Int J Colorectal Dis (2003) 18:413–417
DOI 10.1007/s00384-003-0484-1

ORIGINAL ARTICLE

Jochen Hampe
Kerstin Heymann
Michael Krawczak
Stefan Schreiber

Association of inflammatory bowel disease with indicators for childhood antigen and infection exposure

Accepted: 7 February 2003
Published online: 10 April 2003

J. Hampe · S. Schreiber (✉)
First Department of Medicine,
Christian Albrecht University,
Schittenhelmstrasse 12,
24105 Kiel, Germany
e-mail: s.schreiber@mucosa.de
Tel.: +49-431-5972350
Fax: +49-431-5971842

K. Heymann
Department of Surgery,
Franziskus-Krankenhaus,
Berlin, Germany

M. Krawczak
Institute for Medical Informatics
and Statistics,
Christian Albrecht University,
Kiel, Germany

Abstract *Background and aims:* Genetic susceptibility plays an important role in the predisposition to inflammatory bowel disease (IBD), but genetics alone cannot explain the six- to eightfold rise in incidence accompanying postwar socioeconomic changes in developed countries. It is presently unclear how environmental factors either trigger or modify the risk for and course of IBD in the presence of genetic susceptibility. *Patients and methods:* We evaluated 2,351 consecutive responses from IBD patients and from 3,364 of their unaffected first-degree relatives (parents/siblings) who completed a multi-item questionnaire with their physicians as part of a study of IBD genetics. All single-patient families were excluded from the analysis to avoid ascertainment bias, resulting in 120–133 independent cases for the analysis of environmental factors and 1,685 patients to be included in the analysis of birth rank. *Results:* No statistically significant association was observed between the presence of IBD and the availability of either (warm) tap water, water toilets, or central heating during childhood. However, higher birth rank (≥3) was significantly associated with a lower risk of IBD (odds ratio 0.68). *Conclusion:* Lower birth rank as a possible indicator of increased childhood infection exposure was associated with a higher risk for IBD. Future studies need to address the interaction of known genetic variations (e.g., in the *NOD2* gene) with environmental factors potentially mediating this effect.

Keywords Crohn's disease · Ulcerative colitis · Disease risk · Environment · Childhood hygiene

Introduction

A worldwide increase in the incidence of inflammatory bowel disease (IBD) has occurred since World War II. As corroborated by (a) the observed leveling of the north-south incidence gradient in Europe [25] and (b) incidence differences noted relative to migrant populations [23], socioeconomic improvements induce a shift from low risk to high risk for IBD (approx. six-eightfold increase). While it is difficult precisely to define "Western life-style", one of the possible explanations for this development involves changes in childhood living conditions that would have served to alter the maturation of the intestinal and systemic immune system. Among the factors involved, the amount and timing (late vs. early) of infection and antigen exposure may play an important role. This process cannot be assessed directly, but surrogate markers such as childhood hygiene, family size, and birth order have been used to measure the associated effects [1, 10, 17].

Individual aspects of the so-called "sheltered child" hypothesis of IBD [6, 11] have been investigated previously. Gent and colleagues [5] found an effect of childhood hygiene on the risk for Crohn's disease. The observed association between high levels of sanitation and hygiene in childhood and the incidence of Crohn's dis-

ease may, however, be indirect and may merely serve as a marker for other life-style facets in industrialized countries. Additional findings concerning family size and birth order have been inconsistent [6, 22] although a more recent publication has reiterated the association of birth order and IBD [15].

Familial clustering has been a consistent finding with IBD [2, 3, 20, 21]. Significant advances have been made towards a delineation of the genetic basis of the disease by defining the first genomic candidate regions [8, 24] and replicating them in independent populations [14, 19]. Recently *NOD2* has been identified as the first susceptibility gene for IBD in four different populations [7, 9, 18]. Homozygous carriers of the C-insertion mutation in the leucine-rich region of the *NOD2* gene have a very high risk of IBD, and no unaffected individual with this genotype had been observed in either of the studies.

It is still unclear how putative environmental risk factors interact with the susceptibility genotype [4]. Our interest was thus to investigate the effect of environmental factors on the relative risk of IBD and on disease type and characteristics. Therefore, a large number of spontaneous and familial IBD cases were collected and compared to unaffected relatives, who served as internal controls. This approach adds to population-based case/control studies in that it allows the investigation of environmental factors in the presence of genetic susceptibility.

Patients and methods

Study population

In July 1996, a letter introducing the present study was sent to all 8,340 members of the German Crohn's and Colitis Foundation (Deutsche Morbus Crohn und Colitis ulcerosa Vereinigung). Patients for whom the diagnosis of IBD had been documented by standard clinical, endoscopic, or radiological criteria, and for whom first-degree relatives (parents or siblings) were available were considered eligible for participation. The first 2,351 consecutive respondents (1,468 Crohn's disease, 651 ulcerative colitis, 232 indeterminate colitis) plus 3,364 of their unaffected family members were enrolled in the study. Patients were coded as indeterminate colitis if they had their diagnosis changed during the course of their illness.

All participants completed a multi-item questionnaire together with their physicians. Neither the physicians nor the patients were aware of the hypotheses to be investigated. From July 1996 to January 1998, a telephone hotline was available for patients and physicians to provide assistance if and when formal questions regarding the questionnaire arose. All patients gave informed consent before inclusion in the study. The study protocol was approved by the institutional ethics committees and by the regional government data protection authorities (Landesdatenschutzbeauftragter).

Clinical and epidemiological information

Information on the factors to be investigated in the present study was obtained through specific items in the questionnaire:

- Availability of warm tap water during the first 3 years of childhood (coded yes/no)
- Classification of community size for both the childhood and current place of residence (village: under 3000 inhabitants; town: 3,000–100,000 inhabitants; city: over 100,000 inhabitants)
- Number, age, and sex of children and siblings

Clinical information was obtained via questions that could be answered reliably by the family physician or the patient himself:

- Type of disease (Crohn's disease, ulcerative colitis, or undetermined colitis)
- Confirmatory diagnostic method used (endoscopy, histology, or radiology)
- Presence of stenoses, fistulae, extraintestinal manifestations (yes/no)
- Total duration of hospital inpatient stays (never, less than a month, 1–3 months, 3–6 months, >6 months)
- Total postdiagnostic duration of steroid medication more than 10 mg prednisolone or equivalent (never, less than 1 month, 1–3 months, 3–6 months, up to 1 year, more than 1 year)
- Total number of surgical operations

Quality management and exclusions of patients

Interaction with the family physician or gastroenterologist was guaranteed by a request for concurrent venipuncture (blood sample used for DNA preparation) at the physician's office. Questionnaires without an accompanying blood sample were not processed. To assess the validity of the answers given to the questionnaire all participants received a second questionnaire after 6–9 months repeating core items of the first survey in a different order and in different wording. Based upon the second survey additional reasons for exclusion were defined as (a) lack of completion of the second questionnaire, either because the patient was enrolled too recently (less than 6 months previously) or because they failed to complete the second questionnaire, and (b) irresolvable differences in answers given to the two questionnaires.

Risk analysis of environmental factors and family structure

For the sake of comparability over generations, the present study had to focus on the epidemiology of IBD among patients and their siblings. This means that only data from sibships were used, and that parental data were neglected unless a parent himself was affected or was a sibling of a patient. Furthermore, sibships were included only if in their questionnaires all members had given consistent answers regarding number, age, and sex of the siblings. In this case all healthy siblings were included in the analysis even if they had not themselves filled out a questionnaire. Families without a questionnaire from at least one affected member were excluded from the analysis. The above criteria resulted in the inclusion of 1,954 IBD patients and 289 unaffected siblings in the analysis. Single children and other small family structures were not found to be overrepresented in the sample compared with German birth rate statistics from 1960 to the present [26].

Statistics

Potential disease associations were assessed for statistical significance using the χ^2 test. Relative disease risks were quantified in

Table 1 Effect of environmental factors on the risk for inflammatory bowel disease (*OR* odds ratio, *CI* confidence interval)

Environmental factor	Patients		Unaffected sibs		OR	95% CI
	Present	Absent	Present	Absent		
Tap water	106	27	184	44	0.94	0.53–1.66
Warm tap water	67	55	109	112	1.25	0.78–2.00
Water toilet	102	30	155	64	1.40	0.83–2.39
Central heating	45	75	74	161	1.31	0.80–2.12

Table 2 Effect of sibship size on the risk for inflammatory bowel disease

Sibship size	Patients	Nonaffected relatives	Total
2 siblings[a]	94	143	237
>2 siblings[a]	71	124	195
Total	165	267	432

[a] Including index patient; only families with at least one patient and one additional sibling were included in this analysis; in addition, the index patients themselves were excluded from the analysis

the form of odds ratios with associated 95% confidence intervals. Family recruitment was carried out through index patients and was therefore not random. Any causal relationship between mere family size and one of the environmental factors studied here would have led to a spurious association with the disease state. To control for this possible ascertainment bias, one patient per family was excluded from the analysis of the environmental factors. This lead to a substantial reduction in the number of individuals available for analysis, leaving 120–133 patients with IBD and 219 – 235 unaffected siblings for inclusion. A similar reduction in sample size had to be applied in the analysis of sibship size. In the analysis of birth order, however, all patients were included as long as they had at least one sibling in the data set, i.e., only one-child families were excluded. Ascertainment bias is a serious issue with respect to birth order only if the patient is the sole child of a couple (in which case their birth rank would always be equal to 1). The exact numbers of individuals included in each of the analyses are given in Tables 1, 2, 3.

Results

Neither the availability of tap water, warm water, water toilet, nor the presence of central heating during childhood were significantly associated with the risk of IBD (Table 1). Nevertheless, a trend towards a higher risk was associated with the availability of warm water (odds ratio 1.25, 95% CI: 0.78 – 2.00), water toilet (odds ratio 1.40, 95% CI: 0.83 – 2.39), and central heating (odds ratio 1.31, 95% CI: 0.80 – 2.12).

Sibship size in families with two or more siblings was not significantly associated with the risk for IBD (Table 2, χ^2=0.48, 1 df, P>0.20). In the analysis of birth rank and disease risk, however, birth later in the sibling order was associated with a significantly lower risk for IBD (Table 3). A global χ^2 test (Table 3) showed that this trend was significant at the 95% level (χ^2=9.96, 4 df, P=0.04). When birth ranks are categorized as "low" (birth rank <3) or "high" (birth rank ≥3), an odds ratio of 0.68 for higher birth ranks was obtained (95% CI: 0.51–0.91). A collapsed χ^2 analysis showed that this risk reduction was highly significant (χ^2=7.13, 1 df, P=0.008).

Discussion

Population-based case-control studies including that of Gent and colleagues [5] have suggested that environmental factors (e.g., hygiene and sanitation in childhood) are major modifiers of the individual risk for IBD. These earlier studies indicated that the risk of developing Crohn's disease can increase by as much as fivefold in the presence of hot water supply, as opposed to a lack of warm tap water.

Surrogate markers of improved sanitation including (warm) tap water, water toilets, and central heating were

Table 3 Effect of birth rank on the risk for inflammatory bowel disease; only sibships with at least one patient and one additional sibling were included in the analysis (*OR* odds ratio, *CI* confidence interval)

Birth rank	Patients	Unaffected sibs	Given rank vs. higher ranks		Given rank vs. lower ranks	
			OR	95% CI	OR	95% CI
1	585	71	1.33	0.98–1.80	–	–
2	640	89	1.38	0.99–1.91	0.87	0.62–1.23
3	289	49	1.35	0.83	0.77	0.54–1.10
4	98	24	0.84	2.19	0.56	0.35–0.93
>4	73	15	–	–	0.70	0.39–1.30

also investigated in the present study. In our view, indicators of socioeconomic conditions can serve as surrogate measures of childhood exposure to infections and natural environmental antigens. Firstborn children are usually exposed to infection later in life (e.g., after entering childcare facilities or nurseries) than their younger siblings who might come in contact with common viral and bacterial infections through their siblings [13]. However, none of the investigated parameters was found to be statistically significantly associated with IBD, although a trend towards higher risks was observed for the availability of warm tap water, water toilet and central heating.

The lack of statistical significance of our results regarding environmental exposure is likely to reflect the limited power of the data due to the exclusion of single patient families. This correction, however, was necessary to avoid ascertainment bias due recruitment of families through index patients. This notwithstanding, the observed trends indicate that any potential effect, if it were to exist, is likely to be smaller than previously thought [5].

Higher birth order was found to be associated with a lower risk of IBD in the present study. The odds ratio of 0.68 for birth rank of 3 or higher is in agreement with result from a recent study in Swedish IBD patients [15] which reported an odds ratio of 0.83.

A more northern European, "Westernized" life-style has accompanied the economic development of all southern European countries. Therefore the previous north-south gradient in the incidence of IBD in Europe and its apparent leveling over the past 20 years appear to corroborate our own findings. Effects of small family size and the consequently lower or later exposure to common infections have been invoked for a number of conditions such as asthma, atopy [16, 17, 27], and multiple sclerosis [1, 10] to explain their increased incidence in Western countries. All these conditions, at least in part, have an autoimmune pathogenesis, and their increased frequency might reflect altered maturation processes of the immune system in childhood. However, the molecular mechanisms of these processes remain to be elucidated [12, 28].

In summary, our study adds evidence to the notion that indicators of childhood infection exposure are associated with an increased risk for IBD. The recently identified susceptibility gene for Crohn's disease, *NOD2*, is activated in response to lipopolysaccharide. This finding also supports our results in that an altered intestinal flora or an altered immune maturation of the intestinal lymphatic system may be the consequence of the life-style changes investigated in the present study. Overall the effects, however, are moderate and indicate the effect of other environmental factors that need to be studied more specifically in future studies.

Acknowledgements The authors thank all patients and colleagues who made this study possible through their cooperation. In particular, the following persons are gratefully acknowledged: Prof. Raedler, Krankenhaus Tabea, Hamburg; Dr. Theuer, Heilbronn; Prof. Kruis, Krankenhaus Kalk, Cologne; Dr. Meckler, Schlossbergklinik, Gedern; Dr. Herchenbach, Prosper-Hospital, Recklinghausen; Prof. Scheurlen, Uni-Klinikum, Würzburg; Dr. Demharter, Zentralklinikum, Augsburg; Dr. Simon, Munich; Dr. Purrmann, Moers; Dr. Jessen, Kiel; Dr. Zehnter, Dortmund; Drs. Lübke and Weismüller, Coblenz; Dr. Eiche, Denkendorf; Dr. Schönfelder, Uni-Klinikum, Aachen, all from Germany. This work was supported by the Deutsche Forschungsgemeinschaft, the German Crohn's and Colitis Association, the German National Genome Research Network, and a competence network for inflammatory bowel disease.

References

1. Alter M, Zhen-xin Z, Davanipour Z, Sobel E, Min Lai S, LaRue L (1987) Does delay in acquiring childhood infection increase risk of multiple sclerosis? Ital J Neurol Sci 8:23–28
2. Bayless TM, Tokayer AZ, Polito 2nd JM, Quaskey SA, Mellits ED, Harris ML (1996) Crohn's disease: concordance for site and clinical type in affected family members-potential hereditary influences. Gastroenterology 111:573–579
3. Colombel JF, Grandbastien B, Gower-Rousseau C, Plegat S, Evrard JP, Dupas JL, Gendre JP, Modigliani R, Belaiche J, Hostein J, Hugot JP, van Kruiningen H, Cortot A (1996) Clinical characteristics of Crohn's disease in 72 families. Gastroenterology 111:604–607
4. Ekbom A (1997) Epidemiology of inflammatory bowel disease. Curr Opin Gastroenterol 13:289–292
5. Gent AE, Hellier MD, Grace RH, Swarbrick ET, Coggon D (1994) Inflammatory bowel disease and domestic hygiene in infancy. Lancet 343:766–767
6. Gilat T, Hacohen D, Lilos P, Langman MJ (1987) Childhood factors in ulcerative colitis and Crohn's disease. An international cooperative study. Scand J Gastroenterol 22:1009–1024
7. Hampe J, Grebe J, Nikolaus S, Solberg C, Croucher PJ, Mascheretti S, Jahnsen J, Moum B, Klump B, Krawczak M, Mirza MM, Foelsch UR, Vatn M, Schreiber S (2002) Association of NOD2 (CARD 15) genotype with clinical course of Crohn's disease: a cohort study. Lancet 359:1661–1665
8. Hugot JP, Laurentpuig P, Gower-Rousseau C, Olson JM, Lee JC, Beaugerie L, Naom I, Dupas JL, Vangossum A, Orholm M, Bonaitipellie C, Weissenbach J, Mathew CG, Lennardjones JE, Cortot A, Colombel JF, Thomas G (1996) Mapping of a susceptibility locus for Crohn's disease on chromosome 16. Nature 379:821–823
9. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G (2001) Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 411:599–603

10. James WH (1988) Further evidence in support of the hypothesis that one cause of multiple sclerosis is childhood infection. Neuroepidemiology 7:130–133
11. Koutroubakis I, Manousos ON, Meuwissen SG, Pena AS (1996) Environmental risk factors in inflammatory bowel disease. Hepatogastroenterology 43:381–93
12. Maggi E, Parronchi P, Manetti R, Simonelli C, Piccinni F, Rugiu FS, et al (1992) Reciprocal regulatory effects of IFN-gamma and IL-4 on the in vitro development of human TH1 and TH2 clones. J Immunol 148:2142–2147
13. Martinez FD, Wright AL, Holberg CJ, Morgan WJ, Tassig LM (1992) Maternal age as a risk factor for wheezing lower respiratory illnesses in the first year of life. Am J Epidemiol 136:1258–1268
14. Mirza MM, Lee J, Teare D, Hugot JP, Laurent-Puig P, Colombel JF, Hodgson SV, Thomas G, Easton DF, Lennard-Jones JE, Mathew CG (1998) Evidence of linkage of the inflammatory bowel disease susceptibility locus on chromosome 16 (IBD1) to ulcerative colitis. J Med Genet 35:218–221
15. Montgomery SM, Lambe M, Wakefield AJ, Pounder RE, Ekbom A (2002) Siblings and the risk of inflammatory bowel disease. Scand J Gastroenterol 37:1301–1308
16. Mutius E von, Fritzsch C, Weiland SK, Roll G, Magnussen H (1992) Prevalence of asthma and allergic disorders among children in united Germany: a descriptive comparison. BMJ 305:1395–1399
17. Mutius E von, Martinez FD, Fritzsch C, Nicolai T, Reitmeir P, Thiemann HH (1994) Skin test reactivity and number of siblings. BMJ 308:692–695
18. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nunez G, Cho JH (2001) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 411:603–606
19. Ohmen JD, Yang HY, Yamamoto KK, Zhao HY, Ma Y, Bentley LG, Huang Z, Gerwehr S, Pressman S, McElree C, Targan S, Rotter JI, Fischel Ghodsian N (1996) Susceptibility locus for inflammatory bowel disease on chromosome 16 has a role in Crohn's disease, but not in ulcerative colitis. Hum Mol Genet 5:1679–1683
20. Orholm M, Munkholm P, Langholz E, Nielsen OH, Sorensen IA, Binder V (1991) Familial occurrence of inflammatory bowel disease. N Engl J Med 324:84–88
21. Peeters M, Nevens H, Baert F, Hiele M, de Meyer AM, Vlietinck R, Rutgeerts P (1996) Familial aggregation in Crohn's disease: increased age-adjusted risk and concordance in clinical characteristics. Gastroenterology 111:597–603
22. Persson PG, Leijonmarck CE, Bernell O, Hellers G, Ahlbom A (1993) Risk indicators of inflammatory bowel disease. Int J Epidemiol 22:555–560
23. Probert CS, Jayanthi V, Rampton DS, Mayberry JF (1996) Epidemiology of inflammatory bowel disease in different ethnic and religious groups: limitations and aetiological clues. Int J Colorectal Dis 11:25–28
24. Satsangi J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell JI, Jewell DP (1996) Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. Nat Genet 14:199–202
25. Shivananda S, Lennard Jones J, Logan R, Fear N, Price A, Carpenter L, van Blankenstein M (1996) Incidence of inflammatory bowel disease across Europe: is there a difference between north and south? Results of the European Collaborative Study on Inflammatory Bowel Disease (EC-IBD). Gut 39:690–697
26. Statistisches Bundesamt (1997) Haushalte und Familien. Metzler-Poeschel, Stuttgart
27. Strachan DP (1989) Hay fever, hygiene and household size. BMJ 299:1259–1260
28. Wisniewski HM, Kieth AB (1977) Chronic relapsing experimental allergic encephalomyelitis: an experimental model of multiple sclerosis. Ann Neurol 1:144–148