Genetics and epidemiology may contribute to understanding the pathogenesis of IBD – a new approach is now indicated

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AS Peña, JBA Crusius, M Oudkerk Pool, et al. Genetics and epidemiology may contribute to understanding the pathogenesis of IBD – a new approach is now indicated. Can J Gastroenterol 1993:7(2):71-75. The present article summarizes the genetic and epidemiological factors contributing to the pathogenesis of inflammatory bowel disease (IBD) in which a consensus seems to have been reached. The factors identified so far strongly support the involvement of the immune system in disease predisposition to IBD. The models that have resulted from segregation analysis of families with multiple cases of IBD have led to propose a multifactorial inheritance for Crohn's disease and ulcerative colitis – a recessive gene in Crohn's disease and a dominant gene for ulcerative colitis. At present, the lack of genetic markers does not allow the acceptance of any one of these models. Some environmental factors, such as perinatal infections, absence of breastfeeding, smoking in Crohn's disease, influence of transfusion in diminishing relapses in Crohn's disease and drugs that are active in controlling these diseases, may imply the existence of an abnormal immunoregulation in the control of inflammation. New technology is available to approach the problem.

Key Words: Crohn's disease, Epidemiology, Genetics, Human leucocyte antigens, Immunogenetics, Inflammatory bowel disease, Tumour necrosis factor, Ulcerative colitis

Considérations génétiques et épidémiologiques dans la compréhension de la pathogènese de la maladie intestinale inflammatoire: indications d'une nouvelle approche

RÉSUMÉ: Le présent article résume les facteurs génétiques et épidémiologiques qui contribuent à la pathogènèse de la maladie intestinale inflammatoire au sujet duquel un consensus semble avoir été atteint. Les facteurs identifiés jusqu'à présent appuient nettement le rôle du système immunitaire dans la prédisposition.

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There is overwhelming evidence that genetic factors play a role in causing inflammatory bowel disease (IBD) (1-5). The observations supporting hereditary factors are increased familial incidence, higher prevalence of the disease in monozygotic versus dizygotic twins, higher rates in first degree relatives versus spouses and varied frequency found in different ethnic groups (Table 1). Since IBD has a variable age of onset, usually starting after 10 years of age, risks are dependent on the age-specific incidence; these have been calculated for patients in Baltimore, Maryland, USA (6) and for the Ashkenazi Jewish population from Los Angeles, California (7). This Jewish population has a three to four times higher risk of developing IBD than their non-Jewish neighbours. Ashkenazi Jews of middle European origin have an excess risk in relation to Jews of Polish/Russian origin (8,9).

The association of IBD with diseases of known genetic predisposition also supports the genetic predisposition. The diseases associated with IBD (such as ankylosing spondylitis, psoriasis, atopy and eczema, celiac disease, multiple sclerosis, autoimmune hemolytic anemia and primary sclerosing cholangitis) are linked to genes of the major histocompatibility complex (MHC).
à cette maladie. Les modèles qui ont résulté de l’analyse des familles ou se présentaient plusieurs cas de maladie intestinale inflammatoire ont permis de formuler une hypothèse multi-factorielle héréditaire de la maladie de Crohn et de la colite ulcéreuse, soit un gène récessif dans la maladie de Crohn et un gène dominant dans la colite ulcéreuse. À l’heure actuelle, le manque de marqueurs génétiques ne permet pas de confirmer l’un ou l’autre de ces modèles. Certains facteurs environnementaux, comme les infections péri-natales, l’absence d’allaitement maternel, le tabagisme dans la maladie de Crohn, l’influence des transfusions sur la diminution des rechutes de la maladie de Crohn et les médicaments actifs dans la maîtrise de ces maladies, peuvent permettre de supposer l’existence d’une immuno-régulation anormale dans le contrôle de l’inflammation. Une nouvelle technologie est disponible pour aborder le problème.

Some of these diseases are associated with class II antigens. For example, ankylosing spondylitis is associated with human lymphocyte antigen (HLA)-B27 and HLA-Bw60 (10). Recent studies from Germany showed a special type of genetic heterogeneity of patients with both ankylosing spondylitis and Crohn’s disease. The phenotype HLA-B27, -B44 was markedly increased when both diseases coincided. Individuals with this phenotype have a relative risk of 68.8 for the concurrent manifestation of Crohn’s disease and ankylosing spondylitis (11, 12). Other diseases found in association with IBD are linked to HLA class II antigens. Celiac disease primarily is associated with the HLA-DQA1*0501 and HLA-DQB1*0201 (13,14). These observations suggest that genes at the short arm of chromosome 6 are also of importance in the disease susceptibility to IBD.

Although in some families ulcerative colitis and Crohn’s disease coexist, there is strong disease concordance among family members. In the majority of the studies reported so far (1-5), the familial prevalence of Crohn’s disease and ulcerative colitis is higher when the patient has Crohn’s disease. However, a recent study in Italy (15) found no significant differences; the prevalence of ulcerative colitis in first degree relatives of patients with this disease is seven per 1000 and the prevalence of Crohn’s disease among first degree relatives of Crohn’s disease patients is five per 1000 (15).

The observations of multiple cases in families of patients with IBD have led to the postulation of different forms of segregation (3). Several models have resulted from these analyses. Some investigators propose multifactorial inheritance for Crohn’s disease and ulcerative colitis, while others have put forward that segregation analysis of data in families of patients with Crohn’s disease supports a recessive gene in Crohn’s disease and a dominant gene for ulcerative colitis. No genetic markers exist to test the validity of these models. The numbers of pedigrees with multiple cases collected by any centre are not large enough to permit a valid statistical analysis. Drs G Hellers and L Iselius, from the Huddinge University Hospital, Huddinge, Sweden, are collecting pedigrees of families with at least two cases of Crohn’s disease or ulcerative colitis to perform complex segregation analysis.

**EPIDEMIOLOGICAL STUDIES**

The different epidemiological factors which may be relevant in disease susceptibility to Crohn’s disease and ulcerative colitis are shown in Table 2 (16-18). There are many studies showing that the relative risk of ulcerative colitis developing in nonsmokers definitely is higher compared with smokers (ex-smokers usually develop their disease after stopping smoking). Epidemiological studies (19-22) consistently support a different association of cigarette smoking with ulcerative colitis and Crohn’s disease. It has been suggested that in genetically predisposed individuals, smoking exposure may determine the type of IBD that will develop. A study of identical twins in Sweden showed that the smoking pattern was similar in concordant and discordant twins; after diagnosis two healthy monozygotic twins had given up smoking during a mean period of 6.5 years without developing ulcerative colitis, implying that the sharing of identical genes and smoking patterns are not enough to develop IBD and indicating that other environmental factors are important (23).

Heavy smoking produces a reduced ratio of T helper-inducer to T suppressor cells (24). There is also some evidence that smoking increases production of colonic mucus in vitro (25). Prytz et al (26) have shown that intestinal permeability is decreased as measured by the oral tracer 51Cr-EDTA in patients who smoke and have ulcerative colitis. This protective effect of smoking seems to be due to nicotine. Srivastava et al (27) have shown that 15 or 30 mg of nicotine administered transdermally for four weeks to patients suffering from mild ulcerative colitis produced a significant improvement (10 of 16 patients treated improved histologically). In Crohn’s disease, vascular abnormalities are more severe.

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**TABLE 1**

**Observations supporting a genetic predisposition to IBD**

- Increased familial aggregations
- Higher concordance rate in identical twins
- If both parents are affected, higher prevalence in children
- Low prevalence in spouses
- Increased prevalence in some ethnic groups
- Association to diseases with known genetic predisposition

**TABLE 2**

**Environmental factors in IBD**

- Perinatal infections
- Lack of breast feeding or early weaning
- Higher urban prevalence
- Low socioeconomic status
- Smoking
- Use of oral contraceptives
- Viral and bacterial infections
- Increased incidence over too short a time span
than in ulcerative colitis (28). The pro-
thrombotic effects of smoking predominate since it is well known that
smoking induces endothelial cell
damage (29). Smoking could worsen
the ischemic process. The thrombo-
genic vasoconstrictive effects would
explain the higher susceptibility to
tobacco in women using oral contra-
ceptives (30,31). The risk of develop-
ing IBD in oral contraceptive users may
be increased for Crohn’s disease but not
for ulcerative colitis (this issue, how-
ever, has not been settled).

The environmental factors iden-
tified so far, such as dietary factors,
smoking patterns and the use of oral
contraceptives, appear to be more im-
portant as risk factors for developing
Crohn’s disease than for ulcerative colitis.

Some of the environmental factors
found in epidemiological studies, eg,
increased perinatal infections in a
cohort of patients with IBD, lack of
breastfeeding and influence of blood
transfusion (the number of relapses in
Crohn’s disease diminishes after blood
transfusion [32]), may imply the exist-
ence of an abnormal immunoregula-
tion in the control of inflammation.

The kind of drugs active in controlling
diseases supports this idea.

THE IMMUNE SYSTEM, THE
ENVIRONMENT AND IBD

In the interaction between genes
and the environment, the ultimate
defence mechanisms to eliminate
pathogens, infected or malignant cells,
is generation of a specific immune
response. The immune system of the
gastrointestinal tract consists of a com-
plex network which has to distinguish
between foreign proteins (the majority
of which are beneficial to the or-
ganism) and the toxic proteins which
have to be eliminated. To deal with
these special environmental condi-
tions, the gut has developed a specific
way to recognize foreign proteins
without mounting an immune response
(tolerance) or producing secretory im-
munoglobulin A, which is not able to
activate complement. T cells in the gut
do not respond by proliferating but
rather by producing a variety of
cytokines. Many genes are involved in
the development of specific immune
function. A set of closely linked genes
at the short arm of chromosome 6 plays
a unique role in regulating the immune
system: HLA classes I and II are in-
volved in immune recognition; CD4-
positive T lymphocytes recognize
antigens processed by the antigen
presenting cells (eg, macrophages) and
these are presented together with Class
II molecules, resulting in proliferation
and lymphokine production; CD8-
positive T lymphocytes recognize pep-
tides presented by class I molecules,
resulting in killing of the target cell;
and the region between class I and class
II genes, the ‘central region’, encodes at
least 20 genes whose macrophage
products regulate B cell proliferation
and antibody production (33,34).

Several characteristics of the genes
responsible for regulation of the im-
mune response may explain the epide-
miology of IBD. The polymor-
phism of the HLA system, ie, the oc-
currence of different alleles in each loci
of the MHC, evolves through natural
selection. It is believed that certain
MHC alleles have provided protection
of the species during evolution by
protecting against fatal infections
during childhood. These MHC alleles
are transmitted from generation to
generation. Evidence to support this
suggestion comes from data obtained in
Marek’s disease of chickens (35), in
Dutch survivors of typhoid and yellow
fever epidemics in Surinam (36), and in
malaria in west Africa. HLA-Bw53 and
DRB1*1302 diminish the risk of severe
malaria (37). In the latter, tumour
necrosis factor (TNF) may be involved
in selection of people with diminished
risk to autoimmune disease (38). IBD is
less frequent in areas where autoimmune
disease is unusual. The immune
system at birth is immature; immunity
in the baby is partly obtained through
breastfeeding, and perinatal infections
may therefore play a role in the predis-
position to IBD by exerting its
effects when the immune system is not
fully developed, probably by interfering
with the normal mechanisms of
tolerance. The immune system also is
influenced by emotional factors, per-
haps through neuropeptides and other
hormones.

REGULATION OF
GENE EXPRESSION

Gene transcription in the cells of the
immune system can be induced by
viral infections, heat shock and hor-
mones. Many of these external and in-
ternal factors lead to the binding of
positive regulatory molecules to
specific sequences in the enhancer and
promoter segments. For example, in-
terferon (IFN)-γ and TNF induce class II
expression. The regulatory molecules
interact with DNA and activate the
RNA polymerase, resulting in augmenta-
tion of RNA synthesis (34).

### TABLE 3

Working hypothesis to understand the interaction between genetic predisposition and environmental factors in IBD

- A specific combination of genes on chromosome 6
- Immune system under stress due to abnormal mucus composition, abnormal permeability
- Defect in control of inflammation, dysregulated cytokine production (other genes may be involved)
- In ulcerative colitis autoimmunity predominates, while in Crohn’s disease defect in control of inflammation predominates

### TABLE 4

Approach to the study of the genetic predisposition in IBD

Select patient subgroup

<table>
<thead>
<tr>
<th>Immunological marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil antibodies (pANCA) in a subset of patients with ulcerative colitis</td>
</tr>
<tr>
<td>Antibodies to Mr 40,000 epithelial cell antigen in ulcerative colitis</td>
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</tbody>
</table>

Proposed gene markers to be studied

- HLA (class I and II), central region of the HLA system
- Complement haplotypes
- T cell receptor genes
- Indirect markers
  - Intestinal permeability
  - Mucus glycoproteins

**HLA Human leukocyte antigen: Mr Molecular weight**
A MOLECULAR STUDY OF GENES FOR TNF IN IBD

TNFα ( cachectin) and β (lymphotoxin) are cytokines secreted by activated macrophages, T cells, B cells and natural killer cells (TNFα), and mitogen-stimulated lymphocytes (TNFβ) (39,40). TNFα plays a role in the acute-phase response to inflammation and induces an increase of MHC class II expression in vitro (41). Human T lymphocytes isolated from the lamina propria of colonic specimens triggered by anti-CD3 produce TNFα and IFNγ, which are able to kill cells from human colonic epithelial cell lines (42). These cytokines, therefore, may be important in the immune reactions observed in patients with IBD.

As mentioned above, the human MHC carries the genes for TNFα and β (43). Polymorphism in the TNFα gene has been detected in the New Zealand White mouse strain and appears to be implicated in the susceptibility of (New Zealand Black x New Zealand White) F1 hybrid mice to lupus nephritis. In humans, HLA-DR2 individuals produce less TNFα than individuals who are HLA-DR3 and HLA-DR4 (44,45).

TNF may contribute to the susceptibility of IBD. In a preliminary study of the bi-allelic TNF Nco1 polymorphism in 22 Dutch patients with ulcerative colitis, no major deviation of phenotypes was found when compared with the normal population (unpublished results). Further studies using microsatellite polymorphism will be carried out. TNF alleles in combination with high risk HLA-DR-DQ alleles may contribute to heighten the inflammatory reaction. On the other hand, an insufficient production of TNF may be a risk factor for the development of malignancy. The recent association found between HLA-DR2 and ulcerative colitis and HLA-DQB1 in Crohn’s disease may suggest that the presence of different alleles in other genes of the Class II region or the central part of the short arm of chromosome 6 may determine whether a patient will develop ulcerative colitis (46-48).

The research on HLA antigens, complement system, TNF and T cell receptor genes is at present in an exciting phase. With rapidly developing new technology the search for genetic markers in IBD has reached a new dimension. The possibility to study with new technology two unlinked loci will enhance understanding of the way the immune system fails to modulate or wrongly directs the gastrointestinal tract defense system. A combination of genes within the MHC may predispose the individual to suffer from IBD (Table 3). These are early days in this research and the possibility exists that other (clusters of) genes which regulate production of immunoglobulin, encode TNF-receptor(s), adhesion molecules, integrins and addressins in other parts of the human genome may turn out to be of interest in disease predisposition.

FUTURE STUDIES

A systematic study of the genes which regulate the immune response is indicated. The latest developments in genetics, like the possibility of typing sequence-specific oligonucleotides with the HLA and closely related genes, will undoubtedly help to discover the genes responsible for disease predisposition. However, since clinical features and location of disease at presentation together with the presence of specific complications and evolution of the disease give evidence for the existence of clinical subgroups in patients with IBD, a good integration should exist between the clinicians responsible for managing the patients and the basic scientists in order to select appropriate homogeneous groups (49) (Table 4).

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