA technique provides an assessment of the amount of antibody present.

The strongest phenotypic association of ASCA is with CD involving the small bowel rather than the colon. The group from Edinburgh (22) described a strong association between ASCA positivity and progression type from purely

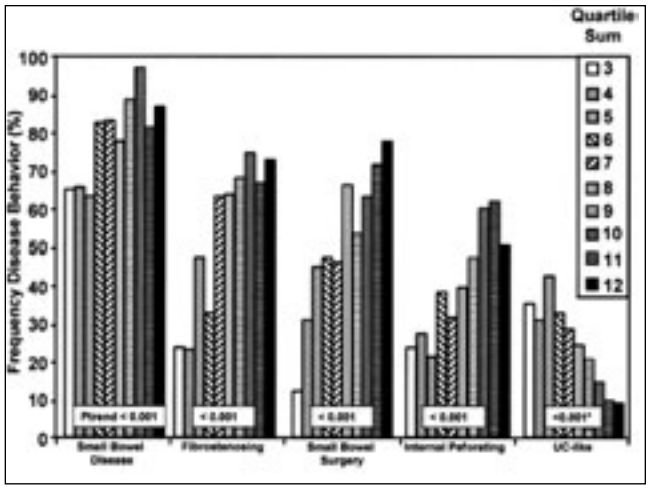

Figure 2) *Frequency of Crohn's disease behaviour according to antibody reactivity. Quartile sum analysis (sum of quartile scores for anti-I2, anti-OmpC and anti-*Saccharomyces cerevisiae *antibodies) was performed to evaluate the association between the combination of the level of antibodies and disease characteristics for any individual patient. By adding individual quartile scores for each antibody, a quartile sum score (range three to 12) was obtained that represented the cumulative immune response toward all three microbial antigens. Patients with high scores had a high likelihood of small bowel, fibrostenotic and perforating disease, were likely to need small bowel surgery, and had a low frequency of ulcerative colitis (UC)-like phenotype. Data from reference 23*

inflammatory to stricturing and penetrating disease with a more severe phenotype and requirement for surgery. Using cluster analysis, Landers et al (5) have shown that CD patients can exhibit a lack of tolerance to specific bacterial antigens and autoantigens, and can be separated into four groups depending on their antibody response patterns: ASCA, OmpC/I2, pANCA or no/low response. The same group was able to stratify patients based on the presence of the markers and levels of the antibody responses (23). Antibodies to I2 were associated with fibrostenotic disease and the need for small bowel surgery, while those to OmpC predicted internal penetrating behaviour. ASCA positivity was associated with small bowel disease, fibrostenosis, internal perforating disease and the need for small bowel surgery, and was negatively associated with UC-like behaviour. Conversely, the presence of pANCA predicted UC-like behaviour and was negatively associated with small bowel disease, fibrostenosis and small bowel surgery. They also found that 72% of patients positive for I2, OmpC and ASCA had undergone small bowel surgery compared with 23% of patients who lacked these antibodies. An association with the level of antibody production was also demonstrated. Levels of ASCA, I2 and OmpC were grouped by quartile. By adding individual quartile scores for each serological marker, a quartile sum score was then calculated. As shown in Figure 2, patients with high quartile sum scores were more likely to have small bowel disease, fibrostenotic and internal perforating disease with an increasing need for small bowel surgery and a decreasing frequency of UC-like phenotype.

Complementary data from an independent European population, using the same methodology, confirmed that the presence and magnitude of serological responses correlated with CD phenotype and severity (24). Multivariate analysis demonstrated independent associations between anti-OmpC and progression of disease type (P=0.005) and long disease duration (P=0.002), and between anti-I2 and long disease duration (P=0.002) and the need for surgery (P=0.033). ASCA positivity was also associated with disease progression (P<0.001). When the presence and magnitude of all antibody responses were considered, reactivity to microbial components was associated with long disease duration (P<0.001), progression of disease type (P<0.001), penetrating disease (P=0.008), small bowel disease (P<0.02), and the need for surgery (P<0.001). However, antibody status was not associated with NOD2/CARD15 genotype. A recent twin study (25) showed a strong correlation between ASCA titres and concordance for CD within monozygotic twin pairs, suggesting that the level of increase is genetically determined but independent of NOD2/CARD15. ASCA may thus be a marker of a response to an environmental antigen. Moreover, specific gene(s) other than NOD2/CARD15 could determine the level of response and perhaps also specific phenotypic characteristics (25).

Serum responses to CBir1 flagellin have been recently shown to independently identify a unique subset of patients with complicated CD: anti-CBir1 expression is independently associated with the presence of internal-penetrating and fibrostenosing disease involving the small bowel (4). There is evidence that patients with abnormalities in both innate and adaptive immunity may develop severe CD (26). Allelic variants of CARD8, which generate a nonfunctioning protein, were associated with internal penetrating CD only among patients who were also OmpC-positive. These data suggest that the combination of abnormalities in the innate and adaptive immune responses in the same individual could result in a severe form of CD.

Serological markers and IBD monitoring and management

Most studies do not support a relationship between UC activity and either the presence of ANCA or their titres. Hence, in contrast with systemic vasculitides, serial measurement of ANCA titres in IBD is not useful for follow-up of disease activity or the prediction of relapses.

The presence of ASCA in CD is generally stable over time and is independent of CD activity and duration. A follow-up study (27) in 25 patients with active CD showed that mesalamine had no influence on ASCA status, and that ASCA levels were decreased but not eliminated by prednisolone therapy. In one pediatric study (28), ASCA titres correlated significantly with disease activity, and a significant reduction of ASCA was observed when clinical remission was achieved. However, Desir et al (29) could not confirm this finding in a study of 61 children with CD that found that changes in ASCA titres over time were unable to predict clinical outcomes.

The association between responses to microbial antigens and CD severity and need for early surgery makes these markers potentially valuable prognostic indicators, but these findings must be confirmed by prospective studies (23). More aggressive medical therapy may be indicated for patients whose serology indicates an increased susceptibility to disease progression.

Another promising field of development for serological markers may be the prediction of response to therapy. Higher clinical response to infliximab has been associated with the presence of 'speckled' ANCA, while lack of response was associated with pANCA (30). Esters et al (31) found no overall relationship between ASCA or ANCA positivity and response to infliximab in 279 patients with CD. Numerically lower response rates were observed in patients with refractory disease with the ANCA+/ASCA– profile, but statistical significance was not achieved. These observations need to be confirmed by other investigators. In a study (32) of 27 patients with CD needing either an ileostomy or a colostomy for refractory proctocolitis or perianal disease, the presence of anti-I2 antibodies predicted a favourable clinical response to fecal diversion. This observation supports the hypothesis that patients who react to particular microbial antigens might respond to manipulation of bacterial flora.

In a prospective study, Fleshner et al (33) found an association between the level of pANCA expression and chronic pouchitis. A long-term follow-up study of 102 UC patients, however, showed no association between pANCA positivity and pouchitis (34). Divergent definitions of pouchitis and differences in data collection methodology (ie, retrospective versus prospective) likely account for these contradictory findings. Furthermore, the fact that only one patient in the retrospective study had a pANCA level above 100 prevents meaningful comparison, and suggests that the population studied had, overall, much less severe disease. A recent study (35) suggested that serological responses in IC patients before IPAA identifies patients who are at risk for developing continuous pouchitis. In that study, the serological expression of any marker alone did not predict the development of continuous pouch inflammation; however, continuous pouch inflammation developed in 10 of 16 patients (63%) who had a positive antibody reactivity profile compared with only two of 12 patients (17%) with a negative profile (P=0.015).

Serological markers and prediction of IBD

Serum autoantibodies, which appear long before onset of clinical disease, are a characteristic feature of autoimmune diseases (36) as emphasized by two recent studies. In a study of patients with rheumatoid arthritis, Nielen et al (37) found antibodies for immunoglobulin M rheumatoid factor or anticyclic-citrullinated-peptide in serum samples taken a median of 4.5 years before disease onset. Arbuckle et al (38), using the United States Armed Serum Repository, identified 132 military personnel diagnosed with systematic lupus erythematosus in whom antinuclear antibodies and anti-Ro appeared as early as 10 years before the onset of the disease. It may thus be possible to identify patients with subclinical disease or a predisposition to its development. Studies have consistently shown an increased frequency of ASCA in first-degree relatives of patients with IBD (1,2). Very interestingly, a recent study by Israeli et al (39) showed for the first time that ASCA may precede by several months the clinical diagnosis of CD.

Recently, anti-OmpC was demonstrated to also have a strong familial aggregation. Mei et al (40) showed that anti-OmpC is detected in 18% of unaffected family members of probands with CD as compared with 6% of control subjects. Whether the expression of anti-OmpC proves to be a pre- and/or subclinical marker of disease will be assessed in ongoing evaluation of these families.

CONCLUSIONS AND RECOMMENDATIONS

Relying exclusively on serum indicators for diagnosis in the clinical setting is not justified. The available serological markers are not sensitive enough to be used for IBD screening in the general population; however, the addition of more markers improves the overall sensitivity. Therapeutic decisions in patients with IC must not rely solely on serological testing, although it might be possible to predict the development of chronic pouchitis. High titres of pANCA in UC patients and of antibodies against microbial antigens in CD patients are associated with worse outcomes, but larger prospective studies are necessary before serology can be used to guide clinical decisions. Recent observations have reinforced the potential utility of serological markers for identifying more homogeneous clusters of CD patients. Ongoing study correlations between serological markers (either individual tests or panels) and genotypes, pathophysiological abnormalities, clinical phenotypes (including natural history) and response to treatment would lead to a clearer understanding of the pathophysiology of different types of IBD. Finally, a key role for these markers is their potential to immunotype clinical cohorts so that results of different, independently executed studies can be compared. Such analysis might shed light on differences in pathophysiology, disease severity and response to treatment.

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NOD2/CARD15 and IBD

Key points 1

- Three NOD2/CARD15 mutations that predispose to the development of CD are established: Cins1007fs, G908R and R702W.
- NOD2/CARD15 variation shows a significant gene-dosage effect, OR genotype MM 17, OR genotype MW 2.4.
- All three mutations show in vitro impairment of muramyl dipeptide (MDP)-driven nuclear factor-kappaB activation.
- The risk of CD with Cins1007fs > G908R ~ R702W.
- Population attributable risk is less for the most northern European populations.
- CD NOD2/CARD15 risk in non-Jewish Caucasians is greater than in Ashkenazi Jews.
- NOD2/CARD15 variants are not present in Pacific Asian populations and are rare in African-Americans.
- Private mutations are increased in CD patients; those with impaired nuclear factor-kappaB activation are likely to predispose to CD.

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Overview of NOD2/CARD15 mutations associated with CD risk and population differences

Three independent NOD2/CARD15-coding mutations (R702W [Arg702Trp], G908R [Gly908Arg] and Cins1007fs [Leu1007fsinsC]) are consistently more common in Caucasian CD patients (30% to 40% of whom are carriers) than healthy Caucasian controls (in whom 10% to 20% are carriers), and therefore these mutations are established genetic risk factors for CD (1). These mutations are absent (2-5) or very rare (6)

in Asians (eg, Japanese, Chinese and Koreans), Arabs (7,8), Africans and African Americans (9,10). The relative risks for developing CD vary widely. In a meta-analysis (11) of 37 studies of NOD2/CARD15 in Caucasian subjects, the overall risk for developing CD in simple heterozygotes (genotype MW, in which there is one normal and one mutant chromosome) was 2.4 (95% CI 2.0 to 2.9), and risk for persons with two mutant chromosomes (genotype MM, simple homozygotes or compound heterozygotes) was 17.1 (95% CI 10.7 to 27.2). The markedly increased risk of CD for MM compared with MW carriers has raised the possibility that NOD2/CARD15 might be recessive and that the risk from the MW genotype might be due to a significant proportion of MW carriers containing a rare mutation of the seemingly wild-type (W) allele. Lesage et al (12) screened for rare coding variants, called private mutations (PM), in 453 CD patients and 103 healthy controls and still found a greater risk of CD in 28 proven MW simple heterozygotes (ie, having one proven wild type allele). The PM allele frequency (*f*) in CD patients ($f_{PM\text{-}CD}$) was 0.13, while that in healthy controls ($f_{PM\text{-}HC}$) was 0.06 (P<0.01). Promoter or splice risk mutations have not been demonstrated in patients with CD.

An intronic variant IVS8+158C/T (JW-1) on the P268S background, was associated with significant risk of CD in Ashkenazi Jews (f_{CD} 20.5%, f_{HC} 6.3%; OR 5.75; 95% CI 2.0 to 16.4) but not in non-Jewish whites (f_{CD} 15.6%, f_{HC} 15.3%) (13). The risk associated with JW-1 remains to be confirmed (14,15), and a functional effect has not been reported. R702W is more prevalent in non-Jewish White patients with CD ($f_{CD\text{-}NJ}$ 10.7, $f_{CD\text{-}AJ}$ 2.6; P=0.00001), while G908R is more prevalent in Ashkenazi Jews with CD ($f_{CD\text{-}NJ}$ 4.3, $f_{CD\text{-}AJ}$ 8.7; P=0.008) (16). In Europeans, population attributable risk of NOD2/CARD15 was observed to be decreased in the most northern European populations (eg, Norwegians, Finns and Scots) compared with those from more southern latitudes. For example, the population attributable risk in Edinburgh, Scotland is 11%, and in Oxford, England is 27%) (17). This suggests that the risk of developing CD may depend on the interaction of NOD2 with unknown risk factors that more often present in southern latitudes.

Differences in risks of specific mutations

The Cins1007fs has been observed to have a greater CD risk than the R702W and G908R variants in the majority of studies. A meta-analysis (11) showed that the ORs for developing CD in non-Jewish Caucasians were 4.1 for Cins1007fs (95% CI 3.2 to 5.2), 2.2 for R702W (95% CI 1.8 to 2.6) and 3.0 for G908R (95% CI 2.4 to 3.7). Complementing this finding, Cins1007fs expressed in vitro in NOD2/CARD15-negative human embryonic kidney 293 cells was unable to activate nuclear factor-kappaB upon stimulation with the bacterial cell wall component, muramyl dipeptide (MDP); whereas R702W and G908R exhibit only reduced stimulation compared with wild-type NOD2/CARD15 transfectants (16). The Cins1007fs variant also showed greater reduction in in vivo human defensin expression compared with R702W and G908R (18). In the Lesage et al study (13), none of the PM alone were significantly more prevalent in CD than healthy controls. However, it is expected that an adequately powered study would prove that some of these rare variants indeed increase the risk of CD, because in vitro MDP-stimulated nuclear factor-kappaB activation is decreased with G978E or nearly absent with E778K (19). Other nonconserved variants, however, have no effect on nuclear factor-kappaB activation (or even have a hyperstimulatory effect, as in the Blau syndrome-associated NOD2 mutations, R334Q and L469F) and are therefore not likely to confer an increased risk of CD. The present literature on the influence of NOD2/CARD15 on CD and its phenotypes is therefore based on incomplete data, because some seemingly wild-type alleles will actually be disease-causing PM alleles. Ultimately, true appreciation of the relevance of NOD2/CARD15 to CD will require large studies of patients whose phenotypes have been carefully assessed and whose genotypes have been examined for rare mutations known for being defective in terms of nuclear factor-kappaB activation.

NOD2/CARD15 and other IBD phenotypes

In general, the allele frequency of NOD2/CARD15 variants has been similar in UC patients and healthy controls and, thus, NOD2/CARD15 variation does not appear to be a risk factor for developing UC. There is evidence, however, that NOD2/CARD15 may interact with the IBD5 (OCTN1/N2) haplotype to increase the risk of UC (20,21). NOD2/CARD15 has not been examined in an adequately powered sample of IC patients. Before NOD2/CARD15 variation can be used to clinically separate CD from UC and IC, it would be necessary to genotype a very large series of UC and IC patients to determine whether NOD2/CARD15 variation influences changes in phenotype to CD, particularly in children.

NOD2/CARD15 and CD site

In nearly all phenotype analyses, NOD2/CARD15 mutations are significantly more prevalent in patients with ileal disease than those with CD limited to the colon. A meta-analysis (11) showed that the OR for ileal compared with colon-only disease was 2.5 (95% CI 2.0 to 3.2). The association between NOD2/CARD15 mutations and ileal disease might be due to the fact that the expression of NOD2 in the intestinal epithelium is localized to Paneth cells, which are numerous in the ileum but not the colon (22,23). This association has important implications for CD phenotype analyses. For example, analysis of CD phenotype using overlapping site categories, such as 'any ileal disease' or 'any colonic disease', is not recommended because most (24), but not all (25), studies have observed similar patterns of NOD2/CARD15 variation with both ileal and ileal-colonic disease. Furthermore, because NOD2 is an innate host determinant of site, NOD2/CARD15 genotypes should be considered in studies in which disease site either is or influences the outcome being measured. Although most studies yielded only numerical trends that did not reach statistical significance, a meta-analysis (11) has shown that NOD2/CARD15 mutations are indeed (weakly) associated with CD limited to the colon (OR 1.5; 95% CI 1.2 to 1.9).

In summary, carriage of NOD2/CARD15 variants is a risk factor for both 'any-ileal' and for 'colonic-only' disease sites, but there is a significantly greater risk for disease involving the ileum. No studies have genotyped large samples of patients with CD involving either the upper GI tract or the jejunum only to determine if NOD2/CARD15 mutations present a risk for disease at these sites.

NOD2/CARD15 and CD behaviour

According to a meta-analysis (11), carriage of NOD2/CARD15 variants is a risk factor for developing intestinal strictures (OR 2.0;

95% CI 1.6 to 2.3). Most strictures occur in the ileum, and some investigators (24,26), but not others (27), have observed this association independent of ileal disease site. NOD2/CARD15 variants have also been associated with nonperianal (intestinal) fistulas (24). Nearly all studies have used the Vienna classification, in which internal and perianal fistulizing disease are considered together (28). NOD2/CARD15 variation tends to be less common in perianal versus nonperianal sites (24). It is noteworthy that perianal fistulas are more commonly found with colonic disease and that internal fistulas and strictures are often observed together. Evidence that NOD2/CARD15 variants predispose to intestinal, but not perianal fistulizing disease, as well as recent evidence that the IBD5 haplotype is associated with perianal disease (29) suggests that separate examination of intestinal and perianal fistulas is important for disease classification.

A well-recognized complicating factor in disease behaviour correlations is that nearly all patients have inflammatory (nonstricturing/nonfistulizing) disease at diagnosis, with complications accumulating over the course of disease. One approach is to compare patients who have complications with those who continue to have a stable inflammatory phenotype for more than seven years after diagnosis (24). Alternatively, Smith et al (30) examined complications as they developed and observed that NOD2/CARD15 variants were associated with delayed rather than rapid progression of strictures and fistulas in patients with CD.

NOD2/CARD15 and other CD phenotype and demographic characteristics

NOD2/CARD15 variation has been associated with increased disease severity, including increased risk for surgery (31,32), acute intestinal obstruction (33), lower weight at diagnosis (34) and younger age at diagnosis (24,27). Patients with ileal disease are more likely to require surgery, and some studies have observed that survival without surgery (24), or without a second operation (27), was not independent of ileal site. Surprisingly, few studies have found an increased presence of NOD2/CARD15 mutations in those with familial as opposed to sporadic CD, although meta-analysis (11,35) has shown that, in non-Jewish populations, the presence of NOD2/CARD15 variants is a risk factor for familial CD (OR 1.4; 95% CI 1.2 to 1.7). It should be noted, however, that ileal disease, young age at onset and family history of CD may all be associated (36). A preliminary population-based report from Manitoba concluded that NOD2/CARD15 variation was a significant risk factor for CD, independent of a family history of IBD (37).

The role of NOD2/CARD15 has been examined for other phenotypes. Two studies have revealed no evidence of a link between NOD2/CARD15 and granulomas in CD patients (38,39), although an earlier study suggested an association between the R702W variant and both stricture formation and granulomas (40). The lack of a clear association is perhaps not surprising given that Blau syndrome is seen with mutations that increase NOD2/CARD15 activity in vitro (19). NOD2/CARD15 has not been shown to be consistently associated with extraintestinal disease manifestations (41). Presently, NOD2/CARD15 has not been shown to influence response to treatment of CD although only infliximab has been evaluated so far (42,43).

Several studies (17,24,27) have demonstrated that more than 90% of patients with two mutant alleles (MM genotype) had ileal disease. Prospective studies of patients will be necessary to determine if this seemingly high positive predictive value is clinically useful. One should note that a significant gene-dosage effect has also been observed for the development of intestinal strictures (24,26).

Conclusions and recommendations from NOD2/CARD15 experience on classification of IBD

The identification of the importance of the NOD2/CARD15 gene has been a major breakthrough in the understanding of this clinically heterogeneous and complex genetic disorder. Mutation of the NOD2/CARD15 gene is now established as an important host determinant of the risk for CD compared with UC, but only in Caucasian populations, and possibly with different influences in Jewish and non-Jewish populations. It is associated with ileal location of disease, stricturing and perhaps internal fistulizing disease, and early age of onset. On the other hand, it is less common in persons with perianal complications of CD. Although NOD2/CARD15 variants have been correlated with severity of disease, it is not clear whether this is independent of their established association with ileal disease. NOD2/CARD15 shows a significant gene-dosage effect for development of CD as well as the site of disease and its complications. The differences in risks associated with individual alleles is relatively minor, although the Cins1007fs has a more profound effect. Finally, a small but potentially significant number of rare NOD2/CARD15 variants (private mutations) have been ignored in nearly all studies.

The important influence of NOD2/CARD15 on IBD disease heterogeneity demands that a uniform approach be taken regarding disease classification; that the classification is structured so that the influence of NOD2/CARD15 and other IBD susceptibility genes can be observed; and that significantly larger studies, and ideally prospective as well as population studies be performed using the updated disease classification guidelines.

We propose the following specific recommendations for the next classification scheme and for future study designs:

1. Retain the nonoverlapping disease site categories, so that comparisons can be made between any ileal disease and disease limited to the colon, and similarly between any colon disease and disease limited to the ileum.
2. Create categories that include any fistulas, as well as separate categories for internal fistulas and perianal fistulas. In addition, create an additional category of "coexisting internal fistulizing and stricturing complications" for the many cases in whom the two complications cannot be separated.
3. When possible, include in the category of "inflammatory" disease behaviour those patients with stable disease for several years. Ideally, stricturing or fistulizing complications would be subclassified into those occurring early in the disease course (perhaps before eight years), moderate disease course (ie, eight to 19 years) and late disease course (ie, after 20 years). Consideration needs to be made, however, that for many retrospective studies, it might not be possible to ascertain the onset of these complications.
4. Match studies for participants' ethnicities, or adjust for differences between groups.
5. Unlike most current genotype/phenotype and

genotype/therapy studies, future studies must take into account the strong NOD2/CARD15 gene-dosage effect.

6. Although they would be costly, the projected development of increasingly comprehensive genetic panels for clinical prediction and genetic counselling (44) demands that more genotype/phenotype studies consider private mutations that likely contribute to CD risk, eg, those that have been shown to decrease NOD2/CARD15 MDP-stimulated activity. This is analogous to what is being done for diseases like cystic fibrosis, in which private mutations are, in aggregate, not uncommon. For comparison purposes, studies that include private mutation genotyping should also perform analyses limited to the three major NOD2/CARD15 mutations.

Instituting the above recommendations would help make future studies more comparable and more useful.

Key points 2

- Germline NOD2/CARD15 variation appears to be a risk factor for CD and not UC.
- NOD2/CARD15 variation is a risk factor for both ileal disease and, to a lesser extent, for CD limited to the colon.
- NOD2/CARD15 variation is a risk factor for stricturing and perhaps intestinal penetrating disease, but more research on disease progression is needed.
- Gene-dosage effects (MM versus MW) need to be considered when assessing overall and disease subtype risks.
- The phenotype and course of UC patients with NOD2/CARD15 variants must be evaluated.
- The overall NOD2/CARD15 risk requires investigation in population-based and community settings, and in prospective studies.

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Role of human leukocyte antigen genes

Key points

- The IBD susceptibility gene(s) within the human leukocyte antigen (HLA) complex remain undiscovered. Strong linkage disequilibrium, extensive polymorphism and high gene density continue to hamper their identification.
- The most consistently replicated associations are with the classical class II gene, HLA-DR. Some HLA-DRB1 alleles are associated with susceptibility while others offer protection. The specific associations with CD and UC are different, with the notable exception that HLA-DRB1*0103 is shared by UC and colonic CD. A hierarchy of association is observed, with HLA-DRB1*0103 conferring the greatest risk (RR two to 10).
- Most HLA associations are population-specific, reflecting ethnic differences in allele frequency and patterns of linkage disequilibrium.
- In CD, the strongest associations are with specific phenotypes defined by the location of disease. DRB1*0103 is a risk factor for CD limited to the colon. DRB1*0701 is a risk factor for ileal (ileal and ileocolonic) CD in Caucasians who lack the common disease-associated NOD2/CARD15 mutations.
- In UC, the strongest associations are seen with overall disease susceptibility, and consistent associations are observed with DRB1*0103 and DRB1*1502. Fewer subgroup associations have been identified than with CD, but the association with DRB1*0103 is particularly strong in patients with extensive or severe disease;
- Specific associations have been described with the extra-intestinal manifestations of CD, but the findings must be replicated by additional studies.
- Stratification of HLA association data by other IBD-associated variants (eg, NOD2/CARD15) reduces genetic heterogeneity and facilitates the identification of important gene-gene interactions.
- The HLA region is likely to harbour more than one IBD susceptibility gene, and recent studies suggest that the second locus is likely to be in the class III region.
- The low sensitivity and specificity of the disease-associated alleles currently limits their use alone in the diagnosis and classification of IBD.

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The HLA complex is a highly polymorphic, gene-dense region on chromosome 6p encompassing at least 130 expressed genes, many of which have immunoregulatory roles (1,2). Several independent studies have provided consistent evidence that both UC (3,4) and CD (3-7) are linked to IBD3 (6p21.1-23), an area which encompasses the HLA complex. These findings have subsequently been confirmed by the IBD consortium in a large replication study of 733 nuclear families (8). A meta-analysis of 10 published genome-wide scans showed that IBD3 was the only locus that had genome-wide significance (9). Although it is difficult to estimate the importance of this region in determining overall genetic susceptibility, calculations derived from studies of the sharing of HLA alleles within families suggest that it contributes between 10% and 33% of the total genetic risk of CD (10), and between 64% and 100% of the total genetic risk of UC (11). Since 1972, more than 100 association studies have investigated the role of HLA genes in determining susceptibility to both UC and CD. Interest has traditionally focused on the classical class II HLA alleles, but recent insights into the biological function of other genes encoded within this region have led investigators to a more comprehensive exploration of this region.

Classical HLA class II genes and IBD

HLA-DRB1 is the most extensively studied gene in IBD research. Convincing evidence of association has been described for a number of alleles, some of which confer risk, while others are protective. Many of these were highlighted in a 1999 meta-analysis (12) of 29 association studies published between 1966 and 1998. Since publication of this report, improvement in study design has enabled the study of accurately characterized, larger patient cohorts. Realization that a

genetic basis might underlie disease heterogeneity has stimulated more detailed genotype-phenotype analyses of this locus.

HLA class II associations with UC

HLA-DRB1*0103 and severe, extensive UC: The most consistently replicated association of UC is with the HLA-DRB1*0103 allele. This is a rare allele that occurs in less than 2% of the European and North American Caucasian and Jewish populations, and is absent in the Japanese. Data from the 1999 meta-analysis (12) demonstrated a moderate disease association in unselected UC patients (OR 3.42), which has subsequently been confirmed in Spanish (13), North American (14), British (15) and Mexican cohorts (16). The association is observed for both the DRB1*0103-DQB1*0301 and DRB1*0103-DQB1*0501 haplotypes, strongly implicating DRB1*0103 in the pathogenesis of the disease. This association is particularly strong in patients with extensive (15-18) or severe disease, as defined by the need for colectomy for failed medical therapy (15,16). Among patients who require colectomy, this allele is associated with a shorter mean time to surgery (15). The low frequency of this allele, however, limits its clinical value in predicting disease course.

HLA-DRB1*1502: HLA-DRB1*1502 is associated with UC in European (15), North American (14), Japanese (19,20) and Korean (21) populations, with similar ORs of 2 to 4.5 despite variable background prevalence (allele carriage rates are 20% to 25% in Japanese but less than 1% in northern European populations). This transracial concordance suggests that this allele, or a nearby one, is a true disease-causing variant. Association with HLA-DRB1*1502, rather than with HLA-DRB1*1501, explains the earlier reported association with the serological antigen DR2 (OR 2.00; 95% CI 1.52 to 2.63) highlighted in the 1999 meta-analysis (12). Limited data suggest HLA-DRB1*1502 is associated with extensive and intractable UC among Japanese (22) but not Korean patients (21). In Japanese (23) and British (24) patients, UC has also been reportedly associated with HLA-B*52, the class I allele in linkage disequilibrium with DRB1*1502. Interestingly HLA-B*52, but not HLA-DRB1*1502, has also been shown to be associated with colonic CD in these populations (25,26), providing suggestive evidence of a shared genetic basis for UC and colonic CD.

HLA-DRB1*04 – UC protection: The HLA-DRB1*04 allele is negatively associated with UC (OR 0.54) (12) but positively associated with CD in northern Europeans and Japanese populations. In the United Kingdom population, the effect is confined to the most prevalent subtype DRB1*0401, and only when present on the two-locus haplotype DRB1*0401 – DQB1*0301 (24). This probably indicates that the true protective polymorphism is found on the associated extended haplotype.

HLA class II associations with CD

HLA-DRB1*07 and NOD2/CARD15 variant-negative ileal CD: The most consistently replicated association of CD with a common HLA allele is with HLA-DRB1*07, which has an allele frequency between 5% and 29% in European and North American populations but less than 1% in Japanese. In the 1999 meta-analysis (12), a weak association was demonstrated in unselected patients with CD (OR 1.42), but three subsequent studies of well-characterized patients from the United Kingdom (25), Canada (27) and Spain (28) showed that the allele is specifically associated with ileal involvement (with or without colonic disease) with ORs of 1.5, 1.9 and 2.6, respectively. These figures are similar to those observed for possession of a single NOD2/CARD15 variant. Importantly, by demonstrating that the association with HLA-DRB1*07 is present only in patients who do not possess one of the three common NOD2/CARD15 variants associated with CD, the United Kingdom and Canadian studies provided evidence for genetic heterogeneity in ileal CD (25,27). The Canadian study found that HLA-DRB1*07 was numerically more prevalent in Jewish patients with ileal CD, although this association did not achieve statistical significance, perhaps because of the small number of patients analyzed (27).

HLA-DRB1*0103 and colonic CD: The association between HLA-DRB1*0103 and CD was not demonstrated until 2000 (14), almost certainly because of the heterogeneity of CD and the low prevalence of this allele, but has now been widely replicated in North American and European patients. DRB1*0103 is strongly associated with colonic location, particularly isolated colonic disease (25,27-29), and the described association with perianal location and fistulizing behaviour appears not to be independent of colonic involvement (25,28). The ORs for isolated colonic disease range from 5.1 to 18.5 in non-Jewish Caucasians (25,27,28). Despite the strength of this association, this allele was present in no more than 32% of patients with isolated colonic disease, thereby limiting its clinical application.

HLA-DRB1*04 and NOD2/CARD15 variant-positive ileal CD?: The association between HLA-DRB1*04 and CD is particularly important in Japanese cohorts, in whom the strongest HLA associations are with DRB1*0405, *0410 and the linked DQB1*0401 – 0402 alleles (20,30,31). This has not been widely observed in European and North American patients, but a weak association with DRB1*04 has recently been identified in Canadian patients with ileal disease (RR 1.7; 95% CI 1.1 to 2.5) (27). This association was stronger in patients possessing one of the three common NOD2/CARD15 variants, which might indicate an epistatic interaction in ileal CD between HLA-DRB1 and NOD2/CARD15 (27). Genotype-phenotype analysis of this allele in Japanese patients is awaited.

HLA-DRB1*1501 – CD protection: HLA-DRB1*1501 is a common allele, with a frequency of 6% to 25% in European and North American Caucasian populations, and 6% to 10% in the Japanese. It is negatively associated with overall CD susceptibility (12), and probably confers protection against CD in all ethnic groups. This allele is encompassed within the serologically defined antigen DR2, explaining the previously reported negative association between DR2 and CD described in the 1999 meta-analysis (12).

HLA-DRB3*0301 – HLA-DRB1*1302: The HLA-DRB3 gene is expressed in less than 50% of European and North American Caucasians, and has not been extensively studied. However, both a meta-analysis of three small studies (12) and a large United Kingdom study (25) support a positive association of CD with DRB3*0301, although it remains unclear whether this is due to linkage disequilibrium with another CD-associated DRB1 allele, DRB1*1302, or the HLA class I allele Cw*0802 (12,25).

HLA class II associations with the extraintestinal manifestations of IBD: A number of HLA associations have been described with extraintestinal manifestations (EIMs) of IBD in small, mixed studies of UC and CD. Type I peripheral arthritis,

a migratory pauciarticular arthritis involving large joints, has been shown to be associated with HLA-DRB1*0103 (as well as with the class I alleles found in linkage disequilibrium, B*27 and B*35) (32). In that study, HLA-DRB1*0103 was found in 35% of patients compared with 3% of controls. In contrast, type II peripheral arthritis, a chronic, small joint, symmetrical arthritis, is associated with HLA-B*44 (32). Uveitis has also been associated with DRB1*0103 and HLA-B*27, and erythema nodosum with the tumour necrosis factor (TNF) promoter single nucleotide polymorphism (SNP)-1031C (33). An increased prevalence of EIMs has previously been reported in patients with colonic CD (34), raising the possibility that the association with DRB1*0103 may merely reflect the documented association with colonic disease. Further work is required to clarify this issue.

A number of important observations should be noted from the reported associations with HLA class II alleles. First, the specific associations with CD and UC are different, with the notable exceptions of HLA-DRB1*0103 and HLA-B*52 with both UC and colonic CD. Second, it is clear that multiple alleles may be associated with any specific phenotype. This may suggest a shared disease-associated peptide motif, as seen in rheumatoid arthritis (35). However, only some disease-associated alleles share significant residue changes in the antigen-binding domain, indicating that this hypothesis cannot entirely explain these associations. Third, it is not known whether a gene-dose effect operates, such that possession of two high-risk HLA alleles confers additional risk. Fourth, while many of the associations appear to be robust, it is clear that the penetrance is low, and the presence of a risk allele is neither necessary nor sufficient for disease to occur. Finally, the reported associations vary with ethnicity and geographical location, reflecting genetic heterogeneity, prevalence of risk alleles in the background population, and population-specific patterns of linkage disequilibrium across the HLA complex.

A second HLA gene for UC and CD?: In IBD, as with a number of other HLA class II-associated diseases, there is increasing evidence for a second susceptibility locus within the HLA (36-40). Such a gene might act independently of the class II locus, or interact with disease-associated class II alleles in *cis* or *trans* positions. Specific additional associations in IBD have been identified with the HLA class I genes, HLA-B and Cw, the nonclassical major histocompatibility complex class I-related (MIC) genes, MICA and MICB, and the three heat shock protein genes (HSPA1L, HSPA1A and HSPA1B). Recent interest has focused on a cluster of immunoregulatory genes centred around TNF in the HLA class III region, including TNF, lymphotoxin alpha, lymphotoxin beta, NFKBIL and BAT1. To date, most studies have investigated only a few SNPs in a single gene, chosen because of previously described associations or for ease of genotyping. Results have generally been inconsistent, even within phenotypically defined subgroups and, as with other HLA-associated diseases, very few studies have fully controlled for linkage disequilibrium with HLA-DRB1.

TNF: The most widely studied gene in the HLA class III region is TNF, which encodes a proinflammatory cytokine found in increased concentrations in the mucosa, serum and stool of patients with IBD. The ΔARE (deletion of AU-rich elements) (41) and TNF–/– mouse (42) models provide experimental evidence of the importance of TNF in IBD. This concept is supported by convincing clinical evidence of dramatic responses in both CD and UC to infusions of the anti-TNF-alpha monoclonal antibody, infliximab. A large number of TNF promoter polymorphisms have been described, although the functional significance of individual variants remains uncertain. The –857 TNF polymorphism has been most widely studied. The C allele is extremely common: 99% of the healthy Caucasian population possess at least one copy and approximately 85% are homozygotes. A modest association with the –857C allele was reported in transmission disequilibrium test and case-control analyses from the United Kingdom (43). RRs for the homozygote TNF-857CC genotype were 2.4 in both UC and in CD patients lacking the three CD-associated NOD2/CARD15 variants (43). This association was replicated in transmission disequilibrium test studies from Australia (44) and the IBD consortium (8). In contrast to the United Kingdom study, the association was stronger in families that carried high-risk NOD2/CARD15 variants. In the latter studies, no association was found with UC, possibly a consequence of the smaller number of UC families included. No specific phenotypic associations have been identified. Although not directly investigated by either of these studies, it should be noted that an important confounder is the linkage disequilibrium between the rare, protective –857T allele and DRB1*0301 (45), an allele shown by several studies to be protective against CD overall. Thus, the TNF-857C association may simply reflect linkage disequilibrium within this highly complex region.

Modest associations have also been reported with many other polymorphic sites in the TNF promoter. In CD, these include TNF-1031C (46), TNF-863A (47) and TNF-308A (25,48,49). The association with TNF-308A appears to be specifically associated with colonic CD (25,48,49); however, once again this finding is confounded by tight linkage disequilibrium across the A1-B8-DR3 ancestral haplotype, the only common haplotype to contain the TNF-308A allele. In small studies, TNF-308A has also been shown to be associated with UC (50,51), providing further evidence of shared genetic susceptibility for UC and colonic CD.

Integrating HLA genotypes into a classification of IBD

The data reviewed in the present report demonstrate that genes in the HLA region are important in determining the susceptibility to and the phenotype of UC and CD. Although the specific disease-causing genes remain unidentified, the strength and consistency of these associations have confirmed the importance of classifying patients both by accurately defined clinical characteristics and by their possession of known genetic risk factors, such as NOD2/CARD15 disease-associated variants. For overall disease susceptibility and clinical phenotype of both CD and UC, the most consistently replicated associations are with the classical class II gene, HLA-DR. In CD, the strongest associations are observed with subgroups defined by the location of disease. No consistent associations have been reported with disease behaviour, which is a less stable phenotypic characteristic, age at diagnosis, disease complications (with the exception of EIMs) or response to treatment. In UC, the strongest associations are seen with overall disease susceptibility, which is consistently associated with DRB1*0103 and DRB1*1502. These alleles vary widely in prevalence across ethnic groups. Fewer subgroup associations have been identified than with CD, but the association with DRB1*0103 is particularly strong in patients with extensive or severe disease.

The low sensitivity and specificity of these associations currently preclude their use as a tool in diagnosis of either CD or UC. For the same reason, it is unlikely that the use of HLA markers alone will be useful in disease screening of asymptomatic individuals. Similarly, HLA markers cannot be reliably used for the discrimination of CD from UC. Indeed the shared association of DRB1*0103 and B*52 with UC and colonic CD suggests the presence of at least one shared HLA susceptibility factor, providing a tantalizing clue about the molecular basis of colonic inflammation. Such shared HLA factors may help to explain clustering of both forms of IBD in the same family. The apparent differential effect of HLA-DRB1*0401 in UC and CD, although unique within the HLA region, is weak, population-specific, and neither sufficiently sensitive nor specific to be clinically useful.

In the near future, the greatest value of HLA genotyping is likely to be in the prediction of disease course in patients with an established diagnosis of UC or CD, but it is currently not sensitive or specific enough to be clinically useful. Integration of an individual's HLA genotype into a panel of other genetic and serological markers may improve the sensitivity and specificity, although ultimately this is likely to require the identification of the primary IBD genes within this complex region.

In common with virtually all other diseases that have HLA associations, the HLA susceptibility genes for IBD remain elusive. Mapping of disease genes within this region is challenging because of the high gene density and degree of polymorphism; the complex haplotype structure and patterns of linkage disequilibrium; the relatively small RRs conferred by individual alleles; and the likely clustering of more than one IBD susceptibility gene either within the HLA complex or within the surrounding IBD3 locus.

The data in the present report indicate that the goal of an integrated classification of IBD would be more easily achieved if the following steps were taken in the design of future studies of the HLA region:

1. Patients need to be rigorously phenotyped, preferably according to the updated World Congress of Gastroenterology 2005 classification guidelines. Further consensus is required regarding the minimum period of follow-up, and how to classify unstable phenotypic characteristics. This type of meticulous approach is likely to require complete case note review for patient inclusion, and would be a dynamic, iterative process. At present, there is debate as to whether disease location should include separate ileal, colonic and ileocolonic subgroups, or whether the last of these is redundant. Furthermore, it is unclear whether perianal and internal fistulizing disease should be classified as separate behavioural entities. Molecular information may help to resolve this issue.
2. Larger studies need be performed to address the problems associated with the low RRs of disease-associated HLA alleles, many of which occur at low frequency, and would also permit the dissection of potential gene-gene HLA interactions involved in the development of a specific phenotype. This might only be possible through multicentre studies, and consensus clinical phenotyping would obviate the problem of variable disease definition. Such studies would require particular attention to ethnic definition.
3. Specific consideration needs to be taken of the complex, variable patterns of linkage disequilibrium across the HLA region, which can currently be addressed only by detailed haplotype studies. Association data from various ethnic cohorts (transracial mapping) may be particularly useful.
4. Stratification of association data by known IBD-associated mutations, such as NOD2/CARD15 variants and specific HLA alleles, might further reduce genetic heterogeneity, facilitating the identification of other disease-associated polymorphisms and important gene-gene interactions.

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New genes for IBD - where are we now?

Key points

- While the IBD5 locus plays an important role in IBD, the definitive gene within the region remains unproven.
- Multidrug resistance-1 (MDR1), Drosophila discs large homolog 5 (DLG5) and toll-like receptor 4 (TLR4) are excellent functional candidate genes with reported associations to IBD.

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Progress towards gene identification

The development of a linkage map of the human genome, involving informative microsatellite markers, has provided the basis for hypothesis-free scanning of the human genome for loci involved in susceptibility to simple monogenic and polygenic diseases (1). Thus far, numerous susceptibility loci have been implicated in IBD with varying degrees of replication and statistical support. Convincing replication of results has been demonstrated for nine such loci, termed IBD 1 to 9 (Table 11 and Figure 3) (2). While some loci appear specific to CD (eg, IBD1 on Ch16q- OMIM 266600) (3) or UC (eg, IBD2 on

TABLE 11
Selected candidate genes with promising inflammatory bowel disease (IBD) genetic association evidence

Gene	Disease	Polymorphisms	Chromosome	Populations	Additional phenotypic features
CARD4/NOD1	IBD	32656*1 ins/del	7p14	British (47)	Early onset and extraintestinal disease (47)
DLG5	IBD	113G/A	10q23	German (28)	
IBD5 locus haplotype and candidate gene (SLC22A4-A5 or organic cation transporter N1 and N2)	CD	600 kb haplotype; L503F and G–207C	5q31-33	Non-Jewish Caucasians (7,9-17,32,48)	Perianal disease (14); Severity/complications (11); Ileal (10); UC (NOD2+) (16); Infliximab-resistant (48)
IL1 RA	UC	Tandem repeat allele 2	2q14	Caucasians (49); Japanese (52)	Pouchitis (50); Ankylosing spondylitis (51); Early onset/pancolitis (52)
MDR1/ABCB1	IBD	A893S/T 3435 C/T	7q21	Caucasians (23,24,27)	UC with 3435T or 3435T-A893 (24,27); IBD/CD with A893 (23); Colitis in mouse MDR1 knockout model (19)
NF-κB1	UC	Promoter –94delATTG	4q24	Caucasians (53,54)	Gene maps to major mouse colitis locus (54)
PPAR-γ	CD	Intron 2 polymorphisms	3p25	Caucasians (55)	Gene also implicated in SAMP1/YitFc mouse model (55)
TLR4	IBD	(Asp299Gly, T399I),	9q32	German (39); Greek (56)	
TLR9	CD	–1237C	3p21	German (57)	Early onset and extraintestinal disease (57)

ABCB1 ATP-binding cassette, subfamily B, member 1; CARD4 Caspase recruitment domain-containing protein 4; CD Crohn's disease; DLG5 Drosophila discs large homologue 5; IL1 RA Interleukin 1 receptor antagonist; Ins/del Insertion/deletion; MDR1 Multidrug resistance gene 1; NF-κB1 Nuclear factor-kappaB subunit 1; NOD1 Nucleotide-binding oligomerization domain 1; PPAR-γ Peroxisome proliferator-activated receptor-gamma; SAMP1 Senescence-accelerated mouse-prone 1; SLC22A4-A5 Solute carrier family 22 member 4 and member 5; TLR Toll-like receptor; UC Ulcerative colitis

Ch12q-OMIM 601458) (4), others seem to confer susceptibility to IBD overall (eg, IBD3 Ch6p- OMIM 604519) (5), supporting the notion that CD and UC are polygenic disorders sharing some, but not all, susceptibility loci.

IBD5 locus

The IBD5 region, on chromosome 5q31-33, is second only to the NOD2/CARD15 region in receiving the most attention in recent years. In Canada, Rioux et al (6) first demonstrated the association between this region and CD, especially in patients diagnosed before the age of 16 years. The same investigators then subjected the IBD5 locus to painstaking analysis, and demonstrated the presence of a number of potentially important candidate genes, including a cytokine gene cluster (7). Progress in narrowing the linkage interval and identifying the primary locus of susceptibility was limited, however, by the extent of linkage disequilibrium within the region. Indeed, Daly et al (8) described in detail the haplotype structure of this region with eleven discrete haplotype blocks punctuated by areas of recombination, as well as the obstacles that these haplotype blocks provided in positional cloning.

The IBD5 locus has been recently re-examined, with particular attention to the contribution of polymorphic markers within the organic cation transporter OCTN1 (SCL22A4 C→T, a missense substitution in exon 9) and OCTN2 (SCL22A5 G→C, a transversion in the promoter region) genes in the IBD5 linkage interval (9). In the original study of the IBD5 region, these genes were initially considered potential candidate genes, but were thought to have little relevance to the pathobiology of CD (7). The OCTN1 and OCTN2 genes are adjacent to each other within a single haplotype block of the IBD5 locus, and Peltekova et al (9) suggest that a haplotype involving two functional OCTN1/2 variants (the TC haplotype) is associated with disease susceptibility. The authors further suggest that the contribution of the OCTN1/2 variants is stronger than, and independent of, the association with risk markers on the IBD5 haplotype. They also found that the TC haplotype interacts positively with the NOD2/CARD15 variants. A genotype-phenotype study from the same group has demonstrated an association between homozygosity for the TC haplotype and ileal disease, especially in the presence of NOD2/CARD15 mutations (10).

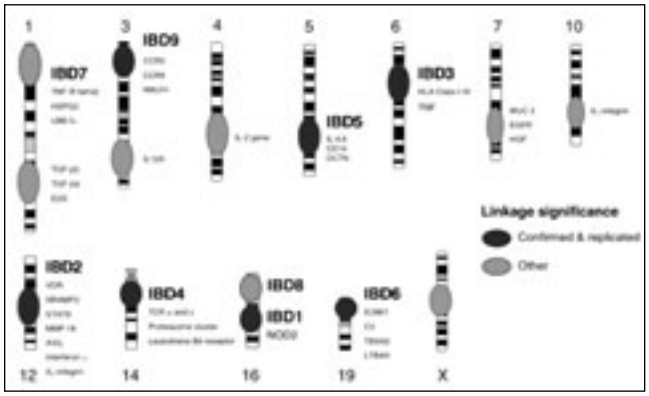

Figure 3) *Susceptibility loci for inflammatory bowel disease (IBD) as identified on genome wide scanning (printed with permission, adapted from reference 58). CCR CC chemokine receptor; EGFR Epidermal growth factor receptor; HGF Hepatocyte growth factor; hMLH1 Human mismatch-repair protein MutL homologue 1; HSPG2 Heparan sulfate proteoglycan 2; ICAM1 Intercellular adhesion molecule 1; IL-12A Interleukin-12A; LTB4H Leukotriene B_4 omega hydroxylase; MUC Mucin; NOD2 Nucleotide-binding oligomerization domain 2; OCTN Organic cation transporter; STAT6 Signal transducer and activator of transcription 6; TBXA Thromboxane A_2; TCR T cell antigen receptor; TGF Transforming growth factor; TNF-R Tumour necrosis factor receptor; UBE1L Ubiquitin-activating enzyme E1-like; VDR Vitamin D receptor*

Yet a number of critical and unanswered questions remain. It appears somewhat implausible, on biological grounds, that

two different variants involved in two adjacent genes are required for disease susceptibility. Given the extent of linkage disequilibrium across the region, are these simply markers for another critical variant? Moreover, no replication data, functional data or expression data in IBD are yet available to bolster the argument that Peltekova et al have identified the critical susceptibility genes. Our own data do demonstrate an association between OCTN1/2 and susceptibility to CD, but suggest that the effect is not independent of the IBD5 haplotype and that OCTN1/2 variants might simply be markers for the haplotype association (11). The latter finding has now been confirmed by several independent groups (12,13). There is now real urgency to resolve this controversy by very large genotyping studies involving several thousand individuals with IBD together with expression and functional studies of OCTN1/2; until then, the causative role of OCTN1/2 variants must be regarded as unproven.

The phenotypic associations of the IBD5 locus with the HLA region and the NOD2/CARD15 gene have been explored in a number of populations, but no consistent pattern has emerged. One study (14) suggested an association with perianal disease, but this was not replicated in other datasets (11,12). In the Edinburgh dataset, we noted an association with disease severity, manifested by progression of the disease and the need for surgery for CD (11). Whether the region has an important part to play in the pathogenesis of UC also remains debatable; the majority of positive studies have involved CD alone. Data from Germany and the United Kingdom have shown an association with UC and evidence for epistasis specifically with the CARD15 702Arg allele (15-17).

MDR-1 gene

The MDR-1 gene maps to chromosome 7q, and is located in a region that was associated with IBD susceptibility in a genome-wide scan in the United Kingdom (18). This gene has generated considerable interest because of its relevance to the pathogenesis of disease susceptibility. The MDR1$^{-/-}$ mouse model develops spontaneous enterocolitis in specific pathogen-free, although not in germ-free, conditions (19). Decreased MDR1 expression has been noted in the colon in the interleukin 10$^{-/-}$ model and the HLA-B27/beta2 microglobulin transgenic rat (20-22). Allelic association studies involving MDR1 variants have concentrated on two SNPs of putative functional significance: the C3435T exonic variant and the A2677G/T variant. To date, positive associations have been demonstrated in five independent datasets (23-27), and the most consistent association is with UC. In the Edinburgh dataset, specific haplotypes involving the two common single nucleotide polymorphisms appear to determine either disease susceptibility, or intriguingly, disease resistance in UC (27). Moreover, phenotypic associations with disease extent are reported. Although the physiological function of the MDR1 protein remains controversial, it appears most likely to play a role in protection of the epithelium against xenobiotics, which is entirely consistent with the involvement of gene-bacterial interactions in the pathogenesis of IBD. This hypothesis is consistent with our own data in the HLA-B27 transgenic model (20).

DLG5

Recent data from Stoll at al (28) in Germany has implicated that germline variation of the Drosophila discs large homolog 5 (DLG5) conveys susceptibility to CD and to IBD in general. The investigators pursued a positional cloning strategy for the 10q23 locus, which had been implicated in their own previous genome-wide scan, but failed to replicate the results in other datasets (29). Given the strength of initial observations, the authors followed the strategy of saturation genotyping and physical mapping, and have generated data implicating variation within this gene, which might have a practical role in maintenance of the epithelial structure or scaffold. DLG5 interacts with vinexin and beta-catenin in immunolocalization studies (30). As for the MDR1 gene, both risk-associated and protective haplotypes were described in the index population from Stoll's group (28). These data have been partially replicated in one study (31), but not in two others (32,33). As with NOD2/CARD15, Japanese patients with CD do not display any of the SNPs of the DLG5 locus described in Caucasian patients, but SNPs unique to the Japanese population show weak associations with CD (34).

TLR-4

After the discovery of the NOD2/CARD15 gene and the realization of the significance of intraluminal bacteria in the pathogenesis of IBD, it was appropriate to look specifically for polymorphisms affecting the receptors of the innate immune system. Lipopolysaccharide, released by Gram-negative bacteria, is a ligand that binds with TLR4 expressed on intestinal epithelial cells. The TLR4 Asp299Gly polymorphism is associated with decreased bronchial responsiveness to lipopolysaccharide in humans, with reduced activation in transfection experiments, similar to some of the NOD2/CARD15 mutations (35). The TLR4 Asp299Gly polymorphism was associated with CD and UC in a Belgian study (36), and with CD in Greek and Dutch populations (37,38). In a German cohort, an association was found between UC and the TLR4 Thr399Ile polymorphism (39). No association was found between the Asp299Gly polymorphism and IBD in German, Hungarian or Scottish datasets (39-41). Whether either of these two SNPs are the causal variants within the TLR4 gene remains controversial, and recent data imply they may simply be in linkage disequilibrium with the causal variants (42).

Other IBD loci

It is likely that a number of the other loci implicated by genome-wide screening will lead to the identification of other risk determinants for IBD. Some may be population-specific and be relevant to only a specific phenotype of disease; the lessons from the IBD1 locus are fresh in our minds. The IBD2 locus was first described in a United Kingdom dataset (18), and more recent data suggest that it is most strongly linked to colonic disease (43). The IBD4 locus (OMIM 606675) appears to be an important determinant of CD in the Flemish population in Belgium (44) and in populations in the United States (45). Most recently, these associations for the IBD4 locus have been replicated by the international genetics consortium (46). Although established criteria for definite linkage have not yet been met, the situation may change once fine mapping of the region and gene identification have been undertaken.

CONCLUSIONS

It is gratifying to consider how much progress has been made in genetic studies in IBD in the past decade. The application of molecular genetics has enhanced our understanding of disease pathobiology and has focused attention on the interaction

between genetic factors and intestinal bacteria. Whether it be through intracellular (NOD2/CARD15) or cell surface (TLR) bacterial recognition, antigen processing (HLA molecules) or a breakdown in epithelial integrity (DLG5), the overwhelming swell of evidence supports the concept that IBD results from a genetic predisposition to excessive or abnormal interaction with an environmental stimulus, most likely part of the normal luminal bacterial flora which, in turn, leads to excessive immune activation and chronic inflammation.

The importance of germline variation of the NOD2/CARD15 gene provides proof of principle for studies of the molecular genetics in complex diseases, but it is still difficult to translate genetic findings to the clinical arena. It is hoped that, in the near future, discoveries in this field will yield a better understanding of the pathogenesis of IBD and lead to novel targeted therapies.

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Discussion – Progress and prospects for an integrated classification system

Crohn's disease

Classification of CD is a difficult task because of the significant heterogeneity in disease presentation and course. In developing a classification system one must consider its purpose. Prior classification schemes have been developed mainly for the purposes of having a simple and practical clinical tool that can be easily recalled and implemented. Indeed, this was the rationale for the revision of the Rome classification to the Vienna classification – to reduce the number of possible classification categories from several hundred to 64. Beyond clinical use, however, a classification scheme is important in standardizing definitions and in developing consistency for research studies. In this case, comprehensiveness is more important than simplicity. It cannot be predicted a priori which genetic, serological or other future biomarkers will be pathophysiologically linked to a particular variation in disease expressivity. Data emerging from molecular and serological studies would suggest that a 'simplified' clinical proposal may not be sufficient to provide adequate information for classification based on genetic and serological markers. To identify the strongest pathophysiological association of a potential biomarker and IBD, the observed variations in phenotypes must be analyzed for the association. Furthermore, to have maximum power to make associations, potential sources of heterogeneity in disease etiology and/or pathogenesis should be reduced.

In the classification described in this Working Party report, the authors have addressed the difficulties and unresolved questions that evolved from the Vienna classification. Specifically, there are data from numerous sources that age of diagnosis is an important factor in disease course. The subclassification by age of diagnosis of 40 years was thought to be insufficient to capture some of these differences and, therefore, the updated classification scheme proposed here further subdivides earlier age of diagnosis into an A1 category to define age of diagnosis of 16 and younger, with A2 now describing age of diagnosis from age 17 to 40 years of age and A3 reserved for diagnosis older than 40 years of age. While this is thought to be a reasonable advance for the clinical classification, it is worthwhile noting that even further subdivisions may be required to address real differences in CD based on age of diagnosis. Some authors have reported unique clinical features for CD diagnosed earlier than eight years of age. More limited data also suggest that late age of diagnosis (ie, over the age of 60 years) may predict a different disease course. Accordingly, as part of a minimal data set that would be useful for studies evaluating the contribution of genetic and serological markers, it is recommended to record the exact age of diagnosis.

The currently proposed classification has also amended the Vienna classification with respect to disease location and behaviour by allowing for the co-classification of L4 with other disease location categories so this site can be examined in isolation and not be confounded by the co-occurrence of upper GI involvement and disease more distally. In addition, an independently documented variable known as the 'p' variable would be added to designate the presence or absence of perianal disease, allowing B3 to be defined as intra-abdominal penetrating disease. Both issues will allow improved accuracy of genotype/serotype correlation with disease phenotype.

While these are significant changes to the clinical classification system, it is worthwhile pointing out that using specific disease location sites as present or absent is an important feature of analysis by genetic and serological typing. For example, association of markers may occur with involvement of the upper GI tract or jejunum or with perianal disease alone. Therefore, it is recommended that a minimal dataset

defining disease location for such studies should be as specific as possible as to the site of disease and presence or absence of inflammation (where absence implies that the site has been examined and found to be free of involvement). For a given site, 'unknown' needs to be recorded when an appropriate examination has not been performed. Areas of difficulty that require further investigation with respect to disease location include how to interpret the presence of microscopic involvement in the absence of gross evidence of disease by endoscopy or diagnostic imaging studies and the utility of recent advances in imaging, such as wireless capsule endoscopy.

Another area of critical importance is the issue of disease severity or disease progression, particularly in view of evolving data implicating genetic and serological markers that predict rapid progression and severe disease. Both in UC, and to a lesser extent in CD, there are good measurements of disease severity for a given 'point-in-time'; however, it has been very difficult to define measurable parameters of what constitutes an overall severe disease course. Need for surgery or disease progression over time have both been used as surrogates in recent studies, but each has limitations. Indications for surgery may vary greatly and, in many centres, there has not been prospective data collection at regular intervals to determine disease progression. While our Working Party has not reached a consensus on an addition of this type of measure to the Montreal classification, it is suggested that future studies in large data sets with access to information about disease course over time be evaluated to arrive at definitions of severe disease and rapid progression.

Areas that have not been included in the clinical classification but are recommended as part of a minimal CD dataset for genetic and serological studies include presence or absence of extraintestinal manifestations and documentation of additional disease features such as ethnicity and country of birth, place of residence, smoking history and family history. All of these are related to some factors that affect the phenotype of CD or the course of disease.

The discovery of the NOD2/CARD15 gene and its involvement in the pathophysiology of CD has provided a paradigm for the integration of genetic markers into clinical classification. As discussed, however, the data currently available with respect to disease heterogeneity and gene penetrance argue against its use in clinical practice or decision making. Future studies will require that detailed phenotypic classification systems be used consistently by investigators throughout the world.

Ulcerative colitis

Many of the issues regarding CD are relevant to UC, in particular, the differing requirements of a purely clinical classification system and of one that allows assessment of integrated serotype/genotype-phenotype relationships. Even from a clinical viewpoint, however, there are several unique aspects to UC that deserve comment. Although UC is arguably a less heterogeneous disorder, it has been somewhat difficult to create a universally acceptable clinical classification scheme that takes into account all of the important aspects of the disease. The authors have developed the Montreal clinical classification for UC, which incorporates the maximal extent of colitis at the most recent assessment. The difficulty inherent in this approach, however, is the need for information on progression, or even regression, of disease over time. As with disease behaviour in CD, extent in UC is clearly a dynamic process whose further elucidation may be required for meaningful correlation with genetic or serum markers.

The severity parameters for UC relate to acute relapses, instead of to changes in severity over time. The latter may be more relevant to molecular and serological markers in UC. Using surrogate measures like the need for surgery, a number of markers have already been implicated, notably HLADRB1*0103 and variants of the MDR1 gene. The historical data from Oxford from Edwards and Truelove (1) strongly suggested that there are distinct patterns of disease activity over time. We see the need to move towards the development of an acceptable classification system that addresses this facet of disease phenotype before the next World Congress. Based on the data from Langholz et al (2), a system that includes the number of flares over time or the time to colectomy might be most useful in this context.

Other areas of critical importance are the occurrence of dysplasia or cancer and the presence of PSC with UC. Some distinct molecular and serological associations have been described in relation to these phenotypes and therefore their presence should be documented as part of a minimal research data set. While somewhat rare, these concurrent diseases result in substantial morbidity and mortality in UC and are important targets for molecular and serological studies using large, collaborative datasets.

We have also discussed a number of other pertinent issues, including age at diagnosis, microscopic versus gross disease extent, and extraintestinal manifestations. As with CD, these parameters might not warrant inclusion as separate entities in an essentially clinically based system, but should be part of the minimal dataset for a research-based analysis of genetic and serological markers. The concept of age-related heterogeneity in UC is supported by data regarding smoking cessation and some preliminary genetic data, while there is strong genetic and epidemiological evidence that extraintestinal manifestations have a distinct genetic basis.

Indeterminate colitis

IC is a confusing entity that is inconsistently understood by clinicians and the IBD pathologists alike. The members of the Working Party strongly contend that definitions should be improved and standardized so that emerging molecular and serological markers could be optimally utilized. Future studies should then enable us to determine whether this disease eventually evolves into CD or UC, is its own unique entity or perhaps is something in between.

The authors of this section of our report have proposed reverting to the original definition for IC in cases of chronic IBD without characteristic features of either UC or CD in a colectomy specimen. The novel term 'colonic IBDU' should be applied to patients who have not undergone surgery, in whom the diagnosis depends on clinical and endoscopic features together with histopathological findings from mucosal biopsies. Use of this term recognizes that IBD has been diagnosed but that additional clinical, imaging, serological and genetic information might be of value in further characterizing the condition. Serological markers promise to identify patients who would eventually progress to definite CD or UC, as well as those who would not (the 'seronegative' group).

In summary, the need for a classification system for IBD has evolved in recent years from the requirement for a simple clinically based scheme to one that allows the integration of the

rapidly evolving data on serotypes and genotypes. Indeed, these data may in turn lead to a transformation of the classification of IBD. At present, the data with respect to serotypes and genotypes are not sufficiently robust for use in clinical practice or in the recommended clinical classification systems for CD, UC or IC. However, any future integrated classification system will depend on standardization of research-related and clinical diagnostic criteria. In turn, minimal datasets must include sufficient relevant clinical data to allow exploration and confirmation of genotype/serotype-phenotype relationships in diverse populations. Our goal for the next five years will be to facilitate this process and move toward an integrated system for the classification of IBD.

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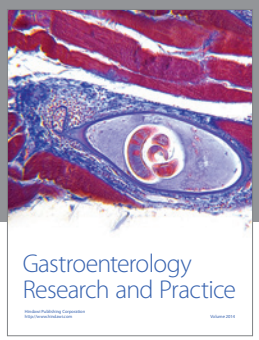

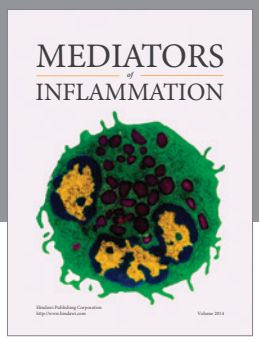

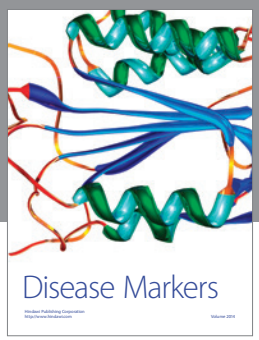

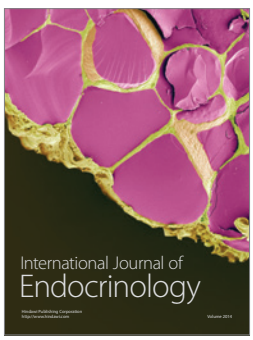

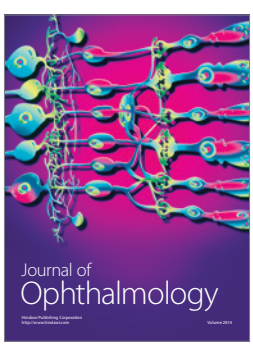

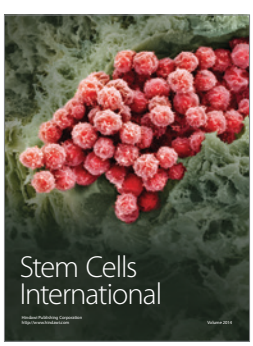

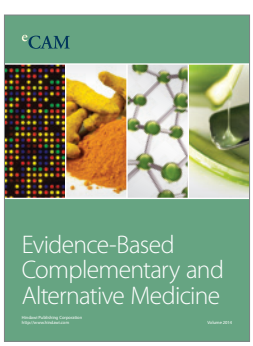

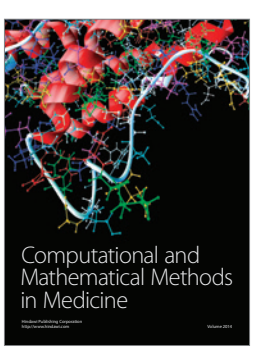

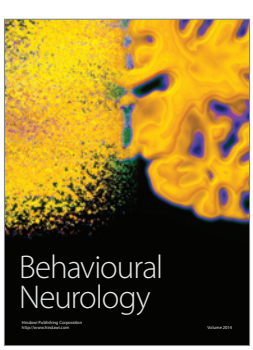

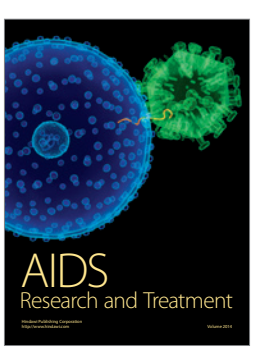

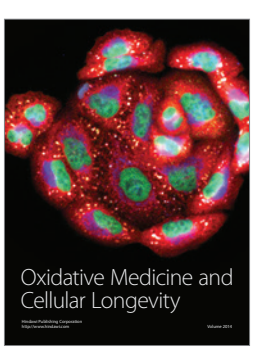

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