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REVIEW

Breastfeeding and genetic factors in the etiology of inflammatory bowel disease in children

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Abstract

Inflammatory bowel disease is a chronic, debilitating disorder of the gastrointestinal tract. The etiology of inflammatory bowel disease has not been elucidated, but is thought to be multifactorial with both environmental and genetic influences. A large body of research has been conducted to elucidate the etiology of inflammatory bowel disease. This article reviews this literature, emphasizing the studies of breastfeeding and the studies of genetic factors, particularly NOD2 polymorphisms.

Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Etiology; Risk factors; Protective factors; NOD2/CARD15; Single nucleotide polymorphisms

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INTRODUCTION

Inflammatory bowel disease (IBD) is an idiopathic condition characterized by chronic destructive inflammation of the gastrointestinal tract. The morbidity of IBD, particularly in younger patients, can be considerable and may include effects on growth and development, reproductive health, education, employment, and psychological health. The pathogenesis of IBD is thought to be a complex interaction between genetic predisposition and inappropriate activation of the mucosal immune system driven by the presence of enteric flora and resulting in tissue injury[1-3]. Genetic factors have been the subject of intense investigation and, at least in some cases, may be involved in inappropriate activation of the mucosal immune system. Discerning other factors that influence the activation of the mucosal immune system or the distribution of enteric flora present in those at risk for IBD is paramount in lessening the impact of IBD, a debilitating condition that affects children and adults throughout the world. One factor that may be important in the pathogenesis of IBD is breastfeeding. Breastfeeding is a protective factor for the development of several chronic disorders[4]. The intent of this article is to review factors involved in the development of IBD in children, with particular emphasis on genetic factors and breastfeeding.

BACKGROUND

IBD is generally considered to include two major disorders, Crohn's disease (CD) and ulcerative colitis (UC). CD and UC are similar conditions, but most experts consider them separate diseases[2,3]. This distinction might have important therapeutic implications. In the individual patient, CD and UC can usually be distinguished on the basis of clinical features (Table 1) and laboratory manifestations, as well as radiographic, endoscopic, and histological features.

Table 1 Clinical and epidemiological features of Crohn's disease and ulcerative colitis

	CD	UC
Region of involvement	Any portion of gastrointestinal tract	Rectum and colon
Typical region of involvement	Ileum and colon	Rectum and extending proximally
Nature of inflammatory process	Segmental, transmural	Continuous, limited to mucosa
Extraintestinal manifestations	Oral aphthous ulcers, peripheral arthritis, erythema nodosum, digital clubbing, episcleritis, renal stones, and gallstones	Pyoderma gangrenosum, sclerosing cholangitis, chronic active hepatitis, and ankylosing spondylitis
Age at presentation	Bimodal; 1st peak in late teens; 2nd peak in late adulthood	Bimodal; 1st peak in late teens; 2nd peak in late adulthood
Gender difference	Women are slightly more likely than men to develop CD	Men are slightly more likely than women to develop UC

In some cases, a definitive diagnosis cannot initially be made. These patients are diagnosed with indeterminate colitis until a definitive diagnosis can be determined[2,5].

The clinical presentation of CD depends on the region of the bowel involved, the degree of inflammation, and the presence of complications. Children with ileocolitis typically present with crampy abdominal pain and diarrhea, which may be bloody. Systemic signs and symptoms such as fever, malaise, easy fatigability, and growth failure, are common in CD. Gastric and duodenal involvement may cause vomiting and epigastric pain. Perianal disease is common in CD. The clinical presentation of UC typically includes bloody diarrhea with mucus. More severe cases may also present with tenesmus, urgency, crampy abdominal pain, and nocturnal bowel movements. Onset is usually insidious with gradual progression of symptoms. Fever, severe anemia, hypoalbuminemia, and leukocytosis may also be present. Presentation may be milder in cases involving only the rectum. Systemic manifestations occur less commonly than in CD. UC is associated with an increased risk of colon cancer. Secondary amenorrhea is common during periods of active disease in both CD and UC[5,6].

Both CD and UC were identified as clinical disorders in the early 20th century. Population-based studies have suggested an uneven distribution of IBD throughout the world with the highest disease rates occurring in "Westernized" countries[6]. The reported incidence of CD is 3-4/100 000 and the prevalence is 30-100/100 000. Earlier age at onset is associated with more severe disease and with increased likelihood of CD in family members. Incidence rates for UC are highest in European countries and the United States (15/100 000) and lowest in Japan and South Africa (1/100 000). The incidence in Israel varies by country of origin with the lowest rates among those from Asia or Africa. The prevalence of UC in European countries and the United States is 100-200/100 000. In Europe and North America, the incidence of IBD has increased steadily since the first half of the 20th century[7,8]. In particular, the incidence of CD has increased although the incidence of UC has generally reached a plateau in the second half of the 20th century (Tables 2 and 3). Of note, the incidence of both CD and UC in children has been increasing.

Rapid changes in the incidence of IBD within the same population can best be explained by changes in environmental factors since changes in genetic predisposition do not occur rapidly[7]. Some studies have demonstrated a predilection of IBD for urban rather than rural populations or a north-south gradient of disease incidence[9]. The incidence of IBD in individuals of Jewish ancestry is higher than in individuals not of Jewish ancestry. Migrant studies have shown that immigrants acquire IBD at a rate consistent with that of the new geographic area[7]. This evidence suggests that there is a significant influence of environmental factors on the development of IBD.

A number of factors, including smoking, oral contraceptive (OC) agents and diet, have been considered as potential risk factors for IBD. Smoking, the most extensively studied environmental factor in the development of IBD, is a risk factor for CD[10], but a protective factor for UC[11]. A meta-analysis of smoking and IBD confirmed these findings (Table 4)[12]. The effect of passive smoke exposure in childhood on the subsequent development of IBD remains inconclusive (Table 4). The relationship between OC agents and the development of IBD is less certain. Epidemiologic investigations of the relationship between OC agents and IBD yielded mixed results. A meta-analysis of these studies showed evidence of a modestly increased risk for CD and UC in OC users (Table 4)[13]. A more recent study confirmed the modestly increased risk of CD and UC in former and current users of OC agents, but the difference was statistically significant only for current users of OC agents and CD [ψ = 3.4 (1.0-11.9), n = 106 age-matched pairs][14]. Because of the direct interface between diet and the gastrointestinal tract, the role of dietary factors in the development of IBD has been extensively investigated. Early studies of diet had serious methodological flaws and their findings have been questioned[15]. A Japanese study of 101 cases and 143 controls found an increased risk of UC associated with consumption of Western foods (P_{trend} = 0.04)[16]. More recently, a well-designed study of diet was conducted in newly diagnosed IBD patients [CD (n = 33), UC (n = 54)] (Table 4)[17]. A decreased risk of CD was associated with increasing consumption of vitamin C. An increased risk of UC was associated with increasing consumption of sucrose, animal fat, cholesterol, and soft drinks.

The role of preceding infections in the development of IBD is unclear (Table 4). In a matched case-control study, patients with CD had a higher rate of gastroenteritis in the first six months of life than controls, but patients with UC did not[18]. In separate studies, children with CD[19] and children with UC[20] were more likely than their unaffected siblings to have had diarrheal illness during infancy. In a large study

Table 2 Incidence of Crohn's disease

Annual incidence per 100 000	Time period	Geographic region	Data collection	Age group studied	References
0.73	1958-1960	Nottingham, England	Retrospective	Adults	[70]
3.63	1970-1972				[70]
6.1	1971-1980	Orebro, Sweden	Retrospective	Children ≤ 16 years old	[71]
[1]3.39 (white males)	1977-1979	Baltimore SMSA	Retrospective	All	[10]
[1]3.54 (white females)					[10]
[1]1.29 (non-white males)					[10]
[1]4.08 (non-white females)					[10]
0.66	1968	Scotland	Retrospective	Children ≤ 16 years old	[72]
2.29[b]	1983				[72]
0.00	1920-1929	Rochester, NY	Retrospective	All	[73]
5.03	1970-1979				[73]
3.90	1980-1989				[73]
< 1.0	1962-1969	Copenhagen	Prospective	All	[74]
4.1	1979-1987				[74]
1.30	1983-1988	South Glamorgan, Wales	Retrospective	Children < 16 years old	[75]
3.11	1989-1993				[75]
[2]1.0	1940-1943	Olmsted County, MN	Retrospective	All	[76]
[2]7.8	1964-1973				[76]
[2]6.9	1984-1993				[76]
14.6	1989-1994	Manitoba, Canada	Retrospective	All	[77]
[2]1.91	1981-1983	Scotland	Retrospective	Children < 19 years old	[78]
[2]2.91	1990-1992				[78]
5.5	1990-1994	Iceland	Prospective	All	[79]
1.2	1984-1986	Sweden	Prospective	Children < 16 years old	[80]
1.3	1993-1995				[80]
[2]5.2	1988-1990	Northern France	Prospective	All	[81]
[2]5.8	1991-1993				[81]
[2]5.9	1994-1996				[81]
[2]6.4	1997-1999				[81]
2.00	1990-1994	Southeastern Norway	Prospective	Children < 16 years old	[82]
4.56	2000-2001	Wisconsin	Prospective	Children < 18 years old	[83]

[1]Age-adjusted; [2]Age- and gender-adjusted; [b]$P < 0.0001$ compared to 1968.

(257 cases of IBD with 2 matched controls per case) of perinatal risk factors for IBD, the greatest risk was associated with postnatal infections in the child [ψ = 5.5 (2.6-11.8)][21]. In a nested case-control study (26 CD, 29 UC, eight randomly selected controls matched for gender and social class) from two national longitudinal birth cohorts, infections during pregnancy or in childhood were associated with an increased risk of CD and UC, but these differences were not statistically significant[22]. In a large, international multicenter study of children with IBD and sex and age-matched controls, recurrent respiratory infections were significantly more common in CD and UC patients than their controls and patients with CD used antibiotics more frequently than their controls[23]. In this study, there were no differences between cases and controls in the frequency of gastroenteritis severe enough to require hospitalization, the age of its occurrence, the frequency of other hospitalizations, other recurrent infections, or tonsillectomy/adenoidectomy[23]. In another study, adults with CD reported an increased frequency of childhood infections compared to neighbor controls as well as more frequent treatment with antibiotics for both otitis and pharyngitis[24]. In this same study, adults with UC reported more frequent childhood infections than neighbor controls, but no increased frequency of antibiotic treatment[24].

The role of perinatal and childhood factors in the development of IBD has been investigated, but very little has been demonstrated (Table 4). No significant differences were found in birth weight, prematurity, birth at home, nursery school attendance, number of children in nursery or first class at school, number of playmates, bedroom sharing with other children, home environment at different ages, birth month, or the number of siblings but children with CD were more likely than controls to be last born ($P < 0.02$). The age interval to the previous sibling did not differ between last-born patients and controls[23]. Two smaller studies found no association between seasonality of birth, maternal age at birth, birth weight, or birth order and either disease[25,26]. In a study of perinatal factors, the occurrence of any perinatal health event increased the risk of both CD and UC[21]. The occurrence of any noninfectious perinatal event was an independent risk factor for IBD [ψ = 3.5 (2.0-6.3)] as was low socioeconomic status [ψ = 2.7 (1.2-5.7)] and low placental weight [ψ = 1.5 (1.0-2.2)]. A large, population-based case-control study from Sweden used siblings as a marker for exposure pattern[27]. Cases were identified through the Swedish Inpatient Register and controls (matched by age and area of residence) through Swedish Census, Birth and Death registers. Analyses were adjusted for sex, multiple birth, maternal age, region, year of birth, and fathers' social class. There was a significant, graded negative association between CD and number of younger siblings. Increasing maternal age was

Table 3 Incidence of ulcerative colitis

Annual incidence per 100 000	Time period	Geographic region	Data collection	Age group studied	References
[1]2.92 (white males)	1977-1979	Baltimore SMSA	Retrospective	All	[10]
[1]1.79 (white females)					[10]
[1]1.29 (non-white males)					[10]
[1]2.90 (non-white females)					[10]
1.91	1968	Scotland	Retrospective	Children ≤ 16 years old	[72]
[3]1.56	1983				[72]
0.06	1920-1929	Rochester, NY	Retrospective	All	[73]
3.51	1970-1979				[73]
2.32	1980-1989				[73]
6.9	1962-1969	Copenhagen	Prospective	All	[84]
9.2	1980-1987				[84]
0.71	1983-1993	South Glamorgan, Wales	Retrospective	Children < 16 years old	[75]
14.3	1989-1994	Manitoba, Canada	Retrospective	All	[77]
16.5	1990-1994	Iceland	Prospective	All	[79]
1.4	1984-1986	Sweden	Prospective	Children < 16 years old	[80]
3.2[a]	1993-1995				[80]
[2]4.2	1988-1990	Northern France	Prospective	All	[81]
[2]4.3	1991-1993				[81]
[2]3.9	1994-1996				[81]
[2]3.5	1997-1999				[81]
2.14	1990-1994	Southeastern Norway	Prospective	Children < 16 years old	[77]
2.14	2000-2001	Wisconsin	Prospective	Children < 18 years old	[83]

[1]Age-adjusted; [2]Age- and gender-adjusted; [3]$P = 0.052$ compared to 1968; [a]$P < 0.05$ compared to 1984-1986.

negatively associated with CD ($P < 0.001$). There was a protective effect of younger siblings that was greatest for those born soon after subjects, with statistical significance disappearing for those born 5 years later than subjects. There was a significant, graded positive association between UC and number of older siblings. Maternal age was not consistently associated with UC. There was no discernible pattern in the relationship with UC risk by age difference between subjects and their older siblings[27].

Several studies have shown an increased risk of IBD in relatives of individuals with CD compared to the general population. Population studies found that first-degree relatives of CD patients had a prevalence of CD that was 10-21 times the population prevalence and a prevalence of UC that was 6-10 times the population prevalence[28,29]. Relatives of a patient with CD had a greater risk of acquiring CD than UC and relatives of a patient with UC had a greater risk of acquiring UC than CD, but both diseases could occur in the same family[28,30]. In general, the familial association has been greater for individuals with CD than for those with UC[28]. Twin studies have demonstrated greater concordance for CD than for UC and greater concordance for monozygotic twins than for dizygotic twins[31-33]. These observations suggest that there is an inherited predisposition to the development of CD and UC.

GENETIC FACTORS

Several groups of investigators have conducted studies to discern the modes of inheritance of CD and UC. In one study, segregation analysis of 265 CD patients and 5387 relatives suggested a recessive susceptibility gene for CD with incomplete penetrance. The model predicted that the proportion of cases explained by the presence of this gene would be very high among those with early onset disease and about 30% of cases would be due to homozygosity for the gene[34]. In a second study, complex segregation analysis of 133 CD patients and their relatives also suggested a recessive major locus, however, with nearly complete penetrance. This model also predicted that 7% of patients would be homozygous for the recessive gene, but 28% of patients under age 20 would be homozygous for the recessive gene[35]. In UC patients, segregation analysis of 65 patients and their relatives suggested a rare additive major gene that would account for 11% of the total phenotypic variance and would have penetrance for heterozygotes of 0.22. The model predicted that affected individuals would be more likely to be heterozygous than homozygous for the additive gene and risk to an offspring of an affected individual would be 11%[36].

Subsequent genome-wide scanning studies have identified a series of IBD susceptibility loci[37-39]. Some of these loci are more strongly associated with CD, others with UC, and some with both. With respect to CD, the most recent genome-wide scanning study confirmed previously established associations at IBD1, IBD5 (5q31), IL23R, ATG16L1, IRGM, TNFSF15, and PTPN2, but also identified 21 new loci associated with CD[40]. The first locus identified, IBD1, has shown evidence for linkage with CD, but not with UC[41]. Definitive evidence for linkage at IBD1 was confirmed in a large, international IBD genetics consortium study that also demonstrated equally increased allele sharing at this locus in Jewish and non-Jewish cohorts[42]. In 2001, three major, relatively uncommon, single nucleotide polymorphisms (SNPs) were identified at a gene in

Table 4 Factors affecting development of IBD

Factor	Effect	Findings
Cigarette smoking	Protective factor for UC	Pooled OR for UC = 0.41 (0.34-0.48); χ^2 = 11.52 (P < 0.001)[12]
	Risk factor for CD	Pooled OR for CD = 2.0 (1.65-2.47); χ^2 = 48.4 (P < 0.001)[12]
Passive cigarette smoke	Uncertain	No effect[25,85]
		UC [ψ = 0.50 (0.25-1.00), *n* = 163][86]
		CD [ψ = 5.32 (1.09-25.9), *n* = 39 age and sex-matched pairs][87]
		UC [ψ = 2.19 (0.75-6.41), *n* = 33 age and sex-matched pairs][87]
Oral contraceptive use	Risk factor for UC	Pooled RR for UC = 1.29 [(0.94-1.77) adjusted for smoking]
	Risk factor for CD	Pooled RR for UC = 1.68 [(0.97-2.88) unadjusted for smoking]
		Pooled RR for CD = 1.44 [(1.12-1.86) adjusted for smoking]
		Pooled RR for CD = 1.68 [(0.97-2.88) unadjusted for smoking]
Diet	Protective factor for CD	
	Vitamin C	ψ = 0.48 and ψ = 0.23 for medium and high intake, respectively, *vs* low intake, P_{trend} = 0.02[17]
	Risk factors for UC	
	Sucrose	[Sucrose] ψ = 2.05 and ψ = 4.22 for medium and high intake, respectively, *vs* low intake, P_{trend} = 0.02
	Animal fat	[Animal fat] ψ = 2.02 and ψ = 4.09 for medium and high intake, respectively, *vs* low intake, P_{trend} = 0.02
	Cholesterol	[Cholesterol] ψ = 2.14 and ψ = 4.57 for medium and high intake, respectively, *vs* low intake, P_{trend} = 0.02
	Soft drinks	[Soft drinks] ψ = 1.84 and ψ = 3.39 for medium and high intake, respectively, *vs* low intake, *vs* P_{trend} = 0.02[17]
Infections	Risk factor for CD/possible risk factor for UC	CD patients had a higher rate of gastroenteritis than did controls (6/57 *vs* 1/114, *P* = 0.005)[8]
		UC patients and controls did not differ (4/51 *vs* 1/102, *P* = NS)[8]
	Gastroenteritis	Children with CD were more likely than unaffected siblings to have had diarrheal illness [RR = 2.7 (95% CI 1.5-5.8) *P* < 0.02, *n* = 294] Children with UC were more likely than unaffected siblings to have had diarrheal illness [RR = 3.2 (95% CI 1.15-8.75), *P* = 0.03, *n* = 231][20]
	Diarrheal illness in infancy	
	Risk factor for CD and UC	Recurrent respiratory infections were significantly more common in CD patients and in UC patients than their controls (102/298 *vs* 156/601 and 73/194 *vs* 106/393, respectively, both *P* < 0.01)[7]
	Recurrent respiratory infections	Adults with CD had an increased frequency of childhood infections compared to neighbor controls [ψ = 4.67, (95% CI 2.65-8.23) *n* = 322 cases, 262 controls][24]
	Childhood infections	Adults with UC had more frequent childhood infections than neighbor controls [ψ = 2.37 (95% CI 1.19-4.71) (*n* = 181 cases, 141 controls)][24]
Antibiotic use	Risk factor for CD	Patients with CD used antibiotics more frequently than controls (*P* < 0.01)[7]
		Adults with CD had more frequent treatment with antibiotics for both otitis [ψ = 2.07 (95% CI 1.03-4.14)] and pharyngitis [ψ = 2.14 (95% CI 1.20-3.84)] than controls[24]
Perinatal factors	Risk factor for UC	For UC, the odds ratios for having one, two, and three or more older siblings were 1.08 (1.03-1.14), 1.09 (1.01-1.16), and 1.12 (1.02-1.23), respectively (*n* = 15 823 cases; 79 546 controls)[27]
	Number of older siblings	
	Protective factor for CD	For CD, the odds ratios for having one, two, and three or more younger siblings were 0.93 (0.88-0.99), 0.89 (0.82-0.96), and 0.83 (0.75-0.92), respectively (*n* = 12 668 cases; 63 035 controls)[27]
	Number of younger siblings	

this locus, the *NOD2/CARD15* gene that conferred susceptibility to CD[41,43]. One group identified three separate SNPs in the *NOD2* gene [a frameshift variant (L1007fsinsC) and two missense variants (R702W and G908R)] which were associated with CD. The genotype relative risks for CD in their sample compared to those with no mutations, for simple heterozygous individuals, homozygous individuals, and compound heterozygous individuals (i.e. those with two different variant alleles) were 3, 38, and 44, respectively. The demonstrated gene-dosage effect suggested a recessive model of inheritance. The other group identified only the frameshift variant SNP. The reported genotype relative risks for heterozygous and homozygous individuals were 1.5 and 17.6, respectively. A third group confirmed the presence of the frameshift variant in two different cohorts of patients[44]. For CD, the mutation was highly associated [heterozygotes and homozygotes *vs* normal, ψ = 2.6 (1.5-4.5), ψ = 42.1 (4.3-∞), respectively]. In all of these studies, the gene mutation was not associated with UC. Subsequent studies by different investigators in different Caucasian cohorts have confirmed that these NOD2 variants were independent risk factors for CD, conferring susceptibility for CD[45-49]. Overall, 27%-32% of CD patients carry one major variant allele compared to 10%-20% of Caucasian controls and 8%-17% of CD patients carry two major variant alleles compared to 1%-5% of Caucasian controls[35,50].

The *NOD2* gene encodes for a protein in monocytes that is involved in the immune-mediated inflammatory response to enteric pathogens. The frameshift variant truncates the NOD2 protein and is associated with a marked hyporesponsiveness of NF-κB activation with lipopolysaccharide treatment. The missense variants yield a NOD2 protein that showed a greater response to lipopolysaccharide, but still a diminished ability to activate NF-κB. How the mutant NOD2 proteins and impaired NF-κB activation confer susceptibility to CD is unknown. However, it is known that NOD2 protein plays a critical role in the detection of bacterial muramyl dipeptide, and can activate the adaptive immune system by acting as an adjuvant receptor for antibody production[37,51].

A number of investigators have attempted to define demographic and clinical features associated with the NOD2 variants known to be associated with CD. Some have identified a younger age of onset of CD associated with the NOD2 variants, particularly the frameshift variant and particularly for homozygotes or compound heterozygotes[45,47] while others have not[46,49,52,53]. No studies have reported any relationship between gender and the NOD2 variants. In one study, the frequency of NOD2 variant alleles was significantly higher in familial

cases of CD than in sporadic cases of CD [30.9% (n = 173) *vs* 19.3% (n = 405), $P < 0.001$][46], but in two other studies the frequency of NOD2 variant alleles did not differ between familial and sporadic cases[47,49]. The reported NOD2 variants confer risk primarily in Caucasians, since they were not found in Asians with CD[54], and were found in much lower frequencies in African-Americans with CD[55]. NOD2 variants have been associated with ileal (or ileocolonic) involvement and stricturing disease[45-47,49,52,56,57]. In most of these studies, homozygous and compound heterozygous patients had increased risk of ileum-specific disease[45], stricturing disease[47,52], or both. Of the known NOD2 variants, the frameshift variant has the strongest association with ileum-specific disease[45] and stricturing disease[52].

BREASTFEEDING

The relationship between breastfeeding in infancy and subsequent development of IBD was first evaluated in the early 1960's by investigators who had observed that some patients with UC demonstrated a striking clinical relationship based on inclusion or exclusion of dairy products from their diet[58]. They conducted a case-control study of 132 adults with UC and 129 controls matched for age and sex. Patients with UC were more likely than controls to never have been breast-fed (χ^2 = 7.42, $0.001 < P < 0.01$) and to have been breast-fed 14 d or less (χ^2 = 9.05, $0.001 < P < 0.005$). Another group of investigators conducted a similar but smaller study with controls matched by age and sex to each of 51 adults with UC and 57 adults with CD. They found that UC patients were more likely never to have been breast-fed than controls (15/51 *vs* 12/102, $P = 0.005$), but there were no differences between CD patients and controls (11/57 *vs* 22/114, P = NS)[18]. However, a population-based case-control study in Sweden demonstrated a significantly shorter duration of breastfeeding in CD patients than in controls matched for sex and age (4.59 mo *vs* 5.76 mo, $P < 0.01$, n = 308 pairs)[59].

One group of investigators conducted two separate studies comparing infant feeding practices among children with IBD and their unaffected siblings. Compared to their unaffected siblings (n = 180), CD patients (n = 114) were less likely to have been breastfed [RR = 3.6 (95% CI 1.4-9.0), $P < 0.01$] and more likely to have received formula food from birth [RR = 3.1 (95% CI 1.3-7.4), $P < 0.02$]. CD patients were younger than their unaffected siblings ($P < 0.01$) but did not differ in gender, birth order, birth month, premature delivery, type of milk used for bottle feeding, age at introduction of solid foods, and length of exclusive and total length of breastfeeding. Multivariate analysis showed that only lack of breastfeeding and diarrheal diseases during infancy were independently associated with later development of CD[19]. In the second study, lack of breastfeeding did not differ significantly between UC patients (n = 93) and unaffected siblings (n = 138) [RR = 1.7 (95% CI 0.77-3.65), $P = 0.19$]. Multivariate analysis showed that children with UC were more likely than their unaffected siblings to be female ($P = 0.01$). UC patients and their unaffected siblings did not differ in age, duration of exclusive breastfeeding, total duration of breastfeeding, age at introduction of solid foods, birth order, or premature delivery[20].

A clinic-based pediatric study of 68 CD patients, 39 UC patients and 202 controls, demonstrated a protective effect of breastfeeding on development of CD [breastfeeding $\leqslant$ 5, 6-11, $\geqslant$ 12 mo *vs* not breastfeeding ψ 0.7 (0.3-1.5), 0.6 (0.2-1.5), 0.1 (0.01-1.10), respectively ($P_{trend} = 0.04$)], and a tendency toward a protective effect of breastfeeding on development of UC [breastfeeding $\leqslant$ 5, 6-11, $\geqslant$ 12 mo *vs* not breastfeeding ψ 0.7 (0.3-1.6), 0.5 (0.2-1.5), 0.2 (0.03-2.20), respectively ($P_{trend} = 0.07$)][25]. Both associations were controlled for maternal smoking. An Italian multi-center study of incident cases (594 UC patients and 225 CD patients) and randomly selected age and gender matched controls (patients with acute disease not related to smoking, OC use, or immunological disorders) showed an increased risk of IBD in those who had not been breastfed compared to those who had [UC ψ = 1.5 (95% CI 1.1-2.1) n = 594 pairs; CD ψ = 1.9 (95% CI 1.1-3.3) n = 225 pairs]. An increased risk of IBD was detected in subjects who had not been breastfed (controlling for smoking status and OC use), but was statistically significant only in females [UC ψ = 2.2 (95% CI 1.2-3.6) n = 240 pairs; CD ψ = 2.5 (95% CI 1.0-4.9) n = 106 pairs][14]. A Japanese study identified incident cases of IBD in children under the age of 15 years from a national epidemiological survey conducted from 1978 to 1993[60]. Healthy controls were matched to cases by age, sex, and block of birth. Children with CD were significantly less likely to have been breastfed during the first 4 mo of life than were healthy children [ψ = 0.3 (95% CI 0.13-0.70) n = 42 cases, 126 controls]. Children with UC were significantly less likely to have been breastfed during the first 4 mo of life than were healthy children [ψ = 0.53 (95% CI 0.31-0.89) n = 133 cases, 266 controls].

Quite a few smaller studies have evaluated the relationship between breastfeeding and development of IBD. Several of these studies showed a trend toward a protective effect of breastfeeding on the development of IBD, but were too small to achieve statistical significance[16,22,26,61]. Three studies conducted as postal questionnaires all showed no association between breastfeeding and either CD or UC[24,62,63]. These postal questionnaire studies in which cases identified their own controls may have suffered from selection bias[24,62,63] and one of these studies had a very poor response rate thus creating a potential for non-respondent bias[63]. Two large studies failed to demonstrate any differences between cases and controls with respect to breastfeeding in infancy[21,23]. Breastfeeding data for one of these studies was limited to that which was obtained from the hospital chart at the time of the child's delivery thus creating potential for differential misclassification bias[21]. Many of these studies that did not demonstrate a protective effect of breastfeeding on the development of IBD did not characterize breastfeeding as exclusive or mixed and did

not report the duration of breastfeeding. Furthermore, many of these studies did not include potential confounders of the relationship between breastfeeding and IBD. These confounders include family history of IBD, cigarette smoking, OC use, preceding infections, antibiotic use, and various perinatal factors.

Recently, a meta-analysis of all these studies was conducted[64]. Studies were graded based on predefined guidelines. Criteria for the highest grade included recruitment of cases and controls by the investigators, confirmation of diagnosis by a physician, confirmation of breastfeeding information by subjects' mothers or other close relatives, and response rate of at least 80% for both cases and controls. Only four studies received the highest grade for CD[19,21,25,59] and four for UC[20,21,25,58]. Based on all studies of the relationship between breastfeeding and IBD, there was a protective effect of breastfeeding on both CD [ψ_{pooled} = 0.67 (95% CI 0.52-0.86) $P < 0.001$ (heterogeneity test)] and UC [ψ_{pooled} = 0.77 (95% CI 0.61-0.96) P = 0.004 (heterogeneity test)]. Based on the highest grade of studies, the effect of breastfeeding was even more pronounced for both CD [ψ_{pooled} = 0.45 (95% CI 0.26-0.79) P = 0.063 (heterogeneity test)] and UC [ψ_{pooled} = 0.56 (95% CI 0.38-0.81) P = 0.268 (heterogeneity test)][65]. The investigators concluded that their meta-analysis supported the hypothesis that breastfeeding is protective for both CD and UC and that the actual effect is probably greater than their analysis demonstrated due to nondifferential misclassification in some of the studies analyzed.

Subsequently, a population-based, pediatric matched case-control study of environmental risk factors and development of IBD was conducted in Northern France[66]. All IBD cases diagnosed between 1988 and 1997 who were under 17 years of age and resident in the study area at the time of diagnosis were recruited for the study. Randomly selected controls were matched to cases by age, sex, and living area. Subjects were interviewed by trained interviewers and answers were validated using the mandatory child health booklet. Controlling for maternal education level, breastfeeding was an independent risk factor for CD [ψ = 2.1 (95% CI 1.3-3.4) P = 0.003, n = 222 pairs] as were family history of IBD, history of eczema, and BCG vaccination[66]. Drinking tap water (*vs* bottled water or well water) was a protective factor for CD. Regarding the unexpected finding of breastfeeding as a risk factor for CD, the investigators speculated that this association might be the result of either delayed infections at weaning or environmental contamination of the breast milk in the highly industrialized region in which the study was conducted. In the same study, there was no association between breastfeeding and development of UC. Controlling for maternal education, risk factors for UC included family history of IBD, disease during pregnancy, and bedroom sharing, but appendectomy was a protective factor for UC[66].

After publication of this case-control study[66], which met the criteria for the highest grade, the meta-analysis was repeated[64]. Including this study, the protective effect of breastfeeding on the development of CD was diminished [ψ_{MH} = 0.62 (95% CI 0.27-1.43)], but the protective effect of breastfeeding on the development of UC was not altered significantly [ψ_{MH} = 0.62 (95% CI 0.43-0.91)]. More importantly, the inclusion of the most recent study resulted in a much higher heterogeneity for the CD studies ($P < 0.001$, chi-square heterogeneity test). The investigators offered several possible explanations for the surprising different results of the highest quality studies. These included differences in genetic characteristics of the studies' populations, subtypes of CD with different etiologies, and variations in the components of breast milk in the different regions studied[64].

SUMMARY

Despite extensive investigation, the etiology of IBD is still unknown. Clearly, a genetic predisposition to IBD exists[28-30]. Genome-wide scanning studies have identified a series of IBD susceptibility loci, some of which are more strongly associated with CD, others with UC, and some with both[38-40]. Three separate mutations in the *NOD2* gene have been identified that confer susceptibility to CD[41,43], but no specific mutations that confer susceptibility to UC have yet been identified. Despite the strong evidence of genetic predisposition to IBD, it is clear that environmental factors also influence the development of IBD. However, only cigarette smoking has a well established association with IBD. Paradoxically, cigarette smoking is a risk factor for CD, but a protective factor for UC[12]. OC use may also play a role in the etiology of CD although, obviously, only in women[13,14]. Numerous dietary components may play a role in the etiology of IBD although these associations are less certain[16,17]. Many investigators have identified associations between preceding infections and the development of IBD[13,18-20,23,24] and some have identified associations between antibiotic use and development of IBD[23,24]. Many perinatal factors have been studied, but no consistent findings have been reported[21-23,25-27]. Although there have been conflicting reports, meta-analysis of these reports indicates that breastfeeding is a protective factor for both CD and UC[65].

Human breast milk contains many substances that may influence growth and development as well as function of the gastrointestinal tract. Some of these factors may have age-dependent effects[67]. Furthermore, the composition of colonic flora differs between breastfed and bottle-fed infants[68]. IBD pathogenesis is presumed to be a complex interaction between genetic predisposition and inappropriate activation of the mucosal immune system driven by the presence of enteric flora and resulting in tissue injury[1-3]. Thus, it seems quite plausible that breastfeeding would have a protective effect on the development of IBD in genetically predisposed individuals, at least in childhood.

The preponderance of evidence suggests that breastfeeding is a protective factor for IBD, with a greater effect for CD than UC[14,18,19,22,25,58-60]. A meta-analysis of all available studies, taking into account the design of the studies, demonstrated this protective effect of breastfeeding on the development of IBD[65]. However,

this relationship has become more tenuous following the most recent study of the relationship between breastfeeding and IBD[64,66]. Why some studies showed a protective effect of breastfeeding, some showed no effect, and two showed that breastfeeding is a risk factor for IBD is unclear. Proposed explanations include differences in genetic characteristics of the populations studied, subtypes of CD with different etiologies, and variations in the components of breast milk in the different regions studied[64]. The heterogeneous findings may also result from differences in study design. Specifically, the heterogeneous findings may be due to the failure to control for genetic predisposition. Since IBD is thought to occur in genetically predisposed hosts, inclusion of subjects whose genetic predisposition is unknown may be inappropriate. Since estimates of the frequency of NOD2 variants in the Caucasian population range from 4% to 20%[37,43,44,69], inclusion of general population controls may confound results of the investigation.

To date, no studies of the relationship between breastfeeding and IBD have incorporated genetic predisposition into the study design. Two studies have been conducted using unaffected siblings of cases as controls[20,21]. The first of these studies found that children with CD were less likely to have been breastfed [RR = 3.6 (95% CI 1.4-9.0) $P < 0.01$] than their unaffected siblings[19]. This is the strongest association between breastfeeding and CD in any of the published studies. The second study found that children with UC were less likely to have been breastfed than their unaffected siblings but the difference was not statistically significant[20]. Genetic predisposition is important in the etiology of both CD and, to a lesser degree, UC. These two studies were completed long before the discovery of the NOD2 variants that confer susceptibility to CD[41,43] and no susceptibility genes for UC have yet been identified. Nevertheless, these two studies may better reflect the true relationship between breastfeeding in infancy and the subsequent development of IBD than any of the other published studies. To better elucidate the relationship between breastfeeding, or any environmental factor, and the development of IBD, future studies should be conducted in such a way that genetic susceptibility to IBD is considered. Specifically, future studies of the etiology of IBD should be designed such that both environmental factors and genetic factors are incorporated in the same study and gene-environment interaction should be assessed.

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