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EDITORIAL

Hygiene hypothesis in inflammatory bowel disease: A critical review of the literature

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Abstract

The hygiene hypothesis is thought to be a significant contributor to the growing incidence of inflammatory bowel disease (IBD) around the world, although the evidence for specific factors that underlie the hygiene hypothesis in IBD is unclear. We aimed to systematically review the literature to determine which hygiene-related factors are associated with the development of IBD. Publications identified from a broad based MEDLINE and Current Contents search between 1966 and 2007 on key terms relevant to the 'hygiene hypothesis' and IBD including *H pylori* exposure, helminths, cold chain hypothesis, measles infection and vaccination, antibiotic use, breastfeeding, family size, sibship, urban upbringing, day care attendance and domestic hygiene were reviewed. The literature suggests that the hygiene hypothesis and its association with decreased microbial exposure in childhood probably plays an important role in the development of IBD, although the strength of the supporting data for each of the factors varies considerably. The most promising factors that may potentially be associated with development of IBD include *H pylori* exposure, helminths, breastfeeding and sibship. However, the vast majority of studies in this area are plagued by serious methodological shortcomings, particularly the reliance on retrospective recall of information making it difficult to truly ascertain the importance of a 'hygiene hypothesis' in IBD. The 'hygiene hypothesis' in IBD is an important area of research that may give clues to the aetiology of this disease. Directions for future research are recommended.

Key words: Inflammatory bowel disease; Hygiene hypothesis; Microbial exposure; Cold chain hypothesis; *H pylori*; Helminths; Measles; Antibiotic; Breastfeeding; Child care

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract[1]. Crohn's disease and ulcerative colitis represent the two most common forms of the condition, with both associated with significant morbidity[2,3]. While the aetiology of IBD remains obscure, it is thought to be the result of a combination of genetic and environmental factors[4]. Although genetic factors including NOD2, IL-23R and ATG16 genes have been implicated in Crohn's disease (CD) and to a lesser degree in and ulcerative colitis (UC)[5,6], they do not account for the striking rise in the incidence of IBD seen over the course of the 20th century[7], raising a strong possibility for an environmental hypothesis in IBD. Only two environmental influences (smoking and appendectomy)[8,9], however, are well established risk factors for IBD, although many others (including oral contraceptives and diet)[10,11] have been proposed to be important in the condition but with inconsistent results[12]. One promising group of environmental factors that may be potentially associated with IBD are those related to the "hygiene hypothesis"[13], which is closely linked to decreased microbial exposure in childhood.

In this review we aim to critically review the evidence for a hygiene hypothesis in IBD by examining factors that are directly linked to microbial exposure, factors that modify the response to infection and proxy measures for microbial exposure. Understanding the role of environmental factors such as these is critical for the possible prevention of IBD in genetically predisposed individuals as well as potentially offering disease improvement to those already suffering with the disease.

THE HYGIENE HYPOTHESIS

The hygiene hypothesis as a potential explanation for IBD comes from observations that the rise in the incidence of IBD, both in developed and developing countries[14,15], has coincided with improvements in hygiene over the 20th century. These improvements in hygiene include access to clean water, a hot water tap, a smaller family size and

thus less crowding, non-contaminated food and hygiene products such as toothpaste[16,17]. There are suggestions however that the rise in allergic and autoimmune disorders[18] over the last century, particularly in developing countries may not be accurate. Based on data from the World Health Organisation it is evident that there is extremely poor coverage of even basic demographic information such as vital registration of deaths[19]. Moreover, so-called Third World economies cannot provide the necessary public infrastructure necessary for diagnosis and reporting of these types of conditions, thus raising doubts as to the importance of a hygiene hypothesis in these disorders.

Nonetheless, Strachan (1989) was the first to link the hygiene hypothesis to the rise in allergic diseases[13]. He reported an inverse relationship between family size and the development of atopic disorders. The hygiene hypothesis is based on the possibility that a child could be overprotected from exposure to common infectious agents in the environment owing to improved hygiene[20]. If the child then comes into contact with a pathogenic infectious agent later in life (delayed exposure), an inappropriate immunologic response is triggered that could lead to the development of an abnormal or ineffective inflammatory process and possibly even IBD.

MICROBIAL EXPOSURE

Improved hygiene is believed to result in a limited exposure to micro-organisms[21]. Such exposure is thought to be necessary in programming the immune system of the gut and mitigating its future inflammatory responses, perhaps even resulting in CD when the immune system is challenged[21]. The underlying premise is that early childhood infection helps to establish the immunological balance between pro-inflammatory Th1 and tolerance-inducing regulatory T cells, preventing the subsequent untoward responses to allergens, microbial or other antigenic stimuli[22]. Thus various childhood circumstances such as day care attendance, presence of siblings, and domestic hygiene-related factors can influence the probability of contracting a "viral infection" at a vulnerable time in immunological development.

We performed a broad based MEDLINE and Current Contents search between 1966 and 2007 on key terms relevant to the "hygiene hypothesis" and IBD including *H pylori* exposure, helminths, cold chain hypothesis, measles infection and vaccination, antibiotic use, breastfeeding, family size, sibship, urban upbringing, day care attendance and domestic hygiene were reviewed. The reference lists from all relevant studies located in this process were then used to trace other studies to provide systematic coverage of relevant studies in this area.

FACTORS DIRECTLY LINKED TO MICROBIAL EXPOSURE

H pylori exposure

Multiple studies since 1994 have found a significantly lower seroprevalence of *H pylori* in patients with IBD compared to both matched controls, and "disease" controls[23-25]. The prevalence of this bacterial infection is lower in CD compared to UC, in the majority of these studies. Potential confounders include the effect of salazopyrine, other 5-ASA compounds, and antibiotics on the carriage and eradication of *H pylori*[26,27]. However, neither salazopyrine nor the other 5-ASA drugs used in IBD reach the concentrations required in the corpus and antrum of the stomach to effect *H pylori* eradication. In addition, studies of IBD patients and disease control groups including patients with COPD[28], indicate that the *H pylori* rates in those individuals exposed to multiple antibiotics are in fact higher compared to those exposed to either no antibiotics or fewer courses.

This reciprocal relationship, similar to that seen between UC and appendectomy[9], has also been linked to subtle changes in disease natural history. Vare *et al*, investigating the relationship between *H pylori* and CD, found that seropositive patients presented at a significantly later age (40 years) compared to seronegative CD patients (30 years, $P < 0.001$)[29]. Modification of phenotype was also identified in a separate study in which seropositive non-smoking CD patients had significantly fewer relapses and a lower risk of bowel resection compared to seronegative non-smoking patients ($P < 0.01$ and $P < 0.05$, respectively)[30].

These observations support a protective role for *H pylori* in both the development and natural history of CD, and need further investigation to determine whether this organism is acting as a surrogate marker of childhood exposure, or may have a more direct and wide-ranging effect on gastrointestinal immune development.

Helminths and IBD

With greater urbanisation and other hygiene practices introduced over the last century has come the decreased acquisition of soil-borne helminths in infants. Helminths are thought to play an important immunoregulatory role in the intestinal flora and as such have been linked to the development of IBD[31]. Firstly helminthic infection is associated with a strong Th2 response, which opposes the Th1 response associated with autoimmune disease and CD[32-34]. Secondly, chronic infection with these organisms may generate a network of regulatory T (Treg) cells that secrete transforming growth factor (TGF)-B and interleukin (IL)-10[33,35]. These cytokines may not only regulate aggressive Th1 responses but also control heightened Th2 responses that contribute to chronic allergic diseases. While there is a wealth of data from animal models to support an immunological role for helminths[32-34] there are limited data to confirm these pathways in the human gut. Moreover helminths infection may bring other anti-colitis mechanisms into play, including increases in mucus and water secretion into the gut lumen[36,37], which may influence the interaction between gut bacteria, their products, and a diseased epithelium, as well as impacting on intestinal motility. Helminths may affect microbial ecology[38] and the neuroendocrine response[39], but none of these factors however have been assessed in human studies. While preliminary results

from human trials in IBD patients using *Trichiuris suis*, or pig whip worm, demonstrate clinical efficacy[40] and no significant allergic disease post infection[41]. Long-term data, particularly after repeated exposure however are urgently needed to confirm these results. Thus, helminths may offer a novel therapeutic avenue in IBD patients, but much more research is needed to fully understand the mechanism behind any potential human association.

Cold chain hypothesis

The cold chain hypothesis shows temporal and geographical coincidences between the development of the refrigerator and the outbreak of Crohn's disease[42]. It is also possible that external factors related to the cold chain hypothesis including machine maintenance procedures, food conservation habits and the availability of electricity may also explain these trends[42]. The potential link between the refrigeration of food and Crohn's disease is *via* exposure to psychrotrophic bacteria with pathogenic properties such as *Listeria monocytogenes*, *Yersinia enterocolitica*, *Clostridium botulinum* and *Bacillus cereus* which are bacteria that are capable of surviving or developing at low temperatures[43]. Some of these bacteria have been identified in CD lesions[44,45]. A recent study by Forbes and Kalantiz (2006) found that the average age of having a fridge was more than 4 years earlier in older IBD patients than in age-matched controls, a difference that was significant[46]. While a greater exposure of the gastrointestinal tract to psychrotrophic bacteria in the early years of life may be a contributory factor to the development of Crohn's disease it is not yet considered to be an independent risk factor for the disease.

Childhood infections

An infectious aetiology has been proposed for IBD based on studies finding a significantly higher frequency of infectious events e.g. gastroenteritis during the first 6 mo after birth especially among future cases of CD[47,48]. A questionnaire-based study examining both adults and children with IBD have reported an association between the incidence of gastroenteritis and diarrhoea in infancy and the later development of CD[49]. However, an international multicenter study found no difference in the frequency of gastroenteritis in patients with IBD versus control subjects[50]. While numerous infectious agents such as paramyxoviruses[51] and Mycobacterium paratuberculosis[52] have been implicated in CD without consistency[53], it is the measles virus that has received the most attention.

The evidence on whether the measles virus increases the risk of IBD has come from several sources. The first is based on the biological plausibility of a measles virus in IBD. It is hypothesized that the measles virus is able to persist within the mesenteric endothelium creating an inflammatory reaction characteristic of CD[54]. While several studies have identified the presence of the measles virus in tissue samples[55,56], this finding has not been confirmed in studies using more sensitive methods such as the polymerase chain reaction (PCR)[57,58].

Another group of studies have examined the association of *in utero* and perinatal measles exposure and the development of IBD. Ekbom *et al* (1996) reviewed the hospital charts from 25 000 deliveries at a Swedish hospital between 1940 and 1949[59]. They found 4 cases of measles infection during pregnancy and of these, three of the offspring had developed CD. This study however suffered from some important biases including the selection of a period of time when it was known that in two cases of measles in the mother were followed by CD in the offspring. Moreover the diagnoses of maternal measles was based on clinical criteria only and the retrospective design makes it impossible to determine whether it is the measles exposure or some other factor during pregnancy that led to the subsequent development of CD in these children. Moreover studies from Denmark[60] and the UK[61] have failed to detect any cases of IBD after exposure to measles virus *in utero*. A history of perinatal infection has been reported to increase the risk of developing CD by five times[62], although the risk for IBD from measles infection specifically is much lower at 1.5 and is not significant[63]. Moreover Haslam *et al* (2000) did not find an increased risk for Crohn's among children born in years with high measles incidence rates versus children born in other years[64].

In summary the evidence supporting an association between measles infection in terms of in situ detection of measles virus, in utero and perinatal exposure and ecological evidence is discordant, but does not appear to be a causative factor in this condition.

FACTORS MODIFYING THE RESPONSE TO INFECTION

Childhood vaccinations

Childhood vaccinations have the ability to alter the maturation of the intestinal and systemic immune system and as such have been implicated in IBD[65]. Thompson *et al*, 1995) were the first to raise the possibility of a link between measles vaccination and IBD[65]. In a cohort analysis of 3545 individuals in the U.K. they showed that individuals with a history of exposure to measles vaccination were 3 fold and 2.5 fold more likely to develop CD and UC, respectively, compared with unvaccinated controls. Methodological shortcomings such as selection of participants from different populations, differential loss to follow-up between the two cohorts, and different ascertainment of outcome by exposure category, however, all dampened the strength of their findings. Subsequent cohort studies were also unable to confirm these initial findings[66,67].

Further controversy was fuelled when Wakefield *et al* reported 8 out of 12 children with non-specific colitis had symptoms attributable by the parents to the measles, mumps and rubella (MMR) vaccination[68]. Several issues related to selection and recall bias, no control group and lack of a clear case definition raised concerns as to the validity of these claims. In response to public concern, a case control study from the Vaccine Safety Datalink Project was conducted to address the link between MMR and risk of IBD[69]. Cases were 142 patients with chart confirmed IBD born 1958 to 1989 who were compared

to 432 controls matched by birth year, gender and health maintenance organisation. There was no evidence that either monovalent measles vaccine or the combination of MMR vaccination was associated with risk of IBD. One study found a significantly increased risk for CD with increasing age, but this was based on three children vaccinated at 2 years or older[67].

Evidence from ecological studies has generally failed to find a link between the incidence of measles vaccination and IBD[70-72]. The incidence of CD has been reported to be increasing since the 1940's, twenty years before the introduction of the measles vaccine.

Although this is a controversial area of research, the findings from well-designed studies do not support a link between vaccines containing measles and the subsequent development of IBD.

Antibiotics and development of IBD

The critical relationship between the human gut and its microflora has been highlighted in the field of IBD, particularly with the development of molecular models of the disorder such as the IL-10 knockout mouse[73]. These models clearly indicate that IBD does not develop in a germ-free environment. The role of gut bacteria has been further underlined by recent genetic discoveries that point to disturbances in the recognition and handling of bacteria in CD[74].

Antibiotics can potentially influence and disturb this relationship, and therefore have been the subject of several case-control studies in IBD[50,75,76]. In addition, some authors have pointed to the temporal relationship between the introduction of antibiotics on a wide scale after the Second World War, and the significant increase in the incidence of IBD seen in the second half of the last century[77].

There are a limited number of studies that address the role of antibiotics in the aetiology of IBD. Two small early studies were both heavily influenced by recall bias and the potential for reverse causation[50,75]. These found a positive association between antibiotic use and CD. In view of the potential lead-in time between onset of symptoms and actual diagnosis of CD, it is perhaps not surprising then that there may be increased prescriptions for antibiotics given to CD patients in the 1-2 years prior to diagnosis. With this confounder in mind, Card *et al* analysed the General Practice Research database (GPRD) in the UK to pull out prospectively collected data on CD cases and controls[76]. This study assessed antibiotic usage, other medication use, and smoking status as well as addressing the above issue of lead-in time, by analysing data 2-5 years pre-diagnosis. The investigators found that 71% of 587 CD patients were prescribed antibiotics 2-5 years prior to diagnosis compared to 58% of 1460 controls (Adjusted OR 1.32 [1.05-1.65], $P < 0.001$). Although there was no obvious confounding by gender, age, or smoking status, there was a lack of specificity with respect to the type of antibiotic prescribed, and the finding of associations with other drug groups including those for neurological conditions and oral contraceptive pills. This indicated that the association with antibiotics may not be causal. However, the strong interaction between cases and use of tetracyclines, particularly in those subjects with no prior gastrointestinal symptoms nor gastrointestinal medications, was intriguing and may provide some guidance as to future studies in this area.

Breastfeeding

The role of infant feeding practices may play a role in the development of IBD by affecting the early exposure to dietary antigens. Some of the important differences between human breast milk and infant formula include immunoglobulins (IG's), lactoferrin, lysozyme, growth factors, allergic factors, carnitine and DHA & ARA. For example Lactoferrin is an iron-binding protein found in human milk but not in formulas. It limits the availability of iron to bacteria in the gut and alters which "healthy" bacteria will thrive in the gut. It has a direct antibiotic effect on bacteria such as *Staphylococcus spp.* and *E. coli*[78].

The majority of the evidence supports a protective effect of breastfeeding in UC and CD[79-81]. A recent study based on an Italian population of 819 IBD patients suggested lack of breastfeeding to be associated with an increased risk of both CD and UC[82]. These results were supported by paediatric studies in Canada and the United States that found paediatric CD patients were less likely to have been breastfed[83,84]. Gilat *et al* performed a 14-center, 9-country study looking at more than 400 IBD patients whose disease started prior to 20 years of age and who were younger than 25 years at the time of the study[50]. These investigators found no significant difference between patients and control subjects in the frequency of breastfeeding as well as consumption of cereal or refined sugar during infancy.

A major problem with studies in this area are their retrospective design and dependence on patient or maternal recall to complete questionnaires. Moreover no studies have examined the potential confounding effects on the development of IBD of environmental factors such as maternal exposure to endocrine disrupting chemicals and the subsequent levels in human breast milk[85]. Further prospective studies using larger sample sizes is necessary to strengthen the validity of these observations.

PROXY MEASURES OF MICROBIAL EXPOSURE

A range of factors including family size, sibship, birth order, urban upbringing, day care attendance and domestic hygiene such as presence of a hot water tap and flush toilets have been examined in IBD studies as a proxy marker of environmental exposures in early life. Table 1 summarises the methodologies of key studies in this area. It can be seen that there is a heavy reliance on adult case-control retrospective designed studies and while many variables have been studied; only a few are significantly consistent.

Family size/ sibship/ birth order

Family size can be used to indicate the level of over-crowding in a home, which has been associated with poor

Table 1 Key studies examining the association between IBD and proxy measures of the 'hygiene hypothesis'

Author	Sample	Study design	Variables examined
Gilat, 1987[50]	14 centres from 9 countries UC = 197; CD = 302 Diagnosis before 20 yr, all patients < 25 yr old Age-sex match controls	Case control Questionnaires	Siblings, birth order, breast feeding, infection, eczema, family history, vaccination, pregnancy factors
Rigas, 1993[84]	New York, USA Diagnosed between 1986-1990 CD = 68; UC = 39 Pediatric gastroenterology controls = 202	Case control Questionnaires	Sibship size, maternal age at birth, month of birth, breastfeeding, maternal smoking one more
Gent *et al*, 1994[92]	UK UC = 231; CD = 133 16-87 yr Age-sex matched controls from same general practice as cases	Case control Home interview	Housing in infancy, presence of a lavatory, hot water tap, separate bathroom
Duggan *et al*, 1998[93]	UK Consecutive weekly attendees at IBD clinics UC = 213, CD = 110 (aged over 15 yr) Aged-Sex match controls from hospital patients undergoing elective surgery	Case control Questionnaire	Previous surgery, childhood domestic circumstances before age 11 yr (heating, day care, toilets, fixed bathroom, bedroom sharing)
Sicilia *et al*, 2001[87]	Spain Gastroenterologists recruited incident cases of CD patients aged 10-79 yr = 103 Outpatient clinic patient controls matched for age, sex and urban/rural.	Case control Population based study Structured interview 95% response rate	Number of persons in home, number of bathrooms, availability of hot water
Montgomery *et al*, 2002[88]	Swedish inpatient register UC = 15 823; CD = 12 668 Controls from Swedish Census, Births & Deaths Register: age and location matched 79 546; 63 035, respectively	Case control Swedish Multi-generation register linking cases and controls with family history information	Siblings
Feeney *et al*, 2002[16]	UK (Grew up in UK) 16-45 yr CD = 139; UC = 137 attendees at Gastroenterology clinics	Case control General hospital Questionnaire	Hp seroprevalence, family, hot water tap, nursery attendance, indoor toilets, swimming pool use, number or cars, number of house moves, urban/rural location, pets
Hampe *et al*, 2003[89]	Germany Consecutive IBD patients identified from German Crohn's & Colitis association = 2351 Controls unaffected first degree relatives = 3364	Case controls Questionnaire	Availability of tap water, toilet, central heating, siblings and community size
Amre *et al*, 2006[22]	Canada Newly diagnosed CD < 20 yr old = 194 Orthopaedic patients controls matched for timing of diagnosis & area of residence	Hospital based Case control Structured questionnaires - mothers mostly answered for patients	Siblings, breastfeeding, day care, place of residency, hot water tap, toilets, number of inhabitants, number of rooms, availability of private bed, personal hygiene, pet ownership, infection, smoking history

hygiene and potential exposure to infection[86]. A small family size and thus a less propensity for exposure to infection has been associated with a lower risk for IBD[86]. Amre *et al* (2006) created a 'crowding index' which refers to the number of rooms in the home divided by the number of inhabitants living in the home and found the mean 'crowding index' to be lower in cases than controls and thus protective against CD[22]. No differences however were found between the numbers of people residing in the home during infancy (< 18 years) in IBD patients versus controls[87], although this study had limited power.

Siblings may influence the development of IBD by altering exposure patterns to microorganisms in early life by affecting acute manifestation of infection by influencing age of transmission and severity[88]. In a large Swedish case control study, Montgomery *et al* (2002) found having older siblings increased the risk for UC, even after controlling for multiple births, sex, maternal age, year of birth, region and socioeconomic class. This may be because older siblings risk exposing younger siblings to infection at a higher dose or at an earlier age. In contrast, having younger siblings was significantly associated with a decreased risk of CD. This effect was most pronounced when younger siblings were born within two years of the patient's birth. It is hypothesised that having younger siblings may prolong or re-expose the older sibling to the presence of micro-organisms, conferring some protective effect against CD through appropriate priming for mucosal immune function[88]. These findings however have not been replicated in smaller studies[22].

Birth order as a potential marker for the hygiene hypothesis in IBD has been examined in several studies with inconsistent results[50,89]. For example, Hampe *et al* (2003) found birth later in the sibling order was significantly associated with a lower risk for IBD (included patients with CD, UC and indeterminate colitis)[89]. This is because first-born children are usually exposed to infection later in life than younger siblings who come into contact with common viral and bacterial infection through older siblings. Rigas *et al* (1993) however did not find birth order to be significantly associated with either disease based on medical records[84].

Urban upbringing

It has been suggested that an urban upbringing represents a more "hygienic" childhood compared with a childhood spent on a farm or a rural location. Several studies report that a higher incidence of IBD in urban as opposed to rural areas[90,91]. Others however have failed to replicate this finding[16,87].

Day care attendance

Day care attendance in young children has the potential for exposing children to greater infections. Amre *et al* (2006) in a hospital- based case control study found day care attendance in the first 6 mo of life to be associated with an increased risk for CD[22]. Feeney *et al* (2002) however found no significant difference between IBD cases and controls in regards to pre-school attendance[16].

Domestic hygiene

Several domestic hygiene-related exposures have been investigated in relation to IBD. This is because good domestic hygiene may protect an individual from exposure to a full range of agents that programme the immune system in the gut during infancy, causing an inappropriate response, perhaps even CD, when exposure occurs at a later age[92]. Gent *et al* (1994) found CD to be 5 times more common in IBD patients whose first house had a hot water tap and a separate bathroom, but no association was reported for mains drainage or access to a flush toilet[92]. Moreover domestic hygiene variables were not associated with UC[93]. A similar finding was reported by Duggan *et al* (1998) who found availability of hot water before age 11 to be significantly associated with CD but not UC[93]. Others however have failed to find a significant association between availability of hot water, toilet facilities, bed sharing, source of drinking water and number of bathrooms and risk for CD[22,50,89] but some of these studies did not evaluate controls that were representative of the case population or had power problems[50]. Pet ownership[22] and animal exposure during childhood[94] have been associated with an increased risk for CD but are not consistent findings[16]. The frequent use of a swimming pool was significantly associated with CD, although the mechanisms behind the association remain unclear and could be more to do with socioeconomic status rather than hygiene related[16]. Most of the studies except one[50], however, rely on the retrospective collection of information *via* questionnaires making it difficult to rule out recall bias (Table 1).

SOCIOECONOMIC STATUS

Earlier studies cited socioeconomic status as an important factor in the development of IBD, in that a higher socioeconomic status was more prevalent in IBD patients versus controls[95,96]. A higher socioeconomic status may also confer less household crowding and better domestic hygiene (hot tap) and thus increase the risk for CD by limiting exposure to micro-organisms[86]. As hygiene practices have improved over the course of the 20th century, socioeconomic status has become less important in IBD. In a recent study by Bernstein *et al* (2001) no differences in socioeconomic status were found between IBD patients and controls[97].

FUTURE DIRECTIONS

Based on a systematic review of the literature it is evident that there is a strong need for future studies in this area to adopt a prospective design in a paediatric sample to avoid the pitfalls associated with retrospective studies such as recall bias. This is crucial as many factors proposed to be important in the hygiene hypothesis such as breastfeeding date back to birth and infancy. Population based studies are also important to overcome problems inherent with selection biases which may be present in the many patient based studies that dominate this area. The evidence suggests that it is also important to look at Crohn's disease and ulcerative colitis separately since exposure risks do not appear to be the same for both disorders. The need to establish and validate better markers for hygiene related factors is crucial as it is impossible to rule out the possibility that the relationship between IBD and a hygiene related factor such as sanitation for example is not an indirect effect that may serve as a mechanism for other yet unknown lifestyle factors. Future research should also be directed towards trying to understand the potential mechanisms underlying associations between hygiene related factors and IBD in the hope of providing clues to the aetiology of this disease.

CONCLUSION

IBD is a growing disease that is costly to the individual and society. The underlying premise of the hygiene hypothesis is that decreased microbial exposure in childhood may lead to the subsequent development of IBD appears to be plausible, although the strength of the evidence supporting this varies between many hygiene-related factors. Helminthic infection, *H pylori* exposure, antibiotic use, breastfeeding and sibship represent the most promising factors supporting the hygiene hypothesis in IBD, but carefully designed prospective evaluation is urgently needed.

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