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REVIEW

Host-microbiome interaction in Crohn's disease: A familiar or familial issue?

Andrea Michielan, Renata D'Incà

Andrea Michielan, Renata D'Incà, Department of Surgical, Oncological and Gastroenterological Sciences, Azienda Ospedaliera - Università degli Studi di Padova, 35128 Padova, Italy

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Correspondence to: Andrea Michielan, MD, Department of Surgical, Oncological and Gastroenterological Sciences, Azienda Ospedaliera - Università degli Studi di Padova, Via Giustiniani 2, 35128 Padova, Italy. andreamichielan@virgilio.it
Telephone: +39-04-98212893
Fax: +39-04-98760820

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Abstract

An impaired interaction between the gut and the intestinal microbiome is likely to be the key element in the pathogenesis of Crohn's disease (CD). Family studies have provided invaluable information on CD pathogenesis and on its etiology. Relatives share the same genetic risk of developing the disease as affected subjects. Relatives also exhibit similar features relating to their host-microbiome interaction, namely genetic variants in loci involved in detecting bacteria, a greater sero-reactivity to microbial components, and an impaired intestinal permeability. The burden of environmental factors such as cigarette smoking and dysbiosis also seems to be particularly relevant in these genetically predisposed subjects. Diet is emerging as an important factor and could account for the changing epidemiology of CD in recent years. Despite the pivotal role of genetics in the disease's pathogenesis (especially in familial CD), screening tests in healthy relatives cannot be recommended.

Key words: Crohn's disease; Genetics; Environment; Microbiome; Relatives

Core tip: Family studies support a host-microbiome interaction in the development of Crohn's disease (CD). Unaffected relatives reveal genetic variants in loci involved in detecting bacteria, a greater sero-reactivity to microbial components, an impaired intestinal permeability, and a greater susceptibility to environmental factors. Whether genetic or environmental factors drive these conditions is still under investigation, but CD pathogenesis is very likely multifactorial. A genetic burden may be hypothesized in familial CD, while environmental factors may be predominant in sporadic CD.

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INTRODUCTION

The pathogenesis of inflammatory bowel disease (IBD) remains unclear, but is likely to be multifactorial and driven by an aberrant immune response to the gut microbiome in genetically susceptible hosts[1]. The genetic hypothesis to explain the pathogenesis of IBD, and Crohn's disease (CD) in particular, has been intriguing researchers ever since familial clustering was first described by Crohn *et al*[2] himself in the 1930s, but the identification of the genes potentially involved has been hampered by the lack of a classical Mendelian inheritance. The rate of concordance in monozygotic twins is relatively low[3]. There is also a growing body of evidence to support an environmental burden in IBD epidemiology, now that the incidence of IBD is increasing in developing countries such as Asia and Africa[4], in migrants in Western countries[5], and in patients' spouses[6].

Family studies have generated invaluable data regarding the pathogenesis of CD, as relatives may share both genetic and environmental factors with patients. These studies have shed light on the role of host-microbiome interaction in the disease's development. The gut microbiome is involved in general homeostasis, with a crucial role in nutrition, energy metabolism and host defense[7]. The relationship between human organisms and their gut microbiome is regulated by the intestinal mucosal barrier, the permeability of which is a functional property that enables coexistence with bacterial symbionts, while preventing penetration by luminal macromolecules and pathogens[8-11]. Changes in the gut microbiome and intestinal permeability lead to an abnormal mucosal immune system response, which is the final step in the pathogenesis of IBD[12-15]. Both innate and acquired gut immunity participate in maintening a state of chronic inflammation, with activated dendritic cells and mucosal T $CD4^+$ cells apparently playing a key part in antigen presentation and response to the gut microbiome[16,17].

Changes in host-microbiome interactions have been well-documented in both CD patients and their unaffected relatives.

FAMILIAL RISK IN CD

Epidemiological studies have shown that almost 30% of IBD patients have a positive family history of the disease[18-21], which is the most important risk factor for the onset of IBD; the lifetime risk for first-degree relatives of CD patients is as high as 7.8% among Jewish people[20,22-25]. It has been demonstrated that having a sibling with CD coincides with a 30-fold increase in the odds of developing the same illness[16,22], or the other major form of IBD, ulcerative colitis (UC), and viceversa[20,26,27]. This cross-disease effect supports the evidence for a common genetic background in the onset of the two forms of IBD. There is also a cumulative effect since the risk increases when more than one relative or both parents are affected[6,28,29]. Familial CD seems to be a distinct entity from sporadic cases of the disease because it becomes manifest at a younger age and has a different phenotypic expression, with a higher prevalence of ileal involvement[23,29-32], a complicated course with penetrating or extraintestinal manifestations[33], and a strong concordance in terms of the site of disease and its behavior[21,33]. Although not all subsequent studies matched these results[34,35], a recent prospective study on more than six thousand CD patients confirmed them[36]. In families with CD, the children affected also have an earlier age of onset and a more aggressive course of the disease than their affected parents. Some authors suggested that genetic anticipation might explain this picture[37], but genetic anticipation is usually associated with monogenic diseases, and several further studies reveal potential biases due to a greater awareness of the condition[38-41], or generated contradictory results[21,42-44]. Irrespective of family history, pediatric-onset CD has a more aggressive behavior, a higher rate of resistance to therapy, and a particular phenotype and genetic susceptibility[45-47]; hence the Paris pediatric modification of the classical Montreal classification of IBD, which takes the influence of a very early onset on the disease's history into account[48].

Twin studies on the concordance in the disease's development and phenotype have identified a genetic predisposition that is stronger for CD than for UC[45,49,50]. The concordance rate ranges from only 30% to 50% in monozygotic twins, however, meaning that environmental factors cannot be overlooked, as discussed below.

FAMILY STUDIES ON *NOD2*/*CARD15* AND OTHER GENES

The nucleotide oligomerization domain 2 (*NOD2*) gene, later termed caspase recruitment domain 15 (CARD15), was the first to be identified as CD-susceptible in 2001[51,52]. Since then, more than a hundred polymorphisms and mutations have been reported in this gene, but only three of them are independently associated with CD, namely alleles *R720W*, *G908R* and *L1007finsC*[53,54]. Alone, these alleles each confer a risk of CD development that ranges from 1.5 to 3 fold, which rises to more than 40 when there are two of them in homozygosis or compound heterozygosis[55]. NOD2/CARD15 is a putative intracellular pattern recognition receptor expressed in several cells (monocytes, macrophages, intestinal epithelial and Paneth cells) and, when mutated, its ability to detect bacteria by recognizing peptidoglycan is impaired[53,56,57].

NOD2/CARD15 probably has a broader range of action in host-microbiome interactions, however, because its genotype affects gut microbiota composition[58], and its mutations have also been associated with defensin deficiency and an increased mucosal permeability in CD patients[59-62].

NOD2/*CARD15* mutations have been seen equally

often in patients with sporadic and familial CD[63-65], with the exception of one report of a higher frequency in the latter[66]. No differences have been found between relatives from multiplex and simplex families either[67], while they carry mutations significantly more frequently than in the general population[63,68-70].

An Italian multicentric study found that, irrespective of family status, CD patients carrying at least one NOD2/CARD15 variant had a clinically aggressive disease that had been diagnosed at a younger age; they featured ileal involvement, a stenosing pattern and a history of surgical resections[54].

It is worth noting that the prevalence of *NOD2/CARD15* mutations in CD patients is less than 50%, while it reaches 20% in healthy controls. This goes to show that, though important, it explains only a minor part of the variance in the development of CD[53]. A recent meta-analysis confirmed that NOD2/CARD15 mutations have little power in predicting the course of the disease[71].

The hypothesis of a genetic predisposition in the onset of CD is nonetheless consistent with the previous-mentioned family studies, and with epidemiological evidence of ethnic differences[72,73]. In recent years, population-based genome-wide association studies (GWAS), and subsequent GWAS meta-analyses have also led to the detection of more than 160 IBD-associated loci, with more than 30 loci related to CD, and nearly 300 potential candidate genes[3,4,45,74].

These genetic studies have underscored the importance of host-microbiome interactions, highlighting the role of genes involved in barrier function, T-cell subsets, cytokine signaling, autophagy and mycobacteria recognition[74-76]. These novel genetic markers have not been studied as extensively as NOD2/CARD15, but current data do not support any familial association[77-80]. On the other hand, a large international multicentric study on IBD4 (a CD-related locus containing several candidate genes) identified a greater genetic concordance in CD families where at least one member smoked than in non-smoking CD families[81]. This important finding again suggests that the expression of CD in a given patient is the result of interaction not only between the gene products of several susceptibility loci, but also between these products and certain environmental factors.

Currently known variants can predict less than 14% of the IBD risk and they are quite common in the general population too, and associated with other inflammatory diseases[82]. In fact, the limited sensitivity and specificity of these mutations make a genetic screening for relatives unfeasible.

FAMILY STUDIES ON SEROLOGICAL MARKERS

A hyper-responsive adaptive immunological response to microbial antigens is characteristic of CD and several antibodies have already been correlated with this condition, including: Anti-glycans (ASCA directed against mannan of *Saccharomyces cervisiae*, ACCA against chitobioside, ALCA against laminaribioside, AMCA against mannobioside, anti-L against laminarin, and anti-C against chitin), anti-bacterial sequence I2 of *Pseudomonas fluorescens* (anti-I2), anti-bacterial flagellins (CBir, A4-Fla2, Fla-X) and anti-outer membrane porin C of *Escherichia coli* (OMPc)[83,84]. Their clinical utility lies in their non-invasiveness, their ability to differentiate IBD phenotypes, and their prognostic value in CD. No current guidelines recommend their routine detection, however, given their low sensitivity, even though recent works have underscored their diagnostic and prognostic value when used in combination[84,85].

Family studies have demonstrated that some of these antibodies are more expressed in unaffected first-degree relatives of CD patients than in the general population, with a prevalence that reaches 20%-25% for ASCA, 15%-19% for anti-OmpC, 62% for ACCA, and 89% for ALCA[83,86-88]. Using a quantitative detection assay, we also found that serum levels of ACCA, ALCA and AMCA were similar in first-degree relatives and CD patients, and significantly higher than in healthy controls[83]. When we tested the magnitude of the total serological response to microbial antigens (the four anti-glycans and anti-OmpC), we found that first-degree relatives had a weaker response than patients, but a stronger response than healthy controls. Being uninfluenced by household conditions, these results support the hypothesis that CD are genetically predisposed to the development of antibodies against microbiota[83]. These antibodies cannot be an epiphenomenon of immune activation because they are not associated with abnormal intestinal permeability or active disease[85,89]. On the other hand, a genetic background as a predisposing factor for sero-reactivity has emerged from studies on ethnicity[90], and on the heritability of ASCA positivity in twins[91] and multiplex families[67], and from works correlating NOD2/CARD15 with serological markers. Several authors have reported that the aforementioned NOD2/CARD15 polymorphisms predispose individuals to the development of anti-microbial antibodies development[54,92-94], and one study even demonstrated that both CD patients and their unaffected relatives carrying any of these genetic variants, had a higher number of positive antibodies and increased serological semi-quantitative levels[95].

The association between serological response and genetics is likely to be more complex, however, and influenced by other factors, as shown by Vasseur *et al*[67], who reported that the ASCA trait in multiplex families is due partly to CD itself, not just to the *NOD2/CARD15* genotype. Two complementary reports have also shown that, while CD patients with a positive family history have a higher prevalence of antibody and serologic responses[96,97], each additional positive antibody increases the risk of CD, whatever the *NOD2/CARD15* genotype[98].

Since a study suggested that ASCA could predict the onset of IBD[99], there has been increasing interest in the sero-reactivity of IBD patients' relatives. No longitudinal studies on serological markers conducted to date have demonstrated which relatives will develop IBD, however[33]. Although antibody response may vary over time, the risk is probably higher the greater the intensity of the response[98], so quantitative tests on a number of antibodies might be helpful for stratifying the risk of disease in relatives.

FAMILY STUDIES ON INTESTINAL PERMEABILITY

An altered mucosal barrier function and greater intestinal permeability contribute to chronic inflammation in IBD, facilitating the interaction between the enteric immune system and the gut microbiome[13,14].

Several changes have been reported in the components of CD patients' mucosal barrier, mainly involving the intercellular adhesion molecules[100,101]. These changes increase paracellular permeability, nearly by as much as 50% when assessed with sugar excretion tests[102].

A greater paracellular permeability may not just a consequence of mucosal inflammation. It can be seen in IBD patients with quiescent disease too, and it correlates with intestinal symptoms even in the absence of any endoscopic disease activity[103].

The hypothesis of a genetic predisposition to barrier impairment in CD is suggested by the association between genes involved in intestinal barrier homeostasis and IBD susceptibility[104], and supported by the observation that up to 40% of relatives have an altered small intestinal permeability[33,102,105]. We found permeability abnormal in both patients and their relatives, with a more frequent occurrence in familial than sporadic cases of CD, and an association with NOD2/CARD15 variants in multiplex patients[61]. Other authors found not such correlation between permeability and genetic polymorphisms[106-108], but such studies mainly involved sporadic cases of CD.

The role of genetics has also been questioned in the light of an increased permeability being observed in spouses of CD patients[33], and after a recent study underscored the importance of age and environmental factors such as age and smoking, rather than genotype, as contributors to permeability in relatives[109]. Oddly enough, relatives who smoked did not seem to have an altered permeability in this latter study. This is a matter that will need further investigation, however, because smoking is a known risk factor for CD and has recently been associated with a greater permeability of the small intestine in experimental models[110].

In conclusion, the abnormal intestinal permeability found in CD patients' relatives further confirms a link between genetics and environmental factors in the development of CD. Thus far, there has been one only reported case of CD occurring in a relative as predicted by an abnormal permeability test[111], so there is still too little evidence to warrant intestinal permeability assessments in relatives for screening purposes.

FAMILY STUDIES ON ENVIRONMENTAL FACTORS

Some environmental factors shared by family members may contribute to modulating the microbiome and its interaction with the gut immune system. CD patients have a particular dysbiosis involving a reduction in *Clostridium* and *Bacteroides* species[12]. Given the symbiotic relationship between the gut microbiome and the mucosal barrier's integrity, this dysbiosis may aggravate any intestinal permeability impairment. In fact, the bacterial strains that diminish in CD are also the main producers of butyrate, which is fundamental to intestinal cell homeostasis and mucosal barrier integrity[12]. Several efforts have been made to manipulate the gut microbiome in order to restore homeostasis: probiotics, prebiotics and fecal transplantation have generated promising results but their efficacy is short-lived in CD, probably because other host characteristics affect the balance of the intestinal flora[112,113].

Siblings have the same dysbiotic features as CD patients, particularly involving a reduction in *Faecalibacterium prausnitzii*[114]. This may be genetically determined[115], to some degree at least, but a study performed on twins showed that the gut microbiome was associated more with disease phenotype (ileal *vs* colonic CD) than with genotype[116].

Similarly, childhood exposure to environmental factors influencing the intestinal microbiome, such as gastrointestinal infections, antibiotic use and hospitalization, may override the role of genetics, even in twins[117,118]. A recent longitudinal study identified a declining role of childhood exposure to such factors, whereas smoking and family history of the disease remained the main risk factors[119]. Smoking has proved particularly harmful in familial CD, raising its incidence and reducing the age of onset[120]. Together with its previously-mentioned effect on intestinal permeability, smoking may also affect the intestinal microbiome, leading to dysbiosis[121].

Epidemiologic data underscore the importance of environment-driven pathways: The incidence of CD is rising (and more rapidly than that of UC)[122], the colonic phenotype is becoming more common than ileal CD[123,124], monozygotic twin concordance is declining, and pediatric studies have shown a reduction in familial CD and an increasing multiethnicity of cases[50,122]. Western diet is increasingly seen as a major contributor to the changing epidemiology of CD because numerous dietary factors may affect the microbiome and intestinal permeability, leading to an acquired bacterial clearance defect that would foster subsequent mucosal inflammation[125]. Several studies have identified highly-

Table 1 Characteristics of familial and sporadic Crohn's disease in patients and healthy relatives

Familial CD	Sporadic CD
Patients	
Younger age at presentation	Onset al the classical peak age for IBD
Predominantly ileal involvement	Predominantly colonic involvement
Penetrating/stenosing phenotype	Less frequently complicated
More frequent extraintestinal manifestations	Less frequent extraintestinal manifestations
More frequent NOD2/CARD15 mutations	NOD2/CARD15 mutations < 50% of patients
Higher prevalence of anti-glycan antibodies	NOD2/CARD15 mutations associated with an increased sero-reactivity to microbial antigens
Impaired intestinal permeability associated with NOD2/CARD15 variants	Impaired intestinal permeability in < 50% of patients
Environmental factors: Smoking	Environmental factors: Smoking, diet?
Healthy relatives	
Genetic concordance of IBD4 locus in families with smokers	No reported genetic concordance
ASCA trait	Increased sero-reactivity to microbial antigens, also correlating with NOD2/CARD15 genotype
Abnormal intestinal permeability	Abnormal intestinal permeability in < 40% of relatives

IBD: Inflammatory bowel disease; NOD2/CARD15: Nucleotide oligomerization domain 2/caspase recruitment domain 15; ASCA: Anti-*Saccharomyces* antibodies; CD: Crohn's disease.

refined sugars as a major culprit[120], but a recent study suggested that the current burden of immune-related diseases (including CD) may also be explained by the increasing consumption of other industrial food additives - *via* an impaired intestinal permeability[126]. There are currently no family studies on the consumption of such dietary components (earlier research mainly addressed cereal intake and produced contradictory results[127]). There is therefore not enough evidence as yet to support a causal effect of diet on CD, although a proinflammatory effect may be postulated for certain dietary components[118]. As one of the environmental factors, diet is likely to be a major contributor to the increasing incidence of colonic CD, as suggested by twin studies[117,128].

CONCLUSION

Despite the accumulating evidence emerging from genetic studies, the numerous susceptibility loci identified to date explain only a part of the variance in CD risk. Host-microbiome interaction has a pivotal role in CD pathogenesis, although the factor capable of turning a symbiotic into a pathogenic relationship remains unknown[75].

Family studies have generated the strongest evidence of genetic and environmental factors being complementary contributors to microbially-driven inflammation in CD. Maybe, familial and sporadic CD should be considered as different entities (Table 1): The genetic burden prevails in familial CD, in which the genetic background influences the disease's phenotype and course, whereas environmental factors could be more important in the pathogenesis of sporadic cases[50].

Some degree of subclinical inflammation has been demonstrated in healthy relatives of CD patients[117,129,130], but it does not necessarily develop into clinical disease over time[131,132]. This limits the value of non-invasive screening tests, even though such tests proved effective in detecting CD even before it becomes symptomatic[133,134].

In conclusion, CD patients' relatives should not undergo screening so long as they are symptom-free, but they deserve special attention because of the invaluable information they can provide on the disease's pathogenesis.

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