Subclinical markers of human inflammatory bowel disease

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H YANG, JI ROTTER. Subclinical markers of human inflammatory bowel disease. Can J Gastroenterol 1995;9(3):161-167. Genetic studies can be greatly aided by the use of subclinical markers that are closer to the basic defect and thus likely to detect more individuals with the abnormal genotype. At least two approaches are generally used to characterize subclinical markers. One is the family study approach. The detection of subclinical abnormalities in unaffected relatives similar to those found in the probands can distinguish between an inherited predisposition and a secondary abnormality due to the disease process. The second approach is the combination of subclinical marker with genetic marker studies. Specific association of a subclinical marker with a genetic marker indicates genetic determination of the subclinical marker. The identification of a genetically determined subclinical marker can help to define a more homogeneous disease group for genetic studies. The most studied subclinical markers in inflammatory bowel disease (IBD) are antineutrophil cytoplasmic antibodies (ANCAs) for ulcerative colitis (UC) and intestinal permeability for Crohn's disease (CD). Even so, for these as well as several other promising subclinical markers, there is an obvious need for more twin, family and genetic marker studies. An elevated intestinal permeability in a proportion of unaffected relatives of CD patients has been observed in the majority of family studies. ANCAs, a highly specific marker for UC, have been found with a significantly increased prevalence in unaffected relatives of UC patients compared with spouses of the patients. Moreover, the distribution of the ANCAs is familial rather than random, suggesting heterogeneity within UC. In combination with genetic marker studies (human leukocyte antigen [HLA] class II genes), the authors observed a differential association: ANCA-positive UC was associated with DR2, while ANCA-negative UC associated with DR4. These data lead to the conclusion that the heterogeneity indicated by ANCA is genetically determined and that this genetic heterogeneity should be taken into consideration in future genetic, clinical and pathophysiological studies. In the aggregate, these data indicate that the subclinical marker approach is a powerful means for demonstrating genetic and etiological heterogeneity, and can be an important tool to define the etiology and natural history of the various diseases that make up IBD. (*Pour résumé, voir page 162*)

Key Words: *Antineutrophil cytoplasmic antibodies, Crohn's disease, Inflammatory bowel disease, Subclinical markers, Ulcerative colitis*

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GENETIC STUDIES USING CLINICAL techniques and clinical disease definition alone have their limits because clinically unaffected individuals with the variant genotype will not be recognized. Such studies can be greatly aided by the use of subclinical markers that are closer to the basic defect and thus likely to detect more individuals with the abnormal genotype (1). Subclinical markers are parameters used to detect the abnormal genotype in the absence of the full phenotype, eg, abnormal glucose tolerance and islet cell antibodies in diabetes or serum cholesterol in coronary artery disease. These markers represent abnormalities having a direct role in the pathogenesis of the disease. They are useful in genetic studies because in many disorders not all individuals with the mutant genotype may manifest the disorder (reduced penetrance), the variability of the phenotype may be so great that the clinical features are too mild to be readily apparent (variable expressivity) or there may be a delayed age at disease onset such that the younger genetically predisposed individuals would be clinically normal (1). Thus, subclinical markers maximize the number of affected individuals that can be detected.

In addition, the detection of subclinical abnormalities in unaffected family members similar to those found in the probands can distinguish between a primary defect indicating the presence of disease pathophysiology, and a secondary abnormality due to the disease process. Therefore finding such abnormalities in clinically unaffected family members helps establish an etiological role for a certain abnormality in

Marqueurs subcliniques de la maladie inflammatoire de l'intestin

RÉSUMÉ : Dans des études de génétique, il peut être très utile de recourir à des marqueurs subcliniques, plus intimement liés aux anomalies de base et ainsi plus susceptibles d'identifier les sujets porteurs du génotype anormal. Au moins deux approches sont habituellement utilisées pour caractériser les marqueurs subcliniques. L'une est l'approche familiale. Le dépistage chez des proches non affectés d'anomalies subcliniques semblables à celles que l'on observe chez le probant peut permettre de distinguer entre prédisposition héréditaire et anomalie secondaire attribuable à un processus pathologique. La deuxième approche est l'association d'un marqueur subclinique et d'épreuves sur les marqueurs génétiques. L'association spécifique d'un marqueur subclinique avec un marqueur génétique indique une détermination génétique du marqueur subclinique. L'identification d'un marqueur subclinique à détermination génétique peut aider à définir un groupe de maladies plus homogène en vue des études génétiques. Les marqueurs subcliniques les plus étudiés dans la maladie inflammatoire de l'intestin sont les anticorps antineutrophiles cytoplasmiques (AANC) pour la colite ulcéreuse (CU) et la perméabilité intestinale dans la maladie de Crohn (MC). À ce chapitre et au sujet de nombreux autres marqueurs subcliniques au potentiel prometteur, il faudrait idéalement procéder à davantage d'études sur les marqueurs gémellaires, familiaux et génétiques. Une perméabilité intestinale accrue, dans une proportion de proches parents non affectés de patients atteints de MC, a été observée dans la majorité des études familiales. Les AANC, marqueurs très spécifiques de la CU, ont été notés avec une prévalence significativement accrue chez des proches non affectés de patients atteints de CU, en comparaison avec les conjoints de ces patients. De plus, la distribution des AANC est familiale plutôt que liée au hasard, ce qui suggère une certaine hétérogénéité dans la CU. En lien avec les épreuves sur les marqueurs génétiques (gène de l'antigène leucocytaire humain de classe II [HLA]), les auteurs ont observé une association différentielle : la CU positive à l'égard de l'AANC serait associée à DR2, alors que la CU négative à l'égard de l'AANC serait associée à DR4. Ces données permettent de conclure que l'hétérogénéité indiquée par la présence d'AANC est déterminée génétiquement et que cette hétérogénéité génétique devrait être prise en ligne de compte lors des épreuves génétiques cliniques et physiopathologiques. Dans l'agrégat, ces données indiquent que l'approche par marqueur subclinique est un moyen puissant de démontrer l'hétérogénéité génétique et étiologique et peut se révéler être un important outil pour définir l'étiologie et l'histoire naturelle de diverses maladies en jeu dans les MII.

TABLE 1
Subclinical marker studies in inflammatory bowel disease

Marker	Observation	Reference/year
↑ Antibody to colonic epithelial cells	UC and CD patients' relatives	Fiocchi et al (2)/1989
↑ Antibody to crude colonic and Escherichia coli	UC patient's female relative	Lagercrantz et al (3)/1971
↓ Mucin species IV	UC patient's twin	Tysk et al (4)/1991
↑ Mucosal cellular IgG_1	UC patient's twin	Helgeland et al (5)/1992
↑ IgA titres against gliadin	UC patient's twin	Lindberg et al (6)/1992
↑ Antineutrophil cytoplasmic antibodies	UC patient's relative	Shanahan et al (7)/1992
↑ Lymphocytotoxic antibody	CD patient's relative	Korsmeyer et al (8)/1975
↑ Complement dysfunction	CD patient's relative	Elmgreen et al (9)/1985
↑ Intestinal permeability	CD patient's relative	Hollander et al (10)/1986
↑ Obligate anaerobic fecal flora (Gram-positive coccoid rods, Gram-negative rods)	CD patient's children	Van de Merwe et al (11)/1988

CD Crohn's disease; Ig Immunoglobulin; UC Ulcerative colitis

a disease. Such an abnormality may either indicate the genetic abnormality predisposing to a disease or identify those in whom an earlier, subclinical phase of the disease process is occurring that may eventuate in clinical disease. Although several abnormalities have been described in inflammatory bowel disease (IBD), only a few have yet been extended beyond the patients to include family members (Table 1) (2-11).

The important characteristics of a subclinical marker for genetic studies include high specificity, constancy, and familiality. Antineutrophil cytoplasmic antibodies (ANCAs) have all of these characteristics.

ANCAs

Specificity: A distinct subset of ANCAs was recently discovered to be highly specific for ulcerative colitis (UC) by Saxon and co-workers (12). Approximately 70% of UC patients are ANCA-positive compared with other forms of colitis, which include Crohn's disease (CD) (as low as 6%) (12-16). ANCA is rare in normal controls. All available ANCA studies in IBD and related diseases are summarized in Table 2 (7,12-23). Although there are differences in the frequency of ANCAs between studies from different countries, the prevalence of ANCAs consistently is significantly increased in UC patients compared with those with CD. ANCAs observed in UC patients have higher titres, with a perinuclear immunofluorescence binding pattern (pANCA), whereas they have lower titres, with cytoplasmic pattern (cANCA), in CD sufferers (12,14). A high specificity indicates that this marker is disease specific, and therefore more likely to be involved in the pathogenesis of the disease.

The prevalence of ANCAs is also significantly increased in patients with primary sclerosing cholangitis (PSC), which is clinically associated with UC. The shared increase in ANCAs in both UC and PSC may indicate that there is a common antigenic target for immune-mediated attack on both colonic and binary epithelial cells. Further support of this concept is that a 40,000 molecular weight colonic epithelial protein

has been identified with a unique epitope(s) that is shared by the skin and biliary tract epithelial cells (24).

Constancy: The presence of ANCAs appears to be independent of disease activity, duration of illness, localization, extent of disease, previous bowel operations or medical treatment (12,15,20, 21). ANCAs are also found in UC patients who are five or more years postcolectomy. ANCAs not only are persistent, but also may have clinical implications. For example, ANCAs have been reported to occur with increased frequency in those postcolectomy UC patients who experience the subsequent inflammation termed 'pouchitis', compared with those who did not develop pouchitis (25,26). The presence of ANCA is not only an indicator of the risk for UC, but also an indicator of the high risk for complications of postcolectomy in UC patients. The presence of ANCAs is thus not simply an epiphenomenon related to active colonic inflammation, but may reflect a fundamental disturbance of immune regulation.

Familiality: Because the relatives of IBD patients have a higher risk than the general population of developing IBD, the prevalence of a subclinical marker is expected to be increased among relatives of IBD patients compared with the general population, an observation termed 'increased familial aggregation' or simply 'familiality'. Family or twin studies can be used to demonstrate familiality.

In a family study of ANCAs, the authors have demonstrated that the clinically healthy relatives of UC patients have an increased frequency of positive ANCAs (16%) compared with environmental controls (3%) (7). They also observed that second degree relatives who are not sharing the same household with the probands have an increased prevalence of ANCAs and that the household controls are not at an increased risk for ANCA (7). These important epidemiological observations suggest that the familial aggregation of ANCAs is due to the shared genetic factors among the family members, and not due to shared environmental factors.

TABLE 2
Antineutrophil cytoplasmic antibodies (ANCAs) in ulcerative colitis and other associated diseases

	% of pANCA					
Location	UC	CD	PSC	Control	Other	Reference/year
USA	68	12	–	0	–	Saxon et al (12)/1990
USA, Canada	61	6	–	0	<2	Duerr et al (14)/1991
USA	68	–	65	–	0	Duerr et al (17)/1991
USA, Canada	68	–	–	3	–	Shanahan et al (7)/1992
Norway	27	0	63	0	–	Zauli et al (18)1992
Germany	83	25	40*	–	–	Seibold et al (13)/1992
France	50	–	–	0	–	Reumaux et al (19)/1992
Sweden	50	8	50	–	–	Peen et al (16)/1993
Netherlands	79	13	–	9	–	Oudkerk-Pool et al (20)/1993
Greece	30	–	–	–	–	Dalekos et al (21)/1993
UK	–	–	80	0†	–	Lo et al (22)/1993
UK	54	10	–	0	–	Cambridge et al (15)/1993
Hong Kong	32	–	–	0	–	Sung et al (23)/1993

**88 primary sclerosing cholangitis (PSC) + ulcerative colitis (UC); †All in children; CD Crohn's disease; pANCA ANCAs with a perinuclear immunofluorescence binding pattern*

A second important finding in this published family study was the significant difference in the frequency of ANCAs in the relatives of probands whose sera were ANCA-positive compared with the relatives of probands whose sera were ANCA-negative (7). This concordant familial distribution indicates heterogeneity within UC.

Genetic marker studies with ANCAs: Although epidemiological observations have suggested the important genetic contributions to the development of ANCAs, genetic marker studies provide definitive evidence for the genetic determination of ANCAs in IBD.

HLA class II genes: It has been shown that UC is associated with the human leukocyte antigen (HLA) DR2 allele (27,28). When UC patients were subdivided into ANCA-positive and -negative groups, ANCA-positive UC patients had a significantly increased frequency of DR2 compared with ANCA-negative controls (44% versus 22%). In contrast, the frequency of DR2 in ANCA-negative UC cases (21%) was virtually identical to that in controls (22%) (29). In addition, the ANCA-negative UC patients had an increase in the DR4 allele compared with ANCA-positive UC patients. Therefore, the heterogeneity within UC indicated by ANCAs has a genetic basis. This genetic marker study further supports the epidemiologic observations: genetic susceptibility is an essential factor for the development of ANCAs.

TABLE 3
Characteristics of ulcerative colitis (UC)-associated antineutrophil cytoplasmic antibodies (ANCAs)

A subset of ANCAs associated with UC (50 to 86% UC)
Specific for UC compared with other forms of colitis
Independent of clinical features
Present in healthy relatives of UC cases
Familial distribution in the presence of ANCAs (ANCA-positive and -negative UC families)
Differential association of human leukocyte antigen class II alleles as a function of ANCA status

Presently, by the combination of family and gene marker studies, ANCAs are the most established subclinical marker for any form of IBD (Table 3).

PERMEABILITY STUDIES

Since the initial family study of permeability in CD (10) there have been several additional studies (Table 4). On first inspection, the results appear somewhat inconsistent. A number of related factors may affect the results of an intestinal permeability study. These may include the type of probes, the

TABLE 4
Permeability studies in relatives of patients with Crohn's disease

	Probes					
	Polyethylene glycol		Lactulose		^{51}Cr-EDTA	
Reference/year	Mean	10%*	Mean	10%*	Mean	10%*
Hollander et al (10)/1986	++	++				
Katz et al (30)/1989			–	++		
Ainsworth et al (31)/1989					–	–
Ruttenberg et al (32)/1992	–	++				
Teahon et al (33)/1992			(	++		++
Valpiani et al (34)/1992			–	++		
Pironi et al (35)/1992†			++	++		
May et al (36)/1993			(	++		

**At least 10% of relatives have a significantly increased permeability compared with controls (estimated in reference 37); †Used prior acetylsalicylic acid to augment permeability test; +Significant increase compared with controls; (Increase in relatives, but not statistically significant; –No difference between relatives and controls*

method of administration of the probe (eg, fasting/nonfasting, with meals/without meals [38]), day urine collection/overnight urine collection, length of urine collection and use of acetylsalicylic acid (ASA) as a challenge (39). It is important for this area of investigation to identify a sensitive and reproducible protocol for permeability testing that reliably separates CD patients (or CD subgroup) from controls. In addition, it has been proposed that some of the statistical methods used to illustrate the increased permeability in the relatives of patients with CD may give misleading results (37). Rather than comparing the means of permeability between the two groups – relatives and controls – one can examine the proportion of the asymptomatic relatives of patients with CD who have permeability values above the upper limits of the range of values in normal controls. The logic of this latter approach is that presumably only a proportion of CD relatives are genetically susceptible. This was recently done (36), and the investigators found that approximately 10% of these relatives had a significant increase in intestinal permeability. When re-examining the published studies by this same approach (ie, defining an increased level as greater than two standard deviations above the mean in controls), Hollander (37) found that the majority of such studies showed a significant increase in intestinal permeability in a fraction of the asymptomatic relatives of patients with CD (Table 4). The possibility of abnormal permeability in relatives remains an attractive hypothesis either as a genetic abnormality or as a marker of early inflammation (40), but this field needs additional studies and methodological standardization. Possibly the most interesting approach was presented by Pironi et al (35). In their study, both healthy relatives of CD patients and healthy controls were given the lactulose/mannitol (L/M) test before and after ASA administration. These investigators concluded that the relatives of CD patients were more sensitive to nonsteroidal anti-inflammatory drugs than healthy controls, ie, the mean percentage increase of above baseline L/M values observed after ASA was greater in relatives than in controls. Thus, these results suggest that an enhanced small bowel mucosal sensitivity to factors increasing permeability can play a primary role in the pathogenesis of the disease. However, more subjects need to be studied to confirm their findings.

OTHER POTENTIAL SUBCLINICAL MARKERS

Both UC and CD – antibodies to colonic epithelial cells: Antibodies to colonic epithelial cells have been reported in both CD and UC patients (28). One study assessed the immune reactivity to gut epithelial cell antigens in healthy members of families of patients with IBD (2). Specific lysis against epithelial cell-associated component antigens (colon-derived) among patients with IBD and among their unaffected first degree family members was significantly higher than in control groups (70% in IBD patients, 56% in relatives and 8% in controls). The possibility has been raised that these antibodies might reflect environmental, in addition to genetic, factors because of their high prevalence in nonrelated family members (41).

In UC – antibodies to crude colonic and *Escherichia coli*: Elevated titres of antibodies to crude colonic and *E coli* 0:14 antigens have been found not only in patients with UC but also in their healthy female relatives (3).

In UC – mucosal production of immunoglobulin G subclasses: The importance of immunoglobulin (Ig) G-mediated immunopathological processes in IBD has been suggested by the increased IgG cell fraction (42) and the elevated secretion of IgG (43) in the affected tissues. It has been also shown that there is a significant difference between UC and CD in terms of IgG subclass production in the mucosal lesion: the proportion of IgG_1 immunocytes has been found to be higher in UC than in CD, while the reverse was true for the IgG_2 cell fraction (44). A recent twin study revealed an interesting result: there was no difference in the cellular IgG subclass pattern between healthy and affected UC twins (ie, where the index twin had UC), and the proportion of IgG_1 in these healthy and diseased twins was significantly correlated (5). In UC, the aberrant mucosal production of IgG_1 and IgG_2 did not depend on active disease and the raised IgG_1 proportion appeared to be disease-specific (5). These findings suggest that genetic mechanisms appear to be involved in the regulation of the IgG subclass response.

In UC – antibodies to gliadin: In a twin study examining antibody (IgG, IgA and IgM) to baker's yeast, yeast mannan, gliadin, ovalbumin and betalactoglobulin, high IgA titres against gliadin were found in healthy and diseased UC monozygotic twins (6). This may indicate a subclinical and/or genetically determined gluten intolerance.

In UC – mucin abnormalities: The gastrointestinal tract is lined with a mucous layer that forms a barrier against exotoxins and microorganisms. One etiological hypothesis is that inborn abnormalities in colonic mucin species may be related to the pathogenesis of UC (45-47).

The content of the chromatographically defined component of colonic mucin designated human colonic mucin species IV has been reported to be reduced in both patients with UC and their apparently healthy twins (4). Composition of the mucins in CD patients and their unaffected twins was not significantly different from controls. These observations suggest that altered profiles of mucin glycoprotein may be present before the onset of UC and therefore may be genetically defined.

In CD – lymphocytotoxic antibodies: Autoantibodies to lymphocyte surface membrane antigens (lymphocytotoxic antibodies) have been found in increased frequency in patients with CD and their relatives, but have also been found in increased frequency in their spouses (8). In addition, these antibodies are not specific to IBD and have been found in a variety of other diseases, such as systemic lupus erythematosus, rheumatoid arthritis and malaria, all involving active immune responses (48).

In CD – complement dysfunction: In a study of CD patients and their clinically unaffected first degree relatives, 38% of cases and 18% of relatives showed subnormal generation of chemotactic activity and decreased use of C3 by the alternative complement pathway (9). All of the relatives with C3 abnormalities were confined to families of probands with similar abnormalities, suggesting that: the abnormalities are not simply secondary to CD; the abnormalities are familial; and because they occurred only in some families and not others, these abnormalities predispose in only a subset of CD.

In CD – obligate anaerobic fecal flora: The obligate aerobic fecal flora of CD patients has been shown to be different from that of healthy controls (CD flora has more Gram-positive coccoid rods and Gram-negative rods than the flora of healthy subjects) (49-51). To investigate whether the abnormal fecal flora is a genetically determined condition that predisposes an individual to the development of CD (52), a family study was conducted (11). The investigators observed that nine of 26 clinically healthy children of CD patients (35%) had abnormal aerobic flora, similar to the frequency in CD patients. In five to seven years of follow-up, three of the nine children with abnormal flora developed symptoms suggestive of CD and one was diagnosed with CD, while none of the 17 children with a normal flora showed symptoms consistent with CD. Thus, the abnormal flora may be indigenous to subjects predisposed to CD. It appears this may be limited to the early onset patient population because the siblings and parents of the CD patients did not show abnormal flora. If the basic observation is confirmed, then it needs to be determined whether the relationship between the abnormal fecal flora and CD is direct (products or cell wall fragments consisting of peptidoglycan and/or lipopolysaccharides could initiate the inflammatory reaction) (53) or indirect (the abnormal flora were less resistant to colonization of the bowel by pathogenic bacteria).

CONCLUSIONS

IBD is a genetically heterogeneous group of disorders. We are still at the beginning stage of using subclinical markers to understand the genetics of IBD. In future genetic studies, these subclinical markers (currently, at the minimum ANCAs) should be taken into consideration for classification of the patients and families to obtain an etiologically homogeneous groups. Natural history studies are needed to understand the role of ANCAs and eventually other subclinical markers in the development of clinical IBD.

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